[0009] Accordingly, use of adeno-associated virus (AAV) vectors for gene therapy is limited due to the single administration to patients (owing to the patient immune response), the limited range of transgene genetic material suitable for delivery in AAV vectors due to minimal viral packaging capacity (about 4.5kb), and slow AAV-mediated gene expression.

[0010] There is large unmet need for disease-modifying therapies in hemophilia B. Current therapies are burdensome and require frequent intravenous (IV) administrations. First, these Factor IX injectables do not provide continuous delivery of factors, with trough levels allowing bleeding episodes. Second, there are no approved gene therapies for hemophilia B, and AAV based therapies cannot be used by 25% to 40% of patients due to pre-existing antibodies. AAV can only be administered once, and the resulting Factor IX levels might not be high enough to be efficacious, or may be supranormal, dose levels cannot be titrated. Third, some hemophilia B patients cannot utilize these therapies because of the development of neutralizing antibodies to these exogenous, artificial clotting factors.

[0011] Accordingly, there is need in the field for a technology that permits expression of a therapeutic FIX protein in a cell, tissue or subject for the treatment of hemophilia B.

BRIEF DESCRIPTION

[0012] The technology described herein relates to methods and compositions for treatment of Hemophilia B by expression of Factor IX (FIX) protein from a capsid-free (*e.g.,* non-viral) DNA vector with covalently-closed ends (referred to herein as a "closed-ended DNA vector" or a "ceDNA vector"), where the ceDNA vector comprises a FIX nucleic acid sequence or codon optimized versions thereof. These ceDNA vectors can be used to produce FIX proteins for treatment, monitoring, and diagnosis. The application of ceDNA vectors expressing FIX to the subject for the treatment of hemophilia B is useful to: (i) provide disease modifying levels of FIX enzyme, be minimally invasive in delivery, be repeatable and dosed-to-effect, have rapid onset of therapeutic effect, result in sustained expression of corrective FIX enzyme in the liver, restoring the coagulation cascade, and/or be titratable to achieve the appropriate pharmacologic levels of the defective enzyme.

[0012a] In one aspect, the present disclosure refers to a capsid-free closed-ended DNA (ceDNA) vector comprising: at least one nucleic acid sequence positioned between flanking inverted terminal repeats (ITRs), wherein the at least one nucleic acid sequence encodes at least one FIX protein.

[0013] In some ~~embodiment~~examples, a ceDNA-vector expressing FIX is optionally present in a liposome nanoparticle formulation (LNP) for the treatment of hemophilia B. A ceDNA LNP formulation described herein can provide one or more benefits, including,: providing disease modifying levels of FIX protein, being minimally invasive in delivery, being repeatable and dosed-to-effect, having a rapid onset of therapeutic effect that is typically within days of therapeutic intervention, having sustained expression of corrective FIX levels in the circulation, be titratable to achieve the appropriate pharmacologic levels of the defective coagulation factor, and/or provide treatments for other types of hemophilia, including but not limited to Factor VII deficiency.

[0014] Accordingly, the present disclosure relates to a capsid-free (*e.g.,* non-viral) DNA vector with covalently-closed ends (referred to herein as a "closed-ended DNA vector" or a "ceDNA vector")

comprising a gene encoding FIX, to permit expression of the FIX therapeutic protein in a cell. In one ~~embodiment~~example, the gene encoding FIX is a heterologous gene.

[0015] The ceDNA vectors for expression of FIX protein production as described herein are capsid-free, linear duplex DNA molecules formed from a continuous strand of complementary DNA with covalently-closed ends (linear, continuous and non-encapsidated structure), which comprise a 5' inverted terminal repeat (ITR) sequence and a 3' ITR sequence, where the 5' ITR and the 3' ITR can have the same symmetrical three-dimensional organization with respect to each other, (*i.e.,* symmetrical or substantially symmetrical), or alternatively, the 5' ITR and the 3' ITR can have different three-dimensional organization with respect to each other (*i.e.,* asymmetrical ITRs). In addition, the ITRs can be from the same or different serotypes. In some ~~embodiment~~examples, a ceDNA vector can comprise ITR sequences that have a symmetrical three-dimensional spatial organization such that their structure is the same shape in geometrical space, or have the same A, C-C' and B-B' loops in 3D space (*i.e.,* they are the same or are mirror images with respect to each other). In some ~~embodiment~~examples, one ITR can be from one AAV serotype, and the other ITR can be from a different AAV serotype.

[0016] Accordingly, some ~~aspect~~examples of the technology described herein relate to a ceDNA vector for improved protein expression and/or production of the above described FIX protein that comprise ITR sequences that flank a nucleic acid sequence comprising any FIX nucleic acid sequence disclosed in Table 1 or any open reading frame sequence included in any ceDNA sequence disclosed in Table 12, the ITR sequences being selected from any of: (i) at least one WT ITR and at least one modified AAV inverted terminal repeat (ITR) (*e.g.,* asymmetric modified ITRs); (ii) two modified ITRs where the mod-ITR pair have a different three-dimensional spatial organization with respect to each other (*e.g.,* asymmetric modified ITRs), or (iii) symmetrical or substantially symmetrical WT-WT ITR pair, where each WT-ITR has the same three-dimensional spatial organization, or (iv) symmetrical or substantially symmetrical modified ITR pair, where each mod-ITR has the same three-dimensional spatial organization. The ceDNA vectors disclosed herein can be produced in eukaryotic cells, thus devoid of prokaryotic DNA modifications and bacterial endotoxin contamination in insect cells.

[0017] The methods and compositions described herein relate, in part, to the discovery of a non-viral capsid-free DNA vector with covalently-closed ends (ceDNA vectors) that can be used to express at least one FIX protein, or more than one FIX protein, from a cell, including but not limited to cells of the liver.

[0018] Provided herein in one ~~aspect~~example are DNA vectors (*e.g.,* ceDNA vectors) comprising at least one nucleic acid sequence, wherein the nucleic acid sequence encodes a transgene, operably linked to a promoter positioned between two different AAV inverted terminal repeat sequences (ITRs), one of the ITRs comprising a functional AAV terminal resolution site and a Rep binding site, and one of the ITRs comprising a deletion, insertion, or substitution relative to the other ITR; wherein the transgene encodes an FIX protein; and wherein the DNA when digested with a restriction enzyme having a single recognition site on the DNA vector has the presence of characteristic bands of linear and

continuous DNA as compared to linear and non-continuous DNA controls when analyzed on a non-denaturing gel. Other ~~aspect~~examples include delivery of the FIX protein by expressing it *in vivo* from a ceDNA vector as described herein and further, the treatment of hemophilia B using ceDNA vectors encoding the FIX protein. Also contemplated herein are cells comprising a ceDNA vector encoding an FIX protein as described herein.

[0019] ~~Aspect~~Examples of the disclosure relate to methods to produce the ceDNA vectors useful for FIX protein expression in a cell as described herein. Other ~~embodiment~~examples relate to a ceDNA vector produced by the methods provided herein. In one ~~embodiment~~example, the capsid free (*e.g.,* non-viral) DNA vector (ceDNA vector) for FIX protein production is obtained from a plasmid (referred to herein as a "ceDNA-plasmid") comprising a polynucleotide expression construct template comprising in this order: a first 5' inverted terminal repeat (*e.g.* AAV ITR); a nucleic acid sequence; and a 3' ITR (*e.g.* AAV ITR), where the 5' ITR and 3'ITR can be asymmetric relative to each other, or symmetric (*e.g.,* WT-ITRs or modified symmetric ITRs) as defined herein.

[0020] The ceDNA vector for expression of the FIX protein as disclosed herein is obtainable by a number of means that would be known to the ordinarily skilled artisan after reading this disclosure. For example, a polynucleotide expression construct template used for generating the ceDNA vectors of the present disclosure can be a ceDNA-plasmid**,** a ceDNA-bacmid, and/or a ceDNA-baculovirus. In one ~~embodiment~~example, the ceDNA-plasmid comprises a restriction cloning site (*e.g.* SEQ ID NO: 123 and/or 124) operably positioned between the ITRs where an expression cassette comprising *e.g.,* a promoter operatively linked to a transgene, *e.g.,* a nucleic acid encoding FIX can be inserted. In some ~~embodiment~~examples, ceDNA vectors for expression of FIX protein are produced from a polynucleotide template (*e.g.,* ceDNA-plasmid, ceDNA-bacmid, ceDNA-baculovirus) containing symmetric or asymmetric ITRs (modified or WT ITRs).

[0021] In a permissive host cell, in the presence of *e.g.,* Rep, the polynucleotide template having at least two ITRs replicates to produce ceDNA vectors expressing the FIX protein. ceDNA vector production undergoes two steps: first, excision ("rescue") of template from the template backbone (*e.g.* ceDNA-plasmid, ceDNA-bacmid, ceDNA-baculovirus genome etc.) via Rep proteins, and second, Rep mediated replication of the excised ceDNA vector. Rep proteins and Rep binding sites of the various AAV serotypes are well known to those of ordinary skill in the art. One of ordinary skill understands to choose a Rep protein from a serotype that binds to and replicates the nucleic acid sequence based upon at least one functional ITR. For example, if the replication competent ITR is from AAV serotype 2, the corresponding Rep would be from an AAV serotype that works with that serotype such as AAV2 ITR with AAV2 or AAV4 Rep but not AAV5 Rep, which does not. Upon replication, the covalently-closed ended ceDNA vector continues to accumulate in permissive cells and ceDNA vector is preferably sufficiently stable over time in the presence of Rep protein under standard replication conditions, *e.g.* to accumulate in an amount that is at least 1 pg/cell, preferably at least 2 pg/cell, preferably at least 3 pg/cell, more preferably at least 4 pg/cell, even more preferably at least 5 pg/cell.

[0022] Accordingly, one ~~aspect~~example of the disclosure relates to a process of producing a ceDNA vector for expression of such FIX proteins comprising the steps of: a) incubating a population of host cells (*e.g.* insect cells) harboring the polynucleotide expression construct template (*e.g.,* a ceDNA-plasmid, a ceDNA-bacmid, and/or a ceDNA-baculovirus), which is devoid of viral capsid coding sequences, in the presence of a Rep protein under conditions effective and for a time sufficient to induce production of the ceDNA vector within the host cells, and wherein the host cells do not comprise viral capsid coding sequences; and b) harvesting and isolating the ceDNA vector from the host cells. The presence of Rep protein induces replication of the vector polynucleotide with a modified ITR to produce the ceDNA vector for expression of FIX protein in a host cell. However, no viral particles (*e.g.* AAV virions) are expressed. Thus, there is no virion-enforced size limitation.

[0023] The presence of the ceDNA vector useful for expression of FIX protein is isolated from the host cells can be confirmed by digesting DNA isolated from the host cell with a restriction enzyme having a single recognition site on the ceDNA vector and analyzing the digested DNA material on denaturing and non-denaturing gels to confirm the presence of characteristic bands of linear and continuous DNA as compared to linear and non-continuous DNA.

[0024] Also provided herein are methods of expressing an FIX protein that has therapeutic uses, in a cell or in a subject, using a ceDNA vector. Such FIX proteins can be used for the treatment of hemophilia B. Accordingly, provided herein are methods for the treatment of hemophilia B comprising administering a ceDNA vector encoding a therapeutic FIX protein to a subject in need thereof.

[0024a] In another aspect, the present disclosure refers to a capsid-free close-ended DNA (ceDNA) vector comprising a nucleic acid sequence selected from the group consisting of the sequences set forth in Table 12. In another aspect, the present disclosure refers to a capsid-free close-ended DNA (ceDNA) vector comprising a nucleic acid sequence at least 85% identical to SEQ ID NO: 404, SEQ ID NO: 405 or SEQ ID NO: 406. In another aspect, the present disclosure refers to a capsid-free close-ended DNA (ceDNA) vector consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NO: 404, SEQ ID NO: 405 and SEQ ID NO: 406.

[0025] In some ~~embodiment~~examples, ~~one aspect of~~ the technology described herein relates to a non-viral capsid-free DNA vector with covalently-closed ends (ceDNA vector), wherein the ceDNA vector comprises at least one nucleic acid sequence, operably positioned between two ITR sequences where the ITR sequences can be asymmetric, or symmetric, or substantially symmetrical as these terms are defined herein, wherein at least one of the ITRs comprises a functional terminal resolution site (trs) and a Rep binding site, and optionally the nucleic acid sequence encodes a transgene (*e.g.,* FIX protein) and wherein the vector is not in a viral capsid.

[0025a] In another aspect, the present disclosure refers to the use of the ceDNA vector as disclosed herein in the manufacture of a medicament for expressing an FIX protein in a cell. In another aspect, the present disclosure refers to the use of the ceDNA vector as disclosed herein in the manufacture of a medicament for treating a subject with hemophilia B. In another aspect, the present disclosure refers to a pharmaceutical composition comprising the ceDNA vector as disclosed herein. In another aspect, the present disclosure refers to a cell containing the ceDNA vector as disclosed herein. In another aspect, the present disclosure refers to a composition comprising the ceDNA vector as disclosed herein and a lipid, wherein the lipid is a lipid nanoparticle. In yet another aspect, the present disclosure refers to a kit comprising the ceDNA vector as disclosed herein or the composition as disclosed herein, or the cell as disclosed herein, and instructions for use.

[0026] These and other aspects of the disclosure are described in further detail below.

DESCRIPTION OF DRAWINGS

[0027] Embodiments of the present disclosure, briefly summarized above and discussed in greater detail below, can be understood by reference to the illustrative embodiments of the disclosure depicted in the appended drawings. However, the appended drawings illustrate only typical embodiments of the disclosure and are therefore not to be considered limiting of scope, for the disclosure may admit to other equally effective embodiments.

[0028] **FIG. 1A** illustrates an exemplary structure of a ceDNA vector for expression of an FIX protein as disclosed herein, comprising asymmetric ITRs. In this embodiment, the exemplary ceDNA vector comprises an expression cassette containing CAG promoter, WPRE, and BGHpA. An open

CLAIMS

1. A capsid-free closed-ended DNA (ceDNA) vector comprising:

at least one nucleic acid sequence positioned between flanking inverted terminal repeats (ITRs), wherein the at least one nucleic acid sequence encodes at least one FIX protein.

2. The ceDNA vector of claim 1, wherein the least one nucleic acid sequence that encodes the at least one FIX protein is selected from the group consisting of: any of the sequences set forth in Table 1 ~~or~~ and any open reading frame (ORF) sequence included in any ceDNA sequence set forth ~~listed~~ in Table 12.

3. The ceDNA vector of claims 1 or 2, wherein:

~~3.~~ the ceDNA vector comprises a promoter sequence selected from the group consisting of any of ~~those~~ the sequences set forth in Table 7 operatively linked to the at least one nucleic acid sequence that encodes the at least one FIX protein~~.~~;

~~4. The ceDNA vector of any of claims 1 to 3, wherein~~ the ceDNA vector comprises an enhancer sequence selected from the group consisting of any of ~~those~~ the sequences set forth in Table 8~~.~~;

~~5. The ceDNA vector of any of claims 1 to 4, wherein~~ the ceDNA vector comprises a 5' UTR and/or an intron sequence selected from the group consisting of any of ~~those~~ the sequences set forth in Table 9A~~.~~;

~~6. The ceDNA vector of any of claims 1 to 5, wherein~~ the ceDNA vector comprises a 3' UTR sequence selected from the group consisting of any of ~~those~~ the sequences set forth in Table 9B~~.~~;

~~7. The ceDNA vector of any of claims 1 to 6, wherein~~ the ceDNA vector comprises at least one poly A sequence selected from ~~any of those~~the group consisting of any of the sequences set forth in Table 10~~.~~; and/or

~~8. The ceDNA vector of any one of claims 1-7, wherein~~ the ceDNA vector comprises at least one promoter sequence operably linked to at least one nucleic acid sequence.

~~9.~~4. The ceDNA vector of any one of claims 1-~~8~~3, wherein the at least one nucleic acid sequence is cDNA.

~~10.~~5. The ceDNA vector of any one of claims 1-~~9~~4, wherein at least one flanking ITR comprises a functional terminal resolution site and a Rep binding site.

~~11.~~6. The ceDNA vector of any one of claims 1-~~10~~5, wherein one or both of the flanking ITRs are derived from a virus selected from the group consisting of: a *Parvovirus*, a *Dependovirus*, and an adeno-associated virus (AAV).

~~12.~~7. The ceDNA vector of any one of claims 1-~~11~~5, wherein: the flanking ITRs are symmetric or asymmetric with respect to ~~one another~~each other; ~~.~~

~~13. The ceDNA vector of claim 12, wherein~~ the flanking ITRs are symmetrical or substantially symmetric~~al~~; or~~.~~

~~14. The ceDNA vector of claim 12, wherein~~ the flanking ITRs are asymmetric.

~~15.~~8. The ceDNA vector of any one of claims 1-~~14~~7, wherein: one ~~or both~~ of the flanking ITRs ~~are~~ is a wild-~~-~~type ITR, or wherein both of the ITRs are wild-type ITRs;~~.~~

one of the flanking ITRs is not a wild-type ITR, or wherein both of the flanking ITRs are not wild-type ITRs;

~~16. The ceDNA vector of any one of claims 1-15, wherein~~ the flanking ITRs are derivd from different viral serotypes;~~.~~

one or both of the flanking ITRs are derived from an AAV serotype selected from the group consisting of: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, and AAV12;

~~17. The ceDNA vector of any one of claims 1-16, wherein~~ the flanking ITRs are selected from the group consisting of any pair of viral serotypes shown in Table 2;~~.~~ and /or

~~18. The ceDNA vector of any one of claims 1-17, wherein~~ one or both of the flanking ITRs comprises a sequence selected from the group consisting of one or more of the sequences set forth in Table 3.

~~19.~~9. The ceDNA vector of any one of claims 1-~~18~~8, wherein at least one of the flanking ITRs is altered from a wild-type AAV ITR sequence by a deletion, an addition, or a substitution that affects the overall three-dimensional conformation of the ITR.

~~20. The ceDNA vector of any one of claims 1-19, wherein one or both of the ITRs are derived from an AAV serotype selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, and AAV12.~~

~~21.~~10. The ceDNA vector of any one of claims 1-~~20~~9, wherein one or both of the ITRs are synthetic.

~~22. The ceDNA vector of any one of claims 1-21, wherein one or both of the ITRs are not a wild type ITR, or wherein both of the ITRs are not wild-type ITRs.~~

11. The ceDNA vector of any one of claims 1-~~22~~10, wherein:

~~23.~~ one or both of the flanking ITRs are modified by a deletion, an insertion, and/or a substitution in at least one of the ITR regions selected from the group consisting of: the A, A', B, B', C, C', D, and D' regions;~~.~~

~~24. The ceDNA vector of claim 23, wherein the deletion, insertion, and/or substitution results in the deletion of all or part of a stem-loop structure normally formed by the A, A', B, B', C, or C' regions.~~

~~25. The ceDNA vector of any one of claims 1-24, wherein~~ one or both of the flanking ITRs are modified by a deletion, an insertion, and/or a substitution that results in the deletion of all or part of a stem-loop structure ~~normally~~ formed by the B and B' regions;~~.~~

~~26. The ceDNA vector of any one of claims 1-24, wherein~~ one or both of the flanking ITRs are modified by a deletion, an insertion, and/or a substitution that results in the deletion of all or part of a stem-loop structure ~~normally~~ formed by the C and C' regions;~~.~~

~~27. The ceDNA vector of any one of claims 1-24, wherein~~ one or both of the flanking ITRs are

modified by a deletion, an insertion, and/or a substitution that results in the deletion of part of a stem-loop structure normally formed by the B and B' regions and/or part of a stem-loop structure ~~normally~~ formed by the C and C' regions;~~.~~

~~28. The ceDNA vector of any one of claims 1-27, wherein~~ one or both of the flanking ITRs comprise a single stem-loop structure in the region that, ~~normally~~ in a wild-type ITR, would comprise~~s~~ a first stem-loop structure formed by the B and B' regions and a second stem-loop structure formed by the C and C' regions; ~~.~~

~~29. The ceDNA vector of any one of claims 1-28, wherein~~ one or both of the flanking ITRs comprise a single stem and two loops in the region that, in a wild-type ITR, would ~~normally~~ comprise~~s~~ a first stem-loop structure formed by the B and B' regions and a second stem-loop structure formed by the C and C' regions; or ~~.~~

~~30. The ceDNA vector of any one of claims 1-29, wherein~~ one or both of the flanking ITRs comprise a single stem and a single loop in the region that, in a wild-type ITR, would ~~normally~~ comprise~~s~~ a first stem-loop structure formed by the B and B' regions and a second stem-loop structure formed by the C and C' regions.

~~31.~~12. The ceDNA vector of any one of claims 1-~~30~~11, wherein both flanking ITRs are altered in a manner that results in an overall three-dimensional symmetry when the flainking ITRs are inverted relative to each other.

~~32.~~13. The ceDNA vector of any one of claims 1-~~31~~12, wherein one or both of the ITRs comprises a nucleic acid sequence selected from the group consisting of the sequences set forth in Tables 3, 5A, 5B, and 6.

~~33.~~14. The ceDNA vector of any one of claims 1-~~32~~13, wherein ~~at~~ the ceDNA vector comprises ~~least one nucleic acid sequence is under the control of~~ at least one regulatory switch.

~~34. The ceDNA vector of claim 33, wherein the at least one regulatory switch is selected from the group consisting of: a binary regulatory switch, a small molecule regulatory switch, a passcode regulatory switch, a nucleic acid-based regulatory switch, a post-transcriptional regulatory switch, a radiation-controlled or ultrasound controlled regulatory switch, a hypoxia-mediated regulatory switch, an inflammatory response regulatory switch, a shear-activated regulatory switch, and a kill switch.~~

~~35.~~15. A capsid-free close-ended DNA (ceDNA) vector comprising a nucleic acid sequence selected from the group consisting of the sequences set forth in Table 12.

~~36.~~16. A capsid-free close-ended DNA (ceDNA) vector comprising a nucleic acid sequence at least 85% identical to SEQ ID NO: 404, SEQ ID NO: 405 or SEQ ID NO: 406.

~~37.~~17. A capsid-free close-ended DNA (ceDNA) vector consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NO: 404, SEQ ID NO: 405 and SEQ ID NO: 406.

~~38.~~18. Use of the ceDNA vector of any one of claims 1-17 in the manufacture of a medicament for ~~A method of~~ expressing an FIX protein in a cell ~~comprising contacting the cell with the ceDNA vector of any one of claims 1-37~~.

~~39. The method of claim 38, wherein the cell is a hepatocyte.~~

~~40. The method of claim 38 or 39, wherein the cell in *in vitro* or *in vivo*.~~

~~41.~~19. The ~~method~~ use of ~~any one of~~ claim~~s 38-40~~18, wherein the at least one nucleic acid sequence is codon optimized for expression in the eukaryotic cell.

~~42.~~20. The ~~method~~ use of ~~any one of~~ claim~~s 38-41~~ 19, wherein the at least one nucleic acid sequence that is codon optimized is selected from the group consisting of any one of the sequences set forth in in Table 1 ~~or~~ and any open reading frame (ORF) sequence included in any ceDNA sequence ~~listed~~ set forth in Table 12.

~~43.~~21. Use of the ~~A method of treating a subject with hemophilia B, comprising administering to the subject a~~ ceDNA vector of any one of claims 1-~~37~~17 in the manufacture of a medicament for treating a subject with hemophilia B~~, wherein at least one nucleic acid sequence encodes at least one FIX protein~~.

~~44.~~22. The ~~method~~ use of claim ~~43~~21, wherein the least one nucleic acid sequence that encodes the at least one FIX protein is selected from the group consisting of any one of the sequences set forth in Table 1.

~~45.~~23. The ~~method~~ use of claim ~~43~~ 21 or ~~44~~22, wherein the ceDNA vector is to be administered to a hepatocyte or the ceDNA vector expresses the FIX protein in a hepatocyte.

~~46. The method of any of claims 44 to 45, wherein the ceDNA vector expresses the FIX protein in a hepatocyte.~~

~~47.~~24. The ~~method~~ use of ~~any of~~ claim~~s 44~~22 or ~~-23~~46, wherein the ceDNA vector is to be administered by intravenous or intramuscular injection.

~~48.~~25. The ~~method~~ use of any one of claims ~~44~~22-24 ~~47~~, further comprising administering to the subject an immune modulating agent.

~~49. The method of claim 48, wherein the immune modulating agent is an immunosuppressant.~~

~~50. The method of claim 49, wherein the immunosuppressant is a tyrosine kinase inhibitor (TKI).~~

~~51. The method of claim 50, wherein the TKI is administered to the subject at a dosage of about 0.5 mg/kg to about 700 mg/kg.~~

~~52.~~26. A pharmaceutical composition comprising the ceDNA vector of any one of claims 1-~~37~~17.

~~53. The pharmaceutical composition of claim 52, further comprising an additional compound.~~

~~54. The pharmaceutical composition of claim 52, wherein the additional compound is an immune modulating agent.~~

~~55. The pharmaceutical composition of claim 54, wherein the immune modulating agent is an immunosuppressant.~~

~~56. The pharmaceutical composition of claim 55, wherein the immunosuppressant is a tyrosine kinase inhibitor (TKI).~~

~~57. The pharmaceutical composition of claim 56, wherein the composition further comprises an excipient or carrier.~~

~~58.~~27. A cell containing ~~a~~ the ceDNA vector of any of claims 1-~~37~~17.

~~59. The cell of claim 58, wherein the cell is a hepatocyte.~~

~~60.~~28. A composition comprising ~~a~~ the ceDNA vector of any of claims 1-~~37~~ 17 and a lipid.~~,~~

~~61. The composition of claim 60,~~ wherein the lipid is a lipid nanoparticle ~~(LNP)~~.

~~62.~~29. A kit comprising the ceDNA vector of any one of claims 1-~~37~~ 17 or the composition of any one of claims ~~52-57~~26, or the cell of claim ~~58~~27, and instructions for use.