

eliminate transferred T cells or stem cells in the event that they trigger serious adverse events (SAEs), or become obsolete following treatment. Yet in some instances, the need for therapy may remain, and there may be a way to reduce the negative effects, while maintaining a sufficient level of therapy.

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In some instances, there is a need to increase the activity of the therapeutic cell. For example, costimulating polypeptides may be used to enhance the activation of T cells, and of CAR-expressing T cells against target antigens, which would increase the potency of adoptive immunotherapy.

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Thus, there is a need for controlled activation or elimination of therapeutic cells, to rapidly enhance the activity of or to remove the possible negative effects of donor cells used in cellular therapy, while retaining part or all of the beneficial effects of the therapy.

15 Summary

Chemical Induction of Dimerization (CID) with small molecules is an effective technology used to generate switches of protein function to alter cell physiology. A high specificity, efficient dimerizer is rimiducid (AP1903), which has two identical, protein-binding surfaces arranged tail-to-tail, each with high affinity and specificity for a mutant or variant of FKBP12: FKBP12(F36V) (FKBP12v36, F_{v36} or F_v), Attachment of one or more F_v domains onto one or more cell signaling molecules that normally rely on homodimerization can convert that protein to rimiducid control. Homodimerization with rimiducid is used in the context of an inducible caspase safety switch, and an inducible activation switch for cellular therapy, where costimulatory polypeptides including MyD88 and CD40 polypeptides are used to stimulate immune activity. Because both of these switches rely on the same ligand inducer, it is difficult to control both functions using these switches within the same cell. In some examples, a molecular switch is provided that is controlled by a distinct dimerizer ligand, based on the heterodimerizing small molecule, rapamycin, or rapamycin analogs ("rapalogs"). Rapamycin binds to FKBP12, and its variants, and can induce heterodimerization of signaling domains that are fused to FKBP12 by binding to both FKBP12 and to polypeptides that contain the FKBP-rapamycin-binding (FRB) domain of mTOR. Provided in some examples of the present application are molecular switches that greatly augment the use of rapamycin, rapalogs and rimiducid as agents for therapeutic applications. In certain examples, the allele specificity of rimiducid is used to allow selective dimerization of F_v-fusions. In other examples,

a rapamycin or rapalog-inducible pro-apoptotic polypeptide, such as, for example, Caspase-9 or a rapamycin or rapalog-inducible costimulatory polypeptide, such as, for example, MyD88/CD40 (MC) is used in combination with a rimiducid-inducible pro-apoptotic polypeptide, such as, for example, Caspase-9, or a rimiducid-inducible chimeric stimulating polypeptide, such as, for example, iMC to produce dual-switches. These dual-switches can be used to control both cell proliferation and apoptosis selectively by administration of either of two distinct ligand inducers.

In other examples, a molecular switch is provided that provides the option to activate a pro-apoptotic polypeptide, such as, for example, Caspase-9, with either rimiducid, or rapamycin or a rapalog, wherein the chimeric pro-apoptotic polypeptide comprises both a rimiducid-induced switch and a rapamycin-, or rapalog-, induced switch. Including both molecular switches on the same chimeric pro-apoptotic polypeptide provides flexibility in a clinical setting, where the clinician can choose to administer the appropriate drug based on its specific pharmacological properties, or for other considerations, such as, for example, availability. These chimeric pro-apoptotic polypeptides may comprise, for example, both a FKBP12-Rapamycin-binding domain of mTOR (FRB), or an FRB variant, and an FKBP12 variant polypeptide, such as, for example, FKBP12v36. By FRB variant polypeptide is meant an FRB polypeptide that binds to a rapamycin analog (rapalog), for example, a rapalog provided in the present application. FRB variant polypeptides comprise one or more amino acid substitutions, bind to a rapalog, and may bind, or may not bind to rapamycin.

In one example of the dual-switch technology, (Fwt.FRBC9/MC.FvFv) a homodimerizer, such as AP1903 (rimiducid), induces activation of a modified cell, and a heterodimerizer, such as rapamycin or a rapalog, activates a safety switch, causing apoptosis of the modified cell. In this example, for example, a chimeric pro-apoptotic polypeptide, such as, for example, Caspase-9, comprising both an FKBP12 and an FRB, or FRB variant region (iFwtFRBC9) is expressed in a cell along with an inducible chimeric MyD88/CD40 costimulating polypeptide, that comprises MyD88 and CD40 polypeptides and at least two copies of FKBP12v36 (MC.FvFv). Upon contacting the cell with a dimerizer that binds to the Fv regions, the MC.FvFv dimerizes or multimerizes, and activates the cell. The cell may, for example, be a T cell that expresses a chimeric antigen receptor directed against a target antigen (CAR ζ). As a safety switch, the cell may be contacted with a heterodimerizer, such as, for example, rapamycin, or a rapalog, that binds to the FRB region on the iFwtFRBC9 polypeptide, as well as the FKBP12 region on the iFwtFRBC9 polypeptide, causing direct dimerization of the Caspase-9 polypeptide, and inducing apoptosis. (Fig. 43 (2), Fig. 57) In another mechanism, the heterodimerizer binds to the FRB region on the iFwtFRBC9

polypeptide, and the Fv region on the MC.FvFv polypeptide, causing scaffold-induced dimerization, due to the scaffold of two FKBP12v36 polypeptides on each MC.FvFv polypeptide (Fig. 43 (1)), and inducing apoptosis. By FKBP12 variant polypeptide is meant an FKBP12 polypeptide that comprises one or more amino acid substitutions and that binds to a ligand such as, for example, rimiducid, with at least 100 times, 500 times, or 1000 times more affinity than the ligand binds to the FKBP12 polypeptide region.

In another example of the dual-switch technology, (FRBFwtMC/FvC9) a heterodimerizer, such as rapamycin or a rapalog, induces activation of a modified cell, and a homodimerizer, such as AP1903 activates a safety switch, causing apoptosis of the modified cell. In this example, for example, a chimeric pro-apoptotic polypeptide, such as, for example, Caspase-9, comprising an Fv region (iFvC9) is expressed in a cell along with an inducible chimeric MyD88/CD40 costimulating polypeptide, that comprises MyD88 and CD40 polypeptides and both an FKBP12 and an FRB or FRB variant region (iFRBFwtMC) (MC.FvFv). Upon contacting the cell with rapamycin or a rapalog that heterodimerizes the FKBP12 and FRB regions, the iFRBFwtMC dimerizes or multimerizes, and activates the cell. The cell may, for example, be a T cell that expresses a chimeric antigen receptor directed against a target antigen (CAR ζ). As a safety switch, the cell may be contacted with a homodimerizer, such as, for example, AP1903, that binds to the iFvC9 polypeptide, causing direct dimerization of the Caspase-9 polypeptide, and inducing apoptosis. (Fig. 57 (right)).

It yet another example of the dual switch compositions and methods of the present application, dual switch apoptotic polypeptides, modified cells that express the dual switch apoptotic polypeptides, and nucleic acids that encode the dual switch apoptotic polypeptides are provided. These dual switch chimeric pro-apoptotic polypeptides allow for a choice of ligand inducer. For example, in one example, modified cells are provided that expresses a FRB.FKBP_v. Δ C9 polypeptide, or a FKBP_v.FRB Δ C9 polypeptide; apoptosis may be induced by contacting the modified cell with either a heterodimer, such as rapamycin or a rapalog, or the homodimer, rimiducid.

Thus, in some examples, modified cells are provided that comprise polynucleotides that encode dual switch chimeric pro-apoptotic polypeptides, for example, FRB.FKBP_v. Δ C9 polypeptide, or a FKBP_v.FRB Δ C9 polypeptides, wherein the FRB polypeptide region may be an FRB variant polypeptide region, such as, for example, FRB_L. It is understood that where FRB is denoted, such as, for example, the table of nomenclature herein, other FRB derivatives may be

used, such as, for example, FRB_L. Similarly, where polypeptides comprising FRB_L is provided as an example of a composition or method of the present application, it is understood that RB or FRB variants or derivatives other than FRB_L may be used, with the appropriate ligand, such as rapamycin or a rapalog. It is also understood that FKBP12 variants other than FKBP12v36 may be substituted for FKBP12v36, as appropriate. The modified cells may further comprise polynucleotides that encode a heterologous protein such as, for example, a chimeric antigen receptor or a recombinant T cell receptor. The modified cells may further comprise polynucleotides that encode a costimulatory polypeptide, such as, for example, a polypeptide that comprises a MyD88 polypeptide region, or a truncated MyD88 polypeptide region lacking the TIR domain, or, for example, a polypeptide that comprises a MyD88 polypeptide region or a truncated MyD88 polypeptide region lacking the TIR domain and a CD40 cytoplasmic polypeptide region lacking the extracellular domain. Also provided in some examples are nucleic acids that comprise polynucleotides that encode dual switch chimeric pro-apoptotic polypeptides, for example, FRB.FKBPV.ΔC9 polypeptide, or a FKBPv.FRBΔC9 polypeptides, wherein the FRB polypeptide region may be an FRB variant polypeptide region, such as, for example, FRB_L. The nucleic acids may further comprise polynucleotides that encode a heterologous protein such as, for example, a chimeric antigen receptor or a recombinant T cell receptor. The nucleic acids may further comprise polynucleotides that encode a costimulatory polypeptide, such as, for example, a polypeptide that comprises a MyD88 polypeptide region, or a truncated MyD88 polypeptide region lacking the TIR domain, or, for example, a polypeptide that comprises a MyD88 polypeptide region or a truncated MyD88 polypeptide region lacking the TIR domain and a CD40 cytoplasmic polypeptide region lacking the extracellular domain.

In some examples of the present application, chimeric polypeptides are provided, wherein a first chimeric polypeptide comprises a first multimerizing region that binds to a first ligand; the first multimerizing region comprises a first ligand binding unit and a second ligand binding unit; the first ligand is a multimeric ligand comprising a first portion and a second portion; the first ligand binding unit binds to the first portion of the first ligand and does not bind significantly to the second portion of the first ligand; and the second ligand binding unit binds to the second portion of the first ligand and does not bind significantly to the first portion of the first ligand. In some examples, a second chimeric polypeptide is also provided, wherein the second chimeric polypeptide comprises a second multimerizing region that binds to a second ligand; the second multimerizing region comprises a third ligand binding unit; the second ligand is a multimeric ligand comprising a third portion; and the third ligand binding unit binds to the third portion of the second ligand and does not

bind significantly to the second portion of the first ligand. Examples of first ligand binding units include, but are not limited to, FKBP12 multimerizing regions, or variants, such as FKBP12v36, examples of second ligand binding units are, for example, FRB or FRB variant multimerizing regions. Examples of a third ligand binding unit include, for example, but are not limited to, FKBP12 multimerizing regions, or variants, such as FKBP12v36. In certain examples, the first ligand binding unit is FKBP12, and the third ligand binding unit is FKBP12v36. In certain examples, the first ligand is rapamycin, or a rapalog, and the second ligand is rimiducid (AP1903).

The multimerizing regions, such as FKBP12/FRB, FRB/FKBP12, and FKBP12v36, may be located amino terminal to the pro-apoptotic polypeptide or costimulatory polypeptide, or, in other examples, may be located carboxyl terminal to the pro-apoptotic polypeptide or costimulatory polypeptide. Additional polypeptides, such as, for example, linker polypeptides, stem polypeptides, spacer polypeptides, or in some examples, marker polypeptides, may be located between the multimerizing region and the pro-apoptotic polypeptide or costimulatory polypeptide, in the chimeric polypeptides.

Thus, provided in some examples are modified cells, comprising a first polynucleotide encoding a chimeric pro-apoptotic polypeptide, wherein the chimeric pro-apoptotic polypeptide comprises (i) a pro-apoptotic polypeptide region; (ii) a FKBP12-Rapamycin-Binding (FRB) domain polypeptide, or FRB variant polypeptide region; and (iii) a FKBP12 or FKBP12 variant polypeptide region (FKBP12v); and a second polynucleotide encoding a chimeric costimulating polypeptide, wherein the chimeric costimulating polypeptide comprises one or more, for example, 1, 2, or 3 FKBP12 variant polypeptide regions and i) a MyD88 polypeptide region or a truncated MyD88 polypeptide region lacking the TIR domain; or ii) a MyD88 polypeptide region or a truncated MyD88 polypeptide region lacking the TIR domain, and a CD40 cytoplasmic polypeptide region lacking the CD40 extracellular domain. In a **first** aspect, there is provided a modified cell, comprising a) a first polynucleotide encoding a chimeric pro-apoptotic polypeptide, wherein the chimeric pro-apoptotic polypeptide comprises (i) a pro-apoptotic polypeptide region; (ii) a FKBP12-Rapamycin-Binding (FRB) polypeptide region, or FRB variant polypeptide region; and (iii) a FKBP12 polypeptide region; and b) a second polynucleotide encoding a chimeric costimulating polypeptide, wherein the chimeric costimulating polypeptide comprises two FKBP12 variant polypeptide regions and i) a MyD88 polypeptide region or a truncated MyD88 polypeptide region lacking the TIR domain; or ii) a MyD88 polypeptide region or a truncated MyD88 polypeptide region lacking the TIR domain, and a CD40 cytoplasmic polypeptide region lacking the CD40 extracellular domain, wherein upon contacting the modified cell with rapamycin or a rapalog, the chimeric pro-apoptotic polypeptide dimerizes or multimerizes, thereby inducing apoptosis of the modified cell; and upon contacting the modified cell with rimiducid, the chimeric costimulating polypeptide dimerizes or multimerizes, thereby activating the modified cell. In some examples, the modified cell further comprises a third polynucleotide encoding a chimeric antigen receptor or a recombinant T cell receptor.

Also provided in some examples is a nucleic acid comprising a promoter operably linked to a first polynucleotide encoding a chimeric pro-apoptotic polypeptide, wherein the chimeric pro-apoptotic polypeptide comprises (i) a pro-apoptotic polypeptide region; (ii) a FKBP12-Rapamycin-Binding (FRB) domain polypeptide, or FRB variant polypeptide region; and (iii) a FKBP12 or FKBP12 variant polypeptide region (FKBP12v); and a second polynucleotide encoding a chimeric

costimulating polypeptide, wherein the chimeric costimulating polypeptide comprises one or more, for example, 1, 2, or 3 FKBP12 variant polypeptide regions and i) a MyD88 polypeptide region or a truncated MyD88 polypeptide region lacking the TIR domain; or ii) a MyD88 polypeptide region or a truncated MyD88 polypeptide region lacking the TIR domain, and a CD40 cytoplasmic polypeptide region lacking the CD40 extracellular domain. In a **second** aspect, there is provided a nucleic acid comprising a promoter operably linked to a) a first polynucleotide encoding a chimeric pro-apoptotic polypeptide, wherein the chimeric pro-apoptotic polypeptide comprises (i) a pro-apoptotic polypeptide region; (ii) a FKBP12-Rapamycin-Binding (FRB) polypeptide region, or FRB variant polypeptide region; and (iii) a FKBP12 polypeptide region; and b) a second polynucleotide encoding a chimeric costimulating polypeptide, wherein the chimeric costimulating polypeptide comprises two FKBP12 variant polypeptide regions and i) a MyD88 polypeptide region or a truncated MyD88 polypeptide region lacking the TIR domain; or ii) a MyD88 polypeptide region or a truncated MyD88 polypeptide region lacking the TIR domain, and a CD40 cytoplasmic polypeptide region lacking the CD40 extracellular domain, wherein upon contacting the chimeric pro-apoptotic polypeptide encoded by said first polynucleotide with rapamycin or a rapalog, the chimeric pro-apoptotic polypeptide dimerizes or multimerizes; and upon contacting the chimeric costimulating polypeptide encoded by said second polynucleotide with rimiducid, the chimeric costimulating polypeptide dimerizes or multimerizes. In some examples, the chimeric costimulating polypeptide comprises a truncated MyD88 polypeptide region lacking the TIR domain and a CD40 cytoplasmic polypeptide region lacking the CD40 extracellular domain. In some examples, the promoter is operably linked to a third polynucleotide, wherein the third polynucleotide encodes a chimeric antigen receptor or a recombinant T cell receptor. In some examples, the pro-apoptotic polypeptide is a Caspase-9 polypeptide, wherein the Caspase-9 polypeptide lacks the CARD domain. In some examples, the cell is a T cell, tumor infiltrating lymphocyte, NK-T cell, or NK cell. In a **third** aspect, there is provided a modified cell, comprising the nucleic acid of the second aspect.

Also provided in some examples are kits or compositions comprising nucleic acid comprising a first polynucleotide encoding a chimeric pro-apoptotic polypeptide, wherein the chimeric pro-apoptotic polypeptide comprises (i) a pro-apoptotic polypeptide region; (ii) a FKBP12-Rapamycin-Binding (FRB) domain polypeptide region, or variant thereof; and (iii) a FKBP12 polypeptide or FKBP12 variant polypeptide region (FKBP12v); and a second polynucleotide encoding a chimeric costimulating polypeptide, wherein the chimeric costimulating polypeptide comprises one or more, for example, 1, 2, or 3 FKBP12 variant polypeptide regions and i) a MyD88 polypeptide region or a truncated MyD88 polypeptide region lacking the TIR domain; or ii) a MyD88 polypeptide region or a truncated MyD88 polypeptide region lacking the TIR domain, and a CD40 cytoplasmic polypeptide region lacking the CD40 extracellular domain.

In some examples, methods are provided for expressing a chimeric pro-apoptotic polypeptide, wherein the chimeric pro-apoptotic polypeptide comprises a pro-apoptotic polypeptide region; a FRB polypeptide or FRB variant polypeptide region; and a FKBP12 polypeptide region of the present examples, comprising contacting a nucleic acid of the present examples with a cell under conditions in which the nucleic acid is incorporated into the cell, whereby the cell expresses the chimeric pro-apoptotic polypeptide from the incorporated nucleic acid.

In some examples, methods are provided for stimulating an immune response in a subject, comprising: transplanting modified cells of the present examples into the subject, and after (a), administering an effective amount of a ligand that binds to the FKBP12 variant polypeptide region of the chimeric costimulating polypeptide to stimulate a cell mediated immune response. In some examples, methods are provided for administering a ligand to a subject who has undergone cell therapy using modified cells, comprising administering a ligand that binds to the FKBP variant

region of the chimeric costimulating polypeptide to the human subject, wherein the modified cells comprise modified cells of the present examples the present examples. Also provided are methods for treating a subject having a disease or condition associated with an elevated expression of a target antigen expressed by a target cell, comprising a) transplanting an effective amount of modified cells into the subject; wherein the modified cells comprise a modified cell of the present examples, wherein the modified cell comprises a chimeric antigen receptor or a recombinant T cell receptor comprising an antigen recognition moiety that binds to the target antigen, and b) after a), administering an effective amount of a ligand that binds to the FKBP12 variant polypeptide region of the chimeric costimulating polypeptide to reduce the number or concentration of target antigen or target cells in the subject. Also provided are methods for reducing the size of a tumor in a subject, comprising a) administering a modified cell of the present examples to the subject, wherein the cell comprises a chimeric antigen receptor or a recombinant T cell receptor comprising an antigen recognition moiety that binds to an antigen on the tumor; and b) after a), administering an effective amount of a ligand that binds to the FKBP12 variant polypeptide region of the chimeric costimulating polypeptide to reduce the size of the tumor in the subject. Also provided are methods for controlling survival of transplanted modified cells in a subject, comprising transplanting modified cells of the present examples into the subject; and administering to the subject rapamycin or a rapalog that binds to the FRB polypeptide or FRB variant polypeptide region of the chimeric pro-apoptotic polypeptide in an amount effective to kill at least 30% of the modified cells that express the chimeric pro-apoptotic polypeptide. In a **fourth** aspect, there is provided use of any of the modified cells of the first or third aspects in the manufacture of a medicament for stimulating an immune response in a subject in need of treatment with said modified cells, wherein:

a) the modified cells are to be transplanted into the subject, and
b) after (a), an effective amount of a ligand that binds to the FKBP12 variant polypeptide region of the chimeric costimulating polypeptide is to be administered to said subject, wherein the ligand that binds to the FKBP12 variant polypeptide region causes the chimeric costimulating polypeptide to dimerize or multimerize, thereby activating the modified cells, wherein the modified cells stimulate a cell mediated immune response, and wherein the modified cells are T cells, tumor infiltrating lymphocytes, NK-T cells, or NK cells.

In a **fifth** aspect, there is provided use of a ligand that binds to the FKBP12 variant region of the chimeric costimulating polypeptide in the manufacture of a medicament for administering a ligand to a subject who has undergone cell therapy using modified cells, wherein the modified cells comprise any of the modified cells of the first or third aspects, wherein the ligand that binds to the FKBP12 variant polypeptide region causes the chimeric costimulating polypeptide to dimerize or multimerize, thereby activating the modified cells, wherein the modified cells stimulate a cell mediated immune response, and wherein the modified cells are T cells, tumor infiltrating lymphocytes, NK-T cells, or NK cells.

In a **sixth** aspect, there is provided use of any of the modified cells of the first or third aspects in the manufacture of a medicament for treating a subject having a disease or condition associated with an elevated expression of a target antigen expressed by a target cell, wherein:

a) an effective amount of the modified cells is to be transplanted into the subject; wherein the modified cell comprises a chimeric antigen receptor or a recombinant T cell receptor comprising an antigen recognition moiety that binds to the target antigen, and

b) after a), an effective amount of a ligand that binds to the FKBP12 variant polypeptide region of the chimeric costimulating polypeptide is to be administered to reduce the number or concentration of target antigen or target cells in the subject,

wherein the modified cells are T cells, tumor infiltrating lymphocytes, NK-T cells, or NK cells.

In a **seventh** aspect, there is provided use of any of the modified cells of the first or third aspects in the manufacture of a medicament for reducing the size of a tumor in a subject, wherein:

a) the modified cell of the first or third aspects is to be administered to the subject, wherein the cell comprises a chimeric antigen receptor or a recombinant T cell receptor comprising an antigen recognition moiety that binds to an antigen on the tumor; and

b) after a), an effective amount of a ligand that binds to the FKBP12 variant polypeptide region of the chimeric costimulating polypeptide is to be administered to reduce the size of the tumor in the subject, wherein the modified cells are T cells, tumor infiltrating lymphocytes, NK-T cells, or NK cells.

In an **eighth** aspect, there is provided use of rapamycin or a rapalog that binds to the FRB polypeptide or FRB variant polypeptide region of the chimeric pro-apoptotic polypeptide in the manufacture of a medicament for controlling survival of transplanted modified cells in a subject, wherein:

a) any of the modified cells of the first or third aspects are to be transplanted into the subject; and

b) after a), the rapamycin or rapalog is to be administered in an amount effective to kill at least 30% of the modified cells that express the chimeric pro-apoptotic polypeptide.

In other examples, modified cells are provided comprising a first polynucleotide encoding a chimeric pro-apoptotic polypeptide, wherein the chimeric pro-apoptotic polypeptide comprises i) a pro-apoptotic polypeptide region; and ii) a FKBP12 variant polypeptide region; and a second polynucleotide encoding a chimeric costimulating polypeptide, wherein the chimeric costimulating polypeptide comprises a FKBP12-Rapamycin Binding (FRB) domain polypeptide or FRB variant polypeptide region; a FKBP12 polypeptide or FKBP12 variant polypeptide region; and a MyD88 polypeptide region or a truncated MyD88 polypeptide region lacking the TIR domain, or a MyD88 polypeptide region, or a truncated MyD88 polypeptide region lacking the TIR domain and a CD40 cytoplasmic polypeptide region lacking the CD40 extracellular domain. In a **ninth** aspect, there is provided a modified cell comprising a) a first polynucleotide encoding a chimeric pro-apoptotic polypeptide, wherein the chimeric pro-apoptotic polypeptide comprises i) a pro-apoptotic polypeptide region; and ii) a FKBP12 variant polypeptide region; and b) a second polynucleotide encoding a chimeric costimulating polypeptide, wherein the chimeric costimulating polypeptide comprises i) a FKBP12-Rapamycin Binding (FRB) polypeptide region or FRB variant polypeptide region; ii) a FKBP12 polypeptide region; and iii) a MyD88 polypeptide region or a truncated MyD88 polypeptide region lacking the TIR domain, or a MyD88 polypeptide region, or a truncated MyD88 polypeptide region lacking the TIR domain and a CD40 cytoplasmic polypeptide region lacking the CD40 extracellular domain, wherein upon contacting the modified cell with rimiducid, the chimeric pro-apoptotic polypeptide dimerizes or multimerizes, thereby inducing apoptosis of the modified cell; and upon contacting the modified cell with rapamycin or a rapalog, the chimeric costimulating polypeptide dimerizes or multimerizes, thereby activating the modified cell.

In some examples, the chimeric costimulating polypeptide comprises a truncated MyD88 polypeptide region lacking the TIR domain and a CD40 cytoplasmic polypeptide region lacking the CD40 extracellular domain. In some examples, the cell further comprises a third polynucleotide, wherein the third polynucleotide encodes a chimeric antigen receptor or a recombinant T cell receptor.

In some examples, nucleic acids are provided, wherein the nucleic acids comprise a promoter operably linked to a first polynucleotide encoding a chimeric pro-apoptotic polypeptide, wherein the chimeric pro-apoptotic polypeptide comprises i) a pro-apoptotic polypeptide region; and i) a FKBP12 variant polypeptide region; and a second polynucleotide encoding a chimeric costimulating polypeptide, wherein the chimeric costimulating polypeptide comprises i) a FKBP12-Rapamycin Binding (FRB) domain polypeptide or FRB variant polypeptide region; ii) a FKBP12 polypeptide region; and ii) a MyD88 polypeptide region or a truncated MyD88 polypeptide region lacking the TIR domain, or a MyD88 polypeptide region or a truncated MyD88 polypeptide region lacking the TIR domain and a CD40 cytoplasmic polypeptide region lacking the CD40 extracellular domain. In a **tenth** aspect, there is provided a nucleic acid comprising a promoter operably linked to a) a first polynucleotide encoding a chimeric pro-apoptotic polypeptide, wherein the chimeric pro-apoptotic polypeptide comprises i) a pro-apoptotic polypeptide region; and ii) a FKBP12 variant polypeptide region; and b) a second polynucleotide encoding a chimeric costimulating polypeptide, wherein the chimeric costimulating polypeptide comprises i) a FKBP12-Rapamycin Binding (FRB) polypeptide region or FRB variant polypeptide region; ii) a FKBP12 polypeptide region; and iii) a MyD88 polypeptide region or a truncated MyD88 polypeptide region lacking the TIR domain, or a MyD88 polypeptide region or a truncated MyD88 polypeptide region lacking the TIR domain and a CD40 cytoplasmic polypeptide region lacking the CD40 extracellular domain, wherein upon contacting the chimeric pro-apoptotic polypeptide encoded by said first polynucleotide with rimiducid, the chimeric pro-apoptotic polypeptide dimerizes or multimerizes; and upon contacting the chimeric costimulating polypeptide encoded by said second polynucleotide with rapamycin or a rapalog, the chimeric costimulating polypeptide, dimerizes or multimerizes.

In some examples, the chimeric costimulating polypeptide comprises a truncated MyD88 polypeptide region lacking the TIR domain and a CD40 cytoplasmic polypeptide region lacking the CD40 extracellular domain. In some examples, the promoter is operably linked to a third polynucleotide, wherein the third polynucleotide encodes chimeric antigen receptor or a recombinant T cell receptor. In some examples, the pro-apoptotic polypeptide is a Caspase-9 polypeptide, wherein the Caspase-9 polypeptide lacks the CARD domain. In some examples, the cell is a T cell, tumor infiltrating lymphocyte, NK-T cell, or NK cell. Also provided are kits or compositions comprising nucleic acids comprising polynucleotides of the present examples. Also provided are methods for expressing a chimeric pro-apoptotic polypeptide and a chimeric costimulating polypeptide, wherein a) the chimeric pro-apoptotic polypeptide comprises i) a pro-apoptotic polypeptide region; and ii) a FKBP12 variant polypeptide region; and b) the chimeric costimulating polypeptide comprises a FRB or FRB variant polypeptide region; a FKBP12 polypeptide region; and a MyD88 polypeptide region or a truncated MyD88 polypeptide region lacking the TIR domain, or a MyD88 polypeptide region or a truncated MyD88 polypeptide region lacking the TIR domain and a CD40 cytoplasmic polypeptide region lacking the CD40 extracellular domain comprising contacting a nucleic acid is a nucleic acid comprising a promoter operably linked to a polynucleotide coding for a chimeric pro-apoptotic polypeptide, wherein the chimeric pro-apoptotic polypeptide comprises a) a pro-apoptotic polypeptide region; b) a FKBP12-Rapamycin binding domain (FRB) polypeptide or FRB variant polypeptide region; and c) a FKBP12 variant polypeptide region, with a cell under conditions in which the nucleic acid is incorporated into the cell, whereby the cell expresses the chimeric pro-apoptotic polypeptide and the chimeric costimulating polypeptide from the incorporated nucleic acid.

In an **eleventh** aspect, there is provided a modified cell comprising a nucleic acid of the tenth aspect.

In a **twelfth** aspect, there is provided use of any of the modified cells of the ninth or eleventh aspects in the manufacture of a medicament for stimulating an immune response in a subject in need thereof, wherein:

5 a) the modified cells are to be transplanted into the subject, and
 b) after (a), an effective amount of a rapamycin or a rapalog that binds to the FRB polypeptide or FRB variant polypeptide region of the chimeric costimulating polypeptide is to be administered to said subject, wherein rapamycin or the rapalog causes the chimeric costimulating polypeptide to dimerize or multimerize, thereby activating the modified cells, wherein the modified cells stimulate a cell mediated immune response, and wherein the modified cells are
10 T cells, tumor infiltrating lymphocytes, NK-T cells, or NK cells.

In a **thirteenth** aspect, there is provided use of rapamycin or a rapalog in the manufacture of a medicament for administering a ligand to a subject who has undergone cell therapy using modified cells, wherein the modified cells comprise any of the modified cells of the ninth or eleventh aspects, wherein rapamycin or the rapalog causes the chimeric costimulating polypeptide to
15 dimerize or multimerize, thereby activating the modified cells, wherein the modified cells stimulate a cell mediated immune response, and wherein the modified cells are T cells, tumor infiltrating lymphocytes, NK-T cells, or NK cells.

In a **fourteenth** aspect, there is provided use of any of the modified cells of the ninth or eleventh aspects in the manufacture of a medicament for treating a subject having a disease or condition
20 associated with an elevated expression of a target antigen expressed by a target cell, wherein:

 a) an effective amount of modified cells is to be transplanted into the subject; wherein the modified cell comprises a chimeric antigen receptor or a recombinant T cell receptor comprising an antigen recognition moiety that binds to the target antigen, and

25 b) after a), an effective amount of rapamycin or a rapalog that binds to the FRB polypeptide or FRB variant region of the chimeric costimulating polypeptide is to be administered to reduce the number or concentration of target antigen or target cells in the subject, wherein the modified cells are T cells, tumor infiltrating lymphocytes, NK-T cells, or NK cells.

In a **fifteenth** aspect, there is provided use of any of the modified cells of the ninth or eleventh aspects in the manufacture of a medicament for reducing the size of a tumor in a subject, wherein:

30 a) the modified cell of the ninth or eleventh aspect is to be administered to the subject, wherein the cell comprises a chimeric antigen receptor or a recombinant T cell receptor comprising an antigen recognition moiety that binds to an antigen on the tumor; and

 b) after a), an effective amount of rapamycin or a rapalog that binds to the FRB or FRB variant polypeptide region of the chimeric costimulating polypeptide is to be administered to reduce
35 the size of the tumor in the subject, wherein the modified cells are T cells, tumor infiltrating lymphocytes, NK-T cells, or NK cells.

In a **sixteenth** aspect, there is provided use of a ligand that binds to the FKBP12 variant polypeptide region of the chimeric pro-apoptotic polypeptide in the manufacture of a medicament for controlling survival of transplanted modified cells in a subject, wherein:

40 a) any of the modified cells of the ninth or eleventh aspects are to be transplanted into the subject,
and

 b) after (a), the ligand is to be administered in an amount effective to kill at least 90% of the modified cells that express the chimeric pro-apoptotic polypeptide.

In some examples, methods are provided of stimulating an immune response in a subject, comprising: a) transplanting modified cells of the present examples into the subject, and b) after (a), administering an effective amount of a rapamycin or a rapalog that binds to the FRB polypeptide or FRB variant polypeptide region of the chimeric stimulating polypeptide to stimulate a cell mediated immune response. In some examples, methods are provided of administering a ligand to a subject who has undergone cell therapy using modified cells, comprising administering rapamycin or a rapalog to the subject, wherein the modified cells comprise modified cells of the present examples. In some examples, methods are provided for treating a subject having a disease or condition associated with an elevated expression of a target antigen expressed by a target cell, comprising a) transplanting an effective amount of modified cells into the subject; wherein the modified cells comprise a modified cell of the present examples, wherein the modified cell comprises a chimeric antigen receptor or a recombinant T cell receptor comprising an antigen recognition moiety that binds to the target antigen, and b) after a), administering an effective amount of rapamycin or a rapalog that binds to the FRB polypeptide or FRB variant region of the chimeric stimulating polypeptide to reduce the number or concentration of target antigen or target cells in the subject. In some examples, methods are provided for reducing the size of a tumor in a subject, comprising a) administering a modified cell of the present examples to the subject, wherein the cell comprises a chimeric antigen receptor or a recombinant T cell receptor comprising an antigen recognition moiety that binds to an antigen on the tumor; and b) after a), administering an effective amount of rapamycin or a rapalog that binds to the FRB or FRB variant polypeptide region of the chimeric stimulating polypeptide to reduce the size of the tumor in the subject. In some examples, methods are provided for controlling survival of transplanted modified cells in a subject, comprising a) transplanting modified cells of the present examples into the subject, and after (a), administering to the subject a ligand that binds to the FKBP12 variant polypeptide region of the chimeric pro-apoptotic polypeptide in an amount effective to kill at least 90% of the modified cells that express the chimeric pro-apoptotic polypeptide.

In some examples of the present application, the chimeric costimulating polypeptide comprises two FKBP12 variant polypeptide regions, and a truncated MyD88 polypeptide region lacking the TIR domain. In some examples, the chimeric costimulating polypeptide further comprises a CD40 cytoplasmic polypeptide region lacking the CD40 extracellular domain. In some examples of the present application, the chimeric costimulating polypeptide comprises 2 FKBP12 variant polypeptide regions.

Also provided in the present application is a nucleic acid comprising a promoter operably linked to a polynucleotide coding for a chimeric pro-apoptotic polypeptide, wherein the chimeric pro-apoptotic polypeptide comprises a) a pro-apoptotic polypeptide region; b) a FKBP12-Rapamycin binding domain (FRB) polypeptide or FRB variant polypeptide region; and c) a FKBP12 variant polypeptide region. In one example, there is provided a nucleic acid comprising a promoter operably linked to a polynucleotide coding for a chimeric pro-apoptotic polypeptide, wherein the chimeric pro-apoptotic polypeptide comprises a) a pro-apoptotic polypeptide region; b) a FKBP12-Rapamycin binding (FRB) polypeptide region or FRB variant polypeptide region; and c) a FKBP12 variant polypeptide region. In some examples, wherein the FKBP12 variant comprises an amino acid substitution at amino acid residue 36. In some examples, the FKBP12 variant polypeptide region is a FKBP12v36 polypeptide region. In some examples, the FRB variant polypeptide region is selected from the group consisting of KLV (T2098L) (FRBL), KTF (W2101F), and KLF (T2098L, W2101F). In some examples, a chimeric pro-apoptotic polypeptide encoded by a nucleic acid of the present examples is provided. In some examples, modified cells are provided that are transfected or transduced with a nucleic acid of the present examples. In some examples, the modified cells comprise a polynucleotide that encodes a chimeric antigen receptor or a recombinant TCR. In some examples, methods are provided of controlling survival of transplanted modified cells in a subject, comprising: a) transplanting modified cells of the present examples, wherein the modified cells comprise a nucleic acid comprising a promoter operably linked to a polynucleotide coding for a chimeric pro-apoptotic polypeptide, wherein the chimeric pro-apoptotic polypeptide comprises a) a pro-apoptotic polypeptide region; b) a FKBP12-Rapamycin binding domain (FRB) polypeptide or FRB variant polypeptide region; and c) a FKBP12 variant polypeptide region. of the present examples into the subject; and b) after (a), administering to the subject i) a first ligand that binds to the FRB or FRB variant polypeptide region of the chimeric pro-apoptotic polypeptide; or ii) a second ligand that binds to the FKBP12 variant polypeptide region of the chimeric pro-apoptotic polypeptide wherein the first ligand or the second ligand are administered in an amount effective to kill at least 30% of the modified cells that express the chimeric pro-apoptotic polypeptide.

Autologous T cells expressing chimeric antigen receptors (CARs) directed toward tumor-associated antigens (TAAs) have had a transformational effect in initial clinical trials on the treatment of certain types of leukemias ("liquid tumors") and lymphomas with objective response (OR) rates approaching 90%. Despite their great clinical promise and the predictable accompanying enthusiasm, this success is tempered by the observed high level of on-target, off-tumor adverse events, typical of a cytokine release syndrome (CRS). To maintain the benefit of these revolutionary treatments while minimizing the risk, a tunable safety switch has been developed, in order to control the activity level of CAR-expressing T cells. An inducible costimulatory chimeric polypeptide allows for a sustained, modulated control of a chimeric antigen

receptor (CAR) that is co-expressed in the cell. The ligand inducer activates the CAR-expressing cell by multimerizing the inducible chimeric signaling molecules, which, in turn, induces NF- κ B and other intracellular signaling pathways, leading to the activation of the target cells, for example, a T cell, a tumor-infiltrating lymphocyte (TIL), a natural killer (NK) cell, or a natural killer T (NK-T) cell.

5 In the absence of the ligand inducer, the T cell is quiescent, or has a basal level of activity.

At the second level of control, a “dimmer” switch may allow for continued cell therapy, while reducing or eliminating significant side effects by eliminating the therapeutic cells from the subject, as needed. This dimmer switch is dependent on a second ligand inducer. In some examples,
10 where there is a need to rapidly eliminate the therapeutic cells, an appropriate dose of the second ligand inducer is administered in order to eliminate over 90% or 95% of the therapeutic cells from the patient. This second level of control may be “tunable,” that is, the level of removal of the therapeutic cells may be controlled so that it results in partial removal of the therapeutic cells. This second level of control may include, for example, a chimeric pro-apoptotic polypeptide.

15 In some examples, the chimeric apoptotic polypeptide comprises a binding site for rapamycin, or a rapamycin analog (rapalog); also present in the therapeutic cell is an inducible chimeric polypeptide that, upon induction by a ligand inducer, activates the therapeutic cell; in some examples, the inducible chimeric polypeptide provides costimulatory activity to the therapeutic cell.
20 The CAR may be present on a separate polypeptide expressed in the cell. In other examples, the CAR may be present as part of the same polypeptide as the inducible chimeric polypeptide. Using this controllable first level, the need for continued therapy, or the need to stimulate therapy, may be balanced with the need to eliminate or reduce the level of negative side effects.

25 In some examples, a rapamycin analog, or “rapalog”, is administered to the patient, which then binds to both the caspase polypeptide and the chimeric antigen receptor, thus recruiting the caspase polypeptide to the location of the CAR, and aggregating the caspase polypeptide. Upon aggregation, the caspase polypeptide induces apoptosis. The amount of rapamycin or rapamycin analog administered to the patient may vary; if the removal of a lower level of cells by apoptosis is
30 desired in order to reduce side effects and continue CAR therapy, a lower level of rapamycin or rapalog may be administered to the patient.

At the second level of therapeutic cell elimination, selective apoptosis may be induced in cells that express a chimeric Caspase-9 polypeptide fused to a dimeric ligand binding polypeptide, such as,

for example, the AP1903-binding polypeptide FKBP12v36, by administering rimiducid (AP1903). In some examples, the Caspase-9 polypeptide includes amino acid substitutions that result in a lower level of basal apoptotic activity as part of the inducible chimeric polypeptide, than the wild type Caspase-9 polypeptide.

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In some examples, the nucleic acid encoding the chimeric polypeptides of the present application further comprise a polynucleotide encoding a chimeric antigen receptor, a T cell receptor, or a T cell receptor-based chimeric antigen receptor. In some examples, the chimeric antigen receptor comprises (i) a transmembrane region, (ii) a T cell activation molecule, and (iii) an antigen
10 recognition moiety. Also provided are modified cells transfected or transduced with a nucleic acid discussed herein

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In some examples of the present application, the cells are transduced or transfected with a viral vector. The viral vector may be, for example, but not limited to, a retroviral vector, such as, for example, but not limited to, a murine leukemia virus vector; an SFG vector; and adenoviral vector, or a lentiviral vector.

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In some examples, the cell is isolated. In some examples, the cell is in a human subject. In some examples, the cell is transplanted in a human subject.

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In some examples, personalized treatment is provided wherein the stage or level of the disease or condition is determined before administration of the multimeric ligand, before the administration of an additional dose of the multimeric ligand, or in determining method and dosage involved in the administration of the multimeric ligand. These methods may be used in any of the methods of any

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of the diseases or conditions of the present application. Where these methods of assessing the patient before administering the ligand are discussed in the context of graft versus host disease, it is understood that these methods may be similarly applied to the treatment of other conditions and diseases. Thus, for example, in some examples of the present application, the method comprises administering therapeutic cells to a patient, and further comprises identifying a presence or
35 absence of a condition in the patient that requires the removal of transfected or transduced therapeutic cells from the patient; and administering a multimeric ligand that binds to the multimerizing region, maintaining a subsequent dosage of the multimeric ligand, or adjusting a subsequent dosage of the multimeric ligand to the patient based on the presence or absence of the condition identified in the patient. And, for example, in other examples of the present

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application, the method further comprises determining whether to administer an additional dose or additional doses of the multimeric ligand to the patient based upon the appearance of graft versus host disease symptoms in the patient. In some examples, the method further comprises identifying the presence, absence or stage of graft versus host disease in the patient, and administering a

5 multimeric ligand that binds to the multimerizing region, maintaining a subsequent dosage of the multimeric ligand, or adjusting a subsequent dosage of the multimeric ligand to the patient based on the presence, absence or stage of the graft versus host disease identified in the patient. In some examples, the method further comprises identifying the presence, absence or stage of graft versus host disease in the patient, and determining whether a multimeric ligand that binds to the

10 multimerizing region should be administered to the patient, or the dosage of the multimeric ligand subsequently administered to the patient is adjusted based on the presence, absence or stage of the graft versus host disease identified in the patient. In some examples, the method further comprises receiving information comprising the presence, absence or stage of graft versus host disease in the patient; and administering a multimeric ligand that binds to the multimerizing region,

15 maintaining a subsequent dosage of the multimeric ligand, or adjusting a subsequent dosage of the multimeric ligand to the patient based on the presence, absence or stage of the graft versus host disease identified in the patient. In some examples, the method further comprises identifying the presence, absence or stage of graft versus host disease in the patient, and transmitting the presence, absence or stage of the graft versus host disease to a decision maker who administers a

20 multimeric ligand that binds to the multimerizing region, maintains a subsequent dosage of the multimeric ligand, or adjusts a subsequent dosage of the multimeric ligand administered to the patient based on the presence, absence or stage of the graft versus host disease identified in the subject. In some examples, the method further comprises identifying the presence, absence or stage of graft versus host disease in the patient, and transmitting an indication to administer a

25 multimeric ligand that binds to the multimeric binding region, maintain a subsequent dosage of the multimeric ligand or adjust a subsequent dosage of the multimeric ligand administered to the patient based on the presence, absence or stage of the graft versus host disease identified in the subject.

30 Also provided is a method for administering donor T cells to a human patient, comprising administering a transduced or transfected T cell of the present application to a human patient, wherein the cells are non-allodepleted human donor T cells.

In some examples, the therapeutic cells are administered to a subject having a non-malignant disorder, or where the subject has been diagnosed with a non-malignant disorder, such as, for example, a primary immune deficiency disorder (for example, but not limited to, Severe Combined Immune Deficiency (SCID), Combined Immune Deficiency (CID), Congenital T-cell

- 5 Defect/Deficiency, Common Variable Immune Deficiency (CVID), Chronic Granulomatous Disease, IPEX (Immune deficiency, polyendocrinopathy, enteropathy, X-linked) or IPEX-like, Wiskott-Aldrich Syndrome, CD40 Ligand Deficiency, Leukocyte Adhesion Deficiency, DOCK 8 Deficiency, IL-10 Deficiency/IL-10 Receptor Deficiency, GATA 2 deficiency, X-linked lymphoproliferative disease (XLP), Cartilage Hair Hypoplasia, and the like), Hemophagocytosis Lymphohistiocytosis (HLH) or
10 other hemophagocytic disorders, Inherited Marrow Failure Disorders (such as, for example, but not limited to, Shwachman Diamond Syndrome, Diamond Blackfan Anemia, Dyskeratosis Congenita, Fanconi Anemia, Congenital Neutropenia, and the like), Hemoglobinopathies (such as, for example, but not limited to, Sickle Cell Disease, Thalassemia, and the like), Metabolic Disorders (such as, for example, but not limited to, Mucopolysaccharidosis, Sphingolipidoses, and the like),
15 or an Osteoclast disorder (such as, for example, but not limited to Osteopetrosis).

- The therapeutic cells may be, for example, any cell administered to a patient for a desired therapeutic result. The cells may be, for example, T cells, natural killer cells, B cells, macrophages, peripheral blood cells, hematopoietic progenitor cells, bone marrow cells, or tumor
20 cells. The modified Caspase-9 polypeptide can also be used to directly kill tumor cells. In one application, vectors comprising polynucleotides coding for the inducible modified Caspase-9 polypeptide would be injected into a tumor and after 10-24 hours (to permit protein expression), the ligand inducer, such as, for example, AP1903, would be administered to trigger apoptosis, causing the release of tumor antigens to the microenvironment. To further improve the tumor
25 microenvironment to be more immunogenic, the treatment may be combined with one or more adjuvants (e.g., IL-12, TLRs, IDO inhibitors, etc.). In some examples, the cells may be delivered to treat a solid tumor, such as, for example, delivery of the cells to a tumor bed. In some examples, a polynucleotide encoding the chimeric Caspase-9 polypeptide may be administered as part of a vaccine, or by direct delivery to a tumor bed, resulting in expression of the chimeric Caspase-9
30 polypeptide in the tumor cells, followed by apoptosis of tumor cells following administration of the ligand inducer. Thus, also provided in some examples are nucleic acid vaccines, such as DNA vaccines, wherein the vaccine comprises a nucleic acid comprising a polynucleotide that encodes an inducible, or modified inducible Caspase-9 polypeptide of the present application. The vaccine may be administered to a subject, thereby

transforming or transducing target cells in vivo. The ligand inducer is then administered following the methods of the present application.

- 5 In some examples, the modified Caspase-9 polypeptide is a truncated modified Caspase-9 polypeptide. In some examples, the modified Caspase-9 polypeptide lacks the Caspase recruitment domain. In some examples, the Caspase-9 polypeptide comprises the amino acid sequence of SEQ ID NO: 9, or a fragment thereof, or is encoded by the nucleotide sequence of SEQ ID NO: 8, or a fragment thereof.
- 10 In some examples, the methods further comprise administering a multimeric ligand that binds to the multimeric ligand binding region. In some examples, the multimeric ligand binding region is selected from the group consisting of FKBP, cyclophilin receptor, steroid receptor, tetracycline receptor, heavy chain antibody subunit, light chain antibody subunit, single chain antibodies comprised of heavy and light chain variable regions in tandem separated by a flexible linker
- 15 domain, and mutated sequences thereof. In some examples, the multimeric ligand binding region is an FKBP12 region. In some examples, the multimeric ligand is an FK506 dimer or a dimeric FK506-like analog ligand. In some examples, the multimeric ligand is AP1903. In some examples, the number of therapeutic cells is reduced by from about 60% to 99%, about 70% to 95%, from 80% to 90% or about 90% or more after administration of the multimeric ligand. In some
- 20 examples, after administration of the multimeric ligand, donor T cells survive in the patient that are able to expand and are reactive to viruses and fungi. In some examples, after administration of the multimeric ligand, donor T cells survive in the patient that are able to expand and are reactive to tumor cells in the patient.
- 25 In some examples, the suicide gene used in the second level of control is a caspase polypeptide, for example, Caspase 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14. In certain examples, the caspase polypeptide is a Caspase-9 polypeptide. In certain examples, the Caspase-9 polypeptide comprises an amino acid sequence of a catalytically active (not catalytically dead) caspase variant polypeptide provided in Table 5 or 6 herein. In other examples, the Caspase-9 polypeptide
- 30 consists of an amino acid sequence of a catalytically active (not catalytically dead) caspase variant polypeptide provided in Table 5 or 6 herein. In other examples, a caspase polypeptide may be used that has a lower basal activity in the absence of the ligand inducer. For example, when included as part of a chimeric inducible caspase polypeptide, certain modified Caspase-9 polypeptides may have lower basal activity compared to

wild type Caspase-9 in the chimeric construct. For example, the modified Caspase-9 polypeptide may comprise an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 9, and may comprise at least one amino acid substitution.

- 5 Certain examples are described further in the following description, examples, claims and drawings.

Brief Description of the Drawings

- 10 The drawings illustrate embodiments of the technology and are not limiting. For clarity and ease of illustration, the drawings are not made to scale and, in some instances, various aspects may be shown exaggerated or enlarged to facilitate an understanding of particular embodiments.

- Fig. 1A illustrates various iCasp9 expression vectors as discussed herein. FIG. 1B illustrates a
15 representative western blot of full length and truncated Caspase-9 protein produced by the expression vectors shown in FIG. 1A.

Fig. 2 is a schematic of the interaction of the suicide gene product and the CID to cause apoptosis.

- 20 Fig. 3 is a schematic depicting a two-tiered regulation of apoptosis. The left section depicts rapalog-mediated recruitment of an inducible caspase polypeptide to FRBI-modified CAR. The right section depicts a rimiducid (AP1903)-mediated inducible caspase polypeptide.

- Fig. 4 is a plasmid map of a vector encoding FRB_L-modified CD19-MC-CAR and inducible
25 Caspase-9. pSFG-iCasp9-2A-CD19-Q-CD28stm-MCz-FRB_L2.

Fig. 5 is a plasmid map of a vector encoding FRB_L-modified Her2-MC-CAR and an inducible Caspase-9 polypeptide. pSFG-iCasp9-2A-aHer2-Q_CD28stm-mMCz-FRB_L2.

- 30 Figs. 6A and 6B provide the results of an assay of two-tiered activation of apoptosis. Fig. 6A shows recruitment of an inducible Caspase-9 polypeptide (iC9) with rapamycin, leading to more gradual apoptosis titration. Fig. 6B shows complete apoptosis using rimiducid (AP1903).

What is claimed is:

1. A modified cell, comprising
 - a) a first polynucleotide encoding a chimeric pro-apoptotic polypeptide, wherein the chimeric pro-apoptotic polypeptide comprises
 - (i) a pro-apoptotic polypeptide region;
 - (ii) a FKBP12-Rapamycin-Binding (FRB) polypeptide region, or FRB variant polypeptide region; and
 - (iii) a FKBP12 polypeptide region; and
 - b) a second polynucleotide encoding a chimeric costimulating polypeptide, wherein the chimeric costimulating polypeptide comprises two FKBP12 variant polypeptide regions and
 - i) a MyD88 polypeptide region or a truncated MyD88 polypeptide region lacking the TIR domain; or
 - ii) a MyD88 polypeptide region or a truncated MyD88 polypeptide region lacking the TIR domain, and a CD40 cytoplasmic polypeptide region lacking the CD40 extracellular domain,wherein upon contacting the modified cell with rapamycin or a rapalog, the chimeric pro-apoptotic polypeptide dimerizes or multimerizes, thereby inducing apoptosis of the modified cell; and upon contacting the modified cell with rimiducid, the chimeric costimulating polypeptide dimerizes or multimerizes, thereby activating the modified cell.
2. The modified cell of claim 1, wherein the cell comprises a third polynucleotide encoding a chimeric antigen receptor or a recombinant T cell receptor.
3. A nucleic acid comprising a promoter operably linked to
 - a) a first polynucleotide encoding a chimeric pro-apoptotic polypeptide, wherein the chimeric pro-apoptotic polypeptide comprises
 - (i) a pro-apoptotic polypeptide region;
 - (ii) a FKBP12-Rapamycin-Binding (FRB) polypeptide region, or FRB variant polypeptide region; and
 - (iii) a FKBP12 polypeptide region; and
 - b) a second polynucleotide encoding a chimeric costimulating polypeptide, wherein the chimeric costimulating polypeptide comprises two FKBP12 variant polypeptide regions and
 - i) a MyD88 polypeptide region or a truncated MyD88 polypeptide region lacking the TIR domain; or
 - ii) a MyD88 polypeptide region or a truncated MyD88 polypeptide region lacking the TIR domain, and a CD40 cytoplasmic polypeptide region lacking the CD40 extracellular domain,wherein upon contacting the chimeric pro-apoptotic polypeptide encoded by said first polynucleotide with rapamycin or a rapalog, the chimeric pro-apoptotic polypeptide dimerizes or multimerizes; and upon contacting the chimeric costimulating polypeptide encoded by said second polynucleotide with rimiducid, the chimeric costimulating polypeptide dimerizes or multimerizes.

4. The nucleic acid of claim 3, wherein the promoter is operably linked to a third polynucleotide that encodes a chimeric antigen receptor or a recombinant T cell receptor.
5. A modified cell, comprising a nucleic acid of claim 4.
6. The modified cell of any one of claims 1, 2, or 5, or the nucleic acid of any one of claims 3 or 4, wherein the chimeric costimulating polypeptide comprises a truncated MyD88 polypeptide region lacking the TIR domain and a CD40 cytoplasmic polypeptide region lacking the CD40 extracellular domain.
7. The modified cell of any one of claims 1, 2, 5, or 6, or the nucleic acid of any one of claims 3, 4, or 6, wherein the pro-apoptotic polypeptide is a Caspase-9 polypeptide that lacks the CARD domain.
8. The modified cell of any one of claims 1, 2, or 5 to 7, wherein the cell is a T cell, tumor infiltrating lymphocyte, NK-T cell, or NK cell.
9. Use of modified cells of any one of claims 1, 2, or 5 to 8 in the manufacture of a medicament for stimulating an immune response in a subject in need of treatment with said modified cells, wherein:
 - a) the modified cells are to be transplanted into the subject, and
 - b) after (a), an effective amount of a ligand that binds to the FKBP12 variant polypeptide region of the chimeric costimulating polypeptide is to be administered to said subject, wherein the ligand that binds to the FKBP12 variant polypeptide region causes the chimeric costimulating polypeptide to dimerize or multimerize, thereby activating the modified cells, wherein the modified cells stimulate a cell mediated immune response, and wherein the modified cells are T cells, tumor infiltrating lymphocytes, NK-T cells, or NK cells.
10. Use of a ligand that binds to the FKBP12 variant region of the chimeric costimulating polypeptide in the manufacture of a medicament for administering a ligand to a subject who has undergone cell therapy using modified cells, wherein the modified cells comprise modified cells of any one of claims 1, 2, or 5 to 8, wherein the ligand that binds to the FKBP12 variant polypeptide region causes the chimeric costimulating polypeptide to dimerize or multimerize, thereby activating the modified cells, wherein the modified cells stimulate a cell mediated immune response, and wherein the modified cells are T cells, tumor infiltrating lymphocytes, NK-T cells, or NK cells.
11. Use of a modified cell of any one of claims 1, 2, or 5 to 8 in the manufacture of a medicament for treating a subject having a disease or condition associated with an elevated expression of a target antigen expressed by a target cell, wherein:
 - a) an effective amount of the modified cells is to be transplanted into the subject; wherein the modified cell comprises a chimeric antigen receptor or a recombinant T cell receptor comprising an antigen recognition moiety that binds to the target antigen, and
 - b) after a), an effective amount of a ligand that binds to the FKBP12 variant polypeptide region of the chimeric costimulating polypeptide is to be administered to reduce the number or concentration of target antigen or target cells in the subject, wherein the modified cells are T cells, tumor infiltrating lymphocytes, NK-T cells, or NK cells.

12. Use of a modified cell of any one of claims 1, 2, or 5 to 8 in the manufacture of a medicament for reducing the size of a tumor in a subject, wherein:

a) the modified cell of any one of claims 1, 2, or 5 to 8 is to be administered to the subject, wherein the cell comprises a chimeric antigen receptor or a recombinant T cell receptor comprising an antigen recognition moiety that binds to an antigen on the tumor; and

b) after a), an effective amount of a ligand that binds to the FKBP12 variant polypeptide region of the chimeric costimulating polypeptide is to be administered to reduce the size of the tumor in the subject,

wherein the modified cells are T cells, tumor infiltrating lymphocytes, NK-T cells, or NK cells.

13. Use of rapamycin or a rapalog that binds to the FRB polypeptide or FRB variant polypeptide region of the chimeric pro-apoptotic polypeptide in the manufacture of a medicament for controlling survival of transplanted modified cells in a subject, wherein:

a) modified cells of any one of claims 1, 2, or 5 to 8 are to be transplanted into the subject; and

b) after a), the rapamycin or rapalog is to be administered in an amount effective to kill at least 30% of the modified cells that express the chimeric pro-apoptotic polypeptide.

14. A modified cell comprising

a) a first polynucleotide encoding a chimeric pro-apoptotic polypeptide, wherein the chimeric pro-apoptotic polypeptide comprises

- i) a pro-apoptotic polypeptide region; and
- ii) a FKBP12 variant polypeptide region; and

b) a second polynucleotide encoding a chimeric costimulating polypeptide, wherein the chimeric costimulating polypeptide comprises

- i) a FKBP12-Rapamycin Binding (FRB) polypeptide region or FRB variant polypeptide region;
- ii) a FKBP12 polypeptide region; and
- iii) a MyD88 polypeptide region or a truncated MyD88 polypeptide region lacking the TIR domain, or a MyD88 polypeptide region, or a truncated MyD88 polypeptide region lacking the TIR domain and a CD40 cytoplasmic polypeptide region lacking the CD40 extracellular domain,

wherein upon contacting the modified cell with rimiducid, the chimeric pro-apoptotic polypeptide dimerizes or multimerizes, thereby inducing apoptosis of the modified cell; and upon contacting the modified cell with rapamycin or a rapalog, the chimeric costimulating polypeptide dimerizes or multimerizes, thereby activating the modified cell.

15. The modified cell of claim 14, wherein the cell comprises a third polynucleotide, wherein the third polynucleotide encodes a chimeric antigen receptor or a recombinant T cell receptor.

16. A nucleic acid comprising a promoter operably linked to
- a) a first polynucleotide encoding a chimeric pro-apoptotic polypeptide, wherein the chimeric pro-apoptotic polypeptide comprises
 - i) a pro-apoptotic polypeptide region; and
 - ii) a FKBP12 variant polypeptide region; and
 - b) a second polynucleotide encoding a chimeric costimulating polypeptide, wherein the chimeric costimulating polypeptide comprises
 - i) a FKBP12-Rapamycin Binding (FRB) polypeptide region or FRB variant polypeptide region;
 - ii) a FKBP12 polypeptide region; and
 - iii) a MyD88 polypeptide region or a truncated MyD88 polypeptide region lacking the TIR domain, or a MyD88 polypeptide region or a truncated MyD88 polypeptide region lacking the TIR domain and a CD40 cytoplasmic polypeptide region lacking the CD40 extracellular domain,wherein upon contacting the chimeric pro-apoptotic polypeptide encoded by said first polynucleotide with rimiducid, the chimeric pro-apoptotic polypeptide dimerizes or multimerizes; and upon contacting the chimeric costimulating polypeptide encoded by said second polynucleotide with rapamycin or a rapalog, the chimeric costimulating polypeptide, dimerizes or multimerizes.
17. The nucleic acid of claim 16, wherein the promoter is operably linked to a third polynucleotide that encodes chimeric antigen receptor or a recombinant T cell receptor.
18. A modified cell comprising a nucleic acid of claim 17.
19. The modified cell of any one of claims 14 or 15, or the nucleic acid of any one of claims 16 or 17, wherein the chimeric costimulating polypeptide comprises a truncated MyD88 polypeptide region lacking the TIR domain and a CD40 cytoplasmic polypeptide region lacking the CD40 extracellular domain.
20. The modified cell of any one of claims 14, 15, 18, or 19, or the nucleic acid of any one of claims 16, 17 or 19, wherein the pro-apoptotic polypeptide is a Caspase-9 polypeptide that lacks CARD domain.
21. The modified cell of any one of claims 14, 15, or 18 to 20, wherein the cell is a T cell, tumor infiltrating lymphocyte, NK-T cell, or NK cell.
22. Use of modified cells of any one of claims 14, 15, or 18 to 21 in the manufacture of a medicament for stimulating an immune response in a subject in need thereof, wherein:
- a) the modified cells are to be transplanted into the subject, and
 - b) after (a), an effective amount of a rapamycin or a rapalog that binds to the FRB polypeptide or FRB variant polypeptide region of the chimeric costimulating polypeptide is to be administered to said subject, wherein rapamycin or the rapalog causes the chimeric costimulating polypeptide to dimerize or multimerize, thereby activating the modified cells, wherein the modified cells stimulate a cell mediated immune response, and wherein the modified cells are T cells, tumor infiltrating lymphocytes, NK-T cells, or NK cells.

23. Use of rapamycin or a rapalog in the manufacture of a medicament for administering a ligand to a subject who has undergone cell therapy using modified cells, wherein the modified cells comprise modified cells of any one of claims 14, 15, or 18 to 21, wherein rapamycin or the rapalog causes the chimeric costimulating polypeptide to dimerize or multimerize, thereby activating the modified cells, wherein the modified cells stimulate a cell mediated immune response, and wherein the modified cells are T cells, tumor infiltrating lymphocytes, NK-T cells, or NK cells.

24. Use of a modified cell of any one of claims 14, 15, or 18 to 21 in the manufacture of a medicament for treating a subject having a disease or condition associated with an elevated expression of a target antigen expressed by a target cell, wherein:

a) an effective amount of modified cells is to be transplanted into the subject; wherein the modified cell comprises a chimeric antigen receptor or a recombinant T cell receptor comprising an antigen recognition moiety that binds to the target antigen, and

b) after a), an effective amount of rapamycin or a rapalog that binds to the FRB polypeptide or FRB variant region of the chimeric costimulating polypeptide is to be administered to reduce the number or concentration of target antigen or target cells in the subject,

wherein the modified cells are T cells, tumor infiltrating lymphocytes, NK-T cells, or NK cells.

25. Use of a modified cell of any one of claims 14, 15, or 18 to 21 in the manufacture of a medicament for reducing the size of a tumor in a subject, wherein:

a) the modified cell of any one of claims 14, 15, or 18 to 21 is to be administered to the subject, wherein the cell comprises a chimeric antigen receptor or a recombinant T cell receptor comprising an antigen recognition moiety that binds to an antigen on the tumor; and

b) after a), an effective amount of rapamycin or a rapalog that binds to the FRB or FRB variant polypeptide region of the chimeric costimulating polypeptide is to be administered to reduce the size of the tumor in the subject,

wherein the modified cells are T cells, tumor infiltrating lymphocytes, NK-T cells, or NK cells.

26. Use of a ligand that binds to the FKBP12 variant polypeptide region of the chimeric pro-apoptotic polypeptide in the manufacture of a medicament for controlling survival of transplanted modified cells in a subject, wherein:

a) the modified cells of any one of claims 14, 15, or 18 to 21 are to be transplanted into the subject, and

b) after (a), the ligand is to be administered in an amount effective to kill at least 90% of the modified cells that express the chimeric pro-apoptotic polypeptide.