

REGULATABLE CONTROLLED EXPRESSION OF A TRANSGENE IN PRIMATES**CROSS-REFERENCE TO RELATED APPLICATIONS**

[0001] This Application claims benefit under 35 U.S.C. § 119(e) of the U.S. Provisional Application No. 63/459,850 filed April 17, 2023, and No. 63/459,853 filed April 17, 2023, the contents of each of which are incorporated herein by reference in their entireties.

TECHNICAL FILED

[0002] The technology described herein relates generally to methods and compositions for regulatable and controllable expression of a transgene, e.g., via multiple administrations of an inducer(s) to a subject, e.g., a primate subject.

BACKGROUND

[0003] Correcting disease phenotypes by the transfer of genetic material into cells is the very essence of gene therapy (GT). Since the 1st approved gene therapy procedure for severe combined immunodeficiency (SCID) in 1990, GT has been successfully deployed to revert diseased phenotypes in multiple human diseases. The remarkable progress and potential of this technology is reflected by 27 FDA approved cell and gene therapies as well as hundreds more in clinical pipelines. Indications that have approved treatments include spinal muscular atrophy (Zolgensma, Novartis), lymphoma (Kymriah, Novartis), Haemophilia A and B (Roctavin and Hemgenix, CSL Behring LLC) and RPE65 mutation-associated retinal dystrophy (Luxterna). The key component of GT is the delivery of genetic material to a cell; however, in early GT experiments it was discovered that this had the potential to cause toxicity at a cellular and systemic level. Therefore, innovations to improve the expression cassette such as reducing CpG content, codon optimisation, reduction of viral components and tissue specific promoters have been vital in enabling the development of safe and efficacious therapies. Despite these advances all approved DNA-based gene therapies rely on the use of constitutively active promoters which gives you no control over the expression level of the transgene once the therapy has been delivered. This lack of control is the cause of adverse outcomes in patients as overexpression or under expression of the transgene could decrease the efficacy of the treatment or lead to toxicity. Indeed, it is known that overexpression of the B domain deleted factor VIII causes significant protein folding stress in hepatocytes which is known risk factor for hepatocellular carcinoma.

[0004] In addition, there are significant differences in physiology between rodents and primates. The preclinical results obtained in rodents are not easily extrapolated to humans, and preclinical data generated in rodents has limited predictive power in humans. Therefore, to increase the efficacy and safety of GT treatments, methods whereby expression levels could be

exquisitely controlled in a robust and predictable manner, especially in primates, are needed. The present disclosure addresses this need.

SUMMARY

[0005] In one aspect provided herein is a method of regulating expression of a transgene in a subject, e.g., a primate subject. The method comprises: (a) administering to a subject, e.g., primate subject at a first time point a first inducer (e.g., administering a dose of a first inducer at a first time point), wherein the subject, e.g., primate subject comprises at least one cell comprising an exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene, wherein the inducible promoter comprises a cis-regulatory element (CRE) that is capable of being bound and activated by a heterodimer of CAR and RXR (i.e., the inducible promoter comprises at least one PBREM enhancer), and wherein the first dose comprises an amount of the inducer that is sufficient to express a measurable level of the transgene in the subject; and (b) administering to the subject at least at a second time point a second inducer (e.g., administering a dose of a second inducer at a second time point) to continue expression of the transgene at a predetermined level for a predetermined time or to modulate expression of the transgene to a predetermined level. The first and second inducers can be same or different. In some embodiments of any of the aspects, at least the first or the inducers are each, independently, artemisinin, a derivative of artemisinin, or a flavonoid compound. For example, then first and second inducers are each, independently, artemisinin or a derivative thereof.

[0006] In some embodiments, the method further comprises administering to the subject at least at a third time point a third inducer (e.g., administering a dose of a third inducer at a third time point) to continue expression of the transgene at a predetermined level for a predetermined time or to modulate expression of the transgene to a predetermined level. The third inducer can be the same as or different than the first inducer. The third inducer can be same or different than the second inducer. Stated another way, the first, the second and third inducer can be all same, all different, or two same (e.g., first and second same, or first and third same, or second and third same) and one different.

[0007] In some embodiments of any of the aspects, the a) first inducer and b) second inducer and third inducer are different, and the second inducer and third inducer are the same. In some embodiments of any of the aspects, the first inducer is a first artemisinin derivative, and the second inducer and third inducer are a second artemisinin derivative. In some embodiments of any of the aspects, the first inducer is artemisinin, and the second inducer and third inducer are an artemisinin derivative. In some embodiments of any of the aspects, the first inducer is an artemisinin derivative, and the second inducer and third inducer are artemisinin.

[0008] In another aspect provided herein is a method of regulating expression of a transgene in a subject, e.g., a primate subject. The method comprises: administering artemisinin or a derivative thereof to the subject, e.g., primate subject, wherein the subject, e.g., primate subject comprises at least one cell comprising an exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene, wherein the inducible promoter comprises a CRE that is capable of being bound and activated by a heterodimer of CAR and RXR (i.e., the inducible promoter comprises at least one PBREM enhancer), wherein the artemisinin or a derivative thereof is administered in an amount that is sufficient to express a measurable level of the transgene in the subject. In some embodiments of this, artemisinin or a derivative thereof is administered to the subject, e.g., primate subject following the prior administration of an inducer to the subject, e.g., primate subject, and optionally, the inducer is administered in an amount that is sufficient to express a measurable level of the transgene in the subject. In some cases, the inducer of the prior administration is different from the artemisinin or a derivative thereof administered to the subject. Alternatively, the inducer of the prior administration is same as the artemisinin or a derivative thereof administered to the subject but at a different dose.

[0009] In another aspect provided herein is a method of regulating expression of a transgene in a subject, e.g., a primate subject. The method comprises: administering a flavonoid compound to the subject, e.g., primate subject, wherein the subject, e.g., primate subject comprises at least one cell comprising an exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene, wherein the inducible promoter comprises a CRE that is capable of being bound and activated by a heterodimer of CAR and RXR (i.e., the inducible promoter comprises at least one PBREM enhancer), wherein the flavonoid compound is administered in an amount that is sufficient to express a measurable level of the transgene in the subject. In some embodiments of this, the flavonoid compound is administered to the subject, e.g., primate subject following the prior administration of an inducer to the subject, e.g., primate subject, and optionally, the inducer is administered in an amount that is sufficient to express a measurable level of the transgene in the subject. In some cases, the inducer of the prior administration is different from the flavonoid compound administered to the subject. Alternatively, the inducer of the prior administration is same as the flavonoid compound administered to the subject but at a different dose.

[0010] In another aspect provided herein is a first and at least a second inducer for use in a method of regulating expression of a transgene in a subject, e.g., a primate subject, the method comprising: (a) administering to the subject at a first time point a first inducer (e.g., administering a dose of a first inducer at a first time point), wherein the subject, e.g., primate subject comprises at least one cell comprising an exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene, wherein the synthetic inducible promoter comprises a CRE that is

capable of being bound and activated by a heterodimer of CAR and RXR (i.e., the inducible promoter comprises at least one PBREM enhancer), wherein the first dose comprises an amount of the inducer that is sufficient to express a measurable level of the transgene in the subject; and (b) administering to the subject at least at a second time point a second inducer (e.g., administering a dose of a second inducer at a second time point) to continue expression of the transgene at a predetermined level for a predetermined time or to modulate expression of the transgene to a predetermined level. In some embodiments, the first and second inducer are independently artemisinin, a derivative of artemisinin, or a flavonoid compound. In some embodiments, the first and second inducer are independently artemisinin or a derivative of artemisinin,

[0011] It is noted that the first and second inducers can be same or different.

[0012] In some embodiments, the first inducer is artemisinin or a derivative thereof. For example, the first inducer is artemisinin, artesunate, dihydroartemisinin, artemether or artemether. In some embodiments, the first inducer is artemisinin or artesunate.

[0013] In some embodiments, the first inducer is a flavonoid compound. For example, the first inducer is flavone. For example, the first inducer is flavone.

[0014] In some embodiments, the second inducer is artemisinin; derivative of artemisinin; a flavonoid compound, e.g. flavone, chrysin, baicalein, or galangin; 1,4-Bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP); phenobarbital (PB); 6-(4Chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde-O-(3,4-dichlorobenzyl)oxime (CITCO); Acetaminophen; Buprenorphine; Phenytoin; Carbamazepine; Valproic Acid; Chlorpromazine; Efavirenz; Nevirapine; Rilpivirine; Etravirine; Diazepam; Cyclophosphamid; Ifosfamide; Cerivastatin; Simvastatin; lovastatin; substituted sulphonamides; Thiazolidin-4-one; Estradiol; Estrone and analogues; 17 α -ethinyl-3,17 β -estradiol (EE2); Dehydroepiandrosterone (DHEA); 5 β -pregnane-3,20-dione; Diethylstilbestrol; Ginkgo *biloba* extract; Galangin; chrysin; baicalein; Diallyl sulphide; Ellagic acid; Resveratrol; Squalestatin-1; Bilobalide; Triclocarban; Triclosan; Dichlorodiphenyltrichloroethane (DDT); Dieldrin; Methoxychlor; Metofluthrin; Permethrin; Pyrethrins; Sulfoxaflor; Diethylhexyl phthalate (DEHP); Cyproconazole; Fluconazole; Propiconazole; FL81; Tri-p-methylphenyl phosphate (TMPP); UM104; or UM145.

[0015] In some embodiments, the second inducer is artemisinin or a derivative thereof. For example, the second inducer is artemisinin, artesunate, dihydroartemisinin, artemether or artemether. In some embodiments, the second inducer is artemisinin or artesunate.

[0016] In some embodiments, the second inducer is a flavonoid compound. For example, the second inducer is flavone. For example, the second inducer is flavone.

[0017] In some embodiments, the third inducer or a subsequent inducer is artemisinin; derivative of artemisinin; a flavonoid compound, e.g. flavone, chrysin, baicalein, or galangin; 1,4-

Bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP); phenobarbital (PB); 6-(4Chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde-O-(3,4-dichlorobenzyl)oxime (CITCO); Acetaminophen; Buprenorphine; Phenytoin; Carbamazepine; Valproic Acid; Chlorpromazine; Efavirenz; Nevirapine; Rilpivirine; Etravirine; Diazepam; Cyclophosphamid; Ifosfamide; Cerivastatin; Simvastatin; lovastatin; substituted sulphonamides; Thiazolidin-4-one; Estradiol; Estrone and analogues; 17 α -ethinyl-3;17 β -estradiol (EE2); Dehydroepiandrosterone (DHEA); 5 β -pregnane-3,20-dione; Diethylstilbestrol; Ginkgo *biloba* extract; Galangin; chrysin; baicalein; Diallyl sulphide; Ellagic acid; Resveratrol; Squalestatin-1; Bilobalide; Triclocarban; Triclosan; Dichlorodiphenyltrichloroethane (DDT); Dieldrin; Methoxychlor; Metofluthrin; Permethrin; Pyrethrins; Sulfoxaflor; Diethylhexyl phthalate (DEHP); Cyproconazole; Fluconazole; Propiconazole; FL81; Tri-p-methylphenyl phosphate (TMPP); UM104; or UM145.

[0018] In some embodiments, the third or a subsequent inducer is artemisinin or a derivative thereof. For example, the third or a subsequent inducer is artemisinin, artesunate, dihydroartemisinin, artemether or artemether. In some embodiments, the third or a subsequent inducer is artemisinin or artesunate.

[0019] In some embodiments, the third or a subsequent inducer is a flavonoid compound. For example, the third or a subsequent inducer is flavone. For example, the third inducer is flavone.

[0020] In some embodiments of any of the aspects, the time between administrations is at least 24 hours, 48 hours, 72 hours, 1 week, 10 days, 2 weeks, 3 weeks, or 4 weeks. In some embodiments of any of the aspects, the time between administrations is at least 24 hours, 48 hours, or 72 hours. In some embodiments of any of the aspects, the time between administrations is no more than 2 months, 3 months, 4 months, 5 months, or 6 months. In some embodiments of any of the aspects, the time between administrations is no more than 2 months.

[0021] In some embodiments of any of the aspects, the time between administrations is 5-7 days, 5-10 days, 7-10 days, 7-14 days, 10-14 days, 10-20 days, 14-21 days, 14-28 days, 21-28 days, or 21-34 days.

[0022] In some embodiments, the time between administration is about 24 hours. For example, an inducer is administered the subject every day (e.g., about every 24 hours). In some embodiments, an inducer is administered the subject every day (e.g., about every 24 hours) for a period of about one week to about 6 months. In some embodiments, an inducer is administered the subject every day (e.g., about every 24 hours) for a period of about two weeks to about 3 months. For example, an inducer is administered to the subject every day (e.g., about every 24 hours) for a period of about two weeks, about three weeks, about 4 weeks, about 5 weeks or about 6 weeks. In some embodiments, an inducer is administered the subject every day (e.g., about every 24 hours) for a period of about 15 days, about 30 days, about 40 days, about 45 days, about 50 days, about

65 days, about 75 days, about 80 days, or about 90 days. When the subject an inducer is administered to the subject every day (e.g., about every 24 hours), such dosing is also referred to as continuous dosing herein. Without wishing to be bound by theory, it is noted that continuous dosing shows no toxicity in the subject. Accordingly, in some embodiments, an inducer is administered to the subject every day (i.e., continuous dosing) for a period of about 15 days, about 30 days, about 40 days, about 45 days, about 50 days, about 65 days, about 75 days, about 80 days, or about 90 days, and the subject does not show signs of toxicity. It is noted that the same inducer can be administered every day or different inducers can be administered on different days. Further, amount of the inducer can be same or different on different days.

[0023] In some embodiments of any of the aspects, the inducer at the second time point (e.g., second inducer), or a subsequent time point (e.g. third inducer), is administered when it is determined that the level of expression of the transgene is decreased to 75% or less of the level of expression of the transgene within the 24 hours after administering at the first time point (e.g., the first inducer), or the previous time point (e.g., second time inducer). In some embodiments of any of the aspects, the inducer at the second time point (e.g., second inducer), or a subsequent time point (e.g. third inducer), is administered when it is determined that the level of expression of the transgene is decreased to 50% or less of the level of expression of the transgene within the 24 hours after administering at the first time point (e.g., the first inducer), or the previous time point (e.g., second time inducer). In some embodiments of any of the aspects, the inducer at the second time point (e.g., second inducer), or a subsequent time point (e.g. third inducer), is administered when it is determined that the level of expression of the transgene is decreased to a background level of expression of the transgene following the 24 hours after administering at the first time point (e.g., the first inducer), or the previous time point (e.g., second time inducer). In some embodiments of any of the aspects, the inducer at the second time point (e.g., second inducer), or a subsequent time point (e.g. third inducer), is administered when it is determined that the level of expression of the transgene is decreased to 25% or less of the level of expression of the transgene within the 24 hours after administering at the first time point (e.g., the first inducer), or the previous time point (e.g., second time inducer).

[0024] In some embodiments of any one of the aspects, the artemisinin derivative is of Formula (I) described herein, or a pharmaceutically acceptable salt thereof. For example the artemisinin derivative is a compound of Formula (I), (I-A), (I-B), (I-C), (I-D), (I-E), (I-F), (I-G), (I-H), (I-I), (I-J), (I-K), (I-L), (I-M), (I-M'), (I-N), (I-N'), (I-O) or (I-O'). In some embodiments, the artemisinin derivative is artesunate, dihydroartemisinin, artesunate, or artemether. For example, the artemisinin derivative is artesunate, or dihydroartemisinin.

[0025] In some embodiments of any one of the aspects, the flavonoid compound is flavone.

[0026] In some embodiments, the first and second inducer (or a subsequent inducer) are administered at about the same amount. For example, dose of the inducer in successive administrations remains same.

[0027] In some embodiments, the first inducer and the second inducer (or a subsequent inducer) are administered at different amounts. For example, the second inducer (or a subsequent inducer) is administered at a lower amount relative to the amount of the first inducer (or the previous inducer), e.g., dose of the inducer in successive administrations is reduced from its prior administration. In some embodiments, the second inducer (or a subsequent inducer) is administered in amount about 1/2, 1/3, 1/4, 1/5, 1/6, 1/7, 1/8, 1/9, 1/10, 1/15, 1/20, 1/25, 1/30, 1/35, 1/40, 1/45, 1/50, 1/55, 1/60, 1/65, 1/70, 1/75, 1/80, 1/85, 1/90, 1/95, 1/100, 1/125, 1/150, 1/175, 1/200, 1/500, 1/1000 or even less of the amount of first the inducer (or the previous inducer). For example, the second inducer (or a subsequent inducer) is administered in amount about 1/2, 1/4, 1/5, 1/8, 1/10, 1/20, 1/40, 1/80 or less of the amount of the first inducer (or the prior inducer).

[0028] In some embodiments, the second inducer (or a subsequent inducer) is administered at a higher amount relative to the amount of the first inducer (or the previous inducer). For example, the amount of inducer administered at the second time point (or a subsequent time point) is at least about 25%, 50%, about 75%, about 1-fold, about 1.2-folds, about 1.25-folds, about 1.3-folds, about 1.5-folds, about 1.75-folds, about 2-folds, about 2.25-folds, about 2.5-folds, about 2.5-folds, about 2.75-folds, about 3-folds, 4-fold, 5-fold, 6-folds, 7-fold, 8-fold, 9-folds, 10-folds or higher than the amount of the first inducer (or previous time point).

[0029] In some embodiments, the first and second inducer (or a subsequent inducer, e.g., third inducer) are administered independently at a dose from about 0.5 mg/kg to about 150mg/kg.

[0030] In some embodiments, the first inducer is administered at a dose from about 1 mg/kg to about 100 mg/kg. For example, the first inducer is administered at a dose of about 80mg/kg, about 70mg/kg, about 60mg/kg, about 50mg/kg, about 40mg/kg, about 30mg/kg, about 20mg/kg, about 10mg/kg, about 6mg/kg, about 5mg/kg, or about 1mg/kg.

[0031] In some embodiments, the second inducer is administered at a dose from about 1 mg/kg to about 100 mg/kg. For example, the second inducer is administered at a dose of 80mg/kg, about 70mg/kg, about 60mg/kg, about 50mg/kg, about 40mg/kg, about 30mg/kg, about 20mg/kg, about 10mg/kg, about 6mg/kg, about 5mg/kg, or about 1mg/kg.

[0032] In some embodiments, the third inducer is administered at a dose from about 1 mg/kg to about 100 mg/kg. For example, the third inducer is administered at a dose of 80mg/kg, about 70mg/kg, about 60mg/kg, about 50mg/kg, about 40mg/kg, about 30mg/kg, about 20mg/kg, about 10mg/kg, about 6mg/kg, about 5mg/kg, or about 1mg/kg.

[0033] In some embodiments, the first inducer is administered at a dose of about 80 mg/kg or

less and the second inducer is administered at a dose of about 20mg/kg or less at the second or subsequent time point. In some embodiments, the first inducer is administered at a dose of about 40 mg/kg and the second inducer is administered at a dose of about 80mg/kg or less at the second or subsequent time point. In some embodiments, the first inducer is administered at a dose of about 20mg/kg and the second inducer is administered at a dose of about 80 mg/kg, 40mg/kg, 20mg/kg, 10 mg/kg, 6 mg/kg or 1 mg/kg at the second or subsequent time point. In some embodiments, the first inducer is administered at a dose of about 10 mg/kg and the second inducer is administered at a dose of about 80mg/kg or less at the second or subsequent time point. In some embodiments, the first inducer is administered at a dose of about 6 mg/kg and the second inducer is administered at a dose of about 80mg/kg or less at the second or subsequent time point. In some embodiments, the first inducer is administered at a dose of about 1 mg/kg and the second inducer is administered at a dose of about 80mg/kg or less at the second or subsequent time point. In some embodiments, the second inducer is administered at a dose of about 80 mg/kg and the first inducer is administered at a dose of about 80 mg/kg or less. In some embodiments, the second inducer is administered at a dose of about 40 mg/kg and the first inducer is administered at a dose of about 80 mg/kg or less.

[0034] In some embodiments, the second inducer is administered at a dose of about 20 mg/kg and the first inducer is administered at a dose of about 80 mg/kg or less. In some embodiments, the second inducer is administered at a dose of about 10 mg/kg and the first inducer is administered at a dose of about 80 mg/kg or less. In some embodiments, the second inducer is administered at a dose of about 6 mg/kg and the first inducer is administered at a dose of about 80 mg/kg or less. In some embodiments, the second inducer is administered at a dose of about 1 mg/kg and the first inducer is administered at a dose of about 80 mg/kg or less.

[0035] In some embodiments of any of the aspects, each dose is no more than 10 mg/kg. In some embodiments of any of the aspects, the second dose, and/or a subsequent dose (e.g., third dose) is no more than 10 mg/kg. In some embodiments of any of the aspects, the first dose, the second dose, and/or a subsequent dose (e.g., third dose) is 5 mg/kg or greater. In some embodiments of any of the aspects, the first dose is 1-80 mg/kg, the second dose is 1-40 mg/kg, and/or a subsequent dose (e.g., third dose) 1-40 mg/kg. In some embodiments of any of the aspects, the second dose and subsequent dose (e.g., third dose) is 1-10 mg/kg. In some embodiments of any of the aspects, the first dose and each subsequent dose is independently 1 mg/kg to 80 mg/kg. In some embodiments of any of the aspects, the first dose and each subsequent dose is independently 5 mg/kg to 80 mg/kg. In some embodiments of any of the aspects, the second dose and subsequent dose (e.g., third dose) is independently 5 mg/kg to 20 mg/kg. In some embodiments of any of the aspects, the second dose and subsequent dose (e.g., third dose) is independently 5 mg/kg to 10 mg/kg.

[0036] In some embodiments, the second time point is at least two days after the first time point. For example, the second time point is at least one month after the first time point, such as the second time point is at least two months of the first time point.

[0037] In some embodiments of any of the aspects, the expression of the transgene increases for a predetermined time after each administration. In some embodiments of any of the aspects, the expression of the transgene increases for a predetermined time after each administration and the magnitude of the increase is positively correlated with the size of the dose.

[0038] In some embodiments, the expression of the transgene goes down to a background expression level within one week of administering the inducer.

[0039] In some embodiments, the method further comprises a step of determining the expression level of the transgene after administering the first, second, or subsequent (e.g., third) inducer. For example, the method further comprises a step of determining the expression level prior of the transgene to administering the first, second, or subsequent (e.g., third) inducer.

[0040] In some embodiments, the method further comprises administering the exogenous nucleotide sequence to the subject prior to administering the first, second, or subsequent (e.g., third) inducer. For example, the method further comprises administering the exogenous nucleotide sequence to the subject prior to administering the first inducer.

[0041] In some embodiments, the method is a method of therapy, optionally, the method of therapy is a method of gene therapy.

[0042] In some embodiments, the exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene is a viral vector. For example, the exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene is adeno-associated viral (AAV), lentiviral, adenoviral, herpes viral, or hepatitis viral vector. In some embodiments the exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene is an AAV vector, e.g., an AAV gene therapy vector.

[0043] In some cases, the first and second inducers independently are administered orally or are intraperitoneally. In some embodiments, at least one of the first and second inducer is administered orally. For example, if the inducer is artemisinin or a derivative thereof, the artemisinin or a derivative thereof is administered orally. In some embodiments, the second inducer is administered orally. In some embodiments, the first inducer is orally. In some embodiments, both the first inducer and second inducer are administered orally.

[0044] In some embodiments, least one of the first and second inducer is administered intraperitoneally. For example, if the inducer is artemisinin or a derivative thereof, the artemisinin or a derivative thereof is administered intraperitoneally. In some embodiments, the second inducer is administered intraperitoneally. In some embodiments, the first inducer is intraperitoneally. In

some embodiments, both the first and second inducer are administered intraperitoneally.

[0045] In another aspect, provided herein is artemisinin or a derivative of artemisinin, for use as an inducer in a method of regulating expression of a transgene in a subject, e.g., a primate subject, wherein the subject, e.g., the primate subject comprises at least one cell comprising an exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene, wherein the synthetic inducible promoter comprises a CRE that is capable of being bound and activated by a heterodimer of CAR and RXR, wherein the inducer is administered to the subject at a dose which comprises an amount of the inducer that is sufficient to express a therapeutically effective level of the transgene in the subject. In some embodiments of this, the artemisinin or a derivative of artemisinin is administered to the subject, e.g., the primate subject following the prior administration of an inducer to the subject, e.g., the primate subject. In some cases, the inducer of the prior administration is a different inducer, or the inducer of the prior administration is the same inducer but at a different dose.

[0046] In another aspect, provided herein is a flavonoid compound (e.g., flavone) for use as an inducer in a method of regulating expression of a transgene in a subject, e.g., a primate subject, wherein the subject, e.g., the primate subject comprises at least one cell comprising an exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene, wherein the synthetic inducible promoter comprises a CRE that is capable of being bound and activated by a heterodimer of CAR and RXR, wherein the inducer is administered to the subject at a dose which comprises an amount of the inducer that is sufficient to express a therapeutically effective level of the transgene in the subject. In some embodiments of this, the flavonoid compound (e.g., flavone) is administered to the subject, e.g., the primate subject following the prior administration of an inducer to the subject, e.g., the primate subject. In some cases, the inducer of the prior administration is a different inducer, or the inducer of the prior administration is the same inducer but at a different dose.

[0047] In some embodiments of any one of the aspects described herein, the artemisinin or a derivative of artemisinin is administered at a dose of from about 0.1mg/kg to 80mg/kg, from about 0.1mg/kg to about 70mg/kg, from about 0.1mg/kg to about 60mg/kg, from about 0.1mg/kg to about 50mg/kg, from about 0.1mg/kg to about 40mg/kg, from about 0.1mg/kg to about 30mg/kg, from about 0.1mg/kg to about 20mg/kg, from about 0.1mg/kg to about 10mg/kg, from about 0.1mg/kg to about 6mg/kg, from about 0.1mg/kg to about 5mg/kg, or from about 0.1mg/kg to about 1mg/kg.

[0048] In some embodiments, a first induction with artemisinin or a derivative thereof increases the transgene expression level to a maximum at about 24 hrs and declines thereafter, wherein, second induction and/or, successive inductions with artemisinin or a derivative thereof has similar induction profile as seen with the first induction, for at least about 1 month, or at least

about 2 months, or, at least about 3 months, or, at least about 5 months or, at least about six months or, even more, and optionally, the method further comprises single administration of exogenous nucleotide sequence encoding the transgene operatively linked to the synthetic inducible promoter prior to first administering the inducer to induce said first induction.

[0049] In some embodiments of any one of the aspects described herein, flavonoid compound is administered at a dose of from about 0.1mg/kg to 80mg/kg, from about 0.1mg/kg to about 70mg/kg, from about 0.1mg/kg to about 60mg/kg, from about 0.1mg/kg to about 50mg/kg, from about 0.1mg/kg to about 40mg/kg, from about 0.1mg/kg to about 30mg/kg, from about 0.1mg/kg to about 20mg/kg, from about 0.1mg/kg to about 10mg/kg, from about 0.1mg/kg to about 6mg/kg, from about 0.1mg/kg to about 5mg/kg, or from about 0.1mg/kg to about 1mg/kg.

[0050] In some embodiments, a first induction with the flavonoid compound increases the transgene expression level to a maximum at about 24 hrs and declines thereafter, wherein, second induction and/or, successive inductions with the flavonoid compound has similar induction profile as seen with the first induction, for at least about 1 month, or at least about 2 months, or, at least about 3 months, or, at least about 5 months or, at least about six months or, even more, and optionally, the method further comprises single administration of exogenous nucleotide sequence encoding the transgene operatively linked to the synthetic inducible promoter prior to first administering the inducer to induce said first induction.

[0051] In some embodiments, the transgene expression level at about 24 hrs in the first induction, or, second induction, or successive inductions is at least about 200-fold higher than that of prior to administering the inducer for the first time, or, the second time, or, any number of successive administrations.

[0052] In some embodiments, the first induction, the second induction, and/or, the successive inductions are not complete until about 24 hours, about 48 hours, about 72 hours, about 96 hours, about 120 hours, about 144 hours, about 150 hours, 160 hours, about 168 hours, about 192 hours, about 216 hours, about 240 hours, about 264 hours, or about 290 hours after administering the inducer. In some embodiments of any one of the aspects, the artemisinin or a derivative thereof is administered at a dose from about 0.5 mg/kg to about 150mg/kg. For example, artemisinin or a derivative thereof is administered at a dose from about 1 mg/kg to about 100 mg/kg, from about 1 mg/kg to about 80 mg/kg, from about 1 mg/kg to about 40 mg/kg, from about 1 mg/kg to about 20 mg/kg, or from about 1 mg/kg to about 10 mg/kg. For example, the artemisinin or a derivative thereof is administered at a dose of about 80mg/kg, about 70mg/kg, about 60mg/kg, about 50mg/kg, about 40mg/kg, about 30mg/kg, about 20mg/kg, about 10mg/kg, about 6mg/kg, about 5mg/kg, or about 1mg/kg.

[0053] In some embodiments of any of the aspects, the transgene encodes an antibody or antigen binding portion an antibody, a coagulation factor, a cytokine, a hormone, a growth factor, a virus protein, a cell receptor ligand, a chimeric antigen receptor (CAR) or a CRISPR-Cas nuclease.

[0054] In some embodiments of any one of the aspects, the transgene encodes an antibody, optionally selected from the group consisting of an anti-TNF α , anti-PD1 antibody, anti-HER2 antibody, anti-PCSK9 antibody, anti-VEGFR2 antibody, anti-IL17 antibody, anti-A β 3-42 antibody, or anti-Sema3A antibody or an antigen binding fragment thereof.

[0055] In some embodiments of any one of the aspects, the transgene encodes a coagulation factor, optionally selected from the group consisting of Factor VIII, Factor IX, Factor VII, Factor X, and Factor XI, optionally, the transgene encodes a coagulation factor selected from Factor VIII, Factor IX.

[0056] In some embodiments of any one of the aspects, the transgene encodes a hormone, optionally selected from the group consisting of selected from Erythropoietin (EPO) and parathyroid hormone (PTH).

[0057] In some embodiments of any one of the aspects, the transgene encodes a CRISPR-Cas nuclease, optionally selected from the group consisting of Cas9, Cas12, Cas Phi, CasX, and CasRx.

[0058] In some embodiments of any one of the aspects, the transgene encodes a virus protein, optionally selected from the group consisting of viral envelop protein, viral nucleocapsid protein, viral membrane protein, REP, CAP, REV, VSV-G and RD114.

[0059]

[0060] In some embodiments of any of the aspects, the subject is a primate. In some embodiments of any one of the aspects, the subject is a non-human primate. In some embodiments of any of the aspects, the subject is a human.

BRIEF DESCRIPTION OF THE DRAWINGS

[0061] This patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawings will be provided by the Office upon request and payment of the necessary fee.

[0062] **FIG. 1** is schematic representation of PK/PD of artemisinin and some exemplary derivatives thereof.

[0063] **FIG. 2** is a bar-graph showing the induction of an exemplary inducible promoter by artemisinin, various exemplary derivatives of artemisinin and other compounds. Structures of artemisinin, exemplary derivatives of artemisinin, and other compounds are also shown.

- [0064] FIG. 3A is a schematic representation of protocols for *in vivo* short-term induction in male mice via IP injection.
- [0065] FIGS. 3B and 3C shows live imaging (FIG. 3B) and *ex vivo* imaging (FIG. 3C) of short-term induction in male mice via intraperitoneal (IP) injection.
- [0066] FIG. 4A is a schematic representation of protocols for *in vivo* testing the sex differences on inducible promoters.
- [0067] FIGS. 4B and 4C show imaging (FIG. 4B) and *ex vivo* imaging (FIG. 4C) of differences in background and induction between sex via IP injection.
- [0068] FIG. 5A is a schematic representation of protocols for *in vivo* testing the effects of route of administration on inducible promoters.
- [0069] FIGS. 5B and 5C show live imaging (FIG. 5B) and *ex vivo* imaging (FIG. 5C) of differences in induction between administration via oral gavage and IP injection.
- [0070] FIG. 6 shows dose response in female mice via oral gavage administration.
- [0071] FIG. 7 shows time course of induction in mice.
- [0072] FIG. 8 shows effect of AAV dose on induction.
- [0073] FIG. 9 is a schematic representation of a protocol for testing induction in non-human primates.
- [0074] FIG. 10 shows results of repeated induction in non-human primates using the protocol shown in FIG. 9.
- [0075] FIG. 11 is a schematic representation of another protocol for testing induction in non-human primates.
- [0076] FIG. 12 shows induction in non-human primates using the protocol shown in FIG. 11. Results for the LSP control promoter and saline negative control are also shown. Pre-induction gonadotropin levels are indicated by blue arrows and gonadotropin levels after induction are illustrated by red arrows.
- [0077] FIGS. 13A and 13B are bar-graph (FIG. 13A) and line-graph (FIG. 13B) showing individual results for each of the non-human primate in FIG. 12. M LSP and F LSP are the male and female LSP controls, respectively; M1 Ind and M2 Ind are males with the inducible promoter; F1 Ind and F2 Ind are females with the inducible promoter; M S and F S are Male and Female saline negative controls, respectively.
- [0078] FIG. 14 is a schematic representation of a protocol for testing induction in non-human primates.
- [0079] FIG. 15 is a line graph showing individual results for each of the non-human primate. M(S) and F(S) are male and female saline negative controls, respectively; Con-F and Con-M are

the female and male LSP controls, respectively; and Ind-M(2) and Ind-(F2) are male and female with the inducible promoter, respectively.

[0080] FIG. 16 is a bar graph showing results of repeated induction in non-human primates using the protocol shown in FIG. 14.

[0081] FIG. 17 is a line graph showing results of continuous oral dosing of the inducer. Female and Male are female and male with the inducible promoter, respectively; Control promoter is the the LSP control; and Saline is saline negative control.

DETAILED DESCRIPTION

[0082] It is noted that the section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

[0083] The inducer(s) can be formulated for administration via parenteral or oral routes. For example, the inducer(s) can be formulated for intravenous, intramuscular, intraarterial, intrathecal, intraventricular, intraperitoneal, subcutaneous, subcuticular, intraarticular, sub capsular, subarachnoid, intraspinal, and/or oral administration.

[0084] The technology described herein is directed generally to methods for regulatable and controllable expression of a transgene in a subject, e.g., a primate subject. In particular, the technology described herein relates to methods for regulatable and controlled expression of a transgene, including but not limited to any of, sustained, e.g., long-lasting expression, transient expression of a transgene, controlled expression of a transgene, and/or dose-dependent and/or titratable expression of a transgene in a primate subject. Although gene therapy offers great promise in a number of situations, the constitutive expression of an added gene can have deleterious effects on a cell. For example, the over-expression or constitute expression of certain proteins can be toxic or determinantal to a cell, e.g., it can lead to unfolded protein responses, it can cause immunity problems. Thus, being able to regulate expression of such proteins so it is transient or temporary and provide expression at desired intervals and intensities is extremely desirable.

[0085] The methods disclosed herein permit one to personalize gene therapy throughout a subject's lifespan to express a transgene at a duration and/or level that meets the subject's needs, by sustaining the transgene expression level at a predetermined level for a pre-determined time, or alternatively, increasing the expression of the transgene by inducing expression of the transgene by one or more, e.g., multiple subsequent administrations of an inducer after initial induction of the transgene expression, thereby controlling the transgene expression level to a desired expression level or desired expression level range based on the time period between the administrations of the inducer and/or amount of the inducer in the re-dose administration, allowing for a regulatable and controlled modulation of the expression of the transgene in the subject.

[0086] Accordingly, in one aspect provided herein is a method of regulating expression of a transgene in a primate subject. The subject comprises an exogenous nucleotide sequence comprising an inducible promoter. The inducible promoter is operably linked to a transgene and comprises a cis-regulatory element (CRE) that is capable of being bound and activated by a heterodimer of CAR and RXR. The method comprises administering an effective amount of an inducer, e.g., artemisinin, a derivative of artemisinin, or a flavonoid compound to the subject, thereby inducing the expression of the transgene from the expression cassette, for example, inducing the expression of the transgene to a measurable or therapeutically effective level in the subject. It is noted that the exogenous nucleotide sequence can be inserted into the genome of the subject, or it can be present in an episomal vector.

[0087] In some embodiments of any of the aspects, the expression of the transgene increases for a predetermined time after each administration. In some embodiments of any of the aspects, the expression of the transgene increases for a predetermined time after each administration and the magnitude of the increase is positively correlated with the size of the dose.

[0088] Without wishing to be bound by a theory, the method described herein can permit control over timing of the expression of the transgene. One can create various positive and negative feedback loops to regulate expression of a transgene in a predetermined manner.

[0089] In some embodiments of any of the aspects described herein, the transgene product is detrimental to the liver cell or to the subject when constitutively expressed.

[0090] The inducer can be administered intermittently or continuously to have transgene expression level at a predetermined level for a predetermined time or to provide intermittent expression of the transgene at predetermined times and/or levels. For example, the inducer can be administered periodically (sometimes referred to herein as a “redose” or “booster” administration) to obtain transgene expression level at a predetermined level for a predetermined time, and/or to increase the expression level of the transgene above a reference expression level, e.g., background expression level or an expression level in the absence of the inducer. Accordingly, in some embodiments, the method further comprises administering to the subject at least a second dose of the inducer to continue expression of the transgene at a predetermined level for a predetermined time or to increase expression of the transgene to a predetermined level. Additionally, one can administer one or more inducers. Moreover, the level of inducer and/or use of multiple inducers can be varied depending upon the level of expression, e.g., level of protein, desired for a particular situation.

[0091] Therefore, in some embodiments, the method comprises: administering to the subject a first dose of an inducer in an amount that is sufficient to express a measurable level of the transgene in the subject; and administering to the subject at least a second dose and/or subsequent

doses of the inducer to continue expression of the transgene at a predetermined level for a predetermined time or to increase expression of the transgene to a predetermined level.

[0092] In some embodiments, the method comprises: administering to the subject at a first time point a dose of a first inducer in an amount that is sufficient to express a measurable level of the transgene in the subject; administering to the subject at at least a second time point a dose of a second inducer in an amount that is sufficient to express a measurable level of the transgene in the subject; and, optionally, administering to the subject at at least a third time point a dose of a third inducer in an amount that is sufficient to express a measurable level of the transgene in the subject. And so on as expression is needed. It is noted that the first, second and any subsequent inducers can be same or different.

[0093] The data presented herein show that once the administration of the inducer is stopped, the expression of the transgene goes down to a background expression level rapidly. Further, the background expression level remains relatively steady even after repeated administration of the inducer. This is contrast to the current methods in the art. With the current methods in the art, the background expression levels are seen to be significantly higher even after induction has been stopped for a period of more than one, two, three, four or more weeks. In contrast, in the methods described herein, the expression of the transgene goes down to a background expression level in less than a week once the administration of the inducer is stopped. The background expression level can be an expression level of the transgene prior to first induction or first administration of the inducer.

[0094] In some embodiments, the expression of the transgene goes down to the background level once the administration of the inducer is stopped. For example, once the administration of the inducer is stopped, the expression of the transgene goes down to an expression level that is no more than 50%, no more than 45%, no more than 40%, no more than 35%, no more than 30%, no more than 35%, no more than 30%, no more than 25%, no more than 20%, no more than 15%, no more than 10%, or no more than 5% higher than the background expression level of the transgene prior to first induction.

[0095] In some embodiments, the expression of the transgene goes down to the background level within one week once administration of the inducer is stopped. For example, the expression of the transgene goes to an expression level that is no more than 50%, no more than 45%, no more than 40%, no more than 35%, no more than 30%, no more than 35%, no more than 30%, no more than 25%, no more than 20%, no more than 15%, no more than 10%, or no more than 5% higher than the background expression level of the transgene prior to first induction within one week stopping the administration of the inducer.

[0096] In some embodiments, the inducer (or inducers) is administered two or more (e.g., 3,

4, 5, 6, 7, 8, 9, 10 or more) times and the expression of the transgene goes down to the background level after the last administration of the inducer. For example, the inducer is administered two or more (e.g., 3, 4, 5, 6, 7, 8, 9, 10 or more) times and the expression of the transgene goes to an expression level that is no more than 50%, no more than 45%, no more than 40%, no more than 35%, no more than 30%, no more than 35%, no more than 30%, no more than 25%, no more than 20%, no more than 15%, no more than 10%, or no more than 5% higher than the background expression level of the transgene prior to first induction within one week after the last administration of the inducer.

[0097] In some embodiments, the administration of the first inducer (e.g., artemisinin, a derivative of artemisinin, or a flavonoid compound such as flavone) increases the transgene expression level to a maximum at about 24 hours. For example, the transgene expression reaches a maximum level at about 24 hours after administration of the inducer and declines thereafter, without the administration of additional inducer.

[0098] In some embodiments, the administration of the second inducer (e.g., artemisinin, a derivative of artemisinin, or a flavonoid compound such as flavone) and/or a subsequent administration of inducer (e.g., artemisinin, a derivative of artemisinin, or a flavonoid compound such as flavone) has similar induction profile as seen with the first inducer for at least about 1 month, or at least about 2 months, or at least about 3 months, or at least about 5 months, or at least about six months, or at least one year, or even longer.

[0099] In some embodiments, the transgene expression level after the first or a subsequent administration is at least about 50-fold, at least about 100-fold, at least about 200-fold, at least about 300-fold, at least about 400-fold, at least about 500-fold, at least about 600-fold, at least about 700-fold, at least about 800-fold, at least about 900-fold, at least about 1000-fold, at least about 1100-fold, at least about 1200-fold, at least about 1300-fold, at least about 1400-fold, at least about 1500-fold, at least about 1600-fold, at least about 1700-fold, at least about 1800-fold, at least about 1900-fold, at least about 1000-fold, or higher than the level prior to the first or subsequent administration. In some embodiments, the transgene expression level after the first or a subsequent administration is at least 50-fold, at least 100-fold, at least 200-fold, at least 300-fold, at least 400-fold, at least 500-fold, at least 600-fold, at least 700-fold, at least 800-fold, at least 900-fold, at least 1000-fold, at least 1100-fold, at least 1200-fold, at least 1300-fold, at least 1400-fold, at least 1500-fold, at least 1600-fold, at least 1700-fold, at least 1800-fold, at least about 1900-fold, at least 1000-fold, or higher than the level prior to the first or subsequent administration.

[00100] In some embodiments, the transgene expression level at about 24 hours after the first or a subsequent administration is at least about 25-fold, at least about 50 fold, at least about 75-fold, at least about 100-fold, at least about 125-fold, at least about 150-fold, at least about 175-fold,

at least about 200-fold, at least about 300-fold, at least about 400-fold, at least about 500-fold, at least about 600-fold, at least about 700-fold, at least about 800-fold, at least about 900-fold, at least about 1000-fold, at least about 1100-fold, at least about 1200-fold, at least about 1300-fold, at least about 1400-fold, at least about 1500-fold, at least about 1600-fold, at least about 1700-fold, at least about 1800-fold, at least about 1900-fold, at least about 1000-fold, or higher than the level prior to the first or subsequent administration. In some embodiments, the transgene expression level at about 24 hours after the first or a subsequent administration is at least 25-fold, at least 50 fold, at least 75-fold, at least 100-fold, at least 125-fold, at least 150-fold, at least 175-fold, at least 200-fold, at least 300-fold, at least 400-fold, at least 500-fold, at least 600-fold, at least 700-fold, at least 800-fold, at least 900-fold, at least 1000-fold, at least 1100-fold, at least 1200-fold, at least 1300-fold, at least 1400-fold, at least 1500-fold, at least 1600-fold, at least 1700-fold, at least 1800-fold, at least about 1900-fold, at least 1000-fold, or higher than the level prior to the first or subsequent administration.

[00101] In some embodiments, the predetermined level of transgene expression level is at least about 50-fold, at least about 100-fold, at least about 200-fold, at least about 300-fold, at least about 400-fold, at least about 500-fold, at least about 600-fold, at least about 700-fold, at least about 800-fold, at least about 900-fold, at least about 1000-fold, at least about 1100-fold, at least about 1200-fold, at least about 1300-fold, at least about 1400-fold, at least about 1500-fold, at least about 1600-fold, at least about 1700-fold, at least about 1800-fold, at least about 1900-fold, at least about 1000-fold, or higher than the level prior to the first administration. In some embodiments, the predetermined level of transgene expression level is at least 50-fold, at least 100-fold, at least 200-fold, at least 300-fold, at least 400-fold, at least 500-fold, at least 600-fold, at least 700-fold, at least 800-fold, at least 900-fold, at least 1000-fold, at least 1100-fold, at least 1200-fold, at least 1300-fold, at least 1400-fold, at least 1500-fold, at least 1600-fold, at least 1700-fold, at least 1800-fold, at least about 1900-fold, at least 1000-fold, or higher than the level prior to the first administration.

Dosing

[00102] Where more than one administration of an inducer(s) is administered (e.g., at a second or any subsequent time point), the second time point, or any subsequent time point is at least 12 hours after the inducer administration at the first time point, or the previous time point. For example, where more than one administration of the inducer is administered, the second time point, or any subsequent time point is at least 18 hours, at least 24 hours, at least 30 hours, at least 36 hours, at least 42 hours, at least 48 hours, at least 54 hours, at least 60 hours, at least 66 hours, or at least 72 hours after the first time point, or the previous time point. In some embodiments, where more than one administration of the inducer(s) is administered, the second time point, or any

subsequent time point is at least 24 hours, at least after 36 hours, at least 42 hours, at least 48 hours, at least 72 hours, at least 1 week, at least 10 days, at least 2 weeks, at least 3 weeks, or at least 4 weeks after the first time point, or the previous time point. In some embodiments, where more than one administration of the inducer(s) is administered, the second time point, or any subsequent time point is at least at least 24 hours after the first time point, or the previous time point. In some embodiments, where more than one administration of the inducer(s) is administered, the second time point, or any subsequent time point is at least at least 72 hours after the first time point, or the previous time point. In some embodiments, where more than one administration of the inducer(s) is administered, the second time point, or any subsequent time point is at least at least 1 week after the first time point, or the previous time point. In some embodiments, where more than one administration of the inducer(s) is administered, the second time point, or any subsequent time point is at least at least 2 weeks after the first time point, or the previous time point.

[00103] In some embodiments, the inducer (or inducers) is administered two or more (e.g., 3, 4, 5, 6, 7, 8, 9, 10 or more) times.

[00104] In some embodiments, where more than one administration of the inducer is administered, the second time point, or any subsequent time point is at least after one week, at least two weeks, at least three weeks, at least four weeks, five weeks, six weeks, seven weeks, eight weeks or more after the first time point, or the previous time point. In some embodiments, the subsequent administration is based upon need of the subject such as a flare-up of a condition the transgene expression product treats. For example, a particular antibody, such as a TNF- α antibody. Some exemplary non-limiting examples where the subsequent administration is based upon need of the subject include, but are not limited to inducing anti-inflammatory antibodies when there is a flare up of the inflammatory condition, inducing a transgene product that has on-target toxicity; inducing transient expression of anti-cancer biologic, e.g., antibody, bispecific, or cytokine; inducing GLP1 for diabetes and/or, weight loss; inducing transient expression of growth factors to initiate nerve or other tissue regeneration in acute spinal cord injury or other injuries. For example, where more than one administration of the inducer(s) is administered, the second time point, or any subsequent time point is at least one month, two months, three months, four months, five months, six months, seven months, eight months, nine months, eleven months, a year or longer after the first time point, or the previous time point.

[00105] In some embodiments, where more than one administration of the inducer(s) is administered, the second time point, or a subsequent time point is within two months of the first time point, or the previous time point. For example, where more than one administration of the inducer(s) is administered, the second time point, or any subsequent time point is within one month of the first time point, or the previous time point. In some embodiments, where more than one

administration of the inducer(s) is administered, the second time point, or any subsequent time point is within seven weeks, within six weeks, within five weeks, within four weeks, within three weeks or within two weeks of the first time point, or the previous time point. Subsequent administrations can be longer.

[00106] In some embodiments, where more than one administration of the inducer(s) is administered, the second time point, or a subsequent time point is within 10 days, 9 days, 8 days, 7 days, 6 days, 5 days, 4 days, 3 days or 2 days of the first time point, or the previous time point. In some embodiments of any one of the aspects described herein, where more than one administration of the inducer(s) is administered, the second time point, or any subsequent time point is 96 hours, 90 hours, 84 hours, or 78 hours of the first time point, or the previous time point. For example, where more than one administration of the inducer(s) is administered, the second time point, or a subsequent time point is within 72 hours, within 66 hours, within 60 hours, within 54 hours, within 48 hours, within 42 hours, or within 36 hours of the first time point, or the previous time point. In some embodiments, where more than one administration of the inducer(s) is administered, the second time point, or a subsequent time point is within 12-36 hours, e.g., within 20-28 hours of the first time point, or the previous time point. For example, where more than one administration of the inducer(s) is administered, the second time point, or a subsequent time point is within about 24 hours of the first time point, or the previous time point.

[00107] In some embodiments of any one of the aspects described herein, where more than one administration of the inducer(s) is administered, the second time point, or a subsequent time point is least 24 hours after and within two weeks of the first time point in a given regimen, or the previous time point. For example, where more than one administration of the inducer(s) is administered, the second time point, or any subsequent time point is at least 30 hours, at least 36 hours, at least 42 hours, at least 48 hours, at least 54 hours, at least 60 hours, at least 66 hours, or at least 72 hours after and within 10 days, 9 days, 8 days, 7 days, 6 days, 5 days, 4 days, 3 days or 2 days of the first time point, or the previous time point. In some embodiments, where more than one administration of the inducer(s) is administered, the second time point, or any subsequent time point is at least after 36 hours, e.g., at least 42 hours or at least 48, at least 54 hours, at least 66 hours, at least 72 hours, at least 80 hours, at least 88 hours or at least 96 hours of the first time point, or the previous time point.

[00108] In some embodiments, where more than one administration of the inducer(s) is administered, the second time point, or any subsequent time point is at least 36 hours after the first time point, or the previous time point. For example, where more than one administration of the inducer(s) is administered, the second time point, or any subsequent time point is least 48 hours after or at least 72 hours after the first time point, or the previous time point.

[00109] In some embodiments, where more than one administration of the inducer(s) is administered, the time between administrations is 5-7 days. In some embodiments, where more than one administration of the inducer(s) is administered, the time between administrations is 5-10 days. In some embodiments, where more than one administration of the inducer(s) is administered, the time between administrations is 7-10 days. In some embodiments, where more than one administration of the inducer(s) is administered, the time between administrations is 7-14 days. In some embodiments, where more than one administration of the inducer(s) is administered, the time between administrations is 10-14 days. In some embodiments, where more than one administration of the inducer(s) is administered, the time between administrations is 10-20 days. In some embodiments, where more than one administration of the inducer(s) is administered, the time between administrations is 14-21 days. In some embodiments, where more than one administration of the inducer(s) is administered, the time between administrations is 14-28 days. In some embodiments, where more than one administration of the inducer(s) is administered, the time between administrations is 21-28 days. In some embodiments, where more than one administration of the inducer(s) is administered, the time between administrations is 21-34 days.

[00110] Times of administration can be periodic, as needed, or pulsed. This permits tailoring of both administration and dosing amounts. Administration is designed to prevent accumulation of proteins in a cell that can be detrimental to the cell.

[00111] In some embodiments, the at least second dose or at least third dose are administered when it is determined that the level of expression of the transgene is decreased to 75% or less, or 50% or less, or 25% or less of the level of expression of the transgene within the 24 hours after the first dose. In some embodiments, the at least second dose or at least third dose are administered when it is determined that the level of expression of the transgene is decreased to a background level of expression of the transgene. In some embodiments, the at least second dose or at least third dose are administered when it is determined that the level of expression of the transgene is decreased to a level of expression of the transgene prior to the first dose.

[00112] Generally, the inducer (or inducers) is administered in an amount sufficient to induce expression of the transgene from the expression cassette. In some embodiments, the inducer (or inducers) is administered at a dose from about 50 µg/kg to about 1000 mg/kg. For example, the inducer (or inducers) is used or given at a dose from about 75 µg/kg to about 900 mg/kg, from about 100 µg/kg to about 800 mg/kg, from about 150 µg/kg to about 700 mg/kg, from about 200 µg/kg to about 600 mg/kg, from about 250 µg/kg to about 250 mg/kg, from about 250 µg/kg to about 50 mg/kg, from about 100 µg/kg to about 1 mg/kg, or from about 1 mg/kg to about 80 mg/kg. In some embodiments, the inducer (or inducers) is administered at a dose from about 1 mg/kg to about 40 mg/kg. For example, the inducer (or inducers) is administered at a dose from about 1

mg/kg to about 20 mg/kg, from about 1 mg/kg to about 10 mg/kg, or from about 1 mg/kg to about 6mg/kg. In some embodiments, the inducer (or inducers) is administered at a dose from about 1mg/kg to about 80 mg/kg.

[00113] It is noted that an amount of inducer(s) administered at the second time point, or any subsequent time point can be about the same or different from the amount of the inducer(s) administered at the first time point, or the previous time point. In some embodiments, the second or subsequent dose of the inducer(s) can be determined using a dose-dependent relationship between the first or preceding dose of inducer(s) and the level of transgene expression from the expression cassette to achieve the desired level of expression of the transgene. For example, if the second administration, or any subsequent administration, is within a week or less of the prior administration, in one embodiment, the second or subsequent dose can be at a significant lower dose than the first dose, or the previous dose.

[00114] In some embodiments, the amount of inducer(s) administered at the second time point, or any subsequent time point is about the same as the amount of the inducer(s) administered at the first time point, or the previous time point. For example, the amount of inducer(s) administered at the second time point, or any subsequent time point is within about 10% (wt/wt or mol/mol) of the amount of the inducer administered at the first time point, or the previous time point. In some embodiments, the amount of inducer(s) administered at the second time point, or any subsequent time point is within about 7.5%, about 5%, about 2.5%, about 2%, about 1.5%, about 1%, or about 0.5%, (wt/wt or mol/mol) of the amount of the inducer(s) administered at the first time point, or the previous time point.

[00115] In some embodiments, the amount of inducer(s) administered at the second time point, or any subsequent time point is lower than the amount of the inducer(s) administered at the first time point, or the previous time point. For example, the amount of inducer(s) administered at the second time point, or any subsequent time point is at least about 10% (wt/wt or mol/mol) lower than the amount of the inducer(s) administered at the first time point, or the previous time point.

[00116] In some embodiments, the amount of inducer(s) administered at the second time point, or any subsequent time point is at least about 50% (wt/wt or mol/mol) lower than the amount of the inducer(s) administered at the first time point, or the previous time point. For example, the amount of inducer(s) administered at the second time point, or any subsequent time point is about 55%, at about 60%, about 65%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 98% (wt/wt or mol/mol) lower than the amount of the inducer(s) administered at the first time point, or the previous time point.

[00117] In some embodiments, the amount of inducer(s) administered at the second time point, or any subsequent time point is about half or less than half of the amount of the inducer(s)

administered at the first time point, or the previous time point. For example, the amount of inducer(s) administered at the second time point, or any subsequent time point is about 1/2, 1/3, 1/4, 1/5, 1/6, 1/7, 1/8, 1/9, 1/10, 1/15, 1/20, 1/25, 1/30, 1/35, 1/40, 1/45, 1/50, 1/55, 1/60, 1/65, 1/70, 1/75, 1/80, 1/85, 1/90, 1/95, 1/100, 1/125, 1/150, 1/175, 1/200, 1/500, 1/1000 or less of the amount of the inducer(s) administered at the first time point, or the previous time point. In some embodiments, the amount of inducer(s) administered at the second time point, or any subsequent time point is about 1/2, 1/4, 1/5, 1/8, 1/10, 1/20, 1/40, 1/80 or less than the amount of the inducer(s) administered at the first time point, or the previous time point.

[00118] In some embodiments, the amount of inducer(s) administered at the second time point, or any subsequent time point is decreased by about 5 mg/kg, about 10 mg/kg, about 20 mg/kg, about 30 mg/kg, about 40 mg/kg or more compared to the amount of the inducer(s) administered at the first time point, or the previous time point. In some embodiments, the amount of inducer(s) administered at the second time point, or any subsequent time point is decreased by 5 mg/kg, 10 mg/kg, 20 mg/kg, 30 mg/kg, 40 mg/kg or more compared to the amount of the inducer(s) administered at the first time point, or the previous time point.

[00119] In some embodiments of any of the aspects, the decreased subsequent dose is sufficient to attain the predetermined level of transgene expression.

[00120] In some embodiments, the amount of inducer(s) administered at the second time point, or any subsequent time point is higher than the amount of the inducer(s) administered at the first time point, or the previous time point. For example, the amount of inducer(s) administered at the second time point, or any subsequent time point is at least about 10% (wt/wt or mol/mol) higher than the amount of the inducer(s) administered at the first time point, or the previous time point. In some embodiments, the amount of inducer(s) administered at the second time point, or any subsequent time point is at least 25%, 50%, about 75%, about 1-fold, about 1.2-folds, about 1.25-folds, about 1.3-folds, about 1.5-folds, about 1.75-folds, about 2-folds, about 2.25-folds, about 2.5-folds, about 2.5-folds, about 2.75-folds, about 3-folds, 4-fold, 5-fold, 6-folds, 7-fold, 8-fold, 9-folds, 10-folds or higher than the amount of the inducer(s) administered at the first time point, or the previous time point.

[00121] In some embodiments, the amount of inducer(s) administered at each subsequent time point is lower than the amount of the inducer(s) administered at an immediate previous time point and the amount of inducer(s) administered at the last time point is higher than the amount of the inducer(s) administered at the immediate previous time point. Generally, the inducer (or inducers) is administered in an amount sufficient to induce expression of the transgene from the expression cassette. In some embodiments, the inducer (or inducers) is administered at a dose from about 50 µg/kg to about 1000 mg/kg. For example, the inducer (or inducers) is used or given at a dose from

about 75 µg/kg to about 900 mg/kg, from about 100 µg/kg to about 800 mg/kg, from about 150 µg/kg to about 700 mg/kg, from about 200 µg/kg to about 600 mg/kg, from about 250 µg/kg to about 250 mg/kg, from about 250 µg/kg to about 50 mg/kg, from about 100 µg/kg to about 1 mg/kg, or from about 1 mg/kg to about 80 mg/kg. In some embodiments, the inducer (or inducers) is administered at a dose from about 1 mg/kg to about 40 mg/kg. For example, the inducer (or inducers) is administered at a dose from about 1 mg/kg to about 20 mg/kg, from about 1 mg/kg to about 10 mg/kg, or from about 1 mg/kg to about 6mg/kg. In some embodiments, the inducer (or inducers) is administered at a dose from about 1mg/kg to about 80 mg/kg

[00122] In some embodiments, the inducer(s) is administered at the second time point, or any subsequent time point at a dose from about 1 mg/kg to 80 mg/kg. For example, the inducer(s) can be administered at a dose of about 1 mg/kg, about 5 mg/kg, about 6 mg/kg, about 10 mg/kg, about 20 mg/kg, about 30 mg/kg, about 40 mg/kg, about 50 mg/kg, about 60 mg/kg, about 70 mg/kg, or about 80 mg/kg.

[00123] In some embodiments, the inducer(s) is administered at the first time point, or the previous time point at a dose from about 0.5 mg/kg to 15 mg/kg. For example, the inducer(s) is administered at the first time point, or the previous time point at a dose from about 0.75 mg/kg to about 15 mg/kg or from about 1 mg/kg to 10 mg/kg. In some embodiments the inducer(s) is administered at the first time point, or the previous time point at a dose of about 1 mg/kg, about 1.5 mg/kg, about 2 mg/kg, about 2.5 mg/kg, about 3 mg/kg, about 3.5 mg/kg, about 4 mg/kg, about 4.5 mg/kg, about 5 mg/kg, about 5.5 mg/kg, about 6 mg/kg, about 6.5 mg/kg, about 7 mg/kg, about 7.5 mg/kg, about 8 mg/kg, about 8.5 mg/kg, about 9 mg/kg, about 9.5 mg/kg, about 10 mg/kg, about 10.5 mg/kg, about 11 mg/kg, about 11.5 mg/kg, about 12 mg/kg, 12.5 mg/kg, about 13 mg/kg, about 13.5 mg/kg, about 14 mg/kg, about 14.5 mg/kg, or about 15 mg/kg.

[00124] In some embodiments, the inducer(s) is administered at each administration at a dose of no more than about 10 mg/kg. For example, the inducer(s) is administered at each administration at a dose of no more than about 10 mg/kg, no more than 10mg/kg, no more than about 5 mg/kg, or no more than 5 mg/kg.

[00125] In some embodiments, the first dose, second dose, third dose, and/or subsequent doses of the inducer(s) are administered at a dose of at least about 5 mg/kg. For example, the first dose, second dose, third dose, and/or subsequent doses of the inducer(s) are administered at a dose of at least about 5 mg/kg, of at least 5 mg/kg, of at least about 6 mg/kg, or at least 6 mg/kg.

[00126] In some embodiments, the first dose, second dose, third dose, and/or subsequent doses of the inducer(s) are administered at a dose of about 1 mg/kg to about 80 mg/kg. In some embodiments, the first dose, second dose, third dose, and/or subsequent doses of the inducer(s) are administered at a dose of 1 mg/kg to 80 mg/kg. In some embodiments, the first dose, second dose,

third dose, and/or subsequent doses of the inducer(s) are administered at a dose of about 5 mg/kg to about 80 mg/kg. In some embodiments, the first dose, second dose, third dose, and/or subsequent doses of the inducer(s) are administered at a dose of 5 mg/kg to 80 mg/kg.

[00127] In some embodiments, the second dose, third dose, and/or subsequent doses of the inducer(s) are administered at a dose of no more than about 10 mg/kg. For example, the second dose, third dose, and/or subsequent doses of the inducer(s) are administered at a dose of no more than about 10 mg/kg, no more than 10mg/kg, no more than about 5 mg/kg, or no more than 5 mg/kg.

[00128] In some embodiments, the second dose, third dose, and/or subsequent doses of the inducer(s) are administered at a dose of about 1 mg/kg to about 80 mg/kg. In some embodiments, the second dose, third dose, and/or subsequent doses of the inducer(s) are administered at a dose of 1 mg/kg to 80 mg/kg. In some embodiments, the second dose, third dose, and/or subsequent doses of the inducer(s) are administered at a dose of about 5 mg/kg to about 80 mg/kg. In some embodiments, the second dose, third dose, and/or subsequent doses of the inducer(s) are administered at a dose of 5 mg/kg to 80 mg/kg. In some embodiments, the second dose, third dose, and/or subsequent doses of the inducer(s) are administered at a dose of about 10 mg/kg to about 80 mg/kg. In some embodiments, the second dose, third dose, and/or subsequent doses of the inducer(s) are administered at a dose of 10 mg/kg to 80 mg/kg. In some embodiments, the second dose, third dose, and/or subsequent doses of the inducer(s) are administered at a dose of about 5 mg/kg to about 20 mg/kg. In some embodiments, the second dose, third dose, and/or subsequent doses of the inducer(s) are administered at a dose of 5 mg/kg to 20 mg/kg. In some embodiments, the second dose, third dose, and/or subsequent doses of the inducer(s) are administered at a dose of about 5 mg/kg to about 10 mg/kg. In some embodiments, the second dose, third dose, and/or subsequent doses of the inducer(s) are administered at a dose of 5 mg/kg to 10 mg/kg.

[00129] In some embodiments, the first dose is 1-80 mg/kg, the second dose is 1-40 mg/kg, and/or the subsequent dose (e.g., third dose) is 1-40 mg/kg. In some embodiments, the first dose is 1-80 mg/kg, the second dose is 1-40 mg/kg, and the subsequent dose (e.g., the third dose) is 1-40 mg/kg.

[00130] In some embodiments, the administering comprises: administering a plurality of doses of a first inducer during a period of 2 to 4 weeks; administering 1 or 2 doses of a second inducer; and administering at least 1 dose of a third inducer. In some embodiments, the administering comprises: administering a plurality of doses of a first inducer during a period of 2 to 4 weeks, wherein each dose of the first inducer is 1-200 mg/kg; administering 1 or 2 doses of a second inducer, wherein each dose of the second inducer is 1-100 mg/kg; administering at least 1 dose of a third inducer, wherein each dose of the third inducer is 1-80 mg/kg. In some embodiments, the

administering comprises: administering a plurality of doses of a first inducer during a period of 2 to 4 weeks, wherein each dose of the first inducer is 160 mg/kg; administering 1 or 2 doses of a second inducer, wherein each dose of the second inducer is 80 mg/kg; administering at least 1 dose of a third inducer, wherein each dose of the third inducer is 40 mg/kg.

[00131] In some embodiments, a dose of the third inducer is administered until the subject no longer requires the expression of the transgene. In some embodiments, the method further comprises repeating the first and/or second administering step if the subject becomes non-responsive to the inducer(s). In some embodiments, the method further comprises repeating the administration of the first inducer and/or second inducer if the subject becomes non-responsive to the third inducer.

[00132] It is noted that the amount of inducer(s) administered to the subject can be varied over time by adjusting the dose, adjusting the time period between doses, or both. Thus, for example, to increase the amount of inducer(s) administered to a subject the dose can be increased while the time period between doses is kept constant, the dose can be kept constant while the time period between doses is reduced, or the dose can be increased and the time period between doses is reduced. To decrease the amount of inducer(s) administered to a subject the dose can be decreased while the time period between doses is kept constant, the dose can be kept constant while the time period between doses is reduced, or the dose can be decreased and the time period between doses is increased.

[00133] It is also noted that the inducer(s) is administered at the second time point, or any subsequent time point can be same as the inducer(s) administered at the first time point, or the previous time point. For example, the inducer(s) administered at the first time point, or the previous time point can be artemisinin and the inducer(s) is administered at the second time point, or any subsequent time point can be an artemisinin derivative. Alternatively, the inducer(s) administered at the first time point, or the previous time point can be an artemisinin derivative and the inducer(s) is administered at the second time point, or any subsequent time point can be artemisinin. In another non-limiting example, the inducer(s) administered at the first time point, or the previous time point can be artemisinin or a derivative, and the inducer(s) administered at the second time point, or any subsequent time point can be same or different. For example, the inducer(s) can be a second artemisinin derivative, e.g., artesunate, artemether, dihydroartemisinin, α -dihydroartemisinin β -glucuronide, artemisone, arteether, artenimol, artesunic acid, artelinic acid, deoxyartemisinin, deoxydihydroartemisinin, 9,10-dihydrodeoxyaremisinin, artemotil, and artemiside, or any inducer such as a flavonoid compound, e.g. flavone, chrysin, baicalein, or galangin; 1,4-Bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP); phenobarbital (PB); 6-(4Chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde-O-(3,4-dichlorobenzyl)oxime

(CITCO); Acetaminophen; Buprenorphine; Phenytoin; Carbamazepine; Valproic Acid; Chlorpromazine; Efavirenz; Nevirapine; Rilpivirine; Etravirine; Diazepam; Cyclophosphamid; Ifosfamide; Cerivastatin; Simvastatin; lovastatin; substituted sulphonamides; Thiazolidin-4-one; Estradiol; Estrone and analogues; 17 α -ethinyl-3;17 β -estradiol (EE2); Dehydroepiandrosterone (DHEA); 5 β -pregnane-3,20-dione; Diethylstilbestrol; Ginkgo *biloba* extract; Galangin; chrysin; baicalein; Diallyl sulphide; Ellagic acid; Resveratrol; Squalestatin-1; Bilobalide; Triclocarban; Triclosan; Dichlorodiphenyltrichloroethane (DDT); Dieldrin; Methoxychlor; Metofluthrin; Permethrin; Pyrethrins; Sulfoxaflor; Diethylhexyl phthalate (DEHP); Cyproconazole; Fluconazole; Propiconazole; FL81; Tri-p-methylphenyl phosphate (TMPP); UM104; or UM145.

[00134] In some embodiments, the inducer(s) administered at the first time point, or the previous time point can be a flavonoid compound, and the inducer(s) administered at the second time point, or any subsequent time point can be same or different. For example, the inducer(s) administered at the first time point, or the previous time point can be flavone, baicalein, chrysin, galangin, (OH)₄ flavone, 6-hydroxyluteolin, acacetin, acerosin, alnetin, apigenin, apiin, artocarpetin, balcalein, beta-sitosterol, biochamin A, cannaflavin A, cannflavin A, cannflavin B, cannflavin C, catechin, catechin-3-gallate, cerrosillin, chrysoeriol, chrysoeriol, cirsilioneol, cirsililol, cirsimaritin, corymbosin, cupressuflavone, cyanidine, daidzein, datiscetin, delphinidine, digidrokempferol, dihydroquercetin, diosmetin, diosmin, echiodinin, epicatechin, epicatechin-3-gallate, epigallocatechin, epigallocatechin-3-gallate, eriodictiol, eupatilin, eupatorin, fisetin, fizetin, flavonol, formononetin, fortunelin, gallocatechin, gallocatechin-3-gallate, gardenin D, genistein, genistin, genkwanin, geraldol, geraldone, glycitein, glycitin, gomoeriodictiol, gossypetin, hesperetin, hinokiflavone, hispidulin, hymenoxin, hypolaetin, isoquercetin, isorhamnetin, isorhamnetin, isoscutellarein, isovitexin, jaceosidin, kaempferol, kaempferol, kampferide, largonidin, luteolin, luteolin, malvidine, mikanin, morin, myricetin, myricetin, naringenin, negletein, neodiosmin, nepetin, nevadensin, nobiletin, nodifloretin, norartocarpetin, norwogonin, onopordin, orientin, oroxylin a, pachipodol, pectolinarigenin, pedaltin, peonidin, petunidin, pilloin, pratol, primetin, primuletin, pruentin, quercetagenin, quercetin, ramnazin, rhoifolin, robinetin, rutin, scaposin, scutellarein, serpyllin, sinensetin, sorbifolin, sudachitin, tangeretin, tangeritin, taxifolin, tectochrysin, tithonine, tricetin, tricin, velutin, vitexin, wightin, wogonin, xanthomicrol, zapotin, zapotinin, 6,7,4'-trihydroxy isoflavone, 7,3'4'-trihydroxy isoflavone, or a pharmaceutically acceptable salt thereof. In some embodiments, the inducer(s) administered at the first time point, or the previous time point is flavone.

[00135] In some embodiments, the inducer(s) administered at the first time point, or the previous time point can be a flavonoid compound and the inducer(s) administered at the second time point, or any subsequent time point can be artemisinin. For example, the inducer(s)

administered at the first time point, or the previous time point can be flavone, baicalein, chrysin, galangin, (OH)₄ flavone, 6-hydroxyluteolin, acacetin, acerosin, alnetin, apigenin, apiin, artocarpetin, balcalein, beta-sitosterol, biochamin A, cannaflavin A, cannflavin A, cannflavin B, cannflavin C, catechin, catechin-3-gallate, cerrosillin, chrysoeriol, chrysoeriol, cirsilineol, cirsilinol, cirsimaritin, corymbosin, cupressuflavone, cyanidine, daidzein, datiscetin, delphinidine, digidrokempferol, dihydroquercetin, diosmetin, diosmin, echiodinin, epicatechin, epicatechin-3-gallate, epigallocatechin, epigallocatechin-3-gallate, eriodiktiol, eupatilin, eupatorin, fisetin, fizetin, flavonol, formononetin, fortunelin, gallocatechin, gallocatechin-3-gallate, gardenin D, genistein, genistin, genkwanin, geraldol, geraldone, glycitein, glycitin, gomoeriodiktiol, gossypetin, hesperetin, hinokiflavone, hispidulin, hymenoxin, hypolaetin, isoquercetin, isorhamnetin, isorhamnetin, isoscutellarein, isovitexin, jaceosidin, kaempferol, kaempferol, kampferide, largonidin, luteolin, luteolin, malvidine, mikanin, morin, myricetin, myricetin, naringenin, negletein, neodiosmin, nepetin, nevadensin, nobiletin, nodifloretin, norartocarpetin, norwogonin, onopordin, orientin, oroxylin a, pachipodol, pectolinarigenin, pedaltin, peonidin, petunidin, pilloin, pratol, primetin, primuletin, pruentin, quercetagetin, quercetin, ramnazin, rhoifolin, robinetin, rutin, scaposin, scutellarein, serpyllin, sinensetin, sorbifolin, sudachitin, tangeretin, tangeritin, taxifolin, tectochrysin, tithonine, tricetin, tricin, velutin, vitexin, wightin, wogonin, xanthomicrol, zapotin, zapotin, 6,7,4'-trihydroxy isoflavone, 7,3'4'-trihydroxy isoflavone, or a pharmaceutically acceptable salt thereof; and the inducer(s) administered at the second time point, or any subsequent time point can be artemisinin, optionally, the inducer(s) administered at the first time point, or the previous time point can be flavone and the inducer(s) administered at the second time point, or any subsequent time point can be artemisinin.

[00136] In some embodiments, the inducer(s) administered at the first time point, or the previous time point can be artemisinin and the inducer(s) is administered at the second time point, or any subsequent time point can be a flavonoid compound, e.g., flavone. For example, the inducer(s) administered at the first time point, or the previous time point can be artemisinin and the inducer(s) is administered at the second time point, or any subsequent time point can be flavone, baicalein, chrysin, galangin, (OH)₄ flavone, 6-hydroxyluteolin, acacetin, acerosin, alnetin, apigenin, apiin, artocarpetin, balcalein, beta-sitosterol, biochamin A, cannaflavin A, cannflavin A, cannflavin B, cannflavin C, catechin, catechin-3-gallate, cerrosillin, chrysoeriol, chrysoeriol, cirsilineol, cirsilinol, cirsimaritin, corymbosin, cupressuflavone, cyanidine, daidzein, datiscetin, delphinidine, digidrokempferol, dihydroquercetin, diosmetin, diosmin, echiodinin, epicatechin, epicatechin-3-gallate, epigallocatechin, epigallocatechin-3-gallate, eriodiktiol, eupatilin, eupatorin, fisetin, fizetin, flavonol, formononetin, fortunelin, gallocatechin, gallocatechin-3-gallate, gardenin D, genistein, genistin, genkwanin, geraldol, geraldone, glycitein, glycitin, gomoeriodiktiol,

gossypetin, hesperetin, hinokiflavone, hispidulin, hymenoxin, hypolaetin, isoquercetin, isorhamnetin, isorhamnetin, isoscutellarein, isovitexin, jaceosidin, kaempferol, kaempferol, kampferide, largonidin, luteolin, luteolin, malvidine, mikanin, morin, myricetin, myricetin, naringenin, negletein, neodiosmin, nepetin, nevadensin, nobiletin, nodifloretin, norartocarpetin, norwogonin, onopordin, orientin, oroxylin a, pachipodol, pectolinarigenin, pedalitin, peonidin, petunidin, pilloin, pratol, primetin, primuletin, pruentin, quercetagetin, quercetin, ramnazin, rhoifolin, robinetin, rutin, scaposin, scutellarein, serpyllin, sinensetin, sorbifolin, sudachitin, tangeretin, tangeritin, taxifolin, tectochrysin, tithonine, tricetin, tricin, velutin, vitexin, wightin, wogonin, xanthomicrol, zapotin, zapotin, 6,7,4'-trishydroxy isoflavone, 7,3'4'-trihydroxy isoflavone, or a pharmaceutically acceptable salt thereof, optionally, the inducer(s) administered at the first time point, or the previous time point can be artemisinin and the inducer(s) administered at the second time point, or any subsequent time point can be flavone.

[00137] In some embodiments, the inducer(s) administered at the first time point, or the previous time point can be a flavonoid compound and the inducer(s) is administered at the second time point, or any subsequent time point can be an artemisinin derivative. For example, For example, the inducer(s) administered at the first time point, or the previous time point can be flavone, baicalein, chrysin, galangin, (OH)₄ flavone, 6-hydroxyluteolin, acacetin, acerosin, alnetin, apigenin, apiin, artocarpetin, balcalein, beta-sitosterol, biochamin A, cannaflavin A, cannflavin A, cannflavin B, cannflavin C, catechin, catechin-3-gallate, cerrosillin, chrysoeriol, chrysoeriol, cirsilinole, cirsilinole, cirsimaritin, corymbosin, cupressuflavone, cyanidine, daidzein, datiscetin, delphinidine, digidrokempferol, dihydroquercetin, diosmetin, diosmin, echiodinin, epicatechin, epicatechin-3-gallate, epigallocatechin, epigallocatechin-3-gallate, eriodiktiol, eupatilin, eupatorin, fisetin, fizetin, flavonol, formononetin, fortunelin, gallocatechin, gallocatechin-3-gallate, gardenin D, genistein, genistin, genkwanin, geraldol, geraldone, glycitein, glycitin, gomoeriodiktiol, gossypetin, hesperetin, hinokiflavone, hispidulin, hymenoxin, hypolaetin, isoquercetin, isorhamnetin, isorhamnetin, isoscutellarein, isovitexin, jaceosidin, kaempferol, kaempferol, kampferide, largonidin, luteolin, luteolin, malvidine, mikanin, morin, myricetin, myricetin, naringenin, negletein, neodiosmin, nepetin, nevadensin, nobiletin, nodifloretin, norartocarpetin, norwogonin, onopordin, orientin, oroxylin a, pachipodol, pectolinarigenin, pedalitin, peonidin, petunidin, pilloin, pratol, primetin, primuletin, pruentin, quercetagetin, quercetin, ramnazin, rhoifolin, robinetin, rutin, scaposin, scutellarein, serpyllin, sinensetin, sorbifolin, sudachitin, tangeretin, tangeritin, taxifolin, tectochrysin, tithonine, tricetin, tricin, velutin, vitexin, wightin, wogonin, xanthomicrol, zapotin, zapotin, 6,7,4'-trishydroxy isoflavone, 7,3'4'-trihydroxy isoflavone, or a pharmaceutically acceptable salt thereof; and the inducer(s) administered at the second time point, or any subsequent time point can be an artemisinin

derivative, optionally, the inducer(s) administered at the first time point, or the previous time point can be flavone and the inducer(s) administered at the second time point, or any subsequent time point can be artesunate, artemether, dihydroartemisinin, α -dihydroartemisinin β -glucuronide, artemisone, arteether, arteminol, artesunic acid, artelinic acid, deoxyartemisinin, deoxydihydroartemisinin, 9,10-dihydrodeoxyaremisinin, artemotil, or artemiside,

[00138] In some embodiments, the inducer(s) administered at the first time point, or the previous time point can be an artemisinin derivative and the inducer(s) is administered at the second time point, or any subsequent time point can be a flavonoid compound. For example, the inducer(s) administered at the first time point, or the previous time point can be an artemisinin derivative and the inducer(s) is administered at the second time point, or any subsequent time point can be flavone, baicalein, chrysin, galangin, (OH)₄ flavone, 6-hydroxyluteolin, acacetin, acerosin, alnetin, apigenin, apiin, artocarpetin, balcalein, beta-sitosterol, biochamin A, cannaflavin A, cannflavin A, cannflavin B, cannflavin C, catechin, catechin-3-gallate, cerrosillin, chrysoeriol, chrysoeriol, cirsilinol, cirsiol, cirsimaritin, corymbosin, cupressuflavone, cyanidine, daidzein, datiscetin, delphinidine, digidrokempferol, dihydroquercetin, diosmetin, diosmin, echiodinin, epicatechin, epicatechin-3-gallate, epigallocatechin, epigallocatechin-3-gallate, eriodiktio, eupatilin, eupatorin, fisetin, fizetin, flavonol, formononetin, fortunelin, galocatechin, galocatechin-3-gallate, gardenin D, genistein, genistin, genkwanin, geraldol, geraldone, glycitein, glycitin, gomoeriodiktio, gossypetin, hesperetin, hinokiflavone, hispidulin, hymenoxin, hypolaetin, isoquercetin, isorhamnetin, isorhamnetin, isoscutellarein, isovitexin, jaceosidin, kaempferol, kaempferol, kampferide, largonidin, luteolin, luteolin, malvidine, mikanin, morin, myricetin, myricetin, naringenin, negletein, neodiosmin, nepetin, nevadensin, nobiletin, nodifloretin, norartocarpetin, norwogonin, onopordin, orientin, oroxylin a, pachipodol, pectolarigenin, pedalitin, peonidin, petunidin, pilloin, pratol, primetin, primuletin, pruentin, quercetagenin, quercetin, ramnazin, rhoifolin, robinetin, rutin, scaposin, scutellarein, serpyllin, sinensetin, sorbifolin, sudachitin, tangeretin, tangeritin, taxifolin, tectochrysin, tithonine, tricetin, tricin, velutin, vitexin, wightin, wogonin, xanthomicrol, zapotin, zapotin, 6,7,4'-trihydroxy isoflavone, 7,3,4'-trihydroxy isoflavone, or a pharmaceutically acceptable salt thereof, optionally, the inducer(s) administered at the first time point, or the previous time point can be artesunate, artemether, dihydroartemisinin, α -dihydroartemisinin β -glucuronide, artemisone, arteether, arteminol, artesunic acid, artelinic acid, deoxyartemisinin, deoxydihydroartemisinin, 9,10-dihydrodeoxyaremisinin, artemotil, or artemiside, and the inducer(s) administered at the second time point, or any subsequent time point can be flavone,

[00139] In another non-limiting example, the inducer(s) administered at the first time point, or the previous time point can be a flavonoid compound, e.g., flavone, and the inducer(s)

administered at the second time point, or any subsequent time point can be 1,4-Bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP); phenobarbital (PB); 6-(4Chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde-O-(3,4-dichlorobenzyl)oxime (CITCO); Acetaminophen; Buprenorphine; Phenytoin; Carbamazepine; Valproic Acid; Chlorpromazine; Efavirenz; Nevirapine; Rilpivirine; Etravirine; Diazepam; Cyclophosphamid; Ifosfamide; Cerivastatin; Simvastatin; lovastatin; substituted sulphonamides; Thiazolidin-4-one; Estradiol; Estrone and analogues; 17 α -ethinyl-3,17 β -estradiol (EE2); Dehydroepiandrosterone (DHEA); 5 β -pregnane-3,20-dione; Diethylstilbestrol; Ginkgo *biloba* extract; Galangin; chrysin; baicalein; Diallyl sulphide; Ellagic acid; Resveratrol; Squalastatin-1; Bilobalide; Triclocarban; Triclosan; Dichlorodiphenyltrichloroethane (DDT); Dieldrin; Methoxychlor; Metofluthrin; Permethrin; Pyrethrins; Sulfoxaflor; Diethylhexyl phthalate (DEHP); Cyproconazole; Fluconazole; Propiconazole; FL81; Tri-p-methylphenyl phosphate (TMPP); UM104; or UM145.

[00140] As used herein, the term “administer” refers to the placement of an inducer or expression cassette into a subject by a method or route which results in at least partial localization of the inducer or the expression cassette at a desired site such that desired effect, e.g., expression of the transgene is produced.

[00141] An inducer or exogenous nucleotide sequence (e.g., a vector comprising the exogenous nucleotide sequence) described herein can be administered by any appropriate route known in the art including, but not limited to, oral or parenteral routes, including intravenous, intramuscular, subcutaneous, transdermal, airway (aerosol), pulmonary, nasal, rectal, and topical (including buccal and sublingual) administration. Exemplary modes of administration include, but are not limited to, injection, infusion, instillation, inhalation, or ingestion. “Injection” includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intraventricular, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, sub capsular, subarachnoid, intraspinal, intracerebro spinal, and intrasternal injection and infusion. In some embodiments, administration will generally be systemic. In some other embodiments, administration will be local.

[00142] The phrases “parenteral administration” and “administered parenterally” as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intraventricular, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, sub capsular, subarachnoid, intraspinal, intracerebro spinal, and intrasternal injection, infusion and other injection or infusion techniques, without limitation. The phrases “systemic administration,” “administered systemically,” “peripheral administration” and “administered peripherally” as used herein mean the administration of a

pharmaceutical composition comprising an inducer such that it enters the subject's system and, thus, is subject to metabolism and other like processes, for example, subcutaneous administration.

[00143] In some embodiments, the inducer administration is intraperitoneal administration. In some embodiments, the administration of the inducer(s) administered at the first time point is intraperitoneal administration. In some embodiments, the administration of the inducer(s) administered at the second time point is intraperitoneal administration. In some embodiments, the administration of the inducer(s) administered at the first time point and the administration of the inducer(s) administered at the second time point are intraperitoneal administration. In some embodiments, the administration of the subsequent inducer(s) at subsequent time point(s) is intraperitoneal administration.

[00144] In some embodiments, the inducer administration is oral administration. In some embodiments, the administration of the inducer(s) administered at the first time point is oral administration. In some embodiments, the administration of the inducer(s) administered at the second time point is oral administration. In some embodiments, the administration of the inducer(s) administered at the first time point and the administration of the inducer(s) administered at the second time point are oral administration. In some embodiments, the administration of the subsequent inducer(s) at subsequent time point(s) is oral administration. Without limitations, oral administration can be in the form of solutions, suspensions, tablets, pills, capsules, sustained-release formulations, oral rinses, powders and the like.

[00145] In jurisdictions that forbid the patenting of methods that are practiced on the human body, the meaning of “administering” of an inducer or expression cassette to a human subject shall be restricted to prescribing a controlled substance that a human subject will be administer to the subject by any technique (e.g., orally, inhalation, topical application, injection, insertion, etc.). The broadest reasonable interpretation that is consistent with laws or regulations defining patentable subject matter is intended. In jurisdictions that do not forbid the patenting of methods that are practiced on the human body, the “administering” includes both methods practiced on the human body and also the foregoing activities.

Inducible promoter

[00146] Embodiments of the various aspects described herein include an inducible promoter. Generally, the inducible promoter comprises a cis-regulatory motif (CRE) that is capable of being bound and activated by a heterodimer of constitutive androstane receptor (CAR) and retinoid X receptor (RXR).

[00147] Constitutive androstane receptor (CAR) is a member of the nuclear receptor superfamily, and it is a key regulator of xenobiotic and endobiotic metabolism. Unlike most nuclear

receptors, CAR is constitutively active in the absence of ligand and is regulated by both agonists and inverse agonists. Activation results in translocation of CAR from the cytosol into the nucleus, where the protein can bind to specific DNA sites, called response motifs. Binding occurs both as a monomer and together with the retinoid X receptor (RXR) resulting in activation or repression of target gene transcription. Retinoid X receptor (RXR) is a member of the nuclear receptor superfamily that is activated by 9-cis retinoic acid and 9-cis-13,14-dihydro-retinoic acid.

[00148] When CAR is activated (by either direct or indirect activation), it translocates to the nucleus where CAR and RXR form a heterodimer (referred to herein as “CAR-RXR heterodimer” or “CAR-RXR”) that can bind to an activate genes comprising an appropriate target sequence. The CAR-RXR heterodimer binds to and induces target genes *via* the PB-responsive enhancer module (PBREM). Accordingly, in some embodiments of any one of the aspects described herein, the CRE that is capable of being bound and activated by a CAR-RXR heterodimer comprises a PBREM element/enhancer, or a functional variant thereof. For example, the inducible promoter comprises at least one PBREM enhancer, e.g., a wild-type PBREM enhancer. In some embodiments, the inducible promoter comprises at least two or more (e.g., 2, 3, 4, 5 or more) PBREM enhancers. For example, the inducible promoter comprises two PBREM enhancers. Exemplary PBREM sequences include, but are not limited to, mouse PBREM sequence:

TCTGTACTTTCCTGACCT*TGGCACAGTGCCACCATCAACTTGCCTGACACC*

(SEQ ID NO: 1),

and human PBREM sequence:

ACTGTACTTTCCTGACCCT*GAAAGAGGTGGCAGCATGGACTTTCCTGAACCA*

(SEQ ID NO: 2).

[00149] PBREM is a 51-bp DNA inducible enhancer consisting of two nuclear receptor DR4 motifs (NR1 and NR2) flanking a nuclear factor -1 (NF1) binding site. It has been proposed in the literature that the NR1 sequence is the binding site for the CAR-RXR heterodimer, and the remaining sequences are partially or completely redundant. A triple repeat of NR1 (was shown to remain inducible in Sueyoshi, *et al.* (J. BIOL. CHEM. Vol. 274,10, pp. 6043-6046, 1999). However, it is believed that parts other than NR1 may play a role in making the PBREM motif more specific and inducible (e.g., reducing background, constitutive expression and allowing from a greater magnitude of induction). In **SEQ ID NO: 1** and **SEQ ID NO: 2**, the NR1 motif is underlined, the NF1 motif is in italics, and the NR2 motif is in bold.

[00150] In some embodiments of any one of the aspects described herein, the CRE that is capable of being bound and activated by a CAR-RXR heterodimer comprises each of an NR1 motif, an NF1 motif, and an NR2 motif. It is noted that the NR1 motif, the NF1 motif and the NR2 motif

can be present in any order. For example, the CRE comprises the motifs in the order NR1-NF1-NR2.

NR1

[00151] A CRE that is capable of being bound and activated by a CAR-RXR heterodimer suitably comprises at least one NR1 motif. In some embodiments of any one of the aspects described herein, the NR1 motif comprises the nucleotide sequence TGTACT-X-TGACC[C/T] (**SEQ ID NO: 3**), wherein X represents any sequence which is from 3 to 6 nucleotides in length. For example, X can be 3 to 5 nucleotides in length. In some embodiments, X is 4 nucleotides in length. In some other embodiments, X is 5 nucleotides in length. Some exemplary nucleotide sequences for X include, but are not limited to, TTCC and TTTCC. In some preferred embodiments, X comprises the sequence TTCC. For example, X consists of the nucleotide sequence TTCC or TTTCC.

[00152] Where nucleotides are shown within square brackets with a slash, it indicates that one of the indicated nucleotides within the square brackets is present at that location.

[00153] In some embodiments of any one of the aspects described herein, the NR1 motif comprises a nucleotide sequence that is at least 90% identical to nucleotides 3 to 18 of **SEQ ID NO: 1 or SEQ ID NO: 2**.

[00154] For example, the NR1 motif comprises a nucleotide sequence that is at least 90% identical the sequence TGTACTTTCCTGACCT (**SEQ ID NO: 4**, mouse NR1) or TGTACTTTCCTGACCC (**SEQ ID NO: 5**, human NR1). For example, the NR1 motif comprises a sequence that is at least 95% identical to **SEQ ID NO: 4 or SEQ ID NO: 5**. In some embodiments, the NR1 motif comprises a sequence that is at least 99% identical to **SEQ ID NO: 4 or SEQ ID NO: 5**. In some embodiments of any one of the aspects described herein, the NR1 motif comprises a nucleotide sequence that is at least 90% identical, preferably at least 95% identical, more preferably at least 99%, and most preferably perfectly identical to **SEQ ID NO: 4 or SEQ ID NO: 5**. In some embodiments of any one of the aspects described herein, the NR1 motif comprises the nucleotide sequence TGTACTTTCCTGACCN (**SEQ ID NO: 6**) or CTGTACTTTCCTGACCN (**SEQ ID NO: 7**).

[00155] The functional variant of a motif can comprise two or more operably linked NR1 motif-containing sequences. For example, the functional variant of a PBREM motif can comprise three, four, five or more operably linked NR1 motif-containing sequences. When two or more NR1 motifs are present, they can be immediately adjacent to one another or separated by a spacer. In some embodiments, a CRE that is capable of being bound and activated by a CAR-RXR heterodimer suitably comprises the general structure NR1-S-NR1, wherein NR1 represents any

NR1 motif as discussed herein, and S represents an optional spacer. When two or more NR1 motifs are present, they can be identical NR1 or they can be different to one another. Further, when a spacer is present, it can have any sequence and be of any suitable length. For example, each spacer can be independently from 2 to 50 nucleotides in length. In some embodiments, each spacer is from 3 to 40, from 4 to 30, from 5 to 20, from 6 to 10, or from 7 to 9 nucleotides in length. In some embodiments, each spacer is independently, 5, 6, 7, 8, 9, 10 or 11 nucleotides in length.

[00156] In some embodiments of any one of the aspects described herein, the NR1 motif comprises the nucleotide sequence [TGTACTTTCCTGACCN-S]_n (**SEQ ID NO: 8**), or [CTGTACTTTCCTGACCN-S]_n (**SEQ ID NO: 9**), wherein N is any nucleotide, n is an integer from 1 to 10, and S is absent or a spacer.

[00157] In some embodiments, n is an integer from 1 to 6. For example, n is an integer from 2 to 4. In some embodiments, n is selected from 2, 3, or 4. For example, n is 1, 2 or 3. In some embodiments, n is 1. In some other embodiments, n is 2. In yet some other embodiments, n is 3.

[00158] When present, each spacer, S, can be independently from 2 to 50 nucleotides in length. In some embodiments, each spacer, S, is from 3 to 40, from 4 to 30, from 5 to 20, from 6 to 10, or from 7 to 9 nucleotides in length. In some embodiments, each spacer, S, is independently, 5, 6, 7, 8, 9, 10 or 11 nucleotides in length.

[00159] In some embodiments of any one of the aspects described herein, n is 2 or 3. For example, the functional variant of a PBREM motif comprises the nucleotide sequence:

TGTACTTTCCTGACCN-S-TGTACTTTCCTGACCN (**SEQ ID NO: 10**);

TGTACTTTCCTGACCN-S-TGTACTTTCCTGACCN-S-TGTACTTTCCTGACCN
(**SEQ ID NO: 11**);

CTGTACTTTCCTGACCN-S-CTGTACTTTCCTGACCN (**SEQ ID NO: 12**);

CTGTACTTTCCTGACCN-S-CTGTACTTTCCTGACCN-S-CTGTACTTTCCTGACCN
(**SEQ ID NO: 13**);

NCTGTACTTTCCTGACCNTG-S-NCTGTACTTTCCTGACCNTG (**SEQ ID NO: 14**);

or

NCTGTACTTTCCTGACCNTG-S-NCTGTACTTTCCTGACCNTG-S-
NCTGTACTTTCCTGACCNTG (**SEQ ID NO: 15**),

wherein N is any nucleotide, and S is absent or a spacer.

[00160] In some embodiments of any one of the aspects described herein, n is 3. For example, the functional variant of a PBREM motif comprises the nucleotide sequence:

TCTGTACTTTCCTGACCTTG-S-TCTGTACTTTCCTGACCTTG-S-
TCTGTACTTTCCTGACCTTG (**SEQ ID NO: 16**); or

ACTGTACTTTCCTGACCCTG-S-ACTGTACTTTCCTGACCCTG-S-

ACTGTACTTTCCTGACCCTG (**SEQ ID NO: 17**),

wherein N is any nucleotide, and S is absent or a spacer.

[00161] In some embodiments of any one of the aspects described herein, the functional variant of a PBREM motif comprises the nucleotide sequence:

gacTCTGTACTTTCCTGACCTTGgacgacTCTGTACTTTCCTGACCTTGgacgacTC

TGTACTTTCCTGACCTTGgac (**SEQ ID NO: 18**); or

gacACTGTACTTTCCTGACCCTGgacgacACTGTACTTTCCTGACCCTGgacgacA
CTGTACTTTCCTGACCCTGgac (**SEQ ID NO: 19**).

NF1

[00162] Generally, the NF1 motif comprises a nucleotide sequence that varies by 7 or fewer nucleotides (e.g., 6, 5, 4, 3, 2, or 1 nucleotides) from nucleotides 19 to 31 of SEQ ID NO: 1 or SEQ ID NO: 2. For example, the NF1 motif comprises the nucleotide sequence TGGCACAGTGCCA (**SEQ ID NO: 20**) or TGAAGAGGTGGCA (**SEQ ID NO: 21**), or a nucleotide sequence that varies no more than 7 nucleotides from **SEQ ID NO: 20 or SEQ ID NO: 21**. In some embodiments of any one of the aspects described herein, the NF1 motif comprises a nucleotide sequence that varies by no more than 1 to 6 nucleotides from **SEQ ID NO: 20 or SEQ ID NO: 21**. For example, the NF1 motif comprises a nucleotide sequence that varies by no more than 1, 2, 3, 4 or 5 nucleotides from **SEQ ID NO: 20 or SEQ ID NO: 21**. In some embodiments of any one of the aspects described herein, the NF1 motif comprises a nucleotide sequence that varies by no more than 1 or 2 nucleotides from **SEQ ID NO: 20 or SEQ ID NO: 21**.

[00163] In some embodiments of any one of the aspects described herein, the NF1 motif comprises the nucleotide sequence TGNNNNGTGNCA (**SEQ ID NO: 22**), where N is any nucleotide.

[00164] In some embodiments of any one of the aspects described herein, the NF1 motif consists of **SEQ ID NO: 20 or SEQ ID NO: 21**.

NR2 motif

[00165] Generally, the NR2 motif comprises a nucleotide sequence that varies by 5 or fewer nucleotides (e.g., 4, 3, 2, or 1 nucleotides) from nucleotides 35 to 50 of SEQ ID NO: 1 or SEQ ID NO: 2. For example, the NR2 motif suitably comprises the nucleotide sequence TCAACTTGCCTGACAC (**SEQ ID NO: 23**) or TGGACTTTCCTGAACC (**SEQ ID NO: 24**), or a nucleotide sequence that varies by no more than by 5 nucleotides from **SEQ ID NO: 23 or SEQ**

ID NO: 24. In some embodiments of any one of the aspects described herein, the NR2 motif comprises a nucleotide sequence that varies by no more than 1 to 4 nucleotides from **SEQ ID NO: 23 or SEQ ID NO: 24**. For example, the NR2 motif comprises a nucleotide sequence that varies by no more than 1, 2, or 3 nucleotides from **SEQ ID NO: 23 or SEQ ID NO: 24**. In some embodiments of any one of the aspects described herein, the NR2 motif comprises a nucleotide sequence that varies by no more than 1 or 2 nucleotides from **SEQ ID NO: 23 or SEQ ID NO: 24**.

[00166] In some embodiments of any one of the aspects described herein, the NR2 motif comprises the nucleotide sequence TNNACTTNCCTGANNC (**SEQ ID NO: 25**), where N is any nucleotide.

[00167] In some embodiments of any one of the aspects described herein, the NR2 motif consists of **SEQ ID NO: 23 or SEQ ID NO: 24**.

CRE

[00168] In some embodiments, the CRE that is capable of being bound and activated by a CAR-RXR heterodimer comprises a nucleotide sequence that is at least 60% identical to **SEQ ID NO: 1 or SEQ ID NO: 2**. For example the CRE that is capable of being bound and activated by a CAR-RXR heterodimer comprises a nucleotide sequence that is at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99 % identical to **SEQ ID NO: 1 or SEQ ID NO: 2**. It is noted that mouse PBREM (**SEQ ID NO: 1**) and human PBREM (**SEQ ID NO: 2**) are 71% identical along their entire length (i.e., 51 nucleotides). Mouse PBREM remains functional in human cells, and human function in PBREM remains functional in mouse cells. Thus, at least this level of overall sequence difference across the PBREM motif can be tolerated.

[00169] In some embodiments of any one of the aspects described herein, the CRE comprises a nucleotide sequence having at least 90% identity to **SEQ ID NO: 1 or SEQ ID NO: 2**. For example, the CRE comprises a comprises a nucleotide sequence having at least 95% identity to **SEQ ID NO: 1 or SEQ ID NO: 2**. In some embodiments of any one of the aspects described herein, the CRE comprises a nucleotide sequence having at least 99% identity to **SEQ ID NO: 1 or SEQ ID NO: 2**.

[00170] In some embodiments of any one of the aspects described herein, the CRE comprises a sequence that is at least 90% identical to nucleotides 3 to 18 of **SEQ ID NO: 1 or SEQ ID NO: 2**. For example, the CRE comprises a sequence that is at least 95% identical to nucleotides 3 to 18 of **SEQ ID NO: 1 or SEQ ID NO: 2**. In some embodiments, the CRE comprises a sequence that is at least 99% identical to nucleotides 3 to 18 of **SEQ ID NO: 1 or SEQ ID NO: 2**. In some embodiments of any one of the aspects described herein, the CRE that is capable of being bound and activated by a CAR-RXR heterodimer comprises a nucleotide sequence that is at least 90%

identical, preferably at least 95% identical, more preferably at least 99%, and most preferably perfectly identical to **SEQ ID NO: 1 or SEQ ID NO: 2** in the region spanning nucleotides 3 to 18, and at least 50%, 55%, 60%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identical across the remainder of **SEQ ID NO: 1 or SEQ ID NO: 2** (i.e., nucleotides 1, 2, and 19 to 51, or nucleotides 21 to 51). Nucleotides are of course numbered with reference to **SEQ ID NO: 1 or SEQ ID NO: 2**. Preferably the overall sequence is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identical to either **SEQ ID NO: 1 or SEQ ID NO: 2**.

[00171] In some embodiments of any one of the aspects described herein, the CRE comprises a nucleotide sequence having at least 90% identity to the nucleotide sequence:

NCTGTACTTTTCCTGACCNTGNNNNNGTGNCANCATNNACTTNCCTGANNCN
(**SEQ ID NO: 26**).

[00172] For example, the CRE comprises a nucleotide sequence having at least 95% identity to **SEQ ID NO: 26**. In some embodiments, the CRE comprises a nucleotide sequence having at least 99% identity to **SEQ ID NO: 26**. For example the CRE comprises a nucleotide sequence having 100% identity to **SEQ ID NO: 26**.

[00173] It is noted that % identity is calculated with respect to the specifically defined nucleotides rather than the undefined “Ns”. Functional variants of a PBREM sequence where one or more nucleotides identified as N are deleted are specifically contemplated as part of such embodiments of the invention, for example where up to 7, 6, 5, 4, 3, 2, or 1 nucleotides marked N are deleted. Furthermore, functional variants of a PBREM sequence where one or more nucleotides are inserted are specifically contemplated as part of such embodiments of the invention, for example where up to 7, 6, 5, 4, 3, 2, or 1 nucleotides are inserted. As one specific example, the rat PBREM element from the CYP2B2 gene comprise a T inserted between the T and C at position 10-11, i.e., within NR1 of the PBREM element. Replacement, deletion or insertion of nucleotides in regions outside of the NR1 motif are likely to be well-tolerated.

[00174] It will be noted that the PBREM element or functional variant thereof can be present in either orientation. The reverse complement of the PBREM elements described herein thus form part of disclosure. It is noted that the human PBREM element is naturally present in the reverse orientation compared to the mouse PBREM element, and that the human PBREM element was shown to remain functional in the “reverse” orientation (i.e., the same orientation as mouse PBREM) in Sueyoshi, *et al.* (J. BIOL. CHEM. Vol. 274,10, pp. 6043-6046, 1999).

[00175] In some embodiments of the invention, the inducible promoter comprises a plurality of CREs that are each capable of being bound and activated by a heterodimer of CAR and RXR. Accordingly, in some embodiments of any one of the aspects described herein, the inducible promoter comprises a plurality of CREs that are each capable of being bound and activated by a

heterodimer of CAR and RXR. Stated in another way, the inducible promoter comprises a cis-regulatory module (CRM) that comprises a plurality of CREs that are each capable of being bound and activated by a heterodimer of CAR and RXR. The CREs that are capable of being bound and activated by a heterodimer of CAR and RXR can be identical, or they can be different to one another.

[00176] In some embodiments, the inducible promoter comprises from 2 to 10 CREs that are each capable of being bound and activated by a heterodimer of CAR and RXR. For example, the inducible promoter comprises from 2 to 7 CREs. In some embodiments, the inducible promoter comprises from 2 to 5 CREs. For example, inducible promoter comprises from 2 to 4 CREs. In some embodiments, inducible promoter comprises 2 or 3 CREs. For example, the inducible promoter comprises 2 or 3 CREs.

[00177] When two or more CREs that are each capable of being bound and activated by a heterodimer of CAR and RXR are present, they can be immediately adjacent to one another or separated by a spacer. Accordingly, in some embodiments, the inducible promoter comprises the general structure CRE-L-CRE, wherein CRE represents any CRE as discussed herein, and L represents an optional spacer.

[00178] It is noted the optional spacer, L, can have any suitable length. For example, each spacer, L, can be independently from 2 to 100 nucleotides in length. In some embodiments, each spacer, L, can be independently from 3 to 50 nucleotides, or from 5 to 30 nucleotides, or from 10 to 25 nucleotides in length. For example, each spacer, L, is independently from 15 to 25 nucleotides in length. In some embodiments, each spacer, L, is independently 18, 19, 20, 21 or 22 nucleotides in length. A spacer of approximately 20 nucleotides in length has been found to be particularly suitable. In some non-limiting examples, each spacer, L, is a multiple of 5 nucleotides in length.

[00179] In some embodiments of any one of the aspects described herein, the CRM comprises the amino acid sequence:

[NCTGTACTTTCCTGACCNTGNNNNGTGNCANCATNNACTTNCCTGANNCN-L]_m (SEQ ID NO: 27),

where m is an integer from 1 to 10, N is any nucleotide, and L is absent or a spacer.

[00180] In some embodiments, m is an integer from 1 to 6. For example, m is an integer from 2 to 4. In some embodiments, m is selected from 2, 3, or 4. For example, m is 1, 2 or 3. In some embodiments, m is 1. In some other embodiments, m is 2. In yet some other embodiments, m is 3.

[00181] When present, each spacer, L, can be independently from 2 to 100 nucleotides in length. In some embodiments, each spacer, L, can be independently from 3 to 50 nucleotides, or from 5 to 30 nucleotides, or from 10 to 25 nucleotides in length. For example, each spacer, L, is

independently from 15 to 25 nucleotides in length. In some embodiments, each spacer, L, is independently 18, 19, 20, 21 or 22 nucleotides in length. A spacer of approximately 20 nucleotides in length has been found to be particularly suitable. In some non-limiting examples, each spacer, L, is a multiple of 5 nucleotides in length.

[00182] In some embodiments of any one of the aspects described herein, the CRM comprises the nucleotide sequence:

NCTGTACTTTCCTGACCNTGNNNNNGTGNCANCATNNACTTNCCTGANN CN-L-
NCTGTACTTTCCTGACCNTGNNNNNGTGNCANCATNNACTTNCCTGANN CN

(SEQ ID NO: 28); or

NCTGTACTTTCCTGACCNTGNNNNNGTGNCANCATNNACTTNCCTGANN CN-L-
NCTGTACTTTCCTGACCNTGNNNNNGTGNCANCATNNACTTNCCTGANN CN-L-
NCTGTACTTTCCTGACCNTGNNNNNGTGNCANCATNNACTTNCCTGANN CN

(SEQ ID NO: 29),

wherein N is any nucleotide and L is absent or a spacer.

[00183] In some embodiments of any one of the aspects described herein, the CRM comprises a nucleotide sequence having at least 60% identity to the nucleotide sequence:

TCTGTACTTTCCTGACCTTGGCACAGTGCCACCATCAACTTGCCTGACACC-L-
TCTGTACTTTCCTGACCTTGGCACAGTGCCACCATCAACTTGCCTGACACC

(SEQ ID NO: 30);

TCTGTACTTTCCTGACCTTGGCACAGTGCCACCATCAACTTGCCTGACACC-L-
TCTGTACTTTCCTGACCTTGGCACAGTGCCACCATCAACTTGCCTGACACC-L-
TCTGTACTTTCCTGACCTTGGCACAGTGCCACCATCAACTTGCCTGACACC

(SEQ ID NO: 31);

ACTGTACTTTCCTGACCCTGAAGAGGTGGCAGCATGGACTTTCCTGAACCA-L-
ACTGTACTTTCCTGACCCTGAAGAGGTGGCAGCATGGACTTTCCTGAACCA-L-
ACTGTACTTTCCTGACCCTGAAGAGGTGGCAGCATGGACTTTCCTGAACCA

(SEQ ID NO: 32); or

ACTGTACTTTCCTGACCCTGAAGAGGTGGCAGCATGGACTTTCCTGAACCA-L-
ACTGTACTTTCCTGACCCTGAAGAGGTGGCAGCATGGACTTTCCTGAACCA

(SEQ ID NO: 33),

wherein L is absent or a spacer.

[00184] For example, the CRM comprises a nucleotide sequence that is at least 65%, 70%, 75%, 80%, 85%, or 90% identical to one of **SEQ ID NO: 30-33**. In some embodiments of any one of the aspects described herein, the inducible promoter comprises a nucleotide sequence having at least 95% identity to one of **SEQ ID NO: 30-33**. For example, the inducible promoter comprises

a nucleotide sequence having at least 99% identity to one of **SEQ ID NO: 30-33**. In some embodiments, the inducible promoter comprises a nucleotide sequence having 100% identity to one of **SEQ ID NO: 30-33**.

[00185] In some embodiments of any one of the aspects described herein, the CRM comprises a nucleotide sequence having at least 60% identity to the nucleotide sequence:

TCTGTACTTTTCTGACCTTGGCACAGTGCCACCATCAACTTGCCTGACACCCA
TTACTCGCATCCATTCTCTGTACTTTTCTGACCTTGGCACAGTGCCACCATC
AACTTGCCTGACACC (**SEQ ID NO: 34**, 2x mouse PBREM CREs);

TCTGTACTTTTCTGACCTTGGCACAGTGCCACCATCAACTTGCCTGACACCCA
TTACTCGCATCCATTCTCTGTACTTTTCTGACCTTGGCACAGTGCCACCATC
AACTTGCCTGACACCGCACTGAAGGTCCTCAATCGTCTGTACTTTTCTGACCT
TGGCACAGTGCCACCATCAACTTGCCTGACACC (**SEQ ID NO: 35**, 3x mouse
PBREM CREs);

TCTGTACTTTTCTGACCTTGGCACAGTGCCACCATCAACTTGCCTGACACCCA
TTACTCGCATCCATTCTCTGTACTTTTCTGACCTTGGCACAGTGCCACCATC
AACTTGCCTGACACCGCACTGAAGGTCCTCAATCGTCTGTACTTTTCTGACCT
TGGCACAGTGCCACCATCAACTTGCCTGACACCCTGACCTCCTGCCAGCAATA
TCTGTACTTTTCTGACCTTGGCACAGTGCCACCATCAACTTGCCTGACACC
(**SEQ ID NO: 36**, 4x mouse PBREM CREs);

ACTGTACTTTTCTGACCTGAAGAGGTGGCAGCATGGACTTTTCTGAACCACA
TTACTCGCATCCATTCTCACTGTACTTTTCTGACCTGAAGAGGTGGCAGCAT
GGACTTTTCTGAACCA (**SEQ ID NO: 37**, 2x human PBREM); or

ACTGTACTTTTCTGACCTGAAGAGGTGGCAGCATGGACTTTTCTGAACCACA
TTACTCGCATCCATTCTCACTGTACTTTTCTGACCTGAAGAGGTGGCAGCAT
GGACTTTTCTGAACCAGCACTGAAGGTCCTCAATCGACTGTACTTTTCTGACC
CTGAAGAGGTGGCAGCATGGACTTTTCTGAACCA (**SEQ ID NO: 38**, 3x human
PBREM).

[00186] For example, the CRM comprises a nucleotide sequence that is at least 65%, 70%, 75%, 80%, 85%, or 90% identical to any one of **SEQ ID NO: 34-38**. In some embodiments of any one of the aspects described herein, the inducible promoter comprises a nucleotide sequence having at least 95% identity to any one of **SEQ ID NO: 34-38**. For example, the inducible promoter comprises a nucleotide sequence having at least 99% identity to any one of **SEQ ID NO: 34-38**. In some embodiments, the inducible promoter comprises a nucleotide sequence having 100% identity to any one of **SEQ ID NO: 34-38**.

[00187] In some embodiments of the various aspects described herein, a CRE that is capable of being bound and activated by a CAR-RXR heterodimer comprises a portion of the PBREM elements (e.g., NR1, NF1 and NR2) from two or more different species. For example, a CRE that is capable of being bound and activated by a CAR-RXR heterodimer comprises PBREM elements (e.g., NR1, NF1 and NR2) from species in two or more different genera (e.g., two different mammals). Such motifs can be referred to as “hybrid PBREM motifs”. A hybrid PBREM motif typically comprises all of the motifs of a wild-type PBREM motif (i.e., the NR1, NF1, and NR2 motifs), but these motifs are derived from two or more different species. In some embodiments a hybrid PBREM motif comprises a portion (e.g., the NR1, NF1, and NR2 motifs) from a first species, and a corresponding portion from a second species (e.g., the corresponding NR1, NF1, and NR2 motifs). By way of non-limiting example, a hybrid PBREM motif can comprise portions (e.g., the NR1, NF1, and NR2 motifs) of primate (e.g., human) and rodent (e.g., mouse) PBREM motifs. For example, a hybrid PBREM motif may comprise an NR1 motif from primate (e.g., human) and an NR2 motif from rodent (e.g., mouse), or an NR1 motif from rodent (e.g., mouse) and an NR2 motif from primate (e.g., human). Primate or rodent NR1 and NR2 motifs can also be combined with an NF1 motif from the corresponding species.

[00188] In some embodiments the hybrid PBREM motif comprises one of the following combinations of PBREM motifs: hNR1-mNF1-hNR2; hNR1-mNF1-mNR2; mNR1-hNF1-mNR2; or mNR1-hNF1-hNR2 (wherein “h” indicates the human motif and m indicates the mouse motif).

[00189] Accordingly, in some embodiments of any one of the aspects described herein, the inducible primer comprises a nucleotide sequence having at least 60% identity to the sequence.

ACTGTACTTTCCTGACCCTGGCACAGTGCCACCATGGACTTTCCTGAACCA
(SEQ ID NO: 39, HMM hybrid);

ACTGTACTTTCCTGACCCTGGCACAGTGCCACCATCAACTTGCCTGACACC
(SEQ ID NO: 40, HMM hybrid);

TCTGTACTTTCCTGACCTTGAAGAGGTGGCACCATCAACTTGCCTGACACC
(SEQ ID NO: 41, MHM hybrid); or

TCTGTACTTTCCTGACCTTGAAGAGGTGGCAGCATGGACTTTCCTGAACCA
(SEQ ID NO: 42, MHH hybrid).

[00190] For example, the inducible primer comprises a nucleotide sequence that is at least 65%, 70%, 75%, 80%, 85%, or 90% identical to one of SEQ ID NO: 39-42. In some embodiments of

any one of the aspects described herein, the inducible primer comprises a nucleotide sequence having at least 95% identity to one of **SEQ ID NO: 39-42**. For example, the inducible promoter comprises a nucleotide sequence having at least 99% identity to one of **SEQ ID NO: 39-42**. In some embodiments, the inducible promoter comprises a nucleotide sequence having 100% identity to one of **SEQ ID NO: 39-42**.

[00191] Generally, the inducible promoter comprises no or only minimal nucleic acid sequences that would lead to constitutive expression or expression in cells which do not express CAR and/or RXR, e.g., non-liver cells. Thus, background expression and expression in cells which do not express CAR and RXR, e.g., non-liver cells is minimized or avoided entirely.

[00192] In some embodiments of any one of the aspects described herein, the inducible promoter comprises a CRE that is capable of being bound and activated by a heterodimer of CAR and RXR operably linked to a minimal promoter or proximal promoter. For example, the inducible promoter comprises a CRE that is capable of being bound and activated by a heterodimer of CAR and RXR operably linked to a minimal promoter. Where a proximal promoter is used, it is a cell or organ specific promoter. Generally, a minimal promoter is preferred, as a proximal promoter will tend to drive at least some degree of background expression. However, some amount of background expression may be desirable in some instances.

[00193] In some embodiments of any one of the aspects described herein, the minimal or proximal promoter is a liver-specific promoter. Thus, in some embodiments, the inducible promoter is a liver-specific inducible promoter.

[00194] The minimal promoter can be any suitable minimal promoter. A wide range of minimal promoters are known in the art. Some exemplary minimal promoters include, but are not limited to, HSV thymidine kinase minimal promoter (MinTK), CMV minimal promoter (CMVmp) and SV40 minimal promoter (SV40mp). The minimal promoter can be a synthetic minimal promoter.

[00195] In some embodiments of any one of the aspects described herein, the inducible promoter comprises one or more (e.g., two or three) CREs that are capable of being bound and activated by a heterodimer of CAR and RXR operably linked to the MinTK minimal promoter. Without wishing to be bound by a theory, this combination provides a desirable combination of low background expression and inducibility.

[00196] In some embodiments of any one of the aspects described herein, the inducible promoter comprises one or more (e.g., two or three) CREs that are capable of being bound and activated by a heterodimer of CAR and RXR operably linked to the SV40 minimal promoter. This combination has been shown to provide high levels of inducibility, albeit with some increase in background expression levels.

[00197] In some embodiments of any one of the aspects described herein, the inducible promoter comprises one or more (e.g., two or three) CREs that are capable of being bound and activated by a heterodimer of CAR and RXR operably linked to the CMVmp minimal promoter.

[00198] It is noted that the CRE that is capable of being bound and activated by a heterodimer of CAR and RXR (or, where a plurality of CREs are present, the most proximal CRE) and the minimal or proximal promoter can be immediately adjacent to one another or separated by a spacer. Accordingly, in some embodiments of any one of the aspects described herein, the CRE that is capable of being bound and activated by a heterodimer of CAR and RXR (or, where a plurality of CREs are present, the most proximal CRE) and the minimal or proximal promoter are separated by a spacer. It is noted that the spacer separating the CRE and the minimal or proximal promoter can have any suitable length. For example, spacer can be independently from 10 to 200 nucleotides in length. In some embodiments, spacer can be independently from 15 to 150 nucleotides, or from 20 to 125 nucleotides, or from 25 to 100 nucleotides in length. For example, spacer can be 10, 15, 20, 25, 30, 40, 45, 50, 55, 60, 70, 75, 80, 85, 90, 95 or 100 nucleotides in length. In some embodiments, the spacer is 20, 46, 80, or 100 nucleotides in length.

[00199] In some embodiments of any one of the aspects, the inducible promoter comprises a nucleotide sequence having at least 60% identity to the sequence:

CTGTACTTTCTGACCTTGGCACAGTGCCACCATCAACTTGCCTGACACCGAT
 CCGGCCCCGCCCAGCGTCTTGTCATTGGCGAATTCTGAACACGCAGATGCAGT
 CGGGGCGGCGCGGTCCGAGGTCCACTTCGCATATTAAGGTGACGCGTGTG
GCCTCGAACACCGAGCGACCCTGCAGCGACCCGCTTAACAGCGTCAACAG
 CGTGCCGCAGATCTCGAGGAGCTTGGCGAGATTTTCAGGAGCTAAGGAAGCT
 AAAC (SEQ ID NO: 43, PB1-MinTk)

TCTGTACTTTCTGACCTTGGCACAGTGCCACCATCAACTTGCCTGACACCGC
 TGGGAGTTCGTAGACGGAGCGATTAAATCCATATGCAGGTCTATATAAGCA
GAGCTCGTTTAGTGAACCGTCAGATCGCCTAGATACGCCATCCACGCTG
TTTTGACCTCCATAGAAGATCGCCACCC (SEQ ID NO: 44, PB1-CMV-MP);

TCTGTACTTTCTGACCTTGGCACAGTGCCACCATCAACTTGCCTGACACCGC
 TGGGAGTTCGTAGACGGACTAGCCCGGGCTCGAGATCTGCGATCTGCATCTC
AATTAGTCAGCAACCATAGTCCCGCCCCCTAACTCCGCCCATCCCGCCCCCT
AACTCCGCCAGTTCCGCCCATCTCCGCCCATCGCTGACTAATTTTTT
TTATTTATGCAGAGGCCGAGGCCGCTCGGCCTCTGAGCTATTCCAGAA

GTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTTGGCA
TTCCGGTACTGTTGGTAAAGCCACCC (SEQ ID NO: 45, PB1-SV40-MP);

TCTGTACTTTCCTGACCTTGGCACAGTGCCACCATCAACTTGCCTGACACCCA
TTACTCGCATCCATTCTCTCTGTACTTTCCTGACCTTGGCACAGTGCCACCATC
AACTTGCCTGACACCGCTGGGAGTTCGTAGACGGAGATCCGGCCCCGCCAG
CGTCTTGTCATTGGCGAATTCGAACACGCAGATGCAGTCGGGGCGGCGCGGT
CCGAGGTCCACTTCGCATATTAAGGTGACGCGTGTGGCCTCGAACACCGA
GCGACCCTGCAGCGACCCGCTTAACAGCGTCAACAGCGTGCCGCAGATCTC
GAGGAGCTTGGCGAGATTTTCAGGAGCTAAGGAAGCTAAAC (SEQ ID NO: 46,
PB1-1-MinTk, 2xPBREM);

TCTGTACTTTCCTGACCTTGGCACAGTGCCACCATCAACTTGCCTGACACCCA
TTACTCGCATCCATTCTCTCTGTACTTTCCTGACCTTGGCACAGTGCCACCATC
AACTTGCCTGACACCGCACTGAAGGTCTCAATCGTCTGTACTTTCCTGACCT
TGGCACAGTGCCACCATCAACTTGCCTGACACCGCTGGGAGTTCGTAGACGG
AGATCCGGCCCCGCCAGCGTCTTGTCATTGGCGAATTCGAACACGCAGATG
CAGTCGGGGCGGCGCGGTCCGAGGTCCACTTCGCATATTAAGGTGACGCGT
GTGGCCTCGAACACCGAGCGACCCTGCAGCGACCCGCTTAACAGCGTCA
ACAGCGTGCCGCAGATCTCGAGGAGCTTGGCGAGATTTTCAGGAGCTAAGGA
AGCTAAAC (SEQ ID NO: 47, PB1-2-MinTk, 3xPBREM);

TCTGTACTTTCCTGACCTTGGCACAGTGCCACCATCAACTTGCCTGACACCCA
TTACTCGCATCCATTCTCTCTGTACTTTCCTGACCTTGGCACAGTGCCACCATC
AACTTGCCTGACACCGCACTGAAGGTCTCAATCGTCTGTACTTTCCTGACCT
TGGCACAGTGCCACCATCAACTTGCCTGACACCCCTGACCTCCTGCCAGCAATA
TCTGTACTTTCCTGACCTTGGCACAGTGCCACCATCAACTTGCCTGACACCGC
TGGGAGTTCGTAGACGGAGATCCGGCCCCGCCAGCGTCTTGTCATTGGCGA
ATTCGAACACGCAGATGCAGTCGGGGCGGCGCGGTCCGAGGTCCACTTCGCA
TATTAAGGTGACGCGTGTGGCCTCGAACACCGAGCGACCCTGCAGCGAC
CCGCTTAACAGCGTCAACAGCGTGCCGCAGATCTCGAGGAGCTTGGCGAGAT
TTTCAGGAGCTAAGGAAGCTAAAC (SEQ ID NO: 48, PB1-3-MinTk);

TCTGTACTTTCCTGACCTTGGCACAGTGCCACCATCAACTTGCCTGACACCCA
TTACTCGCATCCATTCTCTCTGTACTTTCCTGACCTTGGCACAGTGCCACCATC

AACTTGCCTGACACCGCTGGGAGTTCGTAGACGGACTAGCCCCGGGCTCGAGA
TCTGCGATCTGCATCTCAATTAGTCAGCAACCATAGTCCCCGCCCTAACTC
CGCCCATCCCCGCCCTAACTCCGCCCAGTTCCGCCCATTTCTCCGCCCAT
CGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTC
TGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTT
GCAAAAAGCTTGGCATTCCGGTACTGTTGGTAAAGCCACCC (SEQ ID NO: 50,
PB-1-SV40);

TCTGTACTTTTCTGACCTTGGCACAGTGCCACCATCAACTTGCCTGACACCCA
TTACTCGCATCCATTCTCTCTGTACTTTTCTGACCTTGGCACAGTGCCACCATC
AACTTGCCTGACACCGCACTGAAGGTCTCAATCGTCTGTACTTTTCTGACCT
TGGCACAGTGCCACCATCAACTTGCCTGACACCGCTGGGAGTTCGTAGACGG
ACTAGCCCCGGGCTCGAGATCTGCGATCTGCATCTCAATTAGTCAGCAACCA
TAGTCCCCGCCCTAACTCCGCCCATCCCCGCCCTAACTCCGCCCAGTTCC
GCCCATTTCTCCGCCCATCGCTGACTAATTTTTTTTATTTATGCAGAGGC
CGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTT
TTTGGAGGCCTAGGCTTTTGCAAAAAGCTTGGCATTCCGGTACTGTTGGTA
AAGCCACCC (SEQ ID NO: 51, PB1-2-SV40);

TCTGTACTTTTCTGACCTTGGCACAGTGCCACCATCAACTTGCCTGACACCCA
TTACTCGCATCCATTCTCTCTGTACTTTTCTGACCTTGGCACAGTGCCACCATC
AACTTGCCTGACACCGCACTGAAGGTCTCAATCGTCTGTACTTTTCTGACCT
TGGCACAGTGCCACCATCAACTTGCCTGACACCGTACCTCTGCCAGCAATA
TCTGTACTTTTCTGACCTTGGCACAGTGCCACCATCAACTTGCCTGACACCGC
TGGGAGTTCGTAGACGGACTAGCCCCGGGCTCGAGATCTGCGATCTGCATCTC
AATTAGTCAGCAACCATAGTCCCCGCCCTAACTCCGCCCATCCCCGCCCT
AACTCCGCCCAGTTCCGCCCATTTCTCCGCCCATCGCTGACTAATTTTTT
TTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAA
GTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTTGGCA
TTCCGGTACTGTTGGTAAAGCCACCC (SEQ ID NO: 52, PB1-3-SV40);

TCTGTACTTTTCTGACCTTGGCACAGTGCCACCATCAACTTGCCTGACACCCA
TTACTCGCATCCATTCTCTCTGTACTTTTCTGACCTTGGCACAGTGCCACCATC
AACTTGCCTGACACCGCTGGGAGTTCGTAGACGGAGCGATTAATCCATATG
CAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTAGAT

ACGCCATCCACGCTGTTTTGACCTCCATAGAAGATCGCCACCC (SEQ ID NO: 53, PB1-1-CMV);

TCTGTACTTTCCTGACCTTGGCACAGTGCCACCATCAACTTGCCTGACACCCA
TTACTCGCATCCATTCTCTCTGTACTTTCCTGACCTTGGCACAGTGCCACCATC
AACTTGCCTGACACCGCACTGAAGGTCCTCAATCGTCTGTACTTTCCTGACCT
TGGCACAGTGCCACCATCAACTTGCCTGACACCGCTGGGAGTTCGTAGACGG
AGCGATTAATCCATATGCAGGTCTATATAAGCAGAGCTCGTTTAGTGAAC
CGTCAGATCGCCTAGATACGCCATCCACGCTGTTTTGACCTCCATAGAAG
ATCGCCACCC (SEQ ID NO: 54, PB1-2-CMV);

TCTGTACTTTCCTGACCTTGGCACAGTGCCACCATCAACTTGCCTGACACCCA
TTACTCGCATCCATTCTCTCTGTACTTTCCTGACCTTGGCACAGTGCCACCATC
AACTTGCCTGACACCGCACTGAAGGTCCTCAATCGTCTGTACTTTCCTGACCT
TGGCACAGTGCCACCATCAACTTGCCTGACACCGCTGACCTCCTGCCAGCAATA
TCTGTACTTTCCTGACCTTGGCACAGTGCCACCATCAACTTGCCTGACACCGC
TGGGAGTTCGTAGACGGAGCGATTAATCCATATGCAGGTCTATATAAGCA
GAGCTCGTTTAGTGAACCGTCAGATCGCCTAGATACGCCATCCACGCTG
TTTTGACCTCCATAGAAGATCGCCACCC (SEQ ID NO: 55, PB1-3-CMV);

ACTGTACTTTCCTGACCCTGAAGAGACTGTACTTTCCTGACCCTGAAGAGACT
GTACTTTCCTGACCCTGAAGAGGATCCGGCCCCGCCAGCGTCTTGTCATTGG
CGAATTTCGAACACGCAGATGCAGTCGGGGCGGCGCGGTCCGAGGTCCACTTC
GCATATTAAGGTGACGCGTGTGGCCTCGAACACCGAGCGACCCTGCAGC
GACCCGCTTAACAGCGTCAACAGCGTGCCGCAGATCTCGAGGAGCTTGGCG
AGATTTTCAGGAGCT (SEQ ID NO: XX, human NR1x3-MinTK);

ACTGTACTTTCCTGACCCTGAAGAGGTGGCAGCATGGACTTTCCTGAACCAGA
TCCGGCCCCGCCAGCGTCTTGTCATTGGCGAATTCGAACACGCAGATGCAGT
CGGGGCGGCGCGGTCCGAGGTCCACTTCGCATATTAAGGTGACGCGTGTG
GCCTCGAACACCGAGCGACCCTGCAGCGACCCGCTTAACAGCGTCAACAG
CGTGCCGCAGATCTCGAGGAGCTTGGCGAGATTTTCAGGAGCTAAGGAAGCT
AAACATGGAAGATGCCAAAAACATTAAG (SEQ ID NO: 56, human PBREM-MinTK);

ACTGTACTTTCTGACCCTGGCACAGTGCCACCATGGACTTTCTGAACCAGA
TCCGGCCCCGCCCAGCGTCTTGTCATTGGCGAATTCGAACACGCAGATGCAGT
CGGGGCGGCGCGGTCCGAGGTCCACTTTCGCATATTAAGGTGACGCGTGTG
GCCTCGAACACCGAGCGACCCTGCAGCGACCCGCTTAACAGCGTCAACAG
CGTGCCGCAGATCTCGAGGAGCTTGGCGAGATTTTCAGGAGCTAAGGAAGCT
AAACATGGAAGATGCCAAAAACATTAAG (SEQ ID NO: 57, hNR1-mNF1-hNR2-
MinTK (HMH MinTK));

ACTGTACTTTCTGACCCTGGCACAGTGCCACCATCAACTTGCCTGACACCGA
TCCGGCCCCGCCCAGCGTCTTGTCATTGGCGAATTCGAACACGCAGATGCAGT
CGGGGCGGCGCGGTCCGAGGTCCACTTTCGCATATTAAGGTGACGCGTGTG
GCCTCGAACACCGAGCGACCCTGCAGCGACCCGCTTAACAGCGTCAACAG
CGTGCCGCAGATCTCGAGGAGCTTGGCGAGATTTTCAGGAGCTAAGGAAGCT
AAACATGGAAGATGCCAAAAACATTAAG (SEQ ID NO: 58, hNR1-mNF1-mNR2-
MinTK (HMM MinTK));

TCTGTACTTTCTGACCTTGAAGAGGTGGCACCATCAACTTGCCTGACACCGA
TCCGGCCCCGCCCAGCGTCTTGTCATTGGCGAATTCGAACACGCAGATGCAGT
CGGGGCGGCGCGGTCCGAGGTCCACTTTCGCATATTAAGGTGACGCGTGTG
GCCTCGAACACCGAGCGACCCTGCAGCGACCCGCTTAACAGCGTCAACAG
CGTGCCGCAGATCTCGAGGAGCTTGGCGAGATTTTCAGGAGCTAAGGAAGCT
AAACATGGAAGATGCCAAAAACATTAAG (SEQ ID NO: 59, mNR1-hNF1-mNR2-
MinTK (MHM MinTK));

TCTGTACTTTCTGACCTTGAAGAGGTGGCAGCATGGACTTTCTGAACCAGA
TCCGGCCCCGCCCAGCGTCTTGTCATTGGCGAATTCGAACACGCAGATGCAGT
CGGGGCGGCGCGGTCCGAGGTCCACTTTCGCATATTAAGGTGACGCGTGTG
GCCTCGAACACCGAGCGACCCTGCAGCGACCCGCTTAACAGCGTCAACAG
CGTGCCGCAGATCTCGAGGAGCTTGGCGAGATTTTCAGGAGCTAAGGAAGCT
AAACATGGAAGATGCCAAAAACATTAAG (SEQ ID NO: 60, mNR1-hNF1-hNR2-
MinTK (MHM MinTK));

ACTGTACTTTCTGACCCTGAAGAGGTGGCAGCATGGACTTTCTGAACCAGC
TGGGAGTTCGTAGACGGACTAGCCCGGGCTCGAGATCTGCGATCTGCATCTC
AATTAGTCAGCAACCATAGTCCCGCCCCCTAACTCCGCCCATCCCGCCCCCT

AACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATCGCTGACTAATTTTTT
TTATTTATGCAGAGGCCGAGGCCGCTCGGCCTCTGAGCTATTCCAGAA
GTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTTGGCA
TTCCGGTACTGTTGGTAAAGCCACCC (SEQ ID NO: 61, hPB-SV40);

TCTGTACTTTCTGACCTTGAAGAGGTGGCACCATCAACTTGCCTGACACCGC
TGGGAGTTCGTAGACGGACTAGCCCGGGCTCGAGATCTGCGATCTGCATCTC
AATTAGTCAGCAACCATAGTCCCGCCCCCTAACTCCGCCCATCCCGCCCCCTAAC
TCCGCCCAGTTCCGCCCATTCTCCGCCCCATCGCTGACTAATTTTTTTTATTTA
TGhPBCAGAGGCCGAGGCCGCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAG
GAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTTGGCATTCCGGTACTG
TTGGTAAAGCCACCC (SEQ ID NO: 62, MHM-SV40);

ACTGTACTTTCTGACCTTGAAGAGGTGGCAGCATGGACTTTCTGAACCACA
TTACTCGCATCCATTCTCACTGTACTTTCTGACCTTGAAGAGGTGGCAGCAT
GGACTTTCTGAACCAGCTGGGAGTTCGTAGACGGACTAGCCCGGGCTCGAG
ATCTGCGATCTGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCCTAACT
CCGCCCATCCCGCCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCA
TCGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCTCGGCCT
CTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTT
TTGCAAAAAGCTTGGCATTCCGGTACTGTTGGTAAAGCCACCC (SEQ ID NO:
63, 2xhPB SV40);

TCTGTACTTTCTGACCTTGAAGAGGTGGCACCATCAACTTGCCTGACACCCA
TTACTCGCATCCATTCTCTCTGTACTTTCTGACCTTGAAGAGGTGGCACCATC
AACTTGCCTGACACCGCTGGGAGTTCGTAGACGGAGATCCGGCCCCGCCAG
CGTCTTGTCATTGGCGAATTCGAACACGCAGATGCAGTCGGGGCGGCGCGGT
CCGAGGTCCACTTCGCATATTAAGGTGACGCGTGTGGCCTCGAACACCGA
GCGACCTGCGAGCGACCCGCTTAACAGCGTCAACAGCGTGCCGCAGATCTC
GAGGAGCTTGGCGAGATTTTCAGGAGCTAAGGAAGCTAAAC (SEQ ID NO: 64,
2xMHM MinTK);

TCTGTACTTTCTGACCTTGAAGAGGTGGCACCATCAACTTGCCTGACACCCA
TTACTCGCATCCATTCTCTCTGTACTTTCTGACCTTGAAGAGGTGGCACCATC
AACTTGCCTGACACCGCTGGGAGTTCGTAGACGGACTAGCCCGGGCTCGAGA

TCTGCGATCTGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCCTAACTC
CGCCCATCCCGCCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCAT
CGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTC
TGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTT
GCAAAAAGCTTGGCATTCCGGTACTGTTGGTAAAGCCACCC (SEQ ID NO: 65,
2xMHM SV40);

ACTGTACTTTCCTGACCCTGAAGAGGTGGCAGCATGGACTTTCCTGAACCACA
TTACTCGCATCCATTCTCACTGTACTTTCCTGACCCTGAAGAGGTGGCAGCAT
GGACTTTCCTGAACCAGCACTGAAGGTCCTCAATCGACTGTACTTTCCTGACC
CTGAAGAGGTGGCAGCATGGACTTTCCTGAACCAGCTGGGAGTTCGTAGACG
GAGATCCGGCCCCGCCAGCGTCTTGTCAATTGGCGAATTCGAACACGCAGAT
GCAGTCGGGGCGGCGCGGTCCGAGGTCCACTTCGCATATTAAGGTGACGCG
TGTGGCCTCGAACACCGAGCGACCCTGCAGCGACCCGCTTAACAGCGTCA
ACAGCGTGCCGCAGATCTCGAGGAGCTTGGCGAGATTTTCAGGAGCTAAGGA
AGCTAAAC (SEQ ID NO: 66, 3xhPB-minTK); or

ACTGTACTTTCCTGACCCTGAAGAGGTGGCAGCATGGACTTTCCTGAACCACA
TTACTCGCATCCATTCTCACTGTACTTTCCTGACCCTGAAGAGGTGGCAGCAT
GGACTTTCCTGAACCAGCACTGAAGGTCCTCAATCGACTGTACTTTCCTGACC
CTGAAGAGGTGGCAGCATGGACTTTCCTGAACCAGCTGGGAGTTCGTAGACG
GACTAGCCCCGGGCTCGAGATCTGCGATCTGCATCTCAATTAGTCAGCAACC
ATAGTCCCGCCCCCTAACTCCGCCCATCCCGCCCCCTAACTCCGCCCAGTTC
CGCCCATTTCTCCGCCCCATCGCTGACTAATTTTTTTTATTTATGCAGAGG
CCGAGGCCGCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTT
TTTTGGAGGCCTAGGCTTTTGCAAAAAGCTTGGCATTCCGGTACTGTTGGT
AAAGCCACCC (SEQ ID NO: 67, 3xhPB-SV40);

[00200] For example, the inducible promoter comprises a nucleotide sequence that is at least 65%, 70%, 75%, 80%, 85%, or 90% identical to one of SEQ ID NO: 43-67. In some embodiments of any one of the aspects described herein, the inducible promoter comprises a nucleotide sequence having at least 95% identity to one of SEQ ID NO: 43-67. For example, the inducible promoter comprises a nucleotide sequence having at least 99% identity to one of SEQ ID NO: 43-67. In some embodiments, the inducible promoter comprises a nucleotide sequence having 100% identity

to one of SEQ ID NO: 43-67. In SEQ ID NO: 43-67, PBREM elements are underlines and minimal promoter is in bold.

[00201] Additional exemplary inducible promoters are described in US Patent Publication No. US20220111078, contents of which are incorporated herein by reference in their entirety.

Transgene

[00202] Embodiments of the various aspects described herein include a transgene. As used herein, the term “transgene” means a nucleic acid sequence (encoding, for example, a desired gene expression product such as a protein or RNA) that is partly or entirely heterologous, i.e., foreign, to the subject into which it is introduced, or is homologous to an endogenous gene of the subject into which it is introduced. A transgene for use in methods described herein typically encodes a desired gene expression product such as a polypeptide (protein) or RNA. The transgene can be a full-length cDNA or genomic DNA sequence, or any fragment, subunit or mutant thereof that has at least some desired biological activity.

[00203] Where the transgene encodes a protein, it can be essentially any type of protein. By way of non-limiting example, the protein can be an enzyme, an antibody or antibody fragment (e.g., a monoclonal antibody), a hormone, a blood clotting factor, a growth factor, a cytokine, a viral protein (e.g., REP, CAP, REV, VSV-G, or RD114), a therapeutic protein, or a toxic protein (e.g., Caspase 3, 8 or 9).

[00204] In some embodiments of the various aspects described herein, the gene encodes a therapeutic expression product. For example, the gene encodes a therapeutic protein suitable for use in treating a disease or condition associated with the liver. Such genes may be referred to as “therapeutic genes”. The therapeutic expression product can be a protein, e.g., a secretable protein such as, e.g., a clotting factor (e.g., factor IX or factor VIII), GAA, a cytokine, a growth factor, an antibody or nanobody, a chemokine, a plasma factor, insulin, erythropoietin, lipoprotein lipase, or a toxic protein. Alternatively, the therapeutic expression product can be RNA, such as an siRNA or miRNA. Some exemplary therapeutic expression products (and sequences encoding them) include, but are not limited to, factor VIII, factor IX, factor VII, factor X, von Willebrand factor, erythropoietin (EPO), interferon- α , interferon-B, interferon- γ , interleukin 1 (IL-1), interleukin 2 (IL-2), interleukin 3 (IL-3), interleukin 4 (IL-4), interleukin 5 (IL-5), interleukin 6 (IL-6), interleukin 7 (IL-7), interleukin 8 (IL-8), interleukin 9 (IL-9), interleukin 10 (IL-10), interleukin 11 (IL-11), interleukin 12 (IL-12), interleukin-37 (IL-37), interleukin-28 (IL-28), chemokine (C-X-C motif) ligand 5 (CXCL5), granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF), stem cell factor (SCF), keratinocyte growth factor (KGF), monocyte chemoattractant protein-1

(MCP-1), tumour necrosis factor (TNF), afamin (AFM), α 1-antitrypsin, α -galactosidase A, α -L-iduronidase, ATP7b, ornithine transcarbamoylase, phenylalanine hydroxylase, lipoprotein lipase, aromatic amino acid decarboxylase (AADC), ATPase Sarcoplasmic/Endoplasmic Reticulum Ca²⁺ Transporting 2 (ATP2A2), cystic fibrosis transmembrane conductance regulator (CTFR), glutamic acid decarboxylase 65 kDa protein (GAD65), glutamic acid decarboxylase 67 kDa protein (GAD67), lipoprotein lipase (LPL), nerve growth factor (NGF), neurturin (NTN), porphobilinogen deaminase (PBGD), sarcoglycan alpha (SGCA), soluble fms-like tyrosine kinase-1 (sFLT-1), apolipoproteins, low-density lipoprotein receptor (LDL-R), albumin, glucose-6-phosphatase, antibodies, nanobodies, aptamers, anti-viral dominant-negative proteins, and functional fragments, subunits or mutants thereof. Preferably the protein is a primate protein, more preferably a human protein. In some embodiments, the therapeutic protein is AADC. In some embodiments, the therapeutic protein is porphobilinogen deaminase (PBGD).

[00205] Proteins or polypeptides of interest can be, for example, antibodies, enzymes or fragments thereof, viral proteins, cytokines, lymphokines, adhesion molecules, receptors and derivatives or fragments thereof, protein antibiotics, toxin fusion proteins, carbohydrate-protein conjugates, structural proteins, regulatory proteins, vaccines and vaccine like proteins or particles, process enzymes, growth factors, hormones, and any other polypeptides that can serve as agonists or antagonists and/or have therapeutic or diagnostic use. Accordingly, in some embodiments of any one of the aspects described herein, the protein is an immunoglobulin, preferably an antibody or antibody fragment, most preferably a Fab or scFv antibody. In some other embodiments, the protein is a viral protein.

[00206] In some embodiments, the transgene encodes an anti-PD1 antibody, anti-HER2 antibody, anti-PCSK9 antibody, anti-VEGFR2 antibody, anti-IL17 antibody, anti-A β 3-42 antibody, anti-Sema3A antibody, anti-4-1BB (CD137) antibody, anti-5'-nucleotidase antibody, anti-5T4 antibody, anti-activin receptor-like kinase 1 antibody, anti-ACVR2B antibody, anti-adenocarcinoma antigen antibody, anti-amyloid antibody, anti-Ang-2 antibody, anti-angiopoietin 2 antibody, anti-angiopoietin 2 antibody, anti-angiopoietin 3 antibody, anti-anthrax toxin antibody, anti-AOC3 (VAP-1) antibody, anti-AXL antibody, anti-BAFF-R antibody, anti-B-cell activating factor (BAFF) antibody, anti-B-cell maturation antigen (BCMA) antibody, anti-B-lymphoma cell antibody, anti-C242 antigen antibody, anti-C5 antibody, anti-C5a receptor (C5a) antibody, anti-CA-125 antibody, anti-calcitonin antibody, anti-calcitonin gene-related peptide alpha antibody, anti-calcitonin gene-related peptide beta antibody, anti-calcitonin gene-related peptide receptor (CGRP) antibody, anti-CanAg (a glycoform of MUC1) antibody, anti-carbonic anhydrase 9 (CA-IX) antibody, anti-Carcinoembryonic antigen (CEA) antibody, anti-Carcinoembryonic antigen (CEA)-related antigen antibody, anti-cardiac myosin antibody, anti-CCL11 antibody, anti-CCR2

antibody, anti-CCR4 antibody, anti-CCR5 antibody, anti-CCR5 antibody, anti-CD11 antibody, antibody, anti-CD123 antibody, anti-CD125 antibody, anti-CD134 antibody, anti-CD134 antibody, anti-CD147 antibody, anti-CD15 antibody, anti-CD152 antibody, anti-CD154 antibody, anti-CD18 antibody, anti-CD19 antibody, anti-CD2 antibody, anti-CD20 antibody, anti-CD20 antibody, anti-CD20 antibody, anti-CD200 antibody, anti-CD22 antibody, anti-CD23 antibody, anti-CD25 antibody, anti-CD27 antibody, anti-CD276 antibody, anti-CD278 antibody, antibody, anti-CD279 antibody, anti-CD28 antibody, anti-CD3 antibody, anti-CD3 antibody, anti-CD3 antibody, anti-CD3 antibody, anti-CD3 antibody, anti-CD30 antibody, anti-CD319 antibody, anti-CD33 antibody, anti-CD37 antibody, anti-CD38 antibody, anti-CD3E antibody, anti-CD3E antibody, anti-CD4 antibody, anti-CD40 antibody, anti-CD41 antibody, anti-CD44 antibody, anti-CD45 antibody, anti-CD5 antibody, anti-CD51 antibody, anti-CD52 antibody, anti-CD56 antibody, anti-CD6 antibody, anti-CD70 antibody, anti-CD74 antibody, anti-CD79B antibody, anti-CD80 antibody, anti-CEACAM5 antibody, anti-Claudin 18 Isoform 2 antibody, anti-clumping factor A antibody, anti-cMet antibody, anti-c-Met antibody, anti-coagulation factor III antibody, anti-complement C5a antibody, anti-complement component 1s (C1s) antibody, anti-Complement factor D (CFD) antibody, anti-connective tissue growth factor (CTGF) antibody, anti-CSF1 antibody, antibody, anti-CSF1R antibody, anti-CSF2 antibody, anti-CTLA-4 antibody, anti-CXCL10 (IP-10) antibody, anti-CXCR4 (CD184) antibody, anti-dabigatran antibody, anti-dendritic cell-associated lectin 2 antibody, anti-DLL3 antibody, anti-DLL4 antibody, anti-DPP4 antibody, anti-DR5 antibody, anti-EGFL7 antibody, anti-EGFR antibody, anti-EGFR antibody, anti-EGFR antibody, anti-EGFR extracellular domain III antibody, anti-endoglin antibody, anti-endotoxin antibody, anti-EpCAM antibody, anti-EPHA3 antibody, anti-ephrin receptor A3 antibody, anti-episialin antibody, anti-ERBB1 antibody, anti-ERBB3 (HER3) antibody, anti-FAP (gene) (FAP) antibody, anti-FCGRT antibody, anti-FGF 23 antibody, anti-FGFR2 antibody, anti-FGFR2 antibody, anti-fibrin II antibody, anti-fibronectin extra domain-B antibody, anti-folate hydrolase antibody, anti-folate receptor 1 antibody, anti-folate receptor alpha antibody, anti-Frizzled receptor antibody, anti-FVIX antibody, anti-FVX antibody, anti-GD2 ganglioside antibody, anti-GD3 ganglioside antibody, anti-GDF-8 antibody, anti-gelatinase B antibody, anti-glucagon receptor (GCGR) antibody, anti-Glutamate carboxypeptidase II antibody, anti-glypican 3 antibody, anti-GMCSF antibody, anti-GPNMB antibody, anti-GPRC5D antibody, antibody, anti-growth differentiation factor 8 antibody, anti-GUCY2C antibody, anti-Hepatocyte growth factor (HGF) antibody, anti-HER1 antibody, anti-HER1 antibody, anti-HER1 antibody, anti-HER2/neu antibody, anti-HGFR antibody, anti-histone complex antibody, anti-HLA-DR antibody, anti-Hsp90 antibody, anti-human scatter factor receptor kinase antibody, anti-ICAM-1 (CD54) antibody, anti-IFN receptor antibody, anti-IFN- α antibody, anti-IFN- α/β receptor antibody, anti-IFN- γ antibody, anti-IgE antibody, anti-IgE Fc region

antibody, anti-IGF-1 receptor (CD221) antibody, anti-IGF-2 antibody, anti-IGHE antibody, anti-IL-1 antibody, anti-IL-12 antibody, anti-IL-13 antibody, anti-IL-17A antibody, anti-IL-17AF antibody, anti-IL-17F antibody, anti-IL-1 α antibody, anti-IL-1 β antibody, anti-IL-2 antibody, anti-IL-20 antibody, anti-IL-22 antibody, anti-IL-23 antibody, anti-IL-23 antibody, anti-IL-23 antibody, anti-IL-23 antibody, anti-IL-23 antibody, anti-IL-23A antibody, anti-IL-3 receptor antibody, anti-IL-31 receptor A antibody, anti-IL-4 antibody, anti-IL-4R α antibody, anti-IL-5 antibody, anti-IL-6 antibody, anti-IL-6 receptor antibody, anti-IL-9 antibody, anti-inducible T-cell co-stimulatory ligand (ICOSL) antibody, anti-integrin α 4 antibody, anti-integrin β 7 antibody, anti-integrin α 4 β 7 antibody, anti-integrin α 4 β 7 antibody, anti-integrin α 5 β 1 antibody, anti-integrin α Ib β 3 antibody, anti-integrin α v β 3 antibody, anti-Interleukin 36 receptor (IL1RL2/IL1RAP) antibody, anti-ITGA2 (CD49b) antibody, anti-ITGB2 (CD18) antibody, anti-kallikrein antibody, anti-KIR2D antibody, anti-LAG3 antibody, anti-Lewis-Y antigen antibody, anti-LFA-1 (CD11a) antibody, anti-LINGO-1 antibody, anti-lipoteichoic acid antibody, anti-LIV-1 antibody, anti-LOXL2 antibody, anti-LRRC15 antibody, anti-L-selectin (CD62L) antibody, anti-lymphotoxin alpha (LTA) antibody, anti-LYPD3 antibody, anti-macrophage colony stimulating factor (MCSF) antibody, anti-macrophage migration inhibitory factor (MIF) antibody, anti-MASP-2 antibody, anti-MCP-1 antibody, anti-melanoma cell adhesion molecule (MCAM) antibody, anti-mesothelin antibody, anti-MS4A1 antibody, antibody, anti-MST1R antibody, anti-MUC1 antibody, anti-MUC5AC antibody, anti-mucosal addressin cell adhesion molecule antibody, anti-myelin-associated glycoprotein antibody, anti-myostatin antibody, anti-NCA-90 (granulocyte antigen) antibody, anti-nectin-4 antibody, anti-nerve growth factor (NGF) antibody, anti-NGNA ganglioside antibody, anti-NKG2A antibody, anti-NOGO-A antibody, anti-Notch 1 antibody, anti-Notch receptor antibody, anti-NRP1 antibody, anti-OX-40 antibody, anti-oxLDL antibody, anti-PCDC1 antibody, anti-PCDP1 antibody, anti-PDGFR α antibody, anti-PDGFR β antibody, anti-PD-L1 antibody, anti-phosphate-sodium co-transporter antibody, anti-phosphatidylserine antibody, anti-PTK7 antibody, anti-RANKL antibody, anti-repulsive guidance molecule A (RGMA) antibody, anti-RHD (gene) (RHD) antibody, anti-Rhesus factor antibody, anti-Rhesus factor antibody, anti-root plate-specific spondin 3 antibody, anti-ROR1 antibody, anti-RTN4 antibody, anti-sclerostin antibody, anti-sclerostin (SOST) antibody, anti-SDC1 antibody, anti-selectin P antibody, anti-serum amyloid A protein antibody, anti-serum amyloid P component antibody, anti-SLAMF7 antibody, anti-SLITRK6 antibody, anti-SOST antibody, anti-sphingosine-1-phosphate antibody, anti-STEAP1 antibody, anti-TAG-72 antibody, anti-TAG-72 antibody, anti-tau protein antibody, anti-T-cell receptor antibody, anti-TEM1 antibody, anti-tenascin C antibody, anti-TGF- β antibody, anti-TGF- β 1 antibody, anti-TGF- β 2 antibody, anti-thymic stromal lymphopoietin (TSLP) antibody, anti-TIGIT antibody, anti-tissue factor pathway inhibitor (TFPI) antibody, anti-TNF antibody, anti-TNF

antibody, anti-TNF- α antibody, anti-TNF- α antibody, anti-TRAIL-R1 antibody, anti-TRAIL-R2 antibody, anti-TROP-2 antibody, anti-tumor antigen CTAA16.88 antibody, anti-tumor necrosis factor receptor (TNFR) antibody, anti-tumor necrosis factor related activation protein (TRAP) antibody, anti-Tumor-associated glycoprotein 72 (TAG-72) antibody, anti-TWEAK receptor antibody, anti-TYRP1 (glycoprotein 75) antibody, anti-vascular endothelial growth factor A (VEGFA) antibody, anti-VEGFA antibody, anti-VEGFR-1 antibody, anti-vimentin antibody, anti-VISTA (protein) (VSIR) antibody, anti-VWF antibody, anti- α -fetoprotein antibody, anti-A-synuclein antibody or anti- β -amyloid antibody. In some embodiments, the transgene encodes an anti-PD1 antibody. In some embodiments, the transgene encodes an anti-HER2 antibody. In some embodiments, the transgene encodes an anti-PCSK9 antibody. In some embodiments, the transgene encodes an anti-VEGFR2 antibody. In some embodiments, the transgene encodes an anti-IL17 antibody. In some embodiments, the transgene encodes an anti-A β 3-42 antibody. In some embodiments, the transgene encodes an anti-Sema3A antibody.

[00207] In some embodiments, the transgene encodes an antibody selected from the group consisting of 3F8, Abagovomab, Abciximab, Abituzumab, Abrezekimab, Abnilumab, Actoxumab, Adalimumab, Adecatumumab, Aducanumab, Afasevikumab, Afelimomab, Alacizumab pegol, Alemtuzumab, Alirocumab, Altumomab pentetate, Amatuximab, Amivantamab, Anatumomab mafenatol, Andecaliximab, Anetumab ravtansine, Anifrolumab, Ansuvimab, Anrukinzumab, (IMA-638), Apolizumab, Aprutumab ixadotin, Arcitumomab, Ascrinivacumab, Aselizumab, Atezolizumab, Atidortoxumab, Atinumab, Atoltivimab, Atoltivimab/maftivimab/odesivimab, Atorolimumab, Avelumab, Azintuxizumab vedotin, Bamlanivimab, Bapineuzumab, Basiliximab, Bavixumab, BCD-100, Bebtelovimab, Bectumomab, Bedinvetmab, Begelomab, Belantamab mafodotin, Belimumab, Bemarituzumab, Benralizumab, Berlimatoxumab, Bermekimab, Bersanlimab, Bertilimumab, Besilesomab, Bevacizumab, Bezlotoxumab, Biciromab, Bimagrumab, Bimekizumab, Birtamimab, Bivatuzumab, Bleselumab, Blinatumomab, Blontuvetmab, Blosozumab, Bococizumab, Brazikumab, Brentuximab vedotin, Briakinumab, Brodalumab, Brolucizumab, Brontictuzumab, Burosumab, Cabiralizumab, Camidanlumab tesirine, Camrelizumab, Canakinumab, Cantuzumab mertansine, Cantuzumab ravtansine, Caplacizumab, Casirivimab, Capromab, Carlumab, , Carotuximab, Catumaxomab, cBR96-doxorubicin immunoconjugate, Cedelizumab, Cemiplimab, Cergutuzumab amunaleukin, Certolizumab pegol, Cetrelimab, Cetuximab, Cibisatamab, Cilgavimab, Cirmtuzumab, Citatuzumab bogatol, Cixutumumab, Clazakizumab, Clenoliximab, Clivatuzumab tetraxetan, Codrituzumab, Cofetuzumab pelidotin, Coltuximab ravtansine, Conatumumab, Concizumab, Cosfroviximab, Crenezumab, Crizanlizumab, Crotedumab, CR6261, Cusatuzumab, Dacetuzumab, Daclizumab, Dalotuzumab, Dapirolizumab pegol, Daratumumab, Dectrekumab, Demcizumab, Denintuzumab

mafodotin, Denosumab, Depatuxizumab mafodotin, Derlotuximab biotin, Detumomab, Dezamizumab, Dinutuximab, Dinutuximab beta, Diridavumab, Domagrozumab, Donanemab, Dorlimomab aritox, Dostarlimab, Drozitumab, DS-8201, Duligotuzumab, Dupilumab, Durvalumab, Dusigitumab, Duvortuxizumab, Echromeximab, Eculizumab, Edobacomab, Edrecolomab, Efalizumab, Efungumab, Eldelumab, Elezanumab, Elgemtumab, Elotuzumab, Elsilimomab, Emactuzumab, Emapalumab, Emibetuzumab, Emicizumab, Enapotamab vedotin, Enavatuzumab, Enfortumab vedotin, Enlimomab pegol, Enoblituzumab, Enokizumab, Enoticumab, Ensituximab, Epcoritamab, Eptumomab cituxetan, Epratuzumab, Eptinezumab, Erenumab, Erlizumab, Ertumaxomab, Etaracizumab, Etesevimab, Etigilimab, Etrolizumab, Evinacumab, Evolocumab, Exbivirumab, Fanolesomab, Faralimomab, Faricimab, Farletuzumab, Fasinumab, FBTAA05, Felvizumab, Fezakinumab, Fibatuzumab, Ficlaturuzumab, Figitumumab, Firivumab, Flanvotumab, Fletikumab, Flotetuzumab, Fontolizumab, Foralumab, Foravirumab, Fremanezumab, Fresolimumab, Frovocimab, Frunevetmab, Fulranumab, Futuximab, Galcanezumab, Galiximab, Gancotamab, Ganitumab, Gantenerumab, Gatipotuzumab, Gavilimomab, Gedivumab, Gemtuzumab ozogamicin, Gevokizumab, Gilvetmab, Gimsilumab, Girentuximab, Glembatumumab vedotin, Glofitamab, Golimumab, Gomiliximab, Gosuranemab, Guselkumab, Ianalumab, Ibalizumab, Sintilimab, Ibritumomab tiuxetan, Icrucumab, Idarucizumab, Ifabotuzumab, Igovomab, Iladatuzumab vedotin, Imalumab, Imaprelimab, Imciromab, Imdevimab, Imgatuzumab, Inclacumab, Indatuximab ravtansine, Indusatumab vedotin, Inebilizumab, Infliximab, Intetumumab, Inolimomab, Inotuzumab ozogamicin, Ipilimumab, Iomab-B, Iratumumab, Isatuximab, Iscalimab, Istiratumab, Itolizumab, Ixekizumab, Keliximab, Labetuzumab, Lacnotuzumab, Ladiratuzumab vedotin, Lampalizumab, Lanadelumab, Landogrozumab, Laprituximab emtansine, Larcaviximab, Lebrikizumab, Lecanemab, Lemalesomab, Lendalizumab, Lenvervimab, Lenzilumab, Lerdelimumab, Leronlimab, Lesofavumab, Letolizumab, Lexatumumab, Libivirumab, Lifestuzumab vedotin, Ligelizumab, Loncastuximab tesirine, Losatuxizumab vedotin, Lilotomab satetraxetan, Lintuzumab, Lirilumab, Lodelcizumab, Lokivetmab, Lorvotuzumab mertansine, Lucatumumab, Lulizumab pegol, Lumiliximab, Lumretuzumab, Lupartumab, Lupartumab amadotin, Lutikizumab, Maftivimab, Mapatumumab, Margetuximab, Marstacimab, Maslimomab, Mavrilimumab, Matuzumab, Mepolizumab, Metelimumab, Milatuzumab, Minretumomab, Mirikizumab, Mirvetuximab soravtansine, Mitumomab, Modotuximab, Mogamulizumab, Monalizumab, Morolimumab, Mosunetuzumab, Motavizumab, Moxetumomab pasudotox, Muromonab-CD3, Nacolumab tafenatox, Namilumab, Naptumomab estafenatox, Naratuximab emtansine, Namatumab, Natalizumab, Navicixizumab, Navivumab, Naxitamab, Nebacumab, Necitumumab, Nemolizumab, NEOD001, Nerelimomab, Nesvacumab, Netakimab, Nimotuzumab, Nirsevimab,

Nivolumab, Nofetumomab merpentan, Obiltoxaximab, Obinutuzumab, Ocaratuzumab, Ocrelizumab, Odesivimab, Odulimomab, Ofatumumab, Olaratumab, Oleclumab, Olendalizumab, Olokizumab, Omalizumab, Omburtamab, OMS721, Onartuzumab, Ontuxizumab, Onvatilimab, Opicinumab, Oportuzumab monatox, Oregovomab, Orticumab, Otelixizumab, Otilimab, Otlertuzumab, Oxelumab, Ozanezumab, Ozoralizumab, Pagibaximab, Palivizumab, Pamrevlumab, Panitumumab, Pankomab, Panobacumab, Parsatuzumab, Pascolizumab, Pasotuxizumab, Pateclizumab, Patritumab, PDR001, Pembrolizumab, Pentumomab, Perakizumab, Pertuzumab, Pexelizumab, Pidilizumab, Pinatuzumab vedotin, Pintumomab, Placulumab, Pozelimab, Prezalumab, Plozalizumab, Pogalizumab, Polatuzumab vedotin, Ponezumab, Porgaviximab, Prasinezumab, Prezalizumab, Priliximab, Pritoxaximab, Pritumumab, PRO 140, Quilizumab, Racotumomab, Radretumab, Rafivirumab, Ralpancizumab, Ramucirumab, Ranevetmab, Ranibizumab, Raxibacumab, Ravagalimab, Ravulizumab, Refanezumab, Regavirumab, Regdanvimab, Relatlimab, Remtolumab, Reslizumab, Retifanlimab, Rilotumumab, Rinucumab, Risankizumab, Rituximab, Rivabazumab pegol, Robatumumab, Rmab, Roledumab, Romilkimab, Romosozumab, Rontalizumab, Rosmantuzumab, Rovalpituzumab tesirine, Rovelizumab, Rozanolixizumab, Ruplizumab, SA237, Sacituzumab govitecan, Samalizumab, Samrotamab vedotin, Sarilumab, Satralizumab, Satumomab pendetide, Secukinumab, Selicrelumab, Seribantumab, Setoxaximab, Setrusumab, Sevirumab, Sibrotuzumab, SGN-CD19A, SHP647, Sifalimumab, Siltuximab, Simtuzumab, Siplizumab, Sirtratumab vedotin, Sirukumab, Sofituzumab vedotin, Solanezumab, Solitomab, Sonepcizumab[104], Sontuzumab, Sotrovimab, Spartalizumab, Spesolimab[107], Stamulumab, Sulesomab, Suptavumab, Sutimlimab, Suvizumab, Suvratoxumab, Tabalumab, Tacatuzumab tetraxetan, Tadocizumab, Tafasitamab, Talacotuzumab, Talizumab, Talquetamab, Tamtuvatmab, Tanezumab, Taplitumomab paptox, Tarextumab, Tavolimab, Teclistamab, Tefibazumab, Telimomab aritox, Telisotuzumab, Telisotuzumab vedotin, Tenatumomab, Teneliximab, Teplizumab, Tepoditamab, Teprotumumab, Tesidolumab, Tetulomab, Tezepelumab, TGN1412, Tibulizumab, Tildrakizumab, Tigatuzumab, Timigutuzumab, Timolumab, tiragolumab, Tiragotumab, Tislelizumab, Tisotumab vedotin, Tixagevimab, TNX-650, Tocilizumab, Tomuzotuximab, Toralizumab, Tosatoxumab, Tositumomab, Tovetumab, Tralokinumab[115], Trastuzumab, Trastuzumab duocarmazine, Trastuzumab emtansine, TRBS07[117], Tregalizumab, Tremelimumab[118], Trevogrumab, Tucotuzumab celmoleukin, Tuvirumab, Ublituximab[120], Ulocuplumab, Urelumab, Urtoxazumab, Ustekinumab, Utomilumab, Vadastuximab talirine, Vanalimab, Vandortuzumab vedotin, Vantictumab, Vanucizumab, Vapaliximab, Varisacumab, Varlilumab, Vatelizumab, Vedolizumab, Veltuzumab, Vepalimomab, Vesencumab, Vilobelimumab, Visilizumab, Vobarilizumab, Volociximab, Vonlerolizumab, Vopratelimumab, Vorsetuzumab mafodotin,

Votumumab, Vunakizumab, Xentuzumab, XMAB-5574, Zalutumumab, Zanolimumab, Zatuximab, Zenocutuzumab, Ziralimumab, Zolbetuximab, and Zolimomab aritox.

[00208] In some embodiments, the transgene encodes a coagulation factor. For example, the transgene encodes coagulation factor VIII (FVIII). In another non-limiting example, the transgene encodes coagulation factor IX (FIX). In still another non-limiting example, the transgene encodes coagulation factor VII (FVII). In yet another non-limiting example, the transgene encodes coagulation factor XI (FXI).

[00209] In some embodiments, the transgene encodes a cytokine, e.g., a protein or peptide cytokine. For example, the transgene encodes interleukin-1 α (IL-1 α), interleukin-1/3 (IL-1/3), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-8 (IL-8/CXCL8), interleukin-10 (IL-10), interleukin-12 (IL-12), interleukin-13 (IL-13), interleukin-15 (IL-15), interleukin-17 (IL-17), interleukin-18 (IL-18), interleukin-37 (IL-37), interleukin-38 (IL-38), tumor necrosis factor- α (TNF- α), interferon- α (INF- α), interferon- β (INF- β), interferon- γ (INF- γ), granulocyte-monocyte colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), monocyte chemoattractant protein-1 (MCP-1/CCL2), macrophage inflammatory protein 1- α (MCP-1/CCL3), macrophage inflammatory protein-1/3, RANTES (CCL5), Eotaxin (CCL11), vascular endothelial growth factor (VEGF), endothelial growth factor (EGF), or fibroblast growth factor (FGF). In some embodiments, the transgene encodes IL-2. In some embodiments, the transgene encodes IL-37. In some embodiments, the transgene encodes IL-38. In some embodiments of any of the aspects, the subject comprises at least one cell, e.g., at least one liver cell, comprising a plurality of exogenous nucleotide sequences, each exogenous nucleotide sequence comprising a synthetic inducible promoter operably linked to a transgene, wherein the transgenes collectively encode IL-37 and IL-38.

[00210] In some embodiments, the transgene encodes a hormone, e.g., a protein or peptide hormone. For example, the transgene encodes erythropoietin (EPO), parathyroid hormone (PTH), adiponectin, adrenaline (or epinephrine), adrenocorticotrophic hormone (ACTH or corticotropin), adropin, aldosterone, amylin (or islet amyloid polypeptide), androstenedione, angiotensin, angiotensinogen, antidiuretic hormone (or vasopressin), anti-müllerian hormone (or müllerian-inhibiting factor/hormone), arginine vasopressin, atrial natriuretic peptide (ANP or atriopeptin), brain natriuretic peptide, calcitonin, calcitriol, cholecystokinin (CCK), corticotropin-releasing hormone, cortisol, cortistatin, dehydroepiandrosterone, dihydrotestosterone, dopamine, endothelin, enkephalin, estradiol, estriol, estrone, follicle-stimulating hormone (FSH), galanin, gastric inhibitory polypeptide, gastrin, ghrelin, glucagon, glucagon-like peptide-1, gonadotropin-releasing hormone, growth hormone, growth hormone-releasing hormone, guanylin, hepcidin, human

chorionic gonadotropin, human placental lactogen, inhibin, insulin, insulin-like growth factor (or somatomedin), leptin, leukotrienes, lipotropin, luteinizing hormone (LH), melanocyte stimulating hormone (MSH), melatonin, motilin, noradrenaline (or norepinephrine), orexin, osteocalcin, oxytocin (or pitocin), pancreatic polypeptide, pituitary adenylate cyclase-activating peptide, progesterone, prolactin (or leuteotropic hormone), prolactin-releasing hormone, prostacyclin, prostaglandins, relaxin, renin, secretin, somatostatin (or growth hormone-inhibiting hormone or growth hormone release-inhibiting hormone or somatotropin release-inhibiting factor or somatotropin release-inhibiting hormone), testosterone, thrombopoietin, thromboxane, thyroid-stimulating hormone (TSH or thyrotropin), thyrotropin-releasing hormone (TRH), thyroxine, triiodothyronine, uroguanylin, vasoactive intestinal peptide (VIP), or vasopressin. In some embodiments of any of the aspects described herein, the transgene encodes erythropoietin (EPO) or parathyroid hormone (PTH). In some embodiments of any of the aspects described herein, the transgene encodes erythropoietin (EPO). In some embodiments of any of the aspects described herein, the transgene encodes parathyroid hormone (PTH).

[00211] In some embodiments, the transgene encodes a growth factor, e.g., a protein or peptide growth factor. For example, the transgene encodes tumor necrosis factor-alpha (TNF- α), adrenomedullin (AM), angiopoietin (Ang), autocrine motility factor, bone morphogenetic protein (BMP), ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), colony-stimulating factor (e.g., macrophage colony-stimulating factor (M-CSF), granulocyte colony-stimulating factor (G-CSF), or granulocyte macrophage colony-stimulating factor (GM-CSF)), epidermal growth factor (EGF), ephrin (e.g., ephrin A1, ephrin A2, ephrin A3, ephrin A4, ephrin A5, ephrin B1, ephrin B2, or ephrin B3), erythropoietin (EPO), fibroblast growth factor (e.g., FGF1, FGF2, FGF3, FGF4, FGF5, FGF6, FGF7, FGF8, FGF9, FGF10, FGF11, FGF12, FGF13, FGF14, FGF15, FGF16, FGF17, FGF18, FGF19, FGF20, FGF21, FGF22 or FGF23), glial cell line-derived neurotrophic factor (GDNF), neurturin, persephin, artemin, growth differentiation factor-9 (GDF9), hepatocyte growth factor (HGF), hepatoma-derived growth factor (HDGF), insulin-like growth factor (e.g., IGF-1 or IGF-2), interleukin (e.g., IL-1, IL-2, IL-3, IL-4, IL-5, IL-6 or IL-7), keratinocyte growth factor (KGF), migration-stimulating factor (MSF), macrophage-stimulating protein (MSP), myostatin (GDF-8), neuregulin (e.g., NRG1, NRG3, NRG3 or NRG4), neurotrophin (e.g., brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-3 (NT-3) or neurotrophin-4 (NT-4)), placental growth factor (PGF), platelet-derived growth factor (PDGF), renalase (RNLS), T-cell growth factor (TCGF), thrombopoietin (TPO), transforming growth factor (e.g., TGF- α or TGF- β), or vascular endothelial growth factor (VEGF).

[00212] Transgenes encoding a viral or virus protein can also be used. Thus, in some embodiments, the transgene encodes a viral protein. Exemplary, viral proteins include, but not

limited to viral envelop proteins, viral nucleocapsid proteins, viral membrane proteins, REP, CAP, REV, VSV-G and RD114. In some embodiments, the transgene encodes a viral spike protein, such as a coronavirus spike protein.

[00213] In some embodiments, the transgene encodes a cell receptor ligand, e.g., a protein or peptide cell receptor ligand. For example, the transgene encodes EGF, insulin, VEGF, cGMP, cGTP, acetylcholine, ATP, dopamine, GABA, glutamate, glycine, serotonin, ketamine, nicotine, strychnine, cereulide, or muscarine. In some embodiments, the transgene encodes an enzyme.

[00214] In some embodiments of any one of the aspects described herein, the exogenous nucleotide sequence comprises a transgene useful for gene editing. For example, the exogenous nucleotide sequence comprises a transgene encoding a site-specific nuclease, such as a meganuclease, zinc finger nuclease (ZFN), transcription activator-like effector-based nuclease (TALEN), or the clustered regularly interspaced short palindromic repeats system (CRISPR-Cas). Typically, the site-specific nuclease is adapted to edit a desired target genomic locus by making a cut (typically a site-specific double-strand break) which is then repaired via non-homologous end-joining (NHEJ) or homology dependent repair (HDR), resulting in a desired edit. The edit can be the partial or complete repair of a gene that is dysfunctional, or the knock-down or knock-out of a functional gene.

[00215] In some embodiments, the nuclease is a CRISPR-Cas nuclease. For example, the transgene encodes Cas9 (also known as Csn1 and Csx12), C2c1, C2c3, Cas1, Cas100, Cas12, Cas12a, Cas12b, Cas12c, Cas12d, Cas12e, Cas13a, Cas13b, Cas13c, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Casl, CaslB, CaslO, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Cpf1, Csa5, Csa5, CsaX, Csb1, Csb2, Csb3, Csc1, Csc2, Cse1, Cse2, Csf1, Csf2, Csf3, Csf4, Csm2, Csm3, Csm4, Csm5, Csm6, Csn2, Csx1, Csx10, Csx14, Csx15, Csx16, Csx17, Csx3, Csy1, Csy2, Csy3, Cas Phi, CasX or CasRx. In some embodiments, the transgene encodes Cas9. In some embodiments, the transgene encodes Cas12. In some embodiments, the transgene encodes CasRx. In some embodiments, the transgene encodes Cas Phi. In some embodiments, the transgene encodes CasX.

[00216] In some embodiments of any of the aspects, described herein, the transgene encodes a chimeric antigen receptor (CAR). As used herein, “chimeric antigen receptor” or “CAR” refers to an artificially constructed hybrid polypeptide comprising an antigen-binding domain (e.g., an antigen-binding portion of an antibody (e.g., a scFv)), a transmembrane domain, and a T-cell signaling and/or T-cell activation domain. Most commonly, the CAR’s extracellular binding domain is composed of a single chain variable fragment (scFv) derived from fusing the variable heavy and light regions of a murine or humanized monoclonal antibody. Alternatively, scFvs may be used that are derived from Fab’s (instead of from an antibody, e.g., obtained from Fab libraries),

in various embodiments, this scFv is fused to a transmembrane domain and then to an intracellular signaling domain. The signaling and/or activation domain of a CAR may provide activation signals (e.g., TCR ζ , FcR γ , FcR β , CD3 γ , CD3 δ , CD3 ϵ , CD3 ζ , CD22, CD79a, CD79b, and/or CD66d) and/or one or more costimulation (e.g., CARD11, CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD134 (OX40), CD137 (4-1BB), CD150 (SLAMF1), CD152 (CTLA4), CD223 (LAG3), CD270 (HVEM), CD273 (PD-L2), CD274 (PD-L1), CD278 (ICOS), DAP10, LAT, NKD2C SLP76, TRIM, and/or ZAP70) signals upon antigen binding. Further discussion of CARs can be found, e.g., in Maus *et al.* Blood 2014 123:2624-35; Reardon *et al.* Neuro-Oncology 2014 16:1441-1458; Hoyos *et al.* Haematologica 2012 97:1622; Byrd *et al.* J Clin Oncol 2014 32:3039-47; Maher *et al.* Cancer Res 2009 69:4559-4562; and Tamada *et al.* Clin Cancer Res 2012 18:6436-6445; each of which is incorporated by reference herein in its entirety. A large number of CARs are known in the art, e.g., 100s have entered clinical trials and/or been approved, as described in Ivica *et al.* Healthcare (Basel) 2021 9(8):1062; which is incorporated by reference herein in its entirety.

[00217] In some embodiments of any of the aspects described herein, the transgene is detrimental to the liver cell or to the subject when constitutively expressed. A transgene is detrimental when it inhibits cellular or tissue function, survival, repair, or persistence. The detrimental effect can contribute to or increase the pathology of an existing condition, e.g., the condition the subject is receiving the transgene to treat, and/or contribute to or cause a new condition. Non-limiting examples of detrimental effects include toxicity and accumulation of misfolded proteins.

[00218] In some embodiments of any one of the aspects described herein, the expression product can be a nucleic acid, for example an RNA. For example, the expression product can be an antisense RNA, microRNA, siRNA, tRNA, rRNAs, guide RNA or any other regulatory, therapeutic or otherwise useful RNA.

[00219] In some embodiments of any one of the aspects described herein, the gene is not a reporter gene. Preferably, the gene encodes a therapeutic expression product (e.g., as discussed above) or another expression product that is useful in industry or research. Thus, in some embodiments of the various aspects described herein, the gene expresses a product which is not a reporter (such as a fluorescent protein, luminescent protein or a chromogenic protein), and preferably expresses a therapeutic expression product.

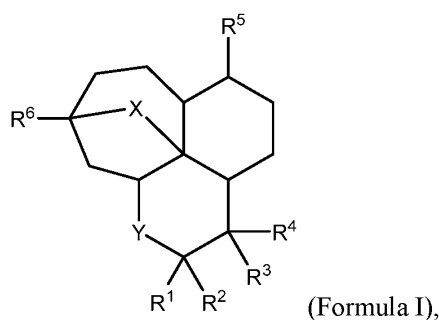
[00220] Suitably the exogenous nucleotide sequence comprises sequences providing or coding for one or more (e.g., 1, 2, 3 or all 4) of a ribosomal binding site, a start codon, a stop codon, and a transcription termination sequence. Accordingly, in some embodiments of any one of the aspects described herein, the exogenous nucleotide sequence comprises a nucleic acid sequence encoding

a ribosomal binding site. In some embodiments, the exogenous nucleotide sequence comprises a nucleic acid sequence encoding a start codon. In some embodiments, the exogenous nucleotide sequence comprises a nucleic acid sequence encoding a stop codon. In some embodiments, the exogenous nucleotide sequence comprises a nucleic acid sequence encoding a transcriptional termination site.

[00221] In some embodiments, the exogenous nucleotide sequence further comprises a nucleic acid sequence encoding a posttranscriptional regulatory element. In some embodiments, the exogenous nucleotide sequence comprises a nucleic acid encoding a polyA element.

Inducer

[00222] In some embodiments of the various aspects described herein, the inducer is artemisinin or a derivative thereof. Exemplary derivatives of artemisinin include, but are not limited to artesunate, arteether, artemether, and dihydroartemisinin (DHA). The derivatives can include those of Formula (I):



wherein:

X is $-(O)_n-$, n is 1 or 2;

Y is O or NR^N ;

where:

R^N is a hydrogen atom or an optionally substituted alkyl, alkenyl, alkynyl, aralkyl or heterocyclalkyl group;

R^1 is hydrogen, halogen, hydroxyl, optionally substituted C_1 - C_6 alkyl, optionally substituted C_2 - C_6 alkenyl, optionally substituted C_2 - C_6 alkynyl, optionally substituted alkoxy, optionally substituted cycloalkyl, optionally substituted aryl, C-linked heteroaryl or heterocyclalkyl group, $-NR^7R^8$, $-X^1-R^9$, $-NR^{10}X^2(=Z^2)-R^{11}$, $-OR^{12}$, $-NHR^{12}$, $-SR^{12}$, $-O(CH_2)_b-CH(X^6)CH(Y^6)-Z^6$,

where:

R^7 and R^8 are independently H, optionally substituted C_1 - C_6 alkyl, optionally substituted C_2 - C_6 alkenyl, optionally substituted C_2 - C_6 alkynyl, optionally

substituted aryl, optionally substituted heteroaryl, optionally substituted heterocyclyl, optionally substituted cycloalkyl; or R⁷ and R⁸ together with the nitrogen atom form optionally substituted heterocyclic group or an amino group derived from an optionally substituted amino acid ester;

X¹ is O, O(CH₂)_p or OCHR^{9A} where R^{9A} is C₁-C₆ alkyl, CN, CO₂H, CO₂Me or CO₂Et, p is an integer of 1 to 4;

R⁹ is Ar—Y—Z or Ar—Z, where Ar is a phenyl group or a naphthyl group, Y is O(CH₂)_q, OCHMe, C₁-C₄ alkyl, wherein q is an integer of 1 to 4; and Z is H, halogen, OR', COOR' or CONHCH₂COOR', wherein R' is H, C₁-C₄ alkyl, o-methoxy phenyl or C₁-C₄ alkylamine group;

R¹⁰ is a hydrogen atom or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, aryl or aralkyl group;

X² is a carbon atom, a sulfur atom, a sulfoxide group S=O, —PR^{X3}, —P—O—R^{X3} or —P—N(R^{X4})—R^{X4} where R^{X3} and R^{X4} each independently represent a hydrogen atom or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, aryl or aralkyl group;

Z² is an oxygen atom, a sulfur atom, or group NR^{X5} where R^{X5} is hydrogen or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, aryl or aralkyl group; and

R¹¹ represents a hydrogen atom or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, aryl, aralkyl, N(R^{X6})₂, NHR^{X6}, NR^{X6}NHR^{X6} or NR^{X6}N(R^{X6})₂, OR^{X6}, SR^{X6}, OR^{X7} or NR^{X6}R^{X7}, where each R^{X6} independently represents a hydrogen atom or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, aryl, aralkyl, or a 10α-dihydroartemisinyl group; R^{X7} represents a bond attached as a substituent to R^{X5} together with the group —X²=Z²— forming an optionally substituted heterocyclic group where Z² represents a group NR^{X5}, or R^{X7} represents a bond attached as a substituent to R¹⁰ together with the group —N—X²(=Z²)— forming an optionally substituted heterocyclic group;

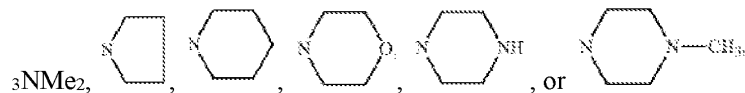
each R¹² is independently H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl optionally substituted cycloalkyl, optionally substituted aryl, or C-linked heteroaryl or heterocyclylalkyl group;

b is 0 or 1;

X^6 is H or OH;

Y^6 is H, CH_3 or phenyl; and

Z^6 is NH_2 , NHR^{6N} , $N(R^{6N})_2$, $NHCH_2CH_2OH$, $NHCH_2CH_2OH$, $NH(CH_2)_2$



where each R^{6N} is independently C_1 - C_6 alkyl;

R^2 is H, halogen, optionally substituted C_1 - C_6 alkyl, optionally substituted C_2 - C_6 alkenyl, optionally substituted C_2 - C_6 alkynyl; or R^1 and R^2 together form a carbonyl ($=O$); or R^1 and R^2 together are $=NCH(R^{13})-(CH_2)_a-C(O)-R^{14}$, where:
a is 0 to 10;

R^{13} is H, optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted hydroxyalkyl, optionally substituted polyhydroxyalkyl, optionally substituted hydroxyaryl, optionally substituted polyhydroxyaryl, optionally substituted heterocyclic-alkyl, optionally substituted mercapto-alkyl, optionally substituted dithio-alkyl, optionally substituted carboxyalkyl, optionally substituted amidoalkyl, or optionally substituted guanidinoalkyl;

R^{14} is OR^{A3} , $NR^{A3}R^{A4}$, and peptide; and

R^{A3} and R^{A4} are independently H, optionally substituted alkyl, or optionally substituted aryl;

R^3 optionally substituted C_1 - C_6 alkyl, optionally substituted C_2 - C_6 alkenyl, optionally substituted C_2 - C_6 alkynyl, H, or halogen;

R^4 is H, halogen, optionally substituted C_1 - C_6 alkyl, optionally substituted C_2 - C_6 alkenyl, optionally substituted C_2 - C_6 alkynyl; or R^2 and R^4 together form a double bond between the carbon atoms they are attached to;

R^5 is optionally substituted C_1 - C_6 alkyl, optionally substituted C_2 - C_6 alkenyl, optionally substituted C_2 - C_6 alkynyl, H, or halogen; and

R^6 is optionally substituted C_1 - C_6 alkyl, optionally substituted C_2 - C_6 alkenyl, optionally substituted C_2 - C_6 alkynyl, H, or halogen, or a pharmaceutically acceptable salt thereof.

[00223] The skilled artisan can readily select any permutation of substituents falling within Formula (I). For example, R^1 can be hydroxyl or optionally substituted C_1 - C_6 alkoxy, R^2 can be hydrogen, R^3 can be optionally substituted C_1 - C_6 alkyl, R^4 can be H, R^5 can be optionally substituted C_1 - C_6 alkyl, X can be $-O-O-$, Y can be O, etc. but any of the various substituents is contemplated herein.

[00224] The inducer for the first or any of the subsequent administration of the inducer can be artemisinin, an artemisinin derivative, or a flavonoid compound. In some embodiments, the first inducer is artemisinin or derivative thereof. For example, the first inducer is artemisinin or a derivative thereof selected from the group consisting of artesunate; artemether; dihydroartemisinin; α -dihydroartemisinin β -glucuronide; artemisone; arteether; artenimol; artesunic acid; artelinic acid; deoxyartemisinin; deoxydihydroartemisinin; 9,10-dihydrodeoxyartemisinin; artemotil; and artemiside. In some embodiments, the first inducer is artemisinin, artesunate, dihydroartemisinin, artesunate, or artemether. For example, the first inducer is artemisinin, artesunate or dihydroartemisinin. In some embodiments, the first inducer is artemisinin.

[00225] In some embodiments, the first inducer is a flavonoid compound. For example, the second inducer is flavone, baicalein, chrysin, galangin, (OH)₄ flavone, 6-hydroxyluteolin, acacetin, acerosin, alnetin, apigenin, apiin, artocarpetin, balcalein, beta-sitosterol, biochamin A, cannaflavin A, cannflavin A, cannflavin B, cannflavin C, catechin, catechin-3-gallate, cerrosillin, chrysoeriol, chrysoeriol, cirsilinole, cirsilinole, cirsimaritin, corymbosin, cupressuflavone, cyanidine, daidzein, datiscetin, delphinidine, digidrokempferol, dihydroquercetin, diosmetin, diosmin, echiodinin, epicatechin, epicatechin-3-gallate, epigallocatechin, epigallocatechin-3-gallate, eriodictiol, eupatilin, eupatorin, fisetin, fizetin, flavonol, formononetin, fortunelin, galocatechin, galocatechin-3-gallate, gardenin D, genistein, genistin, genkwanin, geraldol, geraldone, glycitein, glycitin, gomoeriodictiol, gossypetin, hesperetin, hinokiflavone, hispidulin, hymenoxin, hypolaetin, isoquercetin, isorhamnetin, isorhamnetin, isoscutellarein, isovitexin, jaceosidin, kaempferol, kaempferol, kampferide, largonidin, luteolin, luteolin, malvidine, mikanin, morin, myricetin, myricetin, naringenin, negletein, neodiosmin, nepetin, nevadensin, nobiletin, nodifloretin, norartocarpetin, norwogonin, onopordin, orientin, oroxylin a, pachipodol, pectolarigenin, pedalitin, peonidin, petunidin, pilloin, pratol, primetin, primuletin, pruentin, quercetagenin, quercetin, ramnazin, rhoifolin, robinetin, rutin, scaposin, scutellarein, serpyllin, sinensetin, sorbifolin, sudachitin, tangeretin, tangeritin, taxifolin, tectochrysin, tithonine, tricetin, tricin, velutin, vitexin, wightin, wogonin, xanthomicrol, zapotin, zapotinin, 6,7,4'-trihydroxy isoflavone, 7,3,4'-trihydroxy isoflavone, or a pharmaceutically acceptable salt thereof. In some embodiments, the first inducer is flavone.

[00226] The inducer for the second or any of the subsequent administration of the inducer can be independently artemisinin; an artemisinin derivative; a flavonoid compound, e.g. flavone, chrysin, baicalein, or galangin; 1,4-Bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP); phenobarbital (PB); 6-(4Chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde-O-(3,4-dichlorobenzyl)oxime (CITCO); Acetaminophen; Buprenorphine; Phenytoin; Carbamazepine; Valproic Acid; Chlorpromazine; Efavirenz; Nevirapine; Rilpivirine; Etravirine; Diazepam;

Cyclophosphamid; Ifosfamide; Cerivastatin; Simvastatin; lovastatin; substituted sulphonamides; Thiazolidin-4-one; Estradiol; Estrone and analogues; 17 α -ethinyl-3,17 β -estradiol (EE2); Dehydroepiandrosterone (DHEA); 5 β -pregnane-3,20-dione; Diethylstilbestrol; Ginkgo *biloba* extract; Galangin; chrysin; baicalein; Diallyl sulphide; Ellagic acid; Resveratrol; Squalstatin-1; Bilobalide; Triclocarban; Triclosan; Dichlorodiphenyltrichloroethane (DDT); Dieldrin; Methoxychlor; Metofluthrin; Permethrin; Pyrethrins; Sulfoxaflor; Diethylhexyl phthalate (DEHP); Cyproconazole; Fluconazole; Propiconazole; FL81; Tri-p-methylphenyl phosphate (TMPP); UM104; or UM145.

[00227] In some embodiments of any one of the aspects described herein, the second inducer is artemisinin; artesunate; artemether; dihydroartemisinin; α -dihydroartemisinin β -glucuronide; artemisone; arteether; artenimol; artesunic acid; artelinic acid; deoxyartemisinin; deoxydihydroartemisinin; 9,10-dihydrodeoxyartemisinin; artemotil; artemiside; or flavone. For example, the second inducer is artemisinin, artesunate, dihydroartemisinin, artesunate, artemether, or flavone. In some embodiments, the second inducer is artemisinin, artesunate or dihydroartemisinin.

[00228] In some embodiments of any one of the aspects described herein, the second inducer is a flavonoid compound. For example, the second inducer is flavone, baicalein, chrysin, galangin, (OH)₄ flavone, 6-hydroxyluteolin, acacetin, acerosin, alnetin, apigenin, apiin, artocarpetin, balcain, beta-sitosterol, biochamin A, cannaflavin A, cannflavin A, cannflavin B, cannflavin C, catechin, catechin-3-gallate, cerrosillin, chrysoeriol, chrysoeriol, cirsilin, cirsilin, cirsimarin, corymbosin, cupressuflavone, cyanidine, daidzein, datiscetin, delphinidine, digidrokempferol, dihydroquercetin, diosmetin, diosmin, echiodinin, epicatechin, epicatechin-3-gallate, epigallocatechin, epigallocatechin-3-gallate, eriodictiol, eupatilin, eupatorin, fisetin, fizin, flavonol, formononetin, fortunelin, gallocatechin, gallocatechin-3-gallate, gardenin D, genistein, genistin, genkwanin, geraldol, geraldone, glycitein, glycitin, gomoeriodictiol, gossypetin, hesperetin, hinokiflavone, hispidulin, hymenoxin, hypolaetin, isoquercetin, isorhamnetin, isorhamnetin, isoscutellarein, isovitexin, jaceosidin, kaempferol, kaempferol, kampferide, largonidin, luteolin, luteolin, malvidine, mikanin, morin, myricetin, myricetin, naringenin, negletein, neodiosmin, nepetin, nevadensin, nobiletin, nodifloretin, norartocarpetin, norwogonin, onopordin, orientin, oroxylin a, pachipodol, pectolinarigenin, pedalitin, peonidin, petunidin, pilloin, pratol, primetin, primuletin, pruentin, quercetagenin, quercetin, ramnazin, rhoifolin, robinetin, rutin, scaposin, scutellarein, serpyllin, sinensetin, sorbifolin, sudachitin, tangeretin, tangeritin, taxifolin, tectochrysin, tithonine, tricetin, tricin, velutin, vitexin, wightin, wogonin, xanthomicrol, zapotin, zapotinin, 6,7,4'-trihydroxy isoflavone, 7,3',4'-trihydroxy isoflavone, or a pharmaceutically acceptable salt thereof. In some embodiments, the second inducer is flavone.

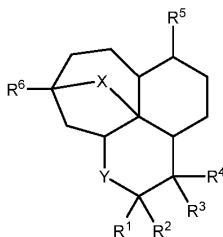
[00229] In some embodiments of any one of the aspects described herein, the first inducer is artemisinin and the second inducer can be artemisinin or a derivative thereof, or the second inducer can be selected from the group consisting of a flavonoid compound, e.g. flavone, chrysin, baicalein, or galangin; 1,4-Bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP); phenobarbital (PB); 6-(4Chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde-O-(3,4-dichlorobenzyl)oxime (CITCO); Acetaminophen; Buprenorphine; Phenytoin; Carbamazepine; Valproic Acid; Chlorpromazine; Efavirenz; Nevirapine; Rilpivirine; Etravirine; Diazepam; Cyclophosphamid; Ifosfamide; Cerivastatin; Simvastatin; lovastatin; substituted sulphonamides; Thiazolidin-4-one; Estradiol; Estrone and analogues; 17 α -ethinyl-3;17 β -estradiol (EE2); Dehydroepiandrosterone (DHEA); 5 β -pregnane-3,20-dione; Diethylstilbestrol; Ginkgo *biloba* extract; Galangin; chrysin; baicalein; Diallyl sulphide; Ellagic acid; Resveratrol; Squalestatin-1; Bilobalide; Triclocarban; Triclosan; Dichlorodiphenyltrichloroethane (DDT); Dieldrin; Methoxychlor; Metofluthrin; Permethrin; Pyrethrins; Sulfoxaflo; Diethylhexyl phthalate (DEHP); Cyproconazole; Fluconazole; Propiconazole; FL81; Tri-p-methylphenyl phosphate (TMPP); UM104; and UM145. For example, the first inducer is artemisinin and the second inducer is an artemisinin, artemisinin derivative or flavone. In some embodiments of any one of the aspects described herein, the first inducer is artemisinin and the second inducer is artemisinin, artesunate, dihydroartemisinin, artesunate, artemether, or flavone. For example, the first inducer is artemisinin and the second inducer is artemisinin, artesunate, dihydroartemisinin, or flavone. In some embodiments, the first inducer is artemisinin and the second inducer is flavone.

[00230] In some embodiments of any one of the aspects described herein, the first inducer is artemisinin and the second inducer is artemisinin, artesunate, dihydroartemisinin, artesunate, artemether, or flavone. For example, the first inducer is artemisinin and the second inducer is artemisinin, artesunate, dihydroartemisinin, or flavone.

[00231] In some embodiments, the first inducer is flavone and the second inducer is artemisinin, or an artemisinin derivative. For example, the first inducer is flavone and the second inducer is artemisinin, artesunate, dihydroartemisinin, artesunate, artemether. In some embodiments, the first inducer is flavone and the second inducer is artemisinin, artesunate or dihydroartemisinin. For example, the first inducer is flavone and the second inducer is artemisinin.

[00232] In some embodiments, the first and second inducers are the same. For example, the first and second inducer are artemisinin. In another non-limiting example, the first and second inducer are flavone. In yet another non-limiting example, the first and second inducer are artesunate. In still another non-limiting example, the first and second inducer are dihydroartemisinin. In another non-limiting example, the first and second inducer are artesunate. In still another non-limiting example, the first and second inducer are artemether.

[00233] In some embodiments of any one of the aspects described herein, artemisinin or a



derivative thereof is of Formula (I):
derivative thereof is of Formula (I):

(Formula I), or a pharmaceutically acceptable salt thereof.

[00234] In some embodiments of any one of the aspects described herein, n is 2, i.e., X is –O–O–. In some other embodiments of any one of the aspects described herein, n is 1, i.e., X is –O–.

[00235] In some embodiments of any one of the aspects described herein, Y is O.

[00236] In some embodiments of any one of the aspects described herein, Y is NR^N.

[00237] In some embodiments of any one of the aspects described herein, R⁶ is methyl.

[00238] In some embodiments of any one of the aspects described herein, R⁵ is methyl.

[00239] In some embodiments of any one of the aspects described herein, R⁴ is H.

[00240] In some embodiments of any one of the aspects described herein R⁴ is halogen. For example, R⁴ is bromo, chloro, fluoro or iodo. In some embodiments, R⁴ is bromo.

[00241] In some embodiments of any one of the aspects described herein, R³ is methyl.

[00242] In some embodiments of any one of the aspects described herein, R³, R⁵ and R⁶ are methyl; and R⁴ are is H.

[00243] In some embodiments of any one of the aspects described herein, R³, R⁵ and R⁶ are methyl; and R⁴ is H.

[00244] In some embodiments of any one of the aspects described herein, n is 2 (i.e., X is –O–O–); R³, R⁵ and R⁶ are methyl; and R⁴ are is H.

[00245] In some embodiments of any one of the aspects described herein, n is 2 (i.e., X is –O–O–); R³, R⁵ and R⁶ are methyl; and R⁴ is H.

[00246] In some embodiments of any one of the aspects described herein, R³, R⁵ and R⁶ are methyl; and R² and R⁴ are H.

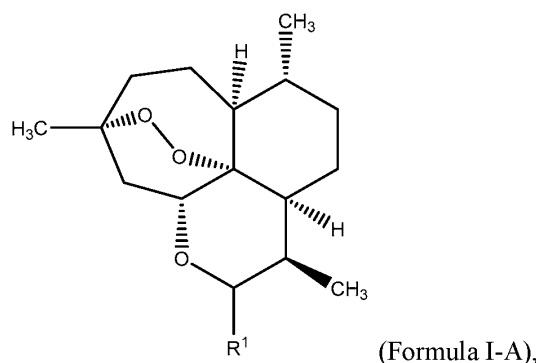
[00247] In some embodiments of any one of the aspects described herein, R² is H; R³, R⁵ and R⁶ are methyl; and R⁴ is halogen.

[00248] In some embodiments of any one of the aspects described herein, n is 2 (i.e., X is –O–O–); R³, R⁵ and R⁶ are methyl; and R² and R⁴ are H.

[00249] In some embodiments of any one of the aspects described herein, n is 2 (i.e., X is –O–O–); R² is H; R³, R⁵ and R⁶ are methyl; and R⁴ is halogen.

[00250] In some embodiments of any one of the aspects described herein, n is 2 (i.e., X is –O–O–); R¹ and R² are carbonyl (=O); R³, R⁵ and R⁶ are methyl; and R⁴ is halogen.

[00251] In some embodiments of any one of the aspects described herein, the artemisinin derivative is of Formula (I-A):



wherein:

R¹ is halogen, —NR^{1A}R^{2A}, or an optionally substituted cycloalkyl, aryl, C-linked heteroaryl or heterocyclalkyl group;

where:

R^{1A} is H or an optionally substituted alkyl, alkenyl or alkynyl group;

R^{2A} is an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, aryl or aralkyl group; or R^{1A} and R^{2A} together with the interjacent nitrogen atom represent an optionally substituted heterocyclic group or an amino group derived from an optionally substituted amino acid ester.

[00252] In some compounds of Formula I-A, R¹ is halogen, optionally substituted cycloalkyl, optionally substituted aryl, C-linked heteroaryl or heterocyclalkyl group, or —NR⁷R⁸.

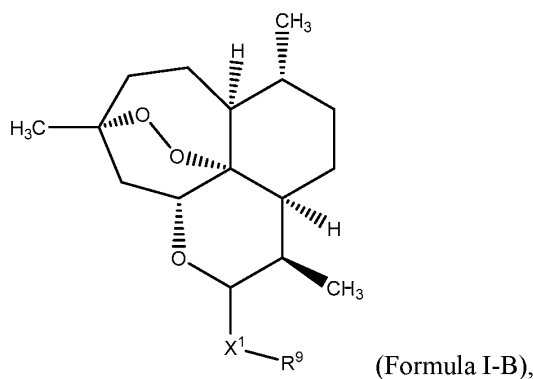
[00253] In some compounds of Formula I-A, R¹ is halogen; phenyl; 2-methoxyphenyl; 2,4-dimethoxyphenyl; 2,4,5-trimethoxyphenyl; 2,4,6-trimethoxyphenyl; 4-F-phenyl; 4-Cl-phenyl; 4-Br-phenyl; 4-I-phenyl; 4-vinylphenyl; 4-C(O)OH-phenyl; 2-naphthyl; 2-hydroxyl-naphthyl; morpholino; thiomorpholino; 2-furyl; 4'-(S,S-dioxothiomorpholin-1'-yl); 4'-benzyl-1'-piperazinyl; 2-pyrrolyl; 4'-benzyl-4'-methylpiperazinium-1'-yl; indolyl; 1,2,3,4-tetrahydroisoquinolyl; N-methylpiperazino; 9-anthryl; 9-phenathryl; 4-phenylpiperazino; 4-(2'-methoxyphenyl)-piperazino; 4-(4'-fluorophenyl)piperazino; 4-(2'-pyridyl)piperazino; 4-(2'-pyridyl)-piperazino; 4-(3'-trifluoromethylphenyl)-piperazino; 1-(2-pyrimidyl)-piperazino; 1-(4-chlorophenyl)-piperazino; 1-(3-chlorophenyl)-piperazino; 1-(2-chlorophenyl)-piperazino; 1-(4-methoxyphenyl)-piperazino; 1-(3-methoxyphenyl)-piperazino; 1-(2-methoxyphenyl)-piperazino; 1-(chloro-tolyl)-piperazino; 4-benzylpiperdino; or methoxynaphthyl.

[00254] In some compounds of Formula I-A, R^1 is $-NR^7R^8$, wherein R^7 is H, methyl, ethyl or propyl; and R^8 is phenyl; 4-F-phenyl; 4-Cl-phenyl; 4-Br-phenyl; 4-I-phenyl; 4-biphenyl; benzyl; 2-F-benzyl; 4-F-benzyl; 3,5-trifluoromethylbenzyl; methyl, ethyl, propyl, butyl, -CH(CH₃)phenyl; -CH(C(O)OCH₃)phenyl; 4-(C(O)OCH₃)phenyl; cyclopentyl; or cyclohexyl. For example, R^7 is H and R^8 is phenyl; 4-F-phenyl; 4-Cl-phenyl; 4-Br-phenyl; 4-I-phenyl; 4-biphenyl; benzyl; 2-F-benzyl; 4-F-benzyl; 3,5-trifluoromethylbenzyl; methyl, ethyl, propyl, butyl, -CH(CH₃)phenyl; -CH(C(O)OCH₃)phenyl; 4-(C(O)OCH₃)phenyl; cyclopentyl; or cyclohexyl.

[00255] In some embodiments of any one of the aspects described herein, the compound of Formula I-A is selected from the group consisting of 10 β -fluoro-10-deoxo-10-dihydroartemisinin, 10 β -phenyl-10-deoxo-10-dihydroartemisinin, 10 α -(4'-fluorobenzylamino)-10-deoxo-10-dihydroartemisinin, 10-(2',4'-dimethoxyphenyl)-10-deoxo-10-dihydroartemisinin, 10 α -(2'-hydroxy-1'-naphthyl)dihydroartemisinin, 10 α -(4'-thiomorpholino-1'-yl)-10-deoxo-10-dihydroartemisinin, 10 α -(4'-(S,S-dioxothiomorpholino-1'-yl)-10-deoxo-10-dihydroartemisinin, 10 α -(4'-benzylpiperazin-1'-yl)-10-deoxo-10-dihydroartemisinin, 10 α -(2'-furyl)-10-deoxo-10-dihydroartemisinin, 10 α -(Pyrrol-2'-yl)-10-deoxo-10-dihydroartemisinin, and 10 α -(4'-Benzyl-4'-methylpiperazin-1'-yl)-10-deoxo-10-dihydroartemisinin iodide salt.

[00256] Compounds of Formula I-A and their synthesis are described, for example, in US Pat. No. 7,43,238, content of which is incorporated herein by reference in its entirety.

[00257] In some embodiments of any one of the aspects described herein, the artemisinin derivative is of Formula (I-B):



wherein:

X^1 is O, O(CH₂)_p or OCHR^{9A}, where R^{9A} is C₁-C₄ alkyl, CN, COOH, COOMe or COOEt, and p is an integer of 1 to 4;

R⁹ is Ar—Y—Z or Ar—Z, where Ar is a phenyl group or a naphthyl group, Y is O(CH₂)_q, OCHMe, C₁-C₄ alkyl; and Z is H, halogen, OR', COOR' or

CONHCH₂COOR', where q is an integer of 1 to 4 and R' is H, C₁-C₄ alkyl, o-methoxy phenyl or C₁-C₄ alkylamine group.

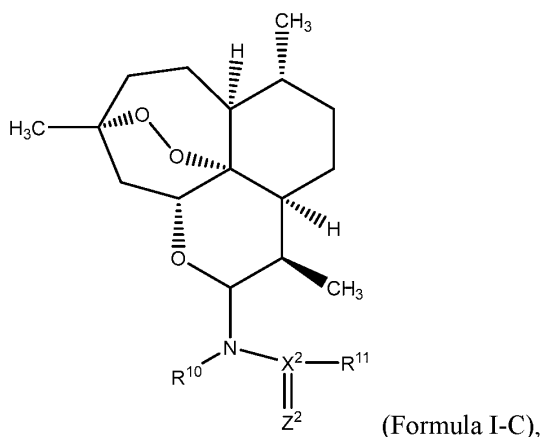
[00258] In some compounds of Formula I-B, X¹ is O, O(CH₂)_p (e.g., p is 1, 2, 3 or 4), or OCHR^{9A} (e.g., R^{9A} is C₁-C₄ alkyl, CN, CO₂H, CO₂Me or CO₂Et.); R⁹ is Ar—Y—Z or Ar—Z (e.g., Ar is a phenyl group or a naphthyl group; Y is O(CH₂)_q (e.g., q is 1, 2, 3, or 4); OCHMe or C₁-C₄ alkyl); and Z is H, halogen, OR', COOR' or CONHCH₂COOR' (wherein R' is H, C₁-C₄ alkyl, o-methoxy phenyl or C₁-C₄ alkylamine group).

[00259] In some compounds of Formula I-B, X is O or OCH₂; R⁹ is Ar—Y—Z; and Y is O(CH₂)_p, OCHMe, or C₁-C₄ hydrocarbyl; and Z is COOR', wherein, p is 1 or 2; Ar is phenyl, and R' is H, Me, Et or o-methoxyphenyl.

[00260] In some embodiments of any one of the aspects described herein, the compound of Formula I-B is selected from the group consisting of methyl 4-(12β-artemisininoxy)phenyl acetate; 4-(12β-artemisininoxy)phenyl acetic acid; ethyl 4-(12β-artemisininoxy)-phenoxy acetate; 3-(12β-artemisininoxy)phenoxy succinic acid; ethyl-[4-(12β-artemisininoxy)benzoyl] aminoacetate; methyl 2-[4-(12β-artemisininoxy)phenyl] propionate; o-methoxy-phenyl 2-[4-(12β-artemisininoxy)phenyl] propionate; ethyl 2-[4-(12β-artemisininoxy)phenoxy] propionate; 2-[4-(12β-artemisininoxy)phenoxy] propanoic acid; o-methoxy-phenyl 2-[4-(12β-artemisininoxy)phenoxy] propionate; o-methoxy-phenyl 2-[3-(12β-artemisininoxy)phenoxy] propionate' and 2-[4-(12β-artemisininoxy)phenyl] propanoic acid.

[00261] Compounds of Formula I-B and their synthesis are described, for example, in US Pat. No. 7,910,750, content of which is incorporated herein by reference in its entirety.

[00262] In some embodiments of any one of the aspects described herein, the artemisinin derivative is of Formula (I-C):



wherein:

R^{10} represents a hydrogen atom or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, aryl or aralkyl group;

X^2 represents a carbon atom, a sulfur atom, a sulfoxide group $S=O$ or a group PR^{X3} , $P-O-R^{X3}$ or $P-N(RX4^4)-R^{X3}$, where R^{X3} and R^{X4} each independently represent a hydrogen atom or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, aryl or aralkyl group;

Z^2 represents an oxygen atom, a sulfur atom or a group NR^{X5} where R^{X5} represents a hydrogen atom or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, aryl or aralkyl group; and

R^{11} represents a hydrogen atom or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, aryl or aralkyl group, or a group $N(R^{X6})_2$, $NHNH_2$, $NR^{X6}NHR^{X6}$ or $NR^{X6}N(R^{X6})_2$, or a group OR^{X6} or SR^{X6} where each R^{X6} independently represents a hydrogen atom or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, aryl or aralkyl group, or a 10α -dihydroartemisinyl group, or R^{X2} represents a group OR^{X6} or $NR^{X6}R^{X7}$ where R^{X6} represents a group as defined above and R^{X7} represents a bond attached as a substituent to R^{X5} together with the group $-X^2=Z^2-$ forming an optionally substituted heterocyclic group where Z^2 represents a group NR^{X5} , or R^{X7} represents a bond attached as a substituent to R^{10} together with the group $-N-X^2(=Z^2)-$ forming an optionally substituted heterocyclic group.

[00263] In some compounds of Formula I-C, R^{10} represents a hydrogen atom, a methyl group, ethyl group or longer chain alkyl group or a branched alkyl group containing up to 9 carbon atoms, preferably a hydrogen atom, a methyl group or an ethyl group.

[00264] In some compounds of Formula I-C, X^2 represents a carbon atom, a sulfur atom, or a group $-PR^{X3}$, $-P-O-R^{X3}$ or $-P-N(RX4^4)-R^{X3}$ where R^{X3} and R^{X4} each independently represent a C_{6-18} aryl group or a 5- to 10-membered C-linked heteroaryl group or a 5- to 10-membered heterocyclyl- C_{1-6} alkyl group optionally substituted by one or more substituents selected from the group consisting of halogen atoms, hydroxyl, C_{1-4} alkyl, C_{2-4} alkenyl, C_{1-4} haloalkyl, C_{1-4} alkoxy, C_{1-4} haloalkoxy, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino and carboxyl groups. Preferably, X^2 represents a carbon atom or a sulfur atom.

[00265] In some compounds of Formula I-C, Z^2 represents an oxygen atom, or a group NR^{X5} where R^{X5} represents a hydrogen atom, a methyl group, ethyl group or longer chain alkyl group or branched alkyl group containing up to 9 carbon atoms, or a C_{6-18} aryl group or a 5- to 10-membered C-linked heteroaryl group or a 5- to 10-membered heterocyclyl- C_{1-6} alkyl group optionally substituted by one or more substituents selected from the group consisting of halogen

atoms, hydroxyl, C₁₋₄ alkyl, C₂₋₄ alkenyl, C₁₋₄ haloalkyl, C₁₋₄ alkoxy, C₁₋₄ haloalkoxy, amino, C₁₋₄ alkylamino, di(C₁₋₄ alkyl)amino and carboxyl groups.

[00266] In some compounds of Formula I-C, R¹¹ represents a hydrogen atom or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, aryl or aralkyl group, or a group OR^{X6}, SR^{X6}, NH₂, NHR^{X6}, or N(R^{X6})₂ where each R^{X6} independently represents a methyl group, ethyl group or longer chain alkyl group or branched alkyl group containing up to 9 carbon atoms, or is a C₆₋₁₈ aryl group or a 5- to 10-membered C-linked heteroaryl group or a 5- to 10-membered heterocyclyl-C₁₋₆ alkyl group optionally substituted by one or more substituents selected from the group consisting of halogen atoms, hydroxyl, C₁₋₄ alkyl, C₂₋₄ alkenyl, C₁₋₄ haloalkyl, C₁₋₄ alkoxy, C₁₋₄ haloalkoxy, amino, C₁₋₄ alkylamino, di(C₁₋₄ alkyl)amino and carboxyl groups. Preferably, R¹¹ represents a group NH₂, or a group NHR^{X6} where R^{X6} represents an alkyl or aryl group, or a group N(R⁶)₂ where R⁶ represent identical or differentiated alkyl groups.

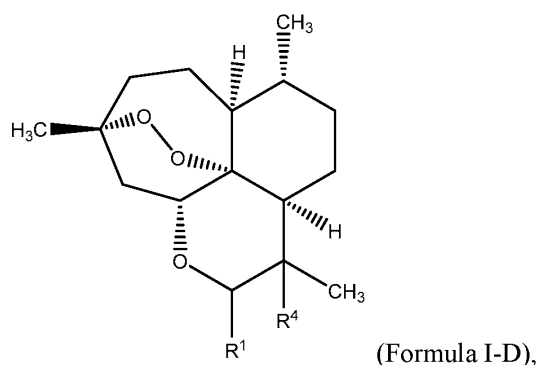
[00267] In some compounds of Formula I-C, R¹¹ represents a hydrogen atom or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, aryl or aralkyl group, preferably a hydrogen atom or an alkyl group, more preferably a hydrogen atom or a methyl group or an ethyl group; X² represents a carbon, phosphorus or sulfur atom, preferably a carbon or sulfur atom; Z² represents an oxygen atom or a group NR^{X5} in where R^{X5} represents a hydrogen atom or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, aryl or aralkyl group, preferably an oxygen atom; and R¹¹ represents a group OR^{X6}, SR^{X6}, NH₂, NHR^{X6}, or N(R^{X6})₂ where each R^{X6} independently represents a hydrogen atom or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, aryl or aralkyl group, or a 10 α -dihydroartemisinyl group, preferably a hydrogen atom or an optionally substituted alkyl or aryl group, more preferably R¹¹ represents a group NH₂, or a group NHR^{X6} where R^{X6} represents an alkyl group, or a group N(R^{X6})₂ where R^{X6} represent identical or differentiated alkyl groups.

[00268] In some compounds of Formula I-C, R¹⁰ represents a hydrogen atom, X² represents a sulfoxide group S=O, Z² represents an oxygen atom, and R¹¹ represents a group NH₂; or R⁹ represents a hydrogen atom, X² represents a carbon atom, Z² represents a group NH, and R¹¹ represents a group NHR^{X6} where R^{X6} represents a hydrogen atom or an optionally substituted alkyl, cycloalkyl, aryl or aralkyl group; or R⁹ represents a hydrogen atom, X² represents a carbon atom, Z² represents an oxygen atom, and R¹¹ represents a group NHR^{X6} where R^{X6} is a hydrogen atom or an optionally substituted alkyl, cycloalkyl, aryl or aralkyl group.

[00269] In some embodiments of any one of the aspects described herein, the compound of Formula I-C is 10 α -(Sulfamino)dihydroartemisinin or Bis[(10 α -dihydroartemisinyl)]sulfamide.

[00270] Compounds of Formula I-C and their synthesis are described, for example, in US Pat. Pub. No. 2005/0119232, content of which is incorporated herein by reference in its entirety.

[00271] In some embodiments of any one of the aspects described herein, the artemisinin derivative is of Formula (I-D):



wherein:

R^4 is halogen (e.g., F, Cl or Br); and

R^1 is H, $-R^{12}$, $-OR^{12}$, $-NHR^{12}$, $-NR^{12}R^{12}$, or $-SR^{12}$, where each R^{12} is independently H, optionally substituted cycloalkyl, optionally substituted aryl, or C-linked heteroaryl or heterocyclylalkyl group.

[00272] In some compounds of Formula I-D, R^1 is $-OCH_3$, $-OCH_2CH_3$, $-OCH_2CH_2OH$, $-NHPh$, $-CH_2N(CH_3)_2$, $-SPh$ or $-CH_2Ph$.

[00273] In some compounds of Formula I-D, R^1 is $-OCH_3$, $-OCH_2CH_3$, $-OCH_2CH_2OH$ and R^4 is Br. For example, R^1 is $-OCH_3$.

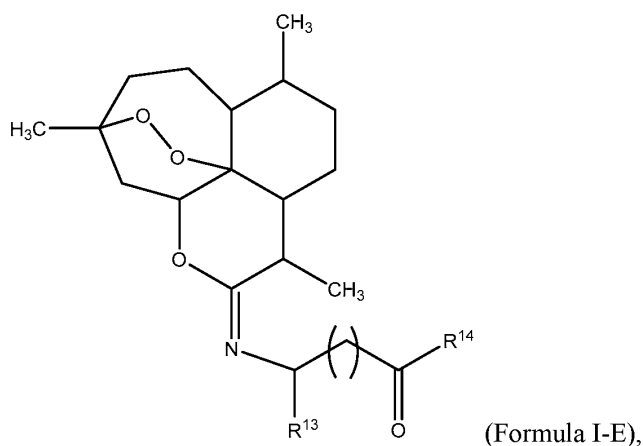
[00274] In some compounds of Formula I-D, R^1 is $-OCH_3$, $-OCH_2CH_3$, $-OCH_2CH_2OH$ and R^4 is Cl. For example, R^1 is $-OCH_2CH_3$.

[00275] In some compounds of Formula I-D, R^1 is $-OCH_3$, $-OCH_2CH_3$, $-OCH_2CH_2OH$ and R^4 is I. For example, R^1 is $-OCH_3$.

[00276] In some compounds of Formula I-D, R^1 is $-OCH_3$, $-OCH_2CH_3$, $-OCH_2CH_2OH$ and R^4 is F. For example, R^1 is $-OCH_3$.

[00277] Compounds of Formula I-D and their synthesis are described, for example, in Chinese Pat. App. No. 10135977 (Pub. No. CN 201310069722), content of which is incorporated herein by reference in its entirety.

[00278] In some embodiments of any one of the aspects described herein, the artemisinin derivative is of Formula (I-E):



wherein:

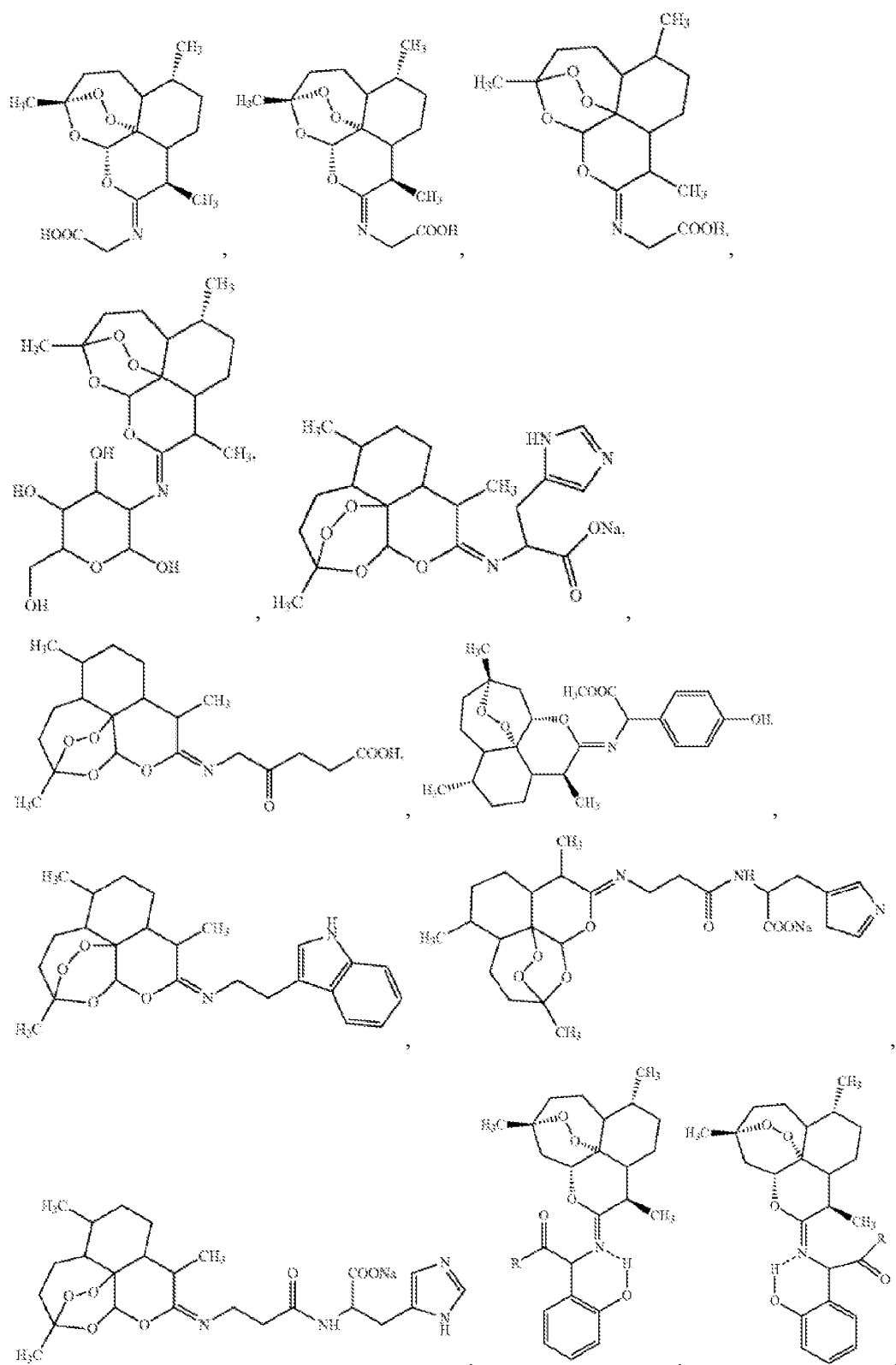
a is 0 to 10;

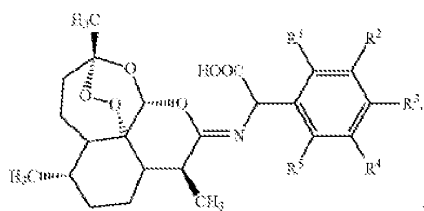
R^{13} is H, optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, hydroxyalkyl, polyhydroxyalkyl, hydroxyaryl, polyhydroxyaryl, heterocyclic-alkyl, mercapto-alkyl, dithio-alkyl, carboxyalkyl, amidoalkyl, or guanidinoalkyl;

R^{14} is OR^{A3} , $NR^{A3}R^{A4}$, or peptide; and

R^{A3} and R^{A4} are independently H, optionally substituted alkyl, or optionally substituted aryl;

[00279] In some embodiments of any one of the aspects described herein, the compound of Formula I-E is selected from the group consisting of:

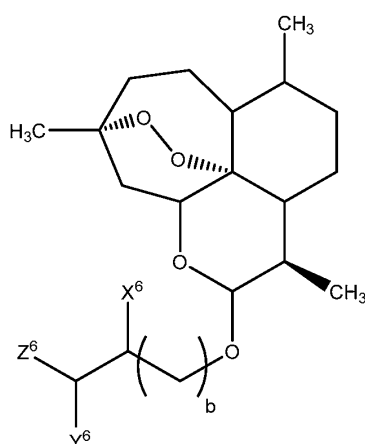




and where R is OR^{AA} or $\text{NR}^{\text{AA}}\text{R}^{\text{AB}}$, where R^{AA} and R^{AB} are independently H, alkyl or aryl; and R^1 , R^2 , R^3 , R^4 , and R^5 are independently H or OH.

[00280] Compounds of Formula I-E and their synthesis are described, for example, in U.S. Pat. App. No. 8,232,417, content of which is incorporated herein by reference in its entirety.

[00281] In some embodiments of any one of the aspects described herein, the artemisinin derivative is of Formula (I-F):



(Formula I-F),

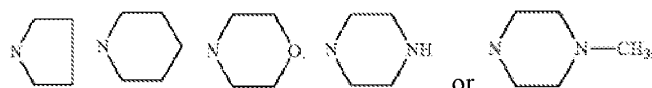
wherein:

b is 0 or 1;

X^6 is H or OH;

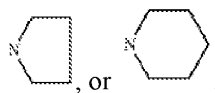
Y^6 is H, CH_3 or phenyl; and

Z^6 is NH_2 , $\text{NHR}^{6\text{N}}$, $\text{N}(\text{R}^{6\text{N}})_2$, $\text{NHCH}_2\text{CH}_2\text{OH}$, $\text{NH}(\text{CH}_2)_2\text{NMe}_2$,

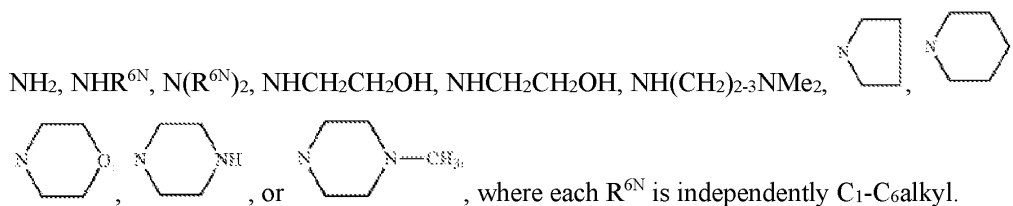
, where each $\text{R}^{6\text{N}}$ is independently C_1 - C_6 alkyl.

[00282] In some compounds of Formula I-F, b is 0; X^6 and Y^6 are H; and Z^6 is NH_2 .

[00283] In some compounds of Formula I-F, b is 1; X^6 is OH; Y^6 is H; and Z^6 is NH_2 , NHMe ,

NHEt , $\text{NHPr}(\text{n})$, NHBu , $\text{NHCH}_2\text{CH}_2\text{OH}$, .

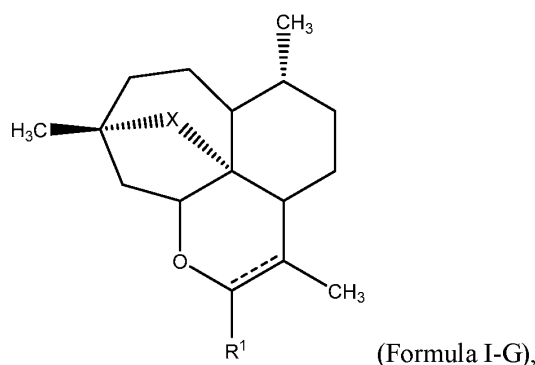
[00284] In some compounds of Formula I-F, b is 1; X⁶ is OH; Y⁶ is CH₃ or phenyl; and Z⁶ is



[00285] In some embodiments of any one of the aspects described herein, the compound of Formula I-F is selected from the group consisting of 2'-amino arteether, 3'-amino-2'-hydroxy-artemisinin propyl ether, 3'-methylamino-2'-hydroxy-artemisinin propyl ether, 3'-methylamino-2'-hydroxy-artemisinin butyl ether, 3'-amino-2'-hydroxy-artemisinin butyl ether, 3'-hydroxyethylamino-2'-hydroxy-artemisinin propyl ether, 3'-phenyl-3'-methylamino-2'-hydroxy-artemisinin propyl ether, 3'-phenyl-3'-amino-2'-hydroxy-artemisinin propyl ether, 3'-hydroxyethylamino-2'-hydroxy-artemisinin butyl ether, 3'-dimethylamino-2'-hydroxy-artemisinin butyl ether, 3'-pyrrolidinyl-2'-hydroxy-artemisinin propyl ether, 3'-piperidyl-2'-hydroxy-artemisinin propyl ether, 3'-methylamino-2'-hydroxy-artemisinin butyl ether, 3'-tert-butylamino-2'-hydroxy-artemisinin propyl ether, 3'-phenyl-3'-pyrrolidinyl-2'-hydroxy-artemisinin propyl ether, 3'-phenyl-3'-piperidyl-2'-hydroxy-artemisinin propyl ether, 3'-phenyl-3'-dimethylamino-2'-hydroxy-artemisinin propyl ether, and 3-phenyl-3'-tert-butylamino-2'-hydroxy-artemisinin propyl ether.

[00286] Compounds of Formula I-F and their synthesis are described, for example, in US Pat. No. 8,129,426, content of which is incorporated herein by reference in its entirety.

[00287] In some embodiments of any one of the aspects described herein, the artemisinin derivative is of Formula (I-G):



wherein:

X is -O- or -O-O-;

R¹ is H, substituted or unsubstituted alkyl, substituted or unsubstituted aryl,

substituted or unsubstituted arylalkyl, substituted or unsubstituted heteroaryl,

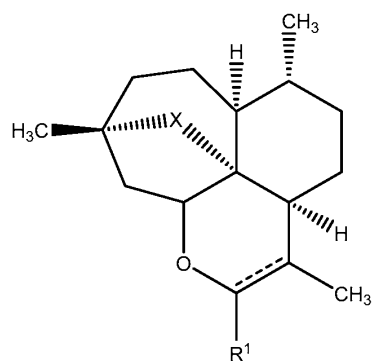
substituted or unsubstituted heteroarylalkyl, $-(CH_2)_jR^{2G}$, $-(CH_2)_jO(CH_2)_kR^{2G}$, $-(CH_2)_jC(O)(CH_2)_kR^{2G}$, $-(CH_2)_jC(O)(CH_2)_kR^{2G}$, $-(CH_2)_jC(O)O(CH_2)_kR^{2G}$, $-(CH_2)_jNR^{3G}R^{4G}$, $-(CH_2)_jC(O)(CH_2)_kNR^{3G}R^{4G}$, $-(CH_2)_jNR^{5G}C(O)(CH_2)_kR^{2G}$, $-(CH_2)_jC(O)(CH_2)_kNR^{3G}R^{4G}$, $-(CH_2)_jNR^{5G}C(O)(CH_2)_kOR^{2G}$, $-(CH_2)_jNR^{5G}C(O)(CH_2)_kNR^{3G}R^{4G}$, wherein each j and each k is independently an integer from 0 to 6; and m is independently an integer from 0 to 2;

R^{2G} is hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, perfluoroalkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted arylalkyl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heteroarylalkyl, substituted or unsubstituted (aryl or heteroaryl)- X^G -(aryl or heteroaryl) wherein X^G is O, S, NH or $N(C_1-C_6)$ alkyl, cholesterol, or a substituted or unsubstituted monosaccharide, and wherein each R^{2G} is optionally independently substituted with 1 to 5 R^{10G} groups;

R^{3G} , R^{4G} , and R^{5G} are independently hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, perfluoroalkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted arylalkyl, substituted or unsubstituted heteroaryl, or substituted or unsubstituted heteroarylalkyl, wherein R^{3G} , R^{4G} , and R^{5G} are each optionally independently substituted with 1 to 5 R^{10G} groups; R^{3G} and R^{4G} , together with the N atom to which they are attached, form substituted or unsubstituted heterocycloalkyl, or substituted or unsubstituted heteroaryl, wherein substituted or unsubstituted heterocycloalkyl, and substituted or unsubstituted heteroaryl are each optionally independently substituted with 1 to 5 R^{10G} groups; and

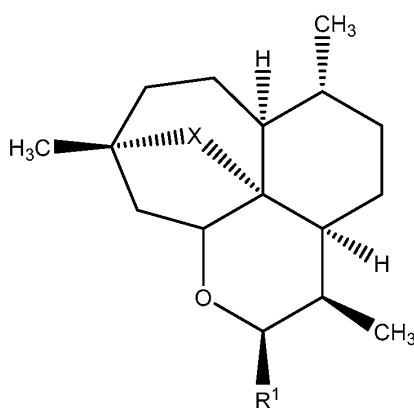
each R^{10G} is independently hydrogen, halogen, hydroxyl, amino, aminoalkyl, aminodialkyl, cyano, nitro, alkyl, $-O$ -alkyl, $-S$ -alkyl, perfluoroalkyl, $-O$ -perfluoroalkyl, oxo, acetyl, or -benzyl.

[00288] In some embodiments of any one of the aspects described herein, the compound of Formula I-G is of Formula I-GA:



(Formula I-GA).

[00289] In some embodiments of any one of the aspects described herein, the compound of Formula I-G is of Formula I-GB:



(Formula I-GB).

[00290] In some compounds of Formula I-G, I-GA or I-GB, R^1 is H, substituted or unsubstituted phenyl, substituted or unsubstituted naphthyl, substituted or unsubstituted biphenyl, substituted or unsubstituted benzyl, substituted or unsubstituted pyrrolyl, substituted or unsubstituted furyl, substituted or unsubstituted thienyl, substituted or unsubstituted pyrazolyl, substituted or unsubstituted imidazolyl, substituted or unsubstituted oxazolyl, substituted or unsubstituted isoxazolyl, substituted or unsubstituted 1,2,4-oxadiazolyl, substituted or unsubstituted 1,3,4-oxadiazolyl, substituted or unsubstituted tetrazolyl, substituted or unsubstituted thiazolyl, substituted or unsubstituted isothiazolyl, substituted or unsubstituted triazolyl, substituted or unsubstituted pyridinyl, substituted or unsubstituted pyridazinyl, substituted or unsubstituted pyrimidinyl, substituted or unsubstituted pyrazinyl, substituted or unsubstituted indolyl, substituted or unsubstituted indazolyl, substituted or unsubstituted benzofuryl, substituted or unsubstituted benzothieryl, substituted or unsubstituted benzopyrazolyl, substituted or unsubstituted benzimidazolyl, substituted or unsubstituted benzoxazolyl, substituted or unsubstituted benzisoxazolyl, substituted or unsubstituted benzothiazolyl, substituted or unsubstituted benzisothiazolyl, substituted or unsubstituted benzotriazolyl, substituted or unsubstituted quinolinyl, substituted or unsubstituted isoquinolinyl,

substituted or unsubstituted phthalazinyl, substituted or unsubstituted quinazolinyl, substituted or unsubstituted quinoxalinyl, $-(CH_2)_jC(O)(CH_2)_kR^{2G}$, $-(CH_2)_jOC(O)(CH_2)_kR^{2G}$, $-(CH_2)_jC(O)O(CH_2)_kR^{2G}$, or $-(CH_2)_jC(O)(CH_2)_kNR^{3G}R^{4G}$.

[00291] In some compounds of Formula I-G, I-GA or I-GB, R^{2G} is hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted phenyl, substituted or unsubstituted naphthyl, substituted or unsubstituted biphenyl, substituted or unsubstituted benzyl, substituted or unsubstituted pyrrolyl, substituted or unsubstituted furyl, substituted or unsubstituted thienyl, substituted or unsubstituted pyrazolyl, substituted or unsubstituted imidazolyl, substituted or unsubstituted oxazolyl, substituted or unsubstituted isoxazolyl, substituted or unsubstituted 1,2,4-oxadiazolyl, substituted or unsubstituted 1,3,4-oxadiazolyl, substituted or unsubstituted tetrazolyl, substituted or unsubstituted thiazolyl, substituted or unsubstituted isothiazolyl, substituted or unsubstituted triazolyl, substituted or unsubstituted pyridinyl, substituted or unsubstituted pyridazinyl, substituted or unsubstituted pyrimidinyl, substituted or unsubstituted pyrazinyl, substituted or unsubstituted indolyl, substituted or unsubstituted indazolyl, substituted or unsubstituted benzofuryl, substituted or unsubstituted benzothienyl, substituted or unsubstituted benzopyrazolyl, substituted or unsubstituted benzimidazolyl, substituted or unsubstituted benzoxazolyl, substituted or unsubstituted benzisoxazolyl, substituted or unsubstituted benzothiazolyl, substituted or unsubstituted benzisothiazolyl, substituted or unsubstituted benzotriazolyl, substituted or unsubstituted quinolinyl, substituted or unsubstituted isoquinolinyl, substituted or unsubstituted phthalazinyl, substituted or unsubstituted quinazolinyl, or substituted or unsubstituted quinoxalinyl.

[00292] In some compounds of Formula I-G, I-GA or I-GB, R^{3G} and R^{4G} are independently hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted phenyl, substituted or unsubstituted benzyl.

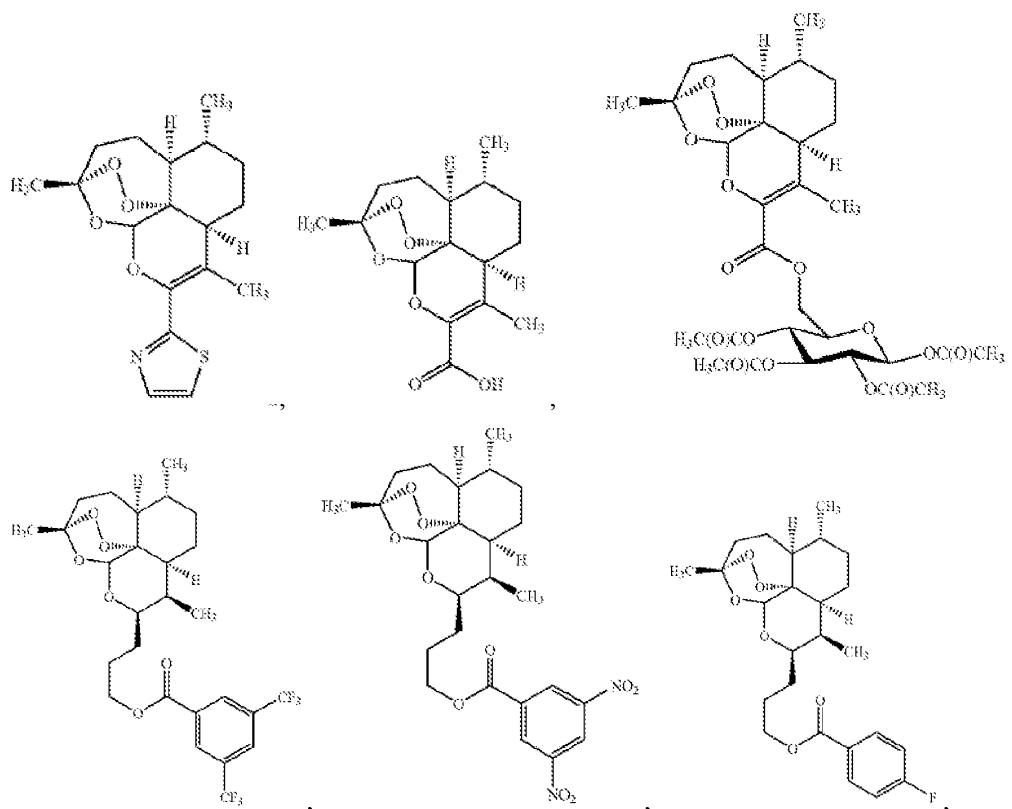
[00293] In some compounds of Formula I-G, I-GA or I-GB, R^{3G} and R^{4G} , together with the N atom to which they are attached, form substituted or unsubstituted pyrrolidinyl, substituted or unsubstituted piperidinyl, substituted or unsubstituted piperazinyl, substituted or unsubstituted morpholinyl, substituted or unsubstituted thiomorpholinyl, substituted or unsubstituted pyrrolyl, or substituted or unsubstituted imidazolyl.

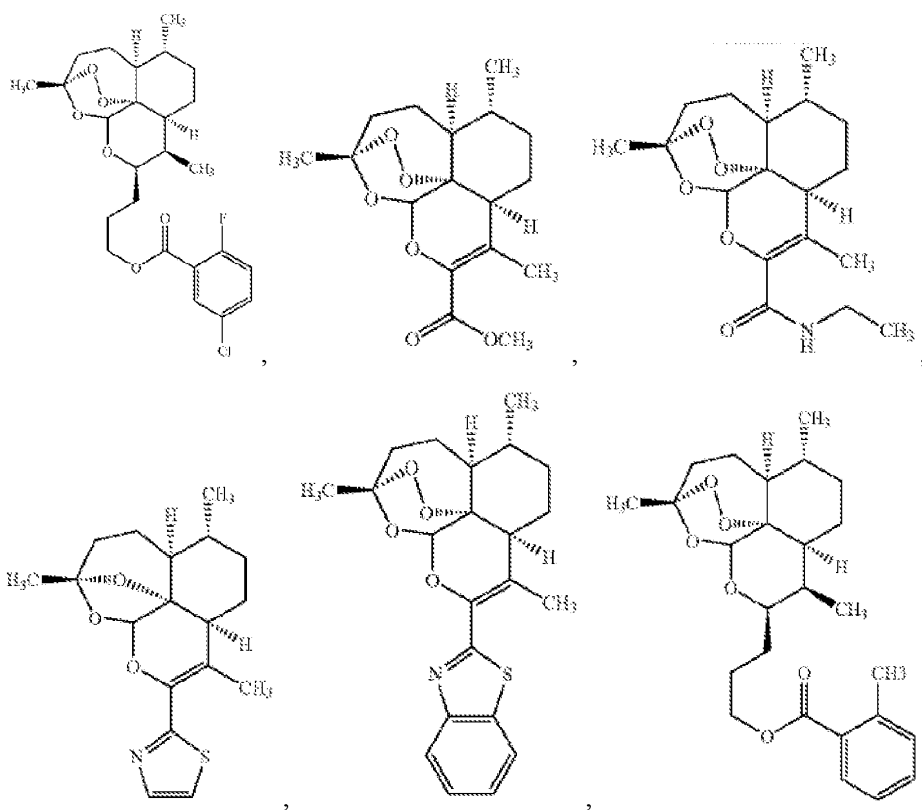
[00294] In some compounds of Formula I-G, I-GA or I-GB, R^1 is $-C(O)R^{2G}$, $-OC(O)R^{2G}$, $-C(O)OR^{2G}$, or $-C(O)NR^{3G}R^{4G}$; R^{2G} is hydrogen, substituted or unsubstituted alkyl, or substituted or unsubstituted phenyl; and R^{3G} and R^{4G} independently are hydrogen, or substituted or unsubstituted alkyl.

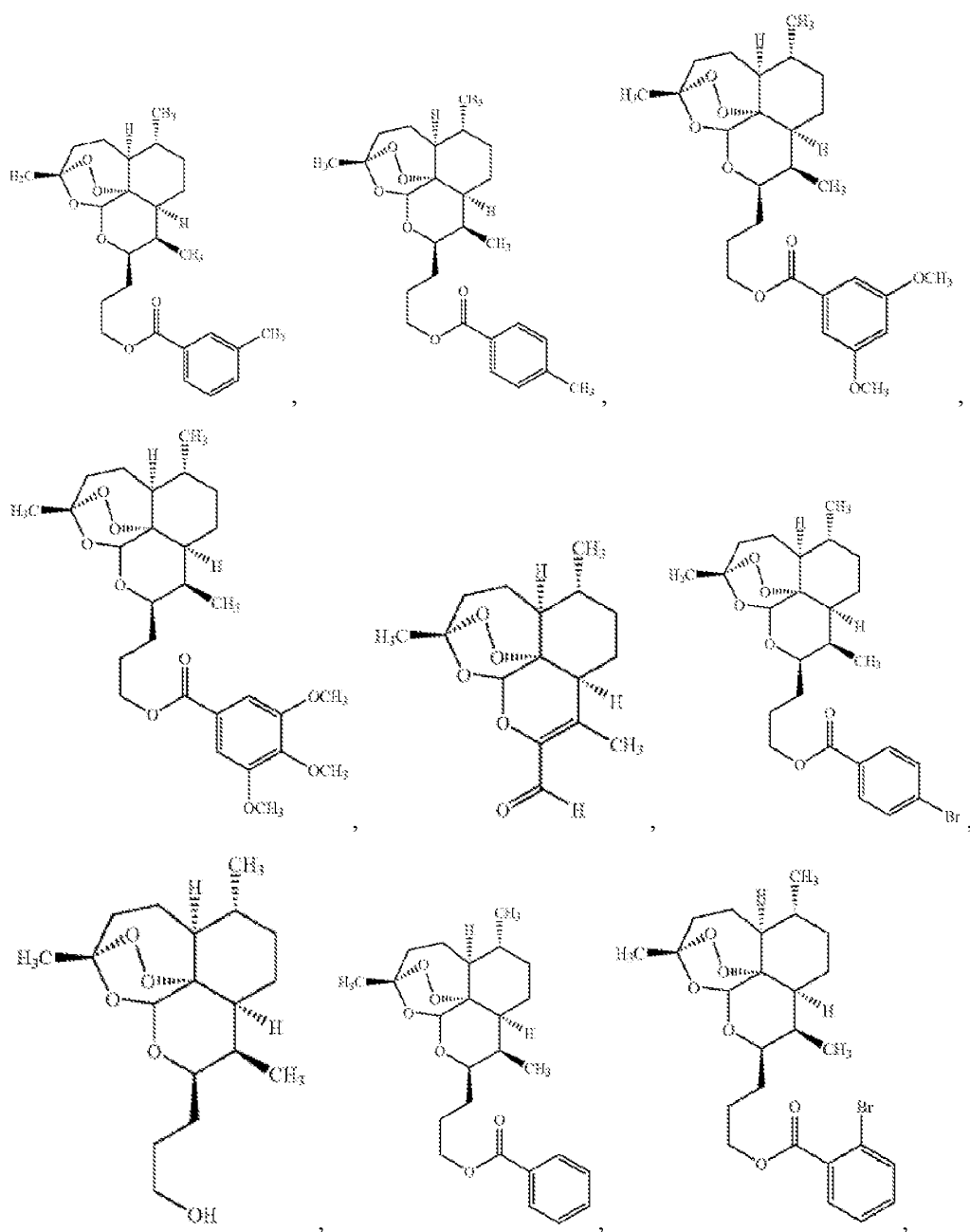
[00295] In some compounds of Formula I-G, I-GA or I-GB, R^1 is $-(CH_2)_jC(O)O(CH_2)_kR^{2G}$, and R^{2G} is substituted or unsubstituted glucose, substituted or unsubstituted galactose, substituted or unsubstituted mannose, substituted or unsubstituted fructose.

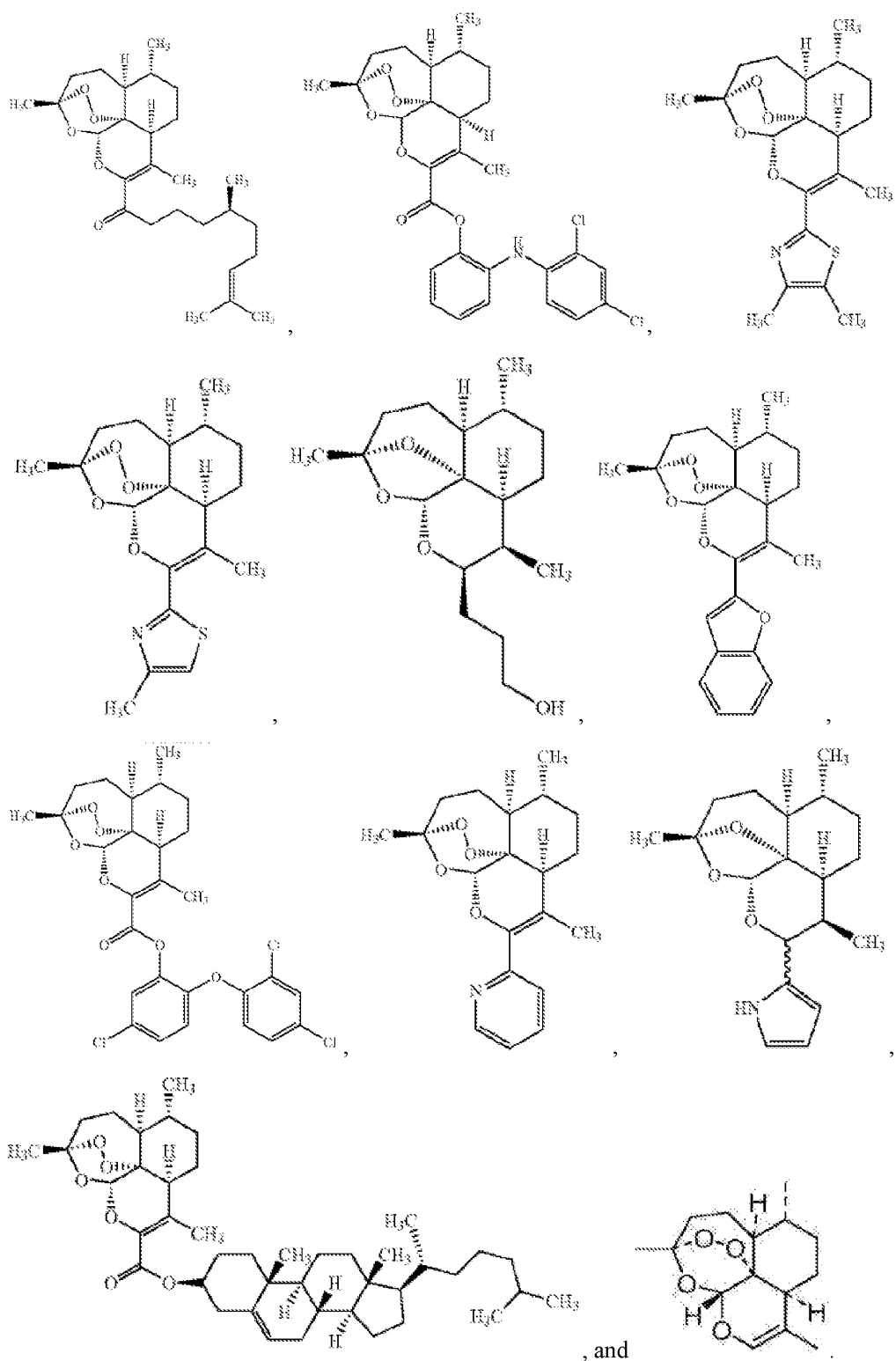
[00296] In some compounds of Formula I-G, I-GA or I-GB, R^1 is $-C(O)OR^2$; and R^{2G} is tetraacetyl α -D glucopyranose, tetraacetyl β -D glucopyranose, tetraacetyl α -D galactose, tetraacetyl β -D galactose, tetraacetyl α -D mannose, tetraacetyl β -D mannose, tetraacetyl α -D fructose, or tetraacetyl β -D fructose.

[00297] In some embodiments of any one of the aspects described herein, the compound of Formula I-G is selected from the group consisting of:



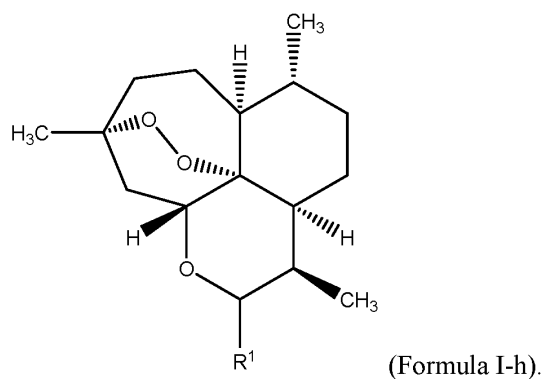






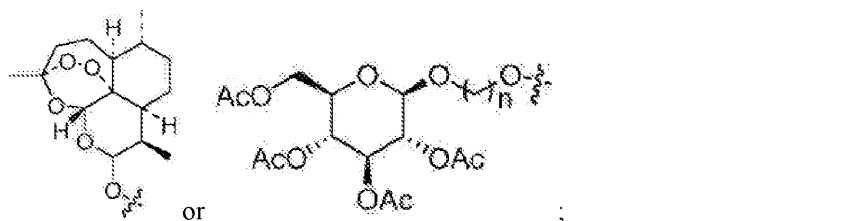
[00298] Compounds of Formula I-XXXX and their synthesis are described, for example, in U.S. Pat. No. 8,431,611, content of which is incorporated herein by reference in its entirety.

[00299] In some embodiments of any one of the aspects described herein, the artemisinin derivative is of Formula (I-H):



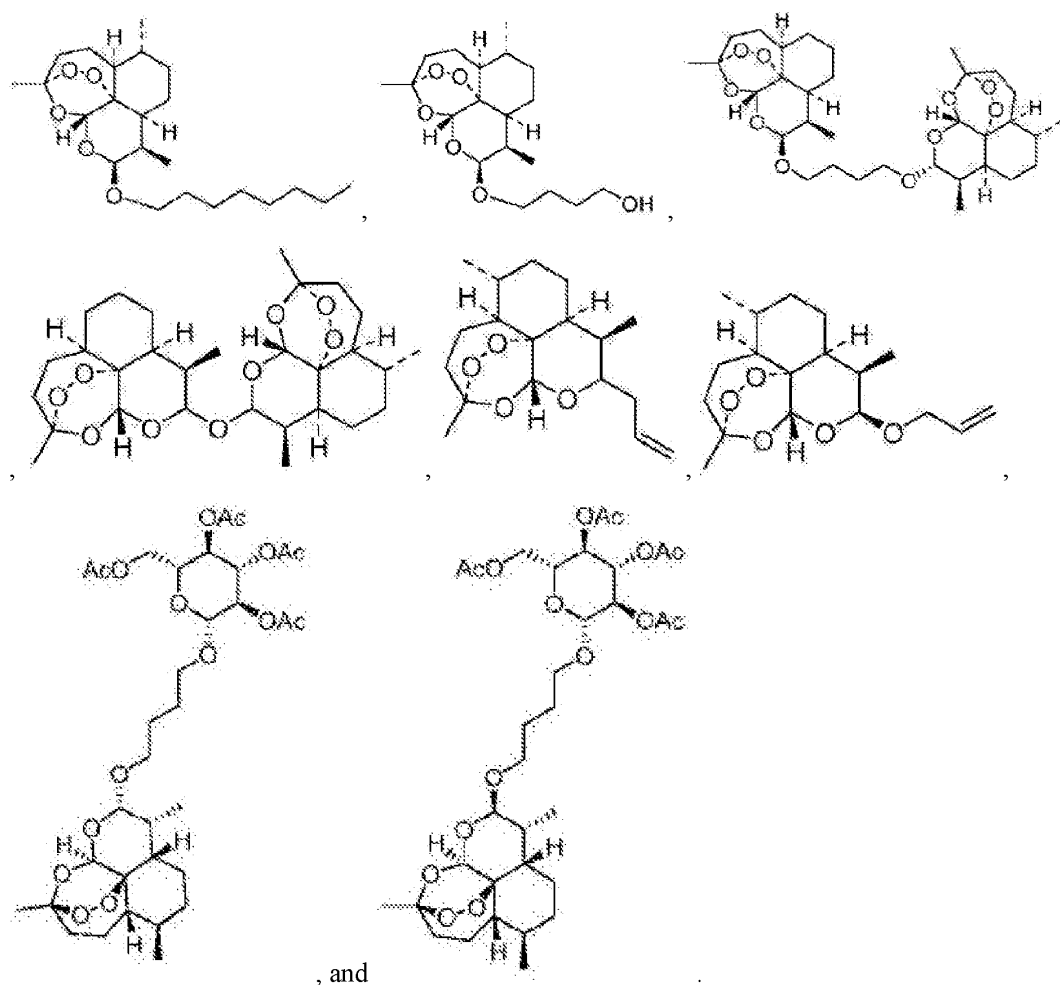
wherein:

R^1 is $-OH$, $-OMe$, $-C_nH_{2n}$, $-OC_nH_{2n+1}$, $-OC_nH_{2n}$, $-OC_nH_{2n}OH$,



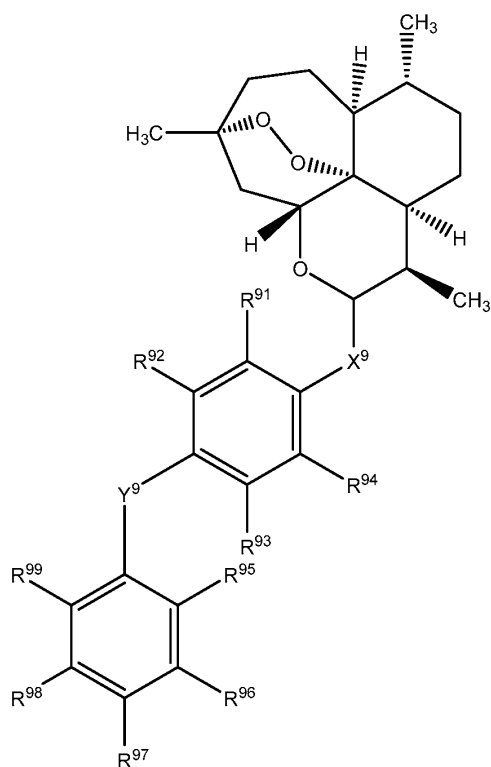
n is an integer from 1 to 10.

[00300] In some embodiments of any one of the aspects described herein, the compound of Formula I-H is selected from the group consisting of:



[00301] Compounds of Formula I-H and their synthesis are described, for example, in Chinese Pat. App. No. 107383048 (Pub. No. CN 201710687057), content of which is incorporated herein by reference in its entirety.

[00302] In some embodiments of any one of the aspects described herein, the artemisinin derivative is of Formula (I-I):

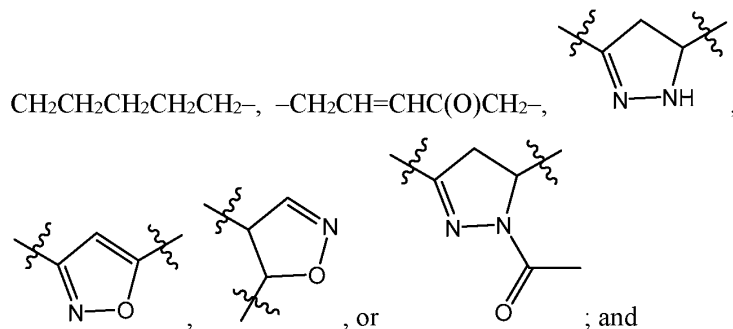


(Formula I-I).

wherein:

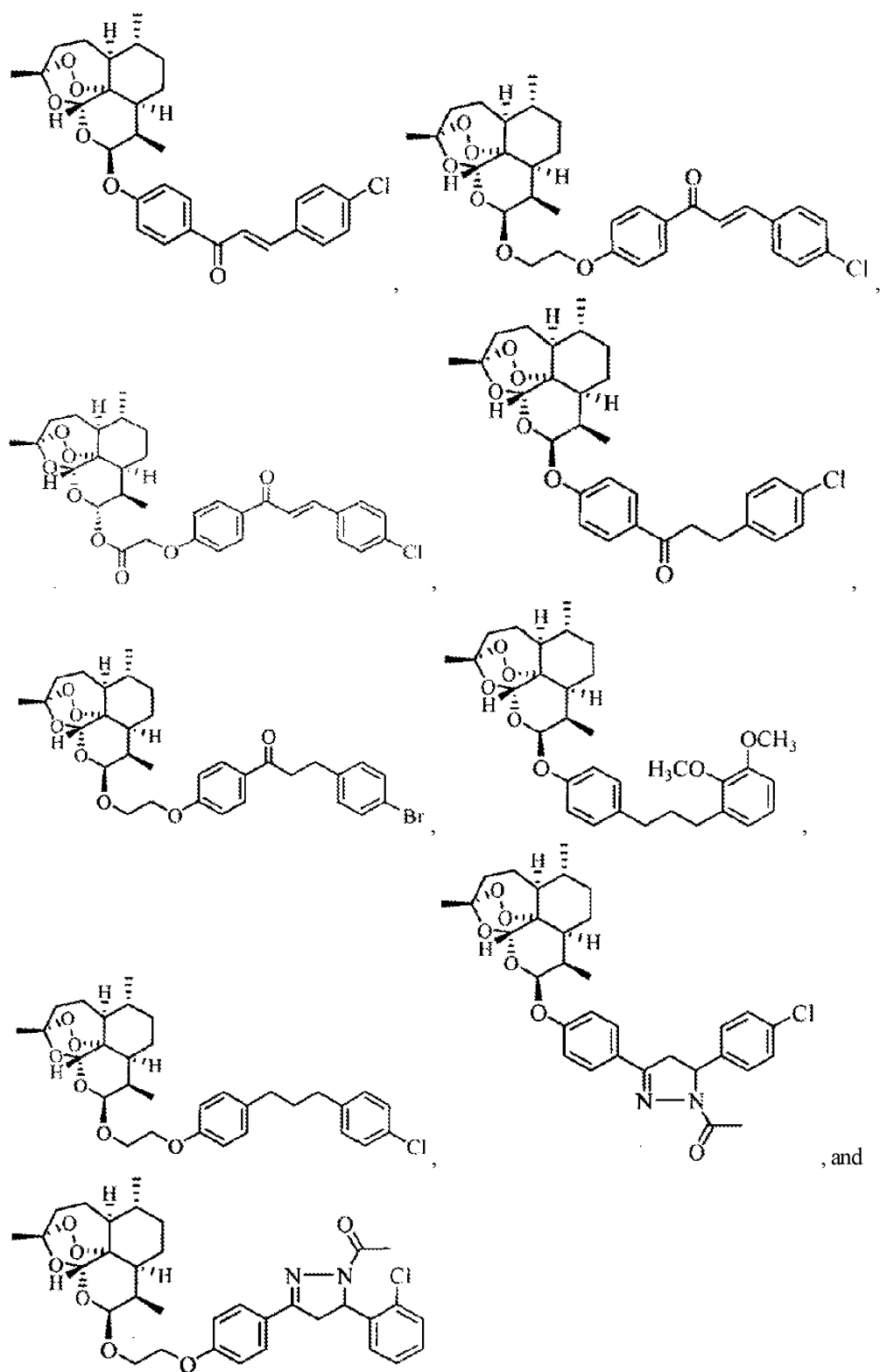
X^9 is $-O-$, $-O(CH_2)_nO-$, $-O(CH_2)_n-$, $-OCO(CH_2)_nO-$ or $-OCO(CH_2)_n-$, where n is an integer from 1 to 5;

Y^9 is $-CH_2C(O)CH=CHCH_2-$, $-CH_2C(O)CH_2CH_2CH_2-$, $-CH_2CH_2CH=CHCH_2-$, $-$



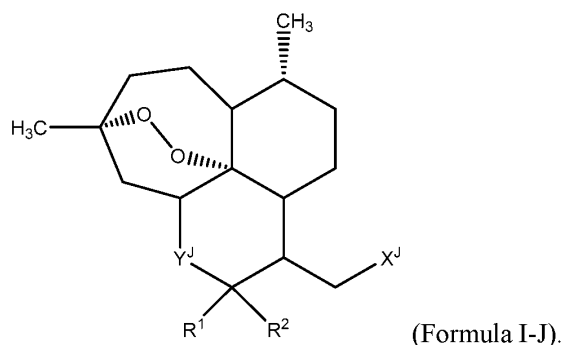
R^{91} - R^{99} are independently hydrogen, halogen, nitro, cyano group, trifluoromethyl, trifluoromethoxy, C_1 - C_4 alkyl, C_1 - C_4 alkoxymethyl, di(C_1 - C_4 alkyl)amino, or C_3 - C_7 cycloalkyl.

[00303] In some embodiments of any one of the aspects described herein, the compound of Formula I-I is selected from the group consisting of:



[00304] Compounds of Formula I-I and their synthesis are described, for example, in Chinese Pat. App. No. 101580510 (Pub. No. CN 200910011753), content of which is incorporated herein by reference in its entirety.

[00305] In some embodiments of any one of the aspects described herein, the artemisinin derivative is of Formula (I-J):



wherein:

Y^J is O or NR^N ;

X^J is H, $-NR^{1J}R^{2J}$, $-CHR^{8J}R^{9J}$ or Ar;

R^1 is H, halogen, OH, an optionally substituted cycloalkyl, aryl, C-linked heteroaryl or heterocyclalkyl, $-NR^{3J}R^{4J}$, $-O-CO-R^{5J}$ or $-OR^{6J}$;

R^2 is H, or R^1 and R^2 together form a carbonyl ($=O$);

R^{1J} and R^{2J} are independently an optionally substituted alkyl, cycloalkyl, aryl, or aralkyl; or R^{1J} and R^{2J} together with the interjacent nitrogen atom represent an optionally substituted heterocyclic group or an amino group derived from an optionally substituted amino acid ester;

R^{3J} is H or an optionally substituted alkyl, alkenyl or alkynyl group;

R^{4J} is an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, aryl or aralkyl; or R^{3J} and R^{4J} together with the interjacent nitrogen atom represent an optionally substituted heterocyclic group or an amino group derived from an optionally substituted amino acid ester;

R^{5J} is an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, aryl, aralkyl, heterocyclic or polycyclic group;

R^{6J} is an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, aryl, aralkyl, heterocyclic or polycyclic group;

R^N is H or an optionally substituted alkyl, alkenyl, alkynyl, aralkyl or heterocyclalkyl group;

R^{8J} is H or an optionally substituted alkyl, alkenyl, alkynyl, aryl or alkoxy carbonyl group;

R^{9J} is a nitro group of an optionally substituted alkyl, alkenyl, alkynyl, aryl, alkanoyl, aroyl, alkoxy carbonyl or aryloxy carbonyl group; or R^{8J} and R^{9J} together with the interjacent carbon atom represent an optionally substituted cycloalkyl or polycyclic group; and

Ar represents an optionally substituted aryl or heteroaryl group.

[00306] In some compounds of Formula I-J, Y^J is O. In some other compounds of Formula I-J, Y^J is NR^N .

[00307] In some compounds of Formula I-J, when X^J is $—NR^1R^2$, then R^1 and R^2 together form a carbonyl ($=O$) group, and Y^J is O.

[00308] In some compounds of Formula I-J, when X^J is NR^N , then X^J is a hydrogen atom and R^1 and R^2 together form a carbonyl ($=O$) group.

[00309] In some compounds of Formula I-J, when X^J is H and Y^J is O, then R^2 is and R^1 is not methoxy, ethoxy or 3-carboxypropanoyloxy group.

[00310] In some compounds of Formula I-J, X^J represents a hydrogen atom or a group $—NR^{1J}R^{2J}$ or $CHR^{8J}R^{9J}$. For example, X^J is H.

[00311] In some compounds of Formula I-J, R^1 is halogen. For example, R^1 is F or Br. bromine.

[00312] In some compounds of Formula I-J, R^1 is a C_{3-8} cycloalkyl group, a C_{6-18} aryl group, a 5- to 10-membered C-linked heteroaryl group or a 5- to 10-membered heterocyclyl- C_{1-6} alkyl group, each group being optionally substituted by one or more substituents selected from the group consisting of halogen atoms, hydroxyl, C_{1-4} alkyl, C_{2-4} alkenyl, C_{1-4} haloalkyl, C_{1-4} alkoxy, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, carboxyl, C_{6-10} aryl, 5 to 10-membered heterocyclic and C_{1-4} alkyl- or phenyl-substituted 5- to 10-membered heterocyclic groups. For example, R^1 is a C_{6-18} aryl group optionally substituted by one or more substituents selected from the group consisting of halogen atoms, hydroxyl, C_{1-4} alkyl, C_{2-4} alkenyl, C_{1-4} haloalkyl, C_{1-4} alkoxy, C_{1-4} haloalkoxy, amino, C_{1-4} alkylamino, di (C_{1-4} alkyl)amino and carboxyl groups. In some compounds of Formula I-J, R^1 is a phenyl, naphthyl, anthryl or phenanthryl group, each group being optionally substituted by one or more substituents selected from the group consisting of halogen atoms and hydroxyl, methyl, vinyl, C_{1-4} alkoxy and carboxyl groups. For example, R^1 can be a phenyl, fluorophenyl, chlorophenyl, bromophenyl, trimethylphenyl, vinylphenyl, methoxyphenyl, dimethoxyphenyl, trimethoxyphenyl, carboxylphenyl, naphthyl, hydroxynaphthyl, methoxynaphthyl, anthryl or phenanthryl group. In some compounds of Formula I-J, R^1 is a dimethoxyphenyl or trimethoxyphenyl.

[00313] In some compounds of Formula I-J, R^1 is $—NR^{3J}R^{4J}$, where R^{3J} is H or a C_{1-6} alkyl group, and R^{4J} is a C_{1-6} alkyl, C_{3-8} cycloalkyl, C_{6-10} aryl or C_{7-16} aralkyl group, or R^{3J} and R^{4J} together with the interjacent nitrogen atom represent a 5- to 10-membered heterocyclic group or an amino group derived from a C_{1-6} alkyl ester of an amino acid, each group being optionally substituted by one or more substituents selected from the group consisting of halogen atoms, C_{1-4} alkyl, C_{1-4} haloalkyl, C_{1-6} alkoxy carbonyl, phenyl, halophenyl, C_{1-4} alkylphenyl, C_{1-4} haloalkylphenyl, C_{1-4} alkoxyphenyl, benzyl, pyridyl and pyrimidinyl groups. For example, R^1 can be $—NR^{3J}R^{4J}$, where R^{3J} is H or a C_{1-4} alkyl group and R^{4J} is a C_{1-4} alkyl, C_{3-6} cycloalkyl, phenyl or benzyl group, or R^{3J} and R^{4J} together with the interjacent nitrogen atom represent a 6- to 10-membered heterocyclic group or an amino group derived from a C_{1-4} alkyl ester of an amino acid, each group being optionally substituted by one or more substituents selected from the group consisting of halogen atoms, C_{1-4} haloalkyl, C_{1-4} alkoxy carbonyl, phenyl, halophenyl, C_{1-4} alkylphenyl, C_{1-4} haloalkylphenyl, C_{1-4} alkoxyphenyl, benzyl, pyridyl and pyrimidinyl groups.

[00314] In some compounds of Formula I-J, R^1 is a propylamino, cyclopentylamino, cyclohexylamino, phenylamino, fluorophenylamino, chlorophenylamino, bromophenylamino, iodophenylamino, methoxycarbonylphenylamino, biphenylamino, benzylamino, fluorobenzylamino, bis(trifluoromethyl)-benzylamino, phenylethylamino, phenylmethoxycarbonyl-methylamino, diethylamino, morpholinyl, thiomorpholinyl, S,S-dioxothiomorpholinyl (morpholinosulphonyl), indolinyl, tetrahydroisoquinolinyl, phenylpiperazinyl, fluorophenylpiperazinyl, chlorophenylpiperazinyl, methylphenylpiperazinyl, trifluoromethylphenylpiperazinyl, methoxyphenylpiperazinyl, benzylpiperazinyl, pyridylpiperazinyl and pyrimidinylpiperazinyl group. For example, R^1 is a phenylamino or fluorophenylamino group.

[00315] In some compounds of Formula I-J, R^1 is $—O—CO—R^{5J}$, where R^{5J} is an optionally substituted alkyl, aryl, aralkyl, heterocyclic or polycyclic group. For example, R^{5J} is a C_{1-6} alkyl, C_{6-18} aryl, 5- to 18- membered heterocyclic or C_{4-26} polycyclic group, each group being optionally substituted by one or more substituents selected from the group consisting of halogen atoms, hydroxyl, C_{1-4} alkyl, C_{1-4} haloalkyl, C_{1-4} alkoxy, C_{1-4} haloalkoxy, oxo, carboxyl and carboxylate groups. In some compounds of Formula I-J, R^{5J} represents a C_{1-4} alkyl, C_{6-14} aryl, 5- to 14-membered heterocyclic or C_{6-14} polycyclic group, each group being optionally substituted by one or more substituents selected from the group consisting of halogen atoms, hydroxyl, C_{1-4} alkyl, C_{1-4} haloalkyl and oxo groups.

[00316] In some compounds of Formula I-J, X^J is H, Y^J is O, R^2 is H, and R^1 is phenyl, 2-methoxyphenyl, 4-methoxyphenyl, 2,4-dimethoxyphenyl, 2,4,6-trimethoxyphenyl, 2-methylphenyl, 3-methylphenyl, 4-methylphenyl, 2,4-dimethylphenyl, 2,4,5-trimethylphenyl,

2,4,6-trimethylphenyl, 2-Br-phenyl, 2-Cl-phenyl, 3-Br-phenyl, 3-Cl-phenyl, 4-Br-phenyl, 4-Cl-phenyl, 4-CO₂H-phenyl, 2-naphthyl, morpholino, indolinyl, 1,2,3,4-tetrahydroisoquinolinyl, N-methyl-piperazino, 4-vinylphenyl, 9-anthryl, or 9-phenylanthryl.

[00317] In some compounds of Formula I-J, X^J is H, Y^J is O, R² is H, R¹ is -OC(O)-R^{5J}, and R^{5J} is isosuinolin-1-yl, anthryl, acridin-9-yl, anthraquinon-2-yl, quinolin-3-yl, quinoxalin-2-yl, 2-hydroxynaphth-1-yl, 1-hydroxynaphth-2-yl, or isoquinolin-4-yl, quinolin-2-yl.

[00318] In some compounds of Formula I-J, X^J is H, Y^J is O, R² is H, R¹ is -OR^{6J}, and R^{6J} is 4-methoxyphenyl, 1-naphthyl, 2-naphthyl, benzyl, 2-F-benzyl, 3-F-benzyl, 4-F-benzyl, -CH₂-(1-naphthyl), -CH₂-(10-anthryl), -CH₂-(1-phenanthryl), -CH₂-(1-pyryl), 4-quinolinyl, cholest-5-en-3-yl.

[00319] In some compounds of Formula I-J, X^J is H, R¹ and R² together form a carbonyl (=O), Y^J is NR^N, and R^N is 4-F-benzyl, 3-F-benzyl, benzyl, methyl, -CH₂CH₂(CH₃)₂, -CH₂CH=CH₂, -CH₂C(O)H, pyrid-2-ylmethyl, thenyl, furfuryl, or -CH₂CH₂OH.

[00320] In some compounds of Formula I-J, R¹ and R² together form a carbonyl (=O), Y^J is O and X^J is indolin-1-yl.

[00321] In some compounds of Formula I-J, R¹ and R² together form a carbonyl (=O), Y^J is O, and X^J is cyclohexyl or adamantyl.

[00322] In some compounds of Formula I-J, R¹ and R² together form a carbonyl (=O), Y^J is O, and X^J is -CHR^{8J}R⁹, where R^{8J} is H and R^{9J} is NO₂, -C(O)-phenyl, or R^{8J} is methyl and R^{9J} is -CO₂H, or R^{8J} and R^{9J} are -C(O)OC₂H₅.

[00323] In some compounds of Formula I-J, R¹ and R² are H, Y^J is O, and X^J is -CHR^{8J}R⁹, where R^{8J} is H and R^{9J} is NO₂.

[00324] In some compounds of Formula I-J, X^J is H, Y^J is O, R² is H and R¹ is -NR^{3J}R^{4J}, where R^{3J} is H and R^{4J} is phenyl, 4-F-phenyl, 4-Cl-phenyl, 4-Br-phenyl, 4-I-phenyl, 4-biphenyl, benzyl, 2-F-benzyl, 3,5-trifluoromethylbenzyl, propyl, -CH(CH₃)phenyl, -CH(CO-OCH₃)phenyl, 4-(CO-OCH₃)phenyl, cyclopentyl, or cyclohexyl.

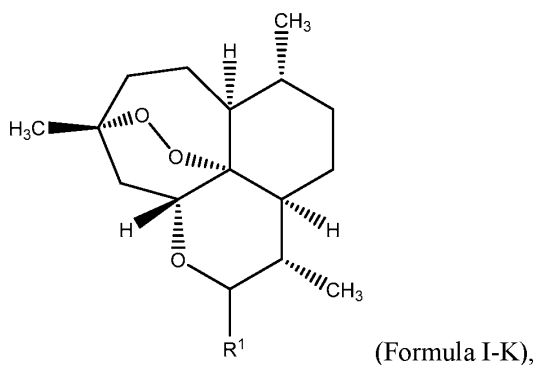
[00325] In some compounds of Formula I-J, X^J is H, Y^J is O, R² is H and R¹ is -NR^{3J}R^{4J}, where R^{3J} and R^{4J} are ethyl.

[00326] In some embodiments of any one of the aspects described herein, the compound of Formula I-J is selected from the group consisting of 10β-fluoro-10-deoxodihydroartemisinin, 10β-(phenyl)dihydroartemisinin, 10α-(4'-fluorobenzylamino)dihydroartemisinin, 10-(2',4'-dimethoxyphenyl)dihydroartemisinin, 10α-(2'-hydroxy-1'-naphthyl)dihydroartemisinin, 10α-(thiomorpholino)dihydroartemisinin, 10α-(4'-S,S-dioxothiomorpholinyl)dihydroartemisinin, 10-dihydroartemisinin ethanoate, 10β-dihydroartemisinin benzoate, 10α-dihydroartemisinin benzoate, 10β-[(2',2',2'-trifluoroethyl)oxy]-dihydroartemisinin, 10β-[4'-(7'-trifluoromethyl)-

quinolinoxy)]dihydroartemisinin, 10 α and β -[(2'-naphthylmethyl)oxy]dihydroartemisinin, 11-azaartemisinin, 11-aza-N-(2-fluorobenzyl)artemisinin, 11-aza-N-(ethoxycarbonyl)ethyl]artemisinin, 9-(N,N-dibenzylaminomethyl)artemisinin, 9-[2,2-di(ethoxycarbonyl)ethyl]-artemisinin, 9-[2-(ethoxycarbonyl)ethyl]artemisinin, 10-desoxo-9-[2-(ethoxycarbonyl)ethyl]-artemisinin, 16-(2'-propyl)artemisinin (9-(2-methylpropyl)artemisinin), 16-(N-1'-indolyl)artemisinin, 10 α -(4'-benzylpiperazin-1'-yl)-10-deoxo-10-dihydroartemisinin, 10 α -(2'-furyl)-10-deoxo-10-dihydroartemisinin, 10 α -(Pyrrol-2'-yl)-10-deoxo-10-dihydroartemisinin, and 10 α -(4'-Benzyl-4'-methylpiperazinium-1'-yl)-10-deoxo-10-dihydroartemisinin iodide salt.

[00327] Compounds of Formula I-J and their synthesis are described, for example, in U.S. Pat. No. 6,649,647, content of which is incorporated herein by reference in its entirety.

[00328] In some embodiments of any one of the aspects described herein, the artemisinin derivative is of Formula (I-K):



wherein:

R^1 is $-B^K-M^K$,

where:

B^K is a linking group; and

M^K is a moiety selected from the group consisting of moieties derived from anti-malarial drugs other than artemisinin and moieties derived from amine compounds, provided that where M^K comprises a quinine the linking group is linked to the quinine via the C₉ hydroxy group of the quinine.

[00329] In some compounds of Formula I-K, the anti-malarial drug is a quinoline derivative. For example, the anti-malarial drug is selected from the group consisting of amodiaquine, n-bisethylamodiaquine, chloroquine, mefloquine, piperazine, primaquine, pamaquine, quinine, isoquine, pyronaridine and tafenoquine. In some compounds of Formula I-K, the anti-malarial drug is not a quinoline derivative. For example, the anti-malarial drug is selected from the group

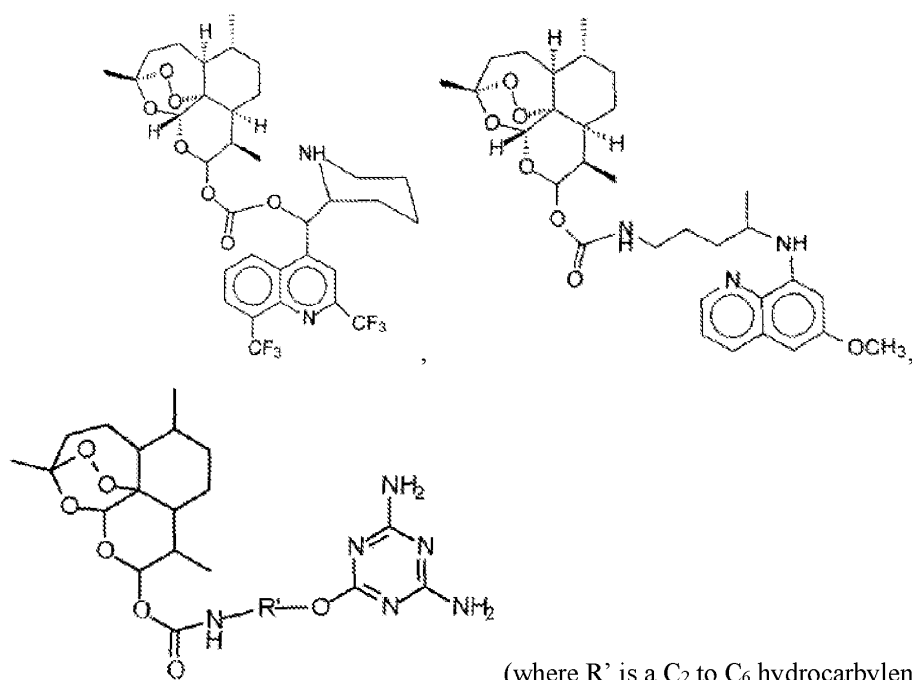
consisting of halofantrine, lumefantrine, proguanil (chlorguanide), cycloguanil and pyrimethamine or their pharmacophores.

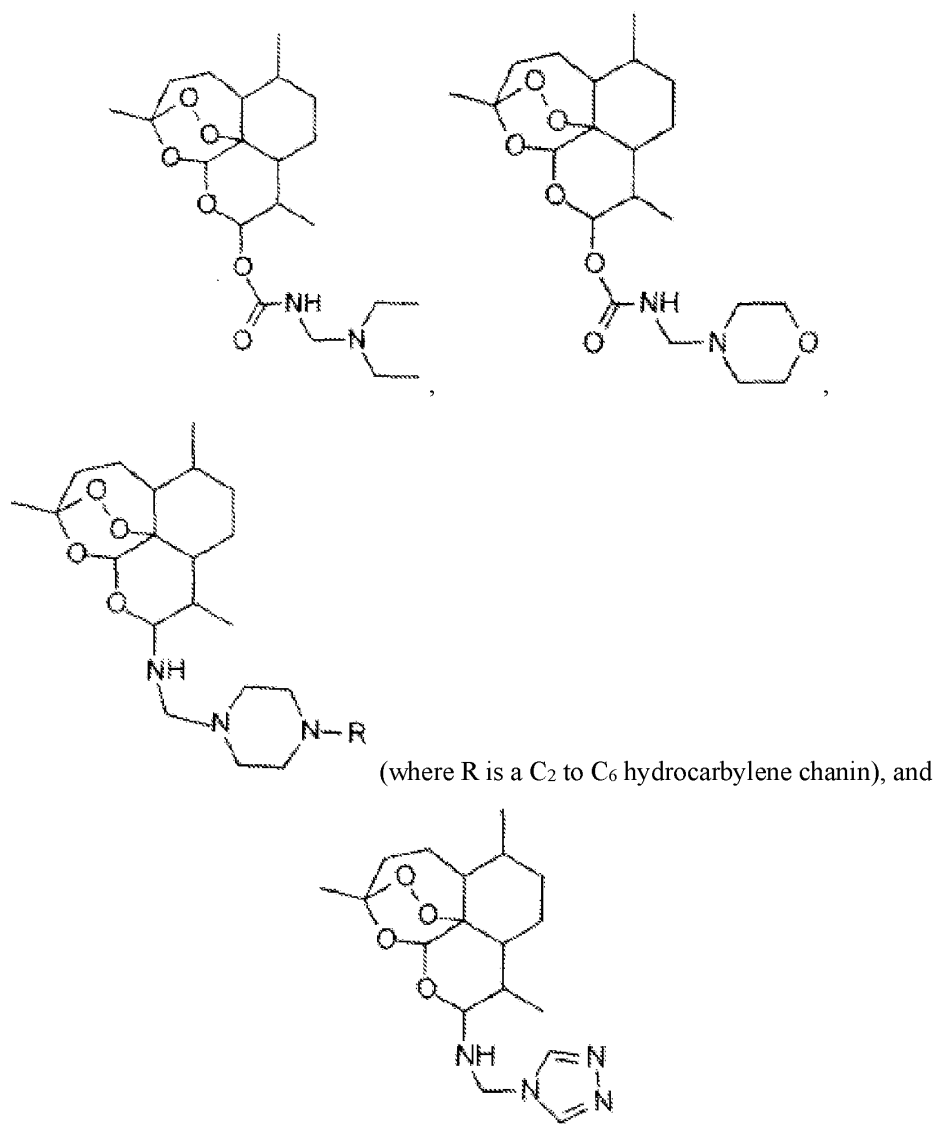
[00330] In some compounds of Formula I-K, M^K is a moiety derived from an amine compound, wherein the the amine compound is selected from the group consisting of 1-amino-4-methylpiperazine, 1 -amino-4-methylpiperazine, 1 (3-aminopropyl)imidazole, 1-aminohydantoin, 1 ,2,4-triazole, 2,4-diamino-6-chloro-pyrimidine, 4-aminomorpholine, 4(2-aminoethyl)morpholine, 4(2-aminomethyl)morpholine, 4-amino-4H-1 ,2,4-triazole, acethydrazide, ethyl 1-piperazine, N,N-dimethylethylenediamine, and N-acetyethylenediamine.

[00331] In some compounds of Formula I-K, R^1 is $-O-C(O)-R^{2K}$, where R^{2K} is an anti-malarial drug comprising a quinolone having a hydroxyl group in its structure, and the carbonyl carbon of R^1 is linked to the hydroxyl group of the anti-malarial drug.

[00332] In some compounds of Formula I-K, R^1 is $-O-C(O)-R^{1K}$, where R^{1K} is an anti-malarial drug comprising a quinolone having a amine group its structure, and the carbonyl carbon of R^1 is linked to the amine group of the anti-malarial drug.

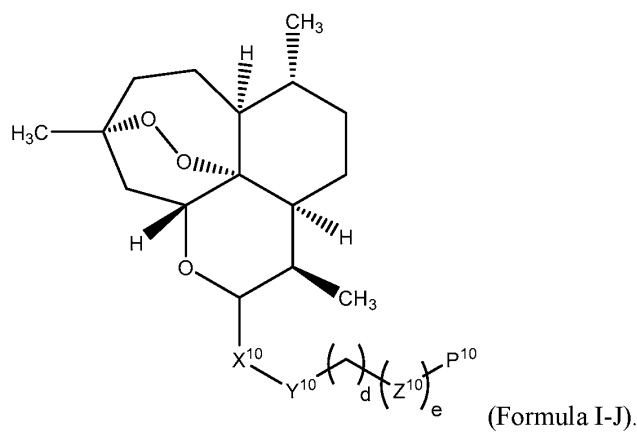
[00333] In some embodiments of any one of the aspects described herein, the compound of Formula I-K is selected from the group consisting of:





[00334] Compounds of Formula I-K and their synthesis are described, for example, in WO2010/032165, content of which is incorporated herein by reference in its entirety.

[00335] In some embodiments of any one of the aspects described herein, the artemisinin derivative is of Formula (I-L):



wherein:

d is an integer from 0 to 5;

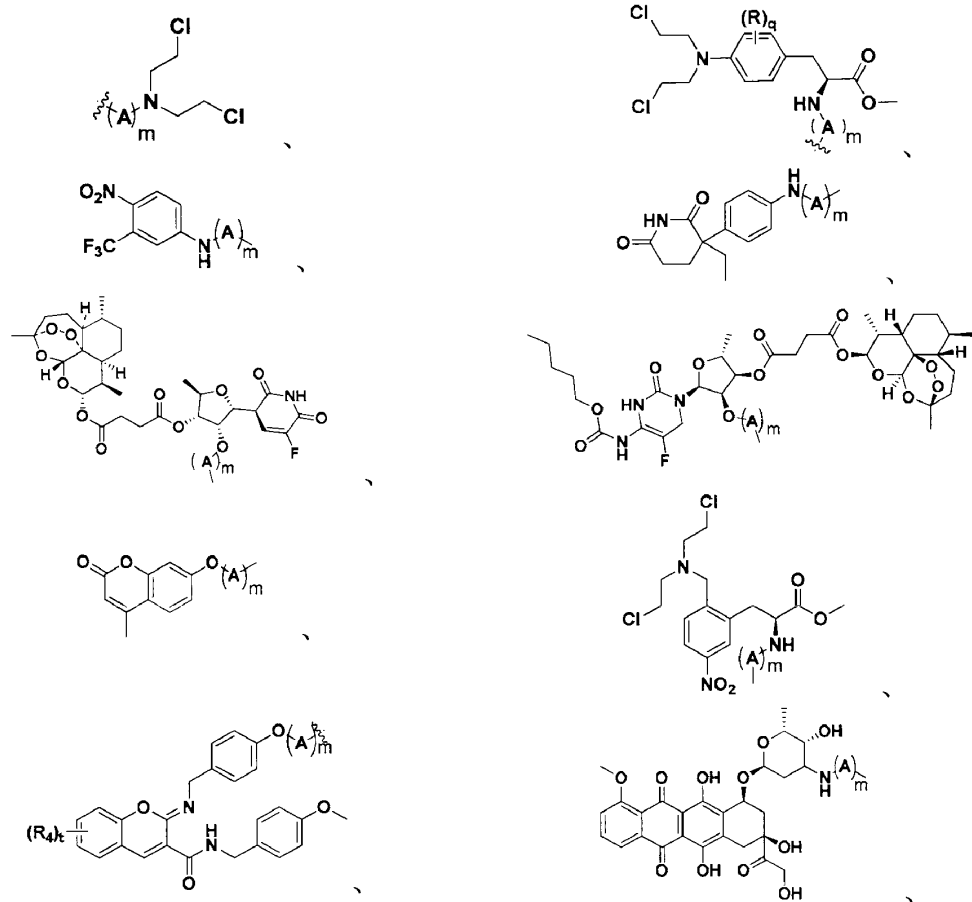
k is 0 or 1;

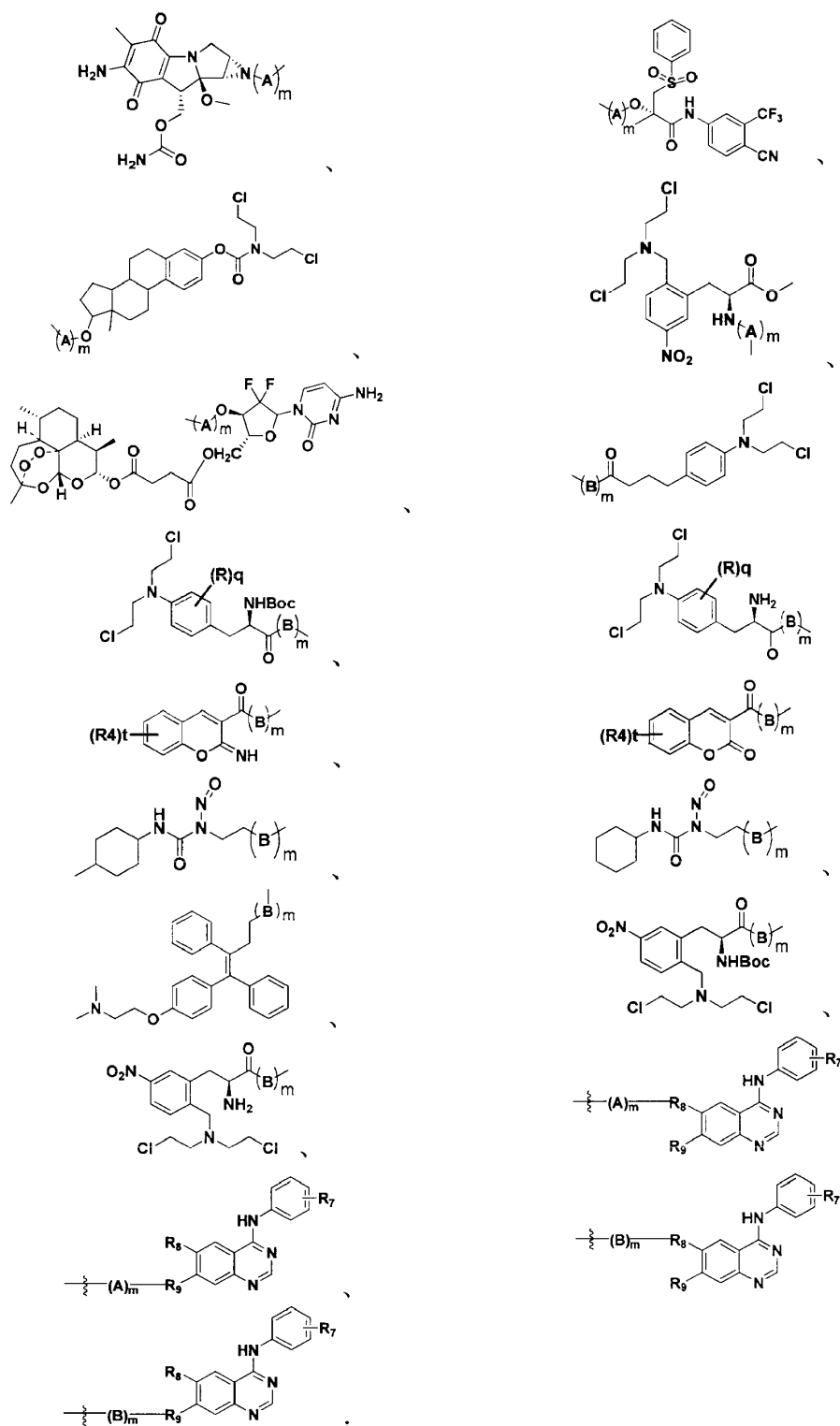
X^{10} is -O-, -S-, -NH- or -CH₂-;

Y^{10} is -CO- or -CH₂-;

Z^{10} is -CH₂-, -O-, -CO-, -CH₂CO-, -CH₂NH-, -CH₂O-, -COCH₂-, -NHCH₂-, -OCH₂- or -NH-; and

P is





wherein R and R₄ are each independently a group selected from the group consisting of: hydrogen, halogen, C₁-C₁₂ straight or branched alkyl, C₂-C₁₂ straight or

branched unsaturated hydrocarbon group, C₃-C₁₂ cycloalkyl group, cyano group, nitro group, amino group or an amine group substituted with a -C₄ fluorenyl group, a hydroxy group, a hydroxymethyl group, a trifluoromethyl group, a trifluoromethoxy group, a carboxyl group, a nonylphenyl group, a substituted phenyl group, a naphthyl group, a biphenyl group, or a substituted group. Or an unsubstituted saturated or unsaturated C₃-C₁₂ heterocyclic group, wherein the substituent is selected from the group consisting of: halogen, trifluoromethyl, ethynyl, hydroxy, amino, hydroxydecyl, carboxy or C₄ amine or aldehyde group; q and t are each independently an integer of 1 to 4;

R₇ is a group selected from the group consisting of one or two at any position on the phenyl ring: hydrogen, halogen, straight or branched fluorenyl, C₂-C₁₂ straight or branched unsaturated hydrocarbyl, C₃-C₁₂ cycloalkyl, ethynyl, cyano, nitro, amino, hydroxy, hydroxymethyl, trifluoromethyl, trifluoromethoxy, carboxy, nonylphenyl, substituted phenyl, naphthalene Base, biphenyl, or substituted or unsubstituted saturated or unsaturated C₃-C₁₂ heterocyclyl, benzyl alcohol, substituted benzyl alcohol, hydrazine, hydrazine-dimethyl, hydrazine, hydrazine-diethyl a substituent, wherein the substituent is selected from the group consisting of: halogen, trifluoromethyl, ethynyl, hydroxy, amino, hydroxyindole, carboxy or amine fluorenyl or aldehyde;

R₈ and R₉ are each independently -W-(D)-E,

wherein

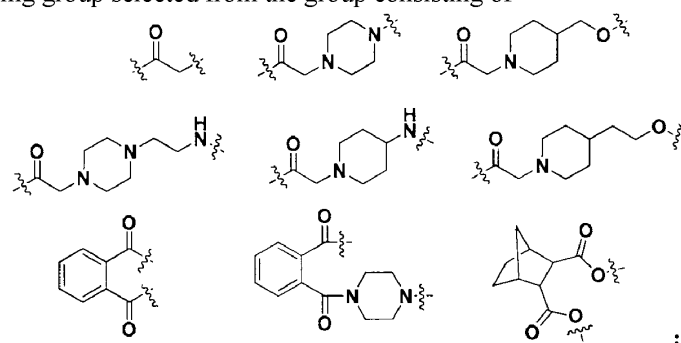
W is hydrogen, oxygen, hydrazine, halogen, linear or branched alkyl or fluorenylene, C₂-C₁₂ linear or branched unsaturated hydrocarbon or alkylene, C₃-C₁₂ cyclic hydrocarbon or Cycloalkyl, cyano, nitro, amino, hydroxy, C₄ hydroxyalkyl, trifluoromethyl, trifluoromethoxy, carboxy, nonylphenyl, substituted phenyl, naphthyl, biphenyl, or a substituted or unsubstituted saturated or unsaturated C₃-C₁₂ heterocyclyl or heterocyclylene, anthracene, fluorenyl-dimethyl, anthracene, fluorenyl-diethyl, wherein the substituent is selected from the group consisting of Halogen, trifluoromethyl, ethynyl, hydroxy, amino, hydroxyalkyl, carboxy or C₄ amine sulfhydryl or aldehyde;

D is carbonyl, straight or branched fluorenyl or alkylene, C₂-C₁₂ linear or branched unsaturated hydrocarbon or alkylene, substituted or unsubstituted saturated or unsaturated C₃-C₄ heterocyclic group or a heterocyclic group, wherein the substituent is selected from the group

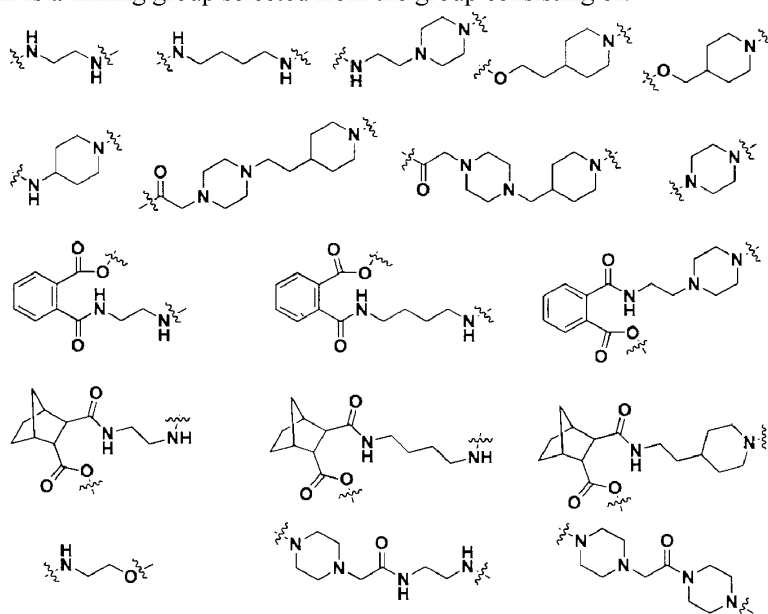
consisting of halogen, trifluoromethyl, ethynyl, hydroxy, amino, C₄hydroxyalkyl, carboxy or C₁-C₄ amine thiol or aldehyde base;

E is a C₁-C₁₂ linear or branched fluorenyl or alkylene group, a C₂-C₁₂ linear or branched unsaturated hydrocarbon group or an alkylene group, a substituted or unsubstituted saturated or unsaturated C₃-C₂ heterocyclic or heterocyclylene group, wherein the substituent is selected from the group consisting of halogen, trifluoromethyl, ethynyl, hydroxy, amino, C₄ hydroxyalkyl, carboxy or d-amine fluorenyl or Aldehyde; j, r are each independently an integer from 0 to 5;

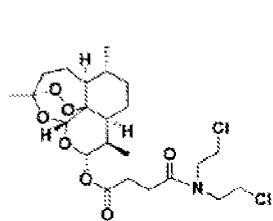
A is a linking group selected from the group consisting of



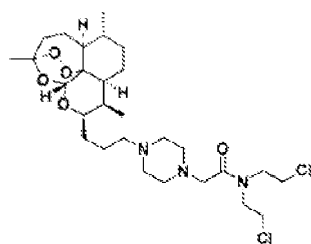
B is a linking group selected from the group consisting of:



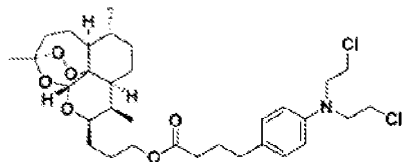
[00336] In some embodiments of any one of the aspects described herein, the compound of Formula I-L is selected from the group consisting of:



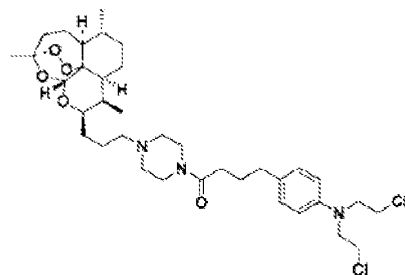
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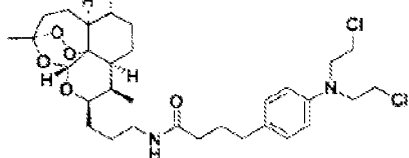
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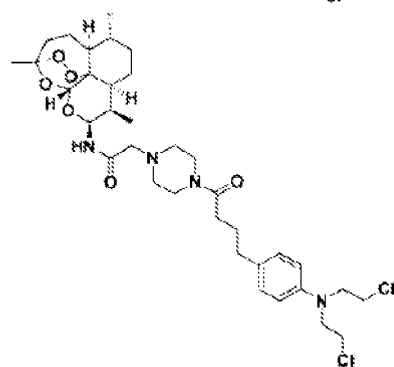
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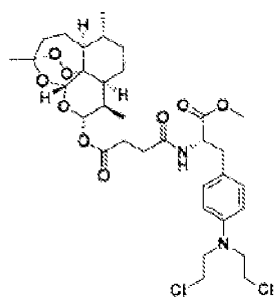
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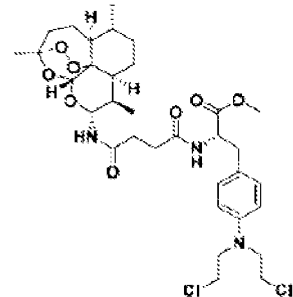
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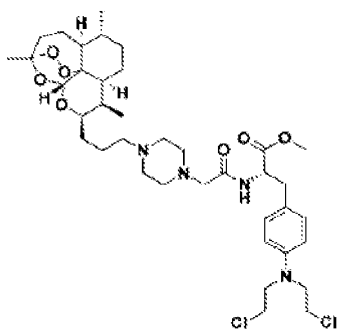
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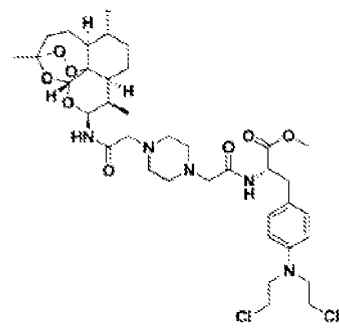
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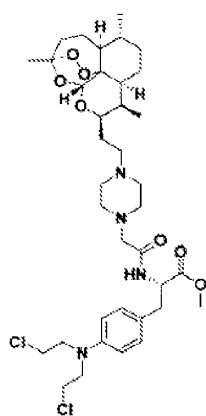
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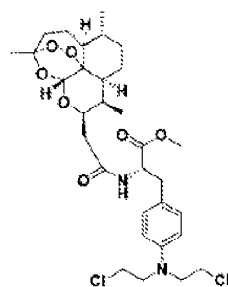
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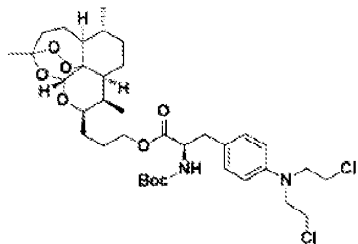
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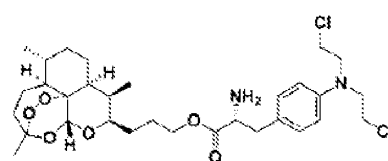
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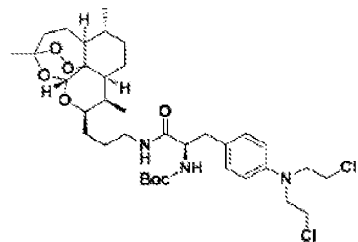
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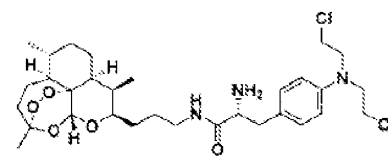
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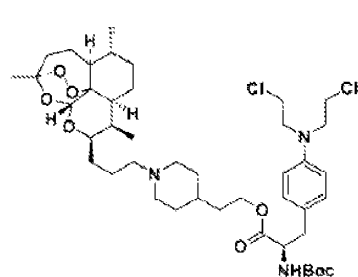
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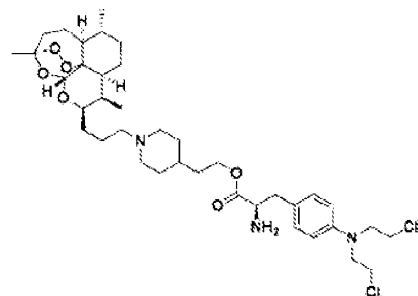
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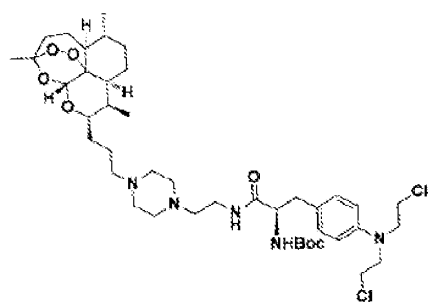
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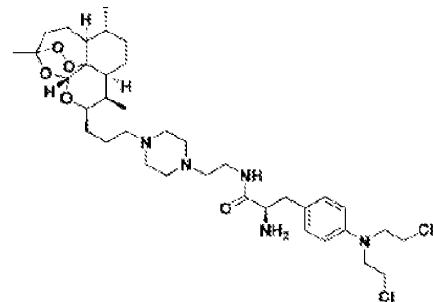
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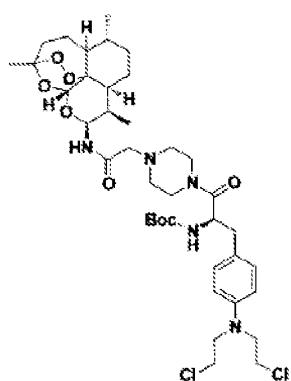
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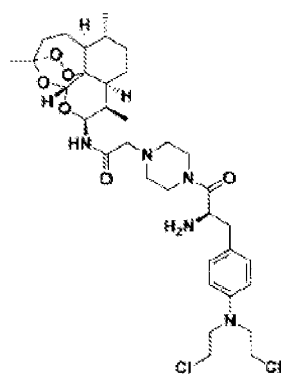
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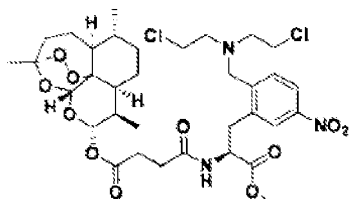
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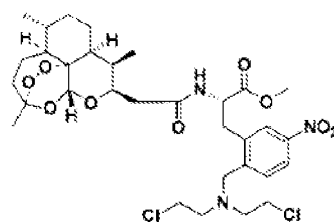
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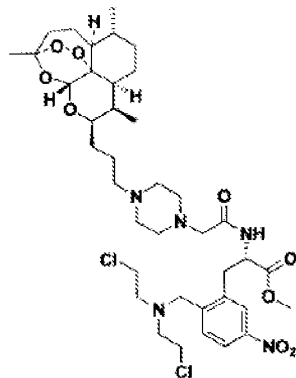
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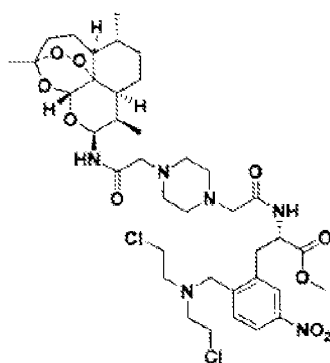
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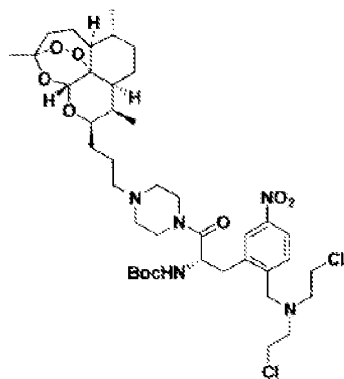
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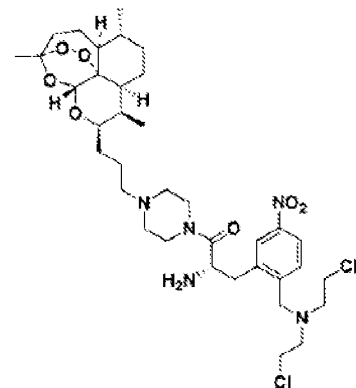
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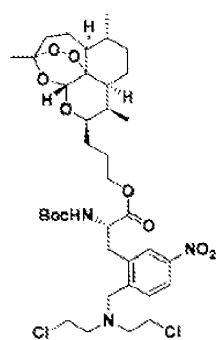
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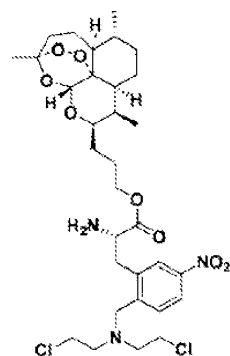
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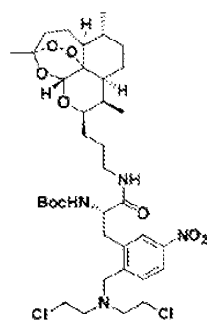
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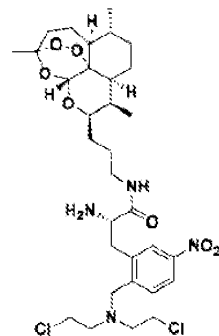
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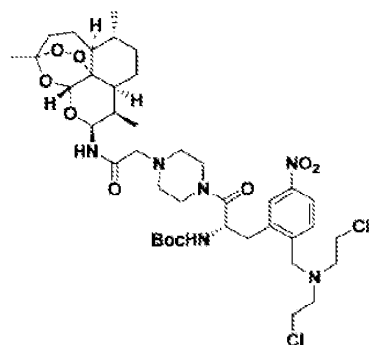
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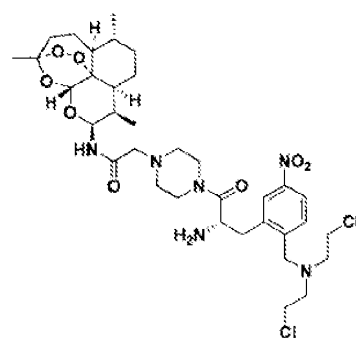
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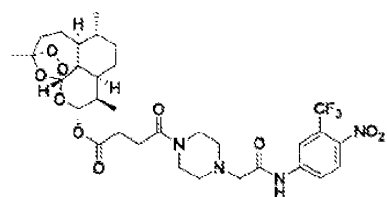
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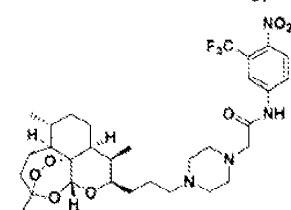
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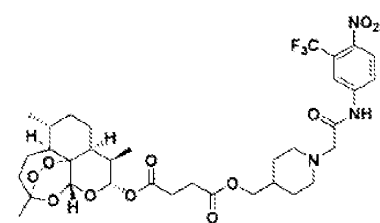
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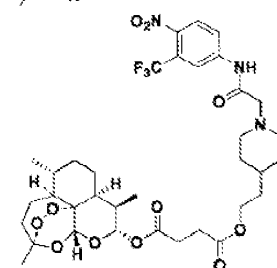
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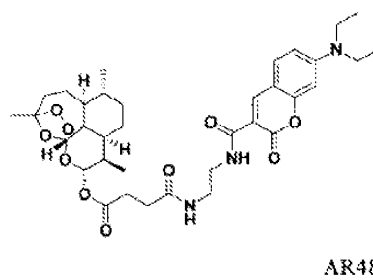
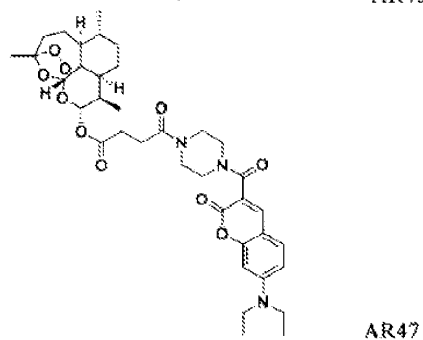
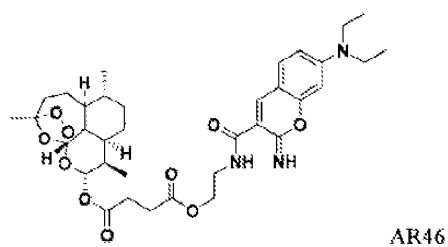
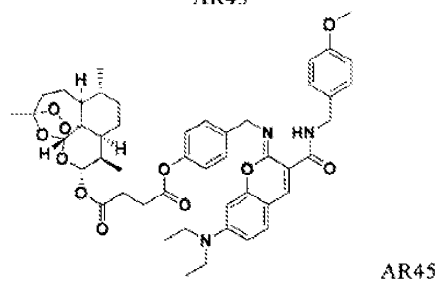
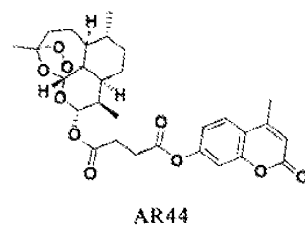
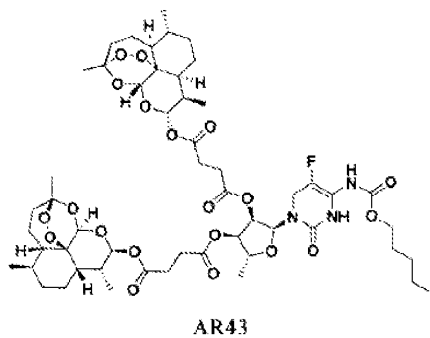
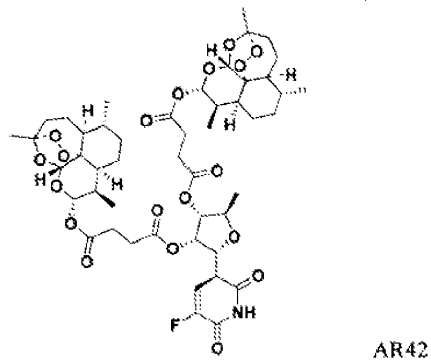
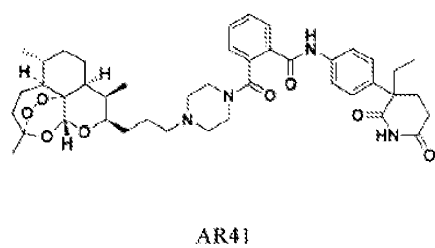
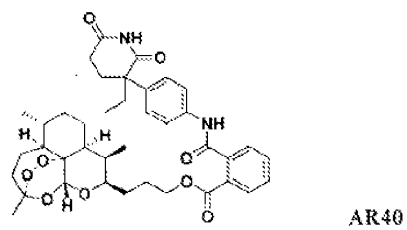
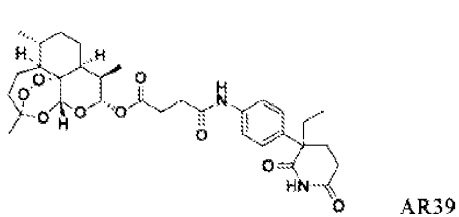
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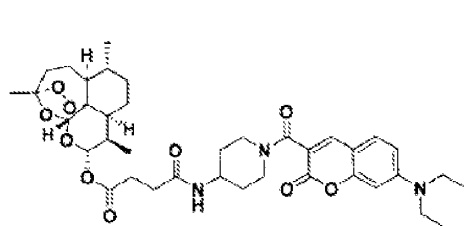


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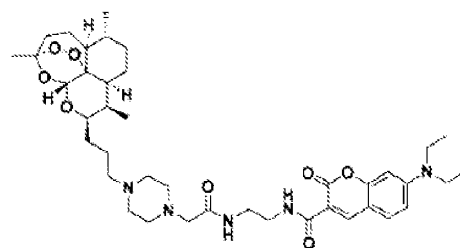


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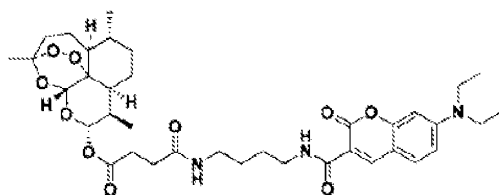




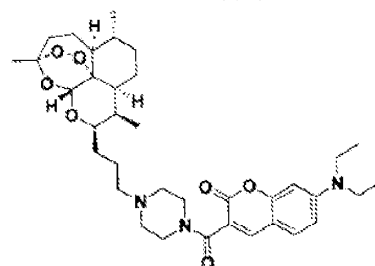
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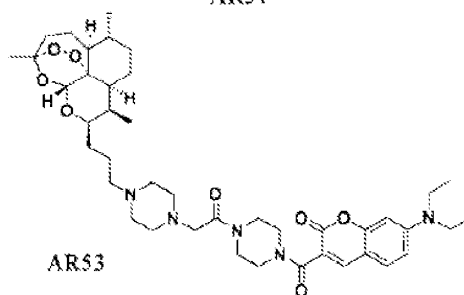
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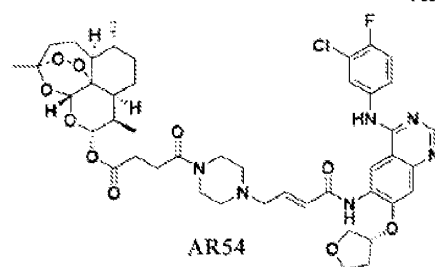
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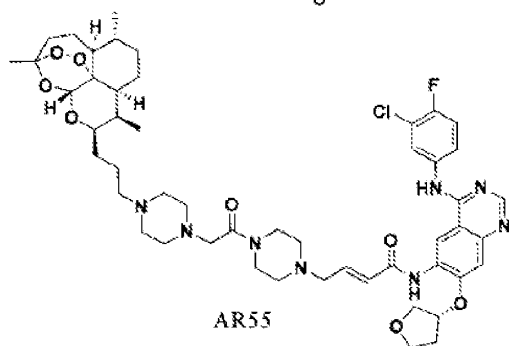
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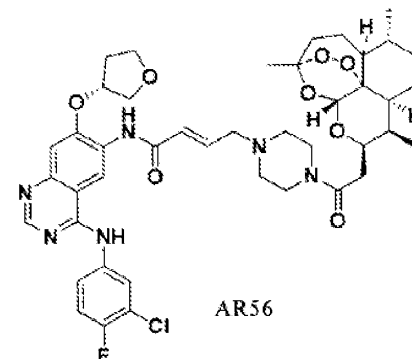
AR53



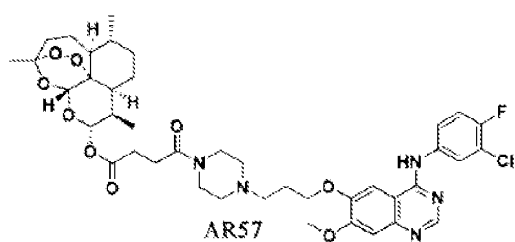
AR54



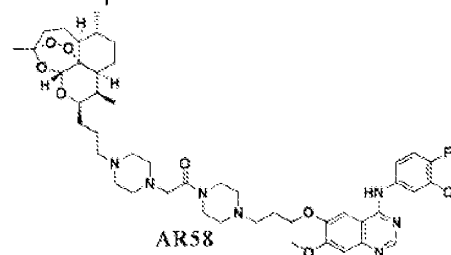
AR55



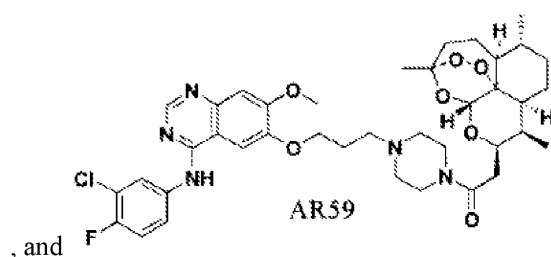
AR56



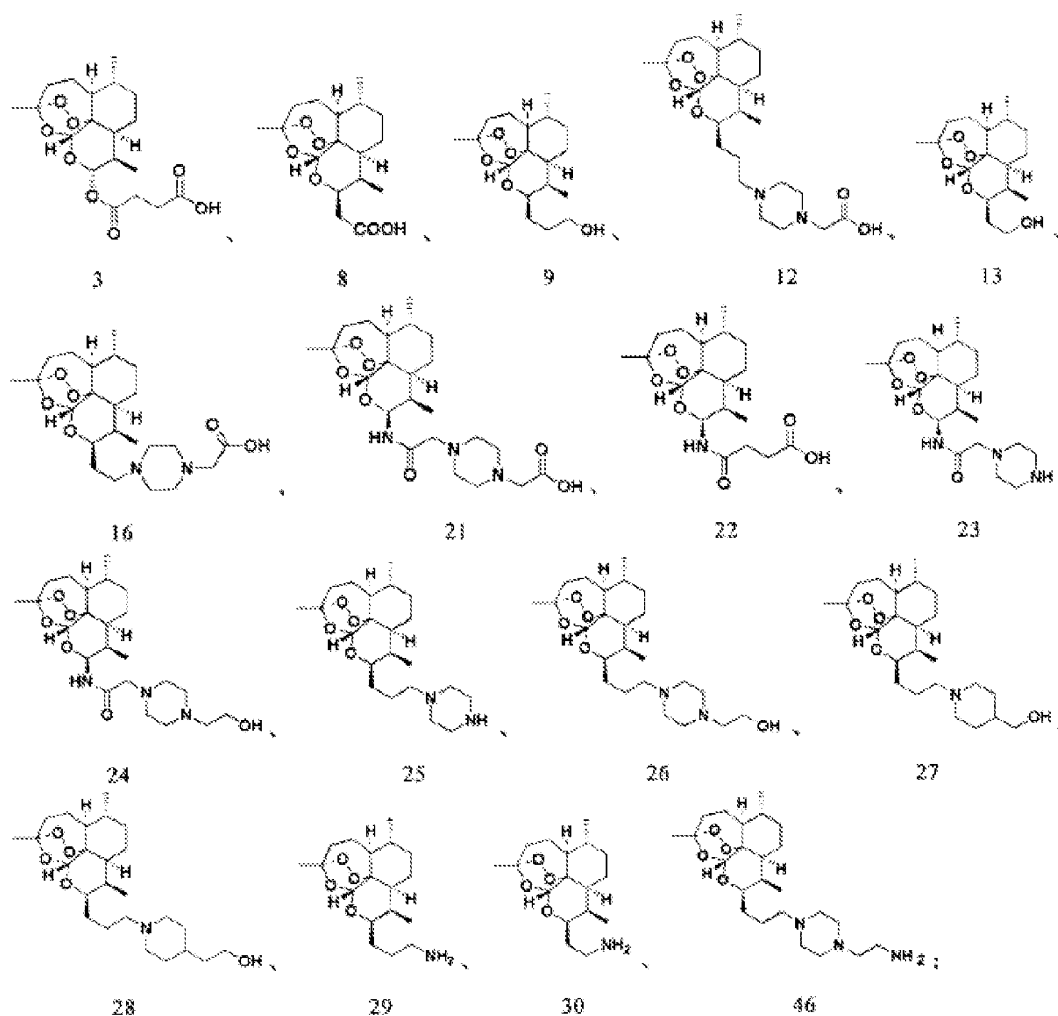
AR57



AR58

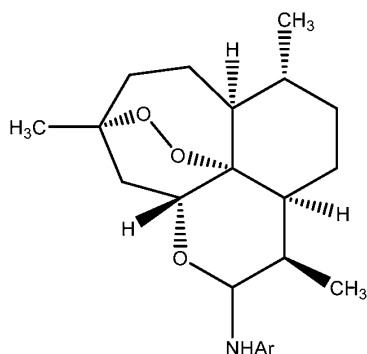


[00337] In some embodiments of any one of the aspects described herein, the compound of Formula I-L is selected from the group consisting of:

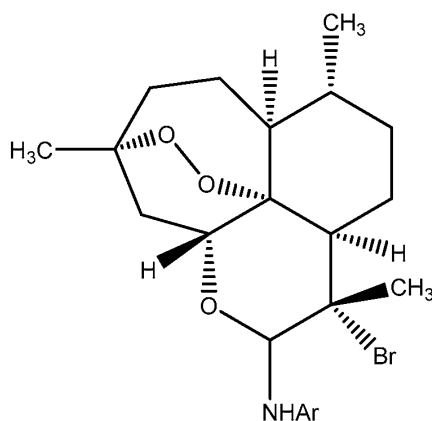


[00338] Compounds of Formula I-L and their synthesis are described, for example, in WO2014/023081, content of which is incorporated herein by reference in its entirety.

[00339] In some embodiments of any one of the aspects described herein, the artemisinin derivative is of Formula (I-M) or (I-M'):



(Formula I-M), or



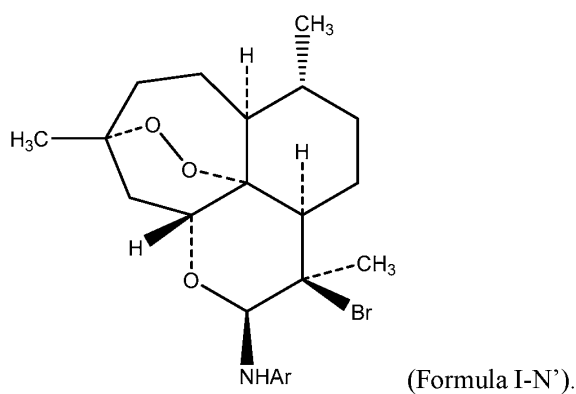
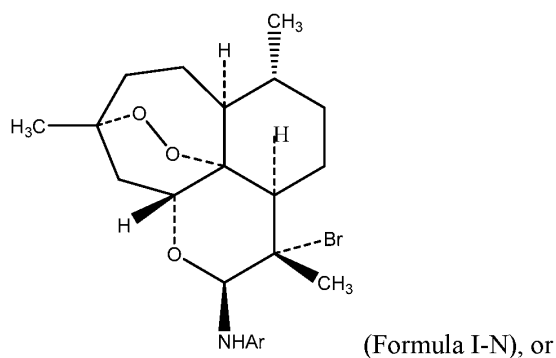
(Formula I-M'),

wherein:

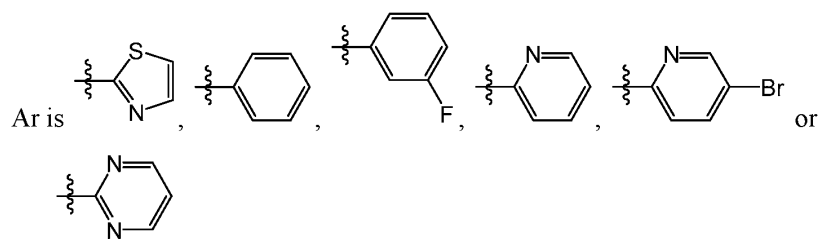
Ar is phenyl, 3-chlorophenyl, 4-chlorophenyl, 3-bromophenyl, 4-bromophenyl, 3-iodophenyl, 4-iodophenyl, 3-fluorophenyl, 4-fluorophenyl, 4-methylphenyl, 4-methoxyphenyl, 3-CO₂H-phenyl, 4-CO₂H-phenyl, or 4-SO₃H-phenyl.

[00340] Compounds of Formula I-M and I-M', and their synthesis are described, for example, in Yang et al., *Biorog. Med. Chem. Lett.*, 1995, vol. 5 (6), pp. 7191-1794, content of which is incorporated herein by reference in its entirety.

[00341] In some embodiments of any one of the aspects described herein, the artemisinin derivative is of Formula (I-N) or (I-N'):

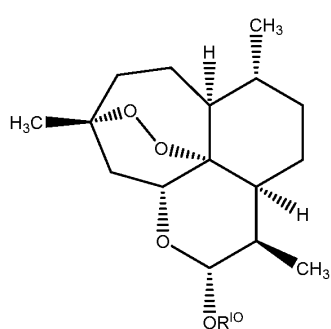


wherein:

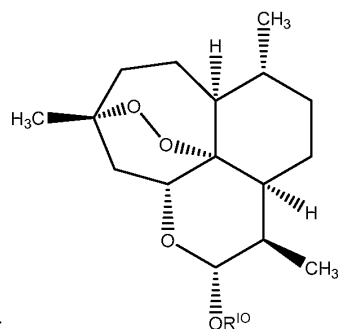


[00342] Compounds of Formula I-N and I-N', and their synthesis are described, for example, in Lin et al., *J. Med. Chem.*, 1999, vol. 33 (9), pp. 2610-2614, content of which is incorporated herein by reference in its entirety.

[00343] In some embodiments of any one of the aspects described herein, the artemisinin derivative is of Formula (I-O) or Formula (I-O')::

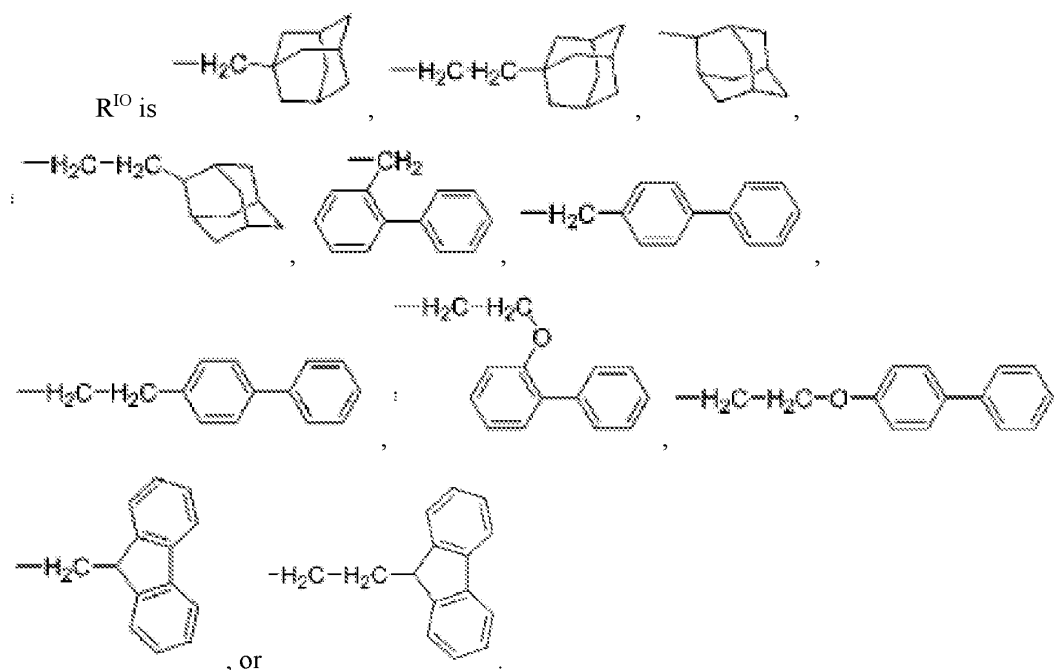


(Formula I-O) or



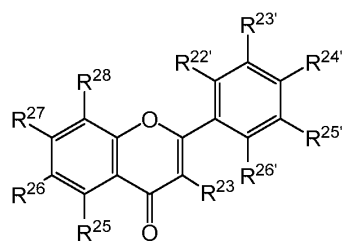
(Formula I-O'):

wherein:

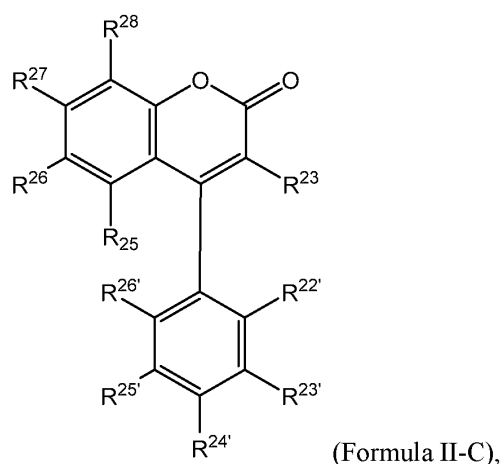
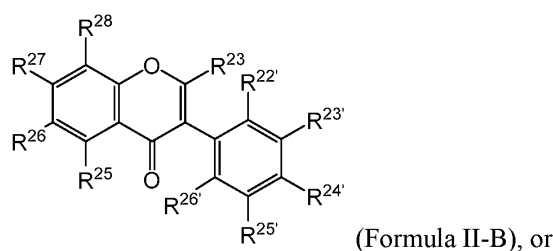


[00344] Compounds of Formula I-O and I-O', and their synthesis are described, for example, in Singh et al., *J. Med. Chem.*, 2006, vol. 49, pp. 7227-7233, content of which is incorporated herein by reference in its entirety.

[00345] In some embodiments of the various aspects described herein, one of the inducers is a flavonoid compound. Generally, flavonoids are of Formula (II-A), (II-B) or (II-C):



(Formula II-A),



wherein:

R^{23} is H, OH, C₁-C₆alkyl; C₂-C₆alkenyl, C₂-C₆alkynyl, haloalkyl, halogen (e.g., F, Cl, Br or I), cyano (-CN), nitro (-NO₂), carboxyl (-CO₂H), amino (-NH₂), alkyl amino (-NH(C₁-C₆alkyl)), dialkylamino (-N(C₁-C₆alkyl)(C₁-C₆alkyl)), or haloalkyl (e.g., CF₃); and

R^{25} , R^{26} , R^{27} , R^{28} , $R^{22'}$, $R^{23'}$, $R^{24'}$, $R^{25'}$ and $R^{26'}$ are independently H, OH, C₁-C₆alkoxy, C₁-C₆alkyl, C₂-C₆alkenyl, C₂-C₆alkynyl, halogen (e.g., F, Cl, Br or I), cyano, nitro, carboxyl, amino, alkyl amino, dialkylamino, thio (-SH), thioalkyl (-S(C₁-C₆alkyl)), or haloalkyl (e.g., CF₃); or

a pharmaceutically acceptable salt thereof.

[00346] In some embodiments, R^{23} is H, OH, C₁-C₆alkyl or halogen. For example, R^{23} is H, OH, methyl, ethyl propyl, fluoro (F), chloro (Cl), bromo (Br) or iodo (I). In some embodiments, R^{23} is H, OH or methyl. For example, R^{23} is H or OH. Preferably R^{23} is H.

[00347] In some embodiments, R^{25} is H, OH, C₁-C₆alkoxy, C₁-C₆alkyl, halogen, haloalkyl, cyano, nitro, amino, alkylamino, dialkylamino, or thio. For example, R^{25} is H, OH, methoxy (OCH₃), ethoxy (OCH₂CH₃), halogen, carboxyl, cyano, amino, CF₃, or thio. In some embodiments, R^{25} is H, OH, methoxy, methyl, fluoro, chloro, bromo, iodo, carboxyl, cyano or thio. In some preferred embodiments, R^{25} is H, OH or methoxy.

[00348] In some embodiments, R^{26} is H, OH, C₁-C₆alkoxy, C₁-C₆alkyl, halogen, haloalkyl, cyano, nitro, amino, alkylamino, dialkylamino, or thio. For example, R^{26} is H, OH, methoxy (OCH₃), ethoxy (OCH₂CH₃), halogen, carboxyl, cyano, amino, CF₃, or thio. In some embodiments, R^{26} is H, OH, methoxy, methyl, fluoro, chloro, bromo, iodo, carboxyl, cyano or thio. In some preferred embodiments, R^{26} is H, OH or methoxy.

[00349] In some embodiments, R^{27} is H, OH, C₁-C₆alkoxy, C₁-C₆alkyl, halogen, haloalkyl, cyano, nitro, amino, alkylamino, dialkylamino, or thio. For example, R^{27} is H, OH, methoxy (OCH₃), ethoxy (OCH₂CH₃), halogen, carboxyl, cyano, amino, CF₃, or thio. In some embodiments, R^{27} is H, OH, methoxy, methyl, fluoro, chloro, bromo, iodo, carboxyl, cyano or thio. In some preferred embodiments, R^{27} is H, OH or methoxy.

[00350] In some embodiments, R^{28} is H, OH, C₁-C₆alkoxy, C₁-C₆alkyl, halogen, haloalkyl, cyano, nitro, amino, alkylamino, dialkylamino, or thio. For example, R^{28} is H, OH, methoxy (OCH₃), ethoxy (OCH₂CH₃), halogen, carboxyl, cyano, amino, CF₃, or thio. In some embodiments, R^{28} is H, OH, methoxy, methyl, fluoro, chloro, bromo, iodo, carboxyl, cyano or thio. In some preferred embodiments, R^{28} is H, OH or methoxy.

[00351] In some embodiments, $R^{22'}$ is H, OH, C₁-C₆alkoxy, C₁-C₆alkyl, halogen, haloalkyl, cyano, nitro, amino, alkylamino, dialkylamino, or thio. For example, $R^{22'}$ is H, OH, methoxy (OCH₃), ethoxy (OCH₂CH₃), halogen, carboxyl, cyano, amino, CF₃, or thio. In some embodiments, $R^{22'}$ is H, OH, methoxy, methyl, fluoro, chloro, bromo, iodo, carboxyl, cyano or thio. In some preferred embodiments, $R^{22'}$ is H, OH or methoxy.

[00352] In some embodiments, $R^{23'}$ is H, OH, C₁-C₆alkoxy, C₁-C₆alkyl, halogen, haloalkyl, cyano, nitro, amino, alkylamino, dialkylamino, or thio. For example, $R^{23'}$ is H, OH, methoxy (OCH₃), ethoxy (OCH₂CH₃), halogen, carboxyl, cyano, amino, CF₃, or thio. In some embodiments, $R^{23'}$ is H, OH, methoxy, methyl, fluoro, chloro, bromo, iodo, carboxyl, cyano or thio. In some preferred embodiments, $R^{23'}$ is H, OH or methoxy.

[00353] In some embodiments, $R^{24'}$ is H, OH, C₁-C₆alkoxy, C₁-C₆alkyl, halogen, haloalkyl, cyano, nitro, amino, alkylamino, dialkylamino, or thio. For example, $R^{24'}$ is H, OH, methoxy (OCH₃), ethoxy (OCH₂CH₃), halogen, carboxyl, cyano, amino, CF₃, or thio. In some embodiments, $R^{24'}$ is H, OH, methoxy, methyl, fluoro, chloro, bromo, iodo, carboxyl, cyano or thio. In some preferred embodiments, $R^{24'}$ is H, OH or methoxy.

[00354] In some embodiments, $R^{25'}$ is H, OH, C₁-C₆alkoxy, C₁-C₆alkyl, halogen, haloalkyl, cyano, nitro, amino, alkylamino, dialkylamino, or thio. For example, $R^{25'}$ is H, OH, methoxy (OCH₃), ethoxy (OCH₂CH₃), halogen, carboxyl, cyano, amino, CF₃, or thio. In some embodiments, $R^{25'}$ is H, OH, methoxy, methyl, fluoro, chloro, bromo, iodo, carboxyl, cyano or thio. In some preferred embodiments, $R^{25'}$ is H, OH or methoxy.

[00355] In some embodiments, $R^{26'}$ is H, OH, C₁-C₆alkoxy, C₁-C₆alkyl, halogen, haloalkyl, cyano, nitro, amino, alkylamino, dialkylamino, or thio. For example, $R^{26'}$ is H, OH, methoxy (OCH₃), ethoxy (OCH₂CH₃), halogen, carboxyl, cyano, amino, CF₃, or thio. In some embodiments, $R^{26'}$ is H, OH, methoxy, methyl, fluoro, chloro, bromo, iodo, carboxyl, cyano or thio. In some preferred embodiments, $R^{26'}$ is H, OH or methoxy.

[00356] In some embodiments, R^{23} is H; and R^{25} , R^{26} , R^{27} , R^{28} , $R^{22'}$, $R^{23'}$, $R^{24'}$, $R^{25'}$ and $R^{26'}$ are independently H, OH, C₁-C₆alkoxy, halogen, amino, cyano, thio, carboxyl or haloalkyl. For example, R^{23} is H; and R^{25} , R^{26} , R^{27} , R^{28} , $R^{22'}$, $R^{23'}$, $R^{24'}$, $R^{25'}$ and $R^{26'}$ are independently H, OH, C₁-C₆alkoxy, halogen, amino, cyano, or CF₃. In some preferred embodiments, R^{23} is H; and R^{25} , R^{26} , R^{27} , R^{28} , $R^{22'}$, $R^{23'}$, $R^{24'}$, $R^{25'}$ and $R^{26'}$ are independently H, OH, or methoxy.

[00357] In some embodiments, all R^{23} , R^{25} , R^{26} , R^{27} , R^{28} , $R^{22'}$, $R^{23'}$, $R^{24'}$, $R^{25'}$ and $R^{26'}$ are H.

[00358] In some embodiments, R^{23} , R^{26} , R^{27} , R^{28} , $R^{22'}$, $R^{23'}$, $R^{24'}$, $R^{25'}$ and $R^{26'}$ are H; and R^{25} is OH.

[00359] In some embodiments, R^{23} , R^{26} , R^{28} , $R^{22'}$, $R^{23'}$, $R^{24'}$, $R^{25'}$ and $R^{26'}$ are H; R^{25} is OH; and R^{27} is OH or methoxy.

[00360] In some embodiments, R^{23} , R^{26} , R^{27} , $R^{22'}$, $R^{23'}$, $R^{24'}$, $R^{25'}$ and $R^{26'}$ are H; and R^{25} and R^{28} are OH.

[00361] In some embodiments, R^{23} , R^{26} , R^{28} , $R^{22'}$, $R^{23'}$, $R^{25'}$ and $R^{26'}$ are H; R^{25} and R^{27} are OH; and $R^{24'}$ is OH or methoxy.

[00362] In some embodiments, R^{23} , R^{26} , R^{28} , $R^{23'}$, $R^{24'}$, $R^{25'}$ and $R^{26'}$ are H; R^{25} and $R^{22'}$ are OH; and R^{27} is methoxy.

[00363] In some embodiments, R^{23} , R^{28} , $R^{22'}$, $R^{23'}$, $R^{24'}$, $R^{25'}$ and $R^{26'}$ are H; and R^{25} , R^{26} and R^{27} are OH.

[00364] In some embodiments, R^{23} , R^{28} , $R^{22'}$, $R^{23'}$, $R^{24'}$, $R^{25'}$ and $R^{26'}$ are H; R^{25} and R^{27} are OH; and R^{26} is methoxy.

[00365] In some embodiments, R^{23} , R^{28} , $R^{22'}$, $R^{23'}$, $R^{24'}$, $R^{25'}$ and $R^{26'}$ are H; R^{25} and R^{26} are OH; and R^{27} is methoxy.

[00366] In some embodiments, R^{23} , R^{26} , $R^{22'}$, $R^{23'}$, $R^{24'}$, $R^{25'}$ and $R^{26'}$ are H; R^{25} and R^{27} are OH; and R^{28} is OH or methoxy.

[00367] In some embodiments, R^{23} , R^{25} , R^{26} , R^{28} , $R^{22'}$, $R^{25'}$ and $R^{26'}$ are H; R^{27} and $R^{24'}$ are OH; and $R^{23'}$ is methoxy.

[00368] In some embodiments, R^{23} , R^{25} , R^{26} , R^{28} , $R^{22'}$, $R^{25'}$ and $R^{26'}$ are H; R^{27} and $R^{24'}$ are methoxy; and $R^{23'}$ is OH.

[00369] In some embodiments, R^{23} , R^{26} , R^{28} , $R^{22'}$, $R^{25'}$ and $R^{26'}$ are H; and R^{25} , R^{27} , $R^{23'}$ and $R^{24'}$ are OH.

- [00370] In some embodiments, R^{23} , R^{28} , $R^{22'}$, $R^{25'}$ and $R^{26'}$ are H; and R^{25} , R^{26} , R^{27} , $R^{23'}$ and $R^{24'}$ are OH.
- [00371] In some embodiments, R^{23} , R^{26} , R^{28} , $R^{22'}$, $R^{25'}$ and $R^{26'}$ are H; R^{25} , R^{27} and $R^{24'}$ are OH; and $R^{23'}$ is methoxy.
- [00372] In some embodiments, R^{23} , R^{26} , R^{28} , $R^{22'}$, $R^{25'}$ and $R^{26'}$ are H; R^{25} , R^{27} and $R^{23'}$ are OH; and $R^{24'}$ is methoxy.
- [00373] In some embodiments, R^{23} , R^{26} , R^{28} , $R^{22'}$, $R^{25'}$ and $R^{26'}$ are H; R^{25} , and $R^{23'}$ are OH; and R^{27} and $R^{24'}$ is methoxy.
- [00374] In some embodiments, R^{23} , R^{26} , R^{28} , $R^{22'}$, $R^{25'}$ and $R^{26'}$ are H; R^{25} and $R^{24'}$ are OH; and R^{27} and $R^{23'}$ is methoxy.
- [00375] In some embodiments, R^{23} , R^{26} , R^{28} , $R^{23'}$, $R^{25'}$ and $R^{26'}$ are H; R^{25} , $R^{22'}$ and $R^{24'}$ are OH; and R^{27} is OH or methoxy.
- [00376] In some embodiments, R^{23} , R^{28} , $R^{22'}$, $R^{23'}$, $R^{25'}$ and $R^{26'}$ are H; R^{25} , R^{27} and $R^{24'}$ are OH; and R^{26} is OH or methoxy.
- [00377] In some embodiments, R^{23} , R^{28} , $R^{22'}$, $R^{23'}$, $R^{25'}$ and $R^{26'}$ are H; R^{25} , R^{26} and $R^{24'}$ are OH; and R^{27} is methoxy.
- [00378] In some embodiments, R^{23} , R^{28} , $R^{22'}$, $R^{23'}$, $R^{25'}$ and $R^{26'}$ are H; R^{25} and R^{27} are OH; and R^{26} and $R^{24'}$ are methoxy.
- [00379] In some embodiments, R^{23} , R^{28} , $R^{22'}$, $R^{23'}$, $R^{25'}$ and $R^{26'}$ are H; R^{25} and $R^{24'}$ are OH; and R^{26} and R^{27} are methoxy.
- [00380] In some embodiments, R^{23} , R^{28} , $R^{22'}$, $R^{23'}$, $R^{25'}$ and $R^{26'}$ are H; R^{25} is OH; and R^{26} , R^{27} and $R^{24'}$ are methoxy.
- [00381] In some embodiments, R^{23} , R^{26} , $R^{22'}$, $R^{23'}$, $R^{25'}$ and $R^{26'}$ are H; and R^{25} , R^{27} , R^{28} and $R^{24'}$ are OH.
- [00382] In some embodiments, R^{23} , R^{27} , R^{28} , $R^{23'}$, $R^{24'}$ and $R^{25'}$ are H; R^{25} is OH or methoxy; and R^{26} , $R^{22'}$, and $R^{26'}$ are methoxy.
- [00383] In some embodiments, R^{23} , R^{27} , R^{28} , $R^{22'}$, $R^{24'}$ and $R^{26'}$ are H; and R^{25} , R^{26} , $R^{23'}$, and $R^{25'}$ are methoxy.
- [00384] In some embodiments, R^{23} , R^{28} , $R^{22'}$, $R^{23'}$, $R^{24'}$, $R^{25'}$ and $R^{26'}$ are H; R^{25} is OH; and R^{25} , R^{26} , R^{27} , and R^{28} are methoxy.
- [00385] In some embodiments, R^{23} , R^{26} , R^{28} , $R^{22'}$, and $R^{26'}$ are H; and R^{25} , R^{27} , $R^{23'}$, $R^{24'}$ and $R^{25'}$ are OH.
- [00386] In some embodiments, R^{23} , R^{26} , R^{28} , $R^{22'}$, and $R^{26'}$ are H; R^{25} , R^{27} , and $R^{24'}$ are OH; and $R^{23'}$ and $R^{25'}$ are methoxy.

- [00387] In some embodiments, R^{23} , R^{26} , R^{28} , $R^{22'}$, and $R^{26'}$ are H; R^{25} is OH; and R^{27} , $R^{23'}$, $R^{24'}$ and $R^{25'}$ are methoxy.
- [00388] In some embodiments, R^{23} , R^{28} , $R^{22'}$, $R^{25'}$ and $R^{26'}$ are H; R^{25} , R^{27} , $R^{23'}$ and $R^{24'}$ are OH; and R^{26} is methoxy.
- [00389] In some embodiments, R^{23} , R^{28} , $R^{22'}$, $R^{25'}$ and $R^{26'}$ are H; R^{25} , R^{26} , $R^{23'}$ and $R^{24'}$ are OH; and R^{27} is methoxy.
- [00390] In some embodiments, R^{23} , R^{28} , $R^{22'}$, $R^{25'}$ and $R^{26'}$ are H; R^{25} , R^{26} , R^{27} and $R^{24'}$ are OH; and $R^{23'}$ is methoxy.
- [00391] In some embodiments, R^{23} , R^{28} , $R^{22'}$, $R^{25'}$ and $R^{26'}$ are H; R^{25} , R^{27} and $R^{24'}$ are OH; and R^{26} and $R^{23'}$ are methoxy.
- [00392] In some embodiments, R^{23} , R^{28} , $R^{22'}$, $R^{25'}$ and $R^{26'}$ are H; R^{25} , $R^{23'}$ and $R^{24'}$ are OH; and R^{26} and R^{27} are methoxy.
- [00393] In some embodiments, R^{23} , R^{28} , $R^{22'}$, $R^{25'}$ and $R^{26'}$ are H; R^{25} and R^{27} are OH; and R^{26} , $R^{23'}$ and $R^{24'}$ are methoxy.
- [00394] In some embodiments, R^{23} , R^{28} , $R^{22'}$, $R^{25'}$ and $R^{26'}$ are H; R^{25} and $R^{24'}$ are OH; and R^{26} , R^{27} and $R^{23'}$ are methoxy.
- [00395] In some embodiments, R^{23} , R^{28} , $R^{22'}$, $R^{23'}$ and $R^{26'}$ are H; R^{25} and $R^{25'}$ are OH; and R^{26} , R^{27} and $R^{24'}$ are methoxy.
- [00396] In some embodiments, R^{23} , R^{28} , $R^{22'}$, $R^{23'}$ and $R^{26'}$ are H; and R^{25} , R^{26} , R^{27} , $R^{24'}$ and $R^{25'}$ are methoxy.
- [00397] In some embodiments, R^{23} , R^{26} , $R^{22'}$, $R^{25'}$ and $R^{26'}$ are H; R^{25} , R^{27} , $R^{23'}$ and $R^{24'}$ are OH; and R^{28} is OH or methoxy.
- [00398] In some embodiments, R^{23} , R^{26} , $R^{24'}$, $R^{25'}$ and $R^{26'}$ are H; R^{25} and $R^{23'}$ are OH; and R^{27} , R^{28} and $R^{22'}$ are methoxy.
- [00399] In some embodiments, R^{23} , $R^{22'}$, $R^{23'}$, $R^{25'}$ and $R^{26'}$ are H; R^{25} and R^{27} are OH; and R^{26} , R^{28} and $R^{24'}$ are methoxy.
- [00400] In some embodiments, R^{23} , $R^{22'}$, $R^{23'}$, $R^{25'}$ and $R^{26'}$ are H; R^{25} and $R^{24'}$ are OH; and R^{26} , R^{27} and R^{28} are methoxy.
- [00401] In some embodiments, R^{23} , $R^{22'}$, $R^{23'}$, $R^{25'}$ and $R^{26'}$ are H; and R^{25} , R^{26} , R^{27} , R^{28} and $R^{24'}$ are methoxy.
- [00402] In some embodiments, R^{23} , R^{26} , $R^{25'}$ and $R^{26'}$ are H; R^{25} is OH; and R^{27} , R^{28} , $R^{22'}$, $R^{23'}$, and $R^{24'}$ are methoxy.
- [00403] In some embodiments, R^{23} , $R^{22'}$, $R^{25'}$ and $R^{26'}$ are H; R^{25} , R^{27} and $R^{24'}$ are OH; and R^{26} , R^{28} , and $R^{23'}$ are methoxy.

[00404] In some embodiments, R^{23} , $R^{22'}$, $R^{25'}$ and $R^{26'}$ are H; R^{25} , R^{27} and $R^{23'}$ are OH; and R^{26} , R^{28} , and $R^{24'}$ are methoxy.

[00405] In some embodiments, R^{23} , $R^{22'}$, $R^{25'}$ and $R^{26'}$ are H; R^{25} and R^{27} are OH; and R^{26} , R^{28} , $R^{23'}$ and $R^{24'}$ are methoxy.

[00406] In some embodiments, R^{23} , $R^{22'}$, $R^{25'}$ and $R^{26'}$ are H; R^{25} and $R^{23'}$ are OH; and R^{26} , R^{28} and $R^{24'}$ are methoxy.

[00407] In some embodiments, R^{23} , $R^{22'}$, $R^{25'}$ and $R^{26'}$ are H; and R^{25} , R^{26} , R^{27} , R^{28} , $R^{23'}$ and $R^{24'}$ are methoxy.

[00408] In some embodiments, R^{23} , $R^{22'}$ and $R^{26'}$ are H; R^{25} , R^{27} and $R^{25'}$ are OH; and R^{26} , R^{28} , $R^{23'}$ and $R^{24'}$ are methoxy.

[00409] Flavonoid compounds are commercially available, for example, from Natland International Corp., Research Triangle Park, North Carolina; MP Biomedicals, Irvine, California; LC Laboratories, Woburn, Mass. Flavonoid compounds can be isolated from plants or seeds, for example, as described in US patent No. 5702752; 5990291 and 6146668. Flavonoid compounds can also be produced by organisms engineered using genetic engineering techniques, such as yeast, as described in Ralston, et al., *Plant Physiology* 737: 1375-88 (2005).

[00410] In some embodiments of any one of the aspects described herein, the flavonoid compound, e.g., the first or second inducer is of Formula (II-A).

[00411] In some embodiments of any one of the aspects described herein, the flavonoid compound, e.g., the first or second inducer is of Formula (II-B).

[00412] In some embodiments of any one of the aspects described herein, the flavonoid compound, e.g., the first or second inducer is of Formula (II-C).

[00413] Some exemplary flavonoid compounds suitable for use as an inducer include, but are not limited to, flavone, baicalein, chrysin, galangin, (OH)₄ flavone, 6-hydroxyluteolin, acacetin, acerosin, alnetin, apigenin, apiin, artocarpetin, balcalein, beta-sitosterol, biochamin A, cannaflavin A, cannflavin A, cannflavin B, cannflavin C, catechin, catechin-3-gallate, cerrosillin, chrysoeriol, chrysoeriol, cirsilinoleol, cirsilinoleol, cirsimaritin, corymbosin, cupressuflavone, cyanidine, daidzein, datiscetin, delphinidine, digidrokempferol, dihydroquercetin, diosmetin, diosmin, echioidinin, epicatechin, epicatechin-3-gallate, epigallocatechin, epigallocatechin-3-gallate, eriodiktin, eupatilin, eupatorin, fisetin, fizetin, flavonol, formononetin, fortunelin, gallocatechin, gallocatechin-3-gallate, gardenin D, genistein, genistin, genkwanin, geraldol, geraldone, glycitein, glycitin, gomoeriodiktin, gossypetin, hesperetin, hinokiflavone, hispidulin, hymenoxin, hypolaetin, isoquercetin, isorhamnetin, isorhamnetin, isoscutellarein, isovitexin, jaceosidin, kaempferol, kaempferol, kampferide, largonidin, luteolin, luteolin, malvidine, mikanin, morin, myricetin, myricetin, naringenin, negletein, neodiosmin, nepetin, nevadensin, nobiletin,

nodifloretin, norartocarpetin, norwogonin, onopordin, orientin, oroxylin a, pachipodol, pectolarigenin, pedalitin, peonidin, petunidin, pilloin, pratol, primetin, primuletin, pruentin, quercetagenin, quercetin, ramnazin, rhoifolin, robinetin, rutin, scaposin, scutellarein, serpyllin, sinensetin, sorbifolin, sudachitin, tangeretin, tangeritin, taxifolin, tectochrysin, tithonine, tricetin, tricin, velutin, vitexin, wightin, wogonin, xanthomicrol, zapotin, zapotinin, 6,7,4'-trihydroxy isoflavone, 7,3,4'-trihydroxy isoflavone, and pharmaceutically acceptable salts thereof. In some preferred embodiments, the flavonoid compound is flavone.

Vectors

[00414] In some embodiments, the method further comprises administering the exogenous nucleotide sequence to the subject prior to administering the inducer. For administering to a subject, the exogenous nucleotide sequence can be comprised in a vector. The term “vector” is well known in the art, and as used herein, refers to a nucleic acid construct designed for delivery to a host cell or for transfer between different host cells. A vector is suitably used to transport an inserted nucleic acid molecule into a suitable host cell. A vector typically contains all of the necessary elements that permit transcribing the insert nucleic acid molecule, and, preferably, translating the transcript into a polypeptide. A vector typically contains all of the necessary elements such that, once the vector is in a host cell, the vector can replicate independently of, or coincidental with, the host chromosomal DNA; several copies of the vector and its inserted nucleic acid molecule may be generated. Vectors described herein can be episomal vectors (i.e., that do not integrate into the genome of a host cell), or can be vectors that integrate into the host cell genome. A vector can include, but is not limited to, a cloning vector, an expression vector, a plasmid, phage, transposon, cosmid, chromosome, virus, virion, etc.

[00415] As used herein, a vector can be viral or non-viral. Non-viral vectors include but are not limited to plasmid vectors (e.g., pMA-RQ, pUC vectors, bluescript vectors (pBS) and pBR322 or derivatives thereof that are devoid of bacterial sequences (minicircles)) transposons-based vectors (e.g., PiggyBac (PB) vectors or Sleeping Beauty (SB) vectors), etc. Larger vectors such as artificial chromosomes (bacteria (BAC), yeast (YAC), or human (HAC)) may be used to accommodate larger inserts.

[00416] In some embodiments, the vector is a viral vector. As used herein, the term “viral vector” refers to a nucleic acid vector construct that includes at least one element of viral origin and has the capacity to be packaged into a viral vector particle. The viral vector can contain the nucleic acid encoding a polypeptide as described herein in place of non-essential viral genes. The vector and/or particle may be utilized for the purpose of transferring any nucleic acids into cells either *in vitro* or *in vivo*. Viral vectors are derived from viruses and include but are not limited to

retroviral, lentiviral, adeno-associated viral, adenoviral, herpes viral, hepatitis viral vectors and the like. Typically, but not necessarily, viral vectors are replication-deficient as they have lost the ability to propagate in a given cell since viral genes essential for replication have been eliminated from the viral vector. However, some viral vectors can also be adapted to replicate specifically in a given cell, such as e.g., a cancer cell, and are typically used to trigger the (cancer) cell-specific (onco)lysis. Virosomes are a non-limiting example of a vector that comprises both viral and non-viral elements, in particular they combine liposomes with an inactivated HIV or influenza virus (Yamada et al., 2003). Another example encompasses viral vectors mixed with cationic lipids.

[00417] In some embodiments, the viral vector is an adeno-associated viral (AAV) vector. In some preferred embodiments the AAV has a serotype suitable for liver transduction. In some embodiments, the AAV is selected from the group consisting of: AAV1, AAV2, AAV3, AAV3A, AAV3B, AAV4, AAV5, AAV6, AAV8, AAV9, AAV10, AAV11, AAV12, AAV2i8, AAVrh10, AAVrh74, avian AAV, bovine AAV, canine AAV, equine AAV, ovine AAV, or chimeras thereof or derivatives thereof. *See, e.g.,* BERNARD N. FIELDS *et al.*, VIROLOGY, volume 2, chapter 69 (4th ed., Lippincott-Raven Publishers). A number of relatively new AAV serotypes and clades have been identified (*see, e.g.,* Gao et al., (2004) *J. Virology* 78:6381-6388; Moris et al., (2004) *Virology* 33-:375- 383); and also Table 1 as disclosed in U.S. Provisional Application 62,937,556, filed on November 19, 2019, and Table 1 and Table 2 in International Applications WO2020/102645 and WO2020/102667, each of which is incorporated herein in their entirety.

[00418] In some embodiments, the vector is recombinant, e.g., it comprises sequences originating from at least two different sources. In some embodiments of any of the aspects, the vector comprises sequences originating from at least two different species. In some embodiments of any of the aspects, the vector comprises sequences originating from at least two different genes, e.g., it comprises a nucleic acid encoding an expression product which is operably linked to at least one non-native (e.g., heterologous) genetic control element (e.g., a promoter, suppressor, activator, enhancer, response element, or the like).

Vectors and Virions

[00419] In some embodiments of any of the aspects, the rAAV vector (also referred to as a rAAV virion) as disclosed herein comprises a capsid protein, and a rAAV genome in the capsid protein. A rAAV capsid of the rAAV virion can be any of those listed in Table 2 herein, or in Table 1 as disclosed in International Applications WO2020/102645, and WO2020/102667, each of which are incorporated herein in their entirety by reference. In some embodiments of any of the aspects, the rAAV described herein comprises AAV capsid proteins that can be polyploid (also referred to as haploid, or rational haploid, or rational polyploid), i.e., they can comprise VP1, VP2, and VP3

capsid proteins from more than one AAV serotypes in a single AAV virion as described in International Application Nos PCT/US2018/022725, PCT/US2018/044632, and US Patent No. 10,550,405; all of which are incorporated here by reference in their entireties. In some embodiments of any of the aspects, rAAV comprises at least one capsid protein of VP1, VP2, and VP3 selected from AAV serotypes listed in **Table A**.

Table A: AAV Serotypes and exemplary Published corresponding capsid sequence

TABLE X	
Serotype and where capsid sequence is published	Serotype and where capsid sequence is published
AAV3.3b (See SEQ ID NO:72 in US20030138772)	AAV3-3 (See SEQ ID NO: 200 US20150315612)
AAV3-3 (See SEQ ID NO:217 US20150315612)	AAV3a ((See SEQ ID NO: 5 in US6156303)
AAV3a (See SEQ ID NO: 9 in US6156303)	AAV3b (See SEQ ID NO: 6 in US6156303)
AAV3b (See SEQ ID NO:10 in US6156303)	AAV3b (See SEQ ID NO: 1 in US6156303)
AAV4 (See SEQ ID NO:17 US20140348794)	AAV4 ((See SEQ ID NO:5 in US20140348794)
AAV4 (See SEQ ID NO: 3 in US20140348794)	AAV4 (See SEQ ID NO:14 in US20140348794)
AAV4 (See SEQ ID NO: 15 in US20140348794)	AAV4 (See SEQ ID NO: 19 in US20140348794)
AAV4 (See SEQ ID NO: 12 in US20140348794)	AAV4 (See SEQ ID NO: 13 in US20140348794)
AAV4 (See SEQ ID NO: 7 in US20140348794)	AAV4 (See SEQ ID NO: 8 in US20140348794)
AAV4 (See SEQ ID NO: 9 in US20140348794)	AAV4 (See SEQ ID NO: 2 in US20140348794)
AAV4 (See SEQ ID NO: 10 in US20140348794)	AAV4 (See SEQ ID NO: 11 in US20140348794)
AAV4 (See SEQ ID NO: 18 in US20140348794)	AAV4 (See SEQ ID NO:63 in US20030138772) and US20160017295 SEQ
ID NO: (See SEQ ID NO: 4 in US20140348794)	AAV4 (See SEQ ID NO: 16 in US20140348794)
AAV4 (See SEQ ID NO: 20 in US20140348794)	AAV4 (See SEQ ID NO: 6 in US20140348794)
AAV4 (See SEQ ID NO: 1 in US20140348794)	AAV42.2 (See SEQ ID NO: 9 in US20030138772)
AAV42.2 (See SEQ ID NO: 102 in US20030138772)	AAV42.3b (See SEQ ID NO: 36 in US20030138772)
AAV42.3B (See SEQ ID NO: 107 in US20030138772)	AAV42.4 (See SEQ ID NO: 33 in US20030138772)
AAV42.4 (See SEQ ID NO: 88 in US20030138772)	AAV42.8 (See SEQ ID NO: 27 in US20030138772)
AAV42.8 (See SEQ ID NO: 85 in US20030138772)	AAV43.1 (See SEQ ID NO: 39 in US20030138772)
AAV43.1 (See SEQ ID NO: 92 in US20030138772)	AAV43.12 (See SEQ ID NO: 41 in US20030138772)

AAV43.12 (See SEQ ID NO: 93 in US20030138772)	AAV8 (See SEQ ID NO: 15 in US20150159173)
AAV8 (See SEQ ID NO: 7 in US20150376240)	AAV8 (See SEQ ID NO: 4 in US20030138772; US20150315612 SEQ
ID NO: 182	AAV8 (See SEQ ID NO: 95 in US20030138772), US20140359799 SEQ
AAV8 (See SEQ ID NO: 31 in US20150159173)	AAV8 (See, e.g., SEQ ID NO: 8 in US20160017295, or SEQ ID NO: 7 in US7198951, or SEQ ID NO: 223 in US20150315612)
AAV8 (See SEQ ID NO: 8 in US20150376240)	AAV8 (See SEQ ID NO: 214 in US20150315612)
AAV-8b (See SEQ ID NO: 5 in US20150376240)	AAV-8b (See SEQ ID NO: 3 in US20150376240)
AAV-8h (See SEQ ID NO: 6 in US20150376240)	AAV-8h (See SEQ ID NO: 4 in US20150376240)
AAV9 (See SEQ ID NO: 5 in US20030138772)	AAV9 (See SEQ ID NO: 1 in US7198951)
AAV9 (See SEQ ID NO: 9 in US20160017295)	AAV9 (See SEQ ID NO: 100 in US20030138772), US7198951 SEQ ID NO: 2
	AAV9 (See SEQ ID NO: 3 in US7198951)
AAV9 (AAVhu.14) (See SEQ ID NO: 3 in US20150315612)	AAV9 (AAVhu.14) (See SEQ ID NO: 123 in US20150315612)
AAVA3.1 (See SEQ ID NO: 120 in US20030138772)	AAVA3.3 (See SEQ ID NO: 57 in US20030138772)
AAVA3.3 (See SEQ ID NO: 66 in US20030138772)	AAVA3.4 (See SEQ ID NO: 54 in US20030138772)
AAVA3.4 (See SEQ ID NO: 68 in US20030138772)	AAVA3.5 (See SEQ ID NO: 55 in US20030138772)
AAVA3.5 (See SEQ ID NO: 69 in US20030138772)	AAVA3.7 (See SEQ ID NO: 56 in US20030138772)
AAVA3.7 (See SEQ ID NO: 67 in US20030138772)	AAV29. (See SEQ ID NO: 11 in (AAVbb.1) 161 US20030138772)
AAVC2 (See SEQ ID NO: 61 in US20030138772)	AAVCh.5 (See SEQ ID NO: 46 in US20150159173); US20150315612 SEQ
ID NO: 234	AAVcy.2 (AAV13.3) (See SEQ ID NO: 15 in US20030138772)
AAV24.1 (See SEQ ID NO: 101 in US20030138772)	AAVcy.3 (AAV24.1) (See SEQ ID NO: 16 in US20030138772)
AAV27.3 (See SEQ ID NO: 104 in US20030138772)	AAVcy.4 (AAV27.3) (See SEQ ID NO: 17 in US20030138772)
AAVcy.5 (See SEQ ID NO: 227 in US20150315612)	AAV7.2 (See SEQ ID NO: 103 in US20030138772)
AAVcy.5 (AAV7.2) (See SEQ ID NO: 18 in US20030138772)	AAV16.3 (See SEQ ID NO: 105 in US20030138772)
AAVcy.6 (AAV16.3) (See SEQ ID NO: 10 in US20030138772)	AAVcy.5 (See SEQ ID NO: 8 in US20150159173)
AAVcy.5 (See SEQ ID NO: 24 in US20150159173)	AAVCy.5R1 (See SEQ ID NO: in US20150159173)
AAVCy.5R2 (See SEQ ID NO: in US20150159173)	AAVCy.5R3 (See SEQ ID NO: in US20150159173)

AAVCy.5R4 (See SEQ ID NO: in US20150159173)	AAVDJ (See SEQ ID NO: 3 in US20140359799) and SEQ ID NO: 2 in US7588772)
	AAVDJ (See SEQ ID NO: 2 in US20140359799; and SEQ ID NO: 1 in US7588772)
	AAVDJ-8 (See SEQ ID NO: in US7588772; Grimm et al 2008
AAVDJ-8 (See SEQ ID NO: in US7588772; Grimm et al 2008	AAVF5 (See SEQ ID NO: 110 in US20030138772)
AAVH2 (See SEQ ID NO: 26 in US20030138772)	AAVH6 (See SEQ ID NO: 25 in US20030138772)
AAVhEl. 1 (See SEQ ID NO: 44 in US9233131)	AAVhErl. 14 (See SEQ ID NO: 46 in US9233131)
AAVhErl. 16 (See SEQ ID NO: 48 in US9233131)	AAVhErl. 18 (See SEQ ID NO: 49 in US9233131)
AAVhErl.23 (AAVhEr2.29) (See SEQ ID NO: 53 in US9233131)	AAVhErl.35 (See SEQ ID NO: 50 in US9233131)
AAVhErl.36 (See SEQ ID NO: 52 in US9233131)	AAVhErl.5 (See SEQ ID NO: 45 in US9233131)
AAVhErl.7 (See SEQ ID NO: 51 in US9233131)	AAVhErl.8 (See SEQ ID NO: 47 in US9233131)
AAVhEr2.16 (See SEQ ID NO: 55 in US9233131)	AAVhEr2.30 (See SEQ ID NO: 56 in US9233131)
AAVhEr2.31 (See SEQ ID NO: 58 in US9233131)	AAVhEr2.36 (See SEQ ID NO: 57 in US9233131)
AAVhEr2.4 (See SEQ ID NO: 54 in US9233131)	AAVhEr3.1 (See SEQ ID NO: 59 in US9233131)
AAVhu.1 (See SEQ ID NO: 46 in US20150315612)	AAVhu.1 (See SEQ ID NO: 144 in US20150315612)
AAVhu.10 (AAV16.8) (See SEQ ID NO: 56 in US20150315612)	AAVhu.10 (AAV16.8) (See SEQ ID NO: 156 in US20150315612)
AAVhu.11 (AAV16.12) (See SEQ ID NO: 57 in US20150315612)	AAVhu.11 (AAV16.12) (See SEQ ID NO: 153 in US20150315612)
AAVhu.12 (See SEQ ID NO: 59 in US20150315612)	AAVhu.12 (See SEQ ID NO: 154 in US20150315612)
AAVhu.13 (See SEQ ID NO: 16 in US2015015917 and ID NO: 71 in US20150315612)	
AAVhu.13 (See SEQ ID NO: 32 in US20150159173 and ID NO: 129 in US20150315612)	
AAVhu.136.1 (See SEQ ID NO: 165 in US20150315612)	AAVhu.140.1 (See SEQ ID NO: 166 in US20150315612)
AAVhu.140.2 (See SEQ ID NO: 167 in US20150315612)	AAVhu.145.6 (See SEQ ID NO: 178 in US20150315612)
AAVhu.15 (See SEQ ID NO: 147 in US20150315612)	AAVhu.15 (AAV33.4) (See SEQ ID NO: 50 in US20150315612)
AAVhu.156.1 (See SEQ ID NO: 179 in US20150315612)	AAVhu.16 (See SEQ ID NO: 148 in US20150315612)

AAVhu.16 (AAV33.8) (See SEQ ID NO: 51 in US20150315612)	AAVhu.17 (See SEQ ID NO: 83 in US20150315612)
AAVhu.17 (AAV33.12) (See SEQ ID NO: 4 in US20150315612)	AAVhu.172.1 (See SEQ ID NO: 171 in US20150315612)
AAVhu.172.2 (See SEQ ID NO: 172 in US20150315612)	AAVhu.173.4 (See SEQ ID NO: 173 in US20150315612)
AAVhu.173.8 (See SEQ ID NO: 175 in US20150315612)	AAVhu.18 (See SEQ ID NO: 52 in US20150315612)
AAVhu.18 (See SEQ ID NO: 149 in US20150315612)	AAVhu.19 (See SEQ ID NO: 62 in US20150315612)
AAVhu.19 (See SEQ ID NO: 133 in US20150315612)	AAVhu.2 (See SEQ ID NO: 48 in US20150315612)
AAVhu.2 (See SEQ ID NO: 143 in US20150315612)	AAVhu.20 (See SEQ ID NO: 63 in US20150315612)
AAVhu.20 (See SEQ ID NO: 134 in US20150315612)	AAVhu.21 (See SEQ ID NO: 65 in US20150315612)
AAVhu.21 (See SEQ ID NO: 135 in US20150315612)	AAVhu.22 (See SEQ ID NO: 67 in US20150315612)
AAVhu.22 239 (See SEQ ID NO: 138 in US20150315612)	AAVhu.23 (See SEQ ID NO: 60 in US20150315612)
AAVhu.23.2 (See SEQ ID NO: 137 in US20150315612)	AAVhu.24 (See SEQ ID NO: 66 in US20150315612)
AAVhu.24 (See SEQ ID NO: 136 in US20150315612)	AAVhu.25 (See SEQ ID NO: 49 in US20150315612)
AAVhu.25 (See SEQ ID NO: 146 in US20150315612)	AAVhu.26 (See SEQ ID NO: 17 in US20150159173 and SEQ ID NO: 61 in US20150315612)
	AAVhu.26 (See SEQ ID NO: 33 in US20150159173), US20150315612 SEQ
	AAVhu.27 (See SEQ ID NO: 64 in US20150315612)
AAVhu.27 (See SEQ ID NO: 140 in US20150315612)	AAVhu.28 (See SEQ ID NO: 68 in US20150315612)
AAVhu.28 (See SEQ ID NO: 130 in US20150315612)	AAVhu.29 (See SEQ ID NO: 69 in US20150315612)
AAVhu.29 (See SEQ ID NO: 42 in US20150159173 and SEQ ID NO: 132 in US20150315612)	
AAVhu.29 (See SEQ ID NO: 225 in US20150315612)	AAVhu.29R (See SEQ ID NO: in US20150159173)
AAVhu.3 (See SEQ ID NO: 44 in US20150315612)	AAVhu.3 (See SEQ ID NO: 145 in US20150315612)
AAVhu.30 (See SEQ ID NO: 70 in US20150315612)	AAVhu.30 (See SEQ ID NO: 131 in US20150315612)
AAVhu.31 (See SEQ ID NO: 1 in US20150315612)	AAVhu.31 (See SEQ ID NO: 121 in US20150315612)
AAVhu.32 (See SEQ ID NO: 2 in US20150315612)	AAVhu.32 (See SEQ ID NO: 122 in US20150315612)
AAVhu.33 (See SEQ ID NO: 75 in US20150315612)	AAVhu.33 (See SEQ ID NO: 124 in US20150315612)

AAVhu.34 (See SEQ ID NO: 72 in US20150315612)	AAVhu.34 (See SEQ ID NO: 125 in US20150315612)
AAVhu.35 (See SEQ ID NO: 73 in US20150315612)	AAVhu.35 (See SEQ ID NO: 164 in US20150315612)
AAVhu.36 (See SEQ ID NO: 74 in US20150315612)	AAVhu.36 (See SEQ ID NO: 126 in US20150315612)
AAVhu.37 (See SEQ ID NO: 34 in US20150159173 and SEQ ID NO: 88 in US20150315612)	
AAVhu.37 (AAV106.1) (See SEQ ID NO: 10 in US20150315612 and SEQ ID NO: 18 in US20150159173)	
AAVhu.38 (See SEQ ID NO: 161 in US20150315612)	AAVhu.39 (See SEQ ID NO: 102 in US20150315612)
AAVhu.39 (AAVLG-9) (See SEQ ID NO: 24 in US20150315612)	AAVhu.4 (See SEQ ID NO: 47 in US20150315612)
AAVhu.4 (See SEQ ID NO: 141 in US20150315612)	AAVhu.40 (See SEQ ID NO: 87 in US20150315612)
AAVhu.40 (AAV114.3) (See SEQ ID NO: 11 in US20150315612)	AAVhu.41 (See SEQ ID NO: 91 in US20150315612)
AAVhu.41 (AAV127.2) (See SEQ ID NO: 6 in US20150315612)	AAVhu.42 (See SEQ ID NO: 85 in US20150315612)
AAVhu.42 (AAV127.5) (See SEQ ID NO: 8 in US20150315612)	AAVhu.43 (See SEQ ID NO: 160 in US20150315612)
AAVhu.43 (See SEQ ID NO: 236 in US20150315612)	AAVhu.43 (AAV128.1) (See SEQ ID NO: 80 in US20150315612)
AAVhu.44 (See SEQ ID NO: 45 in US20150159173 and SEQ ID NO: 158 in US20150315612)	
AAVhu.44 (AAV128.3) (See SEQ ID NO: 81 in US20150315612)	AAVhu.44R1 (See SEQ ID NO: in US20150159173)
AAVhu.44R2 (See SEQ ID NO: in US20150159173)	AAVhu.44R3 (See SEQ ID NO: in US20150159173)
AAVhu.45 (See SEQ ID NO: 76 in US20150315612)	AAVhu.45 (See SEQ ID NO: 127 in US20150315612)
AAVhu.46 (See SEQ ID NO: 82 in US20150315612)	AAVhu.46 (See SEQ ID NO: 159 in US20150315612)
AAVhu.46 (See SEQ ID NO: 224 in US20150315612)	AAVhu.47 (See SEQ ID NO: 77 in US20150315612)
AAVhu.47 (See SEQ ID NO: 128 in US20150315612)	AAVhu.48 (See SEQ ID NO: 38 in US20150159173)
AAVhu.48 (See SEQ ID NO: 157 in US20150315612)	AAVhu.48 (AAV130.4) (See SEQ ID NO: 78 in US20150315612)
AAVhu.48R1 (See SEQ ID NO: in US20150159173)	AAVhu.48R2 (See SEQ ID NO: in US20150159173)
AAVhu.48R3 (See SEQ ID NO: in US20150159173)	AAVhu.49 (See SEQ ID NO: 209 in US20150315612)
AAVhu.49 (See SEQ ID NO: 189 in US20150315612)	AAVhu.5 (See SEQ ID NO: 45 in US20150315612)
AAVhu.5 (See SEQ ID NO: 142 in US20150315612)	AAVhu.51 (See SEQ ID NO: 208 in US20150315612)

AAVhu.51 (See SEQ ID NO: 190 in US20150315612)	AAVhu.52 (See SEQ ID NO: 210 in US20150315612)
AAVhu.52 (See SEQ ID NO: 191 in US20150315612)	AAVhu.53 (See SEQ ID NO: 19 in US20150159173)
AAVhu.53 (See SEQ ID NO: 35 in US20150159173)	AAVhu.53 (AAV145.1) (See SEQ ID NO: 176 in US20150315612)
AAVhu.54 (See SEQ ID NO: 188 in US20150315612)	AAVhu.54 (AAV145.5) (See SEQ ID NO: 177 in US20150315612)
AAVhu.55 (See SEQ ID NO: 187 in US20150315612)	AAVhu.56 (See SEQ ID NO: 205 in US20150315612)
AAVhu.56 (AAV145.6) (See SEQ ID NO: 168 in US20150315612)	AAVhu.56 (AAV145.6) (See SEQ ID NO: 192 in US20150315612)
AAVhu.57 (See SEQ ID NO: 206 in US20150315612)	AAVhu.57 (See SEQ ID NO: 169 in US20150315612)
AAVhu.57 (See SEQ ID NO: 193 in US20150315612)	AAVhu.58 (See SEQ ID NO: 207 in US20150315612)
AAVhu.58 (See SEQ ID NO: 194 in US20150315612)	AAVhu.6 (AAV3.1) (See SEQ ID NO: 5 in US20150315612)
AAVhu.6 (AAV3.1) (See SEQ ID NO: 84 in US20150315612)	AAVhu.60 (See SEQ ID NO: 184 in US20150315612)
AAVhu.60 (AAV161.10) (See SEQ ID NO: 170 in US20150315612)	AAVhu.61 (See SEQ ID NO: 185 in US20150315612)
AAVhu.61 (AAV161.6) (See SEQ ID NO: 174 in US20150315612)	AAVhu.63 (See SEQ ID NO: 204 in US20150315612)
AAVhu.63 (See SEQ ID NO: 195 in US20150315612)	AAVhu.64 (See SEQ ID NO: 212 in US20150315612)
AAVhu.64 (See SEQ ID NO: 196 in US20150315612)	AAVhu.66 (See SEQ ID NO: 197 in US20150315612)
AAVhu.67 (See SEQ ID NO: 215 in US20150315612)	AAVhu.67 (See SEQ ID NO: 198 in US20150315612)
AAVhu.7 (See SEQ ID NO: 226 in US20150315612)	AAVhu.7 (See SEQ ID NO: 150 in US20150315612)
AAVhu.7 (AAV7.3) (See SEQ ID NO: 55 in US20150315612)	AAVhu.71 (See SEQ ID NO: 79 in US20150315612)
AAVhu.8 (See SEQ ID NO: 53 in US20150315612)	AAVhu.8 (See SEQ ID NO: 12 in US20150315612)
AAVhu.8 (See SEQ ID NO: 151 in US20150315612)	AAVhu.9 (AAV3.1) (See SEQ ID NO: 58 in US20150315612)
AAVhu.9 (AAV3.1) (See SEQ ID NO: 155 in US20150315612)	AAV-LK01 (See SEQ ID NO: 2 in US20150376607)
AAV-LK01 (See SEQ ID NO: 29 in US20150376607)	AAV-LK02 (See SEQ ID NO: 3 in US20150376607)
AAV-LK02 (See SEQ ID NO: 30 in US20150376607)	AAV-LK03 (See SEQ ID NO: 4 in US20150376607)
AAV-LK03 (See SEQ ID NO: 12 in WO2015121501 and SEQ ID NO: 31 in US20150376607)	
AAV-LK04 (See SEQ ID NO: 5 in US20150376607)	AAV-LK04 (See SEQ ID NO: 32 in US20150376607)
AAV-LK05 (See SEQ ID NO: 6 in US20150376607)	AAV-LK05 (See SEQ ID NO: 33 in US20150376607)

AAV-LK06 (See SEQ ID NO: 7 in US20150376607)	AAV-LK06 (See SEQ ID NO: 34 in US20150376607)
AAV-LK07 (See SEQ ID NO: 8 in US20150376607)	AAV-LK07 (See SEQ ID NO: 35 in US20150376607)
AAV-LK08 (See SEQ ID NO: 9 in US20150376607)	AAV-LK08 (See SEQ ID NO: 36 in US20150376607)
AAV-LK09 (See SEQ ID NO: 10 in US20150376607)	AAV-LK09 (See SEQ ID NO: 37 in US20150376607)
AAV-LK10 (See SEQ ID NO: 11 in US20150376607)	AAV-LK10 (See SEQ ID NO: 38 in US20150376607)
AAV-LK11 (See SEQ ID NO: 12 in US20150376607)	AAV-LK11 (See SEQ ID NO: 39 in US20150376607)
AAV-LK12 (See SEQ ID NO: 13 in US20150376607)	AAV-LK12 (See SEQ ID NO: 40 in US20150376607)
AAV-LK13 (See SEQ ID NO: 14 in US20150376607)	AAV-LK13 (See SEQ ID NO: 41 in US20150376607)
AAV-LK14 (See SEQ ID NO: 15 in US20150376607)	AAV-LK14 (See SEQ ID NO: 42 in US20150376607)
AAV-LK15 (See SEQ ID NO: 16 in US20150376607)	AAV-LK15 (See SEQ ID NO: 43 in US20150376607)
AAV-LK16 (See SEQ ID NO: 17 in US20150376607)	AAV-LK16 (See SEQ ID NO: 44 in US20150376607)
AAV-LK17 (See SEQ ID NO: 18 in US20150376607)	AAV-LK17 (See SEQ ID NO: 45 in US20150376607)
AAV-LK18 (See SEQ ID NO: 19 in US20150376607)	AAV-LK18 (See SEQ ID NO: 46 in US20150376607)
AAV-LK19 (See SEQ ID NO: 20 in US20150376607)	AAV-LK19 (See SEQ ID NO: 47 in US20150376607)
AAV-PAEC (See SEQ ID NO: 1 in US20150376607)	AAV-PAEC (See SEQ ID NO: 48 in US20150376607)
AAV-PAEC11 (See SEQ ID NO: 26 in US20150376607)	AAV-PAEC11 (See SEQ ID NO: 54 in US20150376607)
AAV-PAEC 12 (See SEQ ID NO: 27 in US20150376607)	AAV-PAEC 12 (See SEQ ID NO: 51 in US20150376607)
AAV-PAEC 13 (See SEQ ID NO: 28 in US20150376607)	AAV-PAEC 13 (See SEQ ID NO: 49 in US20150376607)
AAV-PAEC2 (See SEQ ID NO: 21 in US20150376607)	AAV-PAEC2 (See SEQ ID NO: 56 in US20150376607)
AAV-PAEC4 (See SEQ ID NO: 22 in US20150376607)	AAV-PAEC4 (See SEQ ID NO: 55 in US20150376607)
AAV-PAEC6 (See SEQ ID NO: 23 in US20150376607)	AAV-PAEC6 (See SEQ ID NO: 52 in US20150376607)
AAV-PAEC7 (See SEQ ID NO: 24 in US20150376607)	AAV-PAEC7 (See SEQ ID NO: 53 in US20150376607)
AAV-PAEC8 (See SEQ ID NO: 25 in US20150376607)	AAV-PAEC8 (See SEQ ID NO: 50 in US20150376607)
AAVpi.1 (See SEQ ID NO: 28 in US20150315612)	AAVpi.1 (See SEQ ID NO: 93 in US20150315612; AAVpi.2 408, see SEQ ID NO: 30 in US20150315612)
AAVpi.2 (See SEQ ID NO: 95 in US20150315612)	AAVpi.3 (See SEQ ID NO: 29 in US20150315612)

AAVpi.3 (See SEQ ID NO: 94 in US20150315612)	AAVrh.10 (See SEQ ID NO: 9 in US20150159173)
AAVrh.10 (See SEQ ID NO: 25 in US20150159173)	AAV44.2 (See SEQ ID NO: 59 in US20030138772)
AAVrh.10 (AAV44.2) (See SEQ ID NO: 81 in US20030138772)	AAV42.1B (See SEQ ID NO: 90 in US20030138772)
AAVrh.12 (AAV42.1b) (See SEQ ID NO: 30 in US20030138772)	AAVrh.13 (See SEQ ID NO: 10 in US20150159173)
AAVrh.13 (See SEQ ID NO: 26 in US20150159173)	AAVrh.13 (See SEQ ID NO: 228 in US20150315612)
AAVrh.13R (See SEQ ID NO: in US20150159173)	AAV42.3A (See SEQ ID NO: 87 in US20030138772)
AAVrh.14 (AAV42.3a) (See SEQ ID NO: 32 in US20030138772)	AAV42.5A (See SEQ ID NO: 89 in US20030138772)
AAVrh.17 (AAV42.5a) (See SEQ ID NO: 34 in US20030138772)	AAV42.5B (See SEQ ID NO: 91 in US20030138772)
AAVrh.18 (AAV42.5b) (See SEQ ID NO: 29 in US20030138772)	AAV42.6B (See SEQ ID NO: 112 in US20030138772)
AAVrh.19 (AAV42.6b) (See SEQ ID NO: 38 in US20030138772)	AAVrh.2 (See SEQ ID NO: 39 in US20150159173)
AAVrh.2 (See SEQ ID NO: 231 in US20150315612)	AAVrh.20 (See SEQ ID NO: 1 in US20150159173)
AAV42.10 (See SEQ ID NO: 106 in US20030138772)	AAVrh.21 (AAV42.10) (See SEQ ID NO: 35 in US20030138772)
AAV42.11 (See SEQ ID NO: 108 in US20030138772)	AAVrh.22 (AAV42.11) (See SEQ ID NO: 37 in US20030138772)
AAV42.12 (See SEQ ID NO: 113 in US20030138772)	AAVrh.23 (AAV42.12) (See SEQ ID NO: 58 in US20030138772)
AAV42.13 (See SEQ ID NO: 86 in US20030138772)	AAVrh.24 (AAV42.13) (See SEQ ID NO: 31 in US20030138772)
AAV42.15 (See SEQ ID NO: 84 in US20030138772)	AAVrh.25 (AAV42.15) (See SEQ ID NO: 28 in US20030138772)
AAVrh.2R (See SEQ ID NO: in US20150159173)	AAVrh.31 (AAV223.1) (See SEQ ID NO: 48 in US20030138772)
AAVC1 (See SEQ ID NO: 60 in US20030138772)	AAVrh.32 (AAVC1) (See SEQ ID NO: 19 in 446 US20030138772)
AAVrh.32/33 (See SEQ ID NO: 2 in US20150159173)	AAVrh.51 (AAV2-5) (See SEQ ID NO: 104 in US20150315612)
AAVrh.52 (AAV3-9) (See SEQ ID NO: 18 in US20150315612)	AAVrh.52 (AAV3-9) (See SEQ ID NO: 96 in US20150315612)
AAVrh.53 (See SEQ ID NO: in US20150315612)	AAVrh.53 (AAV3-11) (See SEQ ID NO: 17 in US20150315612)
AAVrh.53 (AAV3-11) (See SEQ ID NO: 186 in US20150315612)	AAVrh.54 (See SEQ ID NO: 40 in US20150315612)
AAVrh.54 (See SEQ ID NO: 49 in US20150159173 and SEQ ID NO: 116 in US20150315612)	
AAVrh.55 (See SEQ ID NO: 37 in US20150315612)	AAVrh.55 (AAV4-19) (See SEQ ID NO: 117 in US20150315612)
AAVrh.56 (See SEQ ID NO: 54 in US20150315612)	AAVrh.56 (See SEQ ID NO: 152 in US20150315612)

AAVrh.57 (See SEQ ID NO: in 497 US20150315612 SEQ ID NO: 26	AAVrh.57 (See SEQ ID NO: 105 in US20150315612)
AAVrh.58 (See SEQ ID NO: 27 in US20150315612)	AAVrh.58 (See SEQ ID NO: 48 in US20150159173 and SEQ ID NO: 106 in US20150315612)
	AAVrh.58 (See SEQ ID NO: 232 in US20150315612)
AAVrh.59 (See SEQ ID NO: 42 in US20150315612)	AAVrh.59 (See SEQ ID NO: 110 in US20150315612)
AAVrh.60 (See SEQ ID NO: 31 in US20150315612)	AAVrh.60 (See SEQ ID NO: 120 in US20150315612)
AAVrh.61 (See SEQ ID NO: 107 in US20150315612)	AAVrh.61 (AAV2-3) (See SEQ ID NO: 21 in US20150315612)
AAVrh.62 (AAV2-15) (See SEQ ID NO: 33 in US20150315612)	AAVrh.62 (AAV2-15) (See SEQ ID NO: 114 in US20150315612)
AAVrh.64 (See SEQ ID NO: 15 in US20150315612)	AAVrh.64 (See SEQ ID NO: 43 in US20150159173 and SEQ ID NO: 99 in US20150315612)
	AAVrh.64 (See SEQ ID NO: 233 in US20150315612)
AAVrh.64R1 (See SEQ ID NO: in US20150159173	AAVrh.64R2 (See SEQ ID NO: in US20150159173
AAVrh.65 (See SEQ ID NO: 35 in US20150315612)	AAVrh.65 (See SEQ ID NO: 112 in US20150315612)
AAVrh.67 (See SEQ ID NO: 36 in US20150315612)	AAVrh.67 (See SEQ ID NO: 230 in US20150315612)
AAVrh.67 (See SEQ ID NO: 47 in US20150159173 and SEQ ID NO: 47 in US20150315612)	
AAVrh.68 (See SEQ ID NO: 16 in US20150315612)	AAVrh.68 (See SEQ ID NO: 100 in US20150315612)
AAVrh.69 (See SEQ ID NO: 39 in US20150315612)	AAVrh.69 (See SEQ ID NO: 119 in US20150315612)
AAVrh.70 (See SEQ ID NO: 20 in US20150315612)	AAVrh.70 (See SEQ ID NO: 98 in US20150315612)
AAVrh.71 (See SEQ ID NO: 162 in US20150315612)	AAVrh.72 (See SEQ ID NO: 9 in US20150315612)
AAVrh.73 (See SEQ ID NO: 5 in US20150159173)	AAVrh.74 (See SEQ ID NO: 6 in US20150159173)
AAVrh.8 (See SEQ ID NO: 41 in US20150159173)	AAVrh.8 (See SEQ ID NO: 235 in US20150315612)
AAVrh.8R (See SEQ ID NO: 9 in US20150159173, WO2015168666)	AAVrh.8R A586R mutant (See SEQ ID NO: 10 in WO2015168666)
AAVrh.8R R533A mutant (See SEQ ID NO: 11 in WO2015168666)	BAAV (bovine AAV) (See SEQ ID NO: 8 in US9193769)
BAAV (bovine AAV) (See SEQ ID NO: 10 in US9193769)	BAAV (bovine AAV) (See SEQ ID NO: 4 in US9193769)
BAAV (bovine AAV) (See SEQ ID NO: 2 in US9193769)	BAAV (bovine AAV) (See SEQ ID NO: 6 in US9193769)
BAAV (bovine AAV) (See SEQ ID NO: 1 in US9193769)	BAAV (bovine AAV) (See SEQ ID NO: 5 in US9193769)

BAAV (bovine AAV) (See SEQ ID NO: 3 in US9193769)	BAAV (bovine AAV) (See SEQ ID NO: 11 in US9193769)
BAAV (bovine AAV) (See SEQ ID NO: 5 in US7427396)	BAAV (bovine AAV) (See SEQ ID NO: 6 in US7427396)
BAAV (bovine AAV) (See SEQ ID NO: 7 in US9193769)	BAAV (bovine AAV) (See SEQ ID NO: 9 in US9193769)
BNP61 AAV (See SEQ ID NO: 1 in US20150238550)	BNP61 AAV (See SEQ ID NO: 2 in US20150238550)
BNP62 AAV (See SEQ ID NO: 3 in US20150238550)	BNP63 AAV (See SEQ ID NO: 4 in US20150238550)
caprine AAV (See SEQ ID NO: 3 in US7427396)	caprine AAV (See SEQ ID NO: 4 in US7427396)
true type AAV (ttAAV) (See SEQ ID NO: 2 in WO2015121501)	AAAV (Avian AAV) (See SEQ ID NO: 12 in US9238800)
AAAV (Avian AAV) (See SEQ ID NO: 2 in US9238800)	AAAV (Avian AAV) (See SEQ ID NO: 6 in US9238800)
AAAV (Avian AAV) (See SEQ ID NO: 4 in US9238800)	AAAV (Avian AAV) (See SEQ ID NO: 8 in US9238800)
AAAV (Avian AAV) (See SEQ ID NO: 14 in US9238800)	AAAV (Avian AAV) (See SEQ ID NO: 10 in US9238800)
AAAV (Avian AAV) (See SEQ ID NO: 15 in US9238800)	AAAV (Avian AAV) (See SEQ ID NO: 5 in US9238800)
AAAV (Avian AAV) (See SEQ ID NO: 9 in US9238800)	AAAV (Avian AAV) (See SEQ ID NO: 3 in US9238800)
AAAV (Avian AAV) (See SEQ ID NO: 7 in US9238800)	AAAV (Avian AAV) (See SEQ ID NO: 11 in US9238800)
AAAV (Avian AAV) (See SEQ ID NO: in US9238800)	AAAV (Avian AAV) (See SEQ ID NO: 1 in US9238800)
AAV Shuffle 100-1 (See SEQ ID NO: 23 in US20160017295)	AAV Shuffle 100-1 (See SEQ ID NO: 11 in US20160017295)
AAV Shuffle 100-2 (See SEQ ID NO: 37 in US20160017295)	AAV Shuffle 100-2 (See SEQ ID NO: 29 in US20160017295)
AAV Shuffle 100-3 (See SEQ ID NO: 24 in US20160017295)	AAV Shuffle 100-3 (See SEQ ID NO: 12 in US20160017295)
AAV Shuffle 100-7 (See SEQ ID NO: 25 in US20160017295)	AAV Shuffle 100-7 (See SEQ ID NO: 13 in US20160017295)
AAV Shuffle 10-2 (See SEQ ID NO: 34 in US20160017295)	AAV Shuffle 10-2 (See SEQ ID NO: 26 in US20160017295)
AAV Shuffle 10-6 (See SEQ ID NO: 35 in US20160017295)	AAV Shuffle 10-6 (See SEQ ID NO: 27 in US20160017295)
AAV Shuffle 10-8 (See SEQ ID NO: 36 in US20160017295)	AAV Shuffle 10-8 (See SEQ ID NO: 28 in US20160017295)
AAV SM 100-10 (See SEQ ID NO: 41 in US20160017295)	AAV SM 100-10 (See SEQ ID NO: 33 in US20160017295)
AAV SM 100-3 (See SEQ ID NO: 40 in US20160017295)	AAV SM 100-3 (See SEQ ID NO: 32 in US20160017295)
AAV SM 10-1 (See SEQ ID NO: 38 in US20160017295)	AAV SM 10-1 (See SEQ ID NO: 30 in US20160017295)
AAV SM 10-2 (See SEQ ID NO: 10 in US20160017295)	AAV SM 10-2 (See SEQ ID NO: 22 in US20160017295)

AAV SM 10-8 (See SEQ ID NO: 39 in US20160017295)	AAV SM 10-8 (See SEQ ID NO: 31 in US20160017295)
AAV CBr-7.1 (See SEQ ID NO: 4 in WO2016065001)	AAV CBr-7.1 (See SEQ ID NO: 54 in WO2016065001)
AAV CBr-7.10 (See SEQ ID NO: 11 in WO2016065001)	AAV CBr-7.10 (See SEQ ID NO: 61 in WO2016065001)
AAV CBr-7.2 (See SEQ ID NO: 5 in WO2016065001)	AAV CBr-7.2 (See SEQ ID NO: 55 in WO2016065001)
AAV CBr-7.3 (See SEQ ID NO: 6 in WO2016065001)	AAV CBr-7.3 (See SEQ ID NO: 56 in WO2016065001)
AAV CBr-7.4 (See SEQ ID NO: 7 in WO2016065001)	AAV CBr-7.4 (See SEQ ID NO: 57 in WO2016065001)
AAV CBr-7.5 (See SEQ ID NO: 8 in WO2016065001)	AAV CHt-6.6 (See SEQ ID NO: 35 in WO2016065001)
AAV CHt-6.6 (See SEQ ID NO: 85 in WO2016065001)	AAV CHt-6.7 (See SEQ ID NO: 36 in WO2016065001)
AAV CHt-6.7 (See SEQ ID NO: 86 in WO2016065001)	AAV CHt-6.8 (See SEQ ID NO: 37 in WO2016065001)
AAV CHt-6.8 (See SEQ ID NO: 87 in WO2016065001)	AAV CHt-PI (See SEQ ID NO: 29 in WO2016065001)
AAV CHt-PI (See SEQ ID NO: 79 in WO2016065001)	AAV CHt-P2 (See SEQ ID NO: 1 in WO2016065001)
AAV CHt-P2 (See SEQ ID NO: 51 in WO2016065001)	AAV CHt-P5 (See SEQ ID NO: 2 in WO2016065001)
AAV CHt-P5 (See SEQ ID NO: 52 in WO2016065001)	AAV CHt-P6 (See SEQ ID NO: 30 in WO2016065001)
AAV CHt-P6 (See SEQ ID NO: 80 in WO2016065001)	AAV CHt-P8 (See SEQ ID NO: 31 in WO2016065001)
AAV CHt-P8 (See SEQ ID NO: 81 in WO2016065001)	AAV CHt-P9 (See SEQ ID NO: 3 in WO2016065001)
AAV CHt-P9 (See SEQ ID NO: 53 in WO2016065001)	AAV CKd-1 (See SEQ ID NO: 57 in US8734809)
AAV CKd-1 (See SEQ ID NO: 131 in US8734809)	AAV CKd-10 (See SEQ ID NO: 58 in US8734809)
AAV CKd-10 (See SEQ ID NO: 132 in US8734809)	AAV CKd-2 (See SEQ ID NO: 59 in US8734809)
AAV CKd-2 (See SEQ ID NO: 133 in US8734809)	AAV CKd-3 (See SEQ ID NO: 60 in US8734809)
AAV CKd-3 (See SEQ ID NO: 134 in US8734809)	AAV CKd-4 (See SEQ ID NO: 61 in US8734809)
AAV CKd-4 (See SEQ ID NO: 135 in US8734809)	AAV CKd-6 (See SEQ ID NO: 62 in US8734809)
AAV CKd-6 (See SEQ ID NO: 136 in US8734809)	AAV CKd-7 (See SEQ ID NO: 63 in US8734809)
AAV CKd-7 (See SEQ ID NO: 137 in US8734809)	AAV CKd-8 (See SEQ ID NO: 64 in US8734809)
AAV CKd-8 (See SEQ ID NO: 138 in US8734809)	AAV CKd-B 1 (See SEQ ID NO: 73 in US8734809)
AAV CKd-B 1 (See SEQ ID NO: 147 in US8734809)	AAV CKd-B2 (See SEQ ID NO: 74 in US8734809)

AAV CKd-B2 (See SEQ ID NO: 148 in US8734809)	AAV CKd-B3 (See SEQ ID NO: 75 in US8734809)
AAV CKd-B3 (See SEQ ID NO: in US8734809)	AAV CKd-B3 (See SEQ ID NO: 149 in US8734809)
AAV CLv-1 (See SEQ ID NO: 65 in US8734809)	AAV CLv-1 (See SEQ ID NO: 139 in US8734809)
AAV CLv1-1 (See SEQ ID NO: 171 in US8734809)	AAV Civ 1-10 (See SEQ ID NO: 178 in US8734809)
AAV CLv1-2 (See SEQ ID NO: 172 in US8734809)	AAV CLv-12 (See SEQ ID NO: 66 in US8734809)
AAV CLv-12 (See SEQ ID NO: 140 in US8734809)	AAV CLv1-3 (See SEQ ID NO: 173 in US8734809)
AAV CLv-13 (See SEQ ID NO: 67 in US8734809)	AAV CLv-13 (See SEQ ID NO: 141 in US8734809)
AAV CLv1-4 (See SEQ ID NO: 174 in US8734809)	AAV Civ 1-7 (See SEQ ID NO: 175 in US8734809)
AAV Civ 1-8 (See SEQ ID NO: 176 in US8734809)	AAV Civ 1-9 (See SEQ ID NO: 177 in US8734809)
AAV CLv-2 (See SEQ ID NO: 68 in US8734809)	AAV CLv-2 (See SEQ ID NO: 142 in US8734809)
AAV CLv-3 (See SEQ ID NO: 69 in US8734809)	AAV CLv-3 (See SEQ ID NO: 143 in US8734809)
AAV CLv-4 (See SEQ ID NO: 70 in US8734809)	AAV CLv-4 (See SEQ ID NO: 144 in US8734809)
AAV CLv-6 (See SEQ ID NO: 71 in US8734809)	AAV CLv-6 (See SEQ ID NO: 145 in US8734809)
AAV CLv-8 (See SEQ ID NO: 72 in US8734809)	AAV CLv-8 (See SEQ ID NO: 146 in US8734809)
AAV CLv-D1 (See SEQ ID NO: 22 in US8734809)	AAV CLv-D1 (See SEQ ID NO: 96 in US8734809)
AAV CLv-D2 (See SEQ ID NO: 23 in US8734809)	AAV CLv-D2 (See SEQ ID NO: 97 in US8734809)
AAV CLv-D3 (See SEQ ID NO: 24 in US8734809)	AAV CLv-D3 (See SEQ ID NO: 98 in US8734809)
AAV CLv-D4 (See SEQ ID NO: 25 in US8734809)	AAV CLv-D4 (See SEQ ID NO: 99 in US8734809)
AAV CLv-D5 (See SEQ ID NO: 26 in US8734809)	AAV CLv-D5 (See SEQ ID NO: 100 in US8734809)
AAV CLv-D6 (See SEQ ID NO: 27 in US8734809)	AAV CLv-D6 (See SEQ ID NO: 101 in US8734809)
AAV CLv-D7 (See SEQ ID NO: 28 in US8734809)	AAV CLv-D7 (See SEQ ID NO: 102 in US8734809)
AAV CLv-D8 (See SEQ ID NO: 29 in US8734809)	AAV CLv-D8 (See SEQ ID NO: 103 in US8734809); AAV CLv-K1 762, see SEQ ID NO: 18 in WO2016065001)
AAV CLv-K1 (See SEQ ID NO: 68 in WO2016065001)	AAV CLv-K3 (See SEQ ID NO: 19 in WO2016065001)
AAV CLv-K3 (See SEQ ID NO: 69 in WO2016065001)	AAV CLv-K6 (See SEQ ID NO: 20 in WO2016065001)
AAV CLv-K6 (See SEQ ID NO: 70 in WO2016065001)	AAV CLv-L4 (See SEQ ID NO: 15 in WO2016065001)

AAV CLv-L4 (See SEQ ID NO: 65 in WO2016065001)	AAV CLv-L5 (See SEQ ID NO: 16 in WO2016065001)
AAV CLv-L5 (See SEQ ID NO: 66 in WO2016065001)	AAV CLv-L6 (See SEQ ID NO: 17 in WO2016065001)
AAV CLv-L6 (See SEQ ID NO: 67 in WO2016065001)	AAV CLv-M1 (See SEQ ID NO: 21 in WO2016065001)
AAV CLv-M1 (See SEQ ID NO: 71 in WO2016065001)	AAV CLv-M11 (See SEQ ID NO: 22 in WO2016065001)
AAV CLv-M1 1 (See SEQ ID NO: 72 in WO2016065001)	AAV CLv-M2 (See SEQ ID NO: 23 in WO2016065001)
AAV CLv-M2 (See SEQ ID NO: 73 in WO2016065001)	AAV CLv-M5 (See SEQ ID NO: 24 in WO2016065001)
AAV CLv-M5 (See SEQ ID NO: 74 in WO2016065001)	AAV CLv-M6 (See SEQ ID NO: 25 in WO2016065001)
AAV CLv-M6 (See SEQ ID NO: 75 in WO2016065001)	AAV CLv-M7 (See SEQ ID NO: 26 in WO2016065001)
AAV CLv-M7 (See SEQ ID NO: 76 in WO2016065001)	AAV CLv-M8 (See SEQ ID NO: 27 in WO2016065001)
AAV CLv-M8 (See SEQ ID NO: 77 in WO2016065001)	AAV CLv-M9 (See SEQ ID NO: 28 in WO2016065001)
AAV CLv-M9 (See SEQ ID NO: 78 in WO2016065001)	AAV CLv-R1 (See SEQ ID NO: 30 in US8734809)
AAV CLv-R1 (See SEQ ID NO: 104 in US8734809)	AAV CLv-R2 (See SEQ ID NO: 31 in US8734809)
AAV CLv-R2 (See SEQ ID NO: 105 in US8734809)	AAV CLv-R3 (See SEQ ID NO: 32 in US8734809)
AAV CLv-R3 (See SEQ ID NO: 106 in US8734809)	AAV CLv-R4 (See SEQ ID NO: 33 in US8734809)
AAV CLv-R4 (See SEQ ID NO: 107 in US8734809)	AAV CLv-R5 (See SEQ ID NO: 34 in US8734809)
AAV CLv-R5 (See SEQ ID NO: 108 in US8734809)	AAV CLv-R6 (See SEQ ID NO: 35 in US8734809)
AAV CLv-R6 (See SEQ ID NO: 109 in US8734809); AAV CLv-R7 802 (see SEQ ID NO: 36 in US8734809)	AAV CLv-R7 (See SEQ ID NO: 110 in US8734809)
AAV CLv-R8 (See SEQ ID NO: 37 in US8734809)	AAV CLv-R8 (See SEQ ID NO: 111 in US8734809)
AAV CLv-R9 (See SEQ ID NO: 38 in US8734809)	AAV CLv-R9 (See SEQ ID NO: 112 in US8734809)
AAV CSp-1 (See SEQ ID NO: 45 in US8734809)	AAV CSp-1 (See SEQ ID NO: 119 in US8734809)
AAV CSp-10 (See SEQ ID NO: 46 in US8734809)	AAV CSp-10 (See SEQ ID NO: 120 in US8734809)
AAV CSp-11 (See SEQ ID NO: 47 in US8734809)	AAV CSp-11 (See SEQ ID NO: 121 in US8734809)
AAV CSp-2 (See SEQ ID NO: 48 in US8734809)	AAV CSp-2 (See SEQ ID NO: 122 in US8734809)
AAV CSp-3 (See SEQ ID NO: 49 in US8734809)	AAV CSp-3 (See SEQ ID NO: 123 in US8734809)
AAV CSp-4 (See SEQ ID NO: 50 in US8734809)	AAV CSp-4 (See SEQ ID NO: 124 in US8734809)

AAV CSp-6 (See SEQ ID NO: 51 in US8734809)	AAV CSp-6 (See SEQ ID NO: 125 in US8734809)
AAV CSp-7 (See SEQ ID NO: 52 in US8734809)	AAV CSp-7 (See SEQ ID NO: 126 in US8734809)
AAV CSp-8 (See SEQ ID NO: 53 in US8734809)	AAV CSp-8 (See SEQ ID NO: 127 in US8734809)
AAV CSp-8.10 (See SEQ ID NO: 38 in WO2016065001)	AAV CSp-8.10 (See SEQ ID NO: 88 in WO2016065001)
AAV CSp-8.2 (See SEQ ID NO: 39 in WO2016065001)	AAV CSp-8.2 (See SEQ ID NO: 89 in WO2016065001)
AAV CSp-8.4 (See SEQ ID NO: 40 in WO2016065001)	AAV CSp-8.4 (See SEQ ID NO: 90 in WO2016065001)
AAV CSp-8.5 (See SEQ ID NO: 41 in WO2016065001)	AAV CSp-8.5 (See SEQ ID NO: 91 in WO2016065001)
AAV CSp-8.6 (See SEQ ID NO: 42 in WO2016065001)	AAV CSp-8.6 (See SEQ ID NO: 92 in WO2016065001)
AAV CSp-8.7 (See SEQ ID NO: 43 in WO2016065001)	AAV CSp-8.7 (See SEQ ID NO: 93 in WO2016065001)
AAV CSp-8.8 (See SEQ ID NO: 44 in WO2016065001)	AAV CSp-8.8 (See SEQ ID NO: 94 in WO2016065001)
AAV CSp-8.9 (See SEQ ID NO: 45 in WO2016065001)	AAV CSp-8.9 (See SEQ ID NO: 95 in WO2016065001)
AAV CSp-9 842 (See SEQ ID NO: 54 in US8734809)	AAV CSp-9 (See SEQ ID NO: 128 in US8734809)
AAV.hu.48R3 (See SEQ ID NO: 183 in US8734809)	AAV.VR-355 (See SEQ ID NO: 181 in US8734809)
AAV3B (See SEQ ID NO: 48 in WO2016065001)	AAV3B (See SEQ ID NO: 98 in WO2016065001)
AAV4 (See SEQ ID NO: 49 in WO2016065001)	AAV4 (See SEQ ID NO: 99 in WO2016065001)
AAV5 (See SEQ ID NO: 50 in WO2016065001)	AAV5 (See SEQ ID NO: 100 in WO2016065001)
AAVF1/HSC1 (See SEQ ID NO: 20 in WO2016049230)	AAVF1/HSC1 (See SEQ ID NO: 2 in WO2016049230)
AAVF11/HSC11 (See SEQ ID NO: 26 in WO2016049230)	AAVF11/HSC11 (See SEQ ID NO: 4 in WO2016049230)
AAVF12/HSC12 (See SEQ ID NO: 30 in WO2016049230)	AAVF12/HSC12 (See SEQ ID NO: 12 in WO2016049230)
AAVF13/HSC13 (See SEQ ID NO: 31 in WO2016049230)	AAVF13/HSC13 (See SEQ ID NO: 14 in WO2016049230)
AAVF14/HSC14 (See SEQ ID NO: 32 in WO2016049230)	AAVF14/HSC14 (See SEQ ID NO: 15 in WO2016049230)
AAVF15/HSC15 (See SEQ ID NO: 33 in WO2016049230)	AAVF15/HSC15 (See SEQ ID NO: 16 in WO2016049230)
AAVF16/HSC16 (See SEQ ID NO: 34 in WO2016049230)	AAVF16/HSC16 (See SEQ ID NO: 17 in WO2016049230)
AAVF17/HSC17 (See SEQ ID NO: 35 in WO2016049230)	AAVF17/HSC17 (See SEQ ID NO: 13 in WO2016049230)
AAVF2/HSC2 (See SEQ ID NO: 21 in WO2016049230)	AAVF2/HSC2 (See SEQ ID NO: 3 in WO2016049230)

AAVF3/HSC3 (See SEQ ID NO: 22 in WO2016049230)	AAVF3/HSC3 (See SEQ ID NO: 5 in WO2016049230)
AAVF4/HSC4 (See SEQ ID NO: 23 in WO2016049230)	AAVF4/HSC4 (See SEQ ID NO: 6 in WO2016049230)
AAVF5/HSC5 (See SEQ ID NO: 25 in WO2016049230)	AAVF5/HSC5 (See SEQ ID NO: 11 in WO2016049230)
AAVF6/HSC6 (See SEQ ID NO: 24 in WO2016049230)	AAVF6/HSC6 (See SEQ ID NO: 7 in WO2016049230)
AAVF7/HSC7 (See SEQ ID NO: 27 in WO2016049230)	AAVF7/HSC7 (See SEQ ID NO: 8 in WO2016049230)
AAVF8/HSC8 (See SEQ ID NO: 28 in WO2016049230)	AAVF8/HSC8 (See SEQ ID NO: 9 in WO2016049230)
AAVF9/HSC9 (See SEQ ID NO: 10 in WO2016049230)	AAVF9/HSC9 882 (see SEQ ID NO: 29 in WO2016049230)

[00420] In some embodiments of any of the aspects, the AAV vector (also referred to as a rAAV virion) as disclosed herein comprises a capsid protein from any of those disclosed in WO2019/241324, which is incorporated herein in its entirety by reference. In some embodiments of any of the aspects, the rAAV vector comprises a liver specific capsid, e.g., a liver specific capsid selected from XL32 and XL32.1, as disclosed in WO2019/241324, which is incorporated herein in its entirety by reference. In some embodiments of any of the aspects, the rAAV vector is a AAVXL32 or AAVXL32.1 as disclosed in WO2019/241324, which is incorporated herein in its entirety by reference.

[00421] Exemplary chimeric or variant capsid proteins that can be used as the AAV capsid in the rAAV vector described herein can be selected from Table 2 from WO 2021/102107; which is incorporated herein by reference herein in its entirety or can be used with any combination with wild type capsid proteins and/or other chimeric or variant capsid proteins now known or later identified and each is incorporated herein. In some embodiments of any of the aspects, the rAAV vector encompassed for use is a chimeric vector, e.g., as disclosed in 9,012,224 and US 7,892,809, which are incorporated herein in their entireties by reference.

[00422] In some embodiments of any of the aspects, the rAAV vector is a haploid rAAV vector, as disclosed in US application US2018/0371496 and PCT/US18/22725, or polyploid rAAV vector, e.g., as disclosed in PCT/US2018/044632 filed on 7/31/2018 and in US application 16/151,110, each of which are incorporated herein in their entirety by reference. In some embodiments of any of the aspects, the rAAV vector is a rAAV3 vector, as disclosed in 9,012,224 and WO 2017/106236 which are incorporated herein in their entirety by reference.

[00423] In some embodiments of any of the aspects, the rAAV is a AAVXL32 or AAVXL32.1 AAV vector as disclosed in WO2019/241324, which is incorporated herein in its entirety by reference. In some embodiments of any of the aspects, the rAAV vector comprises a capsid

disclosed in WO2019241324A1, or International Patent application PCT/US2019/036676, which are incorporated herein in their entireties by reference. In some embodiments of any of the aspects, the AAV vector is a AAV8 vector or a rational haploid comprising an AAV8 capsid protein. In some embodiments of any of the aspects, the recombinant AAV vector is a chimeric AAV vector, haploid AAV vector, a hybrid AAV vector or polyploid AAV vector. In some embodiments of any of the aspects, the recombinant AAV vector is a rational haploid vector, a mosaic AAV vector, a chemically modified AAV vector, or a AAV vector from any AAV serotypes, for example, from any AAV serotype disclosed in Table 1 as disclosed in International Applications WO2020/102645, and WO2020/102667, each of which are incorporated by reference herein in their entirety.

[00424] In some embodiments of any of the aspects, an rAAV vector is an AAV3b capsid. AAV3b capsids encompassed for use are described in 2017/106236, and 9,012,224 and 7,892,809, and International application PCT/US19/61653, filed Nov 15, 2019, and International Applications WO2020/102645, and WO2020/102667, each of which are incorporated herein by reference in their entirety. In addition, AAV3b capsids of the AAV vector for use according to the methods as disclosed herein are disclosed in International Patent Applications WO 2020/102645 and WO2021102107, which are incorporated herein in its entirety by reference herein.

[00425] In some embodiments of any of the aspects, the AAV3b capsid comprises SEQ ID NO: 44 as disclosed in International Patent Applications WO 2020/102645 and WO2021102107, each of which are incorporated by reference herein in its entirety. In some embodiments of any of the aspects, the AAV capsid can be a modified AAV capsid that is derived in whole or in part from the AAV capsid set forth in SEQ ID NO: 44 as disclosed in International Patent Applications WO 2020/102645 and WO2021102107. In some embodiments of any of the aspects, the amino acids from an AAV3b capsid as set forth in SEQ ID NO: 44 as disclosed in International Patent Applications WO 2020/102645 and WO2021102107 can be, or are substituted with amino acids from another capsid of a different AAV serotype, wherein the substituted and/or inserted amino acids can be from any AAV serotype, and can include either naturally occurring or partially or completely synthetic amino acids.

[00426] In some embodiments of any of the aspects, an AAV capsid can be an AAV3b265D capsid. In this particular embodiment, an AAV3b265D capsid comprises a modification in the amino acid sequence of the two-fold axis loop of an AAV3b capsid via replacement of amino acid G265 of the AAV3b capsid with D265. In some embodiments of any of the aspects, an AAV3b265D capsid comprises SEQ ID NO: 46 as set forth in International Patent Applications WO 2020/102645 and WO2021102107, each of which is incorporated by reference herein in its entirety. However, the modified virus capsids of the invention are not limited to AAV capsids set

forth in SEQ ID NO: 46 as set forth in International Patent Applications WO 2020/102645 and WO2021102107. In some embodiments of any of the aspects, the amino acids from AAV3b265D as set forth in SEQ ID NO: 46 can be, or are substituted with amino acids from a capsid from an AAV of a different serotype, wherein the substituted and/or inserted amino acids can be from any AAV serotype, and can include either naturally occurring or partially or completely synthetic amino acids.

[00427] In some embodiments of any of the aspects, an rAAV vector is an AAV3b265D549A capsid. In this particular embodiment, an AAV3b265D549A capsid comprises a modification in the amino acid sequence of the two-fold axis loop of an AAV3b capsid via replacement of amino acid G265 of the AAV3b capsid with D265 and replacement of amino acid T549 of the AAV3b capsid with A549. In some embodiments of any of the aspects, an AAV3b265D549A capsid comprises SEQ ID NO: 50 as disclosed in International Patent Applications WO 2020/102645 and WO2021102107, each of which is incorporated by reference herein in its entirety. However, the modified virus capsids of the invention are not limited to AAV capsids set forth in SEQ ID NO: 50 as disclosed in International Patent Applications WO 2020/102645 and WO2021102107. In some embodiments of any of the aspects, the amino acids from AAV3b265D549A as set forth in SEQ ID NO: 50 as disclosed in International Patent Applications WO 2020/102645 and WO2021102107 can be, or are substituted with amino acids from a capsid from an AAV of a different serotype, wherein the substituted and/or inserted amino acids can be from any AAV serotype, and can include either naturally occurring or partially or completely synthetic amino acids. In some embodiments of any of the aspects, the amino acids from AAV3bSASTG (i.e., a AAV3b capsid comprising Q263A/T265 mutations) can be, or are substituted with amino acids from a capsid from an AAV of a different serotype, wherein the substituted and/or inserted amino acids can be from any AAV serotype, and can include either naturally occurring or partially or completely synthetic amino acids.

[00428] In another embodiment, an rAAV vector is an AAV3b549A capsid. In this particular embodiment, an AAV3b549A capsid comprises a modification in the amino acid sequence of the two-fold axis loop of an AAV3b capsid via replacement of amino acid T549 of the AAV3b capsid with A549. In some embodiments, an AAV3b549A capsid comprises SEQ ID NO: 52 as disclosed in International Patent Applications WO 2020/102645 and WO2021102107, each of which is incorporated by reference herein in its entirety. However, the modified virus capsids of the invention are not limited to AAV capsids set forth in SEQ ID NO: 52 as disclosed in International Patent Applications WO 2020/102645 and WO2021102107. In some embodiments of any of the aspects, the amino acids from AAV3b549A as set forth in SEQ ID NO: 52 as disclosed in International Patent Applications WO 2020/102645 and WO2021102107 can be, or are substituted

with amino acids from a capsid from an AAV of a different serotype, wherein the substituted and/or inserted amino acids can be from any AAV serotype, and can include either naturally occurring or partially or completely synthetic amino acids.

[00429] In another embodiment, an rAAV vector is an AAV3bQ263Y capsid. In this particular embodiment, an AAV3bQ263Y capsid comprises a modification in the amino acid sequence of the two-fold axis loop of an AAV3b capsid via replacement of amino acid Q263 of the AAV3b capsid with Y263. In some embodiments of any of the aspects, an AAV3b549A capsid comprises SEQ ID NO: 54 as disclosed in International Patent Applications WO 2020/102645 and WO2021102107, each of which is incorporated by reference herein in its entirety. However, the modified virus capsids of the invention are not limited to AAV capsids set forth in SEQ ID NO: 54 as disclosed in International Patent Applications WO 2020/102645 and WO2021102107. In some embodiments of any of the aspects, the amino acids from AAV3bQ263Y as set forth in SEQ ID NO: 54 as disclosed in International Patent Applications WO 2020/102645 and WO2021102107 can be, or are substituted with amino acids from a capsid from an AAV of a different serotype, wherein the substituted and/or inserted amino acids can be from any AAV serotype, and can include either naturally occurring or partially or completely synthetic amino acids.

[00430] In another embodiment, an rAAV vector is AAV3bSASTG serotype or comprises a AAV3bSASTG capsid. In this particular embodiment, an AAV3bSASTG capsid comprises a modification in the amino acid sequence to comprise a SASTG mutation, in particular, the AAV3b capsid was modified to resemble AAV2 Q263A/T265 subvariant by introducing these modifications at similar positions in the AAV3b capsid (as disclosed in Messina EL, et al., Adeno-associated viral vectors based on serotype 3b use components of the fibroblast growth factor receptor signaling complex for efficient transduction. Hum. Gene Ther. 2012 Oct; 23(10):1031-4, Piacentino III, Valentino, et al. "X-linked inhibitor of apoptosis protein-mediated attenuation of apoptosis, using a novel cardiac-enhanced adeno-associated viral vector." Human gene therapy 23.6 (2012): 635-646 , which are both incorporated herein in their entirety by reference). Accordingly, in some embodiments of any of the aspects, an rAAV vector is AAV3bSASTG serotype or comprises a AAV3bSASTG capsid comprising a AAV3b Q263A/T265 capsid. In some embodiments of any of the aspects, the amino acids from AAV3bSASTG can be, or are substituted with amino acids from a capsid from an AAV of a different serotype, wherein the substituted and/or inserted amino acids can be from any AAV serotype, and can include either naturally occurring or partially or completely synthetic amino acids.

[00431] One can target desired tissues using the appropriate capsids. For example, the central nervous system using AAV9 or a rhesus capsid or a rational haploid using at least one of a AAV9 or Rhesus viral protein. One can target the muscle using myo AAV, see, e.g., WO2019/2071323

and WO2022/020616, which are each incorporated herein in their entirety by reference.

[00432] In order to facilitate their introduction into a cell, an rAAV vector genome useful in the invention can comprise one or more recombinant nucleic acid constructs that include (1) a heterologous sequence to be expressed (e.g., a polynucleotide as described herein) and (2) viral sequence elements that facilitate integration and expression of the heterologous gene. The viral sequence elements may include those sequences of an AAV vector genome that are required in cis for replication and packaging (e.g., functional ITRs) of the DNA into an AAV capsid. In some embodiments of any of the aspects, such an rAAV vector genome may also contain marker or reporter genes. In some embodiments of any of the aspects, an rAAV vector genome can have one or more of the AAV3b wild-type (WT) cis genes replaced or deleted in whole or in part, but retain functional flanking ITR sequences.

[00433] In some embodiments, the vector is an expression vector. As used herein, the term “expression vector” refers to a vector that directs expression of an RNA or polypeptide from sequences linked to transcriptional regulatory sequences on the vector. The sequences expressed will often, but not necessarily, be heterologous to the cell. An expression vector may comprise additional elements, for example, the expression vector may have two replication systems, thus allowing it to be maintained in two organisms, for example in human cells for expression and in a prokaryotic host for cloning and amplification. Examples of eukaryotic expression vectors include, but are not limited to, pW-LNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; pSVK3, pBPV, pMSG and pSVL available from Amersham Pharmacia Biotech; and pCMVDsRed2-express, pIRES2-DsRed2, pDsRed2-Mito, pCMV-EGFP available from Clontech. Many other vectors are well-known and commercially available. For mammalian cells adenoviral vectors, the pSV and the pCMV series of vectors are particularly well-known non-limiting examples. There are many well-known yeast expression vectors including, without limitation, yeast integrative plasmids (YIp) and yeast replicative plasmids (YRp). For plants the Ti plasmid of agrobacterium is an exemplary expression vector, and plant viruses also provide suitable expression vectors, e.g., tobacco mosaic virus (TMV), potato virus X, and cowpea mosaic virus.

[00434] In some embodiments, the expression vector is a plasmid. Such a plasmid can include a variety of other functional nucleic acid sequences, such as one or more selectable markers, one or more origins of replication, polycloning sites and the like.

[00435] In some embodiments, the method comprises administering the exogenous nucleotide sequence (e.g., a vector comprising the exogenous nucleotide sequence) systemically. Systemic administration can be enteral (e.g., oral, sublingual, and rectal) or parenteral (e.g., injection). Preferred routes of injection for the exogenous nucleotide sequence (e.g., a vector comprising the

exogenous nucleotide sequence) include intravenous, intramuscular, subcutaneous, intra-arterial, intra-articular, intrathecal, and intradermal injections.

[00436] In some embodiments exogenous nucleotide sequence (e.g., a vector comprising the exogenous nucleotide sequence) can be administered concurrently or sequentially with one or more additional therapeutic agents or with one or more saturating agents designed to prevent clearance of the vectors by the reticular endothelial system.

[00437] Where the vector is an AAV vector, the dosage of the vector can be from 1×10^9 gc/kg to 1×10^{15} gc/kg or more, suitably from 5×10^9 gc/kg to 1×10^{13} gc/kg, suitably from 5×10^{10} gc/kg to 5×10^{12} gc/kg. In some embodiments, where the vector is an AAV vector, the dosage of the vector can be from 1×10^{10} gc/kg to 1×10^{15} gc/kg or more, suitably from 1×10^{12} gc/kg to 1×10^{14} gc/kg, suitably from 5×10^{12} gc/kg to 5×10^{13} gc/kg. In some embodiments, the dosage of the vector can be from 1×10^{10} gc/kg to 1×10^{15} gc/kg. In some embodiments, the dosage of the vector can be from 1×10^{12} gc/kg to 1×10^{14} gc/kg. In some embodiments, the dosage of the vector can be from 5×10^{12} gc/kg to 5×10^{13} gc/kg. In some embodiments, the dose of AAV vector administered to a subject is at least about 1.0E9 gc/kg, at least about 1.0E10 gc/kg, at least about 1.0E11 gc/kg, at least about 1.0E12gc/kg, about 1.1E12 gc/kg, about 1.2E12 gc/kg, about 1.3E12 gc/kg, about 1.4E12 gc/kg, about 1.5E12 gc/kg, about 1.6E12 gc/kg, about 1.7E12 gc/kg, about 1.8E12 gc/kg, about 1.9E12 gc/kg, about 2.0E12 gc/kg, about 3.0E12 gc/kg, about 4.0E12 gc/kg, about 5.0E12 gc/kg, about 6.0E12 gc/kg, about 7.0E12 gc/kg, about 8.0E12 gc/kg, about 9.0E12 gc/kg, about 1.0E13 gc/kg, about 1.2E13 gc/kg, about 1.2E13 gc/kg, about 1.2E13 gc/kg, about 1.3E13 gc/kg, about 1.4E13 gc/kg, about 1.5E13 gc/kg, about 1.6E13 gc/kg, about 1.7E13 gc/kg, about 1.8E13 gc/kg, about 1.9E13 gc/kg, about 2.0E13 gc/kg, about 3.0E13 gc/kg, about 4.0E13 gc/kg, about 5.0E13 gc/kg, about 6E13gc/kg, about 7E13gc/kg, about 8E13 gc/kg, about 9E13 gc/kg, about 1.0E14 gc/kg, about 1.2E14 gc/kg, about 1.2E14 gc/kg, about 1.2E14 gc/kg, about 1.3E14 gc/kg, about 1.4E14 gc/kg, about 1.5E14 gc/kg, about 1.6E14 gc/kg, about 1.7E14 gc/kg, about 1.8E14 gc/kg, about 1.9E14 gc/kg, about 2.0E14 gc/kg, about 3.0E14 gc/kg, about 4.0E14 gc/kg, about 5.0E14 gc/kg, about 6E14gc/kg, about 7E14gc/kg, about 8E14 gc/kg, about 9E14 gc/kg, about 1.0E15 gc/kg, about 1.2E15 gc/kg, about 1.2E15 gc/kg, about 1.2E15 gc/kg, about 1.3E15 gc/kg, about 1.4E15 gc/kg, about 1.5E15 gc/kg, about 1.6E15 gc/kg, about 1.7E15 gc/kg, about 1.8E15 gc/kg, about 1.9E15 gc/kg, about 2.0E15 gc/kg, about 3.0E15 gc/kg, about 4.0E15 gc/kg, about 5.0E15 gc/kg, about 6E15gc/kg, about 7E15gc/kg, about 8E15 gc/kg, about 9E15 gc/kg, about 1.0E16 gc/kg, about 1.2E16 gc/kg, about 1.2E16 gc/kg, about 1.2E16 gc/kg, about 1.3E16 gc/kg, about 1.4E16 gc/kg, about 1.5E16 gc/kg, about 1.6E16 gc/kg, about 1.7E16 gc/kg, about 1.8E16 gc/kg, about 1.9E16 gc/kg, about 2.0E16 gc/kg, about 3.0E16 gc/kg, about 4.0E16 gc/kg, about 5.0E16 gc/kg, about 6E16gc/kg, about 7E16gc/kg, about 8E16 gc/kg, or about 9E16 gc/kg. In some

embodiments, exemplary doses for AAV vector are titers of between 1.2×10^{12} and 4.0×10^{12} gc/kg, for example, least about 1.0×10^{12} gc/kg, about 1.1×10^{12} gc/kg, about 1.2×10^{12} gc/kg, about 1.3×10^{12} gc/kg, about 1.4×10^{12} gc/kg, about 1.5×10^{12} gc/kg, about 1.6×10^{12} gc/kg, about 1.7×10^{12} gc/kg, about 1.8×10^{12} gc/kg, about 1.9×10^{12} gc/kg, about 2.0×10^{12} gc/kg, about 2.1×10^{12} gc/kg, about 2.2×10^{12} gc/kg, about 2.3×10^{12} gc/kg, about 2.4×10^{12} gc/kg, about 2.5×10^{12} gc/kg, about 2.6×10^{12} gc/kg, about 2.7×10^{12} gc/kg, about 2.8×10^{12} gc/kg, about 2.9×10^{12} gc/kg, about 3.0×10^{12} gc/kg, about 3.1×10^{12} gc/kg, about 3.2×10^{12} gc/kg, about 3.3×10^{12} gc/kg, about 3.4×10^{12} gc/kg, about 3.5×10^{12} gc/kg, about 3.6×10^{12} gc/kg, about 3.7×10^{12} gc/kg, about 3.8×10^{12} gc/kg, about 3.9×10^{12} gc/kg, about 4.0×10^{12} gc/kg.

[00438] In some embodiments, exemplary doses for AAV vector are titers of between 1×10^{13} gc/kg and 1×10^{15} gc/kg, example, about 1×10^{13} gc/kg, about 3×10^{13} gc/kg, about 5×10^{13} gc/kg, about 6×10^{13} gc/kg, about 8×10^{13} gc/kg, about 9×10^{13} gc/kg, about 1×10^{14} gc/kg, about 3×10^{14} gc/kg, about 6×10^{14} gc/kg, about 9×10^{14} or about 1×10^{15} gc/kg.

[00439] In some embodiments, where the vector is an AAV vector, the dosage of the vector can be within the range of 1.0×10^9 vg/kg to 5.0×10^{12} vg/kg. In some embodiments, the dose of AAV vector administered to a subject is at least about 1.0×10^9 vg/kg, at least about 1.0×10^{10} vg/kg, at least about 1.0×10^{11} vg/kg, at least about 1.0×10^{12} vg/kg, about 1.1×10^{12} vg/kg, about 1.2×10^{12} vg/kg, about 1.3×10^{12} vg/kg, about 1.4×10^{12} vg/kg, about 1.5×10^{12} vg/kg, about 1.6×10^{12} vg/kg, about 1.7×10^{12} vg/kg, about 1.8×10^{12} vg/kg, about 1.9×10^{12} vg/kg, about 2.0×10^{12} vg/kg, about 3.0×10^{12} vg/kg, about 4.0×10^{12} vg/kg, about 5.0×10^{12} vg/kg, about 6.0×10^{12} vg/kg, about 7.0×10^{12} vg/kg, about 8.0×10^{12} vg/kg, about 9.0×10^{12} vg/kg, about 1.0×10^{13} vg/kg, about 1.2×10^{13} vg/kg, about 1.2×10^{13} vg/kg, about 1.2×10^{13} vg/kg, about 1.3×10^{13} vg/kg, about 1.4×10^{13} vg/kg, about 1.5×10^{13} vg/kg, about 1.6×10^{13} vg/kg, about 1.7×10^{13} vg/kg, about 1.8×10^{13} vg/kg, about 1.9×10^{13} vg/kg, about 2.0×10^{13} vg/kg, about 3.0×10^{13} vg/kg, about 4.0×10^{13} vg/kg, about 5.0×10^{13} vg/kg, about 6×10^{13} vg/kg, about 7×10^{13} vg/kg, about 8×10^{13} vg/kg, about 9×10^{13} vg/kg, about 1.0×10^{14} vg/kg, about 1.2×10^{14} vg/kg, about 1.2×10^{14} vg/kg, about 1.2×10^{14} vg/kg, about 1.3×10^{14} vg/kg, about 1.4×10^{14} vg/kg, about 1.5×10^{14} vg/kg, about 1.6×10^{14} vg/kg, about 1.7×10^{14} vg/kg, about 1.8×10^{14} vg/kg, about 1.9×10^{14} vg/kg, about 2.0×10^{14} vg/kg, about 3.0×10^{14} vg/kg, about 4.0×10^{14} vg/kg, about 5.0×10^{14} vg/kg, about 6×10^{14} vg/kg, about 7×10^{14} vg/kg, about 8×10^{14} vg/kg, about 9×10^{14} vg/kg, about 1.0×10^{15} vg/kg, about 1.2×10^{15} vg/kg, about 1.2×10^{15} vg/kg, about 1.2×10^{15} vg/kg, about 1.3×10^{15} vg/kg, about 1.4×10^{15} vg/kg, about 1.5×10^{15} vg/kg, about 1.6×10^{15} vg/kg, about 1.7×10^{15} vg/kg, about 1.8×10^{15} vg/kg, about 1.9×10^{15} vg/kg, about 2.0×10^{15} vg/kg, about 3.0×10^{15} vg/kg, about 4.0×10^{15} vg/kg, about 5.0×10^{15} vg/kg, about 6×10^{15} vg/kg, about 7×10^{15} vg/kg, about 8×10^{15} vg/kg, about 9×10^{15} vg/kg, about 1.0×10^{16} vg/kg, about 1.2×10^{16} vg/kg, about 1.2×10^{16} vg/kg, about 1.2×10^{16} vg/kg, about 1.3×10^{16} vg/kg, about 1.4×10^{16} vg/kg, about 1.5×10^{16} vg/kg, about 1.6×10^{16} vg/kg, about 1.7×10^{16} vg/kg, about 1.8×10^{16} vg/kg, about 1.9×10^{16} vg/kg, about 2.0×10^{16} vg/kg, about 3.0×10^{16} vg/kg, about 4.0×10^{16} vg/kg, about 5.0×10^{16} vg/kg, about 6×10^{16} vg/kg, about 7×10^{16} vg/kg, about 8×10^{16} vg/kg, or about 9×10^{16} vg/kg.

vg/kg. In some embodiments, exemplary doses for AAV vector are titers of between 1.2E12 and 4.0E12 vg/kg, for example, least about 1.0E12 vg/kg, about 1.1E12 vg/kg, about 1.2E12 vg/kg, about 1.3E12 vg/kg, about 1.4E12 vg/kg, about 1.5E12 vg/kg, about 1.6E12 vg/kg, about 1.7E12 vg/kg, about 1.8E12 vg/kg, about 1.9E12 vg/kg, about 2.0E12 vg/kg, about 2.1E12 vg/kg, about 2.2E12 vg/kg, about 2.3E12 vg/kg, about 2.4E12 vg/kg, about 2.5E12 vg/kg, about 2.6E12 vg/kg, about 2.7E12 vg/kg, about 2.8E12 vg/kg, about 2.9E12 vg/kg, about 3.0E12 vg/kg, about 3.1E12 vg/kg, about 3.2E12 vg/kg, about 3.3E12 vg/kg, about 3.4E12 vg/kg, about 3.5E12 vg/kg, about 3.6E12 vg/kg, about 3.7E12 vg/kg, about 3.8E12 vg/kg, about 3.9E12 vg/kg, about 4.0E12 vg/kg.

[00440] In some embodiments, exemplary doses for AAV vector are titers of between 1E13 vg/kg and 1E15 vg/kg, example, about 1e13 vg/kg, about 3e13 vg/kg, about 5e13 vg/kg, about 6e13 vg/kg, about 8e13 vg/kg, about 9e13 vg/kg, about 1e14 vg/kg, about 3e14 vg/kg, about 6e14 vg/kg, about 9e14 or about 1e15vg/kg.

[00441] In some embodiments, where the vector is an AAV vector, the dosage of the vector can be within the range of 1.0E9 vg to 5.0E12vg. In some embodiments, the dose of AAV vector administered to a subject is at least about 1.0E9 vg, at least about 1.0E10 vg, at least about 1.0E11 vg, at least about 1.0E12vg, about 1.1E12 vg, about 1.2E12 vg, about 1.3E12 vg, about 1.4E12 vg, about 1.5E12 vg, about 1.6E12 vg, about 1.7E12 vg, about 1.8E12 vg, about 1.9E12 vg, about 2.0E12 vg, about 3.0E12 vg, about 4.0E12 vg, about 5.0E12 vg, about 6.0E12 vg, about 7.0E12 vg, about 8.0E12 vg, about 9.0E12 vg, about 1.0E13 vg, about 1.2E13 vg, about 1.2E13 vg, about 1.2E13 vg, about 1.3E13 vg, about 1.4E13 vg, about 1.5E13 vg, about 1.6E13 vg, about 1.7E13 vg, about 1.8E13 vg, about 1.9E13 vg, about 2.0E13 vg, about 3.0E13 vg, about 4.0E13 vg, about 5.0E13 vg, about 6E13vg, about 7E13vg, about 8E13 vg, about 9E13 vg, about 1.0E14 vg, about 1.2E14 vg, about 1.2E14 vg, about 1.2E14 vg, about 1.3E14 vg, about 1.4E14 vg, about 1.5E14 vg, about 1.6E14 vg, about 1.7E14 vg, about 1.8E14 vg, about 1.9E14 vg, about 2.0E14 vg, about 3.0E14 vg, about 4.0E14 vg, about 5.0E14 vg, about 6E14vg, about 7E14vg, about 8E14 vg, about 9E14 vg, about 1.0E15 vg, about 1.2E15 vg, about 1.2E15 vg, about 1.2E15 vg, about 1.3E15 vg, about 1.4E15 vg, about 1.5E15 vg, about 1.6E15 vg, about 1.7E15 vg, about 1.8E15 vg, about 1.9E15 vg, about 2.0E15 vg, about 3.0E15 vg, about 4.0E15 vg, about 5.0E15 vg, about 6E15vg, about 7E15vg, about 8E15 vg, about 9E15 vg, about 1.0E16 vg, about 1.2E16 vg, about 1.2E16 vg, about 1.2E16 vg, about 1.3E16 vg, about 1.4E16 vg, about 1.5E16 vg, about 1.6E16 vg, about 1.7E16 vg, about 1.8E16 vg, about 1.9E16 vg, about 2.0E16 vg, about 3.0E16 vg, about 4.0E16 vg, about 5.0E16 vg, about 6E16vg, about 7E16vg, about 8E16 vg, or about 9E16 vg. In some embodiments, exemplary doses for AAV vector are titers of between 1.2E12 and 4.0E12 vg, for example, least about 1.0E12 vg, about 1.1E12 vg, about 1.2E12 vg, about 1.3E12 vg, about 1.4E12 vg, about 1.5E12 vg, about 1.6E12 vg, about 1.7E12 vg, about 1.8E12 vg, about 1.9E12 vg, about

2.0E12 vg, about 2.1E12 vg, about 2.2E12 vg, about 2.3E12 vg, about 2.4E12 vg, about 2.5E12 vg, about 2.6E12 vg, about 2.7E12 vg, about 2.8E12 vg, about 2.9E12 vg, about 3.0E12 vg, about 3.1E12 vg, about 3.2E12 vg, about 3.3E12 vg, about 3.4E12 vg, about 3.5E12 vg, about 3.6E12 vg, about 3.7E12 vg, about 3.8E12 vg, about 3.9E12 vg, about 4.0E12 vg.

[00442] In some embodiments, exemplary doses for AAV vector are titers of between 1E13 vg and 1E15 vg, example, about 1e13 vg, about 3e13 vg, about 5e13 vg, about 6e13 vg, about 8e13 vg, about 9e13 vg, about 1e14 vg, about 3e14 vg, about 6e14 vg, about 9e14 or about 1e15vg.

[00443] Gene therapy protocols for therapeutic gene expression in target cells *in vitro* and *in vivo*, are well-known in the art and will not be discussed in detail here. Briefly, they include intramuscular injection, interstitial injection, instillation in airways, application to endothelium, intra-hepatic parenchyme, and intravenous or intra-arterial administration (e.g., intra-hepatic artery, intra- hepatic vein) of plasmid DNA vectors (naked or in liposomes) or viral vectors. Various devices have been developed for enhancing the availability of DNA to the target cell. While a simple approach is to contact the target cell physically with catheters or implantable materials containing the relevant vector, more complex approaches can use jet injection devices and suchlike. Gene transfer into mammalian liver cells has been performed using both *ex vivo* and *in vivo* procedures. The *ex vivo* approach typically requires harvesting of the cells, *in vitro* transduction with suitable expression vectors, followed by reintroduction of the transduced cells into the subject. *In vivo* gene transfer has been achieved by injecting DNA or viral vectors into the liver parenchyma, hepatic artery, or portal vein.

Expression level

[00444] In some embodiments, the method further comprises a step of determining the expression level after administering the inducer. The step of determining the expression level can comprise determining the amount of the product expressed in the subject and/or assessing the response of a subject to the expression product. Standard laboratory techniques can be used to determine the amount of the expression product.

[00445] After determining the expression lever, if a higher amount of the therapeutic product in the subject is desired, the method comprises increasing the amount of inducer administered to the subject at a subsequent administration of the inducer and/or administering a more potent inducer at a subsequent administration of the inducer. Alternately, if a lower amount of the therapeutic product in the subject is desired, the method comprises stopping administering the inducer, lowering the amount of inducer administered to the subject and/or administering a less potent inducer at a subsequent administration of the inducer.

[00446] Without wishing to be bound by a theory, duration of the expression can be adjusted by using inducers with an appropriate *in-vivo* half-life. If expression for a period of a few days or less is required, inducers with a short *in vivo* half-life can be used. As used herein, the term "*in vivo* half-life" refers to the time taken for the serum concentration of an inducer to reduce by 50%, *in vivo*, for example due to degradation of the inducer and/or clearance or sequestration of the inducer by natural mechanisms. The term "short *in vivo* half-life" as used herein refers to *in vivo* half-life less than about 24, less than about 18 hours, less than about 12 hours, less than about 11, less than about 10 hours, less than about 9 hours, less than about 8 hours, less than about 7 hours, less than about 6 hours, less than about 5 hours, less than about 4 hour, or less than about 3 hours. Accordingly, in some embodiments, the inducer has an *in vivo* half-life of less than less than about 24, less than about 18 hours, less than about 12 hours, less than about 11, less than about 10 hours, less than about 9 hours, less than about 8 hours, less than about 7 hours, less than about 6 hours, less than about 5 hours, less than about 4 hour, or less than about 3 hours.

[00447] In some embodiments, the inducer has an *in vivo* half-life of at least one day. For example, the inducer has an *in vivo* half-life of from about one day to about a month. In some embodiments, the inducer has an *in vivo* half-life of about 1 day, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, about 7 days, about 8 days, about 9 days, about 10 days, about 11 days, about 12 days, about 13 days, about 14 days, about 15 days, about 16 days, about 17 days, about 18 days, about 19 days, about 20 days, about 21 days, about 22 days, about 23 days, about 24 days, about 25 days, about 26 days, about 27 days or about 28 days.

[00448] Expression in the liver is of particular interest as it is involved in a wide range of essential functions in the body, including the synthesis of many proteins involved in metabolism, haemostasis, and protection against infection. Given that many diseases are linked to disruption of gene expression in the liver, there is a significant interest in developing gene therapy strategies that allow expression of a transgene in the liver to produce a therapeutic expression product. Diseases associated with aberrant gene expression include, but are not limited to, haemophilia (including haemophilia A or B), familial hypercholesterolemia, ornithine transcarbamylase deficiency, phenylketonuria, ornithine transcarbamylase deficiency, glycogen storage disease, α_1 -antitrypsin deficiency, hereditary hemochromatosis, tyrosinemia type I, argininosuccinic aciduria, hepatitis virus infection, non-viral hepatitis, liver cancer, genetic cholestasis, Wilson's disease, and various other liver diseases (such as non-alcoholic fatty liver disease (NAFLD), and alcohol-related liver disease (ARLD)). Use for the treatment of haemophilia A or B represent preferred embodiments of the various aspects of the invention.

[00449] The method suitably comprises expressing a therapeutically effective amount of the therapeutic product from the gene in the liver of said subject. The therapeutic product may have a

therapeutic effect in the liver or in another location in the subject. For example, the therapeutic product may be released into the blood stream.

Pharmaceutical compositions

[00450] For administration to a subject, the inducer can be provided in pharmaceutically acceptable compositions. These pharmaceutically acceptable compositions comprise an inducer, formulated together with one or more pharmaceutically acceptable carriers (additives) and/or diluents. As described in detail below, the pharmaceutical compositions described herein can be specially formulated for administration in solid or liquid form, including those adapted for the following: (1) oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), gavages, lozenges, dragees, capsules, pills, tablets (e.g., those targeted for buccal, sublingual, and systemic absorption), boluses, powders, granules, pastes for application to the tongue; (2) parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation; (3) topical application, for example, as a cream, ointment, or a controlled-release patch or spray applied to the skin; (4) intravaginally or intrarectally, for example, as a pessary, cream or foam; (5) sublingually; (6) ocularly; (7) transdermally; (8) transmucosally; or (9) nasally. Additionally, compounds can be implanted into a patient or injected using a drug delivery system. See, for example, Urquhart, et al., Ann. Rev. Pharmacol. Toxicol. 24: 199-236 (1984); Lewis, ed. "Controlled Release of Pesticides and Pharmaceuticals" (Plenum Press, New York, 1981); U.S. Pat. No. 3,773,919; and U.S. Pat. No. 3,270,960, content of all of which is herein incorporated by reference.

[00451] As used here, the term "pharmaceutically acceptable" refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[00452] As used here, the term "pharmaceutically-acceptable carrier" means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, manufacturing aid (e.g., lubricant, talc magnesium, calcium or zinc stearate, or steric acid), or solvent encapsulating material, involved in carrying or transporting the subject compound from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2)

starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, methylcellulose, ethyl cellulose, microcrystalline cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) lubricating agents, such as magnesium stearate, sodium lauryl sulfate and talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol (PEG); (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) pH buffered solutions; (21) polyesters, polycarbonates and/or polyanhydrides; (22) bulking agents, such as polypeptides and amino acids (23) serum component, such as serum albumin, HDL and LDL; (22) C₂-C₁₂ alcohols, such as ethanol; and (23) other non-toxic compatible substances employed in pharmaceutical formulations. Wetting agents, coloring agents, release agents, coating agents, sweetening agents, flavoring agents, perfuming agents, preservative and antioxidants can also be present in the formulation. The terms such as "excipient", "carrier", "pharmaceutically acceptable carrier" or the like are used interchangeably herein.

[00453] Examples of solid carriers include starch, sugar, bentonite, silica, and other commonly used carriers. Further non-limiting examples of carriers and diluents which can be used in the formulations comprising a NR4A inhibitor include saline, syrup, dextrose, and water.

[00454] Pharmaceutically-acceptable antioxidants include, but are not limited to, (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acids, and the like.

[00455] The inducer can be formulated in a gelatin capsule, in tablet form, dragee, syrup, suspension, topical cream, suppository, injectable solution, or kits for the preparation of syrups, suspension, topical cream, suppository or injectable solution just prior to use. Also, compounds can be included in composites, which facilitate its slow release into the blood stream, e.g., silicon disc, polymer beads.

[00456] The formulations can conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Techniques, excipients and formulations generally are found in, e.g., *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, Pa. 1985, 17th edition, Nema et al., *PDA J. Pharm. Sci. Tech.* 1997 51:166-171. Methods to make invention formulations include the step of bringing into association or contacting

an ActRIIB compound with one or more excipients or carriers. In general, the formulations are prepared by uniformly and intimately bringing into association one or more compounds with liquid excipients or finely divided solid excipients or both, and then, if appropriate, shaping the product.

[00457] The preparative procedure may include the sterilization of the pharmaceutical preparations. The compounds may be mixed with auxiliary agents such as lubricants, preservatives, stabilizers, salts for influencing osmotic pressure, etc., which do not react deleteriously with the compounds.

[00458] Examples of injectable forms include solutions, suspensions and emulsions. Injectable forms also include sterile powders for extemporaneous preparation of injectable solutions, suspensions or emulsions. The inducer can be injected in association with a pharmaceutical carrier such as normal saline, physiological saline, bacteriostatic water, Cremophor™ EL (BASF, Parsippany, N.J.), phosphate buffered saline (PBS), Ringer's solution, dextrose solution, ethanol, polyol (e.g., glycerol, propylene glycol, and liquid polyethylene glycol), vegetable oils, and suitable mixtures thereof, and other aqueous carriers known in the art. Appropriate non-aqueous carriers may also be used and examples include fixed oils and ethyl oleate. In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, and sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin. A suitable carrier is 5% dextrose in saline. Frequently, it is desirable to include additives in the carrier such as buffers and preservatives or other substances to enhance isotonicity and chemical stability.

[00459] Conventional dosage forms generally provide rapid or immediate drug release from the formulation. Depending on the pharmacology and pharmacokinetics of the drug, use of conventional dosage forms can lead to wide fluctuations in the concentrations of the drug in a patient's blood and other tissues. These fluctuations can impact a number of parameters, such as dose frequency, onset of action, duration of efficacy, maintenance of therapeutic blood levels, toxicity, side effects, and the like. Advantageously, controlled-release formulations can be used to control a drug's onset of action, duration of action, plasma levels within the therapeutic window,

and peak blood levels. In particular, controlled- or extended-release dosage forms or formulations can be used to ensure that the maximum effectiveness of a drug is achieved while minimizing potential adverse effects and safety concerns, which can occur both from under-dosing a drug (i.e., going below the minimum therapeutic levels) as well as exceeding the toxicity level for the drug. In some embodiments, the composition can be administered in a sustained release formulation.

[00460] Controlled-release pharmaceutical products have a common goal of improving drug therapy over that achieved by their non-controlled release counterparts. Ideally, the use of an optimally designed controlled-release preparation in medical treatment is characterized by a minimum of drug substance being employed to cure or control the condition in a minimum amount of time. Advantages of controlled-release formulations include: 1) extended activity of the drug; 2) reduced dosage frequency; 3) increased patient compliance; 4) usage of less total drug; 5) reduction in local or systemic side effects; 6) minimization of drug accumulation; 7) reduction in blood level fluctuations; 8) improvement in efficacy of treatment; 9) reduction of potentiation or loss of drug activity; and 10) improvement in speed of control of diseases or conditions. Kim, Chong-ju, *Controlled Release Dosage Form Design*, 2 (Technomic Publishing, Lancaster, Pa.: 2000).

[00461] Most controlled-release formulations are designed to initially release an amount of drug (active ingredient) that promptly produces the desired therapeutic effect, and gradually and continually release other amounts of drug to maintain this level of therapeutic or prophylactic effect over an extended period of time. In order to maintain this constant level of drug in the body, the drug must be released from the dosage form at a rate that will replace the amount of drug being metabolized and excreted from the body. Controlled-release of an active ingredient can be stimulated by various conditions including, but not limited to, pH, ionic strength, osmotic pressure, temperature, enzymes, water, and other physiological conditions or compounds.

[00462] A variety of known controlled- or extended-release dosage forms, formulations, and devices can be adapted for use with the salts and compositions of the disclosure. Examples include, but are not limited to, those described in U.S. Pat. Nos.: 3,845,770; 3,916,899; 3,536,809; 3,598,123; 4,008,719; 5,674,533; 5,059,595; 5,591,767; 5,120,548; 5,073,543; 5,639,476; 5,354,556; 5,733,566; and 6,365,185; content of each of which is incorporated herein by reference. These dosage forms can be used to provide slow or controlled-release of one or more active ingredients using, for example, hydroxypropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems (such as OROS® (Alza Corporation, Mountain View, Calif. USA)), or a combination thereof to provide the desired release profile in varying proportions.

[00463] In some embodiments, the inducer is prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible

polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc.

[00464] In the case of oral ingestion, excipients useful for solid preparations for oral administration are those generally used in the art, and the useful examples are excipients such as lactose, sucrose, sodium chloride, starches, calcium carbonate, kaolin, crystalline cellulose, methyl cellulose, glycerin, sodium alginate, gum arabic and the like, binders such as polyvinyl alcohol, polyvinyl ether, polyvinyl pyrrolidone, ethyl cellulose, gum arabic, shellac, sucrose, water, ethanol, propanol, carboxymethyl cellulose, potassium phosphate and the like, lubricants such as magnesium stearate, talc and the like, and further include additives such as usual known coloring agents, disintegrators such as alginic acid and PrimogelTM, and the like. The compounds can be orally administered, for example, with an inert diluent, or with an assimilable edible carrier, or they may be enclosed in hard or soft shell capsules, or they may be compressed into tablets, or they may be incorporated directly with the food of the diet. For oral therapeutic administration, these compounds may be incorporated with excipients and used in the form of tablets, capsules, elixirs, suspensions, syrups, and the like. Such compositions and preparations should contain at least 0.1% of compound. The percentage of the agent in these compositions may, of course, be varied and may conveniently be between about 2% to about 60% of the weight of the unit. The amount of compound in such therapeutically useful compositions is such that a suitable dosage will be obtained. Some preferred compositions are prepared so that an oral dosage unit contains between about 1 mg to about 100 mg of the inducer. Examples of bases useful for formulation of suppositories are oleaginous bases such as cacao butter, polyethylene glycol, lanolin, fatty acid triglycerides, witepsol (trademark, Dynamite Nobel Co. Ltd.) and the like. Liquid preparations may be in the form of aqueous or oleaginous suspension, solution, syrup, elixir and the like, which can be prepared by a conventional way using additives. The compositions can be given as a bolus dose, to maximize the circulating levels for the greatest length of time after the dose. Continuous infusion may also be used after the bolus dose.

[00465] The inducer can also be administrated directly to the airways in the form of an aerosol. For administration by inhalation, the compounds in solution or suspension can be delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or hydrocarbon propellant like propane, butane or isobutene. The compounds can also be administrated in a no-pressurized form such as in an atomizer or nebulizer.

[00466] In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, such as, by way of example only, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound described herein and a suitable powder base such as lactose or starch.

[00467] Representative intranasal formulations are described in, for example, U.S. Pat. Nos. 4,476,116, 5,116,817 and 6,391,452. Formulations that include an inducer are prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, fluorocarbons, and/or other solubilizing or dispersing agents known in the art. See, for example, Ansel, H. C. *et al.*, *Pharmaceutical Dosage Forms and Drug Delivery Systems*, Sixth Ed. (1995). Preferably these compositions and formulations are prepared with suitable nontoxic pharmaceutically acceptable ingredients. These ingredients are known to those skilled in the preparation of nasal dosage forms and some of these can be found in REMINGTON: THE SCIENCE AND PRACTICE OF PHARMACY, 21st edition, 2005. The choice of suitable carriers is dependent upon the exact nature of the nasal dosage form desired, e.g., solutions, suspensions, ointments, or gels. Nasal dosage forms generally contain large amounts of water in addition to the active ingredient. Minor amounts of other ingredients such as pH adjusters, emulsifiers or dispersing agents, preservatives, surfactants, gelling agents, or buffering and other stabilizing and solubilizing agents are optionally present. Preferably, the nasal dosage form should be isotonic with nasal secretions

[00468] The inducer can also be administered parenterally. Solutions or suspensions of these compounds can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof in oils. Illustrative oils are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, or mineral oil. In general, water, saline, aqueous dextrose and related sugar solution, and glycols such as, propylene glycol or polyethylene glycol, are preferred liquid carriers, particularly for injectable solutions. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

[00469] It may be advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. As used herein, “dosage unit” refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

[00470] Administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal

administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

[00471] For use with the methods described herein, oral or enteral formulations tablets can be formulated in accordance with conventional procedures employing solid carriers well-known in the art. Capsules employed for oral formulations to be used with the methods described herein can be made from any pharmaceutically acceptable material, such as gelatin or cellulose derivatives. Sustained release oral delivery systems and/or enteric coatings for orally administered dosage forms are also contemplated, such as those described in U.S. Pat. No. 4,704,295, "Enteric Film-Coating Compositions," issued Nov. 3, 1987; U.S. Pat. No. 4,556,552, "Enteric Film-Coating Compositions," issued Dec. 3, 1985; U.S. Pat. No. 4,309,404, "Sustained Release Pharmaceutical Compositions," issued Jan. 5, 1982; and U.S. Pat. No. 4,309,406, "Sustained Release Pharmaceutical Compositions," issued Jan. 5, 1982. As regards formulations for administering a compound of Formula I as disclosed herein, one particularly useful embodiment

[00472] For intravenous injections or drips or infusions, the inducer can be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art. For other parenteral injections, appropriate formulations include aqueous or nonaqueous solutions, preferably with physiologically compatible buffers or excipients. Such excipients are known.

[00473] Parenteral injections may involve bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The pharmaceutical composition described herein may be in a form suitable for parenteral injection as a sterile suspension, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. In one aspect, the active ingredient is in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

[00474] Some exemplary aspects of the disclosure are described by one or more of following numbered Embodiments:

[00475] Embodiment 1: A method of regulating expression of a transgene in a primate subject, the method comprising: (a) administering to the subject at a first time point a first inducer, wherein the primate subject comprises at least one cell comprising an exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene, wherein the inducible promoter comprises a cis-regulatory element (CRE) that is capable of being bound and activated by a

heterodimer of CAR and RXR, wherein the first inducer is artemisinin or a derivative thereof, and wherein the first dose comprises an amount of the inducer that is sufficient to express a measurable level of the transgene in the subject; (b) administering to the subject at least at a second time point a second inducer to continue expression of the transgene at a predetermined level for a predetermined time or to modulate expression of the transgene to a predetermined level, and wherein the second inducer is artemisinin; derivative of artemisinin; a flavonoid compound, e.g. flavone, chrysin, baicalein, or galangin; 1,4-Bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP); phenobarbital (PB); 6-(4Chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde-O-(3,4-dichlorobenzyl)oxime (CITCO); Acetaminophen; Buprenorphine; Phenytoin; Carbamazepine; Valproic Acid; Chlorpromazine; Efavirenz; Nevirapine; Rilpivirine; Etravirine; Diazepam; Cyclophosphamid; Ifosfamide; Cerivastatin; Simvastatin; lovastatin; substituted sulphonamides; Thiazolidin-4-one; Estradiol; Estrone and analogues; 17 α -ethinyl-3,17 β -estradiol (EE2); Dehydroepiandrosterone (DHEA); 5 β -pregnane-3,20-dione; Diethylstilbestrol; Ginkgo *biloba* extract; Galangin; chrysin; baicalein; Diallyl sulphide; Ellagic acid; Resveratrol; Squalestatin-1; Bilobalide; Triclocarban; Triclosan; Dichlorodiphenyltrichloroethane (DDT); Dieldrin; Methoxychlor; Metofluthrin; Permethrin; Pyrethrins; Sulfoxaflor; Diethylhexyl phthalate (DEHP); Cyproconazole; Fluconazole; Propiconazole; FL81; Tri-p-methylphenyl phosphate (TMPP); UM104; or UM145.

[00476] Embodiment 2: The method of Embodiment 1, wherein the artemisinin derivative is of Formula (I) or a pharmaceutically acceptable salt thereof.

[00477] Embodiment 3: The method of any one of Embodiments 1-2, wherein the first and second inducer are the same.

[00478] Embodiment 4: The method of Embodiment 3, wherein the first and second inducer are artemisinin or a derivative thereof.

[00479] Embodiment 5: The method of Embodiment 3, wherein the first inducer is artemisinin and second inducer is artemisinin or a derivative thereof.

[00480] Embodiment 6: The method of any one of Embodiments 1-2, wherein the first and second inducer are different.

[00481] Embodiment 7: The method of Embodiments 6, wherein the first inducer is a first artemisinin derivative, and the second inducer is artemisinin or a second artemisinin derivative.

[00482] Embodiment 8: The method of Embodiment 6, wherein the first inducer is an artemisinin derivative and the second inducer is artemisinin or a derivative thereof.

[00483] Embodiment 9: The method of Embodiment 6, wherein the first inducer is an artemisinin derivative, and the second inducer is artemisinin.

[00484] Embodiment 10: The method of any one of Embodiments 1-9, wherein the artemisinin derivative is a compound of Formula (I), (I-A), (I-B), (I-C), (I-D), (I-E), (I-F), (I-G), (I-H), (I-I), (I-J), (I-K), (I-L), (I-M), (I-M'), (I-N), (I-N'), (I-O) or (I-O').

[00485] Embodiment 11: The method of any one of Embodiments 1-10, wherein the artemisinin derivative is artesunate, dihydroartemisinin, artesunate, or artemether.

[00486] Embodiment 12: The method of any one of Embodiments 1-11, wherein the artemisinin derivative is artesunate, or dihydroartemisinin.

[00487] Embodiment 13: The method of any one Embodiments 1-12, wherein the first and second inducer are administered at about the same amount.

[00488] Embodiment 14: The method of Embodiment 13, wherein the first inducer and the second inducer are administered at different amounts.

[00489] Embodiment 15: The method of Embodiment 14, wherein the second inducer is administered at a lower amount relative to the amount of the first inducer.

[00490] Embodiment 16: The method of Embodiment 15, wherein the second inducer is administered in amount about 1/2, 1/3, 1/4, 1/5, 1/6, 1/7, 1/8, 1/9, 1/10, 1/15, 1/20, 1/25, 1/30, 1/35, 1/40, 1/45, 1/50, 1/55, 1/60, 1/65, 1/70, 1/75, 1/80, 1/85, 1/90, 1/95, 1/100, 1/125, 1/150, 1/175, 1/200, 1/500, 1/1000 or even less of the amount of first the inducer.

[00491] Embodiment 17: The method of Embodiment 16, wherein the second inducer is administered in amount about 1/2, 1/4, 1/5, 1/8, 1/10, 1/20, 1/40, 1/80 or less of the amount of the first inducer.

[00492] Embodiment 18: The method of Embodiment 14, wherein the second inducer is administered at a higher amount relative to the amount of the first inducer.

[00493] Embodiment 19: The method of Embodiment 18, wherein the amount of inducer administered at the second time point is at least about 25%, 50%, about 75%, about 1-fold, about 1.2-folds, about 1.25-folds, about 1.3-folds, about 1.5-folds, about 1.75-folds, about 2-folds, about 2.25-folds, about 2.5-folds, about 2.5-folds, about 2.75-folds, about 3-folds, 4-fold, 5-fold, 6-folds, 7-fold, 8-fold, 9-folds, 10-folds or higher than the amount of the first inducer.

[00494] Embodiment 20: The method of any one of Embodiments 1-19, wherein the first and second inducer are administered independently at a dose from about 0.5 mg/kg to about 150mg/kg.

[00495] Embodiment 21: The method of any one of Embodiments 1-20, wherein the first inducer is administered at a dose from about 1 mg/kg to about 100 mg/kg.

[00496] Embodiment 22: The method of any one of Embodiments 1-21, wherein the first inducer is administered at a dose of about 80mg/kg, about 70mg/kg, about 60mg/kg, about 50mg/kg, about 40mg/kg, about 30mg/kg, about 20mg/kg, about 10mg/kg, about 6mg/kg, about 5mg/kg, or about 1mg/kg.

[00497] Embodiment 23: The method of any one of Embodiments 1-22, wherein the second inducer is administered at a dose from about 1 mg/kg to about 100 mg/kg.

[00498] Embodiment 24: The method of any one of Embodiments 1-23, wherein the second inducer is administered at a dose of 80mg/kg, about 70mg/kg, about 60mg/kg, about 50mg/kg, about 40mg/kg, about 30mg/kg, about 20mg/kg, about 10mg/kg, about 6mg/kg, about 5mg/kg, or about 1mg/kg.

[00499] Embodiment 25: The method of any one of Embodiments 1-14, wherein the first inducer is administered at a dose of about 80 mg/kg or less and the second inducer is administered at a dose of about 20mg/kg or less at the second or subsequent time point.

[00500] Embodiment 26: The method of any one of Embodiments 1-14, wherein the first inducer is administered at a dose of about 40 mg/kg and the second inducer is administered at a dose of about 80mg/kg or less at the second or subsequent time point.

[00501] Embodiment 27: The method of any one of Embodiments 1-14, wherein the first inducer is administered at a dose of about 20mg/kg and the second inducer is administered at a dose of about 80 mg/kg, 40mg/kg, 20mg/kg, 10 mg/kg, 6 mg/kg or 1 mg/kg at the second or subsequent time point.

[00502] Embodiment 28: The method of any one of Embodiments 1-14, wherein the first inducer is administered at a dose of about 10 mg/kg and the second inducer is administered at a dose of about 80mg/kg or less at the second or subsequent time point.

[00503] Embodiment 29: The method of any one of Embodiments 1-14, wherein the first inducer is administered at a dose of about 6 mg/kg and the second inducer is administered at a dose of about 80mg/kg or less at the second or subsequent time point.

[00504] Embodiment 30: The method of any one of Embodiments 1-14, wherein the first inducer is administered at a dose of about 1 mg/kg and the second inducer is administered at a dose of about 80mg/kg or less at the second or subsequent time point.

[00505] Embodiment 31: The method of any one of Embodiments 1-14, wherein the second inducer is administered at a dose of about 80 mg/kg and the first inducer is administered at a dose of about 80 mg/kg or less.

[00506] Embodiment 32: The method of any one of Embodiments 1-14, wherein the second inducer is administered at a dose of about 40 mg/kg and the first inducer is administered at a dose of about 80 mg/kg or less.

[00507] Embodiment 33: The method of any one of Embodiments 1-14, wherein the second inducer is administered at a dose of about 20 mg/kg and the first inducer is administered at a dose of about 80 mg/kg or less.

[00508] Embodiment 34: The method of any one of Embodiments 1-14, wherein the second inducer is administered at a dose of about 10 mg/kg and the first inducer is administered at a dose of about 80 mg/kg or less.

[00509] Embodiment 35: The method of any one of Embodiments 1-14, wherein the second inducer is administered at a dose of about 6 mg/kg and the first inducer is administered at a dose of about 80 mg/kg or less.

[00510] Embodiment 36: The method of any one of Embodiments 1-14, wherein the second inducer is administered at a dose of about 1 mg/kg and the first inducer is administered at a dose of about 80 mg/kg or less.

[00511] Embodiment 37: The method of any one of Embodiments 1-36, wherein the second time point is at least two days after the first time point.

[00512] Embodiment 38: The method of any one of Embodiments 1-37, wherein the second time point is at least one month after the first time point.

[00513] Embodiment 39: The method of any one of Embodiments 1-38, wherein the second time point is at least two months of the first time point.

[00514] Embodiment 40: The method of any one of Embodiments 1-39, wherein the expression of the transgene goes down to a background expression level within one week of administering the inducer.

[00515] Embodiment 41: The method of any one of Embodiments 1-40, wherein the method further comprises a step of determining the expression level of the transgene after administering the first or second inducer.

[00516] Embodiment 42: The method of any one of Embodiments 1-41, wherein the method further comprises a step of determining the expression level prior of the transgene to administering the first or second inducer.

[00517] Embodiment 43: The method of any one of Embodiments 1-42, wherein the method further comprising administering the exogenous nucleotide sequence to the subject prior to administering the first or second inducer.

[00518] Embodiment 44: The method of any one of Embodiments 1-43, wherein the method is a method of therapy, optionally, the method of therapy is a method of gene therapy.

[00519] Embodiment 45: The method of any one of Embodiments 1-44, wherein the exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene is a viral vector.

[00520] Embodiment 46: The method of any one of Embodiments 1-45, wherein the exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene is adeno-associated viral (AAV), lentiviral, adenoviral, herpes viral, or hepatitis viral vector.

[00521] Embodiment 47: The method of any one of Embodiments 1-46, wherein the exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene is an AAV vector, e.g., an AAV gene therapy vector.

[00522] Embodiment 48: The method of any one of Embodiments 1-47, wherein the first and second inducers independently are administered orally or intraperitoneally.

[00523] Embodiment 49: The method of any one of Embodiments 1-48, wherein at least one of the first and second inducer is administered orally.

[00524] Embodiment 50: The method of Embodiment 49, wherein the second inducer is administered orally.

[00525] Embodiment 51: The method of Embodiment 49, wherein the first inducer is administered orally.

[00526] Embodiment 52: The method of any one of Embodiments 1-48, wherein at least one of the first and second inducer is administered intraperitoneally.

[00527] Embodiment 53: The method of Embodiment 52, wherein the second inducer is administered intraperitoneally.

[00528] Embodiment 54: The method of Embodiment 52, wherein the first inducer is administered intraperitoneally.

[00529]

[00530] Embodiment 55: A first and at least a second inducer as defined in Embodiment 1 or Embodiment 2 for use in a method of regulating expression of a transgene in a primate subject, the method comprising:

[00531] administering to the subject at a first time point a first inducer, wherein the primate subject comprises at least one cell comprising an exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene, wherein the synthetic inducible promoter comprises a cis-regulatory element (CRE) that is capable of being bound and activated by a heterodimer of CAR and RXR, wherein the first inducer is artemisinin or a derivative thereof, and wherein the first dose comprises an amount of the inducer that is sufficient to express a measurable level of the transgene in the subject; and

[00532] administering to the subject at least at a second time point a second inducer to continue expression of the transgene at a predetermined level for a predetermined time or to modulate expression of the transgene to a predetermined level, and

[00533] wherein the second inducer is artemisinin; derivative of artemisinin; a flavonoid compound, e.g. flavone, chrysin, baicalein, or galangin; 1,4-Bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP); phenobarbital (PB); 6-(4Chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde-O-(3,4-dichlorobenzyl)oxime (CITCO); Acetaminophen;

Buprenorphine; Phenytoin; Carbamazepine; Valproic Acid; Chlorpromazine; Efavirenz; Nevirapine; Rilpivirine; Etravirine; Diazepam; Cyclophosphamid; Ifosfamide; Cerivastatin; Simvastatin; lovastatin; substituted sulphonamides; Thiazolidin-4-one; Estradiol; Estrone and analogues; 17 α -ethinyl-3;17 β -estradiol (EE2); Dehydroepiandrosterone (DHEA); 5 β -pregnane-3,20-dione; Diethylstilbestrol; Ginkgo *biloba* extract; Galangin; chrysin; baicalein; Diallyl sulphide; Ellagic acid; Resveratrol; Squalastatin-1; Bilobalide; Triclocarban; Triclosan; Dichlorodiphenyltrichloroethane (DDT); Dieldrin; Methoxychlor; Metofluthrin; Permethrin; Pyrethrins; Sulfoxaflor; Diethylhexyl phthalate (DEHP); Cyproconazole; Fluconazole; Propiconazole; FL81; Tri-p-methylphenyl phosphate (TMPP); UM104; or UM145.

[00534] Embodiment 56: The first and at least a second inducer for use according to Embodiment 55, wherein the method is according to any one of Embodiments 3 to 47.

[00535] Embodiment 57: The first and at least a second inducer for use according to Embodiment 55 or 56, which is a method of therapy, suitably a method of gene therapy.

[00536] Embodiment 58: The first and at least a second inducer for use according to any one of Embodiments 55-57, wherein the exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene is an AAV gene therapy vector.

[00537] Embodiment 59: Artemisinin or a derivative of artemisinin, for use as an inducer in a method of regulating expression of a transgene in a primate subject, wherein the primate subject comprises at least one cell comprising an exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene, wherein the synthetic inducible promoter comprises a cis-regulatory element (CRE) that is capable of being bound and activated by a heterodimer of CAR and RXR, wherein the inducer is administered to the subject at a dose which comprises an amount of the inducer that is sufficient to express a therapeutically effective level of the transgene in the subject.

[00538] Embodiment 60: Artemisinin or a derivative of artemisinin for use according to Embodiment 59, wherein the method is a method of therapy, suitably a method of gene therapy.

[00539] Embodiment 61: Artemisinin or a derivative of artemisinin for use according to Embodiment 59 or 60, wherein the artemisinin derivative is a compound as defined in Embodiment 2, optionally wherein the artemisinin derivative is of Formula (I), (I-A), (I-B), (I-C), (I-D), (I-E), (I-F), (I-G), (I-H), (I-I), (I-J), (I-K), (I-L), (I-M), (I-M'), (I-N), (I-N'), (I-O) or (I-O').

[00540] Embodiment 62: Artemisinin or a derivative of artemisinin for use according to any one of Embodiments 59-61, wherein the derivative of artemisinin is artesunate, dihydroartemisinin, artesunate, or artemether.

[00541] Embodiment 63: Artemisinin or a derivative of artemisinin for use according to any one of Embodiments 59-62, wherein artemisinin or a derivative of artemisinin is administered to the primate subject following the prior administration of an inducer to the primate subject.

[00542] Embodiment 64: Artemisinin or a derivative of artemisinin for use according to Embodiment 63, wherein the inducer of the prior administration was a different inducer, or wherein the inducer of the prior administration was the same inducer but at a different dose.

[00543] Embodiment 65: Artemisinin or a derivative of artemisinin for use according to any one of Embodiments 59-64, wherein the exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene is an AAV gene therapy vector.

[00544] Embodiment 66: Artemisinin or a derivative of artemisinin for use according to any one of Embodiments 59-65, wherein the method comprises prior administration of the exogenous nucleotide sequence to the subject prior to administering the inducer.

[00545] Embodiment 67: Artemisinin or a derivative of artemisinin for use according to any one of Embodiments 59-66, wherein the artemisinin or a derivative of artemisinin is administered at a dose of from about 0.1mg/kg to 80mg/kg, from about 0.1mg/kg to about 70mg/kg, from about 0.1mg/kg to about 60mg/kg, from about 0.1mg/kg to about 50mg/kg, from about 0.1mg/kg to about 40mg/kg, from about 0.1mg/kg to about 30mg/kg, from about 0.1mg/kg to about 20mg/kg, from about 0.1mg/kg to about 10mg/kg, from about 0.1mg/kg to about 6mg/kg, from about 0.1mg/kg to about 5mg/kg, or from about 0.1mg/kg to about 1mg/kg.

[00546] Embodiment 68: Artemisinin or a derivative of artemisinin for use according to any one of Embodiments 55-67, wherein the inducer comprising artemisinin or a derivative of artemisinin is administered orally, optionally wherein the composition comprises artesunate.

[00547] Embodiment 69: The method of any one of Embodiments 1-54, wherein a first induction with artemisinin or a derivative thereof increases the transgene expression level to a maximum at about 24 hrs and declines thereafter, wherein,

[00548] a second induction and/or, successive inductions with artemisinin or a derivative thereof has similar induction profile as seen with the first induction, for at least about 1 month, or at least about 2 months, or, at least about 3 months, or, at least about 5 months or, at least about six months or, even more, and wherein,

[00549] the method further comprises single administration of exogenous nucleotide sequence encoding the transgene operatively linked to the synthetic inducible promoter prior to first administering the inducer to induce said first induction.

[00550] Embodiment 70: The method of Embodiment 69, the transgene expression level at about 24 hrs in the first induction, or, second induction, or successive inductions is at least about

200 fold higher than that of prior to administering the inducer for the first time, or, the second time, or, any number of successive administrations.

[00551] Embodiment 71: The method of Embodiment 69, the first induction, the second induction, and/or, the successive inductions are not complete until about 160 hours after administering the inducer.

[00552] Embodiment 72: The method of Embodiment 69, wherein dose of the inducer in successive administrations is reduced from its prior administration.

[00553] Embodiment 73: The method of Embodiment 69, wherein dose of the inducer in successive administrations remains same.

[00554] Embodiment 74: The method of Embodiment 69, wherein the dose of the inducer ranges from about 1mg/kg to about 80mg/kg.

[00555] Embodiment 75: The method of Embodiment 69, wherein the inducers are administered orally.

[00556] Embodiment 76: The method of Embodiment 69, wherein the inducers are administered intraperitoneally.

[00557]

[00558] Embodiment 77: A method of regulating expression of a transgene in a primate subject, the method comprising: administering artemisinin or a derivative thereof to the primate subject, wherein the primate subject comprises at least one cell comprising an exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene, wherein the inducible promoter comprises a cis-regulatory element (CRE) that is capable of being bound and activated by a heterodimer of CAR and RXR, wherein the artemisinin or a derivative thereof is administered in an amount that is sufficient to express a measurable level of the transgene in the subject.

[00559] Embodiment 78: The method of Embodiment 77, wherein the artemisinin derivative is of Formula (I).

[00560] Embodiment 79: The method of Embodiment 77 or 78, wherein the artemisinin derivative is a compound of Formula (I), (I-A), (I-B), (I-C), (I-D), (I-E), (I-F), (I-G), (I-H), (I-I), (I-J), (I-K), (I-L), (I-M), (I-M'), (I-N), (I-N'), (I-O) or (I-O').

[00561] Embodiment 80: The method of any one of Embodiments 77-79, wherein the derivative of artemisinin is artesunate, dihydroartemisinin, artesunate, or artemether.

[00562] Embodiment 81: The method of any one of Embodiments 77-80, wherein the derivative of artemisinin is artesunate, or dihydroartemisinin.

[00563] Embodiment 82: The method of any one of Embodiments 77-81, wherein the artemisinin or a derivative thereof is administered at a dose from about 0.5 mg/kg to about 150mg/kg.

[00564] Embodiment 83: The method of any one of Embodiments 77-82, wherein the artemisinin or a derivative thereof is administered at a dose from about 1 mg/kg to about 100 mg/kg.

[00565] Embodiment 84: The method of any one of Embodiments 77-83, wherein the artemisinin or a derivative thereof is administered at a dose from about 1 mg/kg to about 80 mg/kg.

[00566] Embodiment 85: The method of any one of Embodiments 77-84, wherein the artemisinin or a derivative thereof is administered at a dose from about 1 mg/kg to about 40 mg/kg.

[00567] Embodiment 86: The method of any one of Embodiments 77-85, wherein the artemisinin or a derivative thereof is administered at a dose from about 1 mg/kg to about 20 mg/kg.

[00568] Embodiment 87: The method of any one of Embodiments 77-86, wherein the artemisinin or a derivative thereof is administered at a dose from about 1 mg/kg to about 10 mg/kg.

[00569] Embodiment 88: The method of any one of Embodiments 77-84, wherein the artemisinin or a derivative thereof is administered at a dose of about 80mg/kg, about 70mg/kg, about 60mg/kg, about 50mg/kg, about 40mg/kg, about 30mg/kg, about 20mg/kg, about 10mg/kg, about 6mg/kg, about 5mg/kg, or about 1mg/kg.

[00570] Embodiment 89: The method of any one of Embodiments 77-88, wherein the method further comprises administering the exogenous nucleotide sequence to the subject prior to administering the artemisinin or a derivative thereof.

[00571] Embodiment 90: The method of any one of Embodiments 77-89, wherein the artemisinin or a derivative thereof is administered to the primate subject following the prior administration of an inducer to the primate subject, and optionally, the inducer is administered in an amount that is sufficient to express a measurable level of the transgene in the subject.

[00572] Embodiment 91: The method of Embodiment 90, wherein the inducer of the prior administration is different from the artemisinin or a derivative thereof administered to the subject.

[00573] Embodiment 92: The method of Embodiment 90, wherein the inducer of the prior administration is same the artemisinin or a derivative thereof administered to the subject but at a different dose.

[00574] Embodiment 93: The method of any one of Embodiments 77-92, wherein the method is a method of therapy, optionally, the method of therapy is a method of gene therapy.

[00575] Embodiment 94: The method of any one of Embodiments 77-93, wherein the exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene is a viral vector.

[00576] Embodiment 95: The method of any one of Embodiments 77-94, wherein the exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene is adeno-associated viral (AAV), lentiviral, adenoviral, herpes viral, or hepatitis viral vector.

[00577] Embodiment 96: The method of any one of Embodiments 77-95, wherein the exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene is an AAV vector, e.g., an AAV gene therapy vector.

[00578] Embodiment 97: The method of any one of Embodiments 77-96, wherein the artemisinin or a derivative thereof is administered orally.

[00579] Embodiment 98: The method of any one of Embodiments 77-96, wherein the artemisinin or a derivative thereof is administered intraperitoneally.

Following were not in the provisional claims

[00580] **Embodiment 99: A method of regulating expression of a transgene in a primate subject, the method comprising: administering a flavonoid compound to the primate subject, wherein the primate subject comprises at least one cell comprising an exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene, wherein the inducible promoter comprises a cis-regulatory element (CRE) that is capable of being bound and activated by a heterodimer of CAR and RXR, wherein the flavonoid compound is administered in an amount that is sufficient to express a measurable level of the transgene in the subject.**

[00581] Embodiment 100: The method of Embodiment 99, wherein the flavonoid compound is of Formula (II-A).

[00582] Embodiment 101: The method of Embodiment 99, wherein the flavonoid compound is of Formula (II-B).

[00583] Embodiment 102: The method of Embodiment 99, wherein the flavonoid compound is of Formula (II-C).

[00584] Embodiment 103: The method of Embodiment 99, wherein the flavonoid compound is flavone, baicalein, chrysin, galangin, (OH)₄ flavone, 6-hydroxyluteolin, acacetin, acerosin, alnetin, apigenin, apiin, artocarpetin, balcalein, beta-sitosterol, biochamin A, cannaflavin A, cannflavin A, cannflavin B, cannflavin C, catechin, catechin-3-gallate, cerrosillin, chrysoeriol, chrysoeriol, cirsilinole, cirsilinole, cirsimaritin, corymbosin, cupressuflavone, cyanidine, daidzein, datiscetin, delphinidine, digidrokempferol, dihydroquercetin, diosmetin, diosmin, echiodinin, epicatechin, epicatechin-3-gallate, epigallocatechin, epigallocatechin-3-gallate, eriodiktin, eupatilin, eupatorin, fisetin, fizetin, flavonol, formononetin, fortunelin, gallocatechin, gallocatechin-3-gallate, gardenin D, genistein, genistin, genkwanin, geraldol, geraldone, glycitein, glycitin, gomoeriodiktin, gossypetin, hesperetin, hinokiflavone, hispidulin, hymenoxin, hypolaetin, isoquercetin, isorhamnetin, isorhamnetin, isoscutellarein, isovitexin, jaceosidin, kaempferol, kaempferol, kampferide, largonidin, luteolin, luteolin, malvidine, mikanin, morin, myricetin, myricetin,

naringenin, negletein, neodiosmin, nepetin, nevadensin, nobiletin, nodifloretin, norartocarpetin, norwogonin, onopordin, orientin, oroxylin a, pachipodol, pectolinarigenin, pedalitin, peonidin, petunidin, pilloin, pratol, primetin, primuletin, pruentin, quercetagetin, quercetin, ramnazin, rhoifolin, robinetin, rutin, scaposin, scutellarein, serpyllin, sinensetin, sorbifolin, sudachitin, tangeretin, tangeritin, taxifolin, tectochrysin, tithonine, tricetin, tricin, velutin, vitexin, wightin, wogonin, xanthomicrol, zapotin, zapotinin, 6,7,4'-trishydroxy isoflavone, 7,3'4'-trihydroxy isoflavone, or a pharmaceutically acceptable salt thereof.

[00585] Embodiment 104: The method of any one of Embodiments 99-103, wherein the flavonoid compound is flavone.

[00586] Embodiment 105: The method of any one of Embodiments 99-104, wherein the flavonoid compound is administered at a dose from about 0.5 mg/kg to about 150mg/kg.

[00587] Embodiment 106: The method of any one of Embodiments 99-105, wherein the flavonoid compound is administered at a dose from about 1 mg/kg to about 100 mg/kg.

[00588] Embodiment 107: The method of any one of Embodiments 99-106, wherein the flavonoid compound is administered at a dose from about 1 mg/kg to about 80 mg/kg.

[00589] Embodiment 108: The method of any one of Embodiments 99-107, wherein the flavonoid compound is administered at a dose from about 1 mg/kg to about 40 mg/kg.

[00590] Embodiment 109: The method of any one of Embodiments 99-108, wherein the flavonoid compound is administered at a dose from about 1 mg/kg to about 20 mg/kg.

[00591] Embodiment 110: The method of any one of Embodiments 99-109, wherein the flavonoid compound is administered at a dose from about 1 mg/kg to about 10 mg/kg.

[00592] Embodiment 111: The method of any one of Embodiments 99-105, wherein the flavonoid compound is administered at a dose of about 80mg/kg, about 70mg/kg, about 60mg/kg, about 50mg/kg, about 40mg/kg, about 30mg/kg, about 20mg/kg, about 10mg/kg, about 6mg/kg, about 5mg/kg, or about 1mg/kg.

[00593] Embodiment 112: The method of any one of Embodiments 99-111, wherein the method further comprises administering the exogenous nucleotide sequence to the subject prior to administering the flavonoid compound.

[00594] Embodiment 113: The method of any one of Embodiments 99-112, wherein the flavonoid compound is administered to the primate subject following the prior administration of an inducer to the primate subject, and optionally, the inducer is administered in an amount that is sufficient to express a measurable level of the transgene in the subject.

[00595] Embodiment 114: The method of Embodiment 115, wherein the inducer of the prior administration is different from the flavonoid compound administered to the subject.

[00596] Embodiment 115: The method of Embodiment 115, wherein the inducer of the prior administration is same as the flavonoid compound administered to the subject but at a different dose.

[00597] Embodiment 116: The method of any one of Embodiments 99-115, wherein the method is a method of therapy, optionally, the method of therapy is a method of gene therapy.

[00598] Embodiment 117: The method of any one of Embodiments 99-116, wherein the exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene is a viral vector.

[00599] Embodiment 118: The method of any one of Embodiments 99-117, wherein the exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene is adeno-associated viral (AAV), lentiviral, adenoviral, herpes viral, or hepatitis viral vector.

[00600] Embodiment 119: The method of any one of Embodiments 99-118, wherein the exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene is an AAV vector, e.g., an AAV gene therapy vector.

[00601] Embodiment 120: The method of any one of Embodiments 99-119, wherein the flavonoid compound is administered orally.

[00602] Embodiment 121: The method of any one of Embodiments 99-120, wherein the flavonoid compound is administered intraperitoneally.

[00603] Embodiment 99: The method of any one of Embodiments 1-54 or 69-121, wherein the at least one cell comprising the exogenous nucleotide sequence is a liver cell.

[00604] Some other exemplary aspects of the disclosure are described by one or more of following numbered Embodiments:

[00605] Embodiment 1: A method of regulating expression of a transgene in the liver of a subject, the method comprising: administering to the subject at at least a first time point a dose of a first inducer; administering to the subject at at least a second time point a second dose of a second inducer, wherein the second inducer can be the same as or different than the first inducer; and administering to the subject at at least a third time point a third dose of a third inducer, wherein the third inducer can be the same as or different than the first inducer and the second inducer; wherein the subject comprises at least one liver cell comprising an exogenous nucleotide sequence comprising a synthetic inducible promoter operably linked to a transgene; wherein the synthetic inducible promoter comprises at least one PBREM enhancer; wherein the transgene is detrimental to the liver cell or to the subject when constitutively expressed.

[00606] Embodiment 2: The method of Embodiment 1, wherein the expression of the transgene increases for a predetermined time after each administration.

- [00607] Embodiment 3: The method of any one of Embodiments 1-2, wherein the expression of the transgene increases for a predetermined time after each administration and the magnitude of the increase is positively correlated with the size of the dose.
- [00608] Embodiment 4: The method of any one of Embodiments 1-3, wherein the time between administrations is at least 24 hours, 48 hours, 72 hours, 1 week, 10 days, 2 weeks, 3 weeks, or 4 weeks.
- [00609] Embodiment 5: The method of any one of Embodiments 1-4, wherein the time between administrations is at least 24 hours, 48 hours, or 72 hours.
- [00610] Embodiment 6: The method of any one of Embodiments 1-5, wherein the time between administrations is no more than 2 months, 3 months, 4 months, 5 months, or 6 months.
- [00611] Embodiment 7: The method of any one of Embodiments 1-6, wherein the time between administrations is no more than 2 months.
- [00612] Embodiment 8: The method of any one of Embodiments 1-7, wherein the time between administrations is 5-7 days, 5-10 days, 7-10 days, 7-14 days, 10-14 days, 10-20 days, 14-21 days, 14-28 days, 21-28 days, or 21-34 days.
- [00613] Embodiment 9: The method of any one of Embodiments 1-8, wherein the at least second dose or at least third dose are administered when it is determined that the level of expression of the transgene is decreased to 75% or less of the level of expression of the transgene within the 24 hours after the first dose.
- [00614] Embodiment 10: The method of any one of Embodiments 1-9, wherein the at least second dose or at least third dose are administered when it is determined that the level of expression of the transgene is decreased to 50% or less of the level of expression of the transgene within the 24 hours after the first dose.
- [00615] Embodiment 11: The method of any one of Embodiments 1-10, wherein the at least second dose or at least third dose are administered when it is determined that the level of expression of the transgene is decreased to 25% or less of the level of expression of the transgene within the 24 hours after the first dose.
- [00616] Embodiment 12: The method of any one of Embodiments 1-11, wherein the subsequent dose is administered when it is determined that the level of expression of the transgene is decreased to a background level of expression of the transgene following the 24 hours after the first dose.
- [00617] Embodiment 13: The method of any one of Embodiments 1-12, wherein each dose is no more than 10 mg/kg.
- [00618] Embodiment 14: The method of any one of 13, wherein the second dose and/or third dose is no more than 10 mg/kg.

- [00619] Embodiment 15: The method of any one of Embodiments 1-14, wherein the first dose, the second dose, and/or the third dose is 5 mg/kg or greater.
- [00620] Embodiment 16: The method of any one of Embodiments 1-15, wherein the first dose is 1-80 mg/kg, the second dose is 1-40 mg/kg, and/or the third dose is 1-40 mg/kg.
- [00621] Embodiment 17: The method of any one of Embodiments 1-16, wherein the second dose and subsequent dose is 1-10 mg/kg.
- [00622] Embodiment 18: The method of any one of Embodiments 1-17, wherein the first dose and subsequent dose is 1 mg/kg to 80 mg/kg.
- [00623] Embodiment 19: The method of any one of Embodiments 1-18, wherein the first dose and subsequent dose is 5 mg/kg to 80 mg/kg.
- [00624] Embodiment 20: The method of any one of Embodiments 1-19, wherein the second dose and subsequent dose is 5 mg/kg to 20 mg/kg.
- [00625] Embodiment 21: The method of any one of Embodiments 1-20, wherein the second dose and subsequent dose is 5 mg/kg to 10 mg/kg.
- [00626] Embodiment 22: The method of any one of Embodiments 1-21, wherein the administering comprises: administering a plurality of doses of a first inducer during a period of 2 to 4 weeks, wherein each dose of the first inducer is 160 mg/kg; administering 1 or 2 doses of a second inducer, wherein each dose of the second inducer is 80 mg/kg; administering at least 1 dose of a third inducer, wherein each dose of the third inducer is 40 mg/kg.
- [00627] Embodiment 23: The method of any one of Embodiments 1-22, wherein the administering comprises: administering a plurality of doses of a first inducer during a period of 2 to 4 weeks, wherein each dose of the first inducer is 160 mg/kg or less; administering 1 or 2 doses of a second inducer, wherein each dose of the second inducer is 80 mg/kg or less; administering at least 1 dose of a third inducer, wherein each dose of the third inducer is 40 mg/kg or less.
- [00628] Embodiment 24: The method of claim 22 or 23, wherein a dose of the third inducer is administered until the subject no longer requires the expression of the transgene.
- [00629] Embodiment 25: The method of any one of claims 22-24, wherein the method further comprises repeating the first or second administering step if the subject becomes non-responsive to the inducer.
- [00630] Embodiment 26: The method of any one of Embodiments 1-25, wherein each of the first dose, second dose, and third dose comprises an amount of the inducer that is sufficient to express a measurable level of the transgene in the subject.
- [00631] Embodiment 27: The method of any one of Embodiments 1-26, wherein the first inducer is artemisinin, a derivative of artemisinin, or flavone.

- [00632] Embodiment 28: The method of any one of Embodiments 1-27, wherein the second inducer is artemisinin, a derivative of artemisinin, or flavone.
- [00633] Embodiment 29: The method of any one of Embodiments 1-28, wherein the third inducer is artemisinin, a derivative of artemisinin, or flavone.
- [00634] Embodiment 30: The method of any one of Embodiments 1-29, wherein the first and second inducer are the same.
- [00635] Embodiment 31: The method of claim 30, wherein the first and second inducer are an artemisinin derivative.
- [00636] Embodiment 32: The method of claim 30, wherein the first and second inducer are artemisinin.
- [00637] Embodiment 33: The method of any one of Embodiments 1-32, wherein the first and second inducer are different.
- [00638] Embodiment 34: The method of claims 33, wherein the first inducer is a first artemisinin derivative, and the second inducer is a second artemisinin derivative.
- [00639] Embodiment 35: The method of claim 33, wherein the first inducer is artemisinin, and the second inducer is an artemisinin derivative.
- [00640] Embodiment 36: The method of claim 33, wherein the first inducer is an artemisinin derivative, and the second inducer is artemisinin.
- [00641] Embodiment 37: The method of any one of Embodiments 1-36, wherein the first inducer, second inducer, and third inducer are the same.
- [00642] Embodiment 38: The method of claim 37, wherein the first inducer, second inducer, and third inducer are an artemisinin derivative.
- [00643] Embodiment 39: The method of claim 37, wherein the first inducer, second inducer, and third inducer are artemisinin.
- [00644] Embodiment 40: The method of any one of Embodiments 1-40, wherein the a) first inducer and b) second inducer and third inducer are different, and the second inducer and third inducer are the same.
- [00645] Embodiment 41: The method of claims 40, wherein the first inducer is a first artemisinin derivative, and the second inducer and third inducer are a second artemisinin derivative.
- [00646] Embodiment 42: The method of claim 40, wherein the first inducer is artemisinin, and the second inducer and third inducer are an artemisinin derivative.
- [00647] Embodiment 43: The method of claim 40, wherein the first inducer is an artemisinin derivative, and the second inducer and third inducer are artemisinin.

- [00648] Embodiment 44: The method of any one of Embodiments 1-43, wherein the artemisinin derivative is a compound of Formula (I), (I-A), (I-B), (I-C), (I-D), (I-E), (I-F), (I-G), (I-H), (I-I), (I-J), (I-K), (I-L), (I-M), (I-M'), (I-N), (I-N'), (I-O) or (I-O').
- [00649] Embodiment 45: The method of any one of Embodiments 1-44, wherein the derivative of artemisinin is artesunate, arteether, artemether, or dihydroartemisinin (DHA).
- [00650] Embodiment 46: The method of any one of Embodiments 1-45, wherein the first or second inducer is, independently, artemisinin or artesunate.
- [00651] Embodiment 47: The method of any one of Embodiments 1-46, wherein the first or second inducer is, independently, arteether, artemether, DHA, or flavone.
- [00652] Embodiment 48: The method of any one of Embodiments 1-46, wherein the synthetic inducible promoter comprises at least two PBREM enhancers.
- [00653] Embodiment 49: The method of any one of Embodiments 1-47, wherein the synthetic inducible promoter comprises at least three PBREM enhancers.
- [00654] Embodiment 50: The method of any one of Embodiments 1-48, wherein the synthetic inducible promoter comprises three PBREM enhancers.
- [00655] Embodiment 51: The method of any one of Embodiments 1-49, wherein the synthetic inducible promoter comprises a MinTK promoter.
- [00656] Embodiment 52: The method of any one of Embodiments 1-50, wherein the synthetic inducible promoter comprises a MinTK minimal promoter.
- [00657] Embodiment 53: The method of any one of Embodiments 1-51, wherein the transgene encodes a chimeric antigen receptor (CAR), hormone, antibody reagent, or nuclease.
- [00658] Embodiment 54: The method of claim 53, wherein the hormone is selected from the group consisting of Erythropoietin (EPO) and parathyroid hormone (PTH).
- [00659] Embodiment 55: The method of claim 53, wherein the antibody reagent is selected from the group consisting of α -PD1, α -HER2, α -PCSK9, α -VEGFR2, α -IL17, and α -A β 3-42.
- [00660] Embodiment 56: The method of claim 53, wherein the nuclease is selected from the group consisting of Cas9, Cas12, Cas Phi, CasX, and CasRx.
- [00661] Embodiment 57: The method of any one of Embodiments 1-56, wherein the subject is a primate.
- [00662] Embodiment 58: The method of any one of Embodiments 1-57, wherein the subject is a human.
- [00663] Embodiment 59: The method of any one of Embodiments 1-58, wherein the administration of the first inducer, the second inducer, and/or the third inducer is oral administration.

[00664] Embodiment 60: The method of any one of Embodiments 1-59, wherein the administration of the first inducer, the second inducer, and/or the third inducer is intraperitoneal administration.

[00665] Embodiment 61: The method of any one of Embodiments 1-60, the method further comprising administering the exogenous nucleotide sequence to the subject prior to administering the first inducer.

[00666] Embodiment 62: A first, a second, and a third inducer for use in a method of regulating expression of a transgene in the liver of a subject, the method comprising: administering to the subject at at least a first time point a dose of a first inducer; administering to the subject at at least a second time point a second dose of a second inducer, wherein the second inducer can be the same as or different than the first inducer; and administering to the subject at at least a third time point a third dose of a third inducer, wherein the third inducer can be the same as or different than the first inducer and the second inducer; wherein the subject comprises at least one liver cell comprising an exogenous nucleotide sequence comprising a synthetic inducible promoter operably linked to a transgene; wherein the synthetic inducible promoter comprises at least one PBREM enhancer; wherein the transgene is detrimental to the liver cell or to the subject when constitutively expressed.

[00667] Embodiment 63: A first, a second and a third inducer for use according to claim 62, wherein the method is as defined in any one of claims 2 to 61.

[00668] Embodiment 64: A first, a second and a third inducer for use according to claim 62 or 63, wherein the method is a method of therapy, suitably a method of gene therapy.

[00669] Embodiment 65: Artemisinin or a derivative thereof for use as a first, a second and/or a third inducer in a method of regulating expression of a transgene in the liver of a subject, the method comprising: administering to the subject at at least a first time point a dose of a first inducer; administering to the subject at at least a second time point a second dose of a second inducer, wherein the second inducer can be the same as or different than the first inducer; and administering to the subject at at least a third time point a third dose of a third inducer, wherein the third inducer can be the same as or different than the first inducer and the second inducer; wherein the subject comprises at least one liver cell comprising an exogenous nucleotide sequence comprising a synthetic inducible promoter operably linked to a transgene; wherein the synthetic inducible promoter comprises at least one PBREM enhancer; wherein the transgene is detrimental to the liver cell or to the subject when constitutively expressed.

[00670] Embodiment 66: Artemisinin or a derivative thereof for use according to claim 65, wherein at least two of the first, second and third inducers are each, independently, artemisinin or a derivative thereof.

[00671] Embodiment 67: Artemisinin or a derivative thereof for use according to claim 65 or 66, wherein the first, second and third inducers are each, independently, artemisinin or a derivative thereof.

[00672] Embodiment 68: Artemisinin or a derivative thereof for use according to any one of claims 65 to 67, wherein the method is as defined in any one of claims 2 to 61.

[00673] Embodiment 69: Artemisinin or a derivative thereof for use according to any one of claims 65 to 68, wherein the method is a method of therapy, suitably a method of gene therapy.

[00674] Some further exemplary aspects of the disclosure are described by one or more of following numbered Embodiments:

[00675] Embodiment 1: A method of regulating expression of a transgene in a primate subject, the method comprising: (a) administering to the subject at a first time point a first inducer, wherein the primate subject comprises at least one cell comprising an exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene, wherein the inducible promoter comprises a PBREM enhancer, wherein the first inducer is artemisinin or a derivative thereof or a flavonoid compound, and wherein the inducer is administered in an amount that is sufficient to express a measurable level of the transgene in the subject; and (b) administering to the subject at least at a second time point a second inducer to continue expression of the transgene at a predetermined level for a predetermined time or to modulate expression of the transgene to a predetermined level, and wherein the second inducer is artemisinin; derivative of artemisinin; a flavonoid compound, e.g. flavone, chrysin, baicalein, or galangin; 1,4-Bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP); phenobarbital (PB); 6-(4Chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde-O-(3,4-dichlorobenzyl)oxime (CITCO); Acetaminophen; Buprenorphine; Phenytoin; Carbamazepine; Valproic Acid; Chlorpromazine; Efavirenz; Nevirapine; Rilpivirine; Etravirine; Diazepam; Cyclophosphamid; Ifosfamide; Cerivastatin; Simvastatin; lovastatin; substituted sulphonamides; Thiazolidin-4-one; Estradiol; Estrone and analogues; 17 α -ethinyl-3,17 β -estradiol (EE2); Dehydroepiandrosterone (DHEA); 5 β -pregnane-3,20-dione; Diethylstilbestrol; Ginkgo *biloba* extract; Galangin; chrysin; baicalein; Diallyl sulphide; Ellagic acid; Resveratrol; Squalestatin-1; Bilobalide; Triclocarban; Triclosan; Dichlorodiphenyltrichloroethane (DDT); Dieldrin; Methoxychlor; Metofluthrin; Permethrin; Pyrethrins; Sulfoxaflor; Diethylhexyl phthalate (DEHP); Cyproconazole; Fluconazole; Propiconazole; FL81; Tri-p-methylphenyl phosphate (TMPP); UM104; or UM145.

[00676] Embodiment 2: The method of Embodiment 1, wherein the expression of the transgene increases for a predetermined time after each administration.

[00677] Embodiment 3: The method of Embodiment 1 or 2, wherein the first and second inducer are the same.

[00678] Embodiment 4: The method of Embodiment 3, wherein the first and second inducer are artemisinin, a derivative of artemisinin, or a flavonoid compound.

[00679] Embodiment 5: The method of Embodiment 3, wherein the first inducer and second inducer are artemisinin.

[00680] Embodiment 6: The method of Embodiment 3, wherein the first inducer and second inducer are a derivative of artemisinin.

[00681] Embodiment 7: The method of Embodiment 3, wherein the first inducer and second inducer are a flavonoid compound, optionally, the flavonoid compound is flavone.

[00682] Embodiment 8: The method of any one of Embodiments 1-2, wherein the first and second inducer are different.

[00683] Embodiment 9: The method of Embodiment 8, wherein the first inducer is artemisinin, and the second inducer is an artemisinin derivative or a flavonoid compound.

[00684] Embodiment 10: The method of Embodiments 8, wherein the first inducer is a first artemisinin derivative, and the second inducer is artemisinin, a second artemisinin derivative, or a flavonoid compound.

[00685] Embodiment 11: The method of Embodiment 8, wherein the first inducer is a flavonoid compound and the second inducer is artemisinin or a derivative of artemisinin.

[00686] Embodiment 12: The method of any one of Embodiments 1-11, wherein the artemisinin derivative is a compound of Formula (I), (I-A), (I-B), (I-C), (I-D), (I-E), (I-F), (I-G), (I-H), (I-I), (I-J), (I-K), (I-L), (I-M), (I-M'), (I-N), (I-N'), (I-O) or (I-O').

[00687] Embodiment 13: The method of any one of Embodiments 1-12, wherein the artemisinin derivative is artesunate, dihydroartemisinin (DHA), artesunate, or artemether, optionally, the artemisinin derivative is artesunate, or dihydroartemisinin, more optionally, the artemisinin derivative is artesunate.

[00688] Embodiment 14: The method of any one of Embodiments 1-13, wherein the flavonoid compound is flavone, baicalein, chrysin, galangin, (OH)₄ flavone, 6-hydroxyluteolin, acacetin, acerosin, alnetin, apigenin, apiin, artocarpetin, balcalein, beta-sitosterol, biochamin A, cannaflavin A, cannflavin A, cannflavin B, cannflavin C, catechin, catechin-3-gallate, cerrosillin, chrysoeriol, chrysoeriol, cirsilinoleol, cirsilinoleol, cirsimaritin, corymbosin, cupressuflavone, cyanidine, daidzein, datiscetin, delphinidine, digidrokempferol, dihydroquercetin, diosmetin, diosmin, echiodinin, epicatechin, epicatechin-3-gallate, epigallocatechin, epigallocatechin-3-gallate, eriodictiol, eupatilin, eupatorin, fisetin, fizetin, flavonol, formononetin, fortunelin, gallocatechin, gallocatechin-3-gallate, gardenin D, genistein, genistin, genkwanin, geraldol, geraldone, glycitein, glycitin, gomoeriodictiol, gossypetin, hesperetin, hinokiflavone, hispidulin, hymenoxin, hypolaetin, isoquercetin, isorhamnetin, isorhamnetin, isoscutellarein, isovitexin, jaceosidin,

kaempferol, kaempferol, kampferide, largonidin, luteolin, luteolin, malvidine, mikanin, morin, myricetin, myricetin, naringenin, negletein, neodiosmin, nepetin, nevadensin, nobiletin, nodifloretin, norartocarpetin, norwogonin, onopordin, orientin, oroxylin a, pachipodol, pectolinarigenin, pedalitin, peonidin, petunidin, pilloin, pratol, primetin, primuletin, pruentin, quercetagetin, quercetin, ramnazin, rhoifolin, robinetin, rutin, scaposin, scutellarein, serpyllin, sinensetin, sorbifolin, sudachitin, tangeretin, tangeritin, taxifolin, tectochrysin, tithonine, tricetin, tricin, velutin, vitexin, wightin, wogonin, xanthomicrol, zapotin, zapotinin, 6,7,4'-trihydroxy isoflavone, or 7,3'4'-trihydroxy isoflavone; optionally, the flavonoid compound is flavone.

[00689] Embodiment 15: The method of any one of Embodiments 1-14, wherein the first and second inducer are administered at about the same amount.

[00690] Embodiment 16: The method of Embodiment 15, wherein the first inducer and the second inducer are administered at different amounts.

[00691] Embodiment 17: The method of Embodiment 16, wherein the second inducer is administered at a lower amount relative to the amount of the first inducer.

[00692] Embodiment 18: The method of Embodiment 17, wherein the second inducer is administered in amount about 1/2, 1/3, 1/4, 1/5, 1/6, 1/7, 1/8, 1/9, 1/10, 1/15, 1/20, 1/25, 1/30, 1/35, 1/40, 1/45, 1/50, 1/55, 1/60, 1/65, 1/70, 1/75, 1/80, 1/85, 1/90, 1/95, 1/100, 1/125, 1/150, 1/175, 1/200, 1/500, 1/1000 or even less of the amount of first the inducer.

[00693] The method of Embodiment 18, wherein the second inducer is administered in amount about 1/2, 1/4, 1/5, 1/8, 1/10, 1/20, 1/40, 1/80 or less of the amount of the first inducer.

[00694] Embodiment 20: The method of Embodiment 16, wherein the second inducer is administered at a higher amount relative to the amount of the first inducer.

[00695] Embodiment 21: The method of Embodiment 20, wherein the amount of inducer administered at the second time point is at least about 25%, 50%, about 75%, about 1-fold, about 1.2-folds, about 1.25-folds, about 1.3-folds, about 1.5-folds, about 1.75-folds, about 2-folds, about 2.25-folds, about 2.5-folds, about 2.5-folds, about 2.75-folds, about 3-folds, 4-fold, 5-fold, 6-folds, 7-fold, 8-fold, 9-folds, 10-folds or higher than the amount of the first inducer.

[00696] Embodiment 22: The method of any one of Embodiments 1-21, wherein the first and second inducer are administered independently at a dose from about 0.5 mg/kg to about 150mg/kg.

[00697] Embodiment 23: The method of any one of Embodiments 1-22, wherein the first inducer is administered at a dose from about 1 mg/kg to about 100 mg/kg.

[00698] Embodiment 24: The method of any one of Embodiments 1-23, wherein the first inducer is administered at a dose of about 80mg/kg, about 70mg/kg, about 60mg/kg, about 50mg/kg, about 40mg/kg, about 30mg/kg, about 20mg/kg, about 10mg/kg, about 6mg/kg, about 5mg/kg, or about 1mg/kg.

[00699] Embodiment 25: The method of any one of Embodiments 1-24, wherein the second inducer is administered at a dose from about 1 mg/kg to about 100 mg/kg.

[00700] Embodiment 26: The method of any one of Embodiments 1-25, wherein the second inducer is administered at a dose of 80mg/kg, about 70mg/kg, about 60mg/kg, about 50mg/kg, about 40mg/kg, about 30mg/kg, about 20mg/kg, about 10mg/kg, about 6mg/kg, about 5mg/kg, or about 1mg/kg.

[00701] Embodiment 27: The method of any one of Embodiments 1-16, wherein the first inducer is administered at a dose of about 80 mg/kg or less and the second inducer is administered at a dose of about 20mg/kg or less at the second or subsequent time point.

[00702] Embodiment 28: The method of any one of Embodiments 1-16, wherein the first inducer is administered at a dose of about 40 mg/kg and the second inducer is administered at a dose of about 80mg/kg or less at the second or subsequent time point.

[00703] Embodiment 29: The method of any one of Embodiments 1-16, wherein the first inducer is administered at a dose of about 20mg/kg and the second inducer is administered at a dose of about 80 mg/kg, 40mg/kg, 20mg/kg, 10 mg/kg, 6 mg/kg or 1 mg/kg at the second or subsequent time point.

[00704] Embodiment 30: The method of any one of Embodiments 1-16, wherein the first inducer is administered at a dose of about 10 mg/kg and the second inducer is administered at a dose of about 80mg/kg or less at the second or subsequent time point.

[00705] Embodiment 31: The method of any one of Embodiments 1-16, wherein the first inducer is administered at a dose of about 6 mg/kg and the second inducer is administered at a dose of about 80mg/kg or less at the second or subsequent time point.

[00706] Embodiment 32: The method of any one of Embodiments 1-16, wherein the first inducer is administered at a dose of about 1 mg/kg and the second inducer is administered at a dose of about 80mg/kg or less at the second or subsequent time point.

[00707] Embodiment 33: The method of any one of Embodiments 1-16, wherein the second inducer is administered at a dose of about 80 mg/kg and the first inducer is administered at a dose of about 80 mg/kg or less.

[00708] Embodiment 34: The method of any one of Embodiments 1-16, wherein the second inducer is administered at a dose of about 40 mg/kg and the first inducer is administered at a dose of about 80 mg/kg or less.

[00709] Embodiment 35: The method of any one of Embodiments 1-16, wherein the second inducer is administered at a dose of about 20 mg/kg and the first inducer is administered at a dose of about 80 mg/kg or less.

[00710] Embodiment 36: The method of any one of Embodiments 1-16, wherein the second inducer is administered at a dose of about 10 mg/kg and the first inducer is administered at a dose of about 80 mg/kg or less.

[00711] Embodiment 37: The method of any one of Embodiments 1-16, wherein the second inducer is administered at a dose of about 6 mg/kg and the first inducer is administered at a dose of about 80 mg/kg or less.

[00712] Embodiment 38: The method of any one of Embodiments 1-16, wherein the second inducer is administered at a dose of about 1 mg/kg and the first inducer is administered at a dose of about 80 mg/kg or less.

[00713] Embodiment 39: The method of any one of Embodiments 1-38, wherein the expression of the transgene goes down to a background expression level within one week of administering the inducer.

[00714] Embodiment 40: The method of any one of Embodiments 1-39, wherein the second inducer is administered when it is determined that the level of expression of the transgene is decreased to 75% or less of the level of expression of the transgene attained after induction with the first inducer.

[00715] Embodiment 41: The method of any one of any one of Embodiments 1-40, wherein the second inducer is administered when it is determined that the level of expression of the transgene is decreased to 50% or less of the level of expression of the transgene attained after induction with the first inducer.

[00716] Embodiment 42: The method of any one of Embodiments 1-41, wherein the second inducer is administered when it is determined that the level of expression of the transgene is decreased to 25% or less of the level of expression of the transgene attained after induction with the first inducer.

[00717] Embodiment 43: The method of any one of Embodiments 1-42, wherein the second inducer is administered when it is determined that the level of expression of the transgene is decreased to a background level of expression of the transgene.

[00718] Embodiment 44: The method of any one of Embodiments 1-43, further comprising: (c) administering to the subject at least at a second time point a second inducer to continue expression of the transgene at a predetermined level for a predetermined time or to modulate expression of the transgene to a predetermined level, optionally, a third inducer wherein the third inducer is same or different from the first and/or second inducer; and (d) optionally, repeating step (c) a one or more times, and where the inducer at each administration is same or different from the inducer at immediate prior administration.

[00719] Embodiment 45: The method of any one of Embodiments 1-44, wherein the time between administrations is at least 12 hours, at least 24 hours, 48 hours, 72 hours, 1 week, 10 days, 2 weeks, 3 weeks, or 4 weeks.

[00720] Embodiment 46: The method of any one of Embodiments 1-45, wherein the time between administrations is at least 24 hours, 48 hours, or 72 hours.

[00721] Embodiment 47: The method of any one of Embodiments 1-46, wherein the time between administrations is no more than 2 months, 3 months, 4 months, 5 months, or 6 months.

[00722] Embodiment 48: The method of any one of Embodiments 1-47, wherein the time between administrations is no more than 2 months.

[00723] Embodiment 49: The method of any one of Embodiments 1-48, wherein the time between administrations is 1-2 days, 3-4 days, 5-7 days, 5-10 days, 7-10 days, 7-14 days, 10-14 days, 10-20 days, 14-21 days, 14-28 days, 21-28 days, or 21-34 days.

[00724] Embodiment 50: The method of any one of Embodiments 1-159, wherein the time between administrations is about a day, e.g., about 24 hours.

[00725] Embodiment 51: The method of any one of Embodiments 1-50, wherein the method further comprises a step of determining the expression level of the transgene after administering the inducer.

[00726] Embodiment 52: The method of any one of Embodiment 1-51, wherein the method further comprises a step of determining the expression level of the transgene prior to administering the inducer.

[00727] Embodiment 53: The method of any one of Embodiments 1-52, wherein the method further comprising administering the exogenous nucleotide sequence to the subject prior to administering the first or second inducer.

[00728] Embodiment 54: The method of any one of Embodiments 1-53, wherein the method is a method of therapy, optionally, the method of therapy is a method of gene therapy.

[00729] Embodiment 55: The method of any one of Embodiments 1-54, wherein the exogenous nucleotide sequence comprising the inducible promoter operably linked to a transgene is a viral vector.

[00730] Embodiment 56: The method of any one of Embodiments 1-55, wherein the exogenous nucleotide sequence comprising the inducible promoter operably linked to a transgene is adeno-associated viral (AAV), lentiviral, adenoviral, herpes viral, or hepatitis viral vector.

[00731] Embodiment 57: The method of any one of Embodiments 1-56, wherein the exogenous nucleotide sequence comprising the inducible promoter operably linked to a transgene is an AAV vector, e.g., an AAV gene therapy vector.

[00732] Embodiment 58: The method of any one of Embodiments 1-57, wherein the inducers independently of other administrations are administered orally or intraperitoneally.

[00733] Embodiment 59: The method of any one of Embodiments 1-58, wherein at least one of the inducers is administered orally.

[00734] Embodiment 60: The method of Embodiment 59, wherein the second inducer is administered orally.

[00735] Embodiment 61: The method of Embodiment 59, wherein the first inducer is administered orally.

[00736] Embodiment 62: The method of any one of Embodiments 1-58, wherein at least one of the inducers is administered intraperitoneally.

[00737] Embodiment 63: The method of Embodiment 62, wherein the second inducer is administered intraperitoneally.

[00738] Embodiment 64: The method of Embodiment 62, wherein the first inducer is administered intraperitoneally.

[00739] Embodiment 65: The method of any one of Embodiment 1-64, wherein the first induction increases the transgene expression level to a maximum at about 24 hrs and declines thereafter, and wherein the second induction and/or successive inductions has similar induction profile as seen with the first induction, for at least about 1 month, or at least about 2 months, or at least about 3 months, or at least about 5 months, or at least about six months, or longer.

[00740] Embodiment 66: The method of Embodiment 65, wherein the transgene expression level at about 24 hrs after the first induction, or second induction, or successive inductions is at least about 200-fold higher than that of prior to administering the inducer for the first time, or, the second time, or any number of successive administrations.

[00741] Embodiment 67: The method of Embodiment 65, wherein the first induction, the second induction, and/or, the successive inductions are not complete until about 160 hours after administering the inducer.

[00742] Embodiment 68: The method of Embodiment 65, wherein dose of the inducer in successive administrations is reduced from its prior administration.

[00743] Embodiment 69: The method of Embodiment 65, wherein dose of the inducer in successive administrations remains same.

[00744] Embodiment 70: The method of Embodiment 65, wherein the dose of the inducer ranges from about 1mg/kg to about 80mg/kg.

[00745] Embodiment 71: The method of Embodiment 65, wherein the inducers are administered orally.

[00746] Embodiment 72: The method of Embodiment 65, wherein the inducers are administered intraperitoneally.

[00747] Embodiment 73: A method of regulating expression of a transgene in a primate subject, the method comprising: administering an inducer to a primate subject, wherein the inducer is artemisinin, an artemisinin derivative or a flavonoid compound, wherein the primate subject comprises at least one cell comprising an exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene, wherein the inducible promoter comprises a PBREM enhancer, wherein the inducer is administered in an amount that is sufficient to express a measurable level of the transgene in the subject.

[00748] Embodiment 74: The method of Embodiment 73, wherein the method comprises administering a plurality of doses of the inducer.

[00749] Embodiment 75: The method of Embodiment 74, wherein the time between administrations is at least 12 hours, at least 24 hours, 48 hours, 72 hours, 1 week, 10 days, 2 weeks, 3 weeks, or 4 weeks.

[00750] Embodiment 76: The method of any one of Embodiments 74-75, wherein the time between administrations is at least 24 hours, 48 hours, or 72 hours.

[00751] Embodiment 77: The method of any one of Embodiments 74-76, wherein the time between administrations is no more than 2 months, 3 months, 4 months, 5 months, or 6 months.

[00752] Embodiment 78: The method of any one of Embodiments 74-77, wherein the time between administrations is no more than 2 months.

[00753] Embodiment 79: The method of any one of Embodiments 74-78, wherein the time between administrations is 1-3 days, 5-7 days, 5-10 days, 7-10 days, 7-14 days, 10-14 days, 10-20 days, 14-21 days, 14-28 days, 21-28 days, or 21-34 days.

[00754] Embodiment 80: The method of any one of Embodiments 74-79, wherein the time between administrations is about 1 day.

[00755] Embodiment 81: The method of any one of Embodiments 74-80, wherein the transgene expression level at about 24 hrs after an induction is at least about 200-fold higher than that of prior to administering the inducer for the first time or any number of prior administrations.

[00756] Embodiment 82: The method of any one of Embodiments 74-81, wherein an induction is not complete until about 160 hours after administering the inducer.

[00757] Embodiment 83: The method of any one of Embodiments 74-82, wherein the transgene expression level increases to a maximum at about 24 hrs after the first administration and declines thereafter and a second induction, and/or successive inductions, has similar induction profile as seen with the first induction, for at least about 1 month, or at least about 2 months, or at least about 3 months, or at least about 5 months, or at least about six months, or longer,

[00758] Embodiment 84: The method of any one of Embodiments 74-83, wherein dose in successive administrations is reduced from a prior administration.

[00759] Embodiment 85: The method of any one of Embodiments 74-84, wherein dose in successive administrations remains same.

[00760] Embodiment 86: The method of any one of Embodiments 73-85, wherein the inducer is artemisinin or an artemisinin derivative.

[00761] Embodiment 87: The method of Embodiment 86, wherein the artemisinin derivative is a compound of Formula (I), (I-A), (I-B), (I-C), (I-D), (I-E), (I-F), (I-G), (I-H), (I-I), (I-J), (I-K), (I-L), (I-M), (I-M'), (I-N), (I-N'), (I-O) or (I-O').

[00762] Embodiment 88: The method of Embodiment 86, wherein the derivative of artemisinin is artesunate, dihydroartemisinin, artesunate, or artemether.

[00763] Embodiment 89: The method of Embodiment 88, wherein the derivative of artemisinin is artesunate, or dihydroartemisinin.

[00764] Embodiment 90: The method of Embodiment 73-85, wherein the inducer is a flavonoid compound.

[00765] Embodiment 91: The method of Embodiment 90, wherein the flavonoid compound is flavone, baicalein, chrysin, galangin, (OH)₄ flavone, 6-hydroxyluteolin, acacetin, acerosin, alnetin, apigenin, apiin, artocarpetin, balcalein, beta-sitosterol, biochamin A, cannaflavin A, cannflavin A, cannflavin B, cannflavin C, catechin, catechin-3-gallate, cerrosillin, chrysoeriol, chrysoeriol, cirsilinole, cirsilinole, cirsimaritin, corymbosin, cupressuflavone, cyanidine, daidzein, datiscetin, delphinidine, digidrokempferol, dihydroquercetin, diosmetin, diosmin, echiodinin, epicatechin, epicatechin-3-gallate, epigallocatechin, epigallocatechin-3-gallate, eriodictiol, eupatilin, eupatorin, fisetin, fizetin, flavonol, formononetin, fortunelin, gallocatechin, gallocatechin-3-gallate, gardenin D, genistein, genistin, genkwanin, geraldol, geraldone, glycitein, glycitin, gomoeriodictiol, gossypetin, hesperetin, hinokiflavone, hispidulin, hymenoxin, hypolaetin, isoquercetin, isorhamnetin, isorhamnetin, isoscutellarein, isovitexin, jaceosidin, kaempferol, kaempferol, kampferide, largonidin, luteolin, luteolin, malvidine, mikanin, morin, myricetin, myricetin, naringenin, negletein, neodiosmin, nepetin, nevadensin, nobiletin, nodifloretin, norartocarpetin, norwogonin, onopordin, orientin, oroxylin a, pachipodol, pectolinarigenin, pedalitin, peonidin, petunidin, pilloin, pratol, primetin, primuletin, pruentin, quercetagenin, quercetin, ramnazin, rhoifolin, robinetin, rutin, scaposin, scutellarein, serpyllin, sinensetin, sorbifolin, sudachitin, tangeretin, tangeritin, taxifolin, tectochrysin, tithonine, tricetin, tricin, velutin, vitexin, wightin, wogonin, xanthomicrol, zapotin, zapotin, zapotin, 6,7,4'-trishydroxy isoflavone, or 7,3,4'-trihydroxy isoflavone; optionally, the flavonoid compound is flavone.

[00766] Embodiment 92: The method of any one of Embodiments 73-91, wherein each dose independently from about 0.5 mg/kg to about 150mg/kg.

[00767] Embodiment 93: The method of any one of Embodiments 73-92, wherein each dose is independently from about 1 mg/kg to about 100 mg/kg.

[00768] Embodiment 94: The method of any one of Embodiments 73-93, wherein each dose is independently from about 1 mg/kg to about 80 mg/kg.

[00769] Embodiment 95: The method of any one of Embodiments 73-94, wherein each dose is independently from about 1 mg/kg to about 40 mg/kg.

[00770] Embodiment 96: The method of any one of Embodiments 73-95, wherein each dose is independently from about 1 mg/kg to about 20 mg/kg.

[00771] Embodiment 97: The method of any one of Embodiments 73-96, wherein each dose is independently from about 1 mg/kg to about 10 mg/kg.

[00772] Embodiment 98: The method of any one of Embodiments 73-94, wherein each dose is independently about 80mg/kg, about 70mg/kg, about 60mg/kg, about 50mg/kg, about 40mg/kg, about 30mg/kg, about 20mg/kg, about 10mg/kg, about 6mg/kg, about 5mg/kg, or about 1mg/kg.

[00773] Embodiment 99: The method of any one of Embodiments 73-98, wherein the method further comprises administering the exogenous nucleotide sequence to the subject prior to administering the inducer for the first time.

[00774] Embodiment 100: The method of any one of Embodiments 73-99, wherein a second inducer or, subsequent inducer is administered to the primate subject following the prior administration of an inducer to the primate subject, and optionally, the inducer of the prior administration is administered in an amount that is sufficient to express a measurable level of the transgene in the subject.

[00775] Embodiment 101: The method of Embodiment 100, wherein the inducer of the prior administration is different from the second or subsequent inducer administered to the subject.

[00776] Embodiment 102: The method of Embodiment 101, wherein the inducer of the prior administration is same as the second or, subsequent inducer administered to the subject but at a different dose.

[00777] Embodiment 103: The method of any one of Embodiments 73-102, wherein the method is a method of therapy, optionally, the method of therapy is a method of gene therapy.

[00778] Embodiment 104: The method of any one of Embodiments 73-103, wherein the exogenous nucleotide sequence comprising the inducible promoter operably linked to a transgene is a viral vector.

[00779] Embodiment 105: The method of any one of Embodiments 73-104, wherein the exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene is adeno-associated viral (AAV), lentiviral, adenoviral, herpes viral, or hepatitis viral vector.

[00780] Embodiment 106: The method of any one of Embodiments 73-105, wherein the exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene is an AAV vector, e.g., an AAV gene therapy vector.

[00781] Embodiment 107: The method of any one of Embodiments 73-106, wherein each administration independently of other administrations is oral administration or intraperitoneal administration.

[00782] Embodiment 108: The method of any one of Embodiments 73-107, wherein each administration is oral administration.

[00783] Embodiment 109: The method of any one of Embodiments 73-107, wherein each administration is intraperitoneal administration.

[00784] Embodiment 110: The method of any one of Embodiments 1-109, wherein the transgene encodes an antibody or antigen binding portion an antibody, a coagulation factor, a cytokine, a hormone, a growth factor, a virus protein, a cell receptor ligand, a chimeric antigen receptor (CAR) or a CRISPR-Cas nuclease.

[00785] Embodiment 111: The method of Embodiment 110, wherein the transgene encodes an antibody or an antigen binding portion thereof, optionally the antibody is an anti-PD1 antibody, anti-HER2 antibody, anti-PCSK9 antibody, anti-VEGFR2 antibody, anti-IL17 antibody, anti-A β 3-42 antibody, anti-Sema3A antibody, anti-4-1BB (CD137) antibody, anti-5'-nucleotidase antibody, anti-5T4 antibody, anti-activin receptor-like kinase 1 antibody, anti-ACVR2B antibody, anti-adenocarcinoma antigen antibody, anti-amyloid antibody, anti-Ang-2 antibody, anti-angiopoietin 2 antibody, anti-angiopoietin 2 antibody, anti-angiopoietin 3 antibody, anti-anthrax toxin antibody, anti-AOC3 (VAP-1) antibody, anti-AXL antibody, anti-BAFF-R antibody, anti-B-cell activating factor (BAFF) antibody, anti-B-cell maturation antigen (BCMA) antibody, anti-B-lymphoma cell antibody, anti-C242 antigen antibody, anti-C5 antibody, anti-C5a receptor (C5a) antibody, anti-CA-125 antibody, anti-calcitonin antibody, anti-calcitonin gene-related peptide alpha antibody, anti-calcitonin gene-related peptide beta antibody, anti-calcitonin gene-related peptide receptor (CGRP) antibody, anti-CanAg (a glycoform of MUC1) antibody, anti-carbonic anhydrase 9 (CA-IX) antibody, anti-Carcinoembryonic antigen (CEA) antibody, anti-Carcinoembryonic antigen (CEA)-related antigen antibody, anti-cardiac myosin antibody, anti-CCL11 antibody, anti-CCR2 antibody, anti-CCR4 antibody, anti-CCR5 antibody, anti-CCR5 antibody, anti-CD11 antibody, antibody, anti-CD123 antibody, anti-CD125 antibody, anti-CD134 antibody, anti-CD134 antibody, anti-CD147 antibody, anti-CD15 antibody, anti-CD152 antibody, anti-CD154 antibody, anti-CD18

antibody, anti-CD19 antibody, anti-CD2 antibody, anti-CD20 antibody, anti-CD20 antibody, anti-CD20 antibody, anti-CD200 antibody, anti-CD22 antibody, anti-CD23 antibody, anti-CD25 antibody, anti-CD27 antibody, anti-CD276 antibody, anti-CD278 antibody, antibody, anti-CD279 antibody, anti-CD28 antibody, anti-CD3 antibody, anti-CD3 antibody, anti-CD3 antibody, anti-CD3 antibody, anti-CD3 antibody, anti-CD30 antibody, anti-CD319 antibody, anti-CD33 antibody, anti-CD37 antibody, anti-CD38 antibody, anti-CD3E antibody, anti-CD3E antibody, anti-CD4 antibody, anti-CD40 antibody, anti-CD41 antibody, anti-CD44 antibody, anti-CD45 antibody, anti-CD5 antibody, anti-CD51 antibody, anti-CD52 antibody, anti-CD56 antibody, anti-CD6 antibody, anti-CD70 antibody, anti-CD74 antibody, anti-CD79B antibody, anti-CD80 antibody, anti-CEACAM5 antibody, anti-Claudin 18 Isoform 2 antibody, anti-clumping factor A antibody, anti-cMet antibody, anti-c-Met antibody, anti-coagulation factor III antibody, anti-complement C5a antibody, anti-complement component 1s (C1s) antibody, anti-Complement factor D (CFD) antibody, anti-connective tissue growth factor (CTGF) antibody, anti-CSF1 antibody, antibody, anti-CSF1R antibody, anti-CSF2 antibody, anti-CTLA-4 antibody, anti-CXCL10 (IP-10) antibody, anti-CXCR4 (CD184) antibody, anti-dabigatran antibody, anti-dendritic cell-associated lectin 2 antibody, anti-DLL3 antibody, anti-DLL4 antibody, anti-DPP4 antibody, anti-DR5 antibody, anti-EGFL7 antibody, anti-EGFR antibody, anti-EGFR antibody, anti-EGFR antibody, anti-EGFR extracellular domain III antibody, anti-endoglin antibody, anti-endotoxin antibody, anti-EpCAM antibody, anti-EPHA3 antibody, anti-ephrin receptor A3 antibody, anti-episialin antibody, anti-ERBB1 antibody, anti-ERBB3 (HER3) antibody, anti-FAP (gene) (FAP) antibody, anti-FCGRT antibody, anti-FGF 23 antibody, anti-FGFR2 antibody, anti-FGFR2 antibody, anti-fibrin II antibody, anti-fibronectin extra domain-B antibody, anti-folate hydrolase antibody, anti-folate receptor 1 antibody, anti-folate receptor alpha antibody, anti-Frizzled receptor antibody, anti-FVIX antibody, anti-FVX antibody, anti-GD2 ganglioside antibody, anti-GD3 ganglioside antibody, anti-GDF-8 antibody, anti-gelatinase B antibody, anti-glucagon receptor (GCGR) antibody, anti-Glutamate carboxypeptidase II antibody, anti-glypican 3 antibody, anti-GMCSF antibody, anti-GPNMB antibody, anti-GPRC5D antibody, antibody, anti-growth differentiation factor 8 antibody, anti-GUCY2C antibody, anti-Hepatocyte growth factor (HGF) antibody, anti-HER1 antibody, anti-HER1 antibody, anti-HER1 antibody, anti-HER2/neu antibody, anti-HGFR antibody, anti-histone complex antibody, anti-HLA-DR antibody, anti-Hsp90 antibody, anti-human scatter factor receptor kinase antibody, anti-ICAM-1 (CD54) antibody, anti-IFN receptor antibody, anti-IFN- α antibody, anti-IFN- α/β receptor antibody, anti-IFN- γ antibody, anti-IgE antibody, anti-IgE Fc region antibody, anti-IGF-1 receptor (CD221) antibody, anti-IGF-2 antibody, anti-IGHE antibody, anti-IL-1 antibody, anti-IL-12 antibody, anti-IL-13 antibody, anti-IL-17A antibody, anti-IL-17AF antibody, anti-IL-17F antibody, anti-IL-1 α antibody, anti-IL-1 β antibody, anti-IL-2 antibody, anti-

IL-20 antibody, anti-IL-22 antibody, anti-IL-23 antibody, anti-IL-23 antibody, anti-IL-23 antibody, anti-IL-23 antibody, anti-IL-23 antibody, anti-IL-23A antibody, anti-IL-3 receptor antibody, anti-IL-31 receptor A antibody, anti-IL-4 antibody, anti-IL-4R α antibody, anti-IL-5 antibody, anti-IL-6 antibody, anti-IL-6 receptor antibody, anti-IL-9 antibody, anti-inducible T-cell co-stimulatory ligand (ICOSL) antibody, anti-integrin α 4 antibody, anti-integrin β 7 antibody, anti-integrin α 4 β 7 antibody, anti-integrin α 4 β 7 antibody, anti-integrin α 5 β 1 antibody, anti-integrin α Ib β 3 antibody, anti-integrin α v β 3 antibody, anti-Interleukin 36 receptor (IL1RL2/IL1RAP) antibody, anti-ITGA2 (CD49b) antibody, anti-ITGB2 (CD18) antibody, anti-kallikrein antibody, anti-KIR2D antibody, anti-LAG3 antibody, anti-Lewis-Y antigen antibody, anti-LFA-1 (CD11a) antibody, anti-LINGO-1 antibody, anti-lipoteichoic acid antibody, anti-LIV-1 antibody, anti-LOXL2 antibody, anti-LRRC15 antibody, anti-L-selectin (CD62L) antibody, anti-lymphotoxin alpha (LTA) antibody, anti-LYPD3 antibody, anti-macrophage colony stimulating factor (MCSF) antibody, anti-macrophage migration inhibitory factor (MIF) antibody, anti-MASP-2 antibody, anti-MCP-1 antibody, anti-melanoma cell adhesion molecule (MCAM) antibody, anti-mesothelin antibody, anti-MS4A1 antibody, antibody, anti-MST1R antibody, anti-MUC1 antibody, anti-MUC5AC antibody, anti-mucosal addressin cell adhesion molecule antibody, anti-myelin-associated glycoprotein antibody, anti-myostatin antibody, anti-NCA-90 (granulocyte antigen) antibody, anti-nectin-4 antibody, anti-nerve growth factor (NGF) antibody, anti-NGNA ganglioside antibody, anti-NKG2A antibody, anti-NOGO-A antibody, anti-Notch 1 antibody, anti-Notch receptor antibody, anti-NRP1 antibody, anti-OX-40 antibody, anti-oxLDL antibody, anti-PCDC1 antibody, anti-PCDP1 antibody, anti-PDGFR α antibody, anti-PDGFR β antibody, anti-PD-L1 antibody, anti-phosphate-sodium co-transporter antibody, anti-phosphatidylserine antibody, anti-PTK7 antibody, anti-RANKL antibody, anti-repulsive guidance molecule A (RGMA) antibody, anti-RHD (gene) (RHD) antibody, anti-Rhesus factor antibody, anti-Rhesus factor antibody, anti-root plate-specific spondin 3 antibody, anti-ROR1 antibody, anti-RTN4 antibody, anti-sclerostin antibody, anti-sclerostin (SOST) antibody, anti-SDC1 antibody, anti-selectin P antibody, anti-serum amyloid A protein antibody, anti-serum amyloid P component antibody, anti-SLAMF7 antibody, anti-SLITRK6 antibody, anti-SOST antibody, anti-sphingosine-1-phosphate antibody, anti-STEAP1 antibody, anti-TAG-72 antibody, anti-TAG-72 antibody, anti-tau protein antibody, anti-T-cell receptor antibody, anti-TEM1 antibody, anti-tenascin C antibody, anti-TGF- β antibody, anti-TGF- β 1 antibody, anti-TGF- β 2 antibody, anti-thymic stromal lymphopoietin (TSLP) antibody, anti-TIGIT antibody, anti-tissue factor pathway inhibitor (TFPI) antibody, anti-TNF antibody, anti-TNF antibody, anti-TNF- α antibody, anti-TNF- α antibody, anti-TRAIL-R1 antibody, anti-TRAIL-R2 antibody, anti-TROP-2 antibody, anti-tumor antigen CTAA16.88 antibody, anti-tumor necrosis factor receptor (TNFR) antibody, anti-tumor necrosis factor related activation protein (TRAP)

antibody, anti-Tumor-associated glycoprotein 72 (TAG-72) antibody, anti-TWEAK receptor antibody, anti-TYRP1 (glycoprotein 75) antibody, anti-vascular endothelial growth factor A (VEGFA) antibody, anti-VEGFA antibody, anti-VEGFR-1 antibody, anti-vimentin antibody, anti-VISTA (protein) (VSIR) antibody, anti-VWF antibody, anti- α -fetoprotein antibody, anti-A-synuclein antibody or anti- β -amyloid antibody. optionally the antibody is selected from the group consisting of an anti-TNF α , anti-PD1 antibody, anti-HER2 antibody, anti-PCSK9 antibody, anti-VEGFR2 antibody, anti-IL17 antibody, anti-A β 3-42 antibody, and anti-Sema3A antibody.

[00786] Embodiment 112: The method of Embodiment 110, wherein the transgene encodes an antibody or an antigen binding portion thereof, and wherein the antibody is selected from the group consisting 3F8, Abagovomab, Abciximab, Abituzumab, Abrezekimab, Abrilumab, Actoxumab, Adalimumab, Adecatumumab, Aducanumab, Afasevikumab, Afelimomab, Alacizumab pegol, Alemtuzumab, Alirocumab, Altumomab pentetate, Amatuximab, Amivantamab, Anatumomab mafenatox, Andecaliximab, Anetumab ravtansine, Anifrolumab, Ansuvimab, Anrukizumab, (IMA-638), Apolizumab, Aprutumab ixadotin, Arcitumomab, Ascrinvacumab, Aselizumab, Atezolizumab, Atidortoxumab, Atinumab, Atoltivimab, Atoltivimab/maftivimab/odesivimab, Atorolimumab, Avelumab, Azintuxizumab vedotin, Bamlanivimab, Bapineuzumab, Basiliximab, Bavixumab, BCD-100, Bebtelovimab, Bectumomab, Bedinvetmab, Begelomab, Belantamab mafodotin, Belimumab, Bemarituzumab, Benralizumab, Berlimatoxumab, Bermekimab, Bersanlimab, Bertilimumab, Besilesomab, Bevacizumab, Bezlotoxumab, Biciromab, Bimagrumab, Bimekizumab, Birtamimab, Bivatuzumab, Bleselumab, Blinatumomab, Blontuvetmab, Blosozumab, Bococizumab, Brazikumab, Brentuximab vedotin, Briakinumab, Brodalumab, Brolucizumab, Brontictuzumab, Burosumab, Cabiralizumab, Camidanlumab tesirine, Camrelizumab, Canakinumab, Cantuzumab mertansine, Cantuzumab ravtansine, Caplacizumab, Casirivimab, Capromab, Carlumab, , Carotuximab, Catumaxomab, cBR96-doxorubicin immunoconjugate, Cedelizumab, Cemiplimab, Cergutuzumab amunaleukin, Certolizumab pegol, Cetrelimab, Cetuximab, Cibisatamab, Cilgavimab, Cirmtuzumab, Citatuzumab bogatox, Cixutumumab, Clazakizumab, Clenoliximab, Clivatuzumab tetraxetan, Codrituzumab, Cofetuzumab pelidotin, Coltuximab ravtansine, Conatumumab, Concizumab, Cosfroviximab, Crenezumab, Crizanlizumab, Crotedumab, CR6261, Cusatuzumab, Dacetuzumab, Daclizumab, Dalotuzumab, Dapirolizumab pegol, Daratumumab, Dectrekumab, Demcizumab, Denintuzumab mafodotin, Denosumab, Depatuxizumab mafodotin, Derlotuximab biotin, Detumomab, Dezamizumab, Dinutuximab, Dinutuximab beta, Diridavumab, Domagrozumab, Donanemab, Dorlimomab aritox, Dostarlimab, Drozitumab, DS-8201, Duligotuzumab, Dupilumab, Durvalumab, Dusigitumab, Duvortuxizumab, Ecomeximab, Eculizumab, Edobacomab, Edrecolomab, Efalizumab, Efungumab, Eldelumab, Elezanumab, Elgemtumab, Elotuzumab,

Elsilimomab, Emactuzumab, Emapalumab, Emibetuzumab, Emicizumab, Enapotamab vedotin, Enavatuzumab, Enfortumab vedotin, Enlimomab pegol, Enoblituzumab, Enokizumab, Enoticumab, Ensituximab, Epcoritamab, Eptumomab cituxetan, Epratuzumab, Eptinezumab, Erenumab, Erlizumab, Ertumaxomab, Etaracizumab, Etesevimab, Etigilimab, Etrolizumab, Evinacumab, Evolocumab, Exbivirumab, Fanolesomab, Faralimomab, Faricimab, Farletuzumab, Fasinumab, FBTA05, Felvizumab, Fezakinumab, Fibatuzumab, Ficlatuzumab, Figitumumab, Firivumab, Flanvotumab, Fletikumab, Flotetuzumab, Fontolizumab, Foralumab, Foravirumab, Fremanezumab, Fresolimumab, Frovocimab, Frunevetmab, Fulranumab, Futuximab, Galcanezumab, Galiximab, Gancotamab, Ganitumab, Gantenerumab, Gatipotuzumab, Gavilimomab, Gedivumab, Gemtuzumab ozogamicin, Gevokizumab, Gilvetmab, Gimsilumab, Girentuximab, Glembatumumab vedotin, Glofitamab, Golimumab, Gomiliximab, Gosuranemab, Guselkumab, Ianalumab, Ibalizumab, Sintilimab, Ibritumomab tiuxetan, Icrucumab, Idarucizumab, Ifabotuzumab, Igovomab, Iladatuzumab vedotin, Imalumab, Imaprelimab, Imciromab, Imdevimab, Imgatuzumab, Inclacumab, Indatuximab ravtansine, Indusatumab vedotin, Inebilizumab, Infliximab, Intetumumab, Inolimomab, Inotuzumab ozogamicin, Ipilimumab, Iomab-B, Iratumumab, Isatuximab, Iscalimab, Istiratumab, Itolizumab, Ixekizumab, Keliximab, Labetuzumab, Lacnotuzumab, Ladiratuzumab vedotin, Lampalizumab, Lanadelumab, Landogrozumab, Laprituximab emtansine, Larcaviximab, Lebrikizumab, Lecanemab, Lemalesomab, Lendalizumab, Lenvervimab, Lenzilumab, Lerdelimumab, Leronlimab, Lesofavumab, Letolizumab, Lexatumumab, Libivirumab, Lifestuzumab vedotin, Ligelizumab, Loncastuximab tesirine, Losatuxizumab vedotin, Lilotomab satetraxetan, Lintuzumab, Lirilumab, Lodelcizumab, Lokivetmab, Lorvotuzumab mertansine, Lucatumumab, Lulizumab pegol, Lumiliximab, Lumretuzumab, Lupartumab, Lupartumab amadotin, Lutikizumab, Maftivimab, Mapatumumab, Margetuximab, Marstacimab, Maslimomab, Mavrilimumab, Matuzumab, Mepolizumab, Metelimumab, Milatuzumab, Minretumomab, Mirikizumab, Mirvetuximab soravtansine, Mitumomab, Modotuximab, Mogamulizumab, Monalizumab, Morolimumab, Mosunetuzumab, Motavizumab, Moxetumomab pasudotox, Muromonab-CD3, Nacolomab tafenatox, Namilumab, Naptumomab estafenatox, Naratuximab emtansine, Narnatumab, Natalizumab, Navicixizumab, Navivumab, Naxitamab, Nebacumab, Necitumumab, Nemolizumab, NEOD001, Nerelimomab, Nesvacumab, Netakimab, Nimotuzumab, Nirsevimab, Nivolumab, Nofetumomab merpentan, Obiltoxaximab, Obinutuzumab, Ocaratuzumab, Ocrelizumab, Odesivimab, Odulimomab, Ofatumumab, Olaratumab, Oleclumab, Olendalizumab, Olokizumab, Omalizumab, Omburtamab, OMS721, Onartuzumab, Ontuxizumab, Onvatilimab, Opicinumab, Oportuzumab monatox, Oregovomab, Orticumab, Otelixizumab, Otilimab, Otlertuzumab, Oxelumab, Ozanezumab, Ozoralizumab, Pagibaximab, Palivizumab, Pamrevlumab,

Panitumumab, Pankomab, Panobacumab, Parsatuzumab, Pascolizumab, Pasotuxizumab, Pateclizumab, Patritumab, PDR001, Pembrolizumab, Pentumomab, Perakizumab, Pertuzumab, Pexelizumab, Pidilizumab, Pinatuzumab vedotin, Pintumomab, Placulumab, Pozelimab, Prezalumab, Plozalizumab, Pogalizumab, Polatuzumab vedotin, Ponezumab, Porgaviximab, Prasinezumab, Prezalizumab, Priliximab, Pritoxaximab, Pritumumab, PRO 140, Quilizumab, Racotumomab, Radretumab, Rafivirumab, Ralpancizumab, Ramucirumab, Ranevetmab, Ranibizumab, Raxibacumab, Ravagalimab, Ravulizumab, Refanezumab, Regavirumab, Regdanvimab, Relatlimab, Remtolumab, Reslizumab, Retifanlimab, Rilotumumab, Rinucumab, Risankizumab, Rituximab, Rivabazumab pegol, Robatumumab, Rmab, Roledumab, Romilkimab, Romosozumab, Rontalizumab, Rosmantuzumab, Rovalpituzumab tesirine, Rovelizumab, Rozanolixizumab, Ruplizumab, SA237, Sacituzumab govitecan, Samalizumab, Samrotamab vedotin, Sarilumab, Satralizumab, Satumomab pendetide, Secukinumab, Selicrelumab, Seribantumab, Setoxaximab, Setrusumab, Sevirumab, Sibrotuzumab, SGN-CD19A, SHP647, Sifalimumab, Siltuximab, Simtuzumab, Siplizumab, Sirtratumab vedotin, Sirukumab, Sofituzumab vedotin, Solanezumab, Solitomab, Sonepcizumab[104], Sontuzumab, Sotrovimab, Spartalizumab, Spesolimab[107], Stamulumab, Sulesomab, Suptavumab, Sutimlimab, Suvizumab, Suvratoxumab, Tabalumab, Tacatuzumab tetraxetan, Tadocizumab, Tafasitamab, Talacotuzumab, Talizumab, Talquetamab, Tamtuvetmab, Tanezumab, Taplitumomab paptax, Tarextumab, Tavolimab, Teclistamab, Tefibazumab, Telimomab aritox, Telisotuzumab, Telisotuzumab vedotin, Tenatumomab, Teneliximab, Teplizumab, Tepoditamab, Teprotumumab, Tesidolumab, Tetulomab, Tezepelumab, TGN1412, Tibulizumab, Tildrakizumab, Tigatuzumab, Timigutuzumab, Timolumab, tiragolumab, Tiragotumab, Tislelizumab, Tisotumab vedotin, Tixagevimab, TNX-650, Tocilizumab, Tomuzotuximab, Toralizumab, Tosatoxumab, Tositumomab, Tovetumab, Tralokinumab[115], Trastuzumab, Trastuzumab duocarmazine, Trastuzumab emtansine, TRBS07[117], Tregalizumab, Tremelimumab[118], Trevogrumab, Tucotuzumab celmoleukin, Tuvirumab, Ublituximab[120], Ulocuplumab, Urelumab, Urtoxazumab, Ustekinumab, Utomilumab, Vadastuximab talirine, Vanalimab, Vandortuzumab vedotin, Vantictumab, Vanucizumab, Vapaliximab, Varisacumab, Varlilumab, Vatelizumab, Vedolizumab, Veltuzumab, Vepalimomab, Vesencumab, Vilobelimumab, Visilizumab, Vobarilizumab, Volociximab, Vonlerolizumab, Vopratelimumab, Vorsetuzumab mafodotin, Votumumab, Vunakizumab, Xentuzumab, XMAB-5574, Zalutumumab, Zanolimumab, Zatuximab, Zenocutuzumab, Ziralimumab, Zolbetuximab, and Zolimomab aritox.

[00787] Embodiment 113: The method of Embodiment 110, wherein the transgene encodes a coagulation factor, optionally the coagulation factor is Factor VIII, Factor IX, Factor VII, Factor X, or Factor XI, preferably the coagulation factor is Factor VIII or Factor IX.

[00788] Embodiment 114: The method of Embodiment 110, wherein the transgene encodes a hormone, optionally the hormone is selected from the group consisting of selected from erythropoietin (EPO), parathyroid hormone (PTH), adiponectin, adrenaline (or epinephrine), adrenocorticotrophic hormone (ACTH or corticotropin), adropin, aldosterone, amylin (or islet amyloid polypeptide), androstenedione, angiotensin, angiotensinogen, antidiuretic hormone (or vasopressin), anti-müllerian hormone (or müllerian-inhibiting factor/hormone), arginine vasopressin), atrial natriuretic peptide (ANP or atriopeptin), brain natriuretic peptide, calcitonin, calcitriol, cholecystokinin (CCK), corticotropin-releasing hormone, cortisol, cortistatin, dehydroepiandrosterone, dihydrotestosterone, dopamine, endothelin, enkephalin, estradiol, estriol, estrone, follicle-stimulating hormone (FSH), galanin, gastric inhibitory polypeptide, gastrin, ghrelin, glucagon, glucagon-like peptide-1, gonadotropin-releasing hormone, growth hormone, growth hormone-releasing hormone, guanylin, hepcidin, human chorionic gonadotropin, human placental lactogen, inhibin, insulin, insulin-like growth factor (or somatomedin), leptin, leukotrienes, lipotropin, luteinizing hormone (LH), melanocyte stimulating hormone (MSH), melatonin, motilin, noradrenaline (or norepinephrine), orexin, osteocalcin, oxytocin (or pitocin), pancreatic polypeptide, pituitary adenylate cyclase-activating peptide, progesterone, prolactin (or leuteotropic hormone), prolactin-releasing hormone, prostacyclin, prostaglandins, relaxin, renin, secretin, somatostatin (or growth hormone-inhibiting hormone or growth hormone release-inhibiting hormone or somatotropin release-inhibiting factor or somatotropin release-inhibiting hormone), testosterone, thrombopoietin, thromboxane, thyroid-stimulating hormone (TSH or thyrotropin), thyrotropin-releasing hormone (TRH), thyroxine, triiodothyronine, uroguanylin, vasoactive intestinal peptide (VIP), or vasopressin, more optionally, the hormone is erythropoietin (EPO) and parathyroid hormone (PTH).

[00789] Embodiment 115: The method of Embodiment 110, wherein the transgene encodes a growth factor, optionally, the growth factor is tumor necrosis factor- α (TNF- α), adrenomedullin (AM), angiopoietin (Ang), autocrine motility factor, bone morphogenetic protein (BMP), ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), colony-stimulating factor (e.g., macrophage colony-stimulating factor (M-CSF), granulocyte colony-stimulating factor (G-CSF), or granulocyte macrophage colony-stimulating factor (GM-CSF)), epidermal growth factor (EGF), ephrin (e.g., ephrin A1, ephrin A2, ephrin A3, ephrin A4, ephrin A5, ephrin B1, ephrin B2, or ephrin B3), erythropoietin (EPO), fibroblast growth factor (e.g., FGF1, FGF2, FGF3, FGF4, FGF5, FGF6, FGF7, FGF8, FGF9, FGF10, FGF11, FGF12, FGF13, FGF14, FGF15, FGF16, FGF17, FGF18, FGF19, FGF20, FGF21, FGF22 or FGF23), glial cell line-derived neurotrophic factor (GDNF), neurturin, persephin, artemin, growth differentiation factor-9 (GDF9), hepatocyte growth factor (HGF), hepatoma-derived growth factor (HDGF), insulin-like

growth factor (e.g., IGF-1 or IGF-2), interleukin (e.g., IL-1, IL-2, IL-3, IL-4, IL-5, IL-6 or IL-7), keratinocyte growth factor (KGF), migration-stimulating factor (MSF), macrophage-stimulating protein (MSP), myostatin (GDF-8), neuregulin (e.g., NRG1, NRG3, NRG3 or NRG4), neurotrophin (e.g., brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-3 (NT-3) or neurotrophin-4 (NT-4)), placental growth factor (PGF), platelet-derived growth factor (PDGF), renalase (RNLS), T-cell growth factor (TCGF), thrombopoietin (TPO), transforming growth factor (e.g., TGF- α or TGF- β), or vascular endothelial growth factor (VEGF).

[00790] Embodiment 116: The method of Embodiment 110, the transgene encodes a CRISPR-Cas nuclease, optionally selected from the group consisting of Cas9, Cas12, Cas Phi, CasX, and CasRx.

[00791] Embodiment 117: The method of Embodiment 110, wherein the transgene encodes a cytokine, optionally, the cytokine is interleukin-1 α (IL-1 α), interleukin-1/3 (IL-1/3), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-8 (IL-8/CXCL8), interleukin-10 (IL-10), interleukin-12 (IL-12), interleukin-13 (IL-13), interleukin-15 (IL-15), interleukin-17 (IL-17), interleukin-18 (IL-18), interleukin-37 (IL-37), interleukin-38 (IL-38), tumor necrosis factor- α (TNF- α), interferon- α (INF- α), interferon-b (INF-b), interferon- γ (INF- γ), granulocyte-monocyte colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), monocyte chemoattractant protein-1 (MCP-1/CCL2), macrophage inflammatory protein 1-ct (MCP-1/CCL3), macrophage inflammatory protein-1/3, RANTES (CCL5), Eotaxin (CCL11), variable endothelial growth factor (VEGF), endothelial growth factor (EGF), or fibroblast growth factor (FGF). In some embodiments, the transgene encodes IL-2. In some embodiments, the transgene encodes IL-37. In some embodiments, the transgene encodes IL-38.

[00792] Embodiment 118: The method of Embodiment 110, wherein the transgene encodes a virus protein, optionally selected from the group consisting of viral envelop protein, viral nucleocapsid protein, viral membrane protein, REP, CAP, REV, VSV-G and RD114

[00793] Embodiment 119: The method of any one of claims 1-110, wherein the transgene is detrimental to the cell or to the subject when constitutively expressed.

[00794] Embodiment 120: The method of any one of Embodiments 1-119, wherein the subject is a non-human primate.

[00795] Embodiment 121: The method of any one of Embodiments 1-120, wherein the subject is a human.

[00796] Embodiment 122: The method of any one of Embodiments 1-121, wherein the cell comprising the cell comprising the exogenous nucleotide sequence is a liver cell.

[00797] Embodiment 123: A first and at least a second inducer for use in a method of regulating expression of a transgene in a primate subject, the method comprising: (a) administering to the subject at a first time point a first inducer, wherein the primate subject comprises at least one cell comprising an exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene, wherein the inducible promoter comprises a PBREM enhancer, wherein the first inducer is artemisinin, a derivative of artemisinin or a flavonoid compound, and wherein the first dose comprises an amount of the inducer that is sufficient to express a measurable level of the transgene in the subject; and (b) administering to the subject at least at a second time point a second inducer to continue expression of the transgene at a predetermined level for a predetermined time or to modulate expression of the transgene to a predetermined level, and wherein the second inducer is artemisinin; derivative of artemisinin; a flavonoid compound, e.g. flavone, chrysin, baicalein, or galangin; 1,4-Bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP); phenobarbital (PB); 6-(4Chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde-O-(3,4-dichlorobenzyl)oxime (CITCO); Acetaminophen; Buprenorphine; Phenytoin; Carbamazepine; Valproic Acid; Chlorpromazine; Efavirenz; Nevirapine; Rilpivirine; Etravirine; Diazepam; Cyclophosphamid; Ifosfamide; Cerivastatin; Simvastatin; lovastatin; substituted sulphonamides; Thiazolidin-4-one; Estradiol; Estrone and analogues; 17 α -ethinyl-3;17 β -estradiol (EE2); Dehydroepiandrosterone (DHEA); 5 β -pregnane-3,20-dione; Diethylstilbestrol; Ginkgo *biloba* extract; Galangin; chrysin; baicalein; Diallyl sulphide; Ellagic acid; Resveratrol; Squalestatin-1; Bilobalide; Triclocarban; Triclosan; Dichlorodiphenyltrichloroethane (DDT); Dieldrin; Methoxychlor; Metofluthrin; Permethrin; Pyrethrins; Sulfoxaflor; Diethylhexyl phthalate (DEHP); Cyproconazole; Fluconazole; Propiconazole; FL81; Tri-p-methylphenyl phosphate (TMPP); UM104; or UM145.

[00798] Embodiment 124: The first and at least a second inducer for use according to Embodiment 123, wherein the method is according to any one of Embodiments 1 to 122.

[00799] Embodiment 125: The first and at least a second inducer for use according to Embodiment 123 or 124, which is a method of therapy, suitably a method of gene therapy.

[00800] Embodiment 126: The first and at least a second inducer for use according to any one of Embodiments 123-125, wherein the exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene is an AAV gene therapy vector.

[00801] Embodiment 127: Artemisinin or a derivative of artemisinin, for use as an inducer in a method of regulating expression of a transgene in a primate subject, wherein the primate subject comprises at least one cell comprising an exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene, wherein the inducible promoter comprises a PBREM enhancer, wherein the inducer is administered to the subject at a dose which comprises an amount

of the inducer that is sufficient to express a therapeutically effective level of the transgene in the subject.

[00802] Embodiment 128: Artemisinin or a derivative of artemisinin for use according to Embodiment 127, wherein the method is a method of therapy, suitably a method of gene therapy.

[00803] Embodiment 129: Artemisinin or a derivative of artemisinin for use according to Embodiment 127 or 128, wherein the artemisinin derivative is a compound as defined in Embodiment 2, optionally wherein the artemisinin derivative is of Formula (I), (I-A), (I-B), (I-C), (I-D), (I-E), (I-F), (I-G), (I-H), (I-I), (I-J), (I-K), (I-L), (I-M), (I-M'), (I-N), (I-N'), (I-O) or (I-O').

[00804] Embodiment 130: Artemisinin or a derivative of artemisinin for use according to any one of Embodiments 127-129, wherein the derivative of artemisinin is artesunate, dihydroartemisinin, artesunate, or artemether.

[00805] Embodiment 131: Artemisinin or a derivative of artemisinin for use according to any one of Embodiments 127-130, wherein artemisinin or a derivative of artemisinin is administered to the primate subject following the prior administration of an inducer to the primate subject.

[00806] Embodiment 132: Artemisinin or a derivative of artemisinin for use according to Embodiment 131, wherein the inducer of the prior administration was a different inducer, or wherein the inducer of the prior administration was the same inducer but at a different dose.

[00807] Embodiment 133: Artemisinin or a derivative of artemisinin for use according to any one of Embodiments 127-132, wherein the exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene is an AAV gene therapy vector.

[00808] Embodiment 134: Artemisinin or a derivative of artemisinin for use according to any one of Embodiments 127 to 133, wherein the method comprises prior administration of the exogenous nucleotide sequence to the subject prior to administering the inducer.

[00809] Embodiment 135: Artemisinin or a derivative of artemisinin for use according to any one of Embodiments 127-134, wherein the artemisinin or a derivative of artemisinin is administered at a dose of from about 0.1mg/kg to 80mg/kg, from about 0.1mg/kg to about 70mg/kg, from about 0.1mg/kg to about 60mg/kg, from about 0.1mg/kg to about 50mg/kg, from about 0.1mg/kg to about 40mg/kg, from about 0.1mg/kg to about 30mg/kg, from about 0.1mg/kg to about 20mg/kg, from about 0.1mg/kg to about 10mg/kg, from about 0.1mg/kg to about 6mg/kg, from about 0.1mg/kg to about 5mg/kg, or from about 0.1mg/kg to about 1mg/kg.

[00810] Embodiment 136: Artemisinin or a derivative of artemisinin for use according to any one of Embodiments 127-135, wherein the inducer comprising artemisinin or a derivative of artemisinin is administered orally, optionally wherein the composition comprises artesunate.

[00811] Embodiment 137: A flavonoid compound, for use as an inducer in a method of regulating expression of a transgene in a primate subject, wherein the primate subject comprises at

least one cell comprising an exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene, wherein the inducible promoter comprises a PBREM enhancer, wherein the inducer is administered to the subject at a dose which comprises an amount of the inducer that is sufficient to express a therapeutically effective level of the transgene in the subject.

[00812] Embodiment 138: Flavonoid compound for use according to Embodiment 137, wherein the method is a method of therapy, suitably a method of gene therapy.

[00813] Embodiment 139: Flavonoid compound for use according to Embodiment 137 or 138, wherein the flavonoid compound is flavone, baicalein, chrysin, galangin, (OH)₄flavone, 6-hydroxyluteolin, acacetin, acerosin, alnetin, apigenin, apiin, artocarpetin, balcalein, beta-sitosterol, biochamin A, cannaflavin A, cannflavin A, cannflavin B, cannflavin C, catechin, catechin-3-gallate, cerrosillin, chrysoeriol, chrysoeriol, cirsilin, cirsilin, cirsimaritin, corymbosin, cupressuflavone, cyanidine, daidzein, datiscetin, delphinidine, digidrokempferol, dihydroquercetin, diosmetin, diosmin, echiodinin, epicatechin, epicatechin-3-gallate, epigallocatechin, epigallocatechin-3-gallate, eriodictiol, eupatilin, eupatorin, fisetin, fizin, flavonol, formononetin, fortunelin, gallocatechin, gallocatechin-3-gallate, gardenin D, genistein, genistin, genkwanin, geraldol, geraldone, glycitein, glycitin, gomoeriodictiol, gossypetin, hesperetin, hinokiflavone, hispidulin, hymenoxin, hypolaetin, isoquercetin, isorhamnetin, isorhamnetin, isoscutellarein, isovitexin, jaceosidin, kaempferol, kaempferol, kampferide, largonidin, luteolin, luteolin, malvidine, mikanin, morin, myricetin, myricetin, naringenin, negletein, neodiosmin, nepetin, nevadensin, nobiletin, nodifloretin, norartocarpetin, norwogonin, onopordin, orientin, oroxylin a, pachipodol, pectolinarigenin, pedalitin, peonidin, petunidin, pilloin, pratol, primetin, primuletin, pruentin, quercetagenin, quercetin, ramnazin, rhoifolin, robinetin, rutin, scaposin, scutellarein, serpyllin, sinensetin, sorbifolin, sudachitin, tangeretin, tangeritin, taxifolin, tectochrysin, tithonine, tricetin, tricin, velutin, vitexin, wightin, wogonin, xanthomicrol, zapotin, zapotinin, 6,7,4'-trihydroxy isoflavone, or 7,3'4'-trihydroxy isoflavone.

[00814] Embodiment 140: Flavonoid compound for use according to any one of Embodiments 137-139, wherein the flavonoid compound is flavone

[00815] Embodiment 141: Flavonoid compound for use according to any one of Embodiments 137-140, wherein the flavonoid compound is administered to the primate subject following the prior administration of an inducer to the primate subject.

[00816] Embodiment 142: Flavonoid compound for use according to Embodiment 141, wherein the inducer of the prior administration was a different inducer, or wherein the inducer of the prior administration was the same inducer but at a different dose.

[00817] Embodiment 143: Flavonoid compound for use according to any one of Embodiments 137-142, wherein the exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene is an AAV gene therapy vector.

[00818] Embodiment 144: Flavonoid compound for use according to any one of Embodiments 137-143, wherein the method comprises prior administration of the exogenous nucleotide sequence to the subject prior to administering the inducer.

[00819] Embodiment 145: Flavonoid compound for use according to any one of Embodiments 137-144, wherein the flavonoid compound is administered at a dose of from about 0.1mg/kg to 80mg/kg, from about 0.1mg/kg to about 70mg/kg, from about 0.1mg/kg to about 60mg/kg, from about 0.1mg/kg to about 50mg/kg, from about 0.1mg/kg to about 40mg/kg, from about 0.1mg/kg to about 30mg/kg, from about 0.1mg/kg to about 20mg/kg, from about 0.1mg/kg to about 10mg/kg, from about 0.1mg/kg to about 6mg/kg, from about 0.1mg/kg to about 5mg/kg, or from about 0.1mg/kg to about 1mg/kg.

[00820] Embodiment 146: Flavonoid compound for use according to any one of Embodiments 137-145, wherein the flavonoid compound is administered orally.

[00821] Embodiment 147: Flavonoid compound for use according to any one of Embodiments 137-145, wherein the flavonoid compound is administered intraperitoneally.

[00822] Embodiment 148: A method of regulating expression of a transgene in the liver of a subject, the method comprising: administering to the subject at least a first time point a dose of a first inducer;

administering to the subject at least a second time point a second dose of a second inducer, wherein the second inducer can be the same as or different than the first inducer; and administering to the subject at least a third time point a third dose of a third inducer, wherein the third inducer can be the same as or different than the first inducer and the second inducer;

wherein the subject comprises at least one liver cell comprising an exogenous nucleotide sequence comprising a synthetic inducible promoter operably linked to a transgene;

wherein the synthetic inducible promoter comprises at least one PBREM enhancer; and

wherein the transgene is detrimental to the liver cell or to the subject when constitutively expressed.

[00823] Embodiment 149: The method of Embodiment 148, wherein the first inducer is selected from the group consisting of artemisinin; derivative of artemisinin; a flavonoid compound, e.g. flavone, chrysin, baicalein, or galangin; 1,4-Bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP); phenobarbital (PB); 6-(4Chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde-O-(3,4-dichlorobenzyl)oxime (CITCO); Acetaminophen; Buprenorphine; Phenytoin; Carbamazepine;

Valproic Acid; Chlorpromazine; Efavirenz; Nevirapine; Rilpivirine; Etravirine; Diazepam; Cyclophosphamid; Ifosfamide; Cerivastatin; Simvastatin; lovastatin; substituted sulphonamides; Thiazolidin-4-one; Estradiol; Estrone and analogues; 17 α -ethinyl-3;17 β -estradiol (EE2); Dehydroepiandrosterone (DHEA); 5 β -pregnane-3,20-dione; Diethylstilbestrol; Ginkgo *biloba* extract; Galangin; chrysin; baicalein; Diallyl sulphide; Ellagic acid; Resveratrol; Squalestatin-1; Bilobalide; Triclocarban; Triclosan; Dichlorodiphenyltrichloroethane (DDT); Dieldrin; Methoxychlor; Metofluthrin; Permethrin; Pyrethrins; Sulfoxaflor; Diethylhexyl phthalate (DEHP); Cyproconazole; Fluconazole; Propiconazole; FL81; Tri-p-methylphenyl phosphate (TMPP); UM104; or UM145.

[00824] Embodiment 150: The method of Embodiment 148 or 149, wherein the second inducer is selected from the group consisting of artemisinin; derivative of artemisinin; a flavonoid compound, e.g. flavone, chrysin, baicalein, or galangin; 1,4-Bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP); phenobarbital (PB); 6-(4Chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde-O-(3,4-dichlorobenzyl)oxime (CITCO); Acetaminophen; Buprenorphine; Phenytoin; Carbamazepine; Valproic Acid; Chlorpromazine; Efavirenz; Nevirapine; Rilpivirine; Etravirine; Diazepam; Cyclophosphamid; Ifosfamide; Cerivastatin; Simvastatin; lovastatin; substituted sulphonamides; Thiazolidin-4-one; Estradiol; Estrone and analogues; 17 α -ethinyl-3;17 β -estradiol (EE2); Dehydroepiandrosterone (DHEA); 5 β -pregnane-3,20-dione; Diethylstilbestrol; Ginkgo *biloba* extract; Galangin; chrysin; baicalein; Diallyl sulphide; Ellagic acid; Resveratrol; Squalestatin-1; Bilobalide; Triclocarban; Triclosan; Dichlorodiphenyltrichloroethane (DDT); Dieldrin; Methoxychlor; Metofluthrin; Permethrin; Pyrethrins; Sulfoxaflor; Diethylhexyl phthalate (DEHP); Cyproconazole; Fluconazole; Propiconazole; FL81; Tri-p-methylphenyl phosphate (TMPP); UM104; or UM145.

[00825] Embodiment 151: The method of any one of Embodiments 148-150, wherein the third inducer is selected from the group consisting of artemisinin; derivative of artemisinin; a flavonoid compound, e.g. flavone, chrysin, baicalein, or galangin; 1,4-Bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP); phenobarbital (PB); 6-(4Chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde-O-(3,4-dichlorobenzyl)oxime (CITCO); Acetaminophen; Buprenorphine; Phenytoin; Carbamazepine; Valproic Acid; Chlorpromazine; Efavirenz; Nevirapine; Rilpivirine; Etravirine; Diazepam; Cyclophosphamid; Ifosfamide; Cerivastatin; Simvastatin; lovastatin; substituted sulphonamides; Thiazolidin-4-one; Estradiol; Estrone and analogues; 17 α -ethinyl-3;17 β -estradiol (EE2); Dehydroepiandrosterone (DHEA); 5 β -pregnane-3,20-dione; Diethylstilbestrol; Ginkgo *biloba* extract; Galangin; chrysin; baicalein; Diallyl sulphide; Ellagic acid; Resveratrol; Squalestatin-1; Bilobalide; Triclocarban; Triclosan; Dichlorodiphenyltrichloroethane (DDT); Dieldrin; Methoxychlor; Metofluthrin; Permethrin;

Pyrethrins; Sulfoxaflor; Diethylhexyl phthalate (DEHP); Cyproconazole; Fluconazole; Propiconazole; FL81; Tri-p-methylphenyl phosphate (TMPP); UM104; or UM145.

[00826] Embodiment 152: The method of any one of Embodiments 1-151, wherein the first inducer is artemisinin, or a derivative thereof.

[00827] Embodiment 153: The method of any one of Embodiments 1-151, wherein first inducer is a flavonoid compound.

[00828] Embodiment 154: The method of any one of Embodiments 1-153, wherein the second inducer or third inducer is artemisinin or, derivative thereof.

[00829] Embodiment 155: The method of any one of Embodiments 1-153, wherein the second inducer is flavonoid compound.

Selected definitions

[00830] For convenience, the meaning of some terms and phrases used in the specification, examples, and appended claims, are provided below. Unless stated otherwise, or implicit from context, the following terms and phrases include the meanings provided below. The definitions are provided to aid in describing particular embodiments, and are not intended to limit the claimed technology, because the scope of the technology is limited only by the claims. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this technology belongs. If there is an apparent discrepancy between the usage of a term in the art and its definition provided herein, the definition provided within the specification shall prevail.

[00831] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, for example, Current Protocols in Molecular Biology (Ausubel, 2000, Wiley and son Inc, Library of Congress, USA); Molecular Cloning: A Laboratory Manual, Third Edition, (Sambrook et al, 2001, Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press); Oligonucleotide Synthesis (M. J. Gait ed., 1984); U.S. Pat. No. 4,683,195; Nucleic Acid Hybridization (Harries and Higgins eds. 1984); Transcription and Translation (Hames and Higgins eds. 1984); Culture of Animal Cells (Freshney, Alan R. Liss, Inc., 1987); Immobilized Cells and Enzymes (IRL Press, 1986); Perbal, A Practical Guide to Molecular Cloning (1984); the series, Methods in Enzymology (Abelson and Simon, eds. -in-chief, Academic Press, Inc., New York), specifically, Vols.154 and 155 (Wu et al. eds.) and Vol. 185, "Gene Expression Technology" (Goeddel, ed.); Gene Transfer Vectors For Mammalian Cells (Miller and Calos eds., 1987, Cold Spring Harbor Laboratory); Immunochemical Methods in Cell and

Molecular Biology (Mayer and Walker, eds., Academic Press, London, 1987); Handbook of Experimental Immunology, Vols. I-IV (Weir and Blackwell, eds., 1986); and Manipulating the Mouse Embryo, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986).

[00832] Definitions of common terms in immunology and molecular biology can be found in The Merck Manual of Diagnosis and Therapy, 19th Edition, published by Merck Sharp & Dohme Corp., 2011 (ISBN 978-0-911910-19-3); Robert S. Porter et al. (eds.), The Encyclopedia of Molecular Cell Biology and Molecular Medicine, published by Blackwell Science Ltd., 1999-2012 (ISBN 9783527600908); and Robert A. Meyers (ed.), Molecular Biology and Biotechnology: a Comprehensive Desk Reference, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8); Immunology by Werner Luttmann, published by Elsevier, 2006; Janeway's Immunobiology, Kenneth Murphy, Allan Mowat, Casey Weaver (eds.), Taylor & Francis Limited, 2014 (ISBN 0815345305, 9780815345305); Lewin's Genes XI, published by Jones & Bartlett Publishers, 2014 (ISBN-1449659055); Michael Richard Green and Joseph Sambrook, Molecular Cloning: A Laboratory Manual, 4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., USA (2012) (ISBN 1936113414); Davis et al., Basic Methods in Molecular Biology, Elsevier Science Publishing, Inc., New York, USA (2012) (ISBN 044460149X); Laboratory Methods in Enzymology: DNA, Jon Lorsch (ed.) Elsevier, 2013 (ISBN 0124199542); Current Protocols in Molecular Biology (CPMB), Frederick M. Ausubel (ed.), John Wiley and Sons, 2014 (ISBN 047150338X, 9780471503385), Current Protocols in Protein Science (CPPS), John E. Coligan (ed.), John Wiley and Sons, Inc., 2005; and Current Protocols in Immunology (CPI) (John E. Coligan, ADA M Kruisbeek, David H Margulies, Ethan M Shevach, Warren Strobe, (eds.) John Wiley and Sons, Inc., 2003 (ISBN 0471142735, 9780471142737), the contents of which are all incorporated by reference herein in their entireties

[00833] As used herein the term “comprising” or “comprises” is used in reference to compositions, methods, and respective component(s) thereof, that are essential to the invention, yet open to the inclusion of unspecified elements, whether essential or not.

[00834] As used herein the term “consisting essentially of” refers to those elements required for a given embodiment. The term permits the presence of additional elements that do not materially affect the basic and novel or functional characteristic(s) of that embodiment of the invention.

[00835] The term “consisting of” refers to compositions, methods, and respective components thereof as provided herein, which are exclusive of any element not recited in that description of the embodiment.

[00836] Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients or reaction conditions used herein should be understood as

modified in all instances by the term “about.” The term “about” when used in connection with percentages can mean $\pm 1\%$.

[00837] The term “statistically significant” or “significantly” refers to statistical significance and generally means at least two standard deviation (2SD) away from a reference level. The term refers to statistical evidence that there is a difference. It is defined as the probability of making a decision to reject the null hypothesis when the null hypothesis is actually true.

[00838] The singular terms “a,” “an,” and “the” include plural referents unless context clearly indicates otherwise. Similarly, the word “or” is intended to include “and” unless the context clearly indicates otherwise. Thus for example, references to “the method” includes one or more methods, and/or steps of the type provided herein and/or which will become apparent to those persons skilled in the art upon reading this disclosure and so forth.

[00839] Although methods and materials similar or equivalent to those provided herein can be used in the practice or testing of this disclosure, suitable methods and materials are described below. The term “comprises” means “includes.” The abbreviation, “e.g.” is derived from the Latin *exempli gratia*, and is used herein to indicate a non-limiting example. Thus, the abbreviation “e.g.” is synonymous with the term “for example.”

[00840] The term “cis-regulatory element” or “CRE”, is a term well-known to the skilled person, and means a nucleic acid sequence such as an enhancer, promoter, insulator, or silencer, that can regulate or modulate the transcription of a neighbouring gene (i.e., *in cis*). CREs are found in the vicinity of the genes that they regulate. CREs typically regulate gene transcription by binding to TFs, i.e., they include TFBS. A single TF may bind to many CREs, and hence control the expression of many genes (pleiotropy). CREs are usually, but not always, located upstream of the transcription start site (TSS) of the gene that they regulate. “Enhancers” are CREs that enhance (i.e., upregulate) the transcription of genes that they are operably associated with, and can be found upstream, downstream, and even within the introns of the gene that they regulate. Multiple enhancers can act in a coordinated fashion to regulate transcription of one gene. “Silencers” in this context relates to CREs that bind TFs called repressors, which act to prevent or downregulate transcription of a gene. The term “silencer” can also refer to a region in the 3' untranslated region of messenger RNA, that bind proteins which suppress translation of that mRNA molecule, but this usage is distinct from its use in describing a CRE. Generally, the CREs described herein are liver-specific inducible enhancers. In the present context, it is preferred that the CRE is located 1500 nucleotides or less from the transcription start site (TSS), more preferably 1000 nucleotides or less from the TSS, more preferably 500 nucleotides or less from the TSS, and suitably 250, 200, 150, or 100 nucleotides or less from the TSS. CREs described herein are preferably comparatively short

in length, preferably 100 nucleotides or less in length, for example they may be 90, 80, 70, 60 nucleotides or less in length. In some embodiments, the CRE is a PBREM enhancer.

[00841] The term “cis-regulatory module” or “CRM” means a functional module made up of two or more CREs. The CREs described herein are typically liver-specific inducible enhancers. Thus, in the present application a CRM typically comprises a plurality of liver-specific inducible CREs. Typically, the multiple CREs within the CRM act together (e.g., additively or synergistically) to enhance the transcription of a gene that the CRM is operably associated with. There is conservable scope to shuffle (i.e., reorder), invert (i.e., reverse orientation), and alter spacing in CREs within a CRM. Accordingly, functional variants of CRMs described herein include variants of the referenced CRMs wherein CREs within them have been shuffled and/or inverted, and/or the spacing between CREs has been altered.

[00842] As used herein, the term “promoter” refers to a region of DNA that generally is located upstream of a nucleic acid sequence to be transcribed that is needed for transcription to occur, i.e., which initiates transcription. Promoters permit the proper activation or repression of transcription of a coding sequence under their control. A promoter typically contains specific sequences that are recognized and bound by plurality of TFs. TFs bind to the promoter sequences and result in the recruitment of RNA polymerase, an enzyme that synthesizes RNA from the coding region of the gene. A great many promoters are known in the art. The inducible described herein typically drive a low level of expression prior to being induced, and upon induction they drive a significantly higher level of expression (e.g., a 2, 3, 4, 5, 6, 7, 8, 9, or even 10-fold increase in expression after induction).

[00843] In some embodiments, the inducible promoters described herein is a synthetic promoter. The term “synthetic promoter” as used herein relates to a promoter that does not occur in nature. In the present context it typically comprises a synthetic CRE and/or CRM described herein operably linked to a minimal (or core) promoter or proximal promoter, e.g., liver-specific proximal promoter. The CREs and/or CRMs described herein serve to provide inducible liver-specific transcription of a gene operably linked to the promoter. Parts of the synthetic promoter can be naturally occurring (e.g., the minimal promoter or one or more CREs in the promoter), but the synthetic promoter as a complete entity is not naturally occurring.

[00844] As used herein, “minimal promoter” (also known as the “core promoter”) refers to a short DNA segment which is inactive or largely inactive by itself, but can mediate transcription when combined with other transcription regulatory elements. Minimum promoter sequence can be derived from various different sources, including prokaryotic and eukaryotic genes. Examples of minimal promoters are discussed above, and include the dopamine beta-hydroxylase gene minimum promoter, cytomegalovirus (CMV) immediate early gene minimum promoter (CMV-

MP), SV40 minimal promoter (SV40-MP), and the herpes thymidine kinase minimal promoter (MinTK). However, the proximal promoter can be synthetic. A minimal promoter typically comprises the transcription start site (TSS) and elements directly upstream, a binding site for RNA polymerase II, and general transcription factor binding sites (often a TATA box).

[00845] As used herein, “proximal promoter” relates to the minimal promoter plus the proximal sequence upstream of the gene that tends to contain primary regulatory elements. It often extends approximately 250 base pairs upstream of the TSS, and includes specific TFBS. In the present case, the proximal promoter is suitably a naturally occurring liver-specific proximal promoter that can be combined with one or more CREs or CRMs described herein. However, the proximal promoter can be synthetic.

[00846] A “functional variant” of a cis-regulatory element, cis-regulatory module, promoter or other nucleic acid sequence in the context of the present disclosure is a variant of a reference sequence that retains the ability to function in the same way as the reference sequence, e.g., as an inducible liver-specific cis-regulatory enhancer element, inducible liver-specific cis-regulatory module or inducible liver-specific promoter. Alternative terms for such functional variants include “biological equivalents” or “equivalents”.

[00847] It will be appreciated that, as discussed above, the ability of a given cis-regulatory element to function as an inducible liver-specific enhancer is determined principally by the ability of the sequence to be bound by the CAR-RXR heterodimer such the expression is induced. Accordingly, in most cases, a functional variant of a cis-regulatory element will contain a suitable binding site for the CAR-RXR heterodimer. The CAR-RXR heterodimer is believed to bind to the NR1 motif in the wild type PBREM element, and thus a sequence which can function as an NR1 motif is desired. There is a very high degree of sequence conservation between mouse and human PBREM in the NR1 motif, and thus it is typically desired that a high level of identity to the NR1 motif is preserved in any functional variant. Additional sequences in the in the wild type PBREM element may contribute to minimising background expression and providing high levels of inducibility, and thus it is generally preferred that a functional variant contains at least some degree of sequence identity in these other areas. Levels of sequence identity between a functional variant and a reference sequence can thus be an indicator of retained functionality. High levels of sequence identity in the NR1 motif of the cis-regulatory element is of generally higher importance than sequence identity in other regions (e.g., NF1 and NR2, where there is considerably less need, if any, for any conservation of sequence).

[00848] The ability of CAR-RXR heterodimer to bind to a given CRE can be determined by any relevant means known in the art, including, but not limited to, electromobility shift assays (EMSA), binding assays, chromatin immunoprecipitation (ChIP), and ChIP-sequencing (ChIP-seq). In a

preferred embodiment the ability of the CAR-RXR heterodimer to bind a given functional variant is determined by EMSA. Methods of performing EMSA are well-known in the art. Suitable approaches are described in Sambrook et al. cited above. Many relevant articles describing this procedure are available, e.g., Hellman and Fried, Nat Protoc. 2007; 2(8): 1849–1861.

[00849] “Liver-specific” or “liver-specific expression” refers to the ability of a cis-regulatory element, cis-regulatory module or promoter to enhance or drive expression of a gene in the liver (or in liver-derived cells) in a preferential or predominant manner as compared to other tissues (e.g., spleen, muscle, heart, lung, and brain). In the case of the present disclosure that expression should be inducible, i.e., expression of the gene only occurs, or increases significantly, when a suitable inducer is administered. Expression of the gene can be in the form of mRNA or protein. In some embodiments, liver-specific expression is such that there is negligible expression in other (i.e., non-liver) tissues or cells, i.e., expression is highly liver-specific.

[00850] The ability of a promoter to function as a liver-specific inducible promoter can be readily assessed by the skilled person. The skilled person can thus easily determine whether any variant of the specific promoters exemplified herein remains functional (i.e., it is a functional variant as defined above). For example, any given CRE to be assessed can be operably linked to a minimal promoter (e.g., positioned upstream of MinTK) and the ability of the cis-regulatory element to provide inducible liver-specific expression of a gene (typically a reporter gene) is measured. Alternatively, a variant of a CRE can be substituted into an inducible liver-specific promoter in place of a reference CRE, and the effects on inducible liver-specific expression driven by said modified promoter can be determined and compared to the unmodified form. Similarly, the ability of a promoter to induce liver-specific expression can be readily assessed by the skilled person (e.g., as described in the examples below). Expression levels and inducibility of a gene driven by a variant of a reference promoter can be compared to the expression levels and inducibility by the reference sequence, and suitable approaches are discussed above. Without wishing to be bound by theory, induction of gene expression via a PBREM element, or variants thereof, is believed to be dependent upon binding thereto of the CAR-RXR heterodimer. The ability of any given CRE that is capable of being bound and activated by a CAR-RXR heterodimer, e.g. a variant of a PBREM element, to function as desired, i.e. to be inducible in the same way as the wild type mouse or human PBREM elements, though not necessarily to the same extent, can readily be determined experimentally. For example, the variant CRE can be inserted into the construct as set out in the examples below in place of SEQ ID NO: 1, and the ability of the construct to be induced compared when SEQ ID NO: 1 was included can be compared. For example, a variant CRE can be provided in place of SEQ ID NO: 1 in the PB1-MinTK construct and tested in liver cells, preferably primary hepatocytes, e.g. AXOL assay-ready expanded (ARE) hepatocytes

(Axol, ax3701) with induction by 1 μ M CITCO, as described in Example 2 of US20220111078. Alternatively, a variant could be inserted into the pAAV-PB1-MinTk construct, as described in Example 4 of US20220111078. Alternatively, the experimental approach used in Sueyoshi, et al. (J. BIOL. CHEM. Vol. 274,10, pp. 6043-6046, 1999) can be used, i.e. the relevant putative PBREM variant sequences can be cloned in front of the tk promoter (BglII site) in the pGL3-Basic vector containing the firefly luciferase reporter gene (Promega), resulting in PBREM VARIANT-tk-luciferase and reporter gene plasmids. Generally, a functional variant of a CRE should provide a level of inducibility that is at least 50%, preferably 75%, more preferably 80%, 85%, 90%, or 95% as inducible when compared to the equivalent construct comprising the wild type PBREM element, e.g. SEQ ID NO: 1 (measured in terms of fold increase in expression as a result of induction, i.e. a 2 fold increase in expression of a reported gene upon induction is considered to be 50% as inducible as a 4-fold increase). Generally, it is desired that the functional variant should provide at least 2-fold induction, more preferably 3-fold, 4-fold, 5-fold, 6-fold or 7-fold induction when provided in place of the wild type PBREM in the PB1-MinTK construct described herein and tested in liver cells, preferably primary hepatocytes, e.g. AXOL assay-ready expanded (ARE) hepatocytes (Axol, ax3701) with induction by 1 μ M CITCO, as described in Example 2 of US20220111078. A functional variant preferably results in a background expression level that is no more than three times as high, preferably no more than twice as high, and preferably no more than 1.5 times as high when compared to the equivalent construct comprising the wild-type mouse PBREM element (e.g. again in the PB1-MinTK construct and tested in ARE hepatocytes).

[00851] Liver-specificity can be identified wherein the expression of a gene (e.g., a therapeutic or reporter gene) when induced occurs preferentially or predominantly in liver-derived cells. Preferential or predominant expression can be defined, for example, where the level of expression when induced is significantly greater in liver-derived cells than in other types of cells (i.e., non-liver-derived cells). For example, expression in liver-derived cells when induced is suitably at least 5-fold higher than non-liver cells, preferably at least 10-fold higher than non-liver cells, and it may be 50-fold higher or more in some cases. For convenience, liver-specific expression can suitably be demonstrated via a comparison of expression levels in a hepatic cell line (e.g., liver-derived cell line such as Huh7 and/or HepG2 cells) or liver primary cells, compared with expression levels in a kidney-derived cell line (e.g., HEK-293), a cervical tissue-derived cell line (e.g., HeLa) and/or a lung-derived cell line (e.g., A549).

[00852] The liver-specific inducible promoters described herein preferably have a reduced expression at a level of at least 4-fold less than the CMV-IE promoter in non-liver-derived cells, suitably in HEK-293, HeLa, and/or A549 cells when induced.

[00853] The liver-specific inducible promoters of described herein are preferably suitable for promoting expression in the liver of a subject, e.g., driving liver-specific expression of a transgene, preferably a therapeutic transgene.

[00854] It should also be noted that the liver-specific promoters of described herein, in some cases, be used in non-liver cells. Generally non-liver cells do not express CAR, and thus the promoters described herein generally do not function in such cells. However, where non-liver cells are engineered to express CAR (and RXR if they do not naturally express RXR) such non-liver cells can induce expression of genes linked to the promoters described herein. In other word, the liver-specific promoters described herein can also function in non-liver cells that have been modified to have a liver-like phenotype in terms of CAR and RXR expression. The term "liver-specific" should be construed accordingly. Where liver cells do not express CAR (e.g., the Huh-7 cell line) or the RXR, they can likewise be modified to express the relevant protein.

[00855] The term "nucleic acid" as used herein typically refers to an oligomer or polymer (preferably a linear polymer) of any length composed essentially of nucleotides. A nucleotide unit commonly includes a heterocyclic base, a sugar group, and at least one, e.g., one, two, or three, phosphate groups, including modified or substituted phosphate groups. Heterocyclic bases may include inter alia purine and pyrimidine bases such as adenine (A), guanine (G), cytosine (C), thymine (T) and uracil (U) which are widespread in naturally occurring nucleic acids, other naturally-occurring bases (e.g., xanthine, inosine, hypoxanthine) as well as chemically or biochemically modified (e.g., methylated), non-natural or derivatised bases. Sugar groups may include inter alia pentose (pentofuranose) groups such as preferably ribose and/or 2-deoxyribose common in naturally occurring nucleic acids, or arabinose, 2-deoxyarabinose, threose or hexose sugar groups, as well as modified or substituted sugar groups. Nucleic acids as intended herein may include naturally occurring nucleotides, modified nucleotides or mixtures thereof. A modified nucleotide may include a modified heterocyclic base, a modified sugar moiety, a modified phosphate group or a combination thereof. Modifications of phosphate groups or sugars may be introduced to improve stability, resistance to enzymatic degradation, or some other useful property. The term "nucleic acid" further preferably encompasses DNA, RNA and DNA RNA hybrid molecules, specifically including hnRNA, pre-mRNA, mRNA, cDNA, genomic DNA, amplification products, oligonucleotides, and synthetic (e.g., chemically synthesised) DNA, RNA or DNA RNA hybrids. A nucleic acid can be naturally occurring, e.g., present in or isolated from nature; or can be non-naturally occurring, e.g., recombinant, i.e., produced by recombinant DNA technology, and/or partly or entirely, chemically or biochemically synthesised. A "nucleic acid" can be double-stranded, partly double stranded, or single-stranded. Where single-stranded, the

nucleic acid can be the sense strand or the antisense strand. In addition, nucleic acid can be circular or linear.

[00856] The terms "identity" and "identical" and the like refer to the sequence similarity between two polymeric molecules, e.g., between two nucleic acid molecules, such as between two DNA molecules. Sequence alignments and determination of sequence identity can be done, e.g., using the Basic Local Alignment Search Tool (BLAST) originally described by Altschul et al. 1990 (J Mol Biol 215: 403-10), such as the "Blast 2 sequences" algorithm described by Tatusova and Madden 1999 (FEMS Microbiol Lett 174: 247-250). Methods for aligning sequences for comparison are well-known in the art. Various programs and alignment algorithms are described in, for example: Smith and Waterman (1981) Adv. Appl. Math. 2:482; Needleman and Wunsch (1970) J. Mol. Biol. 48:443; Pearson and Lipman (1988) Proc. Natl. Acad. Sci. U.S.A. 85:2444; Higgins and Sharp (1988) Gene 73:237-44; Higgins and Sharp (1989) CABIOS 5:151-3; Corpet et al. (1988) Nucleic Acids Res. 16:10881-90; Huang et al. (1992) Comp. Appl. Biosci. 8:155-65; Pearson et al. (1994) Methods Mol. Biol. 24:307-31; Tatiana et al. (1999) FEMS Microbiol. Lett. 174:247-50. A detailed consideration of sequence alignment methods and homology calculations can be found in, e.g., Altschul et al. (1990) J. Mol. Biol. 215:403-10. The National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST™; Altschul et al. (1990)) is available from several sources, including the National Center for Biotechnology Information (Bethesda, MD), and on the internet, for use in connection with several sequence analysis programs. A description of how to determine sequence identity using this program is available on the internet under the "help" section for BLAST™. For comparisons of nucleic acid sequences, the "Blast 2 sequences" function of the BLAST™ (Blastn) program may be employed using the default parameters. Nucleic acid sequences with even greater similarity to the reference sequences will show increasing percentage identity when assessed by this method. Typically, the percentage sequence identity is calculated over the entire length of the sequence. For example, a global optimal alignment is suitably found by the Needleman-Wunsch algorithm with the following scoring parameters: Match score: +2, Mismatch score: -3; Gap penalties: gap open 5, gap extension 2. The percentage identity of the resulting optimal global alignment is suitably calculated by the ratio of the number of aligned bases to the total length of the alignment, where the alignment length includes both matches and mismatches, multiplied by 100.

[00857] As used herein, the term "synthetic" means a nucleic acid molecule that does not occur in nature. Synthetic nucleic acid expression constructs described herein are produced artificially, typically by recombinant technologies. Such synthetic nucleic acids can contain naturally occurring sequences (e.g., promoter, enhancer, intron, and other such regulatory sequences), but these are present in a non-naturally occurring context. For example, a synthetic gene (or portion

of a gene) typically contains one or more nucleic acid sequences that are not contiguous in nature (chimeric sequences), and/or may encompass substitutions, insertions, and deletions and combinations thereof.

[00858] “Complementary” or “complementarity”, as used herein, refers to the Watson-Crick base-pairing of two nucleic acid sequences. For example, for the sequence 5'-AGT-3' binds to the complementary sequence 3'-TCA-5'. Complementarity between two nucleic acid sequences may be “partial”, in which only some of the bases bind to their complement, or it may be complete as when every base in the sequence binds to its complementary base. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridisation between nucleic acid strands.

[00859] “Transfection” in the present application refers broadly to any process of deliberately introducing nucleic acids into cells, and covers introduction of viral and non-viral vectors, and includes transformation, transduction and like terms and processes. Examples include, but are not limited to: transfection with viral vectors; transformation with plasmid vectors; electroporation (Fromm et al. (1986) *Nature* 319:791-3); lipofection (Feigner et al. (1987) *Proc. Natl. Acad. Sci. USA* 84:7413-7); microinjection (Mueller et al. (1978) *Cell* 15:579-85); *Agrobacterium*-mediated transfer (Fraley et al. (1983) *Proc. Natl. Acad. Sci. USA* 80:4803-7); direct DNA uptake; whiskers-mediated transformation; and microprojectile bombardment (Klein et al. (1987) *Nature* 327:70).

[00860] As used herein, the phrase “transgene” refers to an exogenous nucleic acid sequence. In one example, a transgene is a gene encoding an industrially or pharmaceutically useful compound, or a gene encoding a desirable trait. In yet another example, the transgene encodes an antisense nucleic acid sequence, wherein expression of the antisense nucleic acid sequence inhibits expression of a target nucleic acid sequence.

[00861] The term “operably linked”, “operably connected” or equivalent expressions as used herein refer to the arrangement of various nucleic acid elements relative to each such that the elements are functionally connected and are able to interact with each other in the manner intended. Such elements may include, without limitation, a promoter, an enhancer and/or a regulatory element, a polyadenylation sequence, one or more introns and/or exons, and a coding sequence of a gene of interest to be expressed. The nucleic acid sequence elements, when properly oriented or operably linked, act together to modulate the activity of one another, and ultimately may affect the level of expression of an expression product. By modulate is meant increasing, decreasing, or maintaining the level of activity of a particular element. The position of each element relative to other elements may be expressed in terms of the 5' terminus and the 3' terminus of each element, and the distance between any particular elements may be referenced by the number of intervening nucleotides, or base pairs, between the elements. As understood by the skilled person, operably

linked implies functional activity, and is not necessarily related to a natural positional link. Indeed, when used in a vector, cis-regulatory elements will typically be located immediately upstream of the promoter (although this is generally the case, it should definitely not be interpreted as a limitation or exclusion of positions within the vector, but this needs not be the case *in vivo*, e.g., a regulatory element sequence naturally occurring downstream of a gene whose transcription it affects is able to function in the same way when located upstream of the promoter. Hence, according to a specific embodiment, the regulatory or enhancing effect of the regulatory element is position- independent.

[00862] A “spacer sequence” or “spacer” as used herein is a nucleic acid sequence that separates two functional nucleic acid sequences (e.g., TFBS, CREs, CRMs, minimal promoters, etc.). It can have essentially any sequence, provided it does not prevent the functional nucleic acid sequence (e.g., cis-regulatory element) from functioning as desired (e.g., this could happen if it includes a silencer sequence, prevents binding of the desired transcription factor, or suchlike). Typically, it is non-functional, as in it is present only to space adjacent functional nucleic acid sequences from one another.

[00863] “Therapeutically effective amount” and like phrases mean a dose or plasma concentration in a subject that provides the desired specific pharmacological effect, e.g., to express a therapeutic gene. It is emphasized that a therapeutically effective amount may not always be effective in treating the conditions described herein, even though such dosage is deemed to be a therapeutically effective amount by those of skill in the art. The therapeutically effective amount may vary based on the route of administration and dosage form, the age and weight of the subject, and/or the disease or condition being treated.

[00864] The terms “treatment” or “treating” refer to reducing, ameliorating or eliminating one or more signs, symptoms, or effects of a disease or condition.

[00865] The terms “individual,” “subject,” and “patient” are used interchangeably. A subject can be a subject with a disease or condition in need of treatment. Typically, the subject in need thereof will display symptoms characteristic of a disease. The method typically comprises ameliorating the symptoms displayed by the subject in need thereof, by expressing a therapeutic amount of the expression product.

[00866] As used herein, the term “primate” refers to a mammal that falls within the human, ape or monkey family. In some embodiments, the primate is human. In some embodiments, the subject is a non-human primate. As used herein, the term “non-human primate” refers to a mammal that falls within the ape or monkey family and includes, but is not limited to, a baboon, a chimpanzee, a capuchin, a pigtail macaque, a sooty mangabey, a squirrel monkey, a gibbon, a Rhesus monkey, a Cynomolgus monkey, a gorilla, an orangutan, and any other ape or monkey species.

[00867] The description of embodiments of the disclosure is not intended to be exhaustive or to limit the disclosure to the precise form disclosed. While specific embodiments of, and examples for, the disclosure are described herein for illustrative purposes, various equivalent modifications are possible within the scope of the disclosure, as those skilled in the relevant art will recognize. For example, while method steps or functions are presented in a given order, alternative embodiments may perform functions in a different order, or functions may be performed substantially concurrently. The teachings of the disclosure provided herein can be applied to other procedures or methods as appropriate. The various embodiments described herein can be combined to provide further embodiments. Aspects of the disclosure can be modified, if necessary, to employ the compositions, functions and concepts of the above references and application to provide yet further embodiments of the disclosure. Moreover, due to biological functional equivalency considerations, some changes can be made in protein structure without affecting the biological or chemical action in kind or amount. These and other changes can be made to the disclosure in light of the detailed description. All such modifications are intended to be included within the scope of the appended claims.

[00868] Specific elements of any of the foregoing embodiments can be combined or substituted for elements in other embodiments. Furthermore, while advantages associated with certain embodiments of the disclosure have been described in the context of these embodiments, other embodiments may also exhibit such advantages, and not all embodiments need necessarily exhibit such advantages to fall within the scope of the disclosure.

EXAMPLES

[00869] The technology described herein is further illustrated by the following examples which in no way should be construed as being further limiting. It will be apparent to those skilled in the relevant art that various modifications, additions, substitutions, and the like can be performed without altering the spirit or scope of the invention, and such modifications and variations are encompassed within the scope of the invention as defined in the claims which follow.

Example 1: Testing of artemisinin and some exemplary derivatives

[00870] The construct contained 3x human PBREM enhancers together with the MinTK promoter (3xhPB-minTK, **SEQ ID NO: 66**) to drive the expression of luciferase. This construct was tested in the presence and absence of the indicated drugs after transfection into AXOL ARE-hepatocytes.

[00871] Axol Assay-Ready Expanded (ARE) Hepatocytes are primary human hepatocytes that have been expanded in vitro. Large batch sizes (up to 2000 vials) are available, ensuring a reliable, ready-to-use and consistent source of primary hepatocytes. ARE Hepatocytes express CYP enzymes, are metabolically functional, are polarized and can be infected by the Hepatitis C virus. AXOL ARE-hepatocytes express CAR negating the need to transfect with a CAR-expressing plasmid.

[00872] The inducers used are known to act by activating the CAR transcription factor and promoting the translocation to the nucleus. The drugs tested are artemisinin (Omiecinski et al, 2011) and its semi-synthetic derivatives, Artesunate (Li et al, 2003), Arteether (Grace et al, 1998), Artemether (German et al, 2008) and DHA, (Navaratnam et al, 2000). In addition, the Calcium channel blocker nifedipine (approved for use in high blood pressure and angina), (Lynch et al, 2013) and the compound flavone (Yao et al, 2010) were tested.

Materials:

- AXOL assay-ready expanded (ARE) hepatocytes (Axol, ax3701)
- ARE hepatocyte thawing medium (Axol, ax3705)
- ARE maintenance medium (Axol, ax3710)
- Virimer red transfection reagent (Lipocalyx, VR04-02-15)
- Artemisinin (Sigma-Aldrich, 1042747), Artesunate (Sigma-Aldrich, PH2573), Arteether (Sigma-Aldrich, SML2591), DHA (Sigma-Aldrich, 1200520), Artemether (Sigma-Aldrich, 1042780), Nifedipine (Sigma-Aldrich, Y001924), Flavone (Sigma-Aldrich, F2003), Rifampicin (Tcoris 4121), oestradiol (Tcoris 2824), Valproic acid (Tcoris 2815).
- pcDNA6 plasmid which contains the β -galactosidase gene used as internal control for transfection efficiency (ThermoFisher, V22020)
- β -galactosidase substrate solution (ThermoFisher, 75707/75710)
- Pierce BCA kit (23225)
- LARII (Dual Luciferase Reporter 1000 assay system, Promega, E1980)

Methods:

[00873] ARE hepatocytes were cultured and transfected as described in the manufacturer's manual. 200,000 cells were seeded in 2 ml culture medium in collagen-coated 6 well plate. The cells were incubated for 4 h at 37°C and 5% CO₂ for sufficient adhesion. 200 μ l of transfection

mixture containing the DNA to be transfected (3xhPB-MinTK operably linked with luciferase and a β -galactosidase containing plasmid) was added and the cells were incubated on an orbital shaker at 100 rpm for three hours at 37°C and 5% CO₂. 3hrs after transfection the putative inducers were added to the appropriate wells. Cells were incubated under static conditions at 37°C and 5% CO₂ overnight and in the morning the medium was replaced with fresh ARE Hepatocyte Maintenance Medium. Readout was 24hrs after induction. Luciferase expression was measured as per manufacturer's instructions. Luciferase was normalised by total protein and transfection efficiency.

Results:

[00874] Transfection of the ARE hepatocytes was performed with wells aliquoted in triplicate for uninduced (DMSO) or induced (inducer treated samples). There were also additional wells where a CMV-IE-luciferase construct was transfected as a comparator of promoter strength. 3hrs after transfection the appropriate wells were treated with either 10uM artemisinin and related compounds, 25uM nicardipine, 500 uM valproic acid, 10 uM rifampicin, 10 uM oestradiol, or 60uM flavone. The results of 5 independent experiments are shown in **Figure 2**. This figure shows that in the uninduced samples there is a small signal that is comparable to untransfected cells showing that the 3xhPB-minTK has a low background activity. Upon addition of artemisinin or its derivatives an induction can be observed with the most marked response been observed with artemisinin and artesunate (greater than CMV-IE). Artemisinin is at approx. 1.5x as potent as artesunate and is significantly more potent than arteether, DHA and artemether. The results with DHA are particularly interesting as this is the active compound of artemisinin and its derivatives but here it appears to be less potent than both artesunate and artemisinin suggesting the prodrug works best. These results correlate well with the known metabolism of these compounds highlighted in **Figure 1**. Artemisinin is metabolised by cyb2b6 which is the major enzyme involved in CAR activated detoxification, whereas the other drugs are metabolised by different cytochrome P450 enzymes. These data suggest that the 2 most potent artemisinin compounds to use for induction of the 3xhPB promoter are artemisinin and artesunate, however each of the other derivatives could be used if lower expression levels were required (up to CMV-IE).

[00875] Of the 5 drugs tested flavone also showed induction of the promoter. This induction was equivalent to the response observed for DHA and Arteether which was equivalent to CMV-IE. However, this drug was used at 6x the concentration of the anti-malarial compounds. There was no induction observed with nicardipine.

Example 2- Mice *In vivo* experiments using AAV comprising 3xhPB-Min-TK

- [00876] The 3xhPB-MinTK was taken *in vivo* to assess the following attributes:
- Induction with artemisinin via IP injection
 - Induction with Artemisinin via oral gavage
 - Differences in male vs female for induction
 - Long-term expression and induction
 - Dose response curves via induction with different concentrations of artemisinin
 - Kinetics of induction, to obtain response times of the promoter to artemisinin
 - Different doses of AAV to assess maximum inducibility
- [00877] All of these experiments were performed using luciferase as a marker gene

Methods:

- [00878] AAV production was performed as described in Greiger et al, 2015.

Mice experiments

[00879] The AAV serotype chosen was AAV9 as this has a tropism for most tissues and organs and would therefore give an idea of the specificity of our promoters (avoiding issues of AAV tropism). Output from the experiment was luciferase activity measured visually. Promoter activity from the mice were monitored weekly and after 14 – 21 days, once control vector AAV9 with CMV-IE had shown consistent steady results a baseline for induction profiling was established. After this point the inducer was added with measurements taken before and after induction. See results section for more details.

Mice:

[00880] Adults (8 weeks of age) CD1 male or female mice were injected with an AAV9 vector via tail vein with a total of 1×10^{11} vg/mouse. The mice were imaged as follows. They were first anesthetized and received an intraperitoneal injection of luciferin (300 μ l of luciferin stock 15mg/ml). After 5 minutes the mice were placed in the IVIS machine and images were acquired. The exposure time used for images were 1 and 10 seconds. Images were taken once every 7 days until a steady state baseline was achieved with an AAV9 containing CMV-IE. Induction was measured after artemisinin, dissolved in DMSO, was administered at an appropriate concentration depending on the experiment. Delivery was via IP or oral gavage.

Ex vivo luciferase assays:

[00881] The tissue samples were weighed to assure the same amount of tissue was being used for each sample. Samples were kept on ice and then one 3mm QIAGEN carbide bead (Catalog no #69997) was added per tube. Next, 200uL of reporter Lysis buffer (from luciferase assay kit) (1x) per tissue was added before homogenization of the sample at a frequency of 25.0Hz for 75 seconds (x2) was performed ensuring rotation of the adaptor to encourage even homogenization. The samples were then flash frozen at -80 for 15 minutes and then thawed at 37°C for 10 minutes. Once thawed the samples were centrifuged at 10,000g for 3 minutes with the supernatant transferred to a fresh 1.5mL tube. The samples can then be stored at -80°C or directly proceed to BCA and luciferase assay's as per the manufacturer's instructions (Pierce BCA kit (23225) LARII (Dual Luciferase Reporter 1000 assay system, Promega, E1980).

[00882] DNA from tissues for vector copy number analysis was performed using the Maxwell RSC tissue DNA kit (AS1610) as per the manufacturer's instructions. Vector copy number was measured using Taq man probes for qPCR as described in Greiger et al, 2015.

Results

[00883] *Induction of 3xhPB-MinTK via IP and oral administration of artemisinin:* The results of this experiment can be seen in **Figures 3B and 3C** with **Figure 3A** depicting an overview of the experiment. In this experiment male mice (n=5) were injected with 1e11 vg AAV9 containing either 3xhPB-MinTK (promoter 1) or CMV-IE (Con) with each promoter driving the expression of the firefly luciferase gene. There were 3 groups of the 3xhPB-MinTK with one to be given the inducer via IP, one given via oral gavage while the other was to be injected with the vehicle. After 14 days with weekly measurements of luciferase activity via the IVIS, it was determined that the AAV expression from the CMV-IE group had stabilised. The mice were then injected on day 15 with either 80mg/kg of artemisinin or vehicle and luciferase activity was measured at 9, 24 and 48hrs after induction. The results of this can be seen in **Figure 3B**. Here we see that upon addition of artemisinin there was significant activity observed after 9-hrs, a >300-fold induction in both the oral and IP administered mice. This was in fact the peak induction time observed as the level of luciferase activity decreased at 24, and 48hrs with activity almost at non-induced levels by 48hrs. The strength of activity observed at peak expression was 10x that of CMV-IE, showing that upon induction the promoters could produce a therapeutically relevant level of expression. 2 weeks after drug administration the luciferase activity was measured and it was observed that the 3xhPB-MinTK levels had returned to baseline. During this all these measurements the CMV-IE group activity remained constant and did not respond to artemisinin. On day 32 80mg/kg was once more

administered to the mice and after 9hrs the mice were culled. Tissues were extracted from these mice and the ex-vivo luciferase activity was measured in various tissues. The result can be seen in **Figure 3C**. In this figure you can see that only in the liver is activity observed for 3xhPB-MinTK. Indeed, the induction observed above the uninduced (vehicle) group was higher than observed from IVIS measurements, >2,000 fold. Furthermore, a group of mice with no AAV injected as negative control (saline) had comparable values the uninduced mice indicating that the inducible promoter has very low background and the IVIS was overestimating the background while underestimating the inducible level. Overall, these results show that artemisinin at 80mg/kg administered either orally or IP can induce the 3xhPB-MinTK promoters to therapeutically relevant levels from a very low background.

[00884] Induction of 3xhPB-MinTK via IP administration of artemisinin in male and female mice over 6 months: The results of this experiment can be seen in **Figures 4B and 4C** with **Figure 4a** depicting an overview of the experiment. In this experiment male and female mice (n=5) were injected with 1e11vg AAV9 containing either 3xhPB-MinTK (promoter 1) or CMV-IE (Con) with each promoter driving the expression of the firefly luciferase gene. There were 3 groups of the 3xhPB-MinTK one male, and one female group and one male group injected with the vehicle. After 28 days with weekly measurements of luciferase activity via the IVIS, it was determined that the AAV expression from the CMV-IE group had stabilised. The mice were then injected on day 32, 48 and day 134 with either 80mg/kg of artemisinin or vehicle and luciferase activity was measured at 9, 24 and 48hrs after induction. The results of this can be seen in **Figure 4B**. The 1st thing to notice is that the female group have a noticeably lower background than the male mice. It is unsure if this because of the known phenomenon of androgen enhanced transduction or an anomaly of the IVIS measurements. Addition of artemisinin leads to a significant increase activity observed after 9-hrs, with a >300 – 3,000 fold induction in both male and female mice. Again, this time point was peak induction with the level of luciferase activity decreasing at 24, and 48hrs with activity almost at non-induced levels by 48hrs. The strength of activity observed at peak expression was again 10x that of CMV-IE. The kinetics of induction were observed for each repeat injection out to day 134 showing the promoters are still able to be induced many months after AAV injection. Once again the CMV-IE group activity remained constant and did not respond to artemisinin. On day 171 80mg/kg (high dose) was once more administered to the mice and after 9hrs the mice were culled, the female group was split between an induced group and a vehicle group to measure background activity. Tissues were extracted from these mice and the ex-vivo luciferase activity was measured from the liver. The result can be seen in **Figure 4C**. In this figure you can see activity for 3xhPB-MinTK is induced in both male and female mice with the induction being between 4 and 11,000-fold compared to the vehicle (uninduced) mice. Again, the induction observed above the uninduced

(vehicle) group was higher than observed from IVIS measurements. Furthermore, a group of mice with no AAV injected as negative control (saline) had comparable values the uninduced mice indicating that the inducible promoter has very low background and the IVIS was overestimating the background while underestimating the inducible level. Overall, these results show that artemisinin at 80mg/kg administered IP to male and female mice can induce the 3xhPB-MinTK promoters to therapeutically relevant levels from a very low background.

[00885] Induction of 3xhPB-MinTK via oral gavage or IP administration of artemisinin in male mice over 6 months: The results of this experiment can be seen in **Figures 5B and 5C** with **Figure 5A** depicting an overview of the experiment. In this experiment male mice (n=5) were injected with 1e11vg AAV9 containing either 3xhPB-MinTK (promoter 1) or CMV-IE (Con) with each promoter driving the expression of the firefly luciferase gene. There were 3 groups of the 3xhPB-MinTK one oral gavage, and one IP and one group injected with the vehicle. After 28 days with weekly measurements of luciferase activity via the IVIS, it was determined that the AAV expression from the CMV-IE group had stabilised. The mice were then injected on day 32, 48 and day 134 with either 80mg/kg of artemisinin or vehicle and luciferase activity was measured at 9, 24 and 48hrs after induction. The results of this can be seen in **Figure 5B**. Addition of artemisinin leads to a significant increase activity observed after 9-hrs in both oral and IP induced mice, with a >300-fold induction observed. Again, this time point was peak induction with the level of luciferase activity decreasing at 24, and 48hrs with activity almost at non-induced levels by 48hrs. The strength of activity observed at peak expression was again 10x that of CMV-IE. The kinetics of induction were observed for each repeat injection out to day 134 showing the promoters are still able to be induced many months after AAV injection. Once again the CMV-IE group activity remained constant and did not respond to artemisinin. On day 171 the male groups were split and either 80, 40 or 20mg/kg (high, med and low dose) were administered via IP the mice and after 9hrs the mice were culled. Tissues were extracted from these mice and the ex-vivo luciferase activity was measured from the liver. The result can be seen in **Figure 5c**. In this figure one can see activity for 3xhPB-MinTK is induced at all concentrations of artemisinin used with the fold induction being between 100 and 4,000-fold compared to the vehicle (uninduced) mice. Again, the induction observed above the uninduced (vehicle) group was higher than observed from IVIS measurements. Furthermore, a group of mice with no AAV injected as negative control (saline) had comparable values the uninduced mice indicating that the inducible promoter has very low background and the IVIS was overestimating the background while underestimating the inducible level. Overall, these results show that artemisinin at 80mg/kg administered IP or via oral gavage induces expression from 3xhPB-MinTK to therapeutically relevant levels in male mice over a

period of 6 months. Furthermore, the data presented here that the promoters are dose responsive down to 20mg/kg of artemisinin.

[00886] Dose response of 3hPB-MinTK to artemisinin induction: The results of this experiment can be seen in **Figure 6**. In this experiment female mice (n=5) were injected with 1e11vg AAV9 containing either 3xhPB-MinTK (promoter 1) or CMV-IE (Con) with each promoter driving the expression of the firefly luciferase gene. There were 7 groups of the 3xhPB-MinTK one for each concentration of artemisinin to be administered. The drug was delivered via oral gavage, with one group administered the vehicle. After 14 days with weekly measurements of luciferase activity via the IVIS, it was determined that the AAV expression from the CMV-IE group had stabilised. The mice were then treated on day 14 with either 80, 50, 20, 10, or 5mg/kg of artemisinin or vehicle and luciferase activity was measured at 9, 24 and 48hrs after induction. The results of this can be seen in figure 6. Addition of artemisinin at all concentrations leads to a significant increase activity observed after 9-hrs, with a >30 – 5,000-fold induction observed. Again, this time point was peak induction with the level of luciferase activity decreasing at 24, and 48hrs with activity almost at non-induced levels by 48hrs. The strength of activity observed at peak expression with 80mg/kg of artemisinin was 10x that of CMV-IE. Once again, the CMV-IE group activity remained constant and did not respond to artemisinin. The vehicle group had an expression profile that was comparable to a non-AAV injected mouse group indicating the low background activity of these promoters. Overall, this experiment shows that the 3xhPB-MinTK promoter is dose responsive to artemisinin down to 5mg/kg.

[00887] Measuring the time course of induction: The results of this experiment can be seen in **Figure 7**. In this experiment female mice (n=5) were injected with 1e11vg AAV9 containing either 3xhPB-MinTK (promoter 1) or CMV-IE (Con) with each promoter driving the expression of the firefly luciferase gene. There were 2 groups of the 3xhPB-MinTK for 80mg/kg of artemisinin treatment and the other for vehicle treatment. The drug was delivered via oral gavage, with one group administered the vehicle. After 14 days with weekly measurements of luciferase activity via the IVIS, it was determined that the AAV expression from the CMV-IE group had stabilised. The mice were then treated on day 14 with either 80mg/kg of artemisinin or vehicle and luciferase activity was measured at 3, 6, 9, 24 and 48hrs after induction. The results of this can be seen in **Figure 7**. Treatment with artemisinin leads to a significant increase in activity from 3hrs, with activity increasing until the peak was observed after 9-hrs. The level of luciferase activity then decreased at 24, and 48hrs with activity almost at non-induced levels by 48hrs. The strength of activity observed at peak expression with 80mg/kg of artemisinin was 10x that of CMV-IE. Once again, the CMV-IE group activity remained constant and did not respond to artemisinin. The vehicle group had an expression profile that was comparable to a non-AAV injected mouse group

indicating the low background activity of these promoters. Overall, this experiment shows that the 3xhPB-MinTK promoter can respond rapidly to drug administration, 3hrs but that peak induction time is 9hrs.

[00888] *Measuring the effect of AAV dose on induction:* The results of this experiment can be seen in **Figure 8**. In this experiment female mice (n=5) were injected with 1e11vg AAV9 containing either 3xhPB-MinTK (promoter 1) or CMV-IE (Con) with each promoter driving the expression of the firefly luciferase gene. There were 3 groups of the 3xhPB-MinTK one dosed at 1e11vg, one at 5e10vg and one for vehicle at 1e11vg. In addition, there were two CMV-IE groups one for each AAV dose. All artemisinin treatment was at 80mg/kg. The drug was delivered via oral gavage, with one group administered the vehicle. After 14 days with weekly measurements of luciferase activity via the IVIS, it was determined that the AAV expression from the CMV-IE group had stabilised. The mice were then treated on day 14 with either 80mg/kg of artemisinin or vehicle and luciferase activity was measured 9, 24 and 48hrs after induction. The results of this can be seen in **Figure 8**. Treatment with artemisinin leads to a significant increase in activity from with activity peaking after 9-hrs. The level of luciferase activity then decreased at 24, and 48hrs with activity almost at non-induced levels by 48hrs. The different AAV doses showed what was expected, that the lower the dose the lower the overall expression is, however, induction kinetics remained similar regardless of the dose. It should be noted that the lower dose of 3hPB-MinTK promoter was able to reach the expression level of the high dose CMV-IE, whereas at the same dose 3xhPB-MinTK was significantly stronger. At both doses and the vehicle group, the expression profile was comparable to a non-AAV injected mouse group indicating the low background activity of these promoters. Once again, the CMV-IE groups activity remained constant and did not respond to artemisinin.

Example 3- NHP *In vivo* experiments using AAV comprising 3xhPB-Min-TK

[00889] The 3xhPB-MinTK packaged into AAV9 was taken in vivo in NHPs to assess the following attributes:

- Induction with artemisinin via IP injection
- Induction with Artemisinin via oral ingestion
- Differences in male vs female for induction
- Long-term expression and induction
- Dose response curves via induction with different concentrations of artemisinin
- Kinetics of induction, to obtain response times of the promoter to artemisinin

[00890] In this experiment chorionic gonadotropin was used as a marker gene. This is a self-Ag which is not normally expressed in NHPs and which can be measured via ELISA from the serum.

Methods:

[00891] AAV production was performed as described in Greiger et al, 2015.

NHP experiments

[00892] **Figure 9** depicts the study outline for this experiment.

NHPs:

[00893] NHP species used was *Macaca fascicularis* with each monkey pre-screened for neutralising Abs against AAV9 and gonadotropin expression. Each NHP weighed between 2 and 3kg at time of AAV injection.

ELISA:

[00894] Human HCG (Chorionic Gonadotropin) ELISA Kit (HUES01443)

Results

[00895] The results of this experiment can be seen in **Figure 10**. In this experiment male and female NHPs were injected with 1×10^{13} vg/kg of AAV9 containing either 3xhPB-MinTK or LSP with each promoter driving the expression of the chorionic gonadotropin (cGT) gene. LSP was chosen as the comparator promoter here as it has been used in gene therapy clinical trials and is therefore therapeutically relevant. There were an additional 2 NHPs that had no AAV injected into them to determine background level of cGT. After 28 days with weekly measurements of serum cGT concentrations it was determined that the AAV expression from the LSP group had stabilised. The NHPs were then treated on day 30 with 80mg/kg of artemisinin via IP injection and serum levels of cGT were measured at 24, 48 and 72hrs after induction. The results of this can be seen in **Figure 10**. The graph presented is an aggregate of all NHPs and there was no difference in response between male and female NHPs. Pre-induction levels of cGT were equivalent to the no AAV NHP suggesting that the promoters had no background leak. However, treatment with artemisinin leads to a significant increase in cCG in the serum at 24hrs to $>2 \times$ LSP with the concentrations of cCG decreasing at 48 and 72 hrs but not returning to baseline. This observation is correlated with the $\frac{1}{2}$ life of the cGT and therefore in the 2nd induction it was decided to take serum samples 1 week after induction. The described experiment was then repeated on day 74 with results being identical to

the 1st induction with baseline returned to after 1 wk. In the subsequent inductions artemisinin was injected IP at 40, 20, 10 and 1mg/kg. As can be observed from **Figure 10** artemisinin is able to induce a response from the 3xhPB-MinTK promoter at every dose and in a dose dependent manner. Indeed 1mg/kg shows comparable activity to the LSP promoter. In the final induction at 6mg/kg the drug administered was the artemisinin derivative artesunate. This is because artemisinin has poor solubility which made it difficult to get into a form to be ingested by the NHPs. However, artesunate is more soluble and is amenable to oral administration. Also, as seen in **Figure 2**, artesunate is able to activate the promoters. In **Figure 10** you can see that artesunate induces the promoters via oral administration. In this induction, the kinetics are comparable to the IP administration of artemisinin with peak expression observed at 24hrs with it decreasing at 48 and 72hrs. You can also see that 6mg/kg is able to induce the promoter to greater than LSP activity showing therapeutic relevance. Overall, we have shown that the 3xhPB-MinTK promoter can be induced by artemisinin and artesunate and in NHPs via both IP and oral treatment. In addition, these promoters have no detectable background and are induced to therapeutically relevant levels.

Additional NHP Results:

[00896] **Figure 11** depicts the study outline for this experiment. The methodology was otherwise the same as described above. The results of induction of NHPs are shown in **Figures 12** and **13**.

[00897] **Figure 12** shows the collated results of induction in non-human primates using the protocol shown in **Figure 11**, i.e. two cycles of induction with 80 mg/kg IP artemisinin. Results for the LSP control promoter and saline negative control are also shown for comparison. Pre-induction gonadotropin levels are indicated by lower arrows and gonadotropin levels after induction are illustrated by upper arrows. **Figures 13A** and **13B** are bar-graph (**Figure 13A**) and line-graph (**Figure 13B**) showing individual results for each of the non-human primate in **Figure 12**. The individual non-human primates are indicated as follows: M LSP and F LSP are the male and female LSP controls, respectively; M1 Ind and M2 Ind are males with the inducible promoter; F1 Ind and F2 Ind are females with the inducible promoter; M S and F S are male and female saline controls, respectively.

Example 4 – Additional NHP *in vivo* experiments using AAV comprising 3xhPB-Min-TK

[00898] The 3xhPB-MinTK packaged into AAV9 was teste *in vivo* in NHPs to assess the following attributes:

- Dose response of induction with Artemisinin via oral ingestion

- Long-term expression and induction
- Continuous expression over a 30-day induction period.

[00899] In this experiment chorionic gonadotropin was used as a marker gene. This is a self-Ag which is not normally expressed in NHPs and which can be measured via ELISA from the serum.

Methods:

[00900] AAV production was performed as described in Greiger et al., 2015.

NHP experiments

[00901] **Figure 14** depicts the study outline for this experiment.

NHPs:

[00902] NHP species used was *Macaca fascicularis* with each monkey pre-screened for neutralising Abs against AAV9 and gonadotropin expression. Each NHP weighed between 2 and 3kg at time of AAV injection.

ELISA:

[00903] Human HCG (Chorionic Gonadotropin) ELISA Kit (HUES01443)

Results

[00904] In this experiment male and female NHPs were injected with 1e13vg/kg of AAV9 containing either 3xhPB-MinTK or LSP with each promoter driving the expression of the chorionic gonadotropin (cGT) gene. LSP was chosen as the comparator promoter here as it has been used in gene therapy clinical trials and is therefore therapeutically relevant. There were an additional 2 NHPs that had no AAV injected into them to determine background level of cGT. After 28 days with weekly measurements of serum cGT concentrations it was determined that the AAV expression from the LSP group had stabilised. **Figure 15** shows an overview of all inductions that were performed in this study. As can be seen from **Figure 15**, both IP induction via artemisinin and oral induction via artesunate produced a strong response from the promoters. Since it has poor solubility, it was difficult to get artemisinin into a form to be ingested by the NHPs. Thus, artesunate was used for the oral inductions since artesunate is more soluble and is amenable to oral

administration. **Figure 15** also shows that the promoters are dose responsive to IP and oral administration with activity observed with dose as low as 0.1mg/kg, which is far below the recommended dose of artemisinin and artesunate for treatment of malaria. In addition, the promoters once induced produced a therapeutic level of gene expression, even at 1mg/kg, as they expression was equivalent to the control promoter, LSP. There was no observed expression in the preinduction state, with levels here equivalent to non-AAV injected NHPs. Furthermore, induction was observed at almost 600-days after AAV injection. This demonstrates that the promoters and induction was robust over a significant period.

[00905] **Figure 16** shows the kinetics of expression 24, 48, 72 and 168hrs hrs after each induction. The graph presented is an aggregate of all NHPs and there was no difference in response between male and female NHPs (**Figure 15**). Pre-induction levels of cGT were equivalent to the no AAV NHP suggesting that the promoters had no background leak. However, treatment with either artemisinin or artesunate leads to a significant increase in cCG in the serum at 24hrs of cCG decreasing at 48 and 72 hrs but not returning to baseline. This observation is correlated with the half-life of the cGT and baseline was then achieved at 168hrs after induction. Together, **Figures 15 and 16** show that the 3xhPB-MinTK promoter is induced by artemisinin and artesunate in a dose-responsive manner in NHPs via both IP and oral treatment. In addition, these promoters have no detectable background and were induced to therapeutically relevant levels.

[00906] To test whether the promoters could be made to express the cCG continuously and whether administration of the inducer for a prolonged period was toxic. NHPs were dosed for 30 days continuously with 1mg/kg of artesunate orally. Measurements of cCG were taken every 7 days until final dose and then 24, 48, 72 and 168hrs later. The results of this experiment can be seen in **Figure 17**. As seen from **Figure 17**, the 1mg/kg dose elicited a strong expression from the promoters that was visible after 7 days and this expression remained constant for the time course of administration. However, once the dosing was stopped, the levels of serum cCG returned to baseline after 168hrs. There was no observed toxicity in the NHPs during this study. This demonstrates that long-term administration of the inducer is feasible and safe.

Example P0: *In vivo* induction of a marker gene in non-human primates with flavone

[0001] In this example, flavone is used as the inducer to induce the *in vivo* expression in NHPs of chorionic gonadotropin from AAV containing 3xhPB-MinTK driving the expression of a transgene encoding the chorionic gonadotropin to assess the following attributes:

- Induction with via IP injection
- Induction via oral ingestion
- Differences in male vs female for induction

- Long-term expression and induction
- Dose response curves via induction with different concentrations of inducer
- Kinetics of induction, to obtain response times of the promoter to the inducer

Materials and methods:

[0002] A vector as described herein, e.g., 3xhPB-MinTK and encoding chorionic gonadotropin is packaged into AAV9. The AAV9 containing 3xhPB-MinTK driving the expression of the chorionic gonadotropin is produced as described in Greiger et al. 2015. AAV9 containing LSP driving the expression of the chorionic gonadotropin is used as a control.

[0003] Male and/or female NHPs (*Macaca fascicularis*) are pre-screened for neutralizing Abs against AAV9 and injected with 1e13vg/kg of AAV9 containing either 3xhPB-MinTK or LSP with each promoter driving the expression of the chorionic gonadotropin. The levels of the chorionic gonadotropin are measured weekly, e.g., by ELISA or similar assays. Once the AAV expression from the LSP control group is stable, the animals are administered the inducer as follows:

- Day 30: 80mg/kg via IP injection
- Day 74: 80mg/kg via IP injection
- Day 123: 40mg/kg via IP injection
- Day 131: 20mg/kg via IP injection
- Day 160: 10mg/kg via IP injection
- Day 180: 1mg/kg via IP injection
- Day 200: 6mg/kg via oral gavage

[0004] Levels of the chorionic gonadotropin at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of the chorionic gonadotropin from NHPs that had no AAV injected into them are used as background or pre-induction level of the chorionic gonadotropin. Pre-induction levels of the chorionic gonadotropin can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in the chorionic gonadotropin, e.g., in the serum, with a subsequent return to background levels.

Example P1A: *In vivo* induction of an anti-TNF α antibody in non-human primates

[0005] In this example, artemisinin is used as the inducer to induce the *in vivo* expression in NHPs of an anti-TNF α antibody (e.g., adalimumab) from AAV containing 3xhPB-MinTK driving the expression of a transgene encoding the antibody to assess the following attributes:

- Induction with via IP injection
- Induction via oral ingestion
- Differences in male vs female for induction
- Long-term expression and induction
- Dose response curves via induction with different concentrations of inducer
- Kinetics of induction, to obtain response times of the promoter to the inducer

Materials and methods:

[0006] A vector as described herein, e.g., 3xhPB-MinTK and encoding an anti-TNF α antibody is packaged into AAV9. The AAV9 containing 3xhPB-MinTK driving the expression of the antibody is produced as described in Greiger et al. 2015. AAV9 containing LSP driving the expression of the antibody is used a control.

[0007] Male and/or female NHPs (*Macaca fascicularis*) are pre-screened for neutralizing Abs against AAV9 and injected with 1e13vg/kg of AAV9 containing either 3xhPB-MinTK or LSP with each promoter driving the expression of the antibody. The levels of the antibody are measured weekly, e.g., by ELISA or similar assays. Once the AAV expression from the LSP control group is stable, the animals are administered the inducer as follows:

- Day 30: 80mg/kg via IP injection
- Day 74: 80mg/kg via IP injection
- Day 123: 40mg/kg via IP injection
- Day 131: 20mg/kg via IP injection
- Day 160: 10mg/kg via IP injection
- Day 180: 1mg/kg via IP injection
- Day 200: 6mg/kg via oral gavage

[0008] Levels of the antibody at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of the antibody from NHPs that had no AAV injected into them are used as background or pre-induction level of the antibody. Pre-induction levels of the antibody can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in the antibody, e.g., in the serum, with a subsequent return to background levels.

Example P1B: *In vivo* induction of an antibody in non-human primates

[0009] **Example P1A** is repeated with each of the following antibodies: anti-PD1 antibody, anti-HER2 antibody, anti-PCSK9 antibody, anti-VEGFR2 antibody, anti-IL17 antibody, anti-A β 3-42 antibody, anti-Sema3A antibody, anti-4-1BB (CD137) antibody, anti-5'-nucleotidase antibody, anti-5T4 antibody, anti-activin receptor-like kinase 1 antibody, anti-ACVR2B antibody, anti-adenocarcinoma antigen antibody, anti-amyloid antibody, anti-Ang-2 antibody, anti-angiopoietin 2 antibody, anti-angiopoietin 2 antibody, anti-angiopoietin 3 antibody, anti-anthrax toxin antibody, anti-AOC3 (VAP-1) antibody, anti-AXL antibody, anti-BAFF-R antibody, anti-B-cell activating factor (BAFF) antibody, anti-B-cell maturation antigen (BCMA) antibody, anti-B-lymphoma cell antibody, anti-C242 antigen antibody, anti-C5 antibody, anti-C5a receptor (C5a) antibody, anti-CA-125 antibody, anti-calcitonin antibody, anti-calcitonin gene-related peptide alpha antibody, anti-calcitonin gene-related peptide beta antibody, anti-calcitonin gene-related peptide receptor (CGRP) antibody, anti-CanAg (a glycoform of MUC1) antibody, anti-carbonic anhydrase 9 (CA-IX) antibody, anti-Carcinoembryonic antigen (CEA) antibody, anti-Carcinoembryonic antigen (CEA)-related antigen antibody, anti-cardiac myosin antibody, anti-CCL11 antibody, anti-CCR2 antibody, anti-CCR4 antibody, anti-CCR5 antibody, anti-CCR5 antibody, anti-CD11 antibody, antibody, anti-CD123 antibody, anti-CD125 antibody, anti-CD134 antibody, anti-CD134 antibody, anti-CD147 antibody, anti-CD15 antibody, anti-CD152 antibody, anti-CD154 antibody, anti-CD18 antibody, anti-CD19 antibody, anti-CD2 antibody, anti-CD20 antibody, anti-CD20 antibody, anti-CD20 antibody, anti-CD20 antibody, anti-CD200 antibody, anti-CD22 antibody, anti-CD23 antibody, anti-CD25 antibody, anti-CD27 antibody, anti-CD276 antibody, anti-CD278 antibody, antibody, anti-CD279 antibody, anti-CD28 antibody, anti-CD3 antibody, anti-CD3 antibody, anti-CD3 antibody, anti-CD3 antibody, anti-CD3 antibody, anti-CD30 antibody, anti-CD319 antibody, anti-CD33 antibody, anti-CD37 antibody, anti-CD38 antibody, anti-CD3E antibody, anti-CD3E antibody, anti-CD4 antibody, anti-CD40 antibody, anti-CD41 antibody, anti-CD44 antibody, anti-CD45 antibody, anti-CD5 antibody, anti-CD51 antibody, anti-CD52 antibody, anti-CD56 antibody, anti-CD6 antibody, anti-CD70 antibody, anti-CD74 antibody, anti-CD79B antibody, anti-CD80 antibody, anti-CEACAM5 antibody, anti-Claudin 18 Isoform 2 antibody, anti-clumping factor A antibody, anti-cMet antibody, anti-c-Met antibody, anti-coagulation factor III antibody, anti-complement C5a antibody, anti-complement component 1s (C1s) antibody, anti-Complement factor D (CFD) antibody, anti-connective tissue growth factor (CTGF) antibody, anti-CSF1 antibody, antibody, anti-CSF1R antibody, anti-CSF2 antibody, anti-CTLA-4 antibody, anti-CXCL10 (IP-10) antibody, anti-CXCR4 (CD184) antibody, anti-dabigatran antibody, anti-dendritic cell-associated lectin 2 antibody, anti-DLL3 antibody, anti-DLL4 antibody, anti-DPP4 antibody, anti-DR5 antibody, anti-EGFL7 antibody, anti-EGFR antibody, anti-EGFR antibody, anti-EGFR antibody, anti-EGFR extracellular domain III antibody, anti-endoglin antibody, anti-endotoxin antibody, anti-EpCAM

antibody, anti-EPHA3 antibody, anti-ephrin receptor A3 antibody, anti-episialin antibody, anti-ERBB1 antibody, anti-ERBB3 (HER3) antibody, anti-FAP (gene) (FAP) antibody, anti-FCGRT antibody, anti-FGF 23 antibody, anti-FGFR2 antibody, anti-FGFR2 antibody, anti-fibrin II antibody, anti-fibronectin extra domain-B antibody, anti-folate hydrolase antibody, anti-folate receptor 1 antibody, anti-folate receptor alpha antibody, anti-Frizzled receptor antibody, anti-FVIX antibody, anti-FVX antibody, anti-GD2 ganglioside antibody, anti-GD3 ganglioside antibody, anti-GDF-8 antibody, anti-gelatinase B antibody, anti-glucagon receptor (GCGR) antibody, anti-Glutamate carboxypeptidase II antibody, anti-glypican 3 antibody, anti-GMCSF antibody, anti-GPNMB antibody, anti-GPRC5D antibody, antibody, anti-growth differentiation factor 8 antibody, anti-GUCY2C antibody, anti-Hepatocyte growth factor (HGF) antibody, anti-HER1 antibody, anti-HER1 antibody, anti-HER1 antibody, anti-HER2/neu antibody, anti-HGFR antibody, anti-histone complex antibody, anti-HLA-DR antibody, anti-Hsp90 antibody, anti-human scatter factor receptor kinase antibody, anti-ICAM-1 (CD54) antibody, anti-IFN receptor antibody, anti-IFN- α antibody, anti-IFN- α/β receptor antibody, anti-IFN- γ antibody, anti-IgE antibody, anti-IgE Fc region antibody, anti-IGF-1 receptor (CD221) antibody, anti-IGF-2 antibody, anti-IGHE antibody, anti-IL-1 antibody, anti-IL-12 antibody, anti-IL-13 antibody, anti-IL-17A antibody, anti-IL-17AF antibody, anti-IL-17F antibody, anti-IL-1 α antibody, anti-IL-1 β antibody, anti-IL-2 antibody, anti-IL-20 antibody, anti-IL-22 antibody, anti-IL-23 antibody, anti-IL-23 antibody, anti-IL-23 antibody, anti-IL-23 antibody, anti-IL-23 antibody, anti-IL-23 antibody, anti-IL-23A antibody, anti-IL-3 receptor antibody, anti-IL-31 receptor A antibody, anti-IL-4 antibody, anti-IL-4R α antibody, anti-IL-5 antibody, anti-IL-6 antibody, anti-IL-6 receptor antibody, anti-IL-9 antibody, anti-inducible T-cell co-stimulatory ligand (ICOSL) antibody, anti-integrin $\alpha 4$ antibody, anti-integrin $\beta 7$ antibody, anti-integrin $\alpha 4 \beta 7$ antibody, anti-integrin $\alpha 4 \beta 7$ antibody, anti-integrin $\alpha 5 \beta 1$ antibody, anti-integrin $\alpha \text{Ib} \beta 3$ antibody, anti-integrin $\alpha \nu \beta 3$ antibody, anti-Interleukin 36 receptor (IL1RL2/IL1RAP) antibody, anti-ITGA2 (CD49b) antibody, anti-ITGB2 (CD18) antibody, anti-kallikrein antibody, anti-KIR2D antibody, anti-LAG3 antibody, anti-Lewis-Y antigen antibody, anti-LFA-1 (CD11a) antibody, anti-LINGO-1 antibody, anti-lipoteichoic acid antibody, anti-LIV-1 antibody, anti-LOXL2 antibody, anti-LRRC15 antibody, anti-L-selectin (CD62L) antibody, anti-lymphotoxin alpha (LTA) antibody, anti-LYPD3 antibody, anti-macrophage colony stimulating factor (MCSF) antibody, anti-macrophage migration inhibitory factor (MIF) antibody, anti-MASP-2 antibody, anti-MCP-1 antibody, anti-melanoma cell adhesion molecule (MCAM) antibody, anti-mesothelin antibody, anti-MS4A1 antibody, antibody, anti-MST1R antibody, anti-MUC1 antibody, anti-MUC5AC antibody, anti-mucosal addressin cell adhesion molecule antibody, anti-myelin-associated glycoprotein antibody, anti-myostatin antibody, anti-NCA-90 (granulocyte antigen) antibody, anti-nectin-4 antibody, anti-nerve growth factor (NGF) antibody, anti-NGNA ganglioside antibody,

anti-NKG2A antibody, anti-NOGO-A antibody, anti-Notch 1 antibody, anti-Notch receptor antibody, anti-NRPI antibody, anti-OX-40 antibody, anti-oxLDL antibody, anti-PCDC1 antibody, anti-PCDP1 antibody, anti-PDGFR α antibody, anti-PDGFR β antibody, anti-PD-L1 antibody, anti-phosphate-sodium co-transporter antibody, anti-phosphatidylserine antibody, anti-PTK7 antibody, anti-RANKL antibody, anti-repulsive guidance molecule A (RGMA) antibody, anti-RHD (gene) (RHD) antibody, anti-Rhesus factor antibody, anti-Rhesus factor antibody, anti-root plate-specific spondin 3 antibody, anti-ROR1 antibody, anti-RTN4 antibody, anti-sclerostin antibody, anti-sclerostin (SOST) antibody, anti-SDC1 antibody, anti-selectin P antibody, anti-serum amyloid A protein antibody, anti-serum amyloid P component antibody, anti-SLAMF7 antibody, anti-SLITRK6 antibody, anti-SOST antibody, anti-sphingosine-1-phosphate antibody, anti-STEAP1 antibody, anti-TAG-72 antibody, anti-TAG-72 antibody, anti-tau protein antibody, anti-T-cell receptor antibody, anti-TEM1 antibody, anti-tenascin C antibody, anti-TGF- β antibody, anti-TGF- β 1 antibody, anti-TGF- β 2 antibody, anti-thymic stromal lymphopoietin (TSLP) antibody, anti-TIGIT antibody, anti-tissue factor pathway inhibitor (TFPI) antibody, anti-TRAIL-R1 antibody, anti-TRAIL-R2 antibody, anti-TROP-2 antibody, anti-tumor antigen CTAA16.88 antibody, anti-tumor necrosis factor receptor (TNFR) antibody, anti-tumor necrosis factor related activation protein (TRAP) antibody, anti-Tumor-associated glycoprotein 72 (TAG-72) antibody, anti-TWEAK receptor antibody, anti-TYRP1 (glycoprotein 75) antibody, anti-vascular endothelial growth factor A (VEGFA) antibody, anti-VEGFA antibody, anti-VEGFR-1 antibody, anti-vimentin antibody, anti-VISTA (protein) (VSIR) antibody, anti-VWF antibody, anti- α -fetoprotein antibody, anti-A-synuclein antibody or anti- β -amyloid antibody.

[0010] Materials and methods are as described in **Example P1A**, where the anti-TNF α is replaced by one of the above antibodies. Levels of the antibody at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of the antibody from NHPs that had no AAV injected into them are used as background or pre-induction level of the antibody. Pre-induction levels of the antibody can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in the antibody, e.g., in the serum, with a subsequent return to background levels.

Example 1C: *In vivo* induction of antibodies in non-human primates

[0011] **Examples P1A and P1B** are repeated with flavone as the inducer.

[0012] Materials and methods are as described in **Examples P1A and P1B**, where the artemisinin is replaced with flavone. Levels of the antibody at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of the antibody

from NHPs that had no AAV injected into them are used as background or pre-induction level of the antibody. Pre-induction levels of the antibody can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in the antibody, e.g., in the serum, with a subsequent return to background levels.

Example 1D: *In vivo* induction of antibodies in non-human primates

[0013] Examples P1A and P1B are repeated with each of the following inducers: artesunate, arteether, dihydroartemisinin, and artemether.

[0014] Materials and methods are as described in **Examples P1A and P1B**, where the artemisinin is replaced with one of the above inducers. Levels of the antibody at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of the antibody from NHPs that had no AAV injected into them are used as background or pre-induction level of the antibody. Pre-induction levels of the antibody can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in the antibody, e.g., in the serum, with a subsequent return to background levels.

Example P2A: *In vivo* induction of coagulation factor VIII (FVIII) in non-human primates

[0015] In this example, artemisinin is used as the inducer to induce the *in vivo* expression in NHPs of coagulation factor VIII from AAV containing 3xhPB-MinTK driving the expression of a transgene encoding the FVIII to assess the following attributes:

- Induction with via IP injection
- Induction via oral ingestion
- Differences in male vs female for induction
- Long-term expression and induction
- Dose response curves via induction with different concentrations of inducer
- Kinetics of induction, to obtain response times of the promoter to the inducer

Materials and methods:

[0016] A vector as described herein, e.g., 3xhPB-MinTK and encoding the coagulation factor VIII is packaged into AAV9. The AAV9 containing 3xhPB-MinTK driving the expression of the FVIII is produced as described in Greiger et al. 2015. AAV9 containing LSP driving the expression of the cytokine is used a control.

[0017] Male and/or female NHPs (*Macaca fascicularis*) are pre-screened for neutralizing Abs against AAV9 and injected with 1e13vg/kg of AAV9 containing either 3xhPB-MinTK or LSP with each promoter driving the expression of FVIII. The levels of FVIII are measured weekly, e.g., by ELISA or similar assay. Once the AAV expression from the LSP control group is stable, the animals are administered the inducer as follows:

- Day 30: 80mg/kg via IP injection
- Day 74: 80mg/kg via IP injection
- Day 123: 40mg/kg via IP injection
- Day 131: 20mg/kg via IP injection
- Day 160: 10mg/kg via IP injection
- Day 180: 1mg/kg via IP injection
- Day 200: 6mg/kg via oral gavage

[0018] Levels of the FVIII at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of FVIII from NHPs that had no AAV injected into them are used as background or pre-induction level of FVIII. Pre-induction levels of FVIII can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in the FVIII, e.g., in serum with a subsequent return to background levels.

Example P2B: *In vivo* induction of coagulation factor VIII in non-human primates

[0019] **Example P2A** is repeated with flavone as the inducer.

[0020] Materials and methods are as described in **Example P2A**, where the artemisinin is replaced with flavone. Levels of FVIII at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of FVIII from NHPs that had no AAV injected into them are used as background or pre-induction level of FVIII. Pre-induction levels of the antibody can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in FVIII, e.g., in the serum, with a subsequent return to background levels.

Example P2C: *In vivo* induction of coagulation factor VIII in non-human primates

[0021] **Example P2A** is repeated with each of the following inducers: artesunate, arteether, dihydroartemisinin, and artemether.

[0022] Materials and methods are as described in **Example P2A**, where the artemisinin is replaced with one of the above inducers. Levels of FVIII at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of FVIII from NHPs that had no AAV injected into them are used as background or pre-induction level of FVIII. Pre-induction levels of the antibody can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in FVIII, e.g., in the serum, with a subsequent return to background levels.

Example P2D: *In vivo* induction of coagulation factor IX (FIX) in non-human primates

[0023] In this example, artemisinin is used as an inducer to induce the *in vivo* expression in NHPs of coagulation factor IX from AAV containing 3xhPB-MinTK driving the expression of a transgene encoding the FIX to assess the following attributes:

- Induction with via IP injection
- Induction via oral ingestion
- Differences in male vs female for induction
- Long-term expression and induction
- Dose response curves via induction with different concentrations of inducer
- Kinetics of induction, to obtain response times of the promoter to the inducer

Materials and methods:

[0024] A vector as described herein, e.g., 3xhPB-MinTK and encoding the coagulation factor IX is packaged into AAV9. The AAV9 containing 3xhPB-MinTK driving the expression of the FIX is produced as described in Greiger et al. 2015. AAV9 containing LSP driving the expression of the cytokine is used a control.

[0025] Male and/or female NHPs (*Macaca fascicularis*) are pre-screened for neutralizing Abs against AAV9 and injected with 1e13vg/kg of AAV9 containing either 3xhPB-MinTK or LSP with each promoter driving the expression of FIX. The levels of FIX are measured weekly, e.g., by ELISA or similar assay. Once the AAV expression from the LSP control group is stable, the animals are administered the inducer as follows:

- Day 30: 80mg/kg via IP injection
- Day 74: 80mg/kg via IP injection
- Day 123: 40mg/kg via IP injection
- Day 131: 20mg/kg via IP injection

- Day 160: 10mg/kg via IP injection
- Day 180: 1mg/kg via IP injection
- Day 200: 6mg/kg via oral gavage

[0026] Levels of the FIX at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of FIX from NHPs that had no AAV injected into them are used as background or pre-induction level of FIX. Pre-induction levels of FIX can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in the FIX, e.g., in serum with a subsequent return to background levels.

Example P2E: *In vivo* induction of coagulation factor IX in non-human primates

[0027] **Example P3A** is repeated with each flavone as the inducer.

[0028] Materials and methods are as described in **Example P2C**, where the artemisinin is replaced with flavone. Levels of FIX at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of FIX from NHPs that had no AAV injected into them are used as background or pre-induction level of FIX. Pre-induction levels of the antibody can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in FIX, e.g., in the serum, with a subsequent return to background levels.

Example P2F: *In vivo* induction of coagulation factor IX in non-human primates

[0029] **Example P3A** is repeated with each of the following inducers: artesunate, arteether, dihydroartemisinin, and artemether.

[0030] Materials and methods are as described in **Example P2C**, where the artemisinin is replaced with one of the above inducers. Levels of FIX at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of FIX from NHPs that had no AAV injected into them are used as background or pre-induction level of FIX. Pre-induction levels of the antibody can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in FIX, e.g., in the serum, with a subsequent return to background levels.

Example P2G: *In vivo* induction of coagulation factor VII (FVII) in non-human primates

[0031] In this example, artemisinin is used as the inducer to induce the *in vivo* expression in NHPs of coagulation factor VII from AAV containing 3xhPB-MinTK driving the expression of a transgene encoding the FVII to assess the following attributes:

- Induction with via IP injection
- Induction via oral ingestion
- Differences in male vs female for induction
- Long-term expression and induction
- Dose response curves via induction with different concentrations of inducer
- Kinetics of induction, to obtain response times of the promoter to the inducer

Materials and methods:

[0032] A vector as described herein, e.g., 3xhPB-MinTK and encoding the coagulation factor VII is packaged into AAV9. The AAV9 containing 3xhPB-MinTK driving the expression of the FVII is produced as described in Greiger et al. 2015. AAV9 containing LSP driving the expression of the cytokine is used a control.

[0033] Male and/or female NHPs (*Macaca fascicularis*) are pre-screened for neutralizing Abs against AAV9 and injected with 1×10^{13} vg/kg of AAV9 containing either 3xhPB-MinTK or LSP with each promoter driving the expression of FVII. The levels of FVII are measured weekly, e.g., by ELISA or similar assay. Once the AAV expression from the LSP control group is stable, the animals are administered the inducer as follows:

- Day 30: 80mg/kg via IP injection
- Day 74: 80mg/kg via IP injection
- Day 123: 40mg/kg via IP injection
- Day 131: 20mg/kg via IP injection
- Day 160: 10mg/kg via IP injection
- Day 180: 1mg/kg via IP injection
- Day 200: 6mg/kg via oral gavage

[0034] Levels of the FVII at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of FVII from NHPs that had no AAV injected into them are used as background or pre-induction level of FVII. Pre-induction levels of FVII can be equivalent to the no AAV NHP suggesting that the promoters have no background leak.

However, treatment with the inducer leads to a significant increase in the FVII, e.g., in serum with a subsequent return to background levels.

Example P2H: *In vivo* induction of coagulation factor VII in non-human primates

[0035] **Example P4A** is repeated with flavone as the inducer.

[0036] Materials and methods are as described in **Example P2E**, where the artemisinin is replaced with flavone. Levels of FVII at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of FVII from NHPs that had no AAV injected into them are used as background or pre-induction level of FVII. Pre-induction levels of the antibody can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in FVII, e.g., in the serum, with a subsequent return to background levels.

Example P2I: *In vivo* induction of coagulation factor VII in non-human primates

[0037] **Example P4A** is repeated with each of the following inducers: artesunate, arteether, dihydroartemisinin, and artemether.

[0038] Materials and methods are as described in **Example P2E**, where the artemisinin is replaced with one of the above inducers. Levels of FVII at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of FVII from NHPs that had no AAV injected into them are used as background or pre-induction level of FVII. Pre-induction levels of the antibody can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in FVII, e.g., in the serum, with a subsequent return to background levels.

Example P2J: *In vivo* induction of coagulation factor XI (FXI) in non-human primates

[0039] In this example, artemisinin is used as the inducer to induce the *in vivo* expression in NHPs of coagulation factor XI from AAV containing 3xhPB-MinTK driving the expression of a transgene encoding the FXI to assess the following attributes:

- Induction with via IP injection
- Induction via oral ingestion
- Differences in male vs female for induction
- Long-term expression and induction
- Dose response curves via induction with different concentrations of inducer
- Kinetics of induction, to obtain response times of the promoter to the inducer

Materials and methods:

[0040] A vector as described herein, e.g., 3xhPB-MinTK and encoding the coagulation factor XI is packaged into AAV9. The AAV9 containing 3xhPB-MinTK driving the expression of the FXI is produced as described in Greiger et al. 2015. AAV9 containing LSP driving the expression of the cytokine is used as a control.

[0041] Male and/or female NHPs (*Macaca fascicularis*) are pre-screened for neutralizing Abs against AAV9 and injected with 1×10^3 vg/kg of AAV9 containing either 3xhPB-MinTK or LSP with each promoter driving the expression of FXI. The levels of FXI are measured weekly, e.g., by ELISA or similar assay. Once the AAV expression from the LSP control group is stable, the animals are administered the inducer as follows:

- Day 30: 80mg/kg via IP injection
- Day 74: 80mg/kg via IP injection
- Day 123: 40mg/kg via IP injection
- Day 131: 20mg/kg via IP injection
- Day 160: 10mg/kg via IP injection
- Day 180: 1mg/kg via IP injection
- Day 200: 6mg/kg via oral gavage

[0042] Levels of the FXI at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of FXI from NHPs that had no AAV injected into them are used as background or pre-induction level of FXI. Pre-induction levels of FXI can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in the FXI, e.g., in serum with a subsequent return to background levels.

Example P2K: *In vivo* induction of coagulation factor XI in non-human primates

[0043] **Example P4A** is repeated with flavone as the inducer.

[0044] Materials and methods are as described in **Example P2G**, where the artemisinin is replaced with flavone. Levels of FXI at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of FXI from NHPs that had no AAV injected into them are used as background or pre-induction level of FXI. Pre-induction levels of the antibody can be equivalent to the no AAV NHP suggesting that the promoters have no

background leak. However, treatment with the inducer leads to a significant increase in FXI, e.g., in the serum, with a subsequent return to background levels.

Example P2L: *In vivo* induction of coagulation factor XI in non-human primates

[0045] **Example P4A** is repeated with each of the following inducers: artesunate, arteether, dihydroartemisinin, and artemether.

[0046] Materials and methods are as described in **Example P2G**, where the artemisinin is replaced with one of the above inducers. Levels of FXI at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of FXI from NHPs that had no AAV injected into them are used as background or pre-induction level of FXI. Pre-induction levels of the antibody can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in FXI, e.g., in the serum, with a subsequent return to background levels.

Example P3A: *In vivo* induction of interleukin-2 in non-human primates

[0047] In this example, artemisinin is used as the inducer to induce the *in vivo* expression in NHPs of interleukin-2 (IL-2) from AAV containing 3xhPB-MinTK driving the expression of a transgene encoding the IL-2 to assess the following attributes:

- Induction with via IP injection
- Induction via oral ingestion
- Differences in male vs female for induction
- Long-term expression and induction
- Dose response curves via induction with different concentrations of inducer
- Kinetics of induction, to obtain response times of the promoter to the inducer

Materials and methods:

[0048] A vector as described herein, e.g., 3xhPB-MinTK and encoding IL-2 is packaged into AAV9. The AAV9 containing 3xhPB-MinTK driving the expression of the IL-2 is produced as described in Greiger et al. 2015. AAV9 containing LSP driving the expression of the cytokine is used a control.

[0049] Male and/or female NHPs (*Macaca fascicularis*) are pre-screened for neutralizing Abs against AAV9 and injected with 1e13vg/kg of AAV9 containing either 3xhPB-MinTK or LSP with each promoter driving the expression of the IL-2. The levels of the IL-2 are measured weekly,

e.g., by ELISA or similar assay. Once the AAV expression from the LSP control group is stable, the animals are administered the inducer as follows:

- Day 30: 80mg/kg via IP injection
- Day 74: 80mg/kg via IP injection
- Day 123: 40mg/kg via IP injection
- Day 131: 20mg/kg via IP injection
- Day 160: 10mg/kg via IP injection
- Day 180: 1mg/kg via IP injection
- Day 200: 6mg/kg via oral gavage

[0050] Levels of the IL-2 at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of the IL-2 from NHPs that had no AAV injected into them are used as background or pre-induction level of the IL-2. Pre-induction levels of the IL-2 can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in the cytokine, e.g., in serum with a subsequent return to background levels.

Example P3B: *In vivo* induction of a cytokine in non-human primates

[0051] **Example P3A** is repeated with each of the following cytokines: interleukin-1 α (IL-1 α), interleukin-1/3 (IL-1/3), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-8 (IL-8/CXCL8), interleukin-10 (IL-10), interleukin-12 (IL-12), interleukin-13 (IL-13), interleukin-15 (IL-15), interleukin-17 (IL-17), interleukin-18 (IL-18), tumor necrosis factor- α (TNF- α), interferon- α (INF- α), interferon-b (INF-b), interferon- γ (INF- γ), granulocyte-monocyte colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), monocyte chemoattractant protein-1 (MCP-1/CCL2), macrophage inflammatory protein-1 α (MCP-1/CCL3), macrophage inflammatory protein-1/3, RANTES (CCL5), Eotaxin (CCL11), variable endothelial growth factor (VEGF), endothelial growth factor (EGF), and fibroblast growth factor (FGF).

[0052] Materials and methods are as described in **Example P3A**, where the IL-2 replaced by one of the above cytokines. Levels of the cytokine at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of the cytokine from NHPs that had no AAV injected into them are used as background or pre-induction level of the cytokine. Pre-induction levels of the cytokine can be equivalent to the no AAV NHP suggesting that the

promoters have no background leak. However, treatment with the inducer leads to a significant increase in the cytokine, e.g., in the serum, with a subsequent return to background levels.

Example P3D: *In vivo* induction of a cytokine in non-human primates

[0053] Examples P3A and P3B are repeated with flavone as the inducer.

[0054] Materials and methods are as described in **Examples P3A and P3B**, where the artemisinin is replaced with flavone. Levels of the cytokine at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of the cytokine from NHPs that had no AAV injected into them are used as background or pre-induction level of the cytokine. Pre-induction levels of the cytokine can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in the cytokine, e.g., in the serum, with a subsequent return to background levels.

Example P3D: *In vivo* induction of a cytokine in non-human primates

[0055] Examples P3A and P3B are repeated with each of the following inducers: artesunate, arteether, dihydroartemisinin, and artemether.

[0056] Materials and methods are as described in **Examples P3A and P3B**, where the artemisinin is replaced with one of the above inducers. Levels of the cytokine at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of the cytokine from NHPs that had no AAV injected into them are used as background or pre-induction level of the cytokine. Pre-induction levels of the cytokine can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in the cytokine, e.g., in the serum, with a subsequent return to background levels.

Example P4A: *In vivo* induction of erythropoietin in non-human primates

[0057] In this example, artemisinin is used as the inducer to induce the *in vivo* expression in NHPs of erythropoietin (EPO) from AAV containing 3xhPB-MinTK driving the expression of a transgene encoding the EPO to assess the following attributes:

- Induction with via IP injection
- Induction via oral ingestion
- Differences in male vs female for induction
- Long-term expression and induction
- Dose response curves via induction with different concentrations of inducer

- Kinetics of induction, to obtain response times of the promoter to the inducer

Materials and methods:

[0058] A vector as described herein, e.g., 3xhPB-MinTK and encoding EPO is packaged into AAV9. The AAV9 containing 3xhPB-MinTK driving the expression of the EPO is produced as described in Greiger et al. 2015. AAV9 containing LSP driving the expression of the EPO is used as a control.

[0059] Male and/or female NHPs (*Macaca fascicularis*) are pre-screened for neutralizing Abs against AAV9 and injected with 1×10^{13} vg/kg of AAV9 containing either 3xhPB-MinTK or LSP with each promoter driving the expression of the EPO. The levels of the EPO are measured weekly, e.g., by ELISA or similar assays. Once the AAV expression from the LSP control group is stable, the animals are administered the inducer as follows:

- Day 30: 80mg/kg via IP injection
- Day 74: 80mg/kg via IP injection
- Day 123: 40mg/kg via IP injection
- Day 131: 20mg/kg via IP injection
- Day 160: 10mg/kg via IP injection
- Day 180: 1mg/kg via IP injection
- Day 200: 6mg/kg via oral gavage

[0060] Levels of the EPO at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of the EPO from NHPs that had no AAV injected into them are used as background or pre-induction level of the EPO. Pre-induction levels of the EPO can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in the EPO, e.g., in the serum, with a subsequent return to background levels.

Example P4B: *In vivo* induction of a hormone in non-human primates

[0061] **Example P4A** is repeated with each of the following hormones: parathyroid hormone (PTH), insulin-like growth factor (or somatomedin), adrenaline (or epinephrine), melatonin, noradrenaline (or norepinephrine), triiodothyronine, thyroxine, dopamine, prostaglandins, leukotrienes, prostacyclin, thromboxane, amylin (or islet amyloid polypeptide), anti-müllerian hormone (or müllerian-inhibiting factor/hormone), adiponectin, adrenocorticotrophic hormone (or corticotropin), angiotensinogen, angiotensin, antidiuretic hormone (or vasopressin, arginine

vasopressin), atrial natriuretic peptide (or atriopeptin), brain natriuretic peptide, calcitonin, cholecystokinin, corticotropin-releasing hormone, cortistatin, enkephalin, endothelin, follicle-stimulating hormone, galanin, gastric inhibitory polypeptide, gastrin, ghrelin, glucagon, glucagon-like peptide-1, gonadotropin-releasing hormone, growth hormone-releasing hormone, hepcidin, human chorionic gonadotropin, human placental lactogen, growth hormone, inhibin, insulin, leptin, lipotropin, luteinizing hormone, melanocyte stimulating hormone, motilin, orexin, osteocalcin, oxytocin (or pitocin), pancreatic polypeptide, pituitary adenylate cyclase-activating peptide, prolactin (or leuteotropic hormone), prolactin-releasing hormone, relaxin, renin, secretin, somatostatin (or growth hormone-inhibiting hormone or growth hormone release-inhibiting hormone or somatotropin release-inhibiting factor or somatotropin release-inhibiting hormone), thrombopoietin, thyroid-stimulating hormone (or thyrotropin), thyrotropin-releasing hormone, vasoactive intestinal peptide, guanylin, uroguanylin, testosterone, dehydroepiandrosterone, androstenedione, dihydrotestosterone, aldosterone, estradiol, estrone, estriol, cortisol, progesterone, calcitriol, and calcidiol.

[0062] Materials and methods are as described in **Example P4A**, where the EPO replaced by one of the above hormones. Levels of the hormone at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of the hormone from NHPs that had no AAV injected into them are used as background or pre-induction level of the hormone. Pre-induction levels of the hormone can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in the hormone, e.g., in the serum, with a subsequent return to background levels.

Example P4C: *In vivo* induction of a hormone in non-human primates

[0063] **Examples P4A and P4B** are repeated with flavone as the inducer.

[0064] Materials and methods are as described in **Examples P4A and P4B**, where the artemisinin is replaced with flavone. Levels of the hormone at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of the hormone from NHPs that had no AAV injected into them are used as background or pre-induction level of the hormone. Pre-induction levels of the hormone can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in the hormone, e.g., in the serum, with a subsequent return to background levels.

Example P4D: *In vivo* induction of a hormone in non-human primates

[0065] **Examples P4A and P4B** are repeated with each of the following inducers: artesunate, arteether, dihydroartemisinin, and artemether.

[0066] Materials and methods are as described in **Examples P4A and P4B**, where the artemisinin is replaced with one of the above inducers. Levels of the hormone at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of the hormone from NHPs that had no AAV injected into them are used as background or pre-induction level of the hormone. Pre-induction levels of the hormone can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in the hormone, e.g., in the serum, with a subsequent return to background levels.

Example P5A: *In vivo* induction of tumor necrosis factor-alpha in non-human primates

[0067] In this example, artemisinin is used as the inducer to induce the *in vivo* expression in NHPs of tumor necrosis factor-alpha (TNF- α), from AAV containing 3xhPB-MinTK driving the expression of a transgene encoding the TNF- α to assess the following attributes:

- Induction with via IP injection
- Induction via oral ingestion
- Differences in male vs female for induction
- Long-term expression and induction
- Dose response curves via induction with different concentrations of inducer
- Kinetics of induction, to obtain response times of the promoter to the inducer

Materials and methods:

[0068] A vector as described herein, e.g., 3xhPB-MinTK and encoding TNF- α is packaged into AAV9. The AAV9 containing 3xhPB-MinTK driving the expression of the TNF- α is produced as described in Greiger et al. 2015. AAV9 containing LSP driving the expression of the TNF- α is used a control.

[0069] Male and/or female NHPs (*Macaca fascicularis*) are pre-screened for neutralizing Abs against AAV9 and injected with 1e13vg/kg of AAV9 containing either 3xhPB-MinTK or LSP with each promoter driving the expression of the TNF- α . The levels of the TNF- α are measured weekly, e.g., by ELISA or similar assay. Once the AAV expression from the LSP control group is stable, the animals are administered the inducer as follows:

- Day 30: 80mg/kg via IP injection
- Day 74: 80mg/kg via IP injection

- Day 123: 40mg/kg via IP injection
- Day 131: 20mg/kg via IP injection
- Day 160: 10mg/kg via IP injection
- Day 180: 1mg/kg via IP injection
- Day 200: 6mg/kg via oral gavage

[0070] Levels of the TNF- α at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of the TNF- α from NHPs that had no AAV injected into them are used as background or pre-induction level of the TNF- α . Pre-induction levels of the TNF- α can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in the TNF- α , e.g., in serum with a subsequent return to background levels.

Example P5B: *In vivo* induction of a growth factor in non-human primates

[0071] **Example P5A** is repeated with each of the following growth factors: adrenomedullin (AM), angiopoietin (Ang), autocrine motility factor, bone morphogenetic protein (BMP), ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), colony-stimulating factor (e.g., macrophage colony-stimulating factor (M-CSF), granulocyte colony-stimulating factor (G-CSF), or granulocyte macrophage colony-stimulating factor (GM-CSF)), epidermal growth factor (EGF), ephrin (e.g., ephrin A1, ephrin A2, ephrin A3, ephrin A4, ephrin A5, ephrin B1, ephrin B2, or ephrin B3), erythropoietin (EPO), fibroblast growth factor (e.g., FGF1, FGF2, FGF3, FGF4, FGF5, FGF6, FGF7, FGF8, FGF9, FGF10, FGF11, FGF12, FGF13, FGF14, FGF15, FGF16, FGF17, FGF18, FGF19, FGF20, FGF21, FGF22 or FGF23), glial cell line-derived neurotrophic factor (GDNF), neurturin, persephin, artemin, growth differentiation factor-9 (GDF9), hepatocyte growth factor (HGF), hepatoma-derived growth factor (HDGF), insulin-like growth factor (e.g., IGF-1 or IGF-2), interleukin (e.g., IL-1, IL-2, IL-3, IL-4, IL-5, IL-6 or IL-7), keratinocyte growth factor (KGF), migration-stimulating factor (MSF), macrophage-stimulating protein (MSP), myostatin (GDF-8), neuregulin (e.g., NRG1, NRG3, NRG3 or NRG4), neurotrophin (e.g., brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-3 (NT-3) or neurotrophin-4 (NT-4)), placental growth factor (PGF), platelet-derived growth factor (PDGF), renalase (RNLS), T-cell growth factor (TCGF), thrombopoietin (TPO), transforming growth factor (e.g., TGF- α or TGF- β), and vascular endothelial growth factor (VEGF).

[0072] Materials and methods are as described in **Example P5A**, where the TNF- α is replaced by one of the above growth factors. Levels of the growth factor at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of the

growth factor from NHPs that had no AAV injected into them are used as background or pre-induction level of the growth factor. Pre-induction levels of the growth factor can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in the growth factor, e.g., in serum with a subsequent return to background levels.

Example P5C: *In vivo* induction of a growth factor in non-human primates

[0073] **Examples P5A and P5B** are repeated with flavone as the inducer.

[0074] Materials and methods are as described in **Examples P5A and P5B**, where the artemisinin is replaced with flavone. Levels of the growth factor at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of the growth factor from NHPs that had no AAV injected into them are used as background or pre-induction level of the growth factor. Pre-induction levels of the growth factor can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in the growth factor, e.g., in serum with a subsequent return to background levels.

Example P5D: *In vivo* induction of a growth factor in non-human primates

[0075] **Examples P5A and P5B** are repeated with each of the following inducers: artesunate, arteether, dihydroartemisinin, and artemether.

[0076] Materials and methods are as described in **Examples P5A and P5B**, where the artemisinin is replaced with one of the above inducers. Levels of the growth factor at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of the growth factor from NHPs that had no AAV injected into them are used as background or pre-induction level of the growth factor. Pre-induction levels of the growth factor can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in the growth factor, e.g., in serum with a subsequent return to background levels.

Example P6A: *In vivo* induction of a coronavirus spike protein in non-human primates

[0077] In this example, artemisinin is used as the inducer to induce the *in vivo* expression in NHPs of a coronavirus spike protein from AAV containing 3xhPB-MinTK driving the expression of a transgene encoding the spike protein to assess the following attributes:

- Induction with via IP injection
- Induction via oral ingestion

- Differences in male vs female for induction
- Long-term expression and induction
- Dose response curves via induction with different concentrations of inducer
- Kinetics of induction, to obtain response times of the promoter to the inducer

Materials and methods:

[0078] A vector as described herein, e.g., 3xhPB-MinTK and encoding a coronavirus spike protein is packaged into AAV9. The AAV9 containing 3xhPB-MinTK driving the expression of the spike protein is produced as described in Greiger et al. 2015. AAV9 containing LSP driving the expression of the spike protein is used a control.

[0079] Male and/or female NHPs (*Macaca fascicularis*) are pre-screened for neutralizing Abs against AAV9 and injected with 1e13vg/kg of AAV9 containing either 3xhPB-MinTK or LSP with each promoter driving the expression of the spike protein. The levels of the spike protein are measured weekly, e.g., by ELISA, FACS or similar assays. Once the AAV expression from the LSP control group is stable, the animals are administered the inducer as follows:

- Day 30: 80mg/kg via IP injection
- Day 74: 80mg/kg via IP injection
- Day 123: 40mg/kg via IP injection
- Day 131: 20mg/kg via IP injection
- Day 160: 10mg/kg via IP injection
- Day 180: 1mg/kg via IP injection
- Day 200: 6mg/kg via oral gavage

[0080] Levels of the spike protein at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of the spike protein from NHPs that had no AAV injected into them are used as background or pre-induction level of the spike protein. Pre-induction levels of the spike protein can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in the spike protein, e.g., in the serum, with a subsequent return to background levels.

Example P6B: *In vivo* induction of a virus protein in non-human primates

[0081] **Example P6A** is repeated with each of the following virus proteins: viral envelop protein, viral nucleocapsid protein, viral membrane protein, REP, CAP, REV, VSV-G and RD114.

[0082] Materials and methods are as described in **Example P6A**, where the spike protein is replaced by one of the above virus proteins. Levels of the virus protein at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of the virus protein from NHPs that had no AAV injected into them are used as background or pre-induction level of the virus protein. Pre-induction levels of the virus protein can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in the virus protein, e.g., in the serum, with a subsequent return to background levels.

Example P6C: *In vivo* induction of a virus protein in non-human primates

[0083] **Examples P6A and P6B** are repeated with flavone as the inducer.

[0084] Materials and methods are as described in **Examples P6A and P6B**, where the artemisinin is replaced with one of the above inducers. Levels of the virus protein at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of the virus protein from NHPs that had no AAV injected into them are used as background or pre-induction level of the virus protein. Pre-induction levels of the virus protein can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in the virus protein, e.g., in the serum, with a subsequent return to background levels.

Example P6C: *In vivo* induction of a virus protein in non-human primates

[0085] **Examples P6A and P6B** are repeated with each of the following inducers: artesunate, arteether, dihydroartemisinin, and artemether.

[0086] Materials and methods are as described in **Examples P6A and P6B**, where the artemisinin is replaced with one of the above inducers. Levels of the virus protein at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of the virus protein from NHPs that had no AAV injected into them are used as background or pre-induction level of the virus protein. Pre-induction levels of the virus protein can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in the virus protein, e.g., in the serum, with a subsequent return to background levels.

Example P7A: *In vivo* induction of a cell receptor ligand in non-human primates

[0087] In this example, artemisinin is used as the inducer to induce the *in vivo* expression in NHPs of a cell receptor ligand from AAV containing 3xhPB-MinTK driving the expression of a transgene encoding the cell receptor ligand to assess the following attributes:

- Induction with via IP injection
- Induction via oral ingestion
- Differences in male vs female for induction
- Long-term expression and induction
- Dose response curves via induction with different concentrations of inducer
- Kinetics of induction, to obtain response times of the promoter to the inducer

Materials and methods:

[0088] A vector as described herein, e.g., 3xhPB-MinTK and encoding a cell receptor ligand (e.g., acetylcholine, ATP, dopamine, GABA, glutamate, glycine, serotonin, ketamine, nicotine, strychnine, cereulide, EGF, insulin, VEGF, cGMP, cGMP, cGTP, or muscarine) is packaged into AAV9. The AAV9 containing 3xhPB-MinTK driving the expression of the cell receptor ligand is produced as described in Greiger et al. 2015. AAV9 containing LSP driving the expression of the cell receptor ligand is used a control.

[0089] Male and/or female NHPs (*Macaca fascicularis*) are pre-screened for neutralizing Abs against AAV9 and injected with 1e13vg/kg of AAV9 containing either 3xhPB-MinTK or LSP with each promoter driving the expression of the cell receptor ligand. The levels of the cell receptor ligand are measured weekly, e.g., by ELISA or similar assay. Once the AAV expression from the LSP control group is stable, the animals are administered the inducer as follows:

- Day 30: 80mg/kg via IP injection
- Day 74: 80mg/kg via IP injection
- Day 123: 40mg/kg via IP injection
- Day 131: 20mg/kg via IP injection
- Day 160: 10mg/kg via IP injection
- Day 180: 1mg/kg via IP injection
- Day 200: 6mg/kg via oral gavage

[0090] Levels of the cell receptor ligand at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of the cell receptor ligand from NHPs that had no AAV injected into them are used as background or pre-induction level of

the cell receptor ligand. Pre-induction levels of the cell receptor ligand can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in the Cell receptor ligand, e.g., in serum with a subsequent return to background levels.

Example P7B: *In vivo* induction of a cell receptor ligand in non-human primates

[0091] **Example P7A** is repeated with each flavone as the inducer.

[0092] Materials and methods are as described in **Example 7A**, where the artemisinin is replaced with flavone. Levels of the cell receptor ligand at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of the cell receptor ligand from NHPs that had no AAV injected into them are used as background or pre-induction level of the cell receptor ligand. Pre-induction levels of the cell receptor ligand can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in the Cell receptor ligand, e.g., in serum with a subsequent return to background levels.

Example P7C: *In vivo* induction of a cell receptor ligand in non-human primates

[0093] **Example P7A** is repeated with each of the following inducers: artesunate, arteether, dihydroartemisinin, and artemether.

[0094] Materials and methods are as described in **Example 7A**, where the artemisinin is replaced with one of the above inducers. Levels of the cell receptor ligand at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of the cell receptor ligand from NHPs that had no AAV injected into them are used as background or pre-induction level of the cell receptor ligand. Pre-induction levels of the cell receptor ligand can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in the Cell receptor ligand, e.g., in serum with a subsequent return to background levels.

Example P8A: *In vivo* induction of a chimeric antigen receptor in non-human primates

[0095] In this example, artemisinin is used as the inducer to induce the *in vivo* expression in NHPs of a chimeric antigen receptor (CAR) from AAV containing 3xhPB-MinTK driving the expression of a transgene encoding the CAR to assess the following attributes:

- Induction with via IP injection
- Induction via oral ingestion

- Differences in male vs female for induction
- Long-term expression and induction
- Dose response curves via induction with different concentrations of inducer
- Kinetics of induction, to obtain response times of the promoter to the inducer

Materials and methods:

[0096] A vector as described herein, e.g., 3xhPB-MinTK and encoding a CAR, is packaged into AAV9. The AAV9 containing 3xhPB-MinTK driving the expression of the CAR is produced as described in Greiger et al. 2015. AAV9 containing LSP driving the expression of the CAR is used as a control.

[0097] Male and/or female NHPs (*Macaca fascicularis*) are pre-screened for neutralizing Abs against AAV9 and injected with 1×10^{13} vg/kg of AAV9 containing either 3xhPB-MinTK or LSP with each promoter driving the expression of the CAR. The levels of the CAR are measured weekly, e.g., by ELISA, FACS or similar assays. Once the AAV expression from the LSP control group is stable, the animals are administered the inducer as follows:

- Day 30: 80mg/kg via IP injection
- Day 74: 80mg/kg via IP injection
- Day 123: 40mg/kg via IP injection
- Day 131: 20mg/kg via IP injection
- Day 160: 10mg/kg via IP injection
- Day 180: 1mg/kg via IP injection
- Day 200: 6mg/kg via oral gavage

[0098] Levels of the CAR at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of the CAR from NHPs that had no AAV injected into them are used as background or pre-induction level of the CAR. Pre-induction levels of the CAR can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in the CAR, e.g., in the serum, with a subsequent return to background levels.

Example P8B: *In vivo* induction of a CAR in non-human primates

[0099] Example 8A is repeated with flavone as the inducer.

[00100] Materials and methods are as described in **Example 8A**, where the artemisinin is replaced with one of the above inducers. Levels of the CAR at 12 hours, 24 hours, 48 hours, 72

hours, and/or 1 weeks after each administration of the inducer are measured. Levels of the CAR from NHPs that had no AAV injected into them are used as background or pre-induction level of the CAR. Pre-induction levels of the CAR can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in the CAR, e.g., in the serum, with a subsequent return to background levels.

Example P8C: *In vivo* induction of a CAR in non-human primates

[00101] **Example 8A** is repeated with each of the following inducers: artesunate, arteether, dihydroartemisinin, and artemether.

[00102] Materials and methods are as described in **Example 8A**, where the artemisinin is replaced with one of the above inducers. Levels of the CAR at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of the CAR from NHPs that had no AAV injected into them are used as background or pre-induction level of the CAR. Pre-induction levels of the CAR can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in the CAR, e.g., in the serum, with a subsequent return to background levels.

Example P9A: *In vivo* induction of a CRISPR-Cas nuclease 9 in non-human primates

[00103] In this example, artemisinin is used as the inducer to induce the *in vivo* expression in NHPs of a CRISPR-Cas nuclease 9 (Cas9, also known as Csn1 and Csx12) from AAV containing 3xhPB-MinTK driving the expression of a transgene encoding the Cas9 to assess the following attributes:

- Induction with via IP injection
- Induction via oral ingestion
- Differences in male vs female for induction
- Long-term expression and induction
- Dose response curves via induction with different concentrations of inducer
- Kinetics of induction, to obtain response times of the promoter to the inducer

Materials and methods:

[00104] A vector as described herein, e.g., 3xhPB-MinTK and encoding a Cas9 is packaged into AAV9. The AAV9 containing 3xhPB-MinTK driving the expression of the Cas9 is produced as described in Greiger et al. 2015. AAV9 containing LSP driving the expression of the Cas9 is used a control.

[00105] Male and/or female NHPs (*Macaca fascicularis*) are pre-screened for neutralizing Abs against AAV9 and injected with 1e13vg/kg of AAV9 containing either 3xhPB-MinTK or LSP with each promoter driving the expression of the Cas9. The levels of the Cas9 are measured weekly, e.g., by providing a guide RNA (gRNA) and detecting cleavage of the corresponding target. Once the AAV expression from the LSP control group is stable, the animals are administered the inducer as follows:

- Day 30: 80mg/kg via IP injection
- Day 74: 80mg/kg via IP injection
- Day 123: 40mg/kg via IP injection
- Day 131: 20mg/kg via IP injection
- Day 160: 10mg/kg via IP injection
- Day 180: 1mg/kg via IP injection
- Day 200: 6mg/kg via oral gavage

[00106] Levels of the Cas9 at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of the Cas9 from NHPs that had no AAV injected into them are used as background or pre-induction level of the Cas9. Pre-induction levels of the Cas9 can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in the Cas9 with a subsequent return to background levels.

Example P9B: *In vivo* induction of a CRISPR-Cas nuclease in non-human primates

[00107] **Example P9A** is repeated with each of the following CRISPR-Cas nucleases: C2c1, C2c3, Cas1, Cas100, Cas12a, Cas12b, Cas12c, Cas12d, Cas12e, Cas13a, Cas13b, Cas13c, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Casl, CaslB, CaslO, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Cpf1, Csa5, Csa5, CsaX, Csb1, Csb2, Csb3, Csc1, Csc2, Cse1, Cse2, Csf1, Csf2, Csf3, Csf4, Csm2, Csm3, Csm4, Csm5, Csm6, Csn2, Csx1, Csx10, Csx14, Csx15, Csx16, Csx17, Csx3, Csy1, Csy2, and Csy3.

[00108] Materials and methods are as described in **Example P9A**, where the Cas9 is replaced by one of the above Cas nucleases. Levels of the Cas nuclease at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of the Cas nuclease from NHPs that had no AAV injected into them are used as background or pre-induction level of the Cas nuclease. Pre-induction levels of the Cas nuclease can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the

inducer leads to a significant increase in the Cas nuclease with a subsequent return to background levels.

Example P9C: *In vivo* induction of a CRISPR-Cas nuclease in non-human primates

[00109] Examples P9A and P9B are repeated with flavone as the inducer.

[00110] Materials and methods are as described in Examples P9A and P9B where the artemisinin is replaced with flavone. Levels of the Cas nuclease at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of the Cas nuclease from NHPs that had no AAV injected into them are used as background or pre-induction level of the Cas nuclease. Pre-induction levels of the Cas nuclease can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in the Cas nuclease with a subsequent return to background levels.

Example P9C: *In vivo* induction of a CRISPR-Cas nuclease in non-human primates

[00111] Examples P9A and P9B are repeated with each of the following inducers: artesunate, arteether, dihydroartemisinin, and artemether.

[00112] Materials and methods are as described in Examples P9A and P9B where the artemisinin is replaced with one of the above inducers. Levels of the Cas nuclease at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of the Cas nuclease from NHPs that had no AAV injected into them are used as background or pre-induction level of the Cas nuclease. Pre-induction levels of the Cas nuclease can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in the Cas nuclease with a subsequent return to background levels.

Example P10A: *In vivo* induction of an enzyme in non-human primates

[00113] In this example artemisinin is used as the inducer to induce the *in vivo* expression in NHPs of an enzyme from AAV containing 3xhPB-MinTK driving the expression of a transgene encoding the enzyme to assess the following attributes:

- Induction with via IP injection
- Induction via oral ingestion
- Differences in male vs female for induction
- Long-term expression and induction
- Dose response curves via induction with different concentrations of inducer

- Kinetics of induction, to obtain response times of the promoter to the inducer

Materials and methods:

[00114] A vector as described herein, e.g., 3xhPB-MinTK and encoding an enzyme is packaged into AAV9. The AAV9 containing 3xhPB-MinTK driving the expression of the enzyme is produced as described in Greiger et al. 2015. AAV9 containing LSP driving the expression of the enzyme is used a control.

[00115] Male and/or female NHPs (*Macaca fascicularis*) are pre-screened for neutralizing Abs against AAV9 and injected with 1×10^{13} vg/kg of AAV9 containing either 3xhPB-MinTK or LSP with each promoter driving the expression of the enzyme. The levels of the enzyme are measured weekly, e.g., by ELISA or similar assay. Once the AAV expression from the LSP control group is stable, the animals are administered the inducer as follows:

- Day 30: 80mg/kg via IP injection
- Day 74: 80mg/kg via IP injection
- Day 123: 40mg/kg via IP injection
- Day 131: 20mg/kg via IP injection
- Day 160: 10mg/kg via IP injection
- Day 180: 1mg/kg via IP injection
- Day 200: 6mg/kg via oral gavage

[00116] Levels of the enzyme at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of the enzyme from NHPs that had no AAV injected into them are used as background or pre-induction level of the enzyme. Pre-induction levels of the enzyme can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in the Enzyme, e.g., in serum with a subsequent return to background levels.

Example P10B: *In vivo* induction of an enzyme in non-human primates

[00117] **Example 10A** is repeated with flavone as the inducer.

[00118] Materials and methods are as described in **Example 10A**, where the artemisinin is replaced with flavone. Levels of the enzyme at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of the enzyme from NHPs that had no AAV injected into them are used as background or pre-induction level of the enzyme.

Pre-induction levels of the enzyme can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in the Enzyme, e.g., in serum with a subsequent return to background levels.

Example P10C: *In vivo* induction of an enzyme in non-human primates

[00119] Example 10A is repeated with each of the following inducers: artesunate, arteether, dihydroartemisinin, and artemether.

[00120] Materials and methods are as described in Example 10A, where the artemisinin is replaced with one of the above inducers. Levels of the enzyme at 12 hours, 24 hours, 48 hours, 72 hours, and/or 1 weeks after each administration of the inducer are measured. Levels of the enzyme from NHPs that had no AAV injected into them are used as background or pre-induction level of the enzyme. Pre-induction levels of the enzyme can be equivalent to the no AAV NHP suggesting that the promoters have no background leak. However, treatment with the inducer leads to a significant increase in the Enzyme, e.g., in serum with a subsequent return to background levels.

Example P11: Administration of AAV comprising 3XhPB-MinTk to subjects with Hemophilia A

[00121] In a clinical trial with subjects with hemophilia A, the subjects are administered with recombinant AAV having the inducible promoter with PBREM enhancer and also administered with artemisinin or artesunate by IP injection or by oral administration. The trial is divided into the following arms as described below.

Arm 1:

[00122] In one arm, an infusion of AAV5-3XhPB-MinTk to is administered to 15 adults with severe hemophilia A (FVIII ≤ 1 IU/dL) at doses of 6×10^{12} viral genome (vg)/kg (n = 1), 2×10^{13} vg/kg (n = 1), 6×10^{13} vg/kg (n = 7) or 4×10^{13} vg/kg (n = 6). Each of them are administered orally with Artesunate 1mg/kg dose. Four of seven and three of six participants in the 6×10^{13} vg/kg and 4×10^{13} vg/kg cohorts, respectively, maintains median FVIII levels > 15 IU/dL at 4 and 5 years after administration. Individuals in the 6×10^{13} vg/kg cohort has a mean annual bleeding rate (ABR) of 0.7 treated bleeds/year during year 5, resulting in a cumulative mean ABR of 0.8 treated bleeds/year from week 5 onward (95% reduction from baseline). Individuals in the 4×10^{13} vg/kg cohort has a mean ABR of 1.7 treated bleeds/year during year 4, resulting in a cumulative mean ABR of 1.0 treated bleeds/year from week 5 onward, a 92% reduction from baseline. QoL is maintained in patients receiving the higher dose. The most common adverse events are transient, asymptomatic, and mild-to-moderate ALT elevations.

Arm 2:

[00123] In another arm of the study, a single 6×10^{13} vg/kg infusion of AAV5-3XhPB-MinTk are administered to 30 adult males with severe hemophilia A (FVIII ≤ 1 IU/dL) on FVIII prophylaxis (negative for FVIII inhibitors). The patients are also treated with 1mg/kg artemisinin

injection via IP. CSA-assessed FVIII activity increases by a mean of 41.9 IU/dL at weeks 49–52 (baseline FVIII activity is assumed to be 1 IU/dL, as there is no washout of FVIII therapy before AAV infusion). An 85% reduction in mean ABR and 96% reduction in FVIII infusion rate are observed. Data for two years' post-infusion are available for 17 study participants. In these individuals, the mean FVIII activity level is 25 IU/dL at week 104, compared with 42.2 IU/dL at weeks 49–52 after infusion. At week 104, 18% of study participants has a median FVIII activity level of ≥ 40 IU/dL, 80% of participants has an activity level of >5 to <40 IU/dL.

Arm 3:

[00124] 18 men with hemophilia A are enrolled in four dose cohorts where they are treated with AAV3-3XhPB-MinTk; the lowest-dose cohort receives 5×10^{11} vg/kg and the highest-dose cohort receives 2×10^{12} vg/kg, with glucocorticoids administered in cases of suspected immune response. At the time of AAV administration, the participants are administered with 5mg/kg artemisinin by IP route of administration. In 16 men, FVIII expression are maintained, with 12 men followed for >2 years. These men have a 91.5% reduction in ABR after AAV administration. Elevated ALT levels were seen in seven study participants, the majority of which are mild, with the exception of a grade 2 elevation in one participant that requires hospitalization for treatment.

Arm 4:

[00125] 12 Adult women with clinically severe hemophilia A who have not developed FVIII inhibitors and have no AAV neutralizing antibodies are administered with 5×10^{11} vg/kg of AAV XL32.1-3XhPB-MinTk and also are administered orally with 0.5mg/kg of artesunate. Preliminary results demonstrate sustained FVIII levels (10–22%) after 52 weeks in all women. No women develop moderate to severely elevated liver enzyme levels. The patients are not administered with any immunomodulator during the course of the study.

Arm 5:

[00126] 11 women with Hemophilia A are each given three ascending doses: 6×10^{12} vg, 3×10^{13} , and 1×10^{14} vg ($n = 4, 4,$ and 3 patients, respectively) of AAV6-3XhPB-MinTk, and each are given 1mg/kg artemisinin by IP. Patients in the 3×10^{13} vg cohort have available data, which demonstrate mean FVIII activity maintained in the mild to normal range (30% measured by CCA; 45% measured by one-stage assay) to week 104. The ABR is zero for the first-year post-infusion and 0.9 throughout the follow-up, and two patients experience a total of three bleeding events (two traumatic, one unknown; one in a target joint) that necessitated treatment with exogenous FVIII. No patients have resumed prophylaxis. Common treatment-related adverse events are elevated liver enzyme levels. Patients in the 1×10^{14} vg cohort have an ALT elevation that require >7 days of corticosteroid treatment, but these are managed with a tapering course of corticosteroids, and FVIII activity is maintained. No patients develop inhibitors to FVIII.

Arm 6:

[00127] Adult males who have severe hemophilia A with no history of FVIII inhibitors, no detectable immunity to the AAVhu37 capsid, and ≥ 150 exposure days to FVIII products are treated with AAVhu37-3XhPB-MinTk, and also orally administered with 1mg/kg artesunate. Nine patients have received a single infusion of AAV at doses of 0.5, 1.0, and 2.0×10^{13} gene copies/kg ($n = 2, 2,$ and $5,$ respectively). Successful proof-of-concept is achieved with measurable, stable expression

of endogenous FVIII for up to >23 months. Bleeding protection is observed, with patients in the two higher dose groups not requiring prophylaxis with FVIII products from approximately 6–12 weeks after gene transfer, and no spontaneous bleeds requiring treatment being reported once FVIII levels reached >11 IU/dL. Mild-to-moderate ALT elevations are observed in five patients but these are successfully managed with corticosteroids or famotidine.

Arm 7:

[00128] Three men with severe hemophilia A receive a single infusion of AAV8-3XhPB-MinTk -hFVIII, with one patient receiving a dose of 6×10^{11} vg/kg and the other two patients each receiving a dose of 2×10^{12} vg/kg, and each of them receives 1mg/kg artemisinin via IP route of administration. The patients are followed up for 15–52 weeks after vector administration. FVIII activity levels are >15% in all three men, with normalization of FVIII levels in one patient. Serum ALT levels are elevated in two of the patients between 4 and 6 weeks after gene transfer but this responds to corticosteroids, with no loss of transgene expression. No study participants have developed FVIII inhibitors.

Sequences**Table 1 – Exemplary PBREM elements, variants and parts thereof**

Name	Sequence
PBREM (human)	<u>ACTGTACTTTCTGACCCTGAAGAGGTGGCAGCATGGACTTTCTGAAC</u> <u>CCA (SEQ ID NO: 2)</u>

NR1 motif underlined, NF1 motif bold, and NF2 motif double underlined.

Table 2 – Exemplary inducible promoters comprising human PBREM element

Name	Sequence
3xhPB- minTK	<u>ACTGTACTTTCTGACCCTGAAGAGGTGGCAGCATGGACTTTCTGAAC</u> <u>CACATTACTCGCATCCATTCTCACTGTACTTTCTGACCCTGAAGAGGT</u> <u>GGCAGCATGGACTTTCTGAACCAGCACTGAAGGTCCTCAATCGACTG</u> <u>TACTTTCTGACCCTGAAGAGGTGGCAGCATGGACTTTCTGAACCAG</u> <u>CTGGGAGTTCGTAGACGGAGATCCGGCCCCGCCAGCGTCTTGTCATT</u> <u>GGCGAATTCGAACACGCAGATGCAGTCGGGGCGGCGCGGTCCGAGGT</u> <u>CCACTTCGCATATTAAGGTGACGCGTGTTGGCCTCGAACACCGAGC</u> <u>GACCCTGCAGCGACCCGCTTAACAGCGTCAACAGCGTGCCGCAGATC</u> <u>TCGAGGAGCTTGGCGAGATTTTCAGGAGCTAAGGAAGCTAAAC (SEQ</u> <u>ID NO: 66)</u>

PBREM elements are underlined; minimal promoter is in bold; gene sequences are double underlined.

Table 3 – Exemplary constructs and other sequences.

Name	Sequence
LSP-cGT	tctagagctagcatatggatccatcgatttagggataacagggttaattatcagcacacaattgccattatacgcgcgt ataatggactattgtgtgctgatatctgtacacttaagggttagatcttagcttacgtcacttagagggtccacgttagtt ttaaagatccattgatctcctaaacgtgcaagattcgcaacctgggtatacttagcctaggcgctaggtcctagtcag cgggacttttttctaaagtcgttgagaggaggagtcgtcagaccagatagctttgatgtcctgatcggaaggatcggt ggccccctgcaggcagctgttaattaactgcgcgctcgctcgctcactgaggccgcccgggcaaagccgggc gtcgggacgtttgtgcggcctcagtgagcgagcgagcgcgagagaggagtgccaaactccatcact aggggttccttgtagttaatgattaacccgccatgctacttatctacggcgcgccgctaactggccggtacatcta ggagttaattttaaaaagcagtcaaaagtcgaagtcgccttcgagcatttactctctgtttgctctggttaataatct caggagcacaaacattccttactagtttaggaggttaattttaaaaagcagtcaaaagtcgaagtcgccttcgagca ttactctctgtttgctctggttaataatctcaggagcacaaacattccttactagtttagagcgccgccagtggtgc tggaattcggcttttttagggctggaagctacctttgacatcatttctctgcgaatgcatgtataattttacagaaccta ttagaaggatcaccagcctctgctttgtacaactttcccttaaaaaactgccaatcccactgctgtttggcccaata gtgagaacttttctgctgctctgtgtgtgtttgcctatggccctattctgctgctgaagacacttggccagcatg gacttaaacccctccagctctgacaatcctcttctctttgttttacatgaagggtctggcagccaaagcaatcactca aagttcaaaccttatcattttgtgttctcttggccttggtttgtacatcagctttgaaaataccatcccagggttaa

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CCTAGCGCCCGCTCCTTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTTC
GCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTC
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CCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGC
GAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGC
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TCCGCCTTTCTCCCTTCGGGAAGCGTGCGCTTTTCTCATAGCTCACGC

	TGTAGGTATCTCAGTTCGGTGTAGGTCGTTTCGCTCCAAGCTGGGCTGT GTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAA CTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGC AGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTG CTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGA ACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAA AGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGG TGGTTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGAT CTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGA ACGAAAACACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGG ATCTTCACCTAGATCCTTTTAAATTAATAATGAAGTTTTAAATCAATC TAAAGTATATATGAGTAACTTGGTCTGACAGTTACCAATGCTTAATC AGTGAGGCACCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATAGTT GCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACC ATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACC GG CTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGC AGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGT TGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTTCGCAA CGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCGTTTGG TATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATG ATCCCCCATGTTGTGAAAAAAGCGGTTAGCTCCTTCGGTCTCCTCGAT CGTTGTCAGAAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGC AGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTC TGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGC GGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCG CCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAACGTTCTTCG GGGCGAAAACCTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGAT GTAACCCACTCGTGCACCCAAGTATCTTCAGCATCTTTTACTTTTAC CAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAA AAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCT TTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGG ATACATATTTGAATGTATTTAGAAAAATAAACAATAGGGGTTCCGC GCACATTTCCCCGAAAAGTGCCACCTGACGTC (SEQ ID NO: 72)
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[00907] All patents and other publications; including literature references, issued patents, published patent applications, and co-pending patent applications; cited throughout this application are expressly incorporated herein by reference for the purpose of describing and disclosing, for example, the methodologies described in such publications that might be used in connection with the technology described herein. These publications are provided solely for their disclosure prior to the filing date of the present application. Nothing in this regard should be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention or for any other reason. All statements as to the date or representation as to the contents of these

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CLAIMS

What is claimed is:

1. A method of regulating expression of a transgene in a primate subject, the method comprising:
 - (a) administering to the subject at a first time point a first inducer, wherein the primate subject comprises at least one cell comprising an exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene, wherein the inducible promoter comprises a PBREM enhancer, wherein the first inducer is artemisinin or a derivative thereof or a flavonoid compound, and wherein the inducer is administered in an amount that is sufficient to express a measurable level of the transgene in the subject; and
 - (b) administering to the subject at least at a second time point a second inducer to continue expression of the transgene at a predetermined level for a predetermined time or to modulate expression of the transgene to a predetermined level, and wherein the second inducer is artemisinin; derivative of artemisinin; a flavonoid compound, e.g. flavone, chrysin, baicalein, or galangin; 1,4-Bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP); phenobarbital (PB); 6-(4Chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde-O-(3,4-dichlorobenzyl)oxime (CITCO); Acetaminophen; Buprenorphine; Phenytoin; Carbamazepine; Valproic Acid; Chlorpromazine; Efavirenz; Nevirapine; Rilpivirine; Etravirine; Diazepam; Cyclophosphamid; Ifosfamide; Cerivastatin; Simvastatin; lovastatin; substituted sulphonamides; Thiazolidin-4-one; Estradiol; Estrone and analogues; 17 α -ethinyl-3;17 β -estradiol (EE2); Dehydroepiandrosterone (DHEA); 5 β -pregnane-3,20-dione; Diethylstilbestrol; *Ginkgo biloba* extract; Galangin; chrysin; baicalein; Diallyl sulphide; Ellagic acid; Resveratrol; Squalestatin-1; Bilobalide; Triclocarban; Triclosan; Dichlorodiphenyltrichloroethane (DDT); Dieldrin; Methoxychlor; Metofluthrin; Permethrin; Pyrethrins; Sulfoxaflor; Diethylhexyl phthalate (DEHP); Cyproconazole; Fluconazole; Propiconazole; FL81; Tri-p-methylphenyl phosphate (TMPP); UM104; or UM145.
2. The method of claim 1, wherein the expression of the transgene increases for a predetermined time after each administration.
3. The method of claim 1 or 2, wherein the first and second inducer are the same.
4. The method of claim 3, wherein the first and second inducer are artemisinin, a derivative of artemisinin, or a flavonoid compound.

5. The method of claim 3, wherein the first inducer and second inducer are artemisinin.
6. The method of claim 3, wherein the first inducer and second inducer are a derivative of artemisinin.
7. The method of claim 3, wherein the first inducer and second inducer are a flavonoid compound, optionally, the flavonoid compound is flavone.
8. The method of any one of claims 1-2, wherein the first and second inducer are different.
9. The method of claim 8, wherein the first inducer is artemisinin, and the second inducer is an artemisinin derivative or a flavonoid compound.
10. The method of claims 8, wherein the first inducer is a first artemisinin derivative, and the second inducer is artemisinin, a second artemisinin derivative, or a flavonoid compound.
11. The method of claim 8, wherein the first inducer is a flavonoid compound and the second inducer is artemisinin or a derivative of artemisinin.
12. The method of any one of claims 1-11, wherein the artemisinin derivative is a compound of Formula (I), (I-A), (I-B), (I-C), (I-D), (I-E), (I-F), (I-G), (I-H), (I-I), (I-J), (I-K), (I-L), (I-M), (I-M'), (I-N), (I-N'), (I-O) or (I-O').
13. The method of any one of claims 1-12, wherein the artemisinin derivative is artesunate, dihydroartemisinin (DHA), artesunate, or artemether, optionally, the artemisinin derivative is artesunate, or dihydroartemisinin, more optionally, the artemisinin derivative is artesunate.
14. The method of any one of claims 1-13, wherein the flavonoid compound is flavone, baicalein, chrysin, galangin, (OH)₄ flavone, 6-hydroxyluteolin, acacetin, acerosin, alnetin, apigenin, apiin, artocarpetin, balcalein, beta-sitosterol, biochamin A, cannaflavin A, cannflavin A, cannflavin B, cannflavin C, catechin, catechin-3-gallate, cerrosillin, chrysoeriol, chrysoeriol, cirsilinole, cirsilinole, cirsimaritin, corymbosin, cupressuflavone, cyanidine, daidzein, datiscetin, delphinidine, digidrokempferol, dihydroquercetin, diosmetin, diosmin, echiodinin, epicatechin, epicatechin-3-gallate, epigallocatechin, epigallocatechin-3-gallate, eriodictiol, eupatilin, eupatorin, fisetin, fisetin, flavonol, formononetin, fortunelin, gallocatechin, gallocatechin-3-gallate, gardenin D, genistein, genistin, genkwanin, geraldol, geraldol, glycitein, glycitin, gomoeriodictiol, gossypetin, hesperetin, hinokiflavone, hispidulin, hymenoxin, hypolaetin, isoquercetin, isorhamnetin, isorhamnetin, isoscutellarein, isovitexin, jaceosidin, kaempferol, kaempferol, kaempferide, largonidin, luteolin, luteolin, malvidine, mikanin, morin, myricetin, myricetin, naringenin, negletein, neodiosmin, nepetin, nevadensin, nobiletin, nodifloretin, norartocarpetin, norwogonin, onopordin, orientin, oroxylin a, pachipodol, pectolinarigenin, pedalitin, peonidin, petunidin, pilloin, pratol, primetin, primuletin, pruentin, quercetagenin, quercetin,

ramnazin, rhoifolin, robinetin, rutin, scaposin, scutellarein, serpyllin, sinensetin, sorbifolin, sudachitin, tangeretin, tangeritin, taxifolin, tectochrysin, tithonine, tricetin, tricin, velutin, vitexin, wightin, wogonin, xanthomicrol, zapotin, zapotinin, 6,7,4'-trishydroxy isoflavone, or 7,3'4'-trihydroxy isoflavone; optionally, the flavonoid compound is flavone.

15. The method of any one of claims 1-14, wherein the first and second inducer are administered at about the same amount.
16. The method of claim 15, wherein the first inducer and the second inducer are administered at different amounts.
17. The method of claim 16, wherein the second inducer is administered at a lower amount relative to the amount of the first inducer.
18. The method of claim 17, wherein the second inducer is administered in amount about 1/2, 1/3, 1/4, 1/5, 1/6, 1/7, 1/8, 1/9, 1/10, 1/15, 1/20, 1/25, 1/30, 1/35, 1/40, 1/45, 1/50, 1/55, 1/60, 1/65, 1/70, 1/75, 1/80, 1/85, 1/90, 1/95, 1/100, 1/125, 1/150, 1/175, 1/200, 1/500, 1/1000 or even less of the amount of first the inducer.
19. The method of claim 18, wherein the second inducer is administered in amount about 1/2, 1/4, 1/5, 1/8, 1/10, 1/20, 1/40, 1/80 or less of the amount of the first inducer.
20. The method of claim 16, wherein the second inducer is administered at a higher amount relative to the amount of the first inducer.
21. The method of claim 20, wherein the amount of inducer administered at the second time point is at least about 25%, 50%, about 75%, about 1-fold, about 1.2-folds, about 1.25-folds, about 1.3-folds, about 1.5-folds, about 1.75-folds, about 2-folds, about 2.25-folds, about 2.5-folds, about 2.5-folds, about 2.75-folds, about 3-folds, 4-fold, 5-fold, 6-folds, 7-fold, 8-fold, 9-folds, 10-folds or higher than the amount of the first inducer.
22. The method of any one of claims 1-21, wherein the first and second inducer are administered independently at a dose from about 0.5 mg/kg to about 150mg/kg.
23. The method of any one of claims 1-22, wherein the first inducer is administered at a dose from about 1 mg/kg to about 100 mg/kg.
24. The method of any one of claims 1-23, wherein the first inducer is administered at a dose of about 80mg/kg, about 70mg/kg, about 60mg/kg, about 50mg/kg, about 40mg/kg, about 30mg/kg, about 20mg/kg, about 10mg/kg, about 6mg/kg, about 5mg/kg, or about 1mg/kg.
25. The method of any one of claims 1-24, wherein the second inducer is administered at a dose from about 1 mg/kg to about 100 mg/kg.
26. The method of any one of claims 1-25, wherein the second inducer is administered at a dose of 80mg/kg, about 70mg/kg, about 60mg/kg, about 50mg/kg, about 40mg/kg, about 30mg/kg, about 20mg/kg, about 10mg/kg, about 6mg/kg, about 5mg/kg, or about 1mg/kg.

27. The method of any one of claims 1-16, wherein the first inducer is administered at a dose of about 80 mg/kg or less and the second inducer is administered at a dose of about 20mg/kg or less at the second or subsequent time point.
28. The method of any one of claims 1-16, wherein the first inducer is administered at a dose of about 40 mg/kg and the second inducer is administered at a dose of about 80mg/kg or less at the second or subsequent time point.
29. The method of any one of claims 1-16, wherein the first inducer is administered at a dose of about 20mg/kg and the second inducer is administered at a dose of about 80 mg/kg, 40mg/kg, 20mg/kg, 10 mg/kg, 6 mg/kg or 1 mg/kg at the second or subsequent time point.
30. The method of any one of claims 1-16, wherein the first inducer is administered at a dose of about 10 mg/kg and the second inducer is administered at a dose of about 80mg/kg or less at the second or subsequent time point.
31. The method of any one of claims 1-16, wherein the first inducer is administered at a dose of about 6 mg/kg and the second inducer is administered at a dose of about 80mg/kg or less at the second or subsequent time point.
32. The method of any one of claims 1-16, wherein the first inducer is administered at a dose of about 1 mg/kg and the second inducer is administered at a dose of about 80mg/kg or less at the second or subsequent time point.
33. The method of any one of claims 1-16, wherein the second inducer is administered at a dose of about 80 mg/kg and the first inducer is administered at a dose of about 80 mg/kg or less.
34. The method of any one of claims 1-16, wherein the second inducer is administered at a dose of about 40 mg/kg and the first inducer is administered at a dose of about 80 mg/kg or less.
35. The method of any one of claims 1-16, wherein the second inducer is administered at a dose of about 20 mg/kg and the first inducer is administered at a dose of about 80 mg/kg or less.
36. The method of any one of claims 1-16, wherein the second inducer is administered at a dose of about 10 mg/kg and the first inducer is administered at a dose of about 80 mg/kg or less.
37. The method of any one of claims 1-16, wherein the second inducer is administered at a dose of about 6 mg/kg and the first inducer is administered at a dose of about 80 mg/kg or less.

38. The method of any one of claims 1-16, wherein the second inducer is administered at a dose of about 1 mg/kg and the first inducer is administered at a dose of about 80 mg/kg or less.
39. The method of any one of claims 1-38, wherein the expression of the transgene goes down to a background expression level within one week of administering the inducer.
40. The method of any one of claims 1-39, wherein the second inducer is administered when it is determined that the level of expression of the transgene is decreased to 75% or less of the level of expression of the transgene attained after induction with the first inducer.
41. The method of any one of any one of claims 1-40, wherein the second inducer is administered when it is determined that the level of expression of the transgene is decreased to 50% or less of the level of expression of the transgene after the first inducer.
42. The method of any one of claims 1-41, wherein the second inducer is administered when it is determined that the level of expression of the transgene is decreased to 25% or less of the level of expression of the transgene after the first inducer.
43. The method of any one of claims 1-42, wherein the second inducer is administered when it is determined that the level of expression of the transgene is decreased to a background level of expression of the transgene.
44. The method of any one of claims 1-43, further comprising: (c) administering to the subject at least at a second time point a second inducer to continue expression of the transgene at a predetermined level for a predetermined time or to modulate expression of the transgene to a predetermined level, optionally, a third inducer wherein the third inducer is same or different from the first and/or second inducer; and (d) optionally, repeating step (c) a one or more times, and where the inducer at each administration is same or different from the inducer at immediate prior administration.
45. The method of any one of claims 1-44, wherein the time between administrations is at least 12 hours, at least 24 hours, 48 hours, 72 hours, 1 week, 10 days, 2 weeks, 3 weeks, or 4 weeks.
46. The method of any one of claims 1-45, wherein the time between administrations is at least 24 hours, 48 hours, or 72 hours.
47. The method of any one of claims 1-46, wherein the time between administrations is no more than 2 months, 3 months, 4 months, 5 months, or 6 months.
48. The method of any one of claims 1-47, wherein the time between administrations is no more than 2 months.

49. The method of any one of claims 1-48, wherein the time between administrations is 1-2 days, 3-4 days, 5-7 days, 5-10 days, 7-10 days, 7-14 days, 10-14 days, 10-20 days, 14-21 days, 14-28 days, 21-28 days, or 21-34 days.
50. The method of any one of claims 1-159, wherein the time between administrations is about a day, e.g., about 24 hours.
51. The method of any one of claims 1-50, wherein the method further comprises a step of determining the expression level of the transgene after administering the inducer.
52. The method of any one of claim 1-51, wherein the method further comprises a step of determining the expression level of the transgene prior to administering the inducer.
53. The method of any one of claims 1-52, wherein the method further comprising administering the exogenous nucleotide sequence to the subject prior to administering the first or second inducer.
54. The method of any one of claims 1-53, wherein the method is a method of therapy, optionally, the method of therapy is a method of gene therapy.
55. The method of any one of claims 1-54, wherein the exogenous nucleotide sequence comprising the inducible promoter operably linked to a transgene is a viral vector.
56. The method of any one of claims 1-55, wherein the exogenous nucleotide sequence comprising the inducible promoter operably linked to a transgene is adeno-associated viral (AAV), lentiviral, adenoviral, herpes viral, or hepatitis viral vector.
57. The method of any one of claims 1-56, wherein the exogenous nucleotide sequence comprising the inducible promoter operably linked to a transgene is an AAV vector, e.g., an AAV gene therapy vector.
58. The method of any one of claims 1-57, wherein the inducers independently of other administrations are administered orally or intraperitoneally.
59. The method of any one of claims 1-58, wherein at least one of the inducers is administered orally.
60. The method of claim 59, wherein the second inducer is administered orally.
61. The method of claim 59, wherein the first inducer is administered orally.
62. The method of any one of claims 1-58, wherein at least one of the inducers is administered intraperitoneally.
63. The method of claim 62, wherein the second inducer is administered intraperitoneally.
64. The method of claim 62, wherein the first inducer is administered intraperitoneally.

65. The method of any one of claim 1-64, wherein the first induction increases the transgene expression level to a maximum at about 24 hrs and declines thereafter, and wherein the second induction and/or successive inductions has similar induction profile as seen with the first induction, for at least about 1 month, or at least about 2 months, or at least about 3 months, or at least about 5 months, or at least about six months, or longer.
66. The method of claim 65, wherein the transgene expression level at about 24 hrs after the first induction, or second induction, or successive inductions is at least about 200-fold higher than that of prior to administering the inducer for the first time, or, the second time, or, any number of successive administrations.
67. The method of claim 65, wherein the first induction, the second induction, and/or, the successive inductions are not complete until about 160 hours after administering the inducer.
68. The method of claim 65, wherein dose of the inducer in successive administrations is reduced from its prior administration.
69. The method of claim 65, wherein dose of the inducer in successive administrations remains same.
70. The method of claim 65, wherein the dose of the inducer ranges from about 1mg/kg to about 80mg/kg.
71. The method of claim 65, wherein the inducers are administered orally.
72. The method of claim 65, wherein the inducers are administered intraperitoneally.
73. A method of regulating expression of a transgene in a primate subject, the method comprising: administering an inducer to a primate subject, wherein the inducer is artemisinin, an artemisinin derivative or a flavonoid compound, wherein the primate subject comprises at least one cell comprising an exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene, wherein the inducible promoter comprises a PBREM enhancer, wherein the inducer is administered in an amount that is sufficient to express a measurable level of the transgene in the subject.
74. The method of claim 73, wherein the method comprises administering a plurality of doses of the inducer.
75. The method of claim 74, wherein the time between administrations is at least 12 hours, at least 24 hours, 48 hours, 72 hours, 1 week, 10 days, 2 weeks, 3 weeks, or 4 weeks.

76. The method of any one of claims 74-75, wherein the time between administrations is at least 24 hours, 48 hours, or 72 hours.
77. The method of any one of claims 74-76, wherein the time between administrations is no more than 2 months, 3 months, 4 months, 5 months, or 6 months.
78. The method of any one of claims 74-77, wherein the time between administrations is no more than 2 months.
79. The method of any one of claims 74-78, wherein the time between administrations is 1-3 days, 5-7 days, 5-10 days, 7-10 days, 7-14 days, 10-14 days, 10-20 days, 14-21 days, 14-28 days, 21-28 days, or 21-34 days.
80. The method of any one of claims 74-79, wherein the time between administrations is about 1 day.
81. The method of any one of claims 74-80, wherein the transgene expression level at about 24 hrs after an induction is at least about 200-fold higher than that of prior to administering the inducer for the first time or any number of prior administrations.
82. The method of any one of claims 74-81, wherein an induction is not complete until about 160 hours after administering the inducer.
83. The method of any one of claims 74-82, wherein the transgene expression level increases to a maximum at about 24 hrs after the first administration and declines thereafter and a second induction, and/or successive inductions, has similar induction profile as seen with the first induction, for at least about 1 month, or at least about 2 months, or at least about 3 months, or at least about 5 months, or at least about six months, or longer,
84. The method of any one of claims 74-83, wherein dose in successive administrations is reduced from a prior administration.
85. The method of any one of claims 74-84, wherein dose in successive administrations remains same.
86. The method of any one of claims 73-85, wherein the inducer is artemisinin or an artemisinin derivative.
87. The method of claim 86, wherein the artemisinin derivative is a compound of Formula (I), (I-A), (I-B), (I-C), (I-D), (I-E), (I-F), (I-G), (I-H), (I-I), (I-J), (I-K), (I-L), (I-M), (I-M'), (I-N), (I-N'), (I-O) or (I-O').
88. The method of claim 86, wherein the derivative of artemisinin is artesunate, dihydroartemisinin, artesunate, or artemether.
89. The method of claim 88, wherein the derivative of artemisinin is artesunate, or dihydroartemisinin.
90. The method of claim 73-85, wherein the inducer is a flavonoid compound.

91. The method of claim 90, wherein the flavonoid compound is flavone, baicalein, chrysin, galangin, (OH)₄ flavone, 6-hydroxyluteolin, acacetin, acerosin, alnetin, apigenin, apiin, artocarpetin, balcalein, beta-sitosterol, biochamin A, cannaflavin A, cannflavin A, cannflavin B, cannflavin C, catechin, catechin-3-gallate, cerrosillin, chrysoeriol, chrysoeriol, cirsilin, cirsiol, cirsimaritin, corymbosin, cupressuflavone, cyanidine, daidzein, datiscetin, delphinidine, digidrokempferol, dihydroquercetin, diosmetin, diosmin, echiodinin, epicatechin, epicatechin-3-gallate, epigallocatechin, epigallocatechin-3-gallate, eriodictiol, eupatilin, eupatorin, fisetin, fizetin, flavonol, formononetin, fortunelin, galocatechin, galocatechin-3-gallate, gardenin D, genistein, genistin, genkwanin, geraldol, geraldone, glycitein, glycitin, gomoeriodictiol, gossypetin, hesperetin, hinokiflavone, hispidulin, hymenoxin, hypolaetin, isoquercetin, isorhamnetin, isorhamnetin, isoscutellarein, isovitexin, jaceosidin, kaempferol, kaempferol, kampferide, largonidin, luteolin, luteolin, malvidine, mikanin, morin, myricetin, myricetin, naringenin, negletein, neodiosmin, nepetin, nevadensin, nobiletin, nodifloretin, norartocarpetin, norwogonin, onopordin, orientin, oroxylin a, pachipodol, pectolinarigenin, pedalitin, peonidin, petunidin, pilloin, pratol, primetin, primuletin, pruentin, quercetagenin, quercetin, ramnazin, rhoifolin, robinetin, rutin, scaposin, scutellarein, serpyllin, sinensetin, sorbifolin, sudachitin, tangeretin, tangeritin, taxifolin, tectochrysin, tithonine, tricetin, tricin, velutin, vitexin, wightin, wogonin, xanthomicrol, zapotin, zapotinin, 6,7,4'-trihydroxy isoflavone, or 7,3,4'-trihydroxy isoflavone; optionally, the flavonoid compound is flavone.
92. The method of any one of claims 73-91, wherein each dose is independently from about 0.5 mg/kg to about 150mg/kg.
93. The method of any one of claims 73-92, wherein each dose is independently from about 1 mg/kg to about 100 mg/kg.
94. The method of any one of claims 73-93, wherein each dose is independently from about 1 mg/kg to about 80 mg/kg.
95. The method of any one of claims 73-94, wherein each dose is independently from about 1 mg/kg to about 40 mg/kg.
96. The method of any one of claims 73-95, wherein each dose is independently from about 1 mg/kg to about 20 mg/kg.
97. The method of any one of claims 73-96, wherein each dose is independently from about 1 mg/kg to about 10 mg/kg.
98. The method of any one of claims 73-94, wherein each dose is independently about 80mg/kg, about 70mg/kg, about 60mg/kg, about 50mg/kg, about 40mg/kg, about 30mg/kg, about 20mg/kg, about 10mg/kg, about 6mg/kg, about 5mg/kg, or about 1mg/kg.

99. The method of any one of claims 73-98, wherein the method further comprises administering the exogenous nucleotide sequence to the subject prior to administering the inducer for the first time.
100. The method of any one of claims 73-99, wherein a second inducer or, subsequent inducer is administered to the primate subject following the prior administration of an inducer to the primate subject, and optionally, the inducer of the prior administration is administered in an amount that is sufficient to express a measurable level of the transgene in the subject.
101. The method of claim 100, wherein the inducer of the prior administration is different from the second or subsequent inducer administered to the subject.
102. The method of claim 101, wherein the inducer of the prior administration is same as the second or, subsequent inducer administered to the subject but at a different dose.
103. The method of any one of claims 73-102, wherein the method is a method of therapy, optionally, the method of therapy is a method of gene therapy.
104. The method of any one of claims 73-103, wherein the exogenous nucleotide sequence comprising the inducible promoter operably linked to a transgene is a viral vector.
105. The method of any one of claims 73-104, wherein the exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene is adeno-associated viral (AAV), lentiviral, adenoviral, herpes viral, or hepatitis viral vector.
106. The method of any one of claims 73-105, wherein the exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene is an AAV vector, e.g., an AAV gene therapy vector.
107. The method of any one of claims 73-106, wherein each administration independently of other administrations is oral administration or intraperitoneal administration.
108. The method of any one of claims 73-107, wherein each administration is oral administration.
109. The method of any one of claims 73-107, wherein each administration is intraperitoneal administration.
110. The method of any one of claims 1-109, wherein the transgene encodes an antibody or antigen binding portion an antibody, a coagulation factor, a cytokine, a hormone, a growth factor, a virus protein, a cell receptor ligand, a chimeric antigen receptor (CAR) or a CRISPR-Cas nuclease.
111. The method of claim 110, wherein the transgene encodes an antibody or an antigen binding portion thereof, optionally the antibody is an anti-PD1 antibody, anti-HER2 antibody, anti-PCSK9 antibody, anti-VEGFR2 antibody, anti-IL17 antibody, anti-A β 3-42 antibody, anti-Sema3A antibody, anti-4-1BB (CD137) antibody, anti-5'-nucleotidase antibody, anti-5T4

antibody, anti-activin receptor-like kinase 1 antibody, anti-ACVR2B antibody, anti-adenocarcinoma antigen antibody, anti-amyloid antibody, anti-Ang-2 antibody, anti-angiopoietin 2 antibody, anti-angiopoietin 2 antibody, anti-angiopoietin 3 antibody, anti-anthrax toxin antibody, anti-AOC3 (VAP-1) antibody, anti-AXL antibody, anti-BAFF-R antibody, anti-B-cell activating factor (BAFF) antibody, anti-B-cell maturation antigen (BCMA) antibody, anti-B-lymphoma cell antibody, anti-C242 antigen antibody, anti-C5 antibody, anti-C5a receptor (C5a) antibody, anti-CA-125 antibody, anti-calcitonin antibody, anti-calcitonin gene-related peptide alpha antibody, anti-calcitonin gene-related peptide beta antibody, anti-calcitonin gene-related peptide receptor (CGRP) antibody, anti-CanAg (a glycoform of MUC1) antibody, anti-carbonic anhydrase 9 (CA-IX) antibody, anti-Carcinoembryonic antigen (CEA) antibody, anti-Carcinoembryonic antigen (CEA)-related antigen antibody, anti-cardiac myosin antibody, anti-CCL11 antibody, anti-CCR2 antibody, anti-CCR4 antibody, anti-CCR5 antibody, anti-CCR5 antibody, anti-CD11 antibody, antibody, anti-CD123 antibody, anti-CD125 antibody, anti-CD134 antibody, anti-CD134 antibody, anti-CD147 antibody, anti-CD15 antibody, anti-CD152 antibody, anti-CD154 antibody, anti-CD18 antibody, anti-CD19 antibody, anti-CD2 antibody, anti-CD20 antibody, anti-CD20 antibody, anti-CD20 antibody, anti-CD200 antibody, anti-CD22 antibody, anti-CD23 antibody, anti-CD25 antibody, anti-CD27 antibody, anti-CD276 antibody, anti-CD278 antibody, antibody, anti-CD279 antibody, anti-CD28 antibody, anti-CD3 antibody, anti-CD3 antibody, anti-CD3 antibody, anti-CD3 antibody, anti-CD3 antibody, anti-CD30 antibody, anti-CD319 antibody, anti-CD33 antibody, anti-CD37 antibody, anti-CD38 antibody, anti-CD3E antibody, anti-CD3E antibody, anti-CD4 antibody, anti-CD40 antibody, anti-CD41 antibody, anti-CD44 antibody, anti-CD45 antibody, anti-CD5 antibody, anti-CD51 antibody, anti-CD52 antibody, anti-CD56 antibody, anti-CD6 antibody, anti-CD70 antibody, anti-CD74 antibody, anti-CD79B antibody, anti-CD80 antibody, anti-CEACAM5 antibody, anti-Claudin 18 Isoform 2 antibody, anti-clumping factor A antibody, anti-cMet antibody, anti-c-Met antibody, anti-coagulation factor III antibody, anti-complement C5a antibody, anti-complement component 1s (C1s) antibody, anti-Complement factor D (CFD) antibody, anti-connective tissue growth factor (CTGF) antibody, anti-CSF1 antibody, antibody, anti-CSF1R antibody, anti-CSF2 antibody, anti-CTLA-4 antibody, anti-CXCL10 (IP-10) antibody, anti-CXCR4 (CD184) antibody, anti-dabigatran antibody, anti-dendritic cell-associated lectin 2 antibody, anti-DLL3 antibody, anti-DLL4 antibody, anti-DPP4 antibody, anti-DR5 antibody, anti-EGFL7 antibody, anti-EGFR antibody, anti-EGFR antibody, anti-EGFR antibody, anti-EGFR extracellular domain III antibody, anti-endoglin antibody, anti-

endotoxin antibody, anti-EpCAM antibody, anti-EPHA3 antibody, anti-ephrin receptor A3 antibody, anti-episialin antibody, anti-ERBB1 antibody, anti-ERBB3 (HER3) antibody, anti-FAP (gene) (FAP) antibody, anti-FCGRT antibody, anti-FGF 23 antibody, anti-FGFR2 antibody, anti-FGFR2 antibody, anti-fibrin II antibody, anti-fibronectin extra domain-B antibody, anti-folate hydrolase antibody, anti-folate receptor 1 antibody, anti-folate receptor alpha antibody, anti-Frizzled receptor antibody, anti-FVIX antibody, anti-FVX antibody, anti-GD2 ganglioside antibody, anti-GD3 ganglioside antibody, anti-GDF-8 antibody, anti-gelatinase B antibody, anti-glucagon receptor (GCGR) antibody, anti-Glutamate carboxypeptidase II antibody, anti-glypican 3 antibody, anti-GMCSF antibody, anti-GPNMB antibody, anti-GPRC5D antibody, antibody, anti-growth differentiation factor 8 antibody, anti-GUCY2C antibody, anti-Hepatocyte growth factor (HGF) antibody, anti-HER1 antibody, anti-HER1 antibody, anti-HER1 antibody, anti-HER2/neu antibody, anti-HGFR antibody, anti-histone complex antibody, anti-HLA-DR antibody, anti-Hsp90 antibody, anti-human scatter factor receptor kinase antibody, anti-ICAM-1 (CD54) antibody, anti-IFN receptor antibody, anti-IFN- α antibody, anti-IFN- α/β receptor antibody, anti-IFN- γ antibody, anti-IgE antibody, anti-IgE Fc region antibody, anti-IGF-1 receptor (CD221) antibody, anti-IGF-2 antibody, anti-IGHE antibody, anti-IL-1 antibody, anti-IL-12 antibody, anti-IL-13 antibody, anti-IL-17A antibody, anti-IL-17AF antibody, anti-IL-17F antibody, anti-IL-1 α antibody, anti-IL-1 β antibody, anti-IL-2 antibody, anti-IL-20 antibody, anti-IL-22 antibody, anti-IL-23 antibody, anti-IL-23 antibody, anti-IL-23 antibody, anti-IL-23 antibody, anti-IL-23 antibody, anti-IL-23A antibody, anti-IL-3 receptor antibody, anti-IL-31 receptor A antibody, anti-IL-4 antibody, anti-IL-4R α antibody, anti-IL-5 antibody, anti-IL-6 antibody, anti-IL-6 receptor antibody, anti-IL-9 antibody, anti-inducible T-cell co-stimulatory ligand (ICOSL) antibody, anti-integrin $\alpha 4$ antibody, anti-integrin $\beta 7$ antibody, anti-integrin $\alpha 4 \beta 7$ antibody, anti-integrin $\alpha 4 \beta 7$ antibody, anti-integrin $\alpha 5 \beta 1$ antibody, anti-integrin $\alpha \text{IIb} \beta 3$ antibody, anti-integrin $\alpha \text{v} \beta 3$ antibody, anti-Interleukin 36 receptor (IL1RL2/IL1RAP) antibody, anti-ITGA2 (CD49b) antibody, anti-ITGB2 (CD18) antibody, anti-kallikrein antibody, anti-KIR2D antibody, anti-LAG3 antibody, anti-Lewis-Y antigen antibody, anti-LFA-1 (CD11a) antibody, anti-LINGO-1 antibody, anti-lipoteichoic acid antibody, anti-LIV-1 antibody, anti-LOXL2 antibody, anti-LRRC15 antibody, anti-L-selectin (CD62L) antibody, anti-lymphotoxin alpha (LTA) antibody, anti-LYPD3 antibody, anti-macrophage colony stimulating factor (MCSF) antibody, anti-macrophage migration inhibitory factor (MIF) antibody, anti-MASP-2 antibody, anti-MCP-1 antibody, anti-melanoma cell adhesion molecule (MCAM) antibody, anti-mesothelin antibody, anti-MS4A1 antibody, antibody, anti-MST1R

antibody, anti-MUC1 antibody, anti-MUC5AC antibody, anti-mucosal addressin cell adhesion molecule antibody, anti-myelin-associated glycoprotein antibody, anti-myostatin antibody, anti-NCA-90 (granulocyte antigen) antibody, anti-nectin-4 antibody, anti-nerve growth factor (NGF) antibody, anti-NGNA ganglioside antibody, anti-NKG2A antibody, anti-NOGO-A antibody, anti-Notch 1 antibody, anti-Notch receptor antibody, anti-NRP1 antibody, anti-OX-40 antibody, anti-oxLDL antibody, anti-PCDC1 antibody, anti-PCDP1 antibody, anti-PDGFA antibody, anti-PDGFRB antibody, anti-PD-L1 antibody, anti-phosphate-sodium co-transporter antibody, anti-phosphatidylserine antibody, anti-PTK7 antibody, anti-RANKL antibody, anti-repulsive guidance molecule A (RGMA) antibody, anti-RHD (gene) (RHD) antibody, anti-Rhesus factor antibody, anti-Rhesus factor antibody, anti-root plate-specific spondin 3 antibody, anti-ROR1 antibody, anti-RTN4 antibody, anti-sclerostin antibody, anti-sclerostin (SOST) antibody, anti-SDC1 antibody, anti-selectin P antibody, anti-serum amyloid A protein antibody, anti-serum amyloid P component antibody, anti-SLAMF7 antibody, anti-SLITRK6 antibody, anti-SOST antibody, anti-sphingosine-1-phosphate antibody, anti-STEAP1 antibody, anti-TAG-72 antibody, anti-TAG-72 antibody, anti-tau protein antibody, anti-T-cell receptor antibody, anti-TEM1 antibody, anti-tenascin C antibody, anti-TGF- β antibody, anti-TGF- β 1 antibody, anti-TGF- β 2 antibody, anti-thymic stromal lymphopoietin (TSLP) antibody, anti-TIGIT antibody, anti-tissue factor pathway inhibitor (TFPI) antibody, anti-TNF antibody, anti-TNF antibody, anti-TNF- α antibody, anti-TNF- α antibody, anti-TRAIL-R1 antibody, anti-TRAIL-R2 antibody, anti-TROP-2 antibody, anti-tumor antigen CTAA16.88 antibody, anti-tumor necrosis factor receptor (TNFR) antibody, anti-tumor necrosis factor related activation protein (TRAP) antibody, anti-Tumor-associated glycoprotein 72 (TAG-72) antibody, anti-TWEAK receptor antibody, anti-TYRP1 (glycoprotein 75) antibody, anti-vascular endothelial growth factor A (VEGFA) antibody, anti-VEGFA antibody, anti-VEGFR-1 antibody, anti-vimentin antibody, anti-VISTA (protein) (VSIR) antibody, anti-VWF antibody, anti- α -fetoprotein antibody, anti-A-synuclein antibody or anti- β -amyloid antibody. optionally the antibody is selected from the group consisting of an anti-TNF α , anti-PD1 antibody, anti-HER2 antibody, anti-PCSK9 antibody, anti-VEGFR2 antibody, anti-IL17 antibody, anti-A β 3-42 antibody, and anti-Sema3A antibody.

112. The method of claim 110, wherein the transgene encodes an antibody or an antigen binding portion thereof, and wherein the antibody is selected from the group consisting 3F8, Abagovomab, Abciximab, Abituzumab, Abrezekimab, Abrilumab, Actoxumab, Adalimumab, Adecatumumab, Aducanumab, Afasevikumab, Afelimomab, Alacizumab pegol, Alemtuzumab, Alirocumab, Altumomab pentetate, Amatuximab, Amivantamab,

Anatumomab mafenatox, Andecaliximab, Anetumab ravtansine, Anifrolumab, Ansuvimab, Anrukinzumab, (IMA-638), Apolizumab, Aprutumab ixadotin, Arcitumomab, Ascrinvacumab, Aselizumab, Atezolizumab, Atidortoxumab, Atinumab, Atoltivimab, Atoltivimab/maftivimab/odesivimab, Atorolimomab, Avelumab, Azintuxizumab vedotin, Bamlanivimab, Bapineuzumab, Basiliximab, Bavituximab, BCD-100, Bebtelovimab, Bectumomab, Bedinvetmab, Begelomab, Belantamab mafodotin, Belimumab, Bemarituzumab, Benralizumab, Berlimatoxumab, Bermekimab, Bersanlimab, Bertilimumab, Besilesomab, Bevacizumab, Bezlotoxumab, Biciromab, Bimagrumab, Bimekizumab, Birtamimab, Bivatuzumab, Bleselumab, Blinatumomab, Blontuvetmab, Blosozumab, Bococizumab, Brazikumab, Brentuximab vedotin, Briakinumab, Brodalumab, Brolucizumab, Brontictuzumab, Burosumab, Cabiralizumab, Camidanlumab tesirine, Camrelizumab, Canakinumab, Cantuzumab mertansine, Cantuzumab ravtansine, Caplacizumab, Casirivimab, Capromab, Carlumab, , Carotuximab, Catumaxomab, cBR96-doxorubicin immunoconjugate, Cedelizumab, Cempiplimab, Cergutuzumab amunaleukin, Certolizumab pegol, Cetrelimab, Cetuximab, Cibisatamab, Cilgavimab, Cirmtuzumab, Citatuzumab bogatox, Cixutumumab, Clazakizumab, Clenoliximab, Clivatuzumab tetraxetan, Codrituzumab, Cofetuzumab pelidotin, Coltuximab ravtansine, Conatumumab, Concizumab, Cosfroviximab, Crenezumab, Crizanlizumab, Crotedumab, CR6261, Cusatuzumab, Dacetuzumab, Daclizumab, Dalotuzumab, Dapirolizumab pegol, Daratumumab, Dectrekumab, Demcizumab, Denintuzumab mafodotin, Denosumab, Depatuxizumab mafodotin, Derlotuximab biotin, Detumomab, Dezamizumab, Dinutuximab, Dinutuximab beta, Diridavumab, Domagrozumab, Donanemab, Dorlimomab aritox, Dostarlimab, Drozitumab, DS-8201, Duligotuzumab, Dupilumab, Durvalumab, Dusigitumab, Duvortuxizumab, Echromeximab, Eculizumab, Edobacomab, Edrecolomab, Efalizumab, Efungumab, Eldelumab, Elezanumab, Elgentumab, Elotuzumab, Elsilimomab, Emactuzumab, Emapalumab, Emibetuzumab, Emicizumab, Enapotamab vedotin, Enavatuzumab, Enfortumab vedotin, Enlimomab pegol, Enoblituzumab, Enokizumab, Enoticumab, Ensituximab, Epcoritamab, Eptumomab cituxetan, Epratuzumab, Eptinezumab, Erenumab, Erlizumab, Ertumaxomab, Etaracizumab, Etesevimab, Etigilimab, Etrolizumab, Evinacumab, Evolocumab, Exbivirumab, Fanolesomab, Faralimomab, Faricimab, Farletuzumab, Fasinumab, FBTA05, Felvizumab, Fezakinumab, Fibatuzumab, Ficlatuzumab, Figitumumab, Firivumab, Flanvotumab, Fletikumab, Flotetuzumab, Fontolizumab, Foralumab, Foravirumab, Fremanezumab, Fresolimumab, Frovocimab, Frunevetmab, Fulranumab, Futuximab, Galcanezumab, Galiximab, Gancotamab, Ganitumab, Gantenerumab,

Gatipotuzumab, Gavilimomab, Gedivumab, Gemtuzumab ozogamicin, Gevokizumab, Gilvetmab, Gimsilumab, Girentuximab, Glembatumumab vedotin, Glofitamab, Golimumab, Gomiliximab, Gosuranemab, Guselkumab, Ianalumab, Ibalizumab, Sintilimab, Ibritumomab tiuxetan, Icrucumab, Idarucizumab, Ifabotuzumab, Igovomab, Iladatuzumab vedotin, Imalumab, Imaprelimab, Imciromab, Imdevimab, Imgatuzumab, Inclacumab, Indatuximab ravtansine, Indusatumab vedotin, Inebilizumab, Infiximab, Intetumumab, Inolimomab, Inotuzumab ozogamicin, Ipilimumab, Iomab-B, Iratumumab, Isatuximab, Iscalimab, Istiratumab, Itolizumab, Ixekizumab, Keliximab, Labetuzumab, Lacnotuzumab, Ladiratuzumab vedotin, Lampalizumab, Lanadelumab, Landogrozumab, Laprituximab emtansine, Larcaviximab, Lebrikizumab, Lecanemab, Lemalesomab, Lendalizumab, Lenervimab, Lenzilumab, Lerdelimumab, Leronlimab, Lesofavumab, Letolizumab, Lexatumumab, Libivirumab, Lifestuzumab vedotin, Ligelizumab, Loncastuximab tesirine, Losatuxizumab vedotin, Lilotomab satetraxetan, Lintuzumab, Lirilumab, Lodelcizumab, Lokivetmab, Lorvotuzumab mertansine, Lucatumumab, Lulizumab pegol, Lumiliximab, Lumretuzumab, Lupartumab, Lupartumab amadotin, Lutikizumab, Maftivimab, Mapatumumab, Margetuximab, Marstacimab, Maslimomab, Mavrilimumab, Matuzumab, Mepolizumab, Metelimumab, Milatuzumab, Minretumomab, Mirikizumab, Mirvetuximab soravtansine, Mitumomab, Modotuximab, Mogamulizumab, Monalizumab, Morolimumab, Mosunetuzumab, Motavizumab, Moxetumomab pasudotox, Muromonab-CD3, Nacolomab tafenatox, Namilumab, Naptumomab estafenatox, Naratuximab emtansine, Narnatumab, Natalizumab, Navicixizumab, Navivumab, Naxitamab, Nebacumab, Necitumumab, Nemolizumab, NEOD001, Nerelimumab, Nesvacumab, Netakimab, Nimotuzumab, Nirsevimab, Nivolumab, Nofetumomab merpentan, Obiltoxaximab, Obinutuzumab, Ocaratuzumab, Ocrelizumab, Odesivimab, Odulimumab, Ofatumumab, Olaratumab, Oleclumab, Olendalizumab, Olokizumab, Omalizumab, Omburtamab, OMS721, Onartuzumab, Ontuxizumab, Onvatilimab, Opicinumab, Oportuzumab monatox, Oregovomab, Orticumab, Otelixizumab, Otilimab, Otlertuzumab, Oxelumab, Ozanezumab, Ozoralizumab, Pagibaximab, Palivizumab, Pamrevlumab, Panitumumab, Pankomab, Panobacumab, Parsatuzumab, Pascolizumab, Pasotuxizumab, Pateclizumab, Patritumab, PDR001, Pembrolizumab, Pentumomab, Perakizumab, Pertuzumab, Pexelizumab, Pidilizumab, Pinatuzumab vedotin, Pintumomab, Placulumab, Pozelimab, Prezalumab, Plozalizumab, Pogalizumab, Polatuzumab vedotin, Ponezumab, Porgaviximab, Prasinezumab, Prezalizumab, Priliximab, Pritoxaximab, Pritumumab, PRO 140, Quilizumab, Racotumomab, Radretumab, Raffivirumab, Ralpancizumab, Ramucirumab, Ranevetmab, Ranibizumab, Raxibacumab, Ravagalimab,

Ravulizumab, Refanezumab, Regavirumab, Regdanvimab, Relatlimab, Remtolumab, Reslizumab, Retifanlimab, Rilotumumab, Rinucumab, Risankizumab, Rituximab, Rivabazumab pegol, Robatumumab, Rmab, Roledumab, Romilkimab, Romosozumab, Rontalizumab, Rosmantuzumab, Rovalpituzumab tesirine, Rovelizumab, Rozanolixizumab, Ruplizumab, SA237, Sacituzumab govitecan, Samalizumab, Samrotamab vedotin, Sarilumab, Satralizumab, Satumomab pendetide, Secukinumab, Selicrelumab, Seribantumab, Setoxaximab, Setrusumab, Sevirumab, Sibrotuzumab, SGN-CD19A, SHP647, Sifalimumab, Siltuximab, Simtuzumab, Siplizumab, Sirtratumab vedotin, Sirukumab, Sofituzumab vedotin, Solanezumab, Solitomab, Sonepcizumab[104], Sontuzumab, Sotrovimab, Spartalizumab, Spesolimab[107], Stamulumab, Sulesomab, Suptavumab, Sutimlimab, Suvizumab, Suvratouxumab, Tabalumab, Tacatuzumab tetraxetan, Tadocizumab, Tafasitamab, Talacotuzumab, Talizumab, Talquetamab, Tamtvetmab, Tanezumab, Taplitumomab paptox, Tarextumab, Tavolimab, Teclistamab, Tefibazumab, Telimomab aritox, Telisotuzumab, Telisotuzumab vedotin, Tenatumomab, Teneliximab, Teplizumab, Tepoditamab, Teprotumumab, Tesidolumab, Tetulomab, Tezepelumab, TGN1412, Tibulizumab, Tildrakizumab, Tigatuzumab, Timigutuzumab, Timolumab, tiragolumab, Tiragotumab, Tislelizumab, Tisotumab vedotin, Tixagevimab, TNX-650, Tocilizumab, Tomuzotuximab, Toralizumab, Tosatouxumab, Tositumomab, Tovetumab, Tralokinumab[115], Trastuzumab, Trastuzumab duocarmazine, Trastuzumab emtansine, TRBS07[117], Tregalizumab, Tremelimumab[118], Trevogrumab, Tucotuzumab celmoleukin, Tuvirumab, Ublituximab[120], Ulocuplumab, Urelumab, Urtoxazumab, Ustekinumab, Utomilumab, Vadastuximab talirine, Vanalizumab, Vandortuzumab vedotin, Vantictumab, Vanucizumab, Vapaliximab, Varisacumab, Varlilumab, Vatelizumab, Vedolizumab, Veltuzumab, Vepalimomab, Vesencumab, Vilobelimumab, Visilizumab, Vobarilizumab, Volociximab, Vonlerolizumab, Vopratelimumab, Vorsetuzumab mafodotin, Votumumab, Vunakizumab, Xentuzumab, XMAB-5574, Zalutumumab, Zanolimumab, Zatuximab, Zenocutuzumab, Ziralimumab, Zolbetuximab, and Zolimomab aritox.

113. The method of claim 110, wherein the transgene encodes a coagulation factor, optionally the coagulation factor is Factor VIII, Factor IX, Factor VII, Factor X, or Factor XI, preferably the coagulation factor is Factor VIII or Factor IX.
114. The method of claim 110, wherein the transgene encodes a hormone, optionally the hormone is selected from the group consisting of selected from erythropoietin (EPO), parathyroid hormone (PTH), adiponectin, adrenaline (or epinephrine), adrenocorticotrophic hormone (ACTH or corticotropin), adropin, aldosterone, amylin (or islet amyloid

polypeptide), androstenedione, angiotensin, angiotensinogen, antidiuretic hormone (or vasopressin), anti-müllerian hormone (or müllerian-inhibiting factor/hormone), arginine vasopressin), atrial natriuretic peptide (ANP or atriopeptin), brain natriuretic peptide, calcitonin, calcitriol, cholecystokinin (CCK), corticotropin-releasing hormone, cortisol, cortistatin, dehydroepiandrosterone, dihydrotestosterone, dopamine, endothelin, enkephalin, estradiol, estriol, estrone, follicle-stimulating hormone (FSH), galanin, gastric inhibitory polypeptide, gastrin, ghrelin, glucagon, glucagon-like peptide-1, gonadotropin-releasing hormone, growth hormone, growth hormone-releasing hormone, guanylin, hepcidin, human chorionic gonadotropin, human placental lactogen, inhibin, insulin, insulin-like growth factor (or somatomedin), leptin, leukotrienes, lipotropin, luteinizing hormone (LH), melanocyte stimulating hormone (MSH), melatonin, motilin, noradrenaline (or norepinephrine), orexin, osteocalcin, oxytocin (or pitocin), pancreatic polypeptide, pituitary adenylate cyclase-activating peptide, progesterone, prolactin (or leuteotropic hormone), prolactin-releasing hormone, prostacyclin, prostaglandins, relaxin, renin, secretin, somatostatin (or growth hormone-inhibiting hormone or growth hormone release-inhibiting hormone or somatotropin release-inhibiting factor or somatotropin release-inhibiting hormone), testosterone, thrombopoietin, thromboxane, thyroid-stimulating hormone (TSH or thyrotropin), thyrotropin-releasing hormone (TRH), thyroxine, triiodothyronine, uroguanylin, vasoactive intestinal peptide (VIP), or vasopressin, more optionally, the hormone is erythropoietin (EPO) and parathyroid hormone (PTH).

115. The method of claim 110, wherein the transgene encodes a growth factor, optionally, the growth factor is tumor necrosis factor- α (TNF- α), adrenomedullin (AM), angiotensin (Ang), autocrine motility factor, bone morphogenetic protein (BMP), ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), colony-stimulating factor (e.g., macrophage colony-stimulating factor (M-CSF), granulocyte colony-stimulating factor (G-CSF), or granulocyte macrophage colony-stimulating factor (GM-CSF)), epidermal growth factor (EGF), ephrin (e.g., ephrin A1, ephrin A2, ephrin A3, ephrin A4, ephrin A5, ephrin B1, ephrin B2, or ephrin B3), erythropoietin (EPO), fibroblast growth factor (e.g., FGF1, FGF2, FGF3, FGF4, FGF5, FGF6, FGF7, FGF8, FGF9, FGF10, FGF11, FGF12, FGF13, FGF14, FGF15, FGF16, FGF17, FGF18, FGF19, FGF20, FGF21, FGF22 or FGF23), glial cell line-derived neurotrophic factor (GDNF), neurturin, persephin, artemin, growth differentiation factor-9 (GDF9), hepatocyte growth factor (HGF), hepatoma-derived growth factor (HDGF), insulin-like growth factor (e.g., IGF-1 or IGF-2), interleukin (e.g., IL-1, IL-2, IL-3, IL-4, IL-5, IL-6 or IL-7), keratinocyte growth factor (KGF), migration-stimulating factor (MSF), macrophage-stimulating protein (MSP),

myostatin (GDF-8), neuregulin (e.g., NRG1, NRG3, NRG3 or NRG4), neurotrophin (e.g., brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-3 (NT-3) or neurotrophin-4 (NT-4)), placental growth factor (PGF), platelet-derived growth factor (PDGF), renalase (RNLS), T-cell growth factor (TCGF), thrombopoietin (TPO), transforming growth factor (e.g., TGF- α or TGF- β), or vascular endothelial growth factor (VEGF).

116. The method of claim 110, the transgene encodes a CRISPR-Cas nuclease, optionally selected from the group consisting of Cas9, Cas12, Cas Phi, CasX, and CasRx.
117. The method of claim 110, wherein the transgene encodes a cytokine, optionally, the cytokine is interleukin-1 α (IL-1 α), interleukin-1/3 (IL-1/3), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-8 (IL-8/CXCL8), interleukin-10 (IL-10), interleukin-12 (IL-12), interleukin-13 (IL-13), interleukin-15 (IL-15), interleukin-17 (IL-17), interleukin-18 (IL-18), interleukin-37 (IL-37), interleukin-38 (IL-38), tumor necrosis factor- α (TNF- α), interferon- α (INF- α), interferon-b (INF-b), interferon- γ (INF- γ), granulocyte-monocyte colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), monocyte chemoattractant protein-1 (MCP-1/CCL2), macrophage inflammatory protein 1-ct (MCP-1/CCL3), macrophage inflammatory protein-1/3, RANTES (CCL5), Eotaxin (CCL11), variable endothelial growth factor (VEGF), endothelial growth factor (EGF), or fibroblast growth factor (FGF). In some embodiments, the transgene encodes IL-2. In some embodiments, the transgene encodes IL-37. In some embodiments, the transgene encodes IL-38.
118. The method of claim 110, wherein the transgene encodes a virus protein, optionally selected from the group consisting of viral envelop protein, viral nucleocapsid protein, viral membrane protein, REP, CAP, REV, VSV-G and RD114
119. The method of any one of claims 1-110, wherein the transgene is detrimental to the cell or to the subject when constitutively expressed.
120. The method of any one of claims 1-119, wherein the subject is a non-human primate.
121. The method of any one of claims 1-120, wherein the subject is a human.
122. The method of any one of claims 1-121, wherein the cell comprising the cell comprising the exogenous nucleotide sequence is a liver cell.
123. A first and at least a second inducer for use in a method of regulating expression of a transgene in a primate subject, the method comprising:

- (a) administering to the subject at a first time point a first inducer, wherein the primate subject comprises at least one cell comprising an exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene, wherein the inducible promoter comprises a PBREM enhancer, wherein the first inducer is artemisinin, a derivative of artemisinin or a flavonoid compound, and wherein the first dose comprises an amount of the inducer that is sufficient to express a measurable level of the transgene in the subject; and
- (b) administering to the subject at least at a second time point a second inducer to continue expression of the transgene at a predetermined level for a predetermined time or to modulate expression of the transgene to a predetermined level, and wherein the second inducer is artemisinin; derivative of artemisinin; a flavonoid compound, e.g. flavone, chrysin, baicalein, or galangin; 1,4-Bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP); phenobarbital (PB); 6-(4Chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde-O-(3,4-dichlorobenzyl)oxime (CITCO); Acetaminophen; Buprenorphine; Phenytoin; Carbamazepine; Valproic Acid; Chlorpromazine; Efavirenz; Nevirapine; Rilpivirine; Etravirine; Diazepam; Cyclophosphamid; Ifosfamide; Cerivastatin; Simvastatin; lovastatin; substituted sulphonamides; Thiazolidin-4-one; Estradiol; Estrone and analogues; 17 α -ethinyl-3;17 β -estradiol (EE2); Dehydroepiandrosterone (DHEA); 5 β -pregnane-3,20-dione; Diethylstilbestrol; *Ginkgo biloba* extract; Galangin; chrysin; baicalein; Diallyl sulphide; Ellagic acid; Resveratrol; Squalestatin-1; Bilobalide; Triclocarban; Triclosan; Dichlorodiphenyltrichloroethane (DDT); Dieldrin; Methoxychlor; Metofluthrin; Permethrin; Pyrethrins; Sulfoxaflor; Diethylhexyl phthalate (DEHP); Cyproconazole; Fluconazole; Propiconazole; FL81; Tri-p-methylphenyl phosphate (TMPP); UM104; or UM145.
124. The first and at least a second inducer for use according to claim 123, wherein the method is according to any one of claims 1 to 122.
125. The first and at least a second inducer for use according to claim 123 or 124, which is a method of therapy, suitably a method of gene therapy.
126. The first and at least a second inducer for use according to any one of claims 123-125, wherein the exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene is an AAV gene therapy vector.
127. Artemisinin or a derivative of artemisinin, for use as an inducer in a method of regulating expression of a transgene in a primate subject, wherein the primate subject comprises at

least one cell comprising an exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene, wherein the inducible promoter comprises a PBREM enhancer, wherein the inducer is administered to the subject at a dose which comprises an amount of the inducer that is sufficient to express a therapeutically effective level of the transgene in the subject.

128. Artemisinin or a derivative of artemisinin for use according to claim 127, wherein the method is a method of therapy, suitably a method of gene therapy.
129. Artemisinin or a derivative of artemisinin for use according to claim 127 or 128, wherein the artemisinin derivative is a compound as defined in claim 2, optionally wherein the artemisinin derivative is of Formula (I), (I-A), (I-B), (I-C), (I-D), (I-E), (I-F), (I-G), (I-H), (I-I), (I-J), (I-K), (I-L), (I-M), (I-M'), (I-N), (I-N'), (I-O) or (I-O').
130. Artemisinin or a derivative of artemisinin for use according to any one of claims 127-129, wherein the derivative of artemisinin is artesunate, dihydroartemisinin, artesunate, or artemether.
131. Artemisinin or a derivative of artemisinin for use according to any one of claims 127-130, wherein artemisinin or a derivative of artemisinin is administered to the primate subject following the prior administration of an inducer to the primate subject.
132. Artemisinin or a derivative of artemisinin for use according to claim 131, wherein the inducer of the prior administration was a different inducer, or wherein the inducer of the prior administration was the same inducer but at a different dose.
133. Artemisinin or a derivative of artemisinin for use according to any one of claims 127-132, wherein the exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene is an AAV gene therapy vector.
134. Artemisinin or a derivative of artemisinin for use according to any one of claims 127 to 133, wherein the method comprises prior administration of the exogenous nucleotide sequence to the subject prior to administering the inducer.
135. Artemisinin or a derivative of artemisinin for use according to any one of claims 127-134, wherein the artemisinin or a derivative of artemisinin is administered at a dose of from about 0.1mg/kg to 80mg/kg, from about 0.1mg/kg to about 70mg/kg, from about 0.1mg/kg to about 60mg/kg, from about 0.1mg/kg to about 50mg/kg, from about 0.1mg/kg to about 40mg/kg, from about 0.1mg/kg to about 30mg/kg, from about 0.1mg/kg to about 20mg/kg, from about 0.1mg/kg to about 10mg/kg, from about 0.1mg/kg to about 6mg/kg, from about 0.1mg/kg to about 5mg/kg, or from about 0.1mg/kg to about 1mg/kg.

136. Artemisinin or a derivative of artemisinin for use according to any one of claims 127-135, wherein the inducer comprising artemisinin or a derivative of artemisinin is administered orally, optionally wherein the composition comprises artesunate.

137. A flavonoid compound, for use as an inducer in a method of regulating expression of a transgene in a primate subject, wherein the primate subject comprises at least one cell comprising an exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene, wherein the inducible promoter comprises a PBREM enhancer, wherein the inducer is administered to the subject at a dose which comprises an amount of the inducer that is sufficient to express a therapeutically effective level of the transgene in the subject.

138. Flavonoid compound for use according to claim 137, wherein the method is a method of therapy, suitably a method of gene therapy.

139. Flavonoid compound for use according to claim 137 or 138, wherein the flavonoid compound is flavone, baicalein, chrysin, galangin, (OH)₄flavone, 6-hydroxyluteolin, acacetin, acerosin, alnetin, apigenin, apiin, artocarpetin, balcalein, beta-sitosterol, biochamin A, cannaflavin A, cannflavin A, cannflavin B, cannflavin C, catechin, catechin-3-gallate, cerrosillin, chrysoeriol, chrysoeriol, cirsilinoleol, cirsilinoleol, cirsimaritin, corymbosin, cupressuflavone, cyanidine, daidzein, datiscetin, delphinidine, digidrokempferol, dihydroquercetin, diosmetin, diosmin, echiodinin, epicatechin, epicatechin-3-gallate, epigallocatechin, epigallocatechin-3-gallate, eriodictiol, eupatilin, eupatorin, fisetin, fizeitin, flavonol, formononetin, fortunelin, gallocatechin, gallocatechin-3-gallate, gardenin D, genistein, genistin, genkwanin, geraldol, geraldone, glycitein, glycitin, gomoeriodictiol, gossypetin, hesperetin, hinokiflavone, hispidulin, hymenoxin, hypolaetin, isoquercetin, isorhamnetin, isorhamnetin, isoscutellarein, isovitexin, jaceosidin, kaempferol, kaempferol, kampferide, largonidin, luteolin, luteolin, malvidine, mikanin, morin, myricetin, myricetin, naringenin, negletein, neodiosmin, nepetin, nevadensin, nobiletin, nodifloretin, norartocarpetin, norwogonin, onopordin, orientin, oroxylin a, pachipodol, pectolinarigenin, pedalitin, peonidin, petunidin, pilloin, pratol, primetin, primuletin, pruentin, quercetagenin, quercetin, ramnazin, rhoifolin, robinetin, rutin, scaposin, scutellarein, serpyllin, sinensetin, sorbifolin, sudachitin, tangeretin, tangeritin, taxifolin, tectochrysin, tithonine, tricetin, tricin, velutin, vitexin, wightin, wogonin, xanthomicrol, zapotin, zapotin, zapotin, 6,7,4'-trihydroxy isoflavone, or 7,3,4'-trihydroxy isoflavone.

140. Flavonoid compound for use according to any one of claims 137-139, wherein the flavonoid compound is flavone
141. Flavonoid compound for use according to any one of claims 137-140, wherein the flavonoid compound is administered to the primate subject following the prior administration of an inducer to the primate subject.
142. Flavonoid compound for use according to claim 141, wherein the inducer of the prior administration was a different inducer, or wherein the inducer of the prior administration was the same inducer but at a different dose.
143. Flavonoid compound for use according to any one of claims 137-142, wherein the exogenous nucleotide sequence comprising an inducible promoter operably linked to a transgene is an AAV gene therapy vector.
144. Flavonoid compound for use according to any one of claims 137-143, wherein the method comprises prior administration of the exogenous nucleotide sequence to the subject prior to administering the inducer.
145. Flavonoid compound for use according to any one of claims 137-144, wherein the flavonoid compound is administered at a dose of from about 0.1mg/kg to 80mg/kg, from about 0.1mg/kg to about 70mg/kg, from about 0.1mg/kg to about 60mg/kg, from about 0.1mg/kg to about 50mg/kg, from about 0.1mg/kg to about 40mg/kg, from about 0.1mg/kg to about 30mg/kg, from about 0.1mg/kg to about 20mg/kg, from about 0.1mg/kg to about 10mg/kg, from about 0.1mg/kg to about 6mg/kg, from about 0.1mg/kg to about 5mg/kg, or from about 0.1mg/kg to about 1mg/kg.
146. Flavonoid compound for use according to any one of claims 137-145, wherein the flavonoid compound is administered orally.
147. Flavonoid compound for use according to any one of claims 137-145, wherein the flavonoid compound is administered intraperitoneally.
148. A method of regulating expression of a transgene in the liver of a subject, the method comprising: administering to the subject at least a first time point a dose of a first inducer; administering to the subject at least a second time point a second dose of a second inducer, wherein the second inducer can be the same as or different than the first inducer; and administering to the subject at least a third time point a third dose of a third inducer, wherein the third inducer can be the same as or different than the first inducer and the second inducer; wherein the subject comprises at least one liver cell comprising an exogenous nucleotide sequence comprising a synthetic inducible promoter operably linked to a transgene; wherein the synthetic inducible promoter comprises at least one PBREM enhancer; and

wherein the transgene is detrimental to the liver cell or to the subject when constitutively expressed.

149. The method of claim 148, wherein the first inducer is selected from the group consisting of artemisinin; derivative of artemisinin; a flavonoid compound, e.g. flavone, chrysin, baicalein, or galangin; 1,4-Bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP); phenobarbital (PB); 6-(4Chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde-O-(3,4-dichlorobenzyl)oxime (CITCO); Acetaminophen; Buprenorphine; Phenytoin; Carbamazepine; Valproic Acid; Chlorpromazine; Efavirenz; Nevirapine; Rilpivirine; Etravirine; Diazepam; Cyclophosphamid; Ifosfamide; Cerivastatin; Simvastatin; lovastatin; substituted sulphonamides; Thiazolidin-4-one; Estradiol; Estrone and analogues; 17 α -ethinyl-3;17 β -estradiol (EE2); Dehydroepiandrosterone (DHEA); 5 β -pregnane-3,20-dione; Diethylstilbestrol; Ginkgo *biloba* extract; Galangin; chrysin; baicalein; Diallyl sulphide; Ellagic acid; Resveratrol; Squalastatin-1; Bilobalide; Triclocarban; Triclosan; Dichlorodiphenyltrichloroethane (DDT); Dieldrin; Methoxychlor; Metofluthrin; Permethrin; Pyrethrins; Sulfoxaflor; Diethylhexyl phthalate (DEHP); Cyproconazole; Fluconazole; Propiconazole; FL81; Tri-p-methylphenyl phosphate (TMPP); UM104; or UM145.
150. The method of claim 148 or 149, wherein the second inducer is selected from the group consisting of artemisinin; derivative of artemisinin; a flavonoid compound, e.g. flavone, chrysin, baicalein, or galangin; 1,4-Bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP); phenobarbital (PB); 6-(4Chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde-O-(3,4-dichlorobenzyl)oxime (CITCO); Acetaminophen; Buprenorphine; Phenytoin; Carbamazepine; Valproic Acid; Chlorpromazine; Efavirenz; Nevirapine; Rilpivirine; Etravirine; Diazepam; Cyclophosphamid; Ifosfamide; Cerivastatin; Simvastatin; lovastatin; substituted sulphonamides; Thiazolidin-4-one; Estradiol; Estrone and analogues; 17 α -ethinyl-3;17 β -estradiol (EE2); Dehydroepiandrosterone (DHEA); 5 β -pregnane-3,20-dione; Diethylstilbestrol; Ginkgo *biloba* extract; Galangin; chrysin; baicalein; Diallyl sulphide; Ellagic acid; Resveratrol; Squalastatin-1; Bilobalide; Triclocarban; Triclosan; Dichlorodiphenyltrichloroethane (DDT); Dieldrin; Methoxychlor; Metofluthrin; Permethrin; Pyrethrins; Sulfoxaflor; Diethylhexyl phthalate (DEHP); Cyproconazole; Fluconazole; Propiconazole; FL81; Tri-p-methylphenyl phosphate (TMPP); UM104; or UM145.
151. The method of any one of claims 148-150, wherein the third inducer is selected from the group consisting of artemisinin; derivative of artemisinin; a flavonoid compound, e.g. flavone, chrysin, baicalein, or galangin; 1,4-Bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP); phenobarbital (PB); 6-(4Chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde-O-(3,4-dichlorobenzyl)oxime (CITCO); Acetaminophen; Buprenorphine; Phenytoin; Carbamazepine; Valproic Acid; Chlorpromazine; Efavirenz; Nevirapine; Rilpivirine;

Etravirine; Diazepam; Cyclophosphamid; Ifosfamide; Cerivastatin; Simvastatin; lovastatin; substituted sulphonamides; Thiazolidin-4-one; Estradiol; Estrone and analogues; 17 α -ethinyl-3;17 β -estradiol (EE2); Dehydroepiandrosterone (DHEA); 5 β -pregnane-3,20-dione; Diethylstilbestrol; Ginkgo *biloba* extract; Galangin; chrysin; baicalein; Diallyl sulphide; Ellagic acid; Resveratrol; Squalastatin-1; Bilobalide; Triclocarban; Triclosan; Dichlorodiphenyltrichloroethane (DDT); Dieldrin; Methoxychlor; Metofluthrin; Permethrin; Pyrethrins; Sulfoxaflor; Diethylhexyl phthalate (DEHP); Cyproconazole; Fluconazole; Propiconazole; FL81; Tri-p-methylphenyl phosphate (TMPP); UM104; or UM145.

152. The method of any one of claims 1-151, wherein the first inducer is artemisinin, or a derivative thereof.
153. The method of any one of claims 1-151, wherein first inducer is a flavonoid compound.
154. The method of any one of claims 1-153, wherein the second inducer or third inducer is artemisinin or, derivative thereof.
155. The method of any one of claims 1-153, wherein the second inducer is flavonoid compound.