US 20250262313A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2025/0262313 A1**

Low et al. (43) **Pub. Date: Aug. 21, 2025**

(54) **TARGETED LIGAND-PAYLOAD BASED DRUG DELIVERY FOR CELL THERAPY**

(71) Applicant: **Purdue Research Foundation**, West Lafayette, IN (US)

(72) Inventors: **Philip S. Low**, West Lafayette, IN (US); **Madduri SRINIVASARAO**, West Lafayette, IN (US); **Boning Zhang**, West Lafayette, IN (US)

(21) Appl. No.: **19/194,112**

(22) Filed: **Apr. 30, 2025**

Related U.S. Application Data

(62) Division of application No. 16/486,632, filed on Aug. 16, 2019, filed as application No. PCT/US2018/018557 on Feb. 17, 2018, now Pat. No. 12,343,403.

(60) Provisional application No. 62/460,118, filed on Feb. 17, 2017.

Publication Classification

(51) **Int. Cl.**

A61K 47/66	(2017.01)
A61K 38/00	(2006.01)
A61K 40/10	(2025.01)
A61K 40/11	(2025.01)
A61K 40/31	(2025.01)
A61K 40/42	(2025.01)
A61K 47/69	(2017.01)
C07K 14/725	(2006.01)
C12N 9/90	(2006.01)
C12N 15/62	(2006.01)

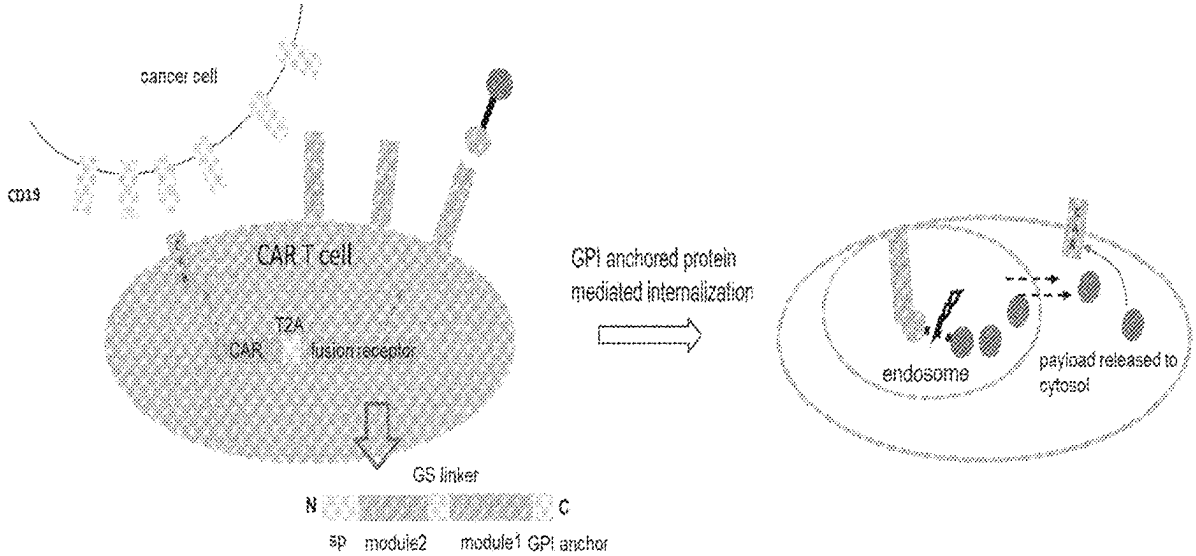
(52) **U.S. Cl.**

CPC ***A61K 47/66*** (2017.08); ***A61K 40/10*** (2025.01); ***A61K 40/11*** (2025.01); ***A61K 40/31*** (2025.01); ***A61K 40/4211*** (2025.01); ***A61K 47/6901*** (2017.08); ***C07K 14/7051*** (2013.01); ***C12N 9/90*** (2013.01); ***C12N 15/62*** (2013.01); *A61K 38/00* (2013.01); *A61K 2239/31* (2023.05); *A61K 2239/38* (2023.05); *A61K 2239/48* (2023.05); *C07K 2319/03* (2013.01); *C07K 2319/20* (2013.01); *C07K 2319/912* (2013.01); *C12Y 502/01008* (2013.01)

(57) **ABSTRACT**

A drug delivery platform providing flexible fine tune of cell therapy is disclosed herein. Particularly, an engineered fusion protein is coupled with a high affinity ligand carrying at least one payload of drug to be internalized by the transplanted cell to observe or regulate transplanted cell therapy effects.

Specification includes a Sequence Listing.



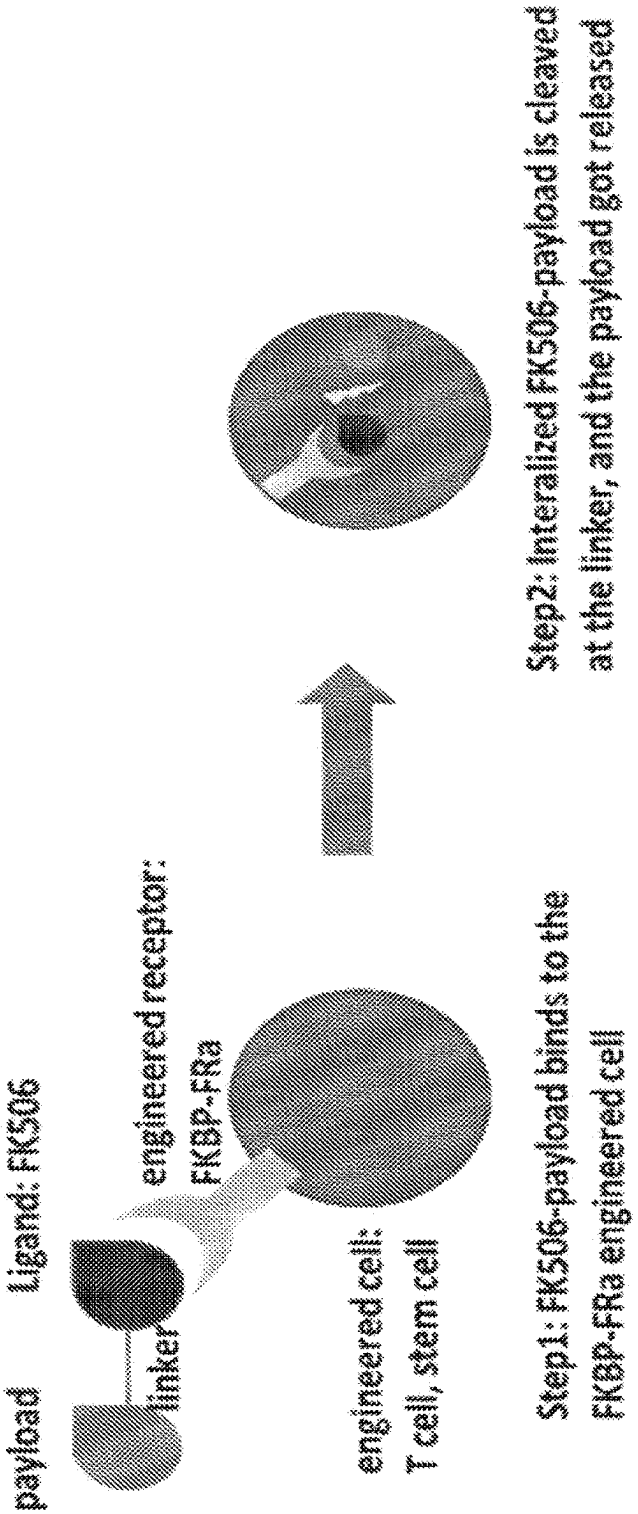

payload
linker
Ligand: FK506
engineered receptor:
FKBP-FRa
engineered cell:
T cell, stem cell
Step1: FK506-payload binds to the
FKBP-FRa engineered cell
Step2: interalized FK506-payload is cleaved
at the linker, and the payload got released

FIG. 1A

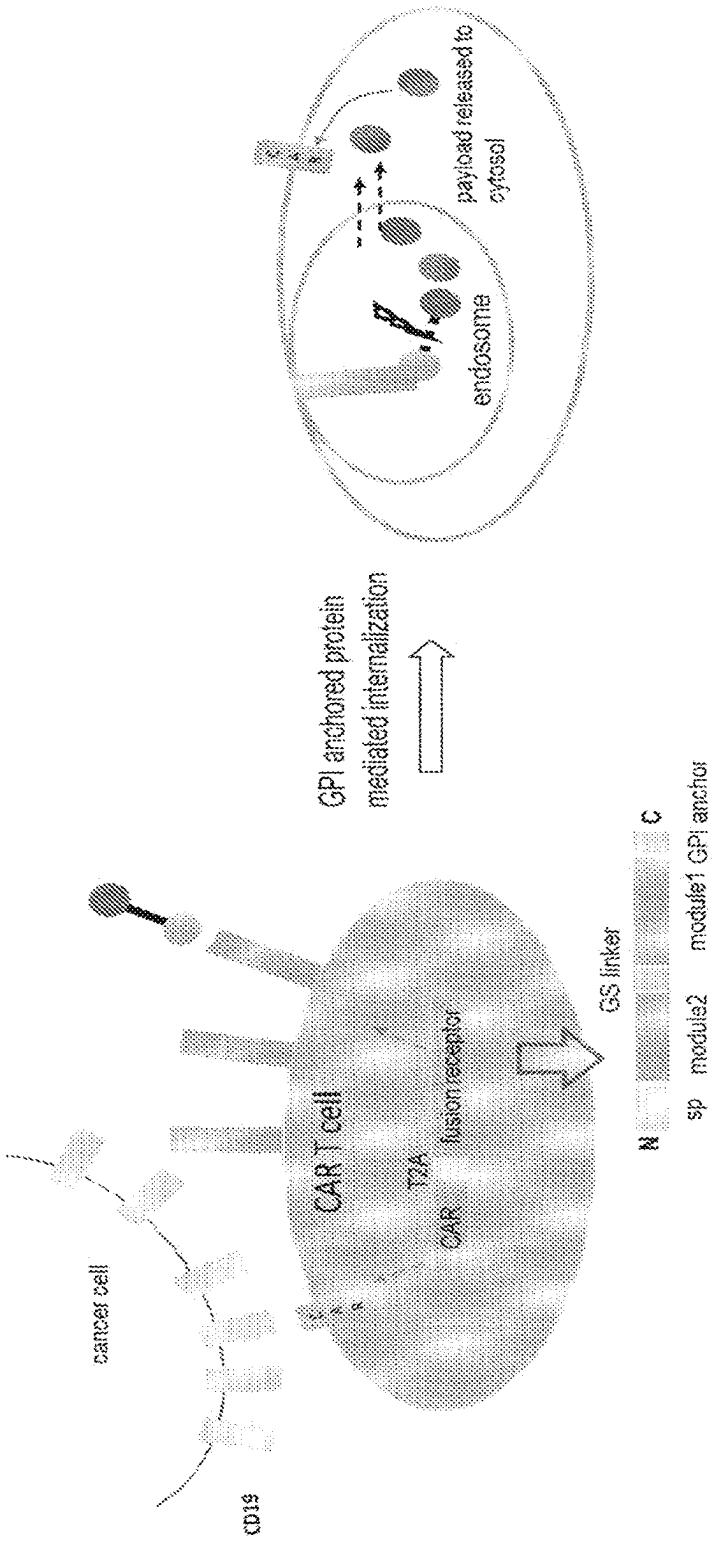

cancer cell
CD19
CAR T cell
T2A
CAR
fusion receptor
GS linker
N
C
sp
module2
module1
GPI anchor
GPI anchored protein
mediated internalization
endosome
payload released to
cytosol

FIG. 1B

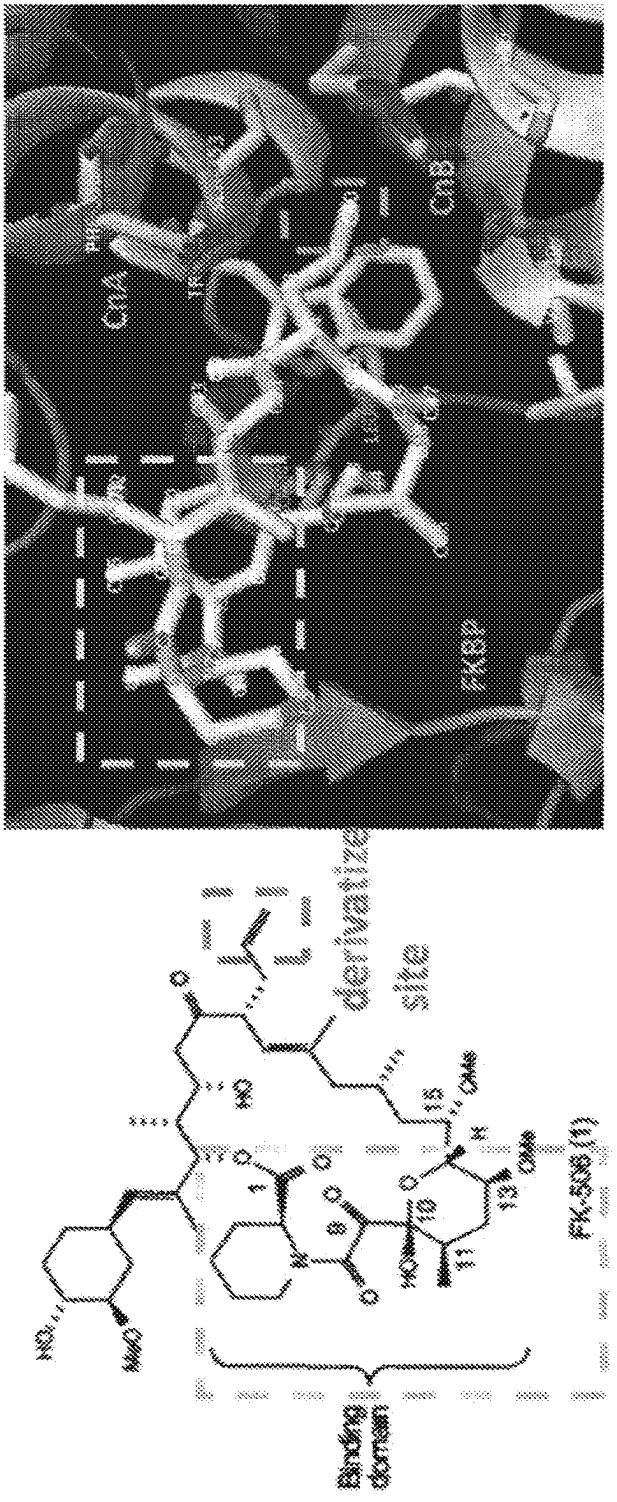


FIG. 2A

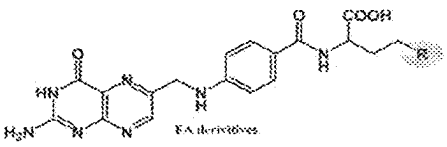
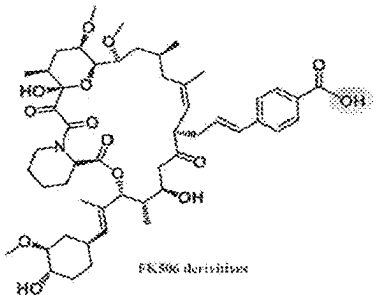
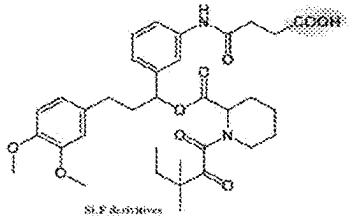
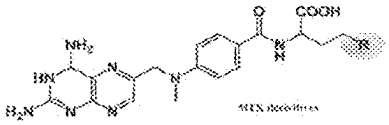
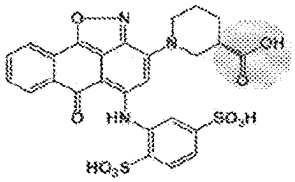
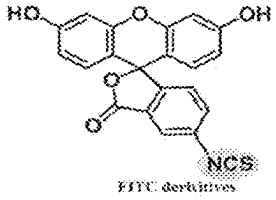
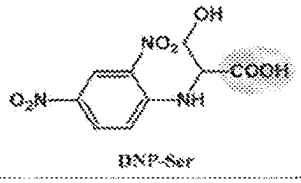
Module1: GPI anchored protein		Module 2	
Protein	Ligand	Protein	Ligand
FRa		FKBP	
			
FRb		DHFR	
uPAR		scFv against FITC	
		scFV against DNP	

FIG. 2B

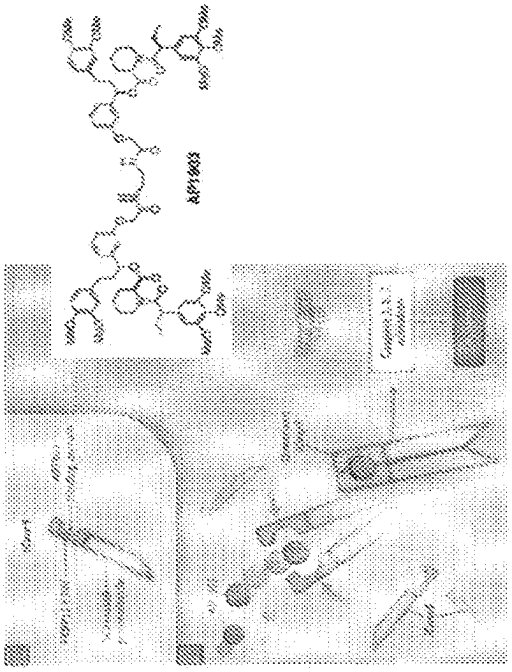
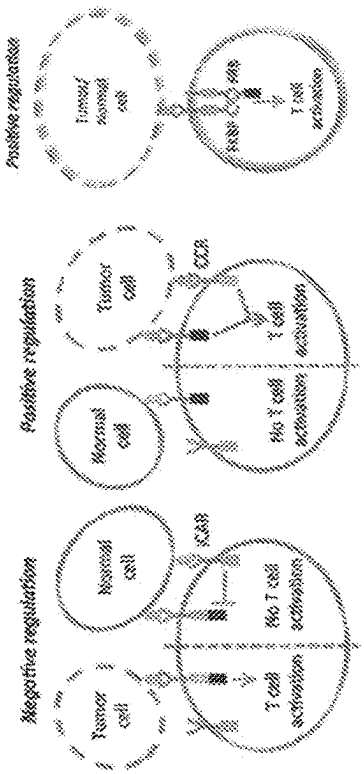


FIG. 3



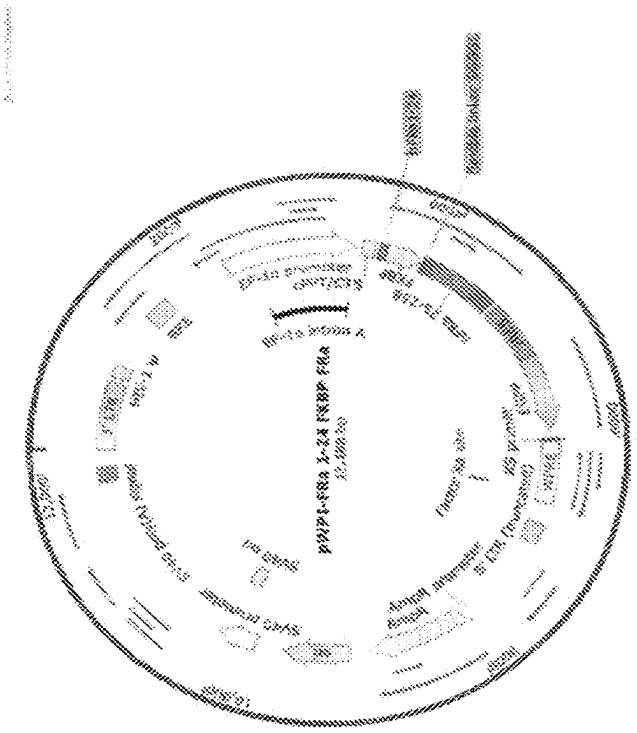


FIG. 4A

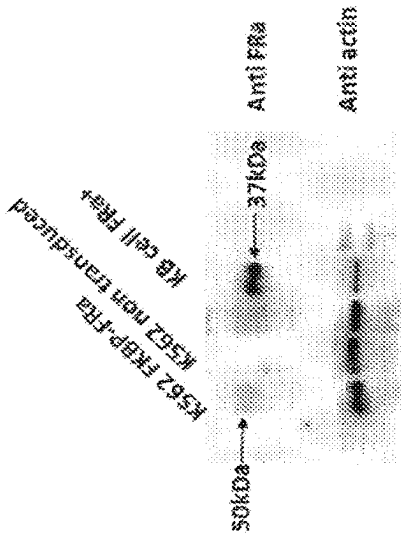

Anti FRa
Anti actin
37kDa
50kDa
K562 FKBP-FRa
K562 mon transduced
K562 cell FRa+

FIG. 4B

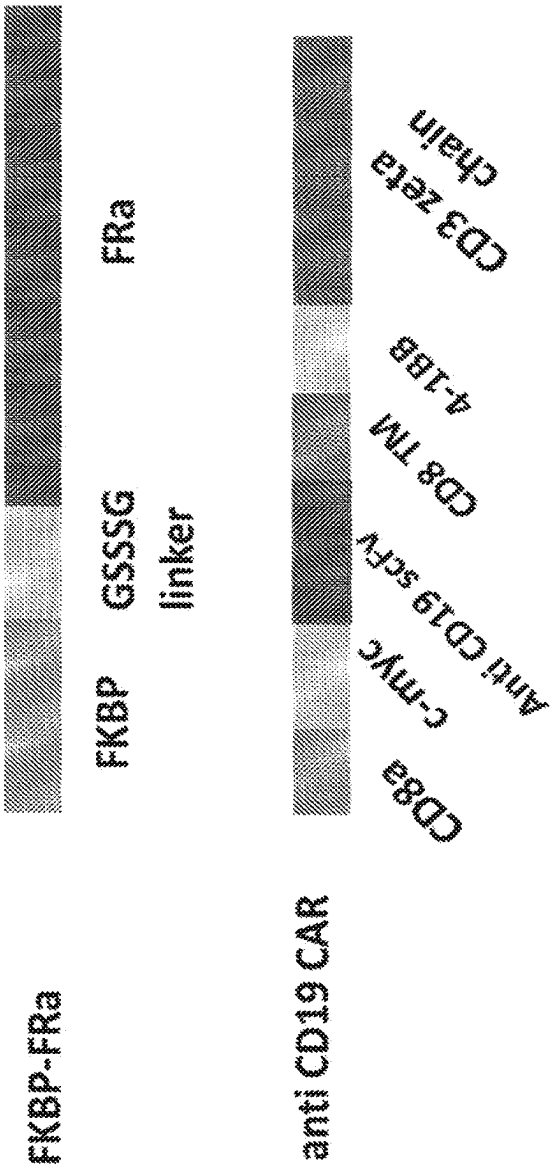

FKBP-FRa
FKBP
GSSG linker
FRa
anti CD19 CAR
CD8a
c-myc
Anti CD19 scFv
CD8 TM
4-1BB
CD3 zeta chain

FIG. 4C

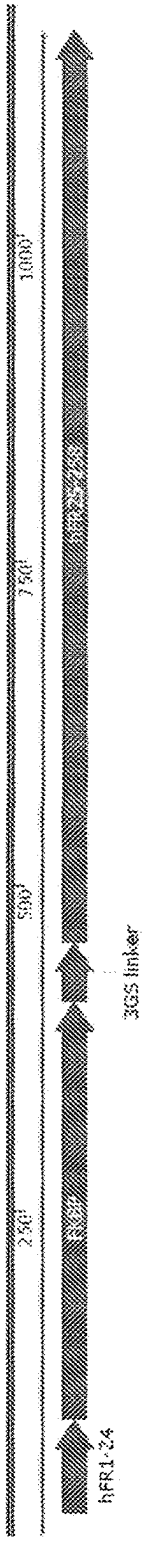

250
500
750
1000
hFR1-24
3GS linker

FIG. 4D

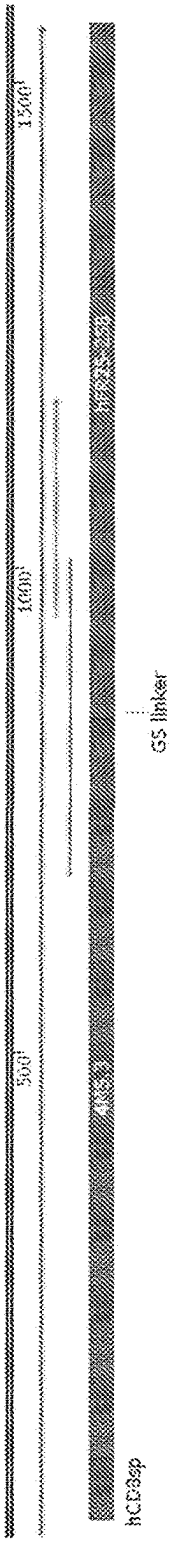

500
1000
1500
GS linker
hCD3ep

FIG. 4E

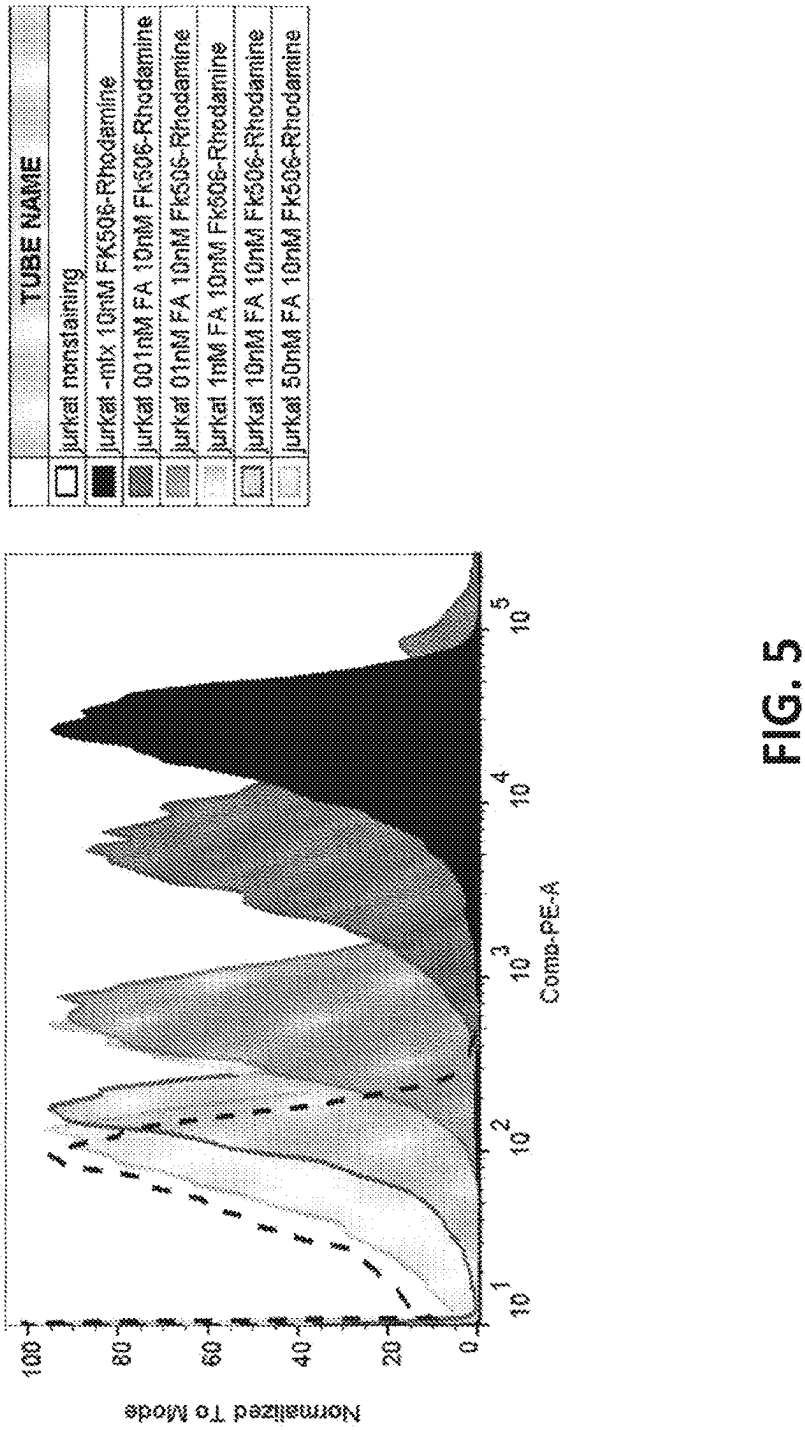

TUBE NAME
Jurkat nonstaining
Jurkat -mix 10nM FK506-Rhodamine
Jurkat 001nM FA 10nM FK506-Rhodamine
Jurkat 01nM FA 10nM FK506-Rhodamine
Jurkat 1nM FA 10nM FK506-Rhodamine
Jurkat 10nM FA 10nM FK506-Rhodamine
Jurkat 50nM FA 10nM FK506-Rhodamine
Normalized To Mode
100
80
60
40
20
0
10^1
10^2
10^3
10^4
10^5
Coma-PE-A

FIG. 5

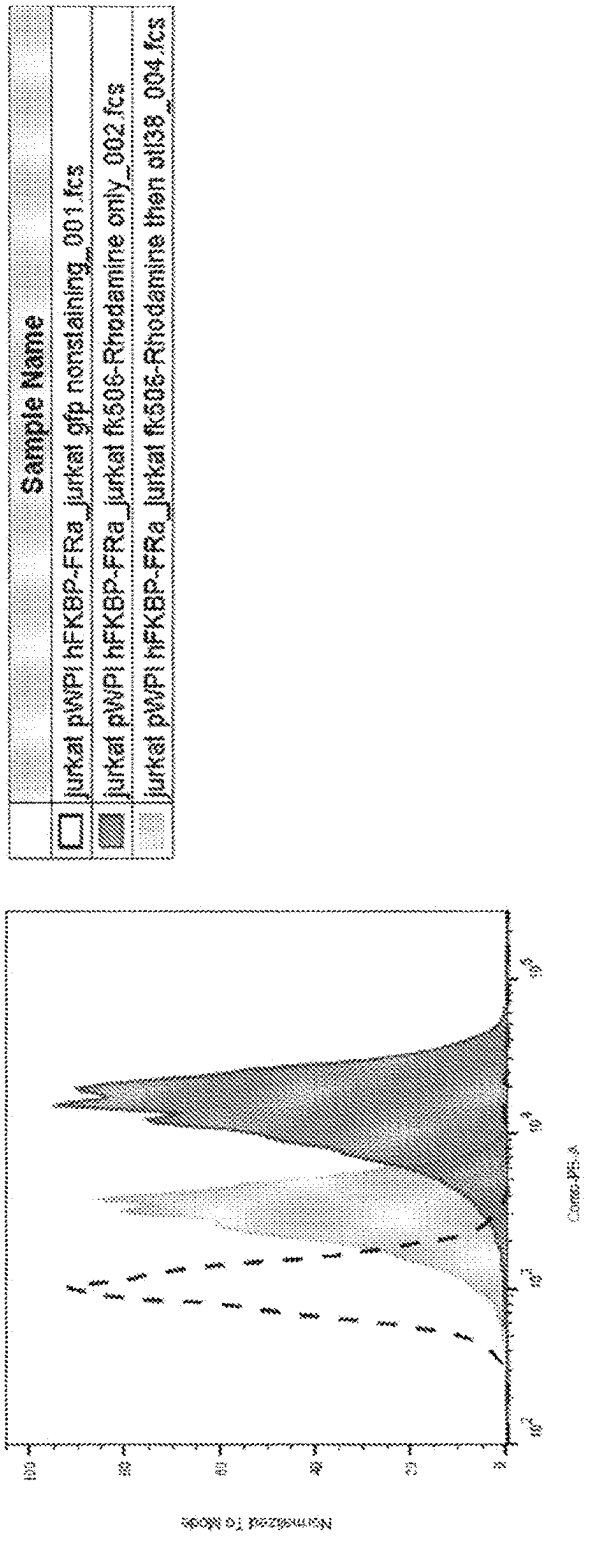

Sample Name
Jurkat pWPI hFKBP-FRa_jurkat gfp nonstaining_001.fcs
Jurkat pWPI hFKBP-FRa_jurkat fk506-Rhodamine only_002.fcs
Jurkat pWPI hFKBP-FRa_jurkat fk506-Rhodamine then ot38_004.fcs
Normalized To Mode
Comp-PE-A

FIG. 6

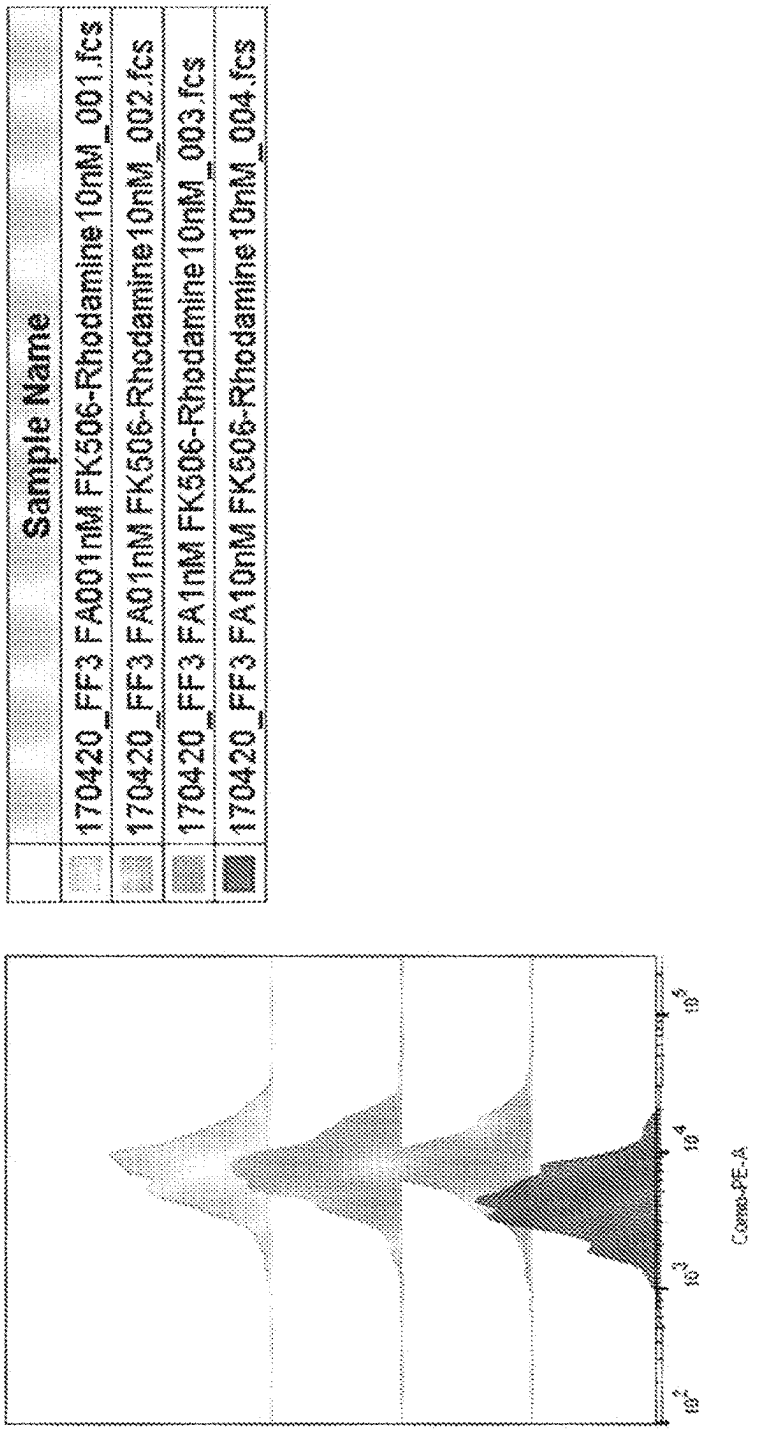

Sample Name
170420_FF3 FA001nM FK506-Rhodamine10nM_001.fcs
170420_FF3 FA01nM FK506-Rhodamine10nM_002.fcs
170420_FF3 FA1nM FK506-Rhodamine10nM_003.fcs
170420_FF3 FA10nM FK506-Rhodamine10nM_004.fcs
Comb-PE-A
10^2
10^3
10^4
10^5

FIG. 7

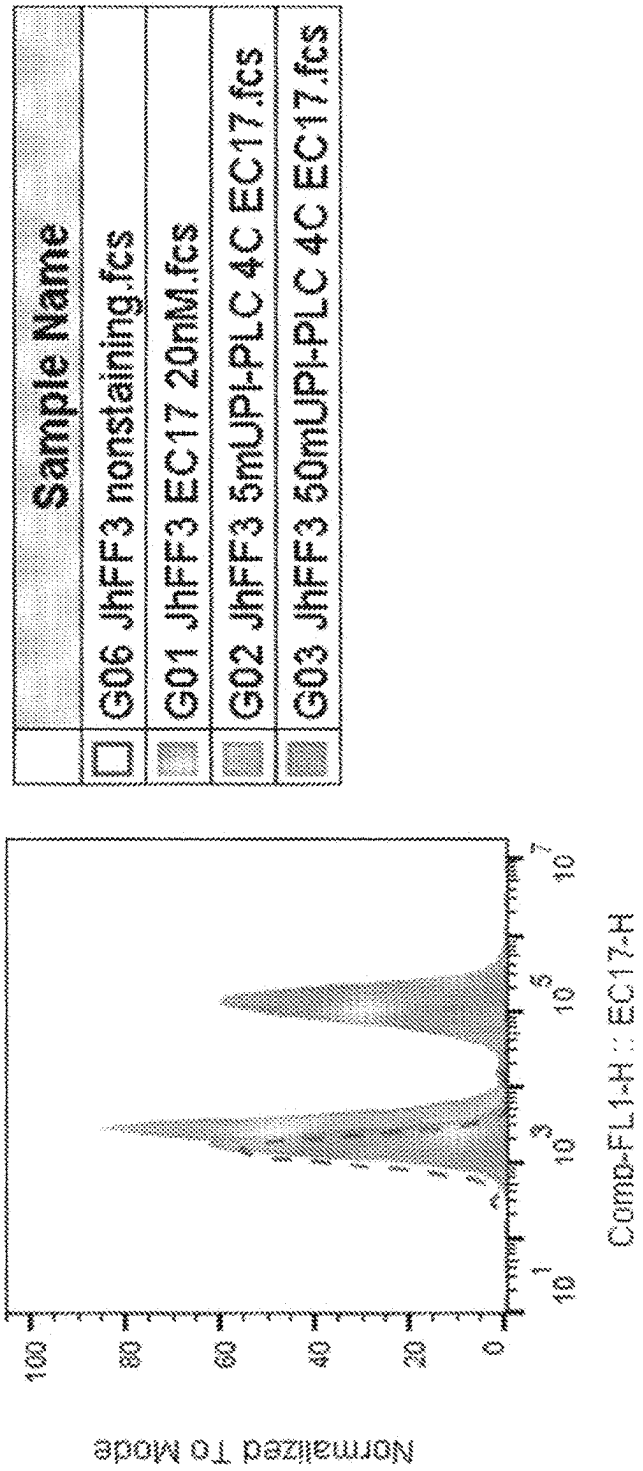

Sample Name
G06 JhFF3 nonstaining.fcs
G01 JhFF3 EC17 20mM.fcs
G02 JhFF3 5mUPI-PLC 4C EC17.fcs
G03 JhFF3 50mUPI-PLC 4C EC17.fcs
Normalized To Mode
100
80
60
40
20
0
10^1
10^3
10^5
10^7
Comp-FL1-H :: EC17-H

FIG. 8

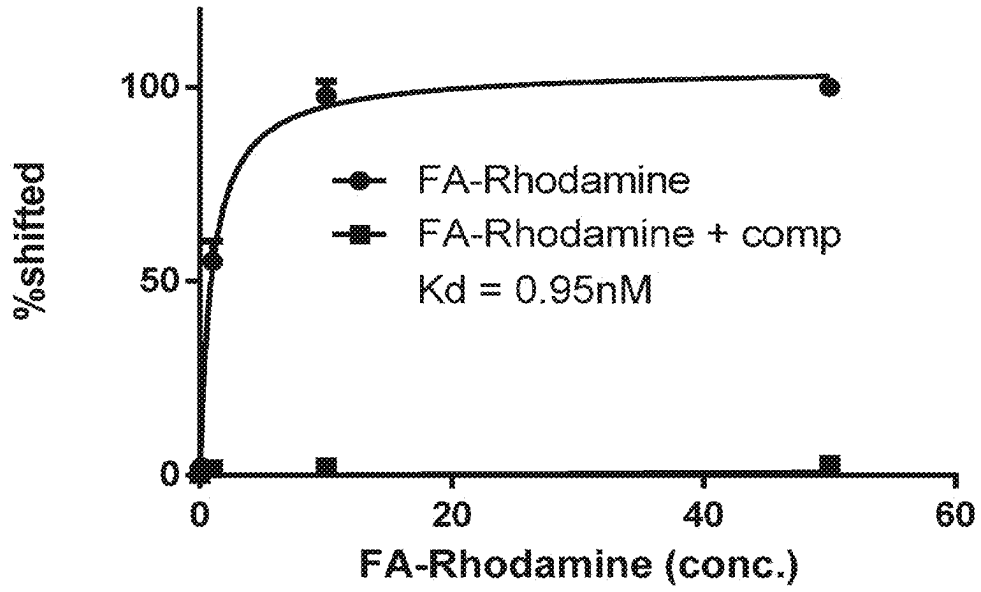

100
50
0
%shifted
FA-Rhodamine
FA-Rhodamine + comp
Kd = 0.95nM
0
20
40
60
FA-Rhodamine (conc.)

FIG. 9

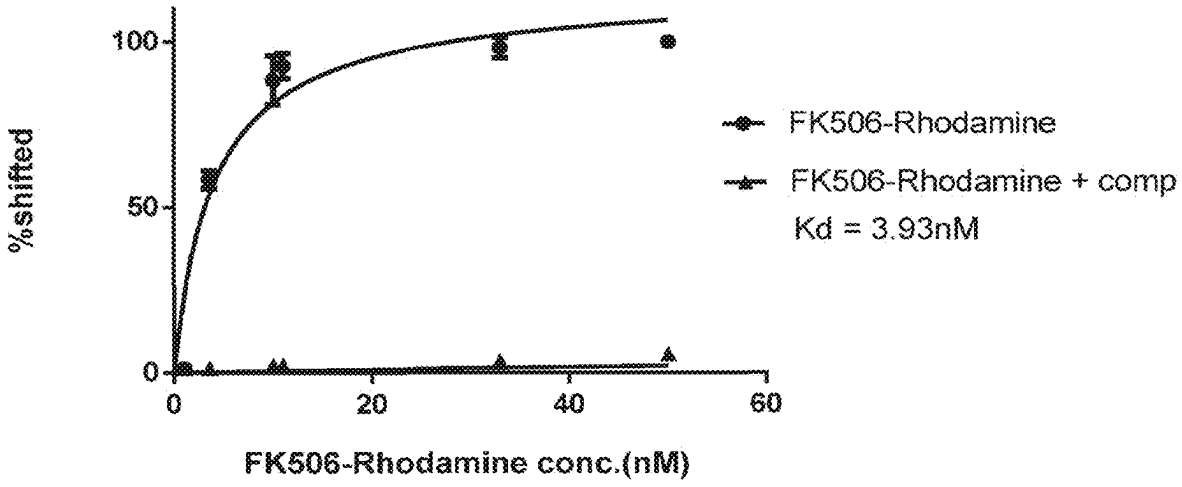

100
50
0
%shifted
0
20
40
60
FK506-Rhodamine conc.(nM)
FK506-Rhodamine
FK506-Rhodamine + comp
Kd = 3.93nM

FIG. 10

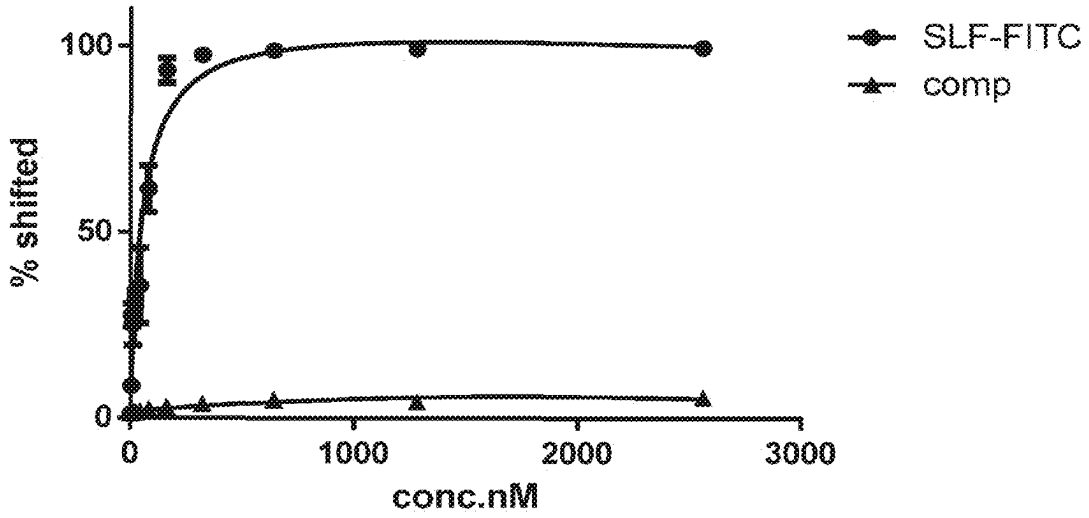


FIG. 11

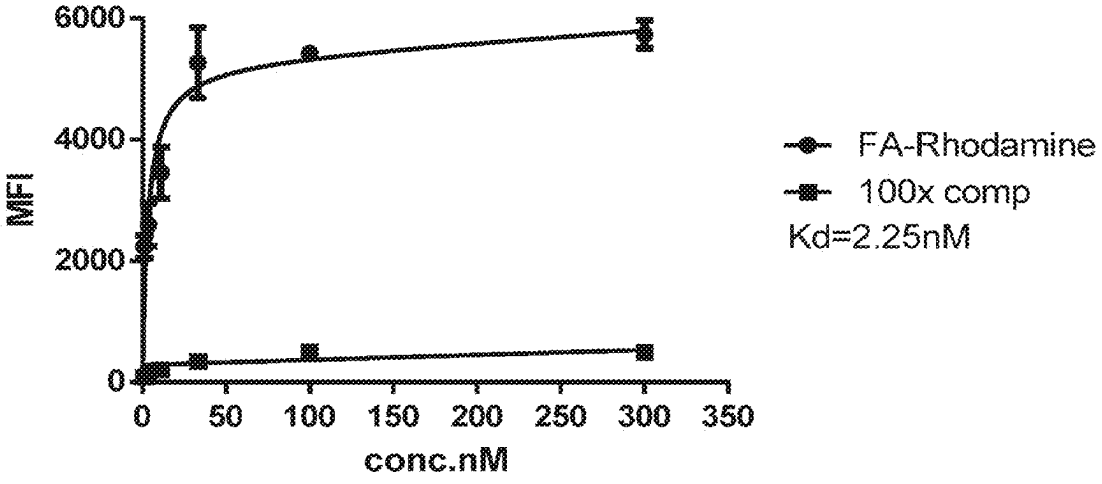

6000
4000
2000
0
MFI
0
50
100
150
200
250
300
350
conc.nM
FA-Rhodamine
100x comp
Kd=2.25nM

FIG. 12

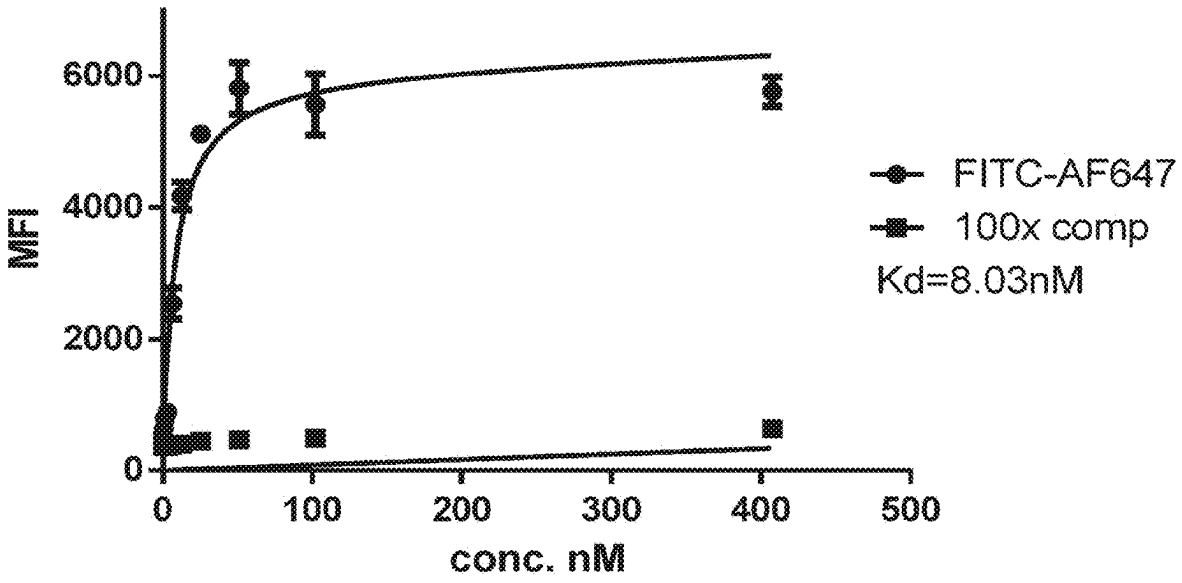

6000
4000
2000
0
MFI
0
100
200
300
400
500
conc. nM
FITC-AF647
100x comp
Kd=8.03nM

FIG. 13

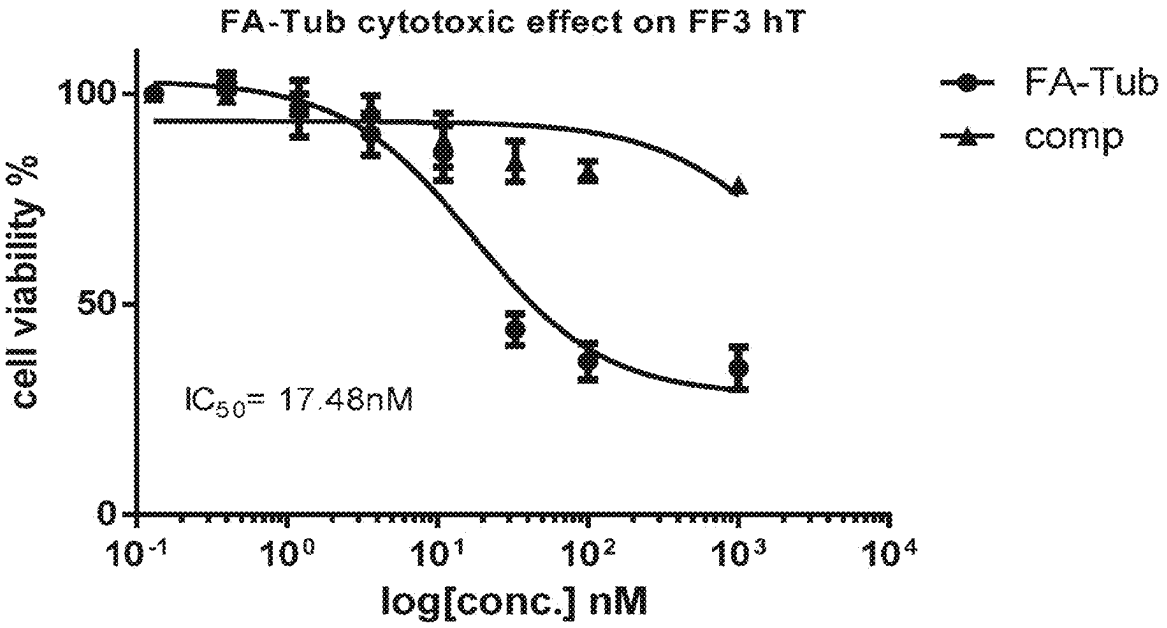

FA-Tub cytotoxic effect on FF3 hT
100
50
0
cell viability %
IC50= 17.48nM
10-1
100
101
102
103
104
log[conc.] nM
FA-Tub
comp

FIG. 14

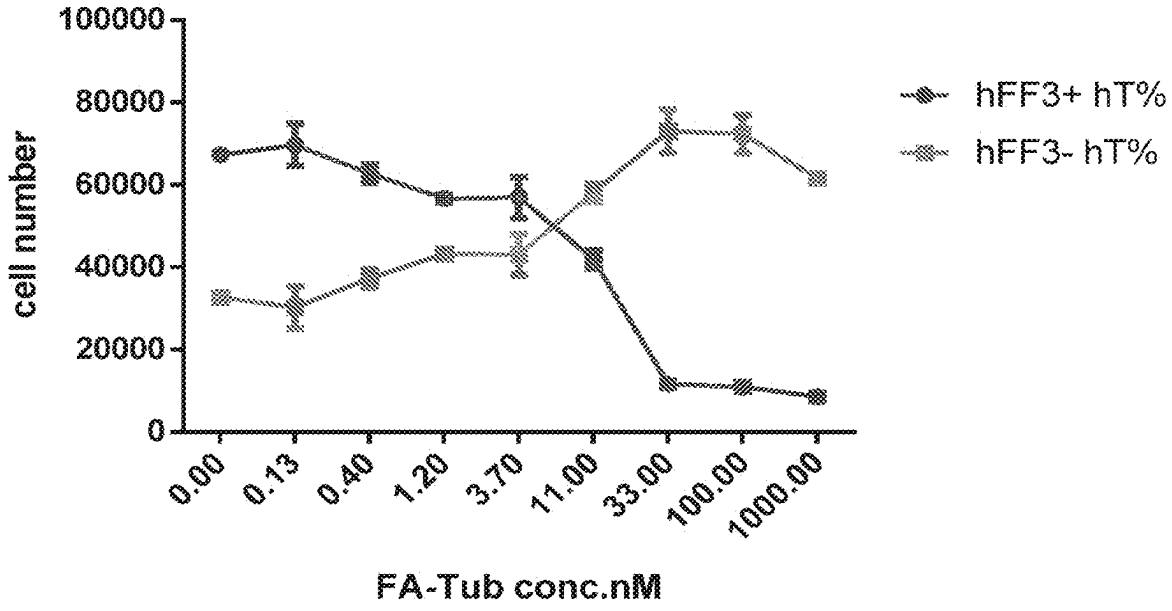

100000
80000
60000
40000
20000
0
cell number
0.00
0.13
0.40
1.20
3.70
11.00
33.00
100.00
1000.00
FA-Tub conc.nM
hFF3+ hT%
hFF3- hT%

FIG. 15

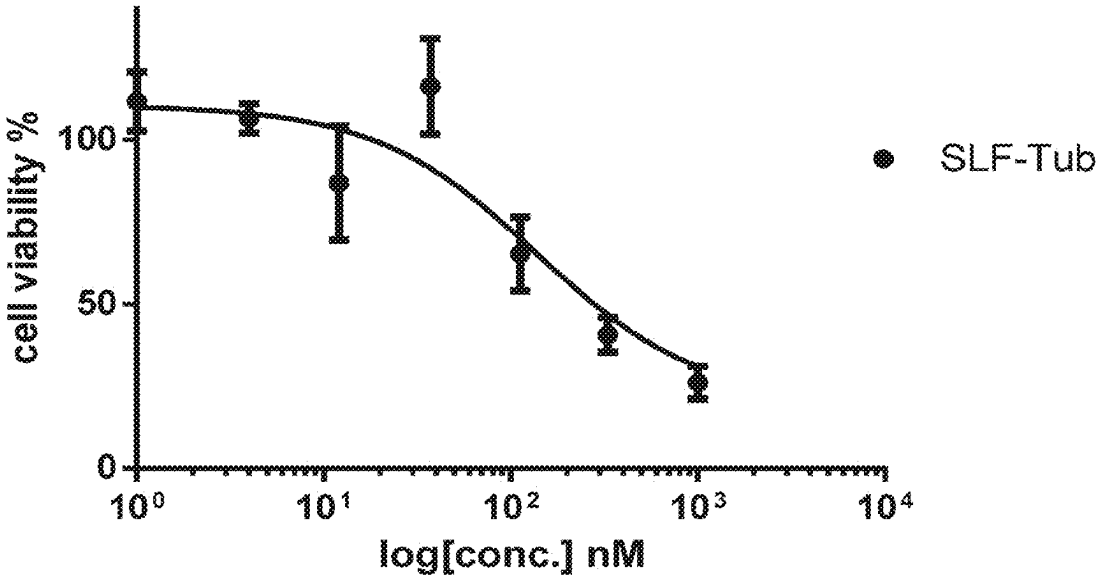


FIG. 16

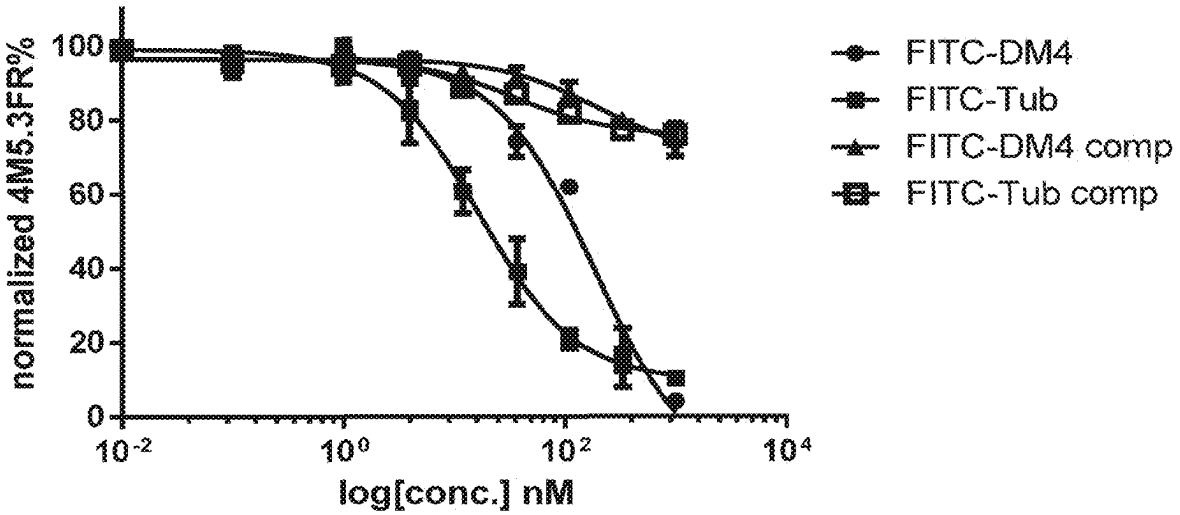

normalized 4M5.3FR%
100
80
60
40
20
0
10-2
100
102
104
log[conc.] nM
FITC-DM4
FITC-Tub
FITC-DM4 comp
FITC-Tub comp

FIG. 17

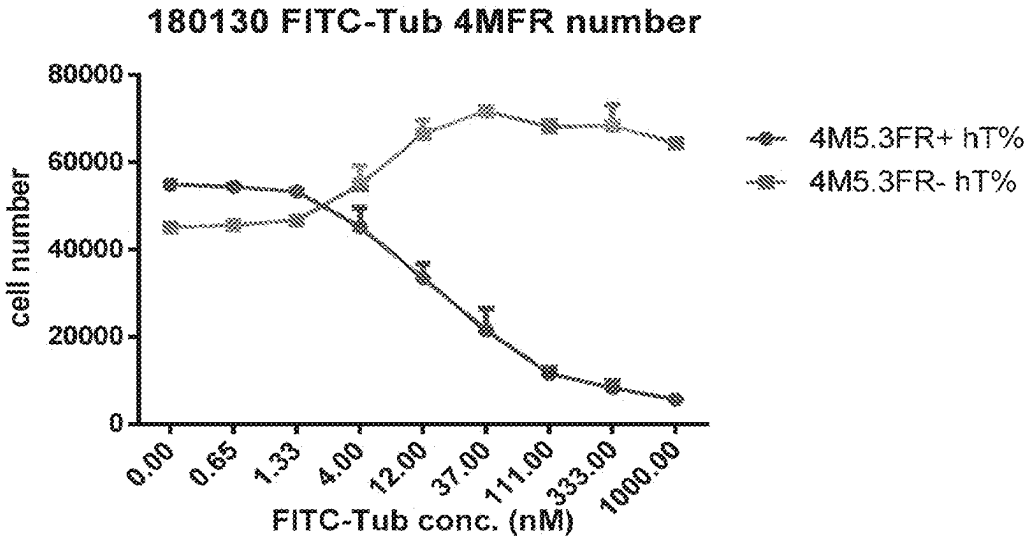

180130 FITC-Tub 4MFR number
cell number
80000
60000
40000
20000
0
0.00
0.65
1.33
4.00
12.00
37.00
111.00
333.00
1000.00
FITC-Tub conc. (nM)
4M5.3FR+ hT%
4M5.3FR- hT%

FIG. 18

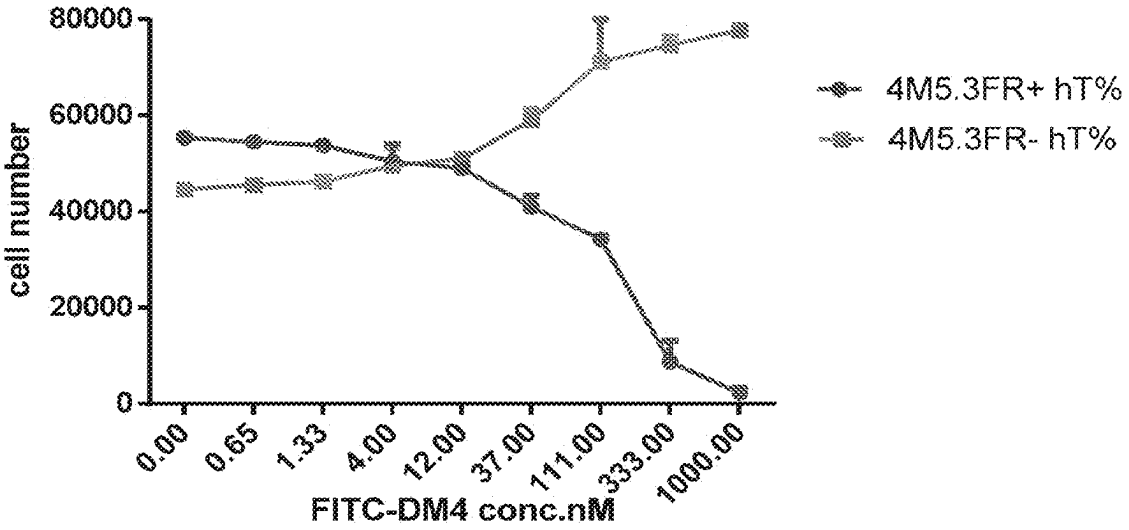

80000
60000
40000
20000
0
cell number
0.00
0.65
1.33
4.00
12.00
37.00
111.00
333.00
1000.00
FITC-DM4 conc.nM
4M5.3FR+ hT%
4M5.3FR- hT%

FIG. 19

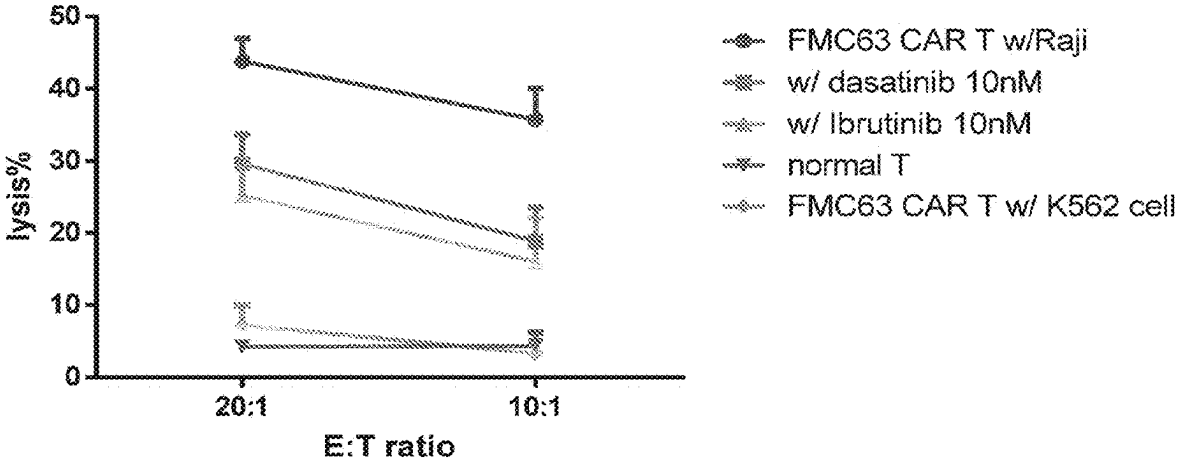

50
40
30
20
10
0
lysis%
20:1
10:1
E:T ratio
FMC63 CAR T w/Raji
w/ dasatinib 10nM
w/ Ibrutinib 10nM
normal T
FMC63 CAR T w/ K562 cell

FIG. 20

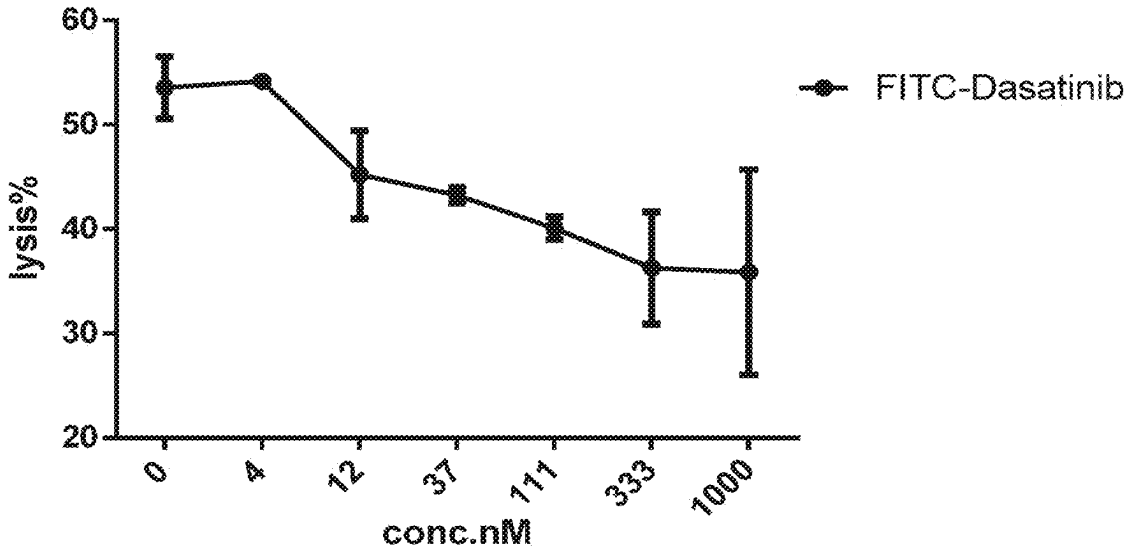

60
50
40
30
20
lysis%
0
4
12
37
111
333
1000
conc.nM
FITC-Dasatinib

FIG. 21

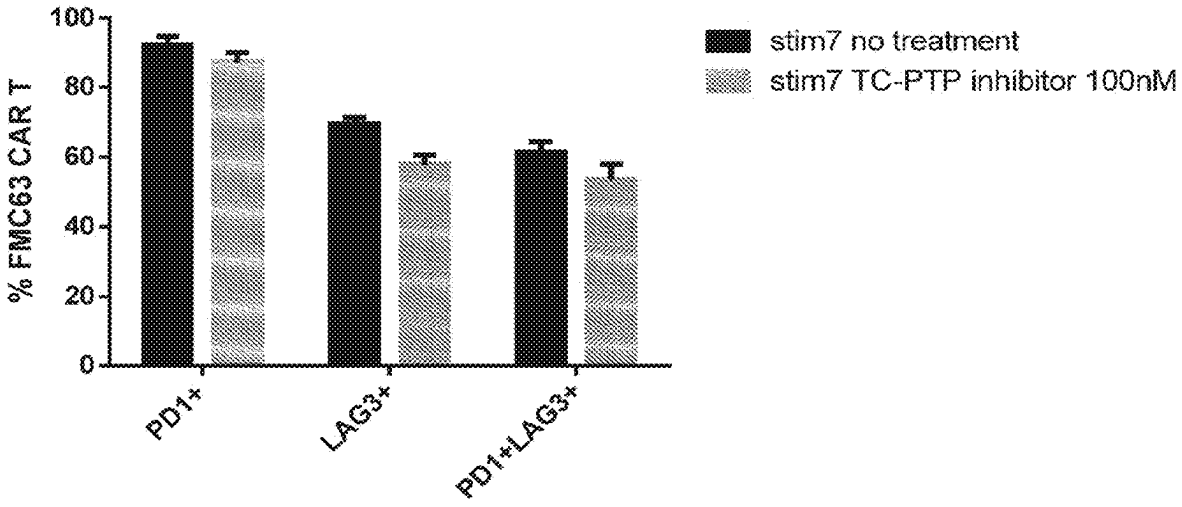

% FMC63 CAR T
100
80
60
40
20
0
PD1+
LAG3+
PD1+LAG3+
stim7 no treatment
stim7 TC-PTP inhibitor 100nM

FIG. 22

TARGETED LIGAND-PAYLOAD BASED DRUG DELIVERY FOR CELL THERAPY

CROSS REFERENCE

[0001] This application is a divisional of and claims the benefit of priority of U.S. application Ser. No. 16/486,632, filed Aug. 16, 2019, which is a U.S. National Stage Filing under 35 U.S.C. § 371 from International Application No. PCT/US2018/018557, filed on Feb. 17, 2018, and published as WO2018/152451 on Aug. 23, 2018, which claims the benefit of U.S. provisional application 62/460,118 under 35 U.S.C. § 119(e), filed on Feb. 17, 2017; the benefit of priority of each of which is hereby claimed herein, and which applications and publication are hereby incorporated herein by reference in their entireties.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

[0002] This application contains a Sequence Listing which has been submitted electronically in ST26 format and hereby incorporated by reference in its entirety. Said ST26 file, created on Apr. 30, 2025, is named 1165083US2.xml and is 101,958 bytes in size.

FIELD OF INVENTION

[0003] This disclosure provides a drug delivery platform for cell therapy. Particularly, an engineered protein is coupled with a high affinity ligand carrying at least one payload of drug to be internalized by the transplanted cell through the engineered protein to regulate transplanted cell therapy effects.

BACKGROUND

[0004] In the last few decades, great advances have been made in this field regarding the cell types, delivery methods and suitable diseases models. In terms of cell types, current cell therapies can be roughly categorized as chimeric antigen receptors (CARs), cell for tumor model and stem cell based regenerative medicine.

[0005] CAR T also known as chimeric T cell receptors, chimeric immunoreceptors or artificial T cell receptors, enable immune effector cells (usually T cells or NK cells) to recognize target cells with corresponding antigen and exercise their cytotoxic activity. The emergence and development of CAR-T technology provides promises to certain types of cancers, which turns CAR-T into a superstar in the field of both biomedical research and clinical studies.

[0006] Regenerative medicine is a game-changing area of medicine with the potential to fully heal damaged tissues and organs, offering solutions and hope for people who have conditions that today are beyond repair. Advances in developmental and cell biology, immunology, and other fields have unlocked new opportunities to refine existing regenerative therapies and develop novel ones.

[0007] Stem cells have the ability to develop—through a process called differentiation—into many different types of cells, such as skin cells, brain cells, lung cells and so on. Stem cells are a key component of regenerative medicine, as they open the door to new clinical applications.

[0008] A variety of stem cells, including adult and embryonic stem cells may be used in regenerative medicine. In addition, various types of progenitor cells, such as those found in umbilical cord blood, and bioengineered cells called induced pluripotent stem cells are used in regenerative medicine. Each type has unique qualities, with some being more versatile than others.

[0009] Many of the regenerative therapies under development begin with the particular patient's own cells. For example, a patient's own skin cells may be collected, reprogrammed in a laboratory to give them certain characteristics, and delivered back to the patient to treat his or her disease.

[0010] Although the anti CD19 CAR T has received great success in clinical applications for leukemia treatment, lethal side effects such as the cytokine storm generated from the fast lysis of tumor cells, as well as the killing of normal CD19+ B cells by the fast proliferating anti-CD19 CAR T cell requires finer control of the CAR T cell. In stem cell based regenerative therapy, efforts have been put to better understand the differentiation process and trophic roles of the transplanted cells in the target tissue. Meanwhile, these processes can be potentially altered by some small molecule drugs that specifically delivered to the stem cell to further contribute to the regeneration of the target tissue.

[0011] Another long lasting concern about the CAR T cells as well as other stem cell based regenerative therapy is the tumorigenic potential of these transplanted cells. In summary, it will be ideal to have a private doorway to control the activity of the transplanted cell, either CAR T cell or stem cell, after they are being transplanted.

SUMMARY OF THE INVENTION

[0012] This disclosure provides a drug delivery platform for fine tuning cell therapy. The drug delivery system comprises:

[0013] a. an engineered protein on a target cell for transplant, wherein the fusion protein comprises a first component and a second component, the first component and the second component are connected by a peptide linker, the first component is a non-membrane protein, the second component is a membrane anchored peptide or protein;

[0014] b. at least one small ligand conjugated to a linker, wherein the at least one small ligand has intrinsic high affinity to at least one component of the engineered protein; and

[0015] c. at least one payload of drug conjugated to the linker, wherein the payload of drug is associated with the target cell when the small ligand binds to at least one component of the engineered protein.

[0016] In some embodiment, the aforementioned drug delivery platform has a payload of drug of imaging agent. Such imaging agent may be sleeted from from the group consisting of fluorescent dye rhodamine, fluorescein, and S0456. Alternatively, such imaging agent is selected from the group consisting of radioisotope chelating imaging moieties, EC 20 chelating head, NOTA and DOTA.

[0017] In some embodiment, the aforementioned drug delivery platform has a payload of drug of cytotoxic drug. Such cytoxic drug may be selected from the group consisting of tubulysin, DM1, DM4, and an auristatin.

[0018] In some embodiment, the aforementioned drug delivery platform has a payload of drug of a modulator of gene expression.

[0019] In some embodiment, the aforementioned drug delivery platform has a payload of drug of modulator of the cell's activity.

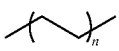
[0020] In some embodiments, the aforementioned modulator may be selected from the group of Dasatinib, MEK1/2 inhibitor, and PI3K inhibitor; group of HDAC inhibitor, kinase inhibitor and metabolic inhibitor; group of GSK3 beta inhibitor, MAO-B inhibitor and Cdk5 inhibitor.

[0021] In some embodiments, the aforementioned modulator is a phosphatase inhibitor, an RORγt agonist or an siRNA mi181a1.

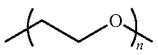
[0022] In some embodiments the aforementioned payload of drug is a phosphatase inhibitor, including but not limited to inhibitors against SHP1/2, TC-PTP.

[0023] In some embodiment, the aforementioned payload of drug in the drug delivery platform is further internalized by the target cell when the small ligand binds to at least one component of the engineered protein.

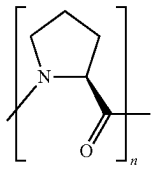
[0024] In some embodiment, the aforementioned drug delivery platform has a releasable linker to connect the small ligand and the payload drug. The linker can be selected from the group consisting of



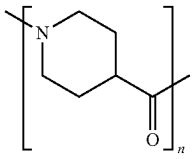
alkyl



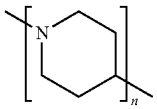
poly ethylene glycol (PEG)



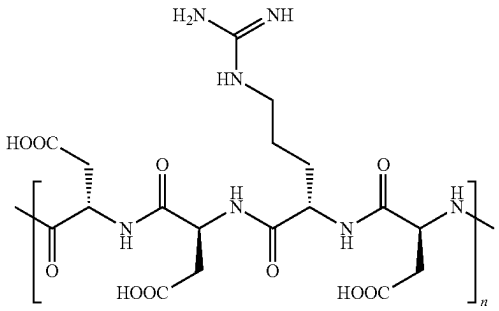
polyproline



oligo-(4-piperidine carboxylic acid)

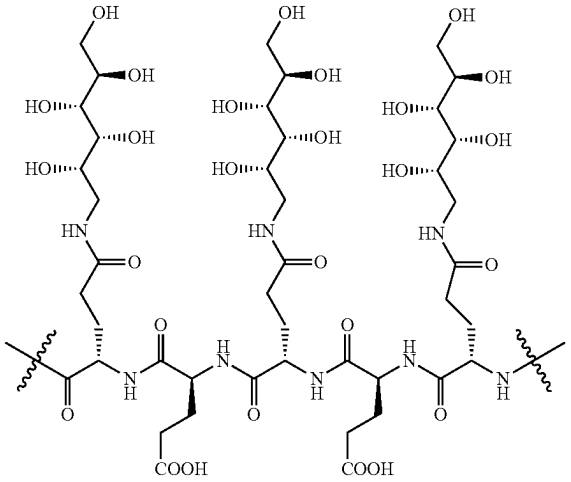


oligo piperidine



peptide

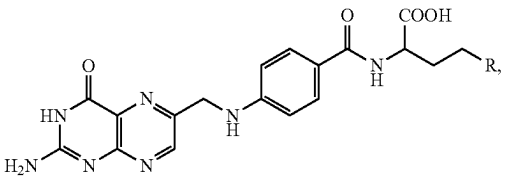
-continued



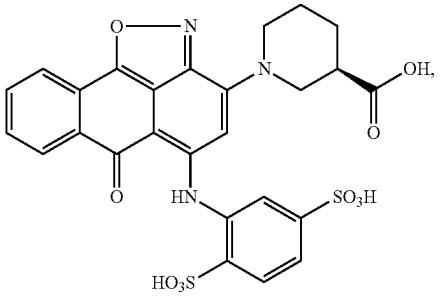
saccharo-peptide

[0025] In some embodiment, the aforementioned engineered protein components are are selected from the group consisting of Folate Receptor alpha (FRa), Folate Receptor beta (FRb), Urokinase receptor (uPAR), FK506 binding protein (FKBP), dihydrofolate reductase (DHFR), Single Chain Fragment Variable against Fluorescein isothiocyanate (scFv against FITC), and Single Chain Fragment Variable against dinitrophenol (scFv against DNP).

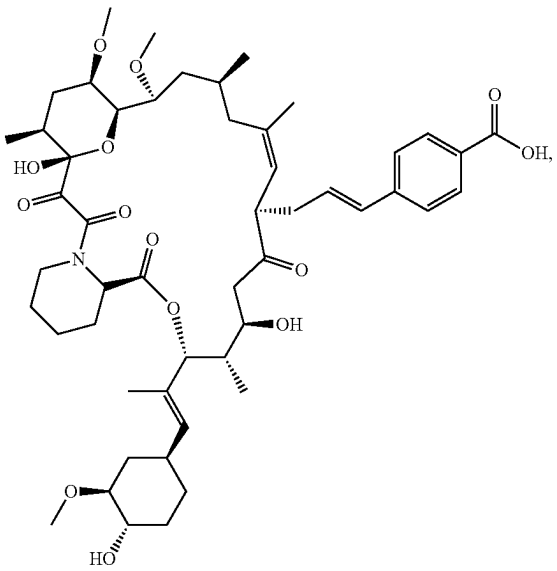
[0026] In some embodiment, the aforementioned small ligand is selected from the group consisting of



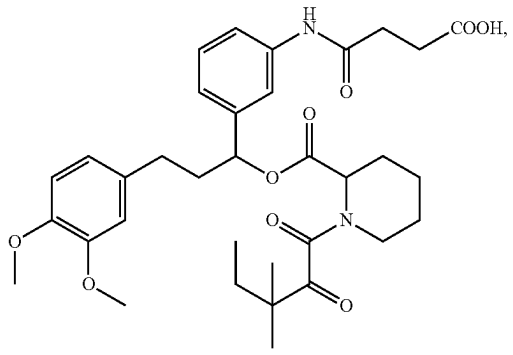
FA derivatives



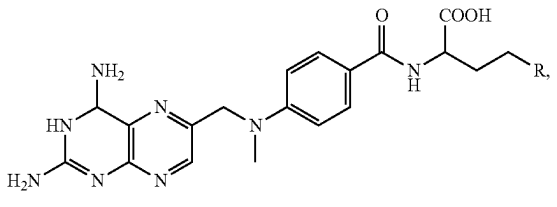
-continued



FK506 derivitives

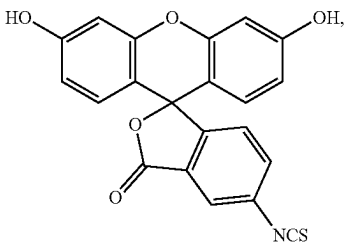


SLF derivitives



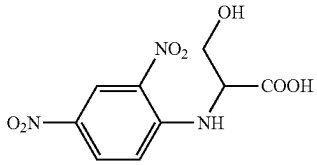
MTX
derivitives

-continued



FTTC derivitives

and



DNP-Ser

[0027] In some embodiment, the aforementioned drug delivery platform has the first component as FKBP, the second component is a peptide that confers a glycosylphosphatidyl inositol (GPI) anchor on the first component, and the small ligand is FK506 or its derivative. In some embodiment, the FK506 derivative abolishes Calcineurin binding site.

[0028] In some embodiment, the aforementioned second component is a full length or truncated Folate Receptor (FR).

[0029] In some embodiment, the aforementioned drug delivery platform has at least one segment of flexible peptide linker SGGGS to connect the first component and the second component of the engineered protein.

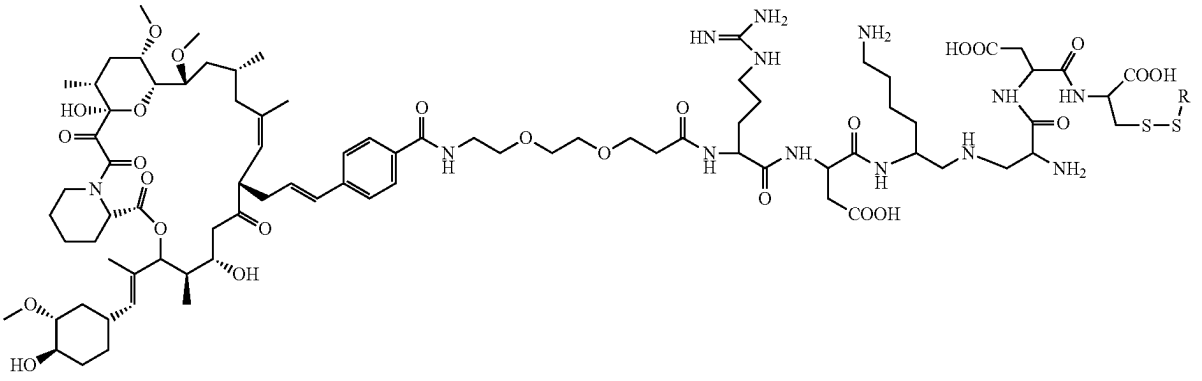
[0030] In some embodiment the aforementioned drug delivery platform comprises an engineered protein selected from the group consisting of SEQ ID NOS: 1-2 (amino acid sequence for mouse FKBP-FRa and amino acid sequence for human FKBP-FRa respectively).

[0031] In some embodiment the aforementioned drug delivery platform comprises an engineered protein selected from the group consisting of SEQ ID NOS: 12-15.

[0032] In some embodiment the aforementioned target cell for transplant is an immune cell. For example, the immune cell can be a NK cell or a Chimeric Antigen Receptor T (CAR T) cell. Such CAR T cell may be expressing amino acid sequence selected from SEQ ID NOS: 3-4.

[0033] In some embodiment the aforementioned drug delivery platform has the small ligand conjugate with formula I.

I



[0034] In some embodiment the aforementioned drug delivery platform has a target cell for transplant as CAR T cell expressing SEQ ID NO:3 (amino acid sequence for mouse antiCD19 CAR T construct) or SEQ ID NO:4 (amino acid sequence for human antiCD19 CAR T construct).

[0035] In some embodiment the aforementioned small ligand is further conjugated to a fluorescent dye or radioactive probe for tracking the drug internalization.

[0036] In some embodiment the aforementioned drug delivery platform comprises a small ligand that is further conjugated to a regulator of endogenous gene expression of a regulator of a transduced transgene expression.

[0037] In some embodiment the aforementioned drug delivery platform, the target cell for transplant is a stem cell, a progenitor cell, or a transplanted cell designed to synthesize a biochemical that is deficient in a patient. This disclosure further provides a CAR T cell comprising a construct expressing amino acid sequences selected from SEQ ID NOS: 12-15.

[0038] This disclosure further provides a DNA construct encoding an amino acid sequence selected from the group consisting of SEQ ID NOS: 12-15.

[0039] This disclosure further provides a DNA construct encoding a FKBP-FRa fusion receptor comprising any of SEQ ID NOS: 1-2 operably linked to an EF1a promoter in a expression vector. In some embodiment, such expression vector is pWPI having SEQ ID NO:5.

[0040] This disclosure further provides a DNA construct comprising any of SEQ ID NOS: 6-8.

[0041] This disclosure further provides a transplanted cell comprising insert genes hFKBP-FR (SEQ ID NO:7) and human antiCD19 CAR (SEQ ID NO:9).

[0042] This disclosure further provides a transplant cell comprising insert genes mFKBP-FR (SEQ ID NO:8) and mouse antiCD19 CAR (SEQ ID NO:10).

[0043] This disclosure further provides a method to modulate cell therapy effect. The method comprises:

[0044] a. Identifying a target cell for transplant, wherein the transplanted target cell has a cell therapy function;

[0045] b. Providing an engineered fusion protein on the surface of the target cell for transplant, the fusion protein comprises a first component and a second component, the first component and the second component are connected by a flexible peptide linker, the first component is a non-membrane protein, the second component is a Glyclosylphosphatidyl inositol (GPI) anchored peptide or protein;

[0046] c. Providing a payload of drug conjugate to the target cell, wherein the payload of drug is conjugated to a small ligand through a linker, and optionally conjugated to a fluorescent dye, wherein the small ligand binds to at least one component of the engineered fusion protein with high affinity and is internalized by the target cell together with the payload of drug;

[0047] d. releasing the drug within the target cell to modulate the target cell therapy function.

[0048] In some embodiment, the aforementioned cell therapy function is to provide optically guided surgery to a subject.

[0049] In some embodiment, the aforementioned cell therapy function is to control the target cell proliferation.

[0050] In some embodiment, the aforementioned cell therapy function is to execute cytotoxicity to the target cell engaged cancer cell.

[0051] In some embodiment, the aforementioned transplanted target cell is an immune cell. For example, the target cell is a CAR T cell.

[0052] In some embodiment, the aforementioned transplanted target cell is a stem cell, a progenitor cell or a transplanted cell designed to synthesize a biochemical that is deficient in a patient.

[0053] In some embodiment, the aforementioned payload of drug is an imaging agent selected from fluorescent dye of Rhodamine and FITC, or a radioisotope imaging agent selected from EC20 chelating head, NOTA and DOTA.

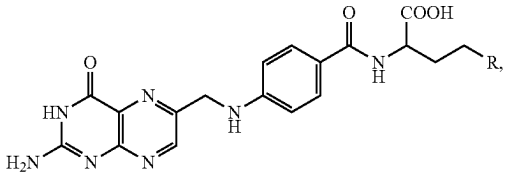
[0054] In some embodiment, the aforementioned payload of drug is a cytotoxic drug selected from the group consisting of Tubulysin, DM1, DM4 and auristatin.

[0055] In some embodiment, the aforementioned payload of drug is a modulator of gene expression selected from kinase inhibitors consisting of Dasatinib, MEK1/2 inhibitor and PI3 Kinase inhibitor, or an siRNA of mi181a1.

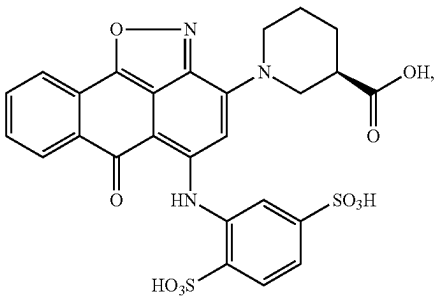
[0056] In some embodiment, the aforementioned transplanted target cell comprises a fusion protein selected from the group consisting of SEQ ID NOS: 12-15.

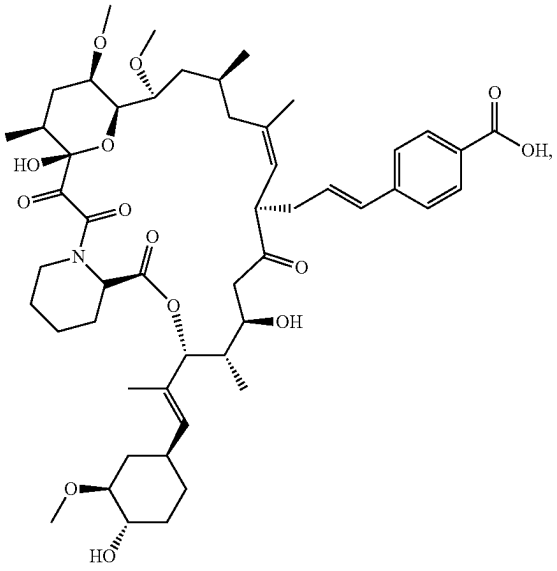
[0057] In some embodiment, the aforementioned engineered protein components are selected from the group consisting of FRa, FRb, uPAR, FKBP, DHFR, scFv against FITC, and scFv against DNP.

[0058] In some some embodiment, the aforementioned small ligand is selected from the group consisting of

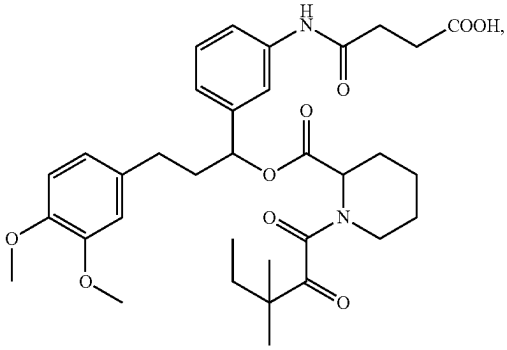


FA derivatives



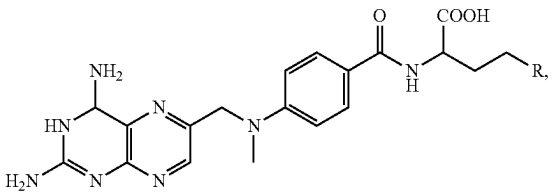


FK506 derivitives

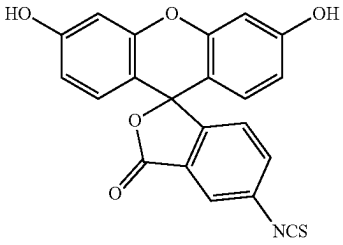


SLF derivitives

-continued

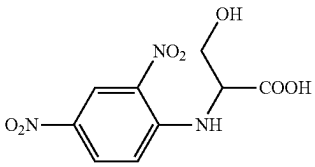


MTX
derivitives



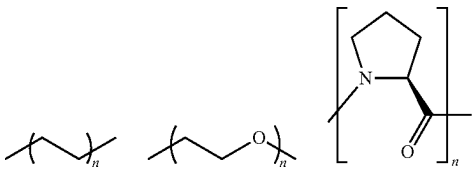
FTTC derivitives

and



DNP-Ser

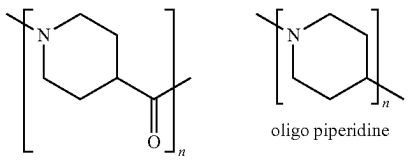
[0059] In some embodiment, the aforementioned linker to connect the small ligand and the payload drug is selected from the group consisting of



alkyl

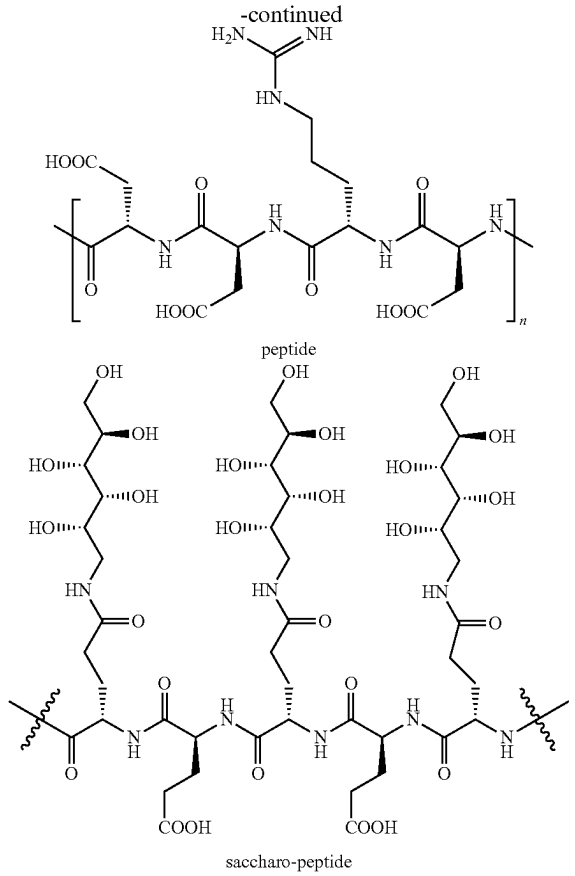
poly ethylene glycol
(PEG)

polyproline



oligo-(4-piperidine
carboxylic acid)

oligo piperidine



[0060] In some embodiment, the aforementioned transplanted target cell comprises an engineered FKBP-LINKER-FRa fusion protein selected from group consisting of SEQ ID NO:1 and SEQ ID NO:2.

[0061] In some embodiment, the aforementioned transplanted target cell is CAR T cell comprising an engineered antiCD19 CAR T construct selected from group consisting of SEQ ID NO:3 and SEQ ID NO:4.

[0062] In some embodiment, the aforementioned drug conjugate is FK506-releasable linker comprising formula I, wherein the binding domain of FK506 has an affinity to FKBP of about 4 pM to about 100 pM.

[0063] In some embodiment the aforementioned transplanted target cell is a CAR T cell and the drug conjugate is selected from the group consisting of GSK3b inhibitor, MAPK inhibitor to control excessive cytokine storm of transplanted CAR T cell.

[0064] In some embodiment the aforementioned transplanted target cell is a CAR T cell and the drug conjugate is a modulator designed to control unwanted T cell proliferation.

[0065] In some embodiment the aforementioned transplanted target cell is a stem cell or progenitor cell and the drug conjugate is GSK3b inhibitor to boost bone fracture repair

[0066] In some embodiment the aforementioned transplanted target cell is a stem cell, a progenitor cell or a transplanted cell designed to synthesize a biochemical that is deficient in a patient; and the drug conjugate is selected from the group consisting of MAO-B inhibitor and cdk5 inhibitor to treat Parkinson disease or other neurodegenerative disease.

[0067] In some embodiment the aforementioned transplanted target cell is a NK cell and the drug conjugate is a RORγt agonist to control Th17 cell mediated immune responses.

[0068] These and other features, aspects and advantages of the present invention will become better understood with reference to the following figures, associated descriptions and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

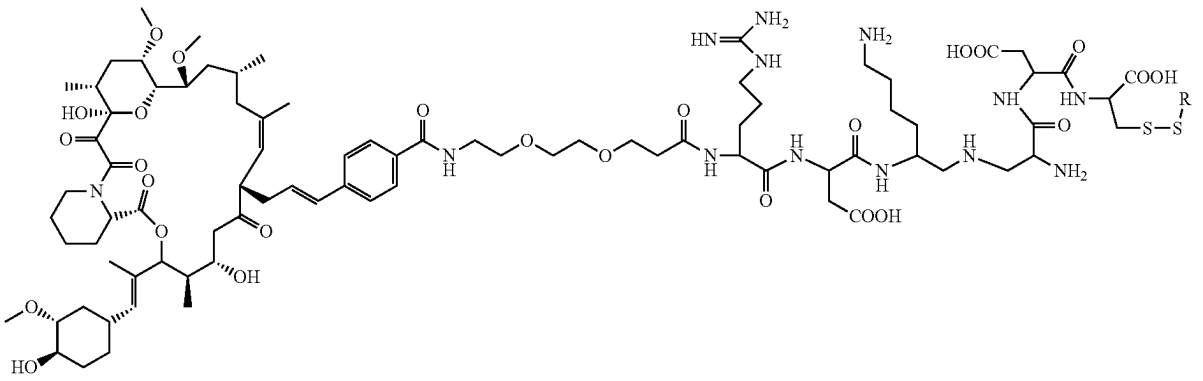
[0069] FIG. **1**A. overview of the FKBP-FRa and FK506-payload based drug delivery platform for cell therapy.

[0070] FIG. **1**B. Graphic illustration of the secret pathway platform for CAR T cells delivery of payload.

[0071] FIG. **2**A. left: chemical structure of FK506 with FKBP binding sites (yellow) and derivatized site (red) highlighted. Right: co-crystal structure of ternary complex of a calcineurin A fragment (green), calcineurin B (cyan), FKBP12 (purple) and the FK506 (yellow), PDB: 1TCO.

[0072] FIG. **2**B. Various combination options of two modules in an engineered fusion protein and their respective ligand choices, with potential derivatization sites highlighted.

I



[0073] FIG. **3**: left: negative and positive regulation of the CAR T cell activity, adapted from *The quest or spatio-temporal control of CAR T cells,* Sun J. etc. 2015. Right: mechanism of AP1903 (FK506 dimer) induced FKBP-caspase9 mediated apoptosis and the structure of AP1903, adapted from *Inducible Apoptosis as a Safety Switch for Adoptive Cell Therapy,* Malcolm K. B. etc. 2011.

[0074] FIG. **4**A. pWPI expression vector map with FKBP-FRa insert (hFRa 1-24: red, FKBP: yellow, hFRa 25-258: red).

[0075] FIG. **4**B. FKBP-FRa transduced K562 cells show a higher band around 50 kDa (37 kDa for FRa plus 12 kDa for FKBP) compare to FRa positive KB cells and non-transduced K562 cells.

[0076] FIG. **4**C. payload carrier construct and CAR T construct design.

[0077] FIG. **4**D. The construct design of FKBPFR3GS (noted as FF3). From N terminal to C terminal, it has 1-24 aa of human FRa as the signal peptide, human FKBP protein, three Gly-Ser linker and then 25-258 aa of human FRa. In FKBPFR1GS (noted as FF1), the three Gly-Ser liner of FF3 is substituted with one Gly-Ser linker with other parts unchanged.

[0078] FIG. **4**E. The construct design of 4m5.3FR. From N terminal to C terminal, it has hCD8 signal peptide, scFv of 4M5.3 antibody against FITC, GS linker, 25-258aa of human Fra.

[0079] FIG. **5**. Interference between the FR and FKBP in FKBPFR1GS fusion receptor. Binding of Folate acid in FKBPFR1GS fusion protein blocks the binding of FK506-Rhodamine at as low as 0.01 nM and totally abolish FK506-Rhodamine binding at 50 nM.

[0080] FIG. **6**. FKBPFR1GS jurkat cell shows decreased FK506-Rhodamine intensity after binding to OTL38. FRET from FK506-Rhodamine (donor) to OTL38 (FA-S0456, acceptor, ex/em:774/794 nm) within the fusion receptor indicates the interaction between FR and FKBP.

[0081] FIG. **7**. Increasing the linker length between FKBP and FR significantly lowers the interference between the two parts. Compare to FF1 (1GS between FKBP and FR), FF3 (3GS between FKBP and FR) preserves the binding of FK506-Rhodamine in the presence of 10 nM FA, which is comparable to the physiological concentration of FA in human body.

[0082] FIG. **8**. PI-PLC treatment releases the GPI anchored fusion receptor FF3. Jurkat T cell with FF3 fusion receptor shows saturated binding with 20 nM FA-FITC (EC17), while after 5 mU PI-PLC or 50 mU PI-PLC treatment, the FA-FITC loses binding to the cell, which indicates the release of the GPI anchored FF3 fusion receptor.

[0083] FIG. **9**. FA-Rhodamine binding curve in FKBPFR3GS fusion receptor. FKBPFR3GS fusion receptor that stably expressed on human T cell can bind to folate acid derivative (FA-Rhodamine) with high affinity (Kd=0.95 nM), which is comparable to the affinity of FA-Rhodamine in FR+KB cell (Kd around 1 nM). Therefore, the binding property of FR in the fusion receptor is preserved.

[0084] FIG. **10**. FK506-Rhodamine binding curve in FKBPFR3GS fusion receptor. FKBPFR3GS fusion receptor that stably expressed on human T cell is able to bind to FK506 derivative (FK506-Rhodamine) with high affinity (Kd=3.93 nM), which means the binding property of FKBP in the fusion receptor is preserved.

[0085] FIG. **11**. SLF-FITC binds to FKBPFR3GS fusion receptor with relatively high binding affinity (Kd=62 nM), while competition with free SLF (100×, preincubation) blocks this binding. SLF, a mimic of FK506, presents a 10 times lower binding affinity to FKBPFR fusion receptor, compare to the parent ligand FK506, which is consistent with previous report.

[0086] FIG. **12**. FA-Rhodamine binding curve in 4M5.3FR fusion receptor. FA-Rhodamine can binds to 4M5.3FR fusion receptor that stably expressed on human T cell with high affinity (Kd=2.25 nM), which is comparable to the affinity of FA-Rhodamine in FR+KB cell (Kd around 1 nM). Therefore, FR binding property is preserved in 4M5.3FR fusion receptor.

[0087] FIG. **13**. FITC-AF647 binding curve in 4M5.3FR fusion receptor. FITC-AF647 can binds to 4M5.3FR fusion receptor that stably expression on human T cell with high affinity (Kd=8.03 nM). 100× comp indicates free FITC sodium. The binding property of scFv 4M5.3 with FITC is preserved in 4M5.3FR fusion receptor.

[0088] FIG. **14**. FA-Tubulysin is able to mediate a receptor specific killing effect against FF3+ human T cell. Compensation with FA (100× preincubation with FA) blocks the effect. This implies the successful internalization and release of the free drug Tubulysin through the FF3 fusion receptor system.

[0089] FIG. **15**. FA-Tubulysin specifically kill the hFF3+ population in a mixed human T cell culture. Absolute number of hFF3+ cell decrease as the FA-Tub concentration increases, while hFF3-cells are killed also through released drugs and bystander effect at high concentration.

[0090] FIG. **16**. SLF-Tub specifically kill the hFF3+ Jurkat cells with a IC_{50}—138 nM. This indicates the successful internalization of SLF-Tub by the FKBPFR3GS fusion receptor and the release of the Tubulysin inside the cell.

[0091] FIG. **17**. Both FITC-DM4 and FITC-Tub can specifically kill the 4M5.3FR+ human T cells, while FITC-Tub has a higher IC_{50}. Compensation of free FITC sodium (100× preincubation) blocks the receptor mediated killing effect. This implies the successful internalization and release of FITC-cytotoxic drug into T cell through 4M5.3FR fusion receptor.

[0092] FIG. **18**. FITC-Tubulysin specifically kill the 4M5.3FR+ population in a mixed human T cell culture. Absolute number of 4M5.3FR+ cell decrease as the FITC-Tub concentration increases, while the 4M5.3FR− cells are killed also through released drugs and bystander effect at high concentration.

[0093] FIG. **19**, FITC-DM4 specifically kill the 4M5.3FR+ population in a mixed human T cell culture. Absolute number of 4M5.3FR+ cell decrease as the FITC-DM4 concentration increases, while the 4M5.3FR− cells are killed also through released drugs and bystander effect at high concentration.

[0094] FIG. **20**. Dasatinib (Lck inhibitor) and Ibrutinib (ITK inhibitor) at 10 nM concentration decrease the lysis effect of antiCD19 CAR T cell (FMC63 CAR T, Effector) against CD19+ Raji tumor cell (Target). Two Effector: Target ratio (E:T) have been tested. Normal T cell and antiCD19 CAR T with CD19−K562 cell were used as negative control.

[0095] FIG. **21**. FITC-Dasatinib can decrease the lysis effect of FMC63+4M5.3FR+hT cell towards Raji cell. This implies the successful internalization and release of FTTC-

Dasatinib into T cell through 4M5.3FR fusion receptor and the release of Dasatinib into T cell.

[0096] FIG. **22**. TC-PTP inhibitor at 100 nM concentration decrease the co-inhibitor molecule population in exhausted antiCD19 CAR T cell (generated by 7 times of stimulation with CD19+Raji cells, see detailed procedure below). Both PD-1, LAG3 and double positive population decreases upon treatment. This implies that phosphatase inhibitors, like TC-PTP inhibitor may be used as a payload for the secret gateway platform for rejuvenating the exhausted CAR T cell.

TABLE 1

Potential applications of the FKBP-FRa cell therapy platform and the corresponding payload.

SEQUENCE LISTINGS
SEQ ID NO: 1 Amino acid sequence for mouse FKBP-FRa
SEQ ID NO: 2 Amino acid sequence for human FKBP-FRa
SEQ ID NO: 3 Amino acid sequence for mouse antiCD19 CAR T construct
SEQ ID NO: 4 Amino acid sequence for human antiCD19 CAR T construct
SEQ ID NO: 5 vector pWPI for human T cell transduction
SEQ ID NO: 6 pMP71 gb NoIIEcoRI mouse antiCD19 for mouse T cell transduction
SEQ ID NO: 7 pWPI-FRa 1-24 FKBP FRa
SEQ ID NO: 8 pWPI niFKBP-mFRa SGGGS
SEQ ID NO: 9 pHR EcorI hAnti cdl9 1D3 myc hinge cd28 cd3zeta
SEQ ID NO: 10 pWPI pmei mAnti cd19 1D3 myc hinge cd28 cd3zeta
SEQ ID NO: 11 FKBP-1SG-FR with GPI anchor amino acid sequence
SEQ ID NO: 12 FKBP-3SG-FR with GPI anchor amino acid sequence
SEQ ID NO: 13 4M5.3-FR with GPI anchor amino acid sequence
SEQ ID NO: 14 FMC63-T2A-FKBP3SGFR
SEQ ID NO: 15 FMC63-T2A-4M5.3SGFR
SEQ ID NO: 16 FRb with signal peptide
SEQ ID NO: 17 uPAR with signal peptide
SEQ ID NO: 18 DHFR
SEQ ID NO: 19 scFv against FITC: 4M5.3(Kd = 200 pM)
SEQ ID NO: 20 scFv against FITC 4D5Flu (Kd = 10 nM)
SEQ ID NO: 21 scFv against DNP SPE7

DETAILED DESCRIPTION

[0097] While the concepts of the present disclosure are illustrated and described in detail in the figures and the description herein, results in the figures and their description are to be considered as exemplary and not restrictive in character; it being understood that only the illustrative embodiments are shown and described and that all changes and modifications that come within the spirit of the disclosure are desired to be protected.

[0098] Unless defined otherwise, the scientific and technology nomenclatures have the same meaning as commonly understood by a person in the ordinary skill in the art pertaining to this disclosure.

[0099] This disclosure provides a novel platform for controlling the transplanted cell activity by genetically incorporating a fusion receptor on the transplanted cell surface. These transplanted cells will then be specifically targeted by a small molecule ligand conjugated-drug payload, using the intrinsic high affinity between the small molecule ligand and the fusion receptor on the transplanted cell surface. One part of the fusion receptor is responsible for internalizing the conjugate and the payload will be released through a releasable linker once it is inside of the transplanted cells. Depending on the transplanted cell type and the desired regulation to be imposed on the transplanted cells, the drug payload can be various functions. By changing the payload in the conjugate, for example, as cytotoxic drug or kinase inhibitors, the drug payload may be used to control the multiple aspects of the transplanted cells, such as proliferation, differentiation or cytokine release profile.

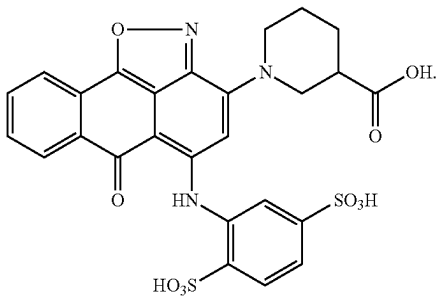
[0100] The peptidyl-proline isomerase (PPIases) family consists of FK506-binding protein (FKBP), cyclophilins and parvulins. In human, there are 18 FKBPs, 24 cyclophilins and 3 parvulins. Among these, FKBP51 and FKBP52 share high to moderate binding affinity of FK506, KD^{FK506}≈104 nM and KD^{FK506}≈23 nM, respectively, comparing to FKBP12 (KD^{FK506}≈0.2 nM). Additionally, none of these two FKBPs are expressed on the cell membrane, resulting in little cross binding activity in our system. Efforts have also been made to design synthetic ligands that have higher affinity to $FKBP12^{F36V}$ than $FKBP^{WT}$, as well as to $FKBP51^{F67V}$, which preserve the overall structure of the wild type proteins. All the homolog and mutated proteins together with their ligands mentioned above, can be adapted to this disclosure.

[0101] Particularly, in one embodiment, an exemplified pair of small molecule ligand and fusion receptor is chosen as FK506-FKBP. The entire process can be generalized in FIG. **1**A, where the FK-506-payload binds to the FKBP-FRa engineered cell first; upon this binding, the transmembrane fusion protein will internalize the payload-linker-FK506. Next, the internalized FK506-payload is cleaved at the linker, and the pay load got released in the cell. Depending on the cell type and the payload type, the released payload drug can exert its desired function.

[0102] In FIG. **1**B, a specific Chimeric Antigen Receptor T cell mediated cell therapy is illustrated. In this model, a CAR T cell expressing a fusion protein with the structure from N terminus to C terminus comprising a suitable signal peptide, a protein module 2 linked to a protein module 1 of GPI anchor is presented to a cancer cell. In some embodiment, the cancer cell has CD19 surface protein, which will be recognized by the CAR T cell and engaged with the payload associated with the CAR T cell, when a targeting ligand binds to at least one module of the fusion receptor. Typically, with high affinity of the targeting ligand toward any one of these modules, the payload associated with the ligand may be internalized into the target cell and be released to engage the cancer cell through the chimeric antigen receptor.

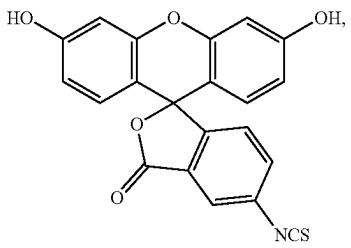
[0103] There can be many different combinations of GPI anchored proteins, represented as Module 1 and its ligands, and Module 2 target cell surface protein and its respective ligands, represented in FIG. **2**B. It is contemplated that either module 1 protein or module 2 protein, or both can engage a high affinity targeting ligand to facilitate the ligand conjugated payload delivery. For example, it is feasible to have a fusion protein comprising FRa-Linker-FKBP structure, wherein FRa engaging with an FA derivative, at the same time, FKBP engaging a FK506 derivative, either FA derivative or FK 506 derivative or both can be linked to a payload, such as a cytotoxic drug, or an imaging agent, or a modulator. With such flexibility of carrying same or different payload, one can achieve some unexpected, synergy or regulatory effect of different or same payload, or to observe the target cell if the payload is an imaging agent. The advantages of flexibility and diversity of payload delivery of this system will be appreciated with more examples.

[0104] Similarly, another embodiment of ligand paired with a GPI anchored protein can be



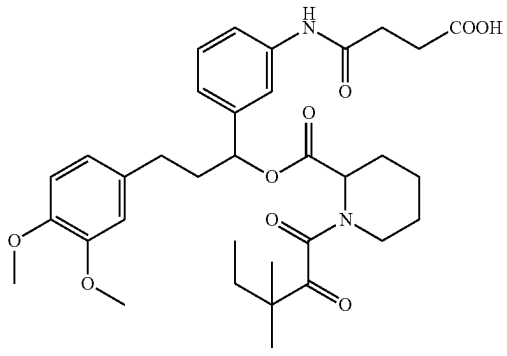
'paired with uPAR

[0105] It is also contemplated that FITC or its derivatives may bind to a single chain fragment of variant (scFv) of an antibody against FITC, for example, a ligand structure of



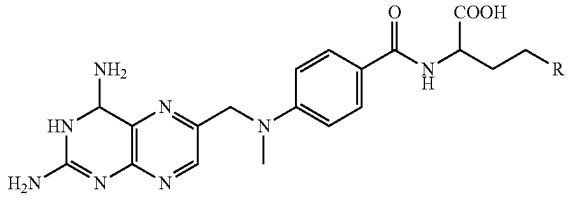
FTTC derivitives

or a structure of



SLF derivitives

paired with FKBP, or

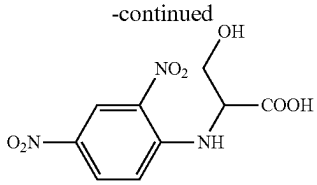


MTX

derivitives

pair with DHFR, or

-continued



DNP-Ser

pair with scFV against DNP.

[0106] It is worth mentioning a few advantages of choosing FK506-FKBP as an exemplary ligand-protein pair in this delivery system. 1. FKBP is not a membrane protein that naturally present on the mammalian cell membrane, so that the FK506-payload conjugate will specifically bind to the target cell; 2. FKBP protein is a relatively small protein with molecular weight of 12 kDa, which makes it easier to be fused with other receptors with minimum perturbation to the receptors' structure and internalization properties. 3. FK506 is not naturally present inside human being, so that the fusion receptor will not be blocked; 4. The binding affinity between FK506-FKBP is around 4 pM so that the payload drug can be delivered with high affinity; 5. The co-crystal structure of FK506-FKBP is available and the well-established derivative site of FK506 preserves the binding of FK506-FKBP while abolishes the unwanted binding between FK506 and calcineurin (See FIG. **2**A).

[0107] It is contemplated that the sequence of FKBP can be modified, and the corresponding FK506 ligand can be modified accordingly to the extent that the modified versions of FKBP and modified version of FK506 still have the desired affinity as exemplified herein or better than the current disclosure.

[0108] For the other portion of the fusion protein, folate receptor (FR) is chosen for its well understood internalization process as a monomer. Prior research shows that 'magic carbonate' linked folic acid conjugate can be internalized through FR and cleaved by the reductive environment inside the cell. Using this mechanism, the FK506-FKBP conjugated drug payload is internalized by FR and will then be released to the cytoplasm.

[0109] Due to the great potential of CAR T therapy as well as the severe side effects, several controlled CAR T cell designs have been reported. Most of them focused on the ON/OFF switch by incorporating either a Boolean gate or a cascade pathway for the T cell activation (see FIG. **3**, left, which depicts negative and positive regulation of the CAR T cell activity, adapted from *The quest for spatio-temporal control of CAR T cells,* Sun J. etc. 2015). Malcolm K. B. group designed the FKBP-caspase9 fusion protein and use FK506 dimer to induce the apoptosis of the target cell (see FIG. **3**, right, which depicts mechanism of AP1903 (FK506 dimer) induced FKBP-caspase9 mediated apoptosis and the structure of AP1903, adapted from *Inducible Apoptosis as a Safety Switch for Adoptive Cell Therapy,* Malcolm K. B. etc. 2011).

[0110] The current disclosure has several advantages over these reported methods: 1. Instead of a binary ON/OFF switch, our platform can delivery multiple kinds of regulating payloads, modifying many aspects of the target cells, thus it has great flexibility compared to binary ON/OFF switch; 2. the controlling moiety, FK506-payload, is a small molecule, which makes it possible for linear control and dosage optimization compared to pre-engineered cells. 3. The platform can be utilized in not only CAR T cell, but many other stem cell based regenerative therapies.

[0111] The greatest novelty and most important part of this platform is the multifunctional payloads, which can be selected to either address the potential side effects or improve the efficiency of the cell therapy. For instance, cytotoxic drugs can be delivered to the transplanted cells if: 1. the cells over-proliferate and affect the normal organ or system, like anti CD19 CAR T cell. 2. The cells become tumorigenic, which lies in the lentivirus based gene modification and the intrinsic characteristic of stem cells.

[0112] On the other hand, some cell therapies receive less success because of the suppressive microenvironment of the target tissue. For instance, in CAR T cell therapy against solid tumor, aside from the low penetration rate, the proliferation and activation of CAR T is highly suppressed by the MDSC and the tumor cells. This can be potentially alleviated by the RORrt agonist or MAP kinase inhibitor induced T cell activation as well as TLR8 agonist induced expression of granzyme B in CAR T cells. Although intracellular targets, like RORrt, may be more suitable for our payload, membrane receptors such as TLR8 may also be accessible due to the proximity on the cell membrane.

[0113] In stem cell regenerative therapy, the payload is more diverse according to the disease models. Instead of delivering a pre-fixed gene, which has been developed by using stem cell as a gene delivery platform, the current disclosure provides a fine tune to transplanted cells and their microenvironment, and obtains the desired phenotype through diverse small molecule payloads. Since the small molecule is conjugated to FK506, which specifically target the FKBP-FRa overexpressed transplant cell, the non-specific targeting of normal tissues of the small molecule is also avoided.

[0114] One of the many examples is to induce the overexpression of BMP2 in mesenchymal stem cell (MSC) for bone fracture repair in skeletal regenerative therapy. Meanwhile, the expression level of BMP2 and/or VEGF can be increased by introducing GSK3 beta inhibitor to the transplanted cells through this drug payload delivery system. GSK3 beta inhibitor is a desired drug for bone fracture repair. Therefore, GSK3 beta inhibitors is ideal as a potential payload for further modulating the function of the transplanted MSC as well as the microenvironment within the bone fracture sites.

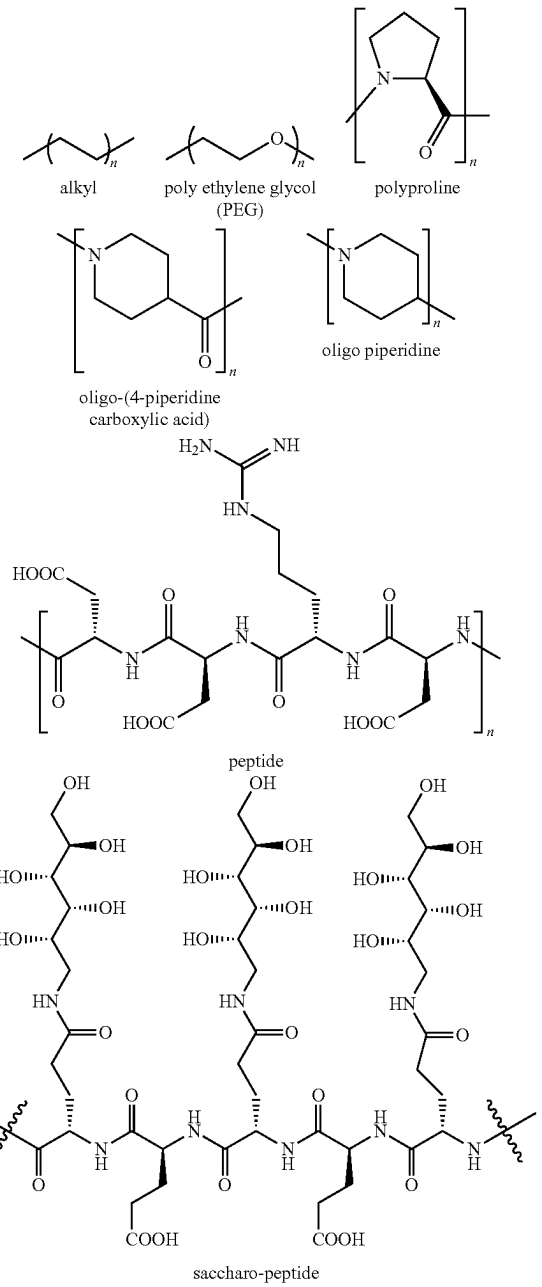
[0115] Another example of this drug payload delivery system goes to the neurodegenerative disease, including Alzheimer, Parkinson disease and etc., where MSC based therapy holds a promising future. MSC has been modified to overexpress GDNF, VEGF and many other cytokines to promote the neuronal regeneration. Meanwhile, small molecules like MAO-B inhibitors has been confirmed to increase the expression of GDNF, NGF and BDNF in astrocytes. Several kinase inhibitors have also been proposed for the treatment of Alzheimer disease, such like PI3K inhibitor (BEZ235), Cdk5 inhibitor (roscovitine) and GSK3b inhibitor (NP-12). Using FK506-FKBP pair targeted specific delivery of these small molecules to MSC in neurodegenerative models will improve the regeneration efficacy while avoiding the side effects of these potent inhibitors and agonists in other non-targeted tissues.

Material and Methods

Compounds and Synthesis Procedure

[0116] Targeting ligand is linked with payload through linkers. The linker optimization options are listed below, payloads are characterized into 3 classes: I imaging, II cytotoxic drug, III regulatory small molecule drug.

Linker Optimization

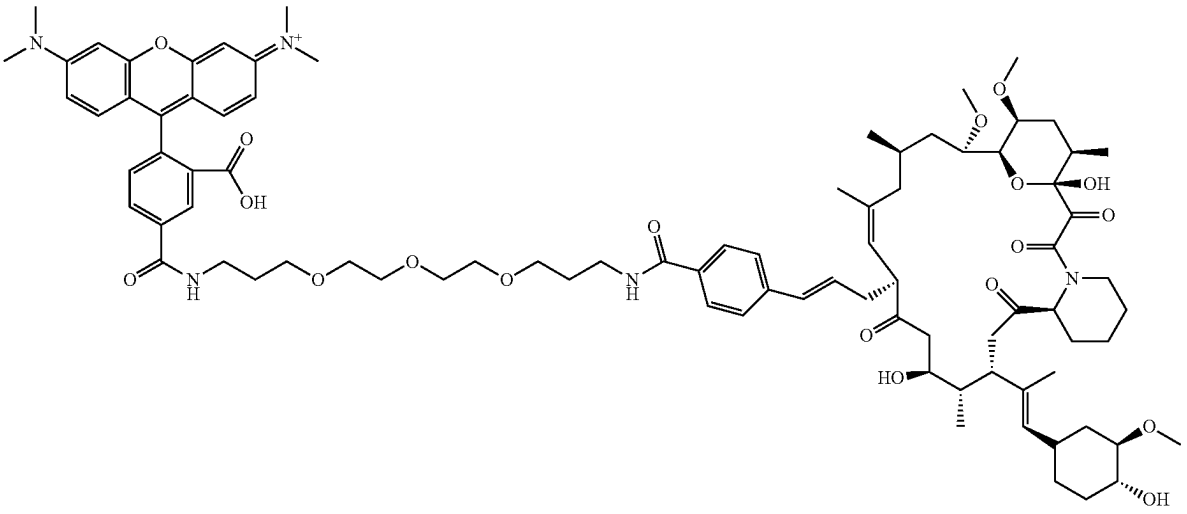


Compounds Classification

Class	function	example
I	Imaging	Fluorescent dye: Rhodamine, FITC Radioisotope imaging: EC20 chelating head, NOTA, DOTA
II	Cytotoxic drug	Anti-microtubule drug: Tubulysin, DM1, DM4
III	Modulator	Kinase inhibitor: Dasatinib, MEK 1/2i, PI3Ki siRNA: mi181a1

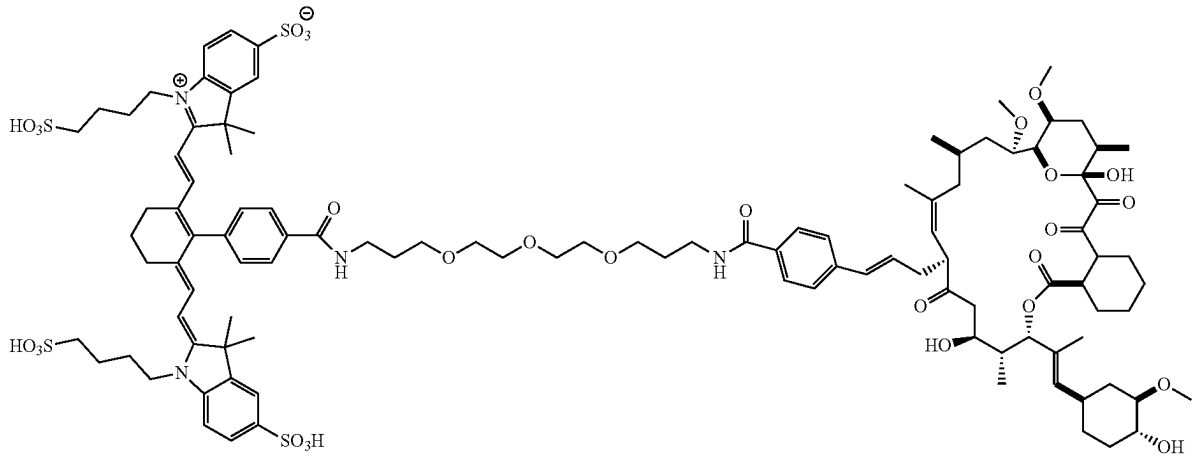
Detailed Compound Structure and Synthesis Route

FK506-Rhodamine



[0117] Procedure: Rhodamine-NHS ester (1.0 equiv.) in dimethylformamide was reacted with Boc-NH-PEG_3-NH_2 (1.2 equiv.) and diisopropylethylamine (3.0 equiv.) for 2 h at room temperature. The product was purified by preparative reverse-phase HPLC with a UV detector. The purified Rhodamine-PEG_3-NH-Boc conjugate (1.0 equiv.) was subjected to Boc deprotection by stirring in a 1:10 TFA-dichloromethane system for 2 h. The crude free amine product was then dissolved in dimethylformamide and activated with EDC (2.0 equiv.), HOBT (2.0 equiv.) in the presence of diisopropylethylamine (3.0 equiv.). After 15 minutes, FK506-CO_2H (1.2 equiv., synthesized using the procedure in the reference: Bioorg. Med. Chem., 17 (2009) 5763-5768) was added and the reaction mixture was stirred overnight. The final FK506-Rhodamine conjugate was isolated after purification on preparative reverse-phase HPLC with a UV detector (monitored at wavelength of 280 nm). The crude product was loaded onto an Xterra RP18 preparative HPLC column (Waters) and eluted with gradient conditions starting with 95% 5 mM sodium phosphate (mobile phase A, pH 7.4) and 5% acetonitrile (mobile phase B) and reaching 0% A and 50% B in 35 min at a flow rate of 12 mL/min. Retention time of the product peak=2.5 min during the gradient (0-50% JB) in a 7 min analytical HPLC-MS analysis. ESI m/z=1539.6. Abbreviations: PEG =polyethylene glycol; EDC=1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide; HOBT=Hydroxybenzotriazole; HPLC=High Performance Liquid Chromatography.

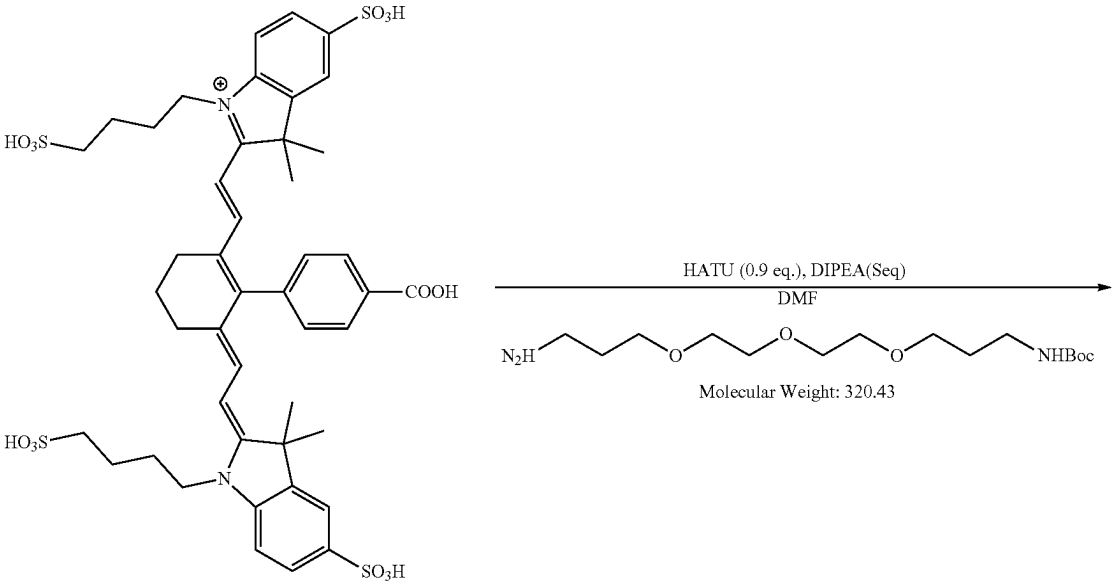
FK506-NIR dye



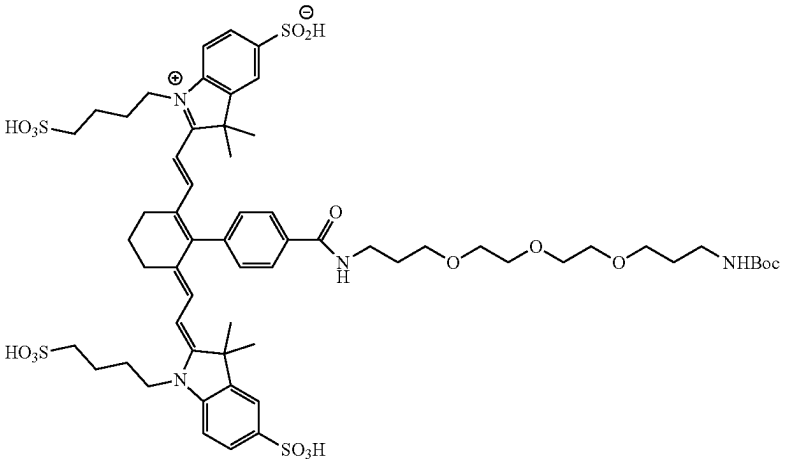
Molecular Weight: 2081.57

-continued

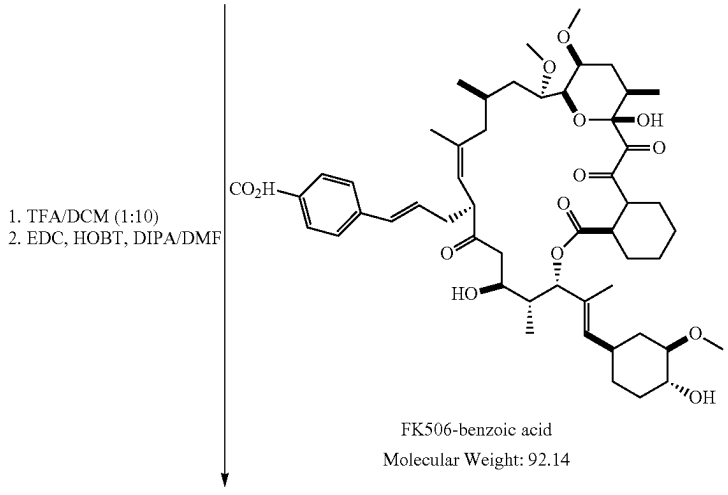
Synthesis procedure



Molecular Weight: 320.43



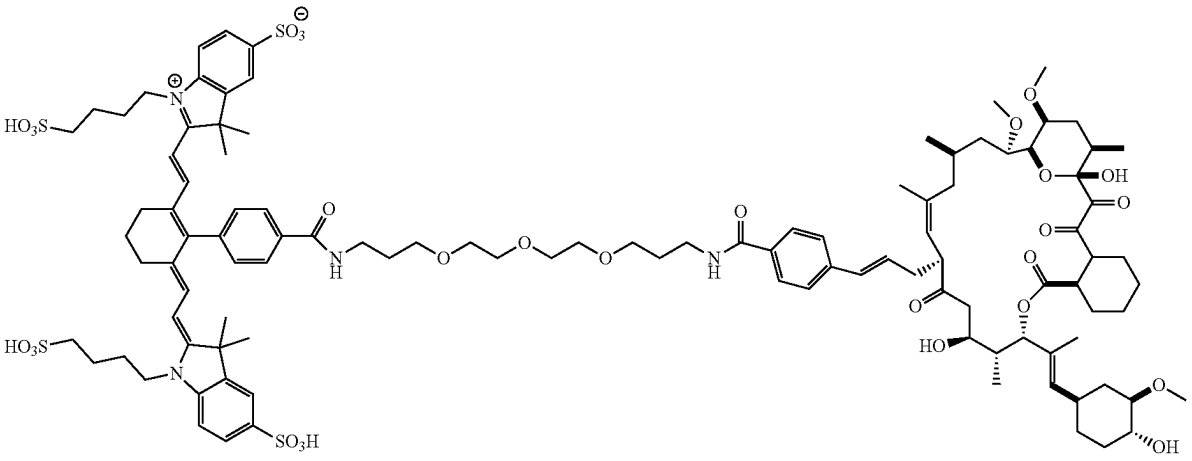
Molecular Weight: 1276.57



FK506-benzoic acid

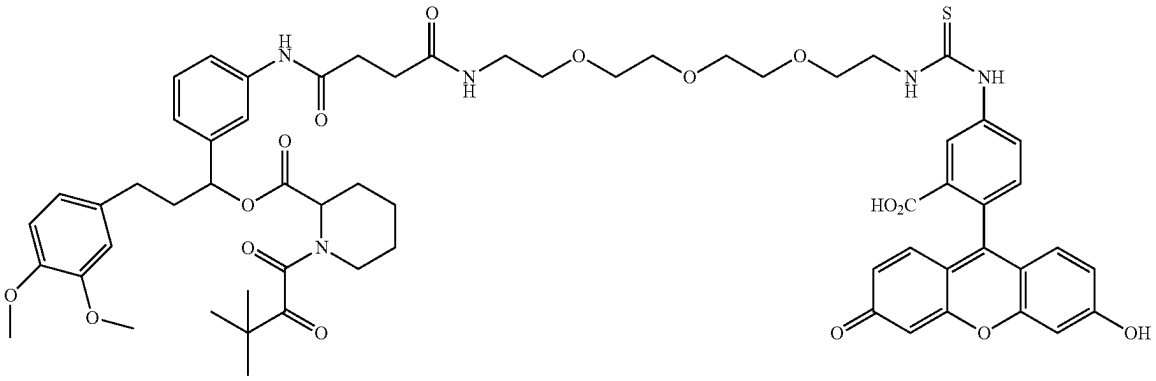
Molecular Weight: 92.14

-continued



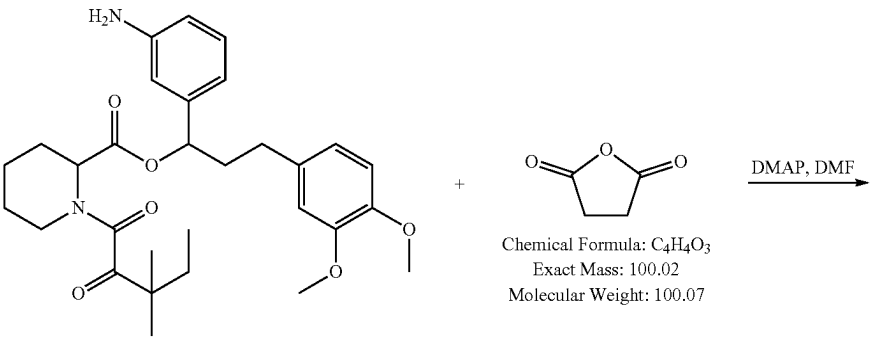
Molecular Weight: 2081.57

SLF-FITC



Chemical Formula: $C_{63}H_{73}N_5O_{16}S$
Exact Mass: 1187.48
Molecular Weight: 1188.36

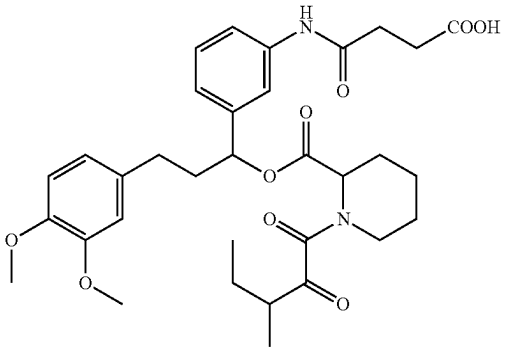
Synthesis procedure:



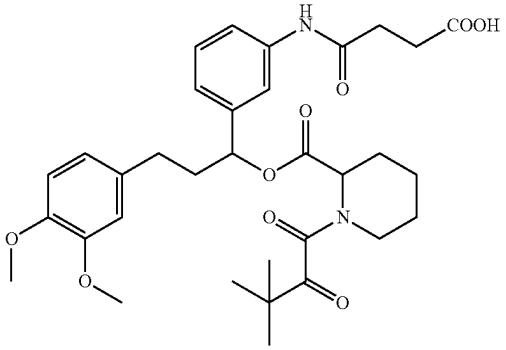
Chemical Formula: $C_{30}H_{40}N_2O_6$
Exact Mass: 524.29
Molecular Weight: 524.66

Chemical Formula: $C_4H_4O_3$
Exact Mass: 100.02
Molecular Weight: 100.07

-continued

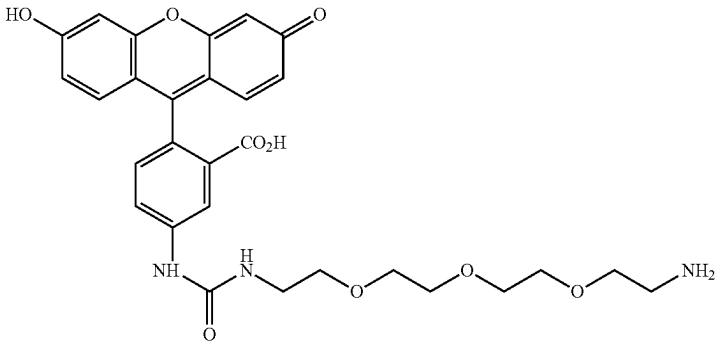


Chemical Formula: $C_{34}H_{44}N_2O_9$
Exact Mass: 624.30
Molecular Weight: 624.73



+

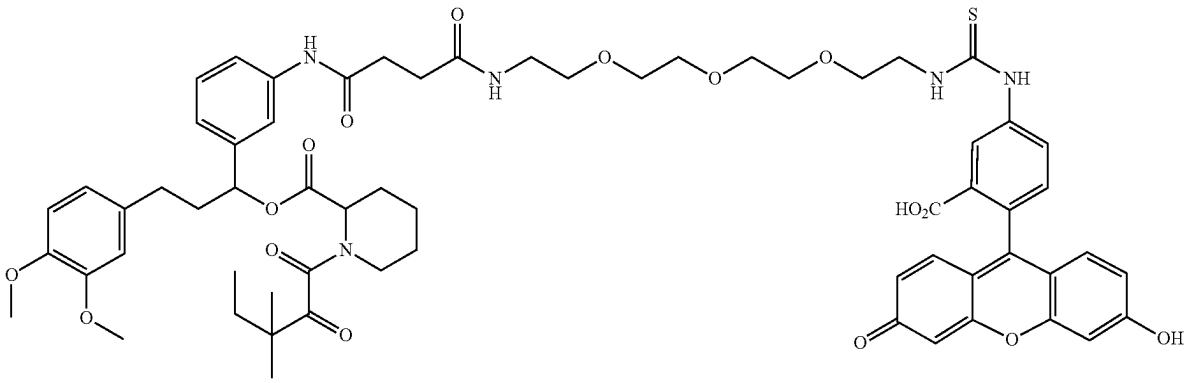
Chemical Formula: $C_{34}H_{44}N_2O_9$
Exact Mass: 624.30
Molecular Weight: 624.73



HATU, DIPEA, DMF

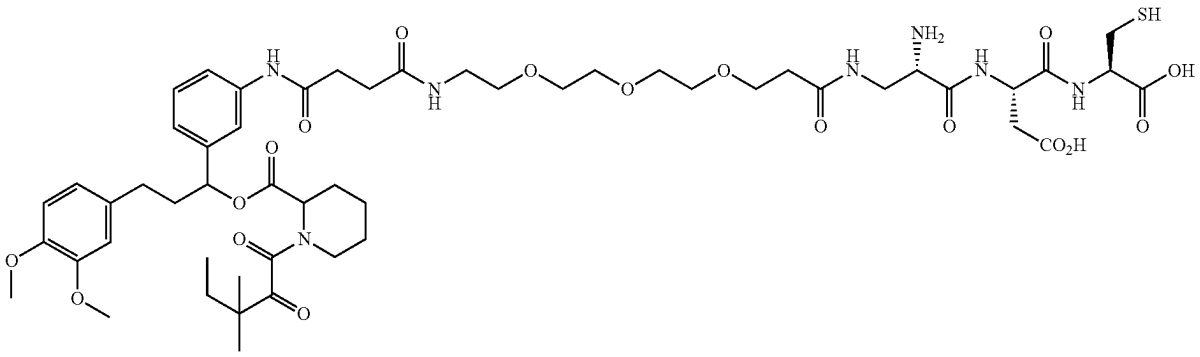
Chemical Formula: $C_{29}H_{31}N_3O_8S$
Exact Mass: 581.18
Molecular Weight: 581.64

-continued



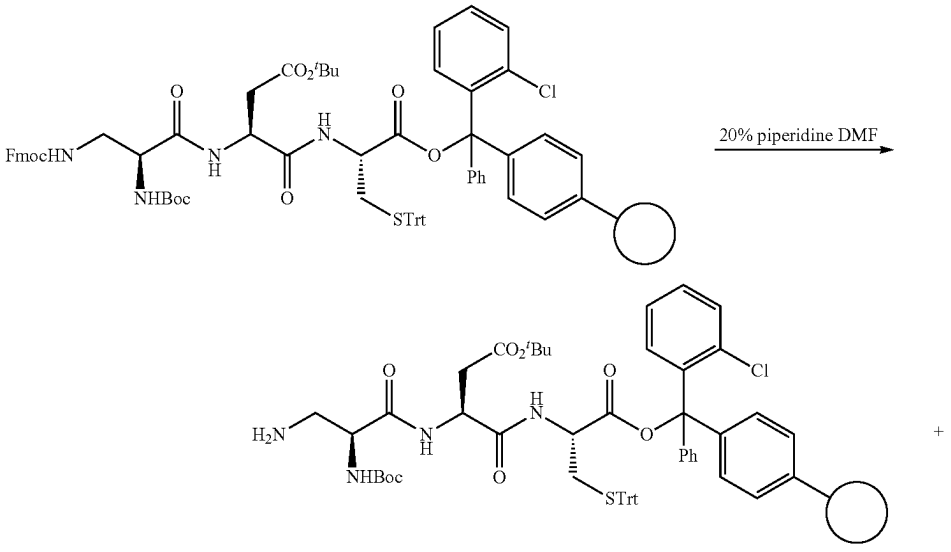
Chemical Formula: $C_{63}H_{73}N_5O_{16}S$
Exact Mass: 1187.48
Molecular Weight: 1188.36

SLF-EC20

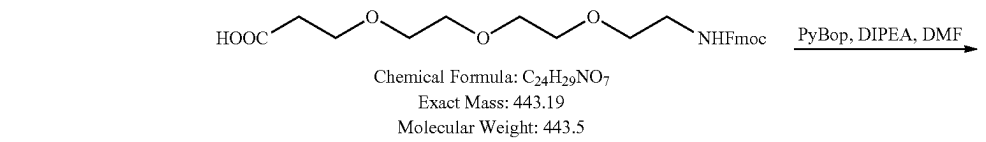


Chemical Formula: $C_{53}H_{77}N_7O_{18}S$
Exact Mass: 1131.50
Molecular Weight: 1132.29

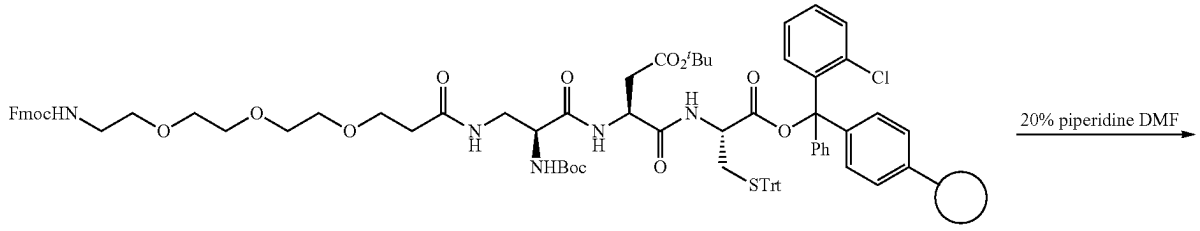
Synthesis procedure

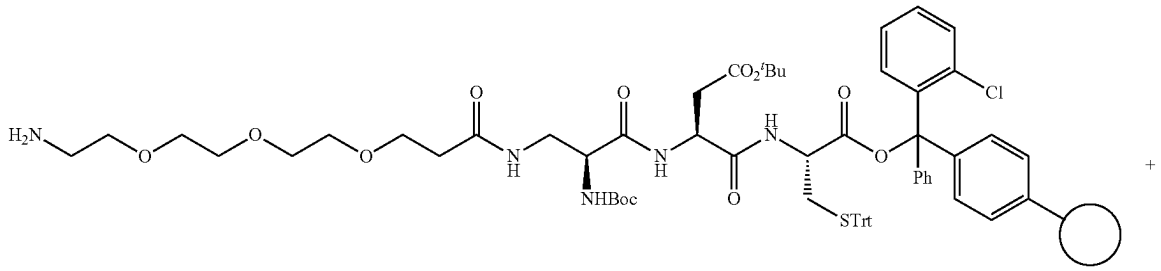


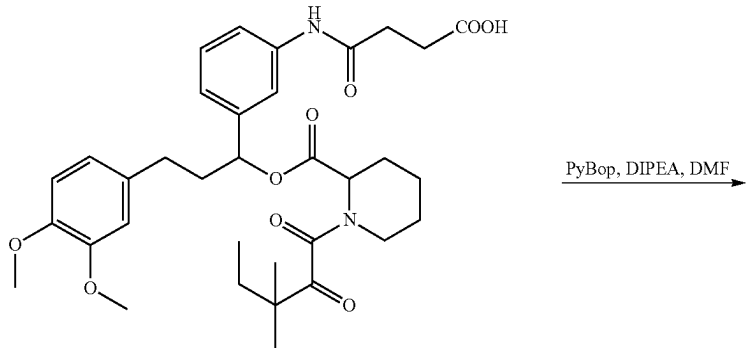
-continued



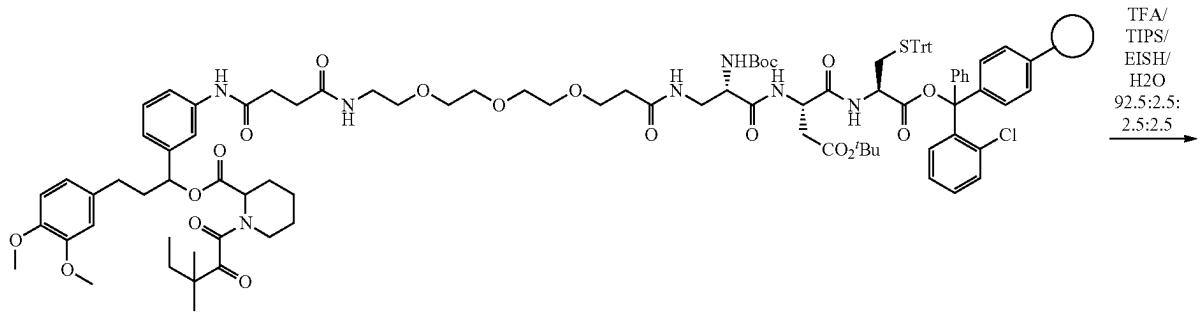
Chemical Formula: $C_{24}H_{29}NO_7$
Exact Mass: 443.19
Molecular Weight: 443.5



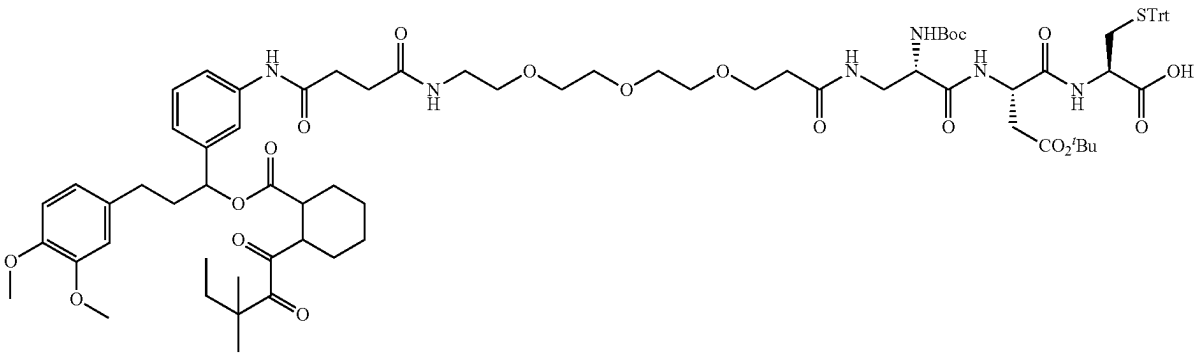




Chemical Formula: $C_{34}H_{44}N_2O_9$
Exact Mass: 624.30
Molecular Weight: 624.73

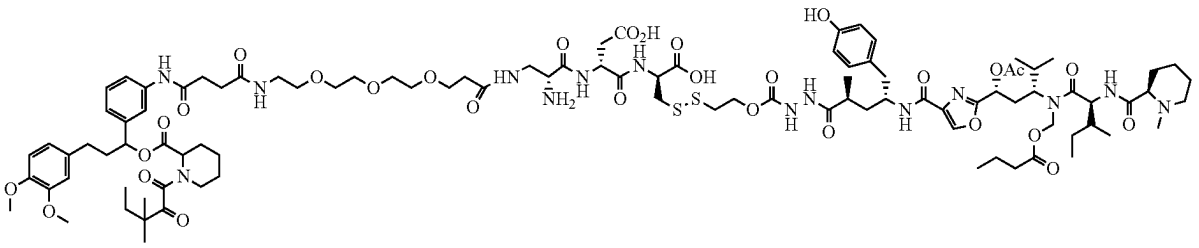


-continued



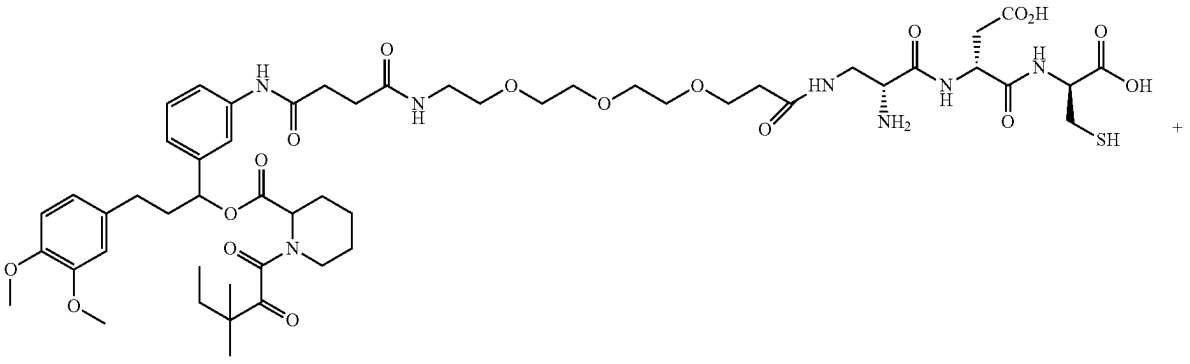
Chemical Formula: $C_{53}H_{77}N_7O_{18}S$
Exact Mass: 1131.50
Molecular Weight: 1132.29

SLF-Tubulysin

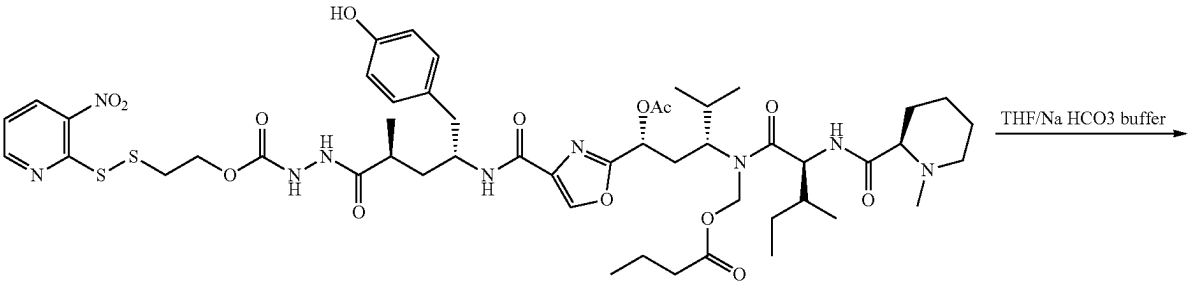


Molecular Weight: 2078.48

Synthesis procedure

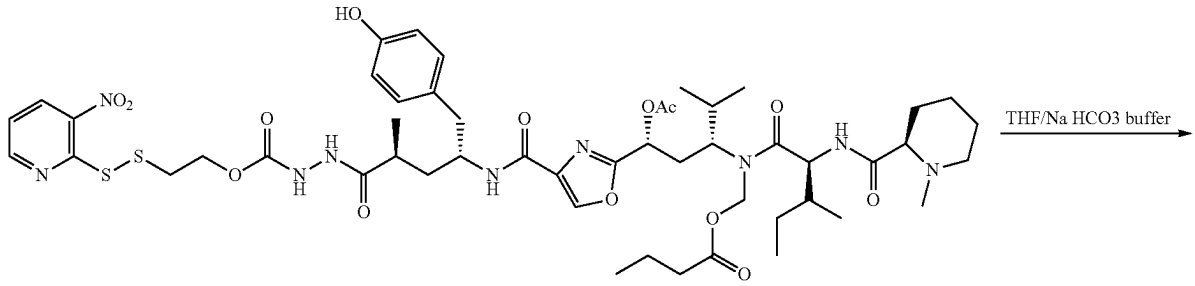


Chemical Formula: $C_{53}H_{77}N_7O_{10}S$

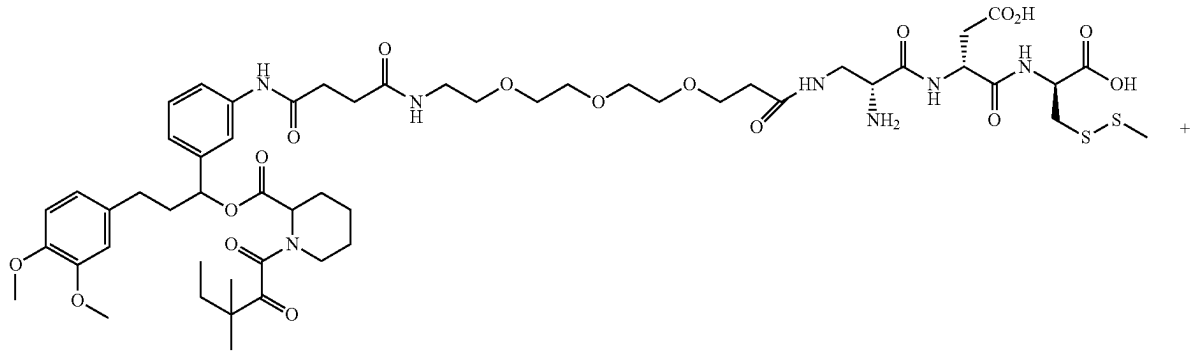


Exact Mass: 1101.43
Molecular Weight: 1102.35

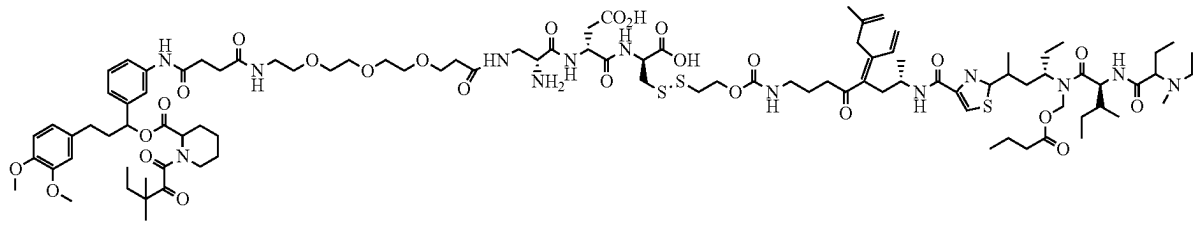
-continued



Exact Mass: 1101.43
Molecular Weight: 1102.35

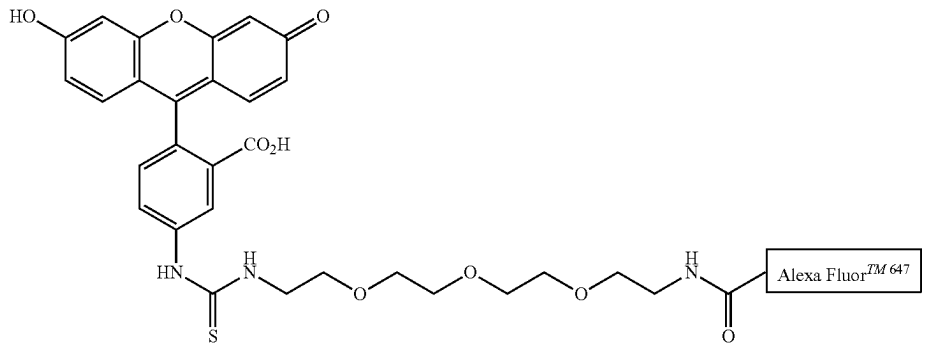


Chemical Formula: $C_{53}H_{77}N_7O_{10}S$
Exact Mass: 1131.50
Molecular Weight: 1132.29



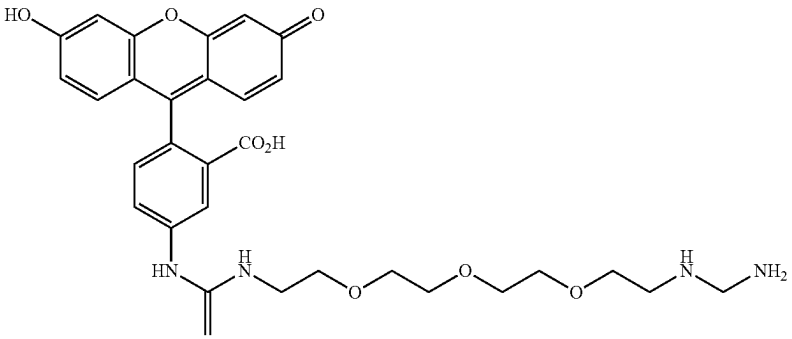
Molecular Weight: 2078.48

FITC-AF647



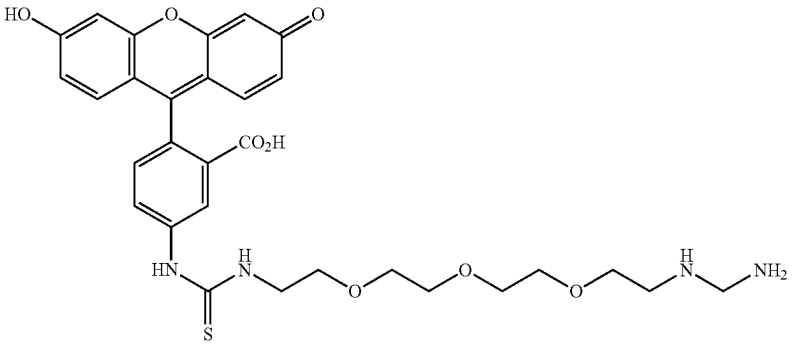
-continued

Synthesis procedure



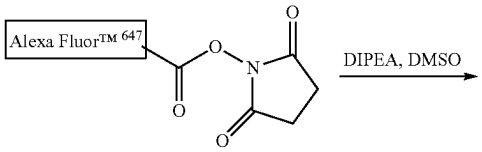
Chemical Formula: $C_{29}H_{31}N_3O_5S$
Exact Mass: 581.18
Molecular Weight: 581.64

Synthesis procedure:

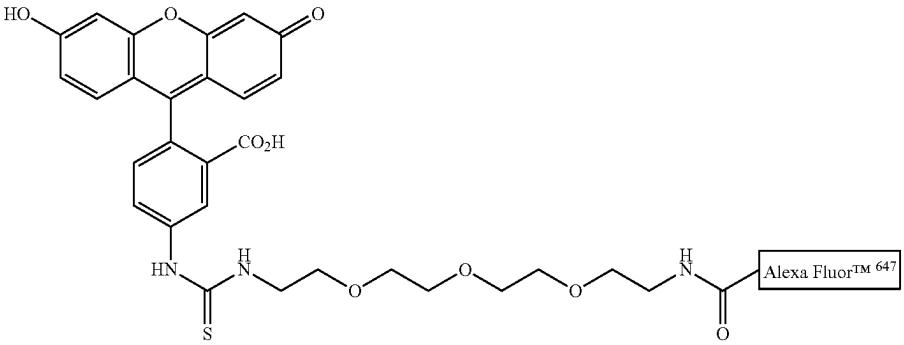


+

Chemical Formula: $C_{29}H_{31}N_3O_5S$
Exact Mass: 581.18
Molecular Weight: 581.64

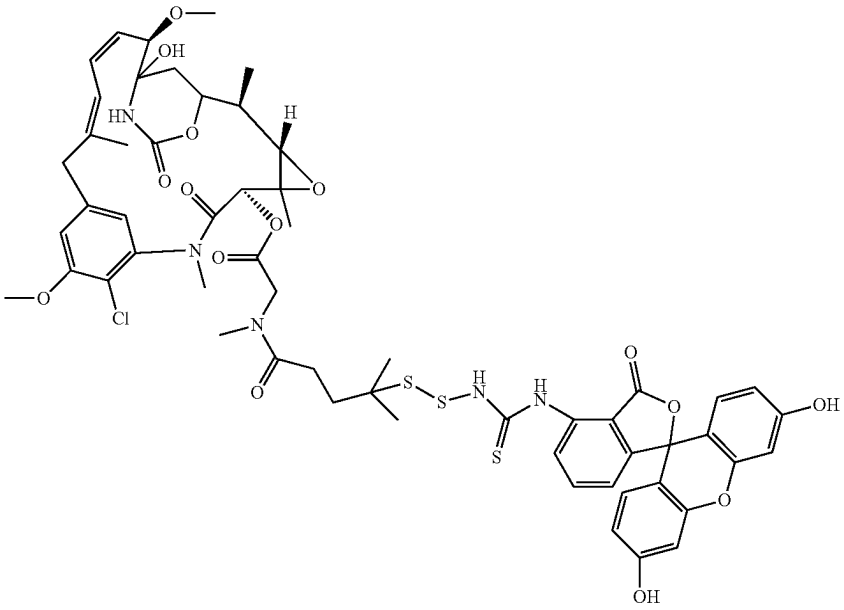


Alexa Fluor™ 647 NHS Ester
(Succinimidyl Ester)

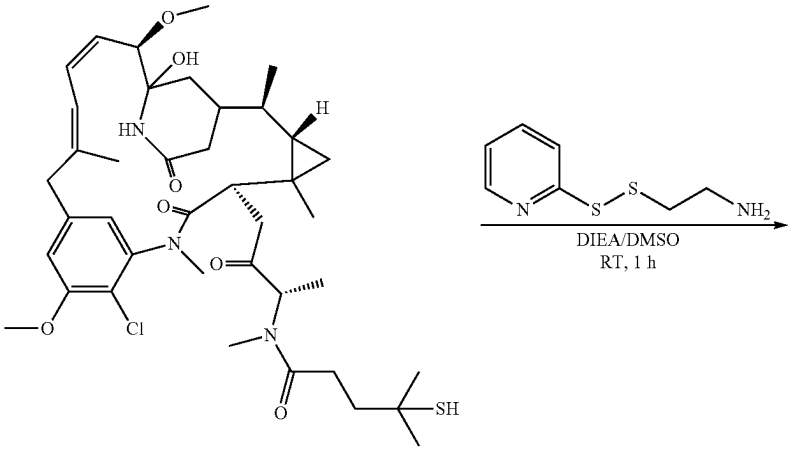


-continued

FITC-DM4:

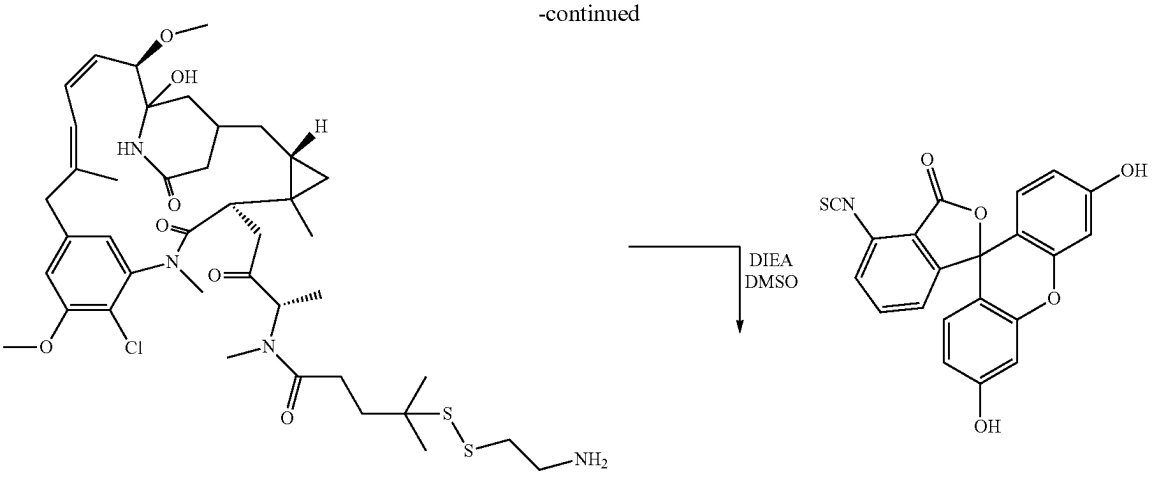


Synthesis procedure:

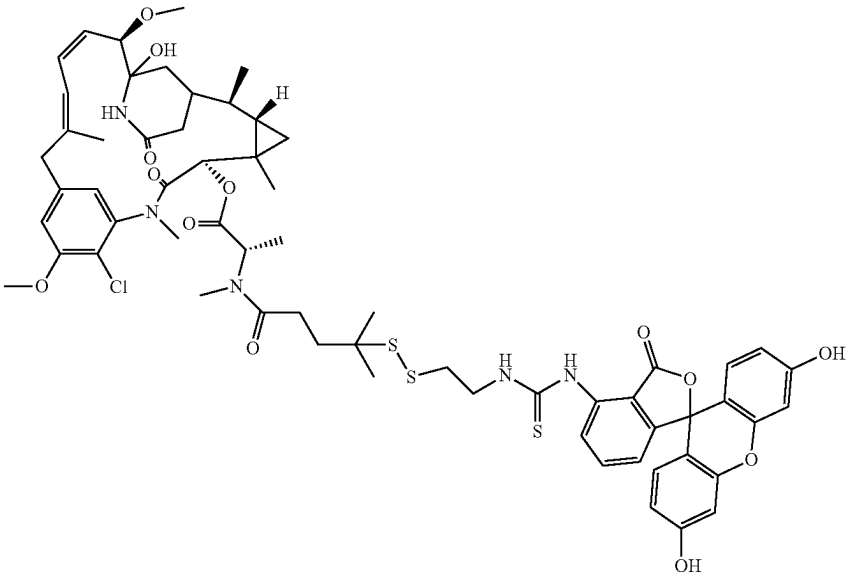


Chemical Formula: $C_{38}H_{54}ClN_3O_{10}S$
Molecular Weight: 780.37

-continued

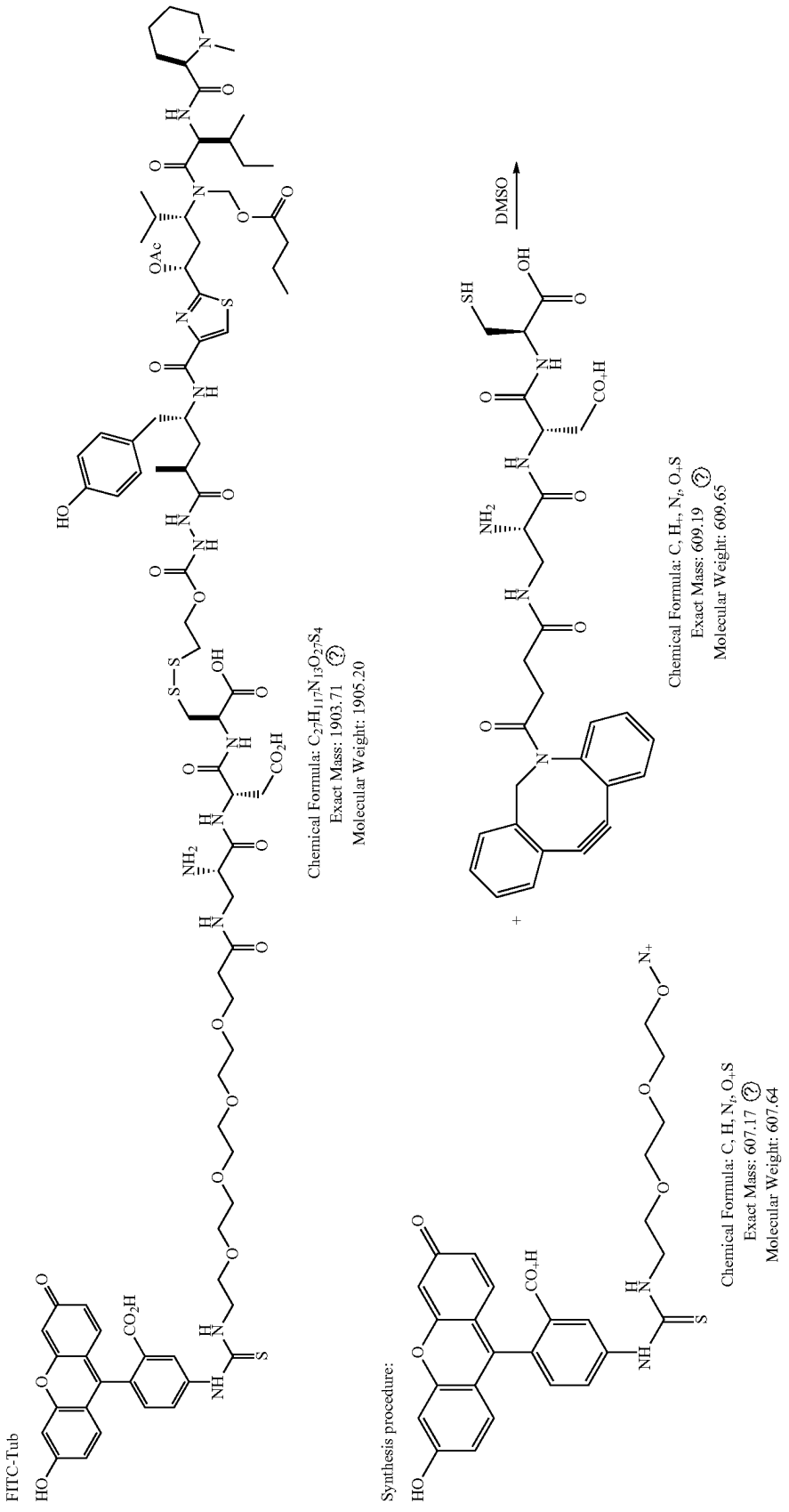


Chemical Formula: $C_{40}H_{59}ClN_4O_{10}S_2$
Molecular Weight: 855.50

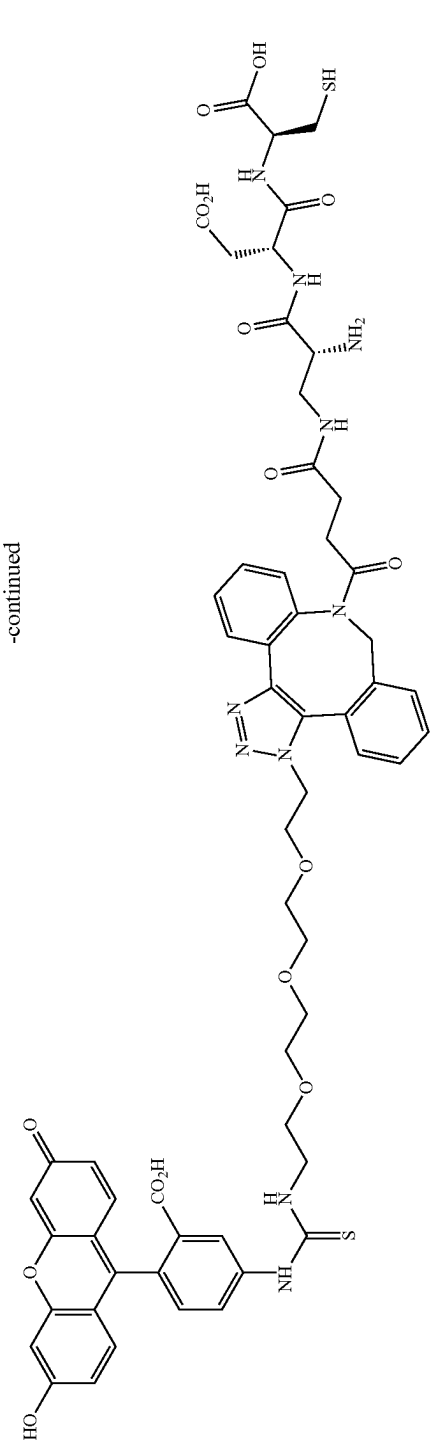


Chemical Formula: $C_{61}H_{70}ClN_5O_{15}S_3$
Molecular Weight: 1244.88

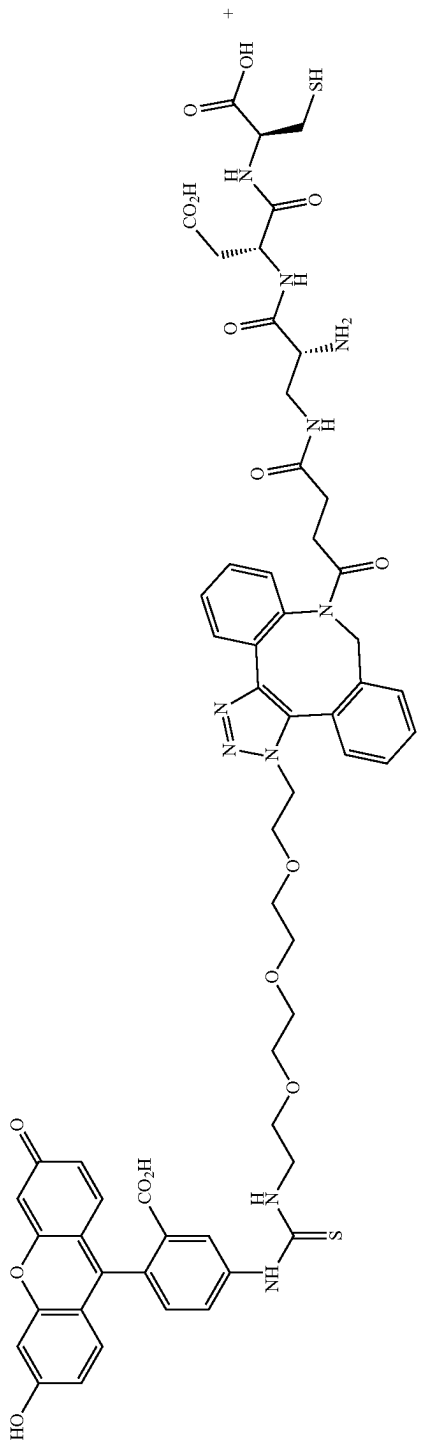
Procedure: DM4 (1.0 equiv.) in dimethylsulfoxide was reacted with 2-(pyridin-2-yldisulfaneyl)ethan-1-amine (1.0 equiv.) and diisopropylethylamine (3.0 equiv.) for 1 h at room temperature. The resulting crude product was then reacted with FITC (1.0 equiv.) and the reaction mixture was stirred for 1 h. The final FITC-DM4 conjugate was isolated after purification on preparative reverse-phase HPLC with a UV detector (monitored at wavelength of 280 nm). The crude product was loaded onto an Xterra RP18 preparative HPLC column (Waters) and eluted with gradient conditions starting with 95% 5 mM sodium phosphate (mobile phase A, pH7.4) and 5% acetonitrile (mobile phase B) and reaching 0% A and 100% B in 10 min at a flow rate of 12 mL/min. Retention time of the product peak = 4.23 min during the gradient (0-100% B) in a 7 min analytical HPLC-MS analysis. ESI m/z = 1244.8. Abbreviations: FITC fluorescein isothiocynanate; HPLC = High Performance Liquid Chromatography.


FITC-Tub
Chemical Formula: $C_{27}H_{117}N_{13}O_{27}S_4$
Exact Mass: 1903.71
Molecular Weight: 1905.20
DMSO
Chemical Formula: $C_{+}H_{+}N_{+}O_{+}S$
Exact Mass: 609.19
Molecular Weight: 609.65
Synthesis procedure:
Chemical Formula: $C_{+}H_{+}N_{+}O_{+}S$
Exact Mass: 607.17
Molecular Weight: 607.64

-continued

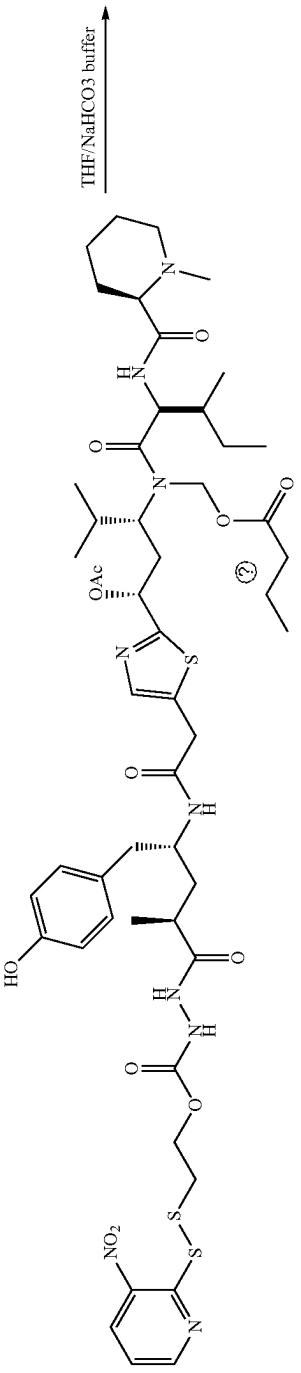


Chemical Formula: $C_{12}H_{11}N, O, S_2$
Exact Mass: 1216.36
Molecular Weight: 1217.29

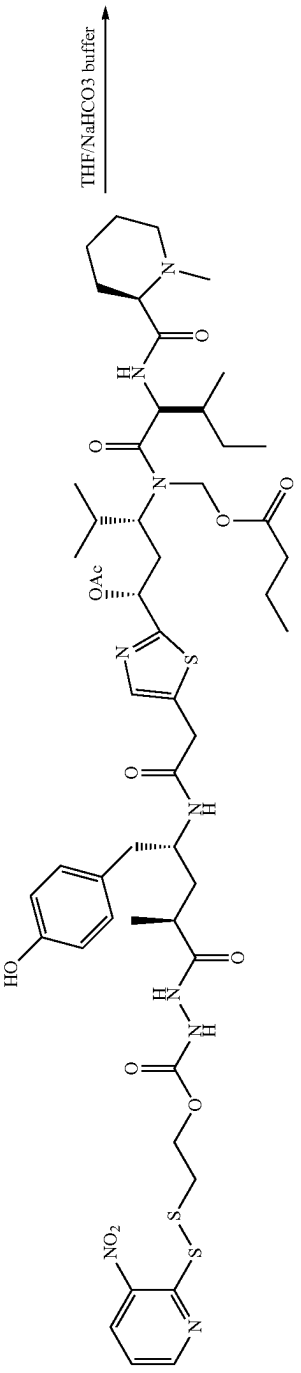


Chemical Formula: $C_{12}H_{11}N, O, S_2$
Exact Mass: 1216.36
Molecular Weight: 1217.29

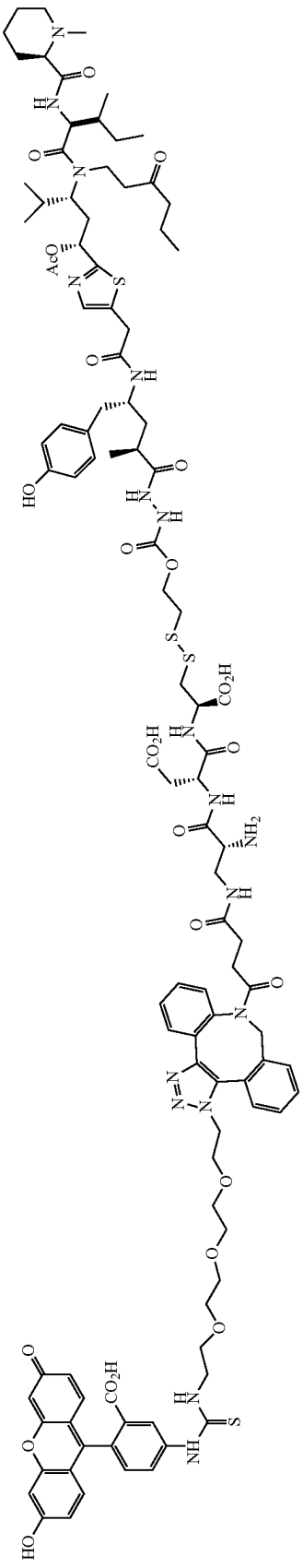
-continued



Exact Mass: 1101.43



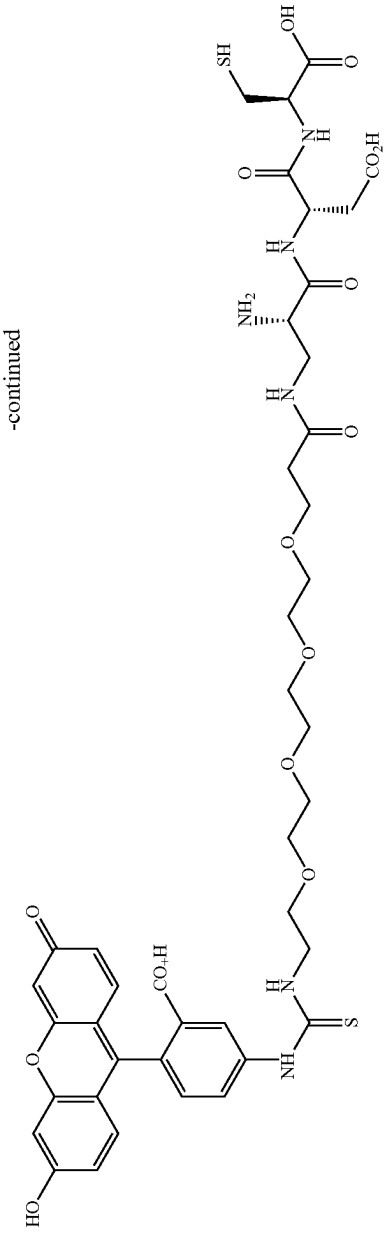
Exact Mass: 1101.43
Molecular Weight: 1102.35



Exact Mass: 2161.80
Molecular Weight: 2183.48

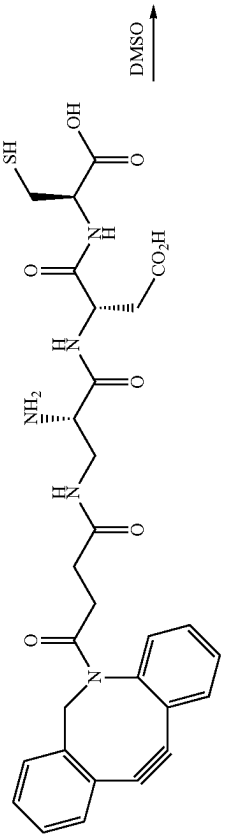
FITC-EC20

-continued



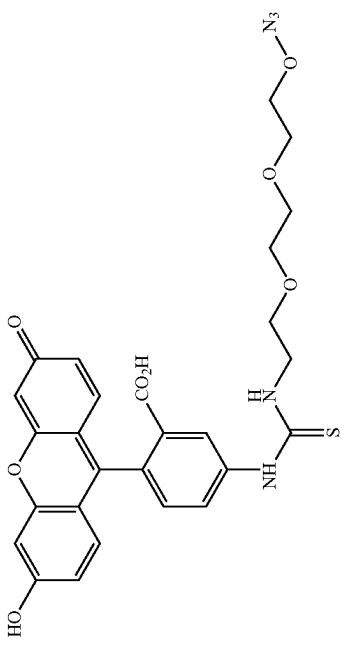
Chemical Formula: $C_{12}H_{50}N_6O_{16}S_2$
Exact Mass: 958.27
Molecular Weight: 959.01

Synthesis procedure:



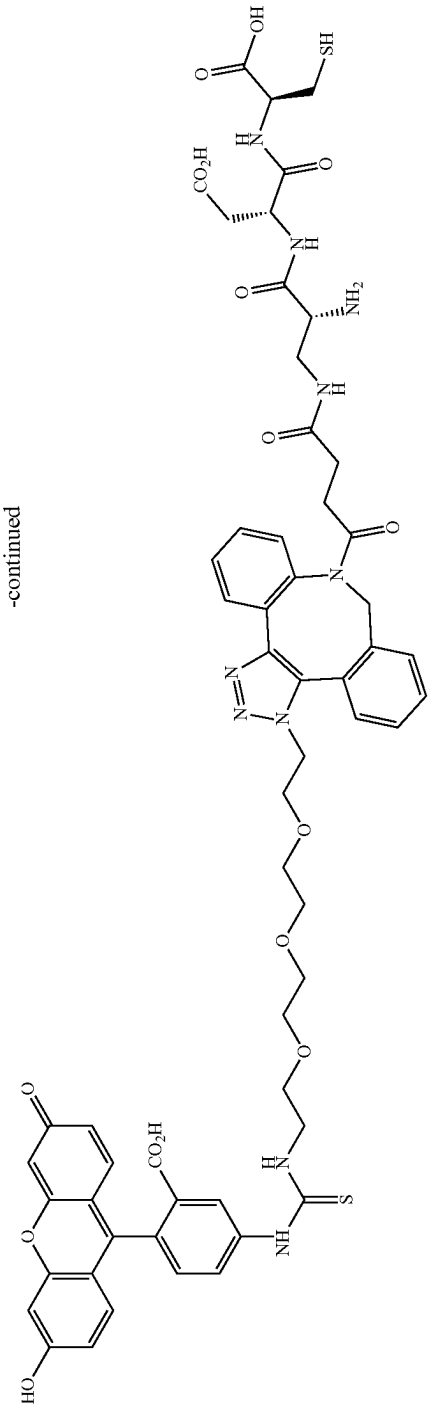
Chemical Formula: $C_{29}H_{31}N_5O_8S$
Exact Mass: 609.19
Molecular Weight: 609.65

DMSO



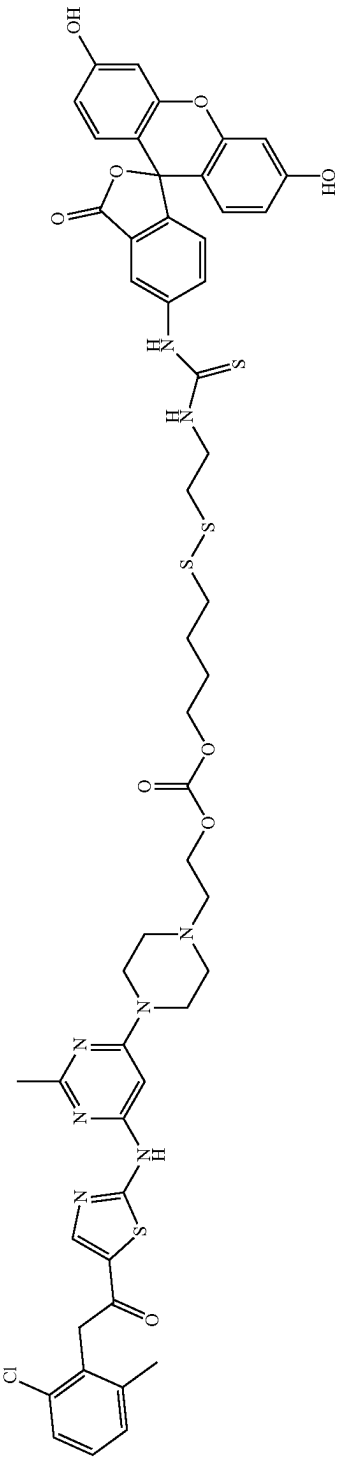
Chemical Formula: $C_{29}H_{16}N_5O_8S$
Exact Mass: 607.17
Molecular Weight: 607.64

-continued



Chemical Formula: $C_{12}H_{11}N_{10}O_{16}S_2$
Exact Mass: 1216.36
Molecular Weight: 1217.29

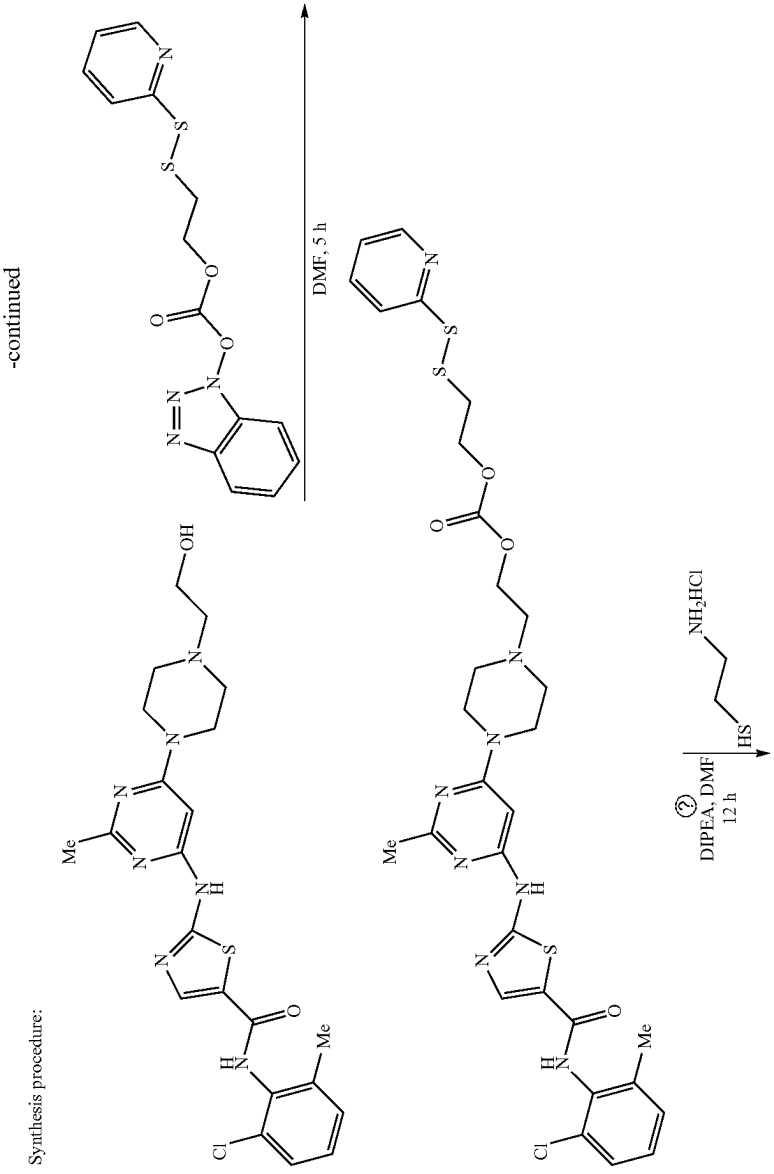
FITC-Dasatinib



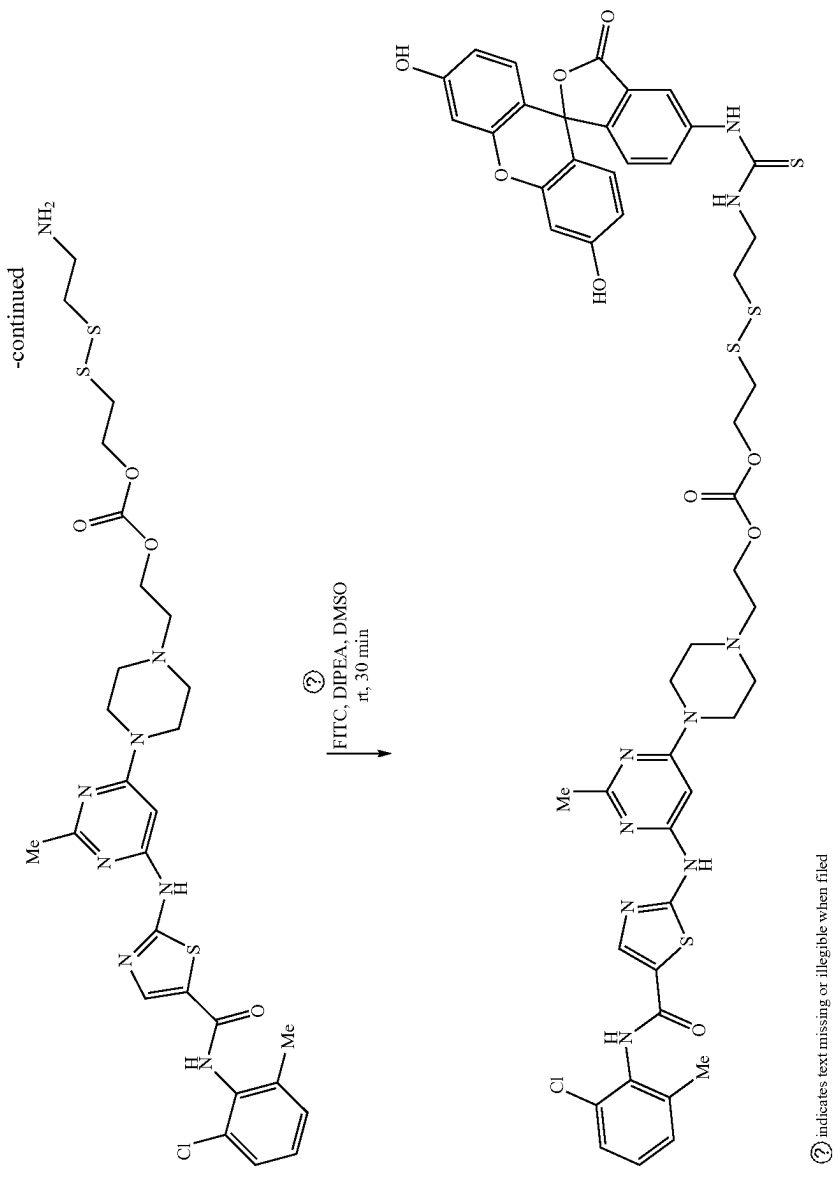
Molecular Weight: 1055.65

-continued

Synthesis procedure:



-continued



? indicates text missing or illegible when filed

The Experiment Procedure

Cell Culture

[0118] 293TN cells were cultured in DMEM with 10% FBS, no antibiotic for lentivirus packaging. Raji and Jurkat cells were cultured in RPMI-1640 with 10% FBS, 10% Penicillin/Streptomycin. Primary human T cells were isolated from hPBMC using ficoll, enriched by negative selection with EasySep™ Human T Cell Enrichment Kit (19051, Stemcell Tech), activated by Dynabeads CD3/CD28 (11161D, Thermo Fisher) for 1 days, cultured with Tex-MACS medium supplemented with 30IU hIL2 (130-097-745, Miltenyi Biotec Inc.). T cells were cryopreserved in RPMI-1640 with 20% human AB serum (HP1022, Valley Biomedical) and 10% DMSO.

Lentivirus Packaging

[0119] Pantropic VSV-G pseudotyped lentivirus was produced via transfection of 293TN cell with the transgene expression vector and packaging plasmid mix (CPCP-K2A, Cellecta) using Lipofectamine 2000. At 24 h, viral supernatant was harvested, concentrated and then added to certain cell lines or the primary T cells that were thawed the same day. For T cell transduction, cells were centrifuged at 2500 rpm for 90 mins, 37 degree after adding the virus supernatant and 8 ug/ml polybrene.

Binding Assay

[0120] For binding assay, cells were incubated with ligand-dye alone or with ligand-dye and free ligands (100×, preincubated for 30 min) for 30 min, at 4 degree. Cells were washed 3 times after incubation, and re-suspended in 2% FBS PBS, 7-AAD were added to gate out the dead cells. FRET imaging of FK506-Rhodamine and FA-S0456: To understand the occupation of the fusion receptor, FKBP-FRa+ jurkat cells are incubated with FA/FA-S0456, FK506/FK506-Rhodamine at the indicated sequence and concentration. FRET is visualized by the loss of intensity of FA-Rhodamine as its energy transferred to FA-OTL38 on the same or nearby receptor, detected by BD Fortessa flow cytometer. Results were analyzed using Flow Jo software.

PI-PLC Treatment to Release the GPI Anchored Protein

[0121] 1×10^5 cells were incubated with 5 mU or 50 mU PI-PLC (P5542-5UN, Sigma) in digestion buffer (2% BSA) at 37 degree for 30 min; after incubation, cells were washed three times by PBS and then incubated with ligand-dye for 30 min on ice.

Cell Viability Assay

[0122] Cells were seeded to 96 well plate and incubated with different concentration of certain ligand-cytotoxic drug for 2 h, with or without 100× preincubation of the free ligand competition. After 2 h incubation, cells were washed by warm medium 3 times and replenished with fresh medium. After 72 h, cell number were tested by CellTiter-Glo® assay (G7570, Promega) or quantified by ligand-dye staining of receptor positive cell.

CAR T cell lysis effect

[0123] 1×10^5 CAR T and certain number of Raji cell were co-incubated in 96 well plate, according to the E:T ratio, with or without the treatment drug. After 24 h, 100 ul supernatant were taken out for LDH assay. Lysis percentage were calculated as (treatment group—CAR T only)/(maximum lysis—CAR T only)%

Exhaustion of CAR T Cell

[0124] 1×10^6 Raji cell were repeated added to 1×10^6 CAR T cell in 24 well plate every 12 h, without changing the medium. Exhaustion status were characterized by lower lysis effect and higher expression of co-inhibitory molecules: PD-1, LAG-3 and Tim-3.

In Vivo Ablation of the Fusion Receptor Positive CAR T by Ligand-Cytotoxic Drug

[0125] 4×10^5 luc+Raji cells were iv injected into the NSG mice. After 6 days, 1×10^7 fusion receptor positive CAR T cells were iv injected. On Day 8, ligand-cytotoxic drug (0.5 umole/kg, 1 umole/kg) were iv injected once. IL2, INFr were measured by ELISA using serum sample taken every 3 days after the CAR T injection. CAR T cell in peripheral blood were counted by flow cytometry.

In Vivo Modulation of Fusion Receptor Positive CAR T by Ligand-Drug

[0126] 4×10^5 luc+Raji cells were iv injected into the NSG mice. After 6 days, 1×10^7 fusion receptor positive CAR T cells were iv injected. On Day 7, ligand-drug drug (0.5 umole/kg, 1 umole/kg) were iv injected once. In the case of CAR T exhaustion model, 1×10^5 CAR T were iv injection on Day 6, once the CAR T population were shown in peripheral blood and tumor burden is not stabilized and continue to increase, ligand-drug (0.5 umole/kg, 1 umole/kg) were iv injected.

In Vivo Imaging and Tracking of Fusion Receptor Positive CAR T

[0127] 2×10^6 Raji were subcutaneously injected to the right should of NSG mice. 14 days later, 1×10^7 fusion receptor positive CAR T were iv injected. On Day 16, animals were administrated 99mTc-bound conjugates (10 nmol, 150 μCi) by iv injection and imaged by SPECT imaging machine.

EXAMPLES

1. Design of Fusion Protein and Its Expression

1.1 Design of FKBP-FR Fusion Receptor (SEQ ID NO:2)

[0128] Synthesis of FK506 derivatives: FK506-Rhodamine and FK506-tubulysin Synthesis was described in materials and methods section. hFRa is a GPI anchored membrane protein, which has 24 amino acids on the N terminal as a signal peptide. In order to preserve the membrane presentation and internalization property, we choose to use the full length of FRa, and incorporate the hFKBP sequence as well as a flexible peptide linker (SGGGS) between T24 and R25 of hFRa (FIG. **4***a*). The flexible linker is chosen to be resistant to common enzyme digestions in

human body. The whole sequence is then inserted into a pWPI lentivirus expression vector, with EF1a as the desired promoter for protein expression in transduced T cell.

1.2 Expression of FKBP-FR Fusion Receptor in Transduced Cells

[0129] Expression of FKBP-FRa is confirmed by western blot in lentivirus transduced K562. Transduced K562 cell lysis shows specific band around 50 kDa compared to non-transduced cells against hFRa antibody (FIG. **4***b*).

1.3 Construction of Fusion Protein of FKBPFR3GS (Noted as FF3)

[0130] See FIG. **4***d* (SEQ ID NO: 12). From N terminal to C terminal it has 1-24 aa of human FRa as the signal peptide, human FKBP protein, three Gly-Ser linker and then 2-258 aa of human FRa. In a construct design of FKBPFR1GS (noted as FF1), the three Gly-Ser linker of FF3 is substituted with one Gly-Ser linker with other parts unchanged. As will be shown in the binding assays, increasing linker length reduces the interference between the two components in the fusion protein.

1.4 Construction of Fusion Protein of FITC-svFv-FR With GS Linker

[0131] See FIG. **4***e*. The construct is also named as 4M5.3 FR (SEQ ID NO: 13). From N-terminal to C-terminal, it has hCD8 signal peptide, svFv of 4M5.3 (against FITC), GS linker, 25-258 aa of human FRa.

1.5. In Vivo Noninvasive Tracking of FKBP-FRa/FKBPtFRa Positive Cells by FK506-99mTc PET Imaging

[0132] For CAR T cell model, 1×10^6 KB cells are subcutaneously implanted in NSG (Jackson laboratory). After the tumor reaches 100 mm^3, 15 million anti-FITC CAR+ FKBP-FRa+ or anti-FITC CAR+ FKBP-FRa− human T cells are intravenously injected to the mice. FITC-FA is injected at the indicated days to induce the proliferation of the CAR T. Mice are imaged every two days after the CAR T implantation by the following procedure. At the day of imaging, FK506-EC20 head is formulated with 99mTc according to previous report. 200 uCi 99mTc in 100 ul solution is i.v. injected to each mouse and whole body image is taken by MiLab PET/CT, focusing on the tumor area, spleen and lymph nodes. 3D images are reconstructed by PMOD software. After the last imaging at around day 10 after CAR T implantation, mice are euthanized and 99mTc distribution in each organ are counted by gamma counter.

[0133] For Hematopoietic Stem Cell transplantation model, humanized NSG mice are generated as reported before. 10 million CD34+ FKBP-FRa+ hHSC are i. v infused into the humanized NSG mice. 4 months later, whole body image is taken using FK506-99mTc as mentioned above, focusing on the bone marrow and spine.

2. FKBP-FRa Fusion Receptor Specifically Binds and Internalizes FK506-Payload

2.1 FKBP-FRa Fusion Receptor Specifically Binds FK506-Rhodamine

[0134] For binding assay, cells were incubated with ligand-dye alone or with ligand-dye and free ligands (100×, preincubated for 30 min) for 30 min, at 4 degree. Cells were washed 3 times after incubation, and re-suspended in 2% FBS PBS, 7-AAD were added to gate out the dead cells. BD Fortessa flow cytometer were used. Results were analyzed using FlowJo software.

2.2. Binding of FK506-Rhodamine by FKBP-FRa Fusion Receptor (FRET Imaging of FK506-Rhodamine and FA-S0456)

[0135] To understand the occupation of the fusion receptor, FKBP-FRa+ jurkat cells are incubated with FA/FA-S0456, FK506/FK506-Rhodamine at the indicated sequence and concentration. FRET is visualized by the loss of intensity of FA-Rhodamine as its energy transferred to FA-OTL38 on the same or nearby receptor, detected by BD Fortessa flow cytometer.

[0136] FIG. **5** shows binding of Folate acid in FKBPFR1GS fusion protein blocks the binding of FK506-Rhodamine at as low as 0.01 nM and totally abolishes FK506 Rhodamine binding at 50 nM.

[0137] FIG. **6** shows FKBPFR1GS jurkat cells have decreased FK506-Rhodamine intensity after binding to OTL38, a folate receptor targeted dye. FRET from FK506-Rhodamine (donor) to OTL38 (FA-S0456, acceptor, ex/em: 774/794 nm) indicates the interaction between FR and FKBP within the fusion receptor.

[0138] FIG. **7** shows increasing the linker length between FKBP and FR significantly lowers the interference between the two components of the fusion protein. Compare to FF1 (1GS between FKBP and FR), FF3 (3GS between FKBP and FR) preserves the binding of FK506-Rhodamine in the presence of 10 nM FA, which is comparable to the physiological concentration of FA in human body.

2.3. Release of GPI Anchored FF3 Fusion Receptor

[0139] Using PI-PLC treat T cells having FF3 fusion protein resulted the release of GPI anchored receptor FF3. Jurkat T cell with FF3 fusion receptor shows saturated binding with 20 nM FA-FITC (EC17), while after 5 mU PI-PLC or 50 mU PI-PLC treatment, the FA-FITC loses binding to the cell, which indicates the release of the GPI anchored FF3 fusion receptor. See FIG. **8**.

2.4 Fusion Protein FKBPFR3GS in Human T Cell Retains FR Binding Property

[0140] FA-Rhodamine binding curve is shown in FKBPFR3GS fusion receptor. FKBPFR3GS fusion receptor that stably expressed on human T cell can bind to folate acid derivative (FA-Rhodamine) with high affinity (Kd=0.95 nM), which is comparable to the affinity of FA-Rhodamine in FR+ KB cell (Kd around 1 nM). Therefore, the binding property of FR in the fusion receptor is preserved. See FIG. **9**.

2.5 Fusion Protein FKBPFR3GS in Human T Cell Retains FKBP Binding Property

[0141] FK506-Rhodamine binding curve in FKBPFR3GS fusion receptor. FKBPFR3GS fusion receptor that stably expressed on human T cell is able to bind to FK506 derivative (FK506-Rhodamine) with high affinity (Kd=3.93 nM), which means the binding property of FKBP in the fusion receptor is preserved. See FIG. **10**.

2.6 SLF-FITC Binds to FKBPFR3GS Fusion Receptor With Relative High Binding Affinity (Kd=62 nM)

[0142] Competition of free SLF (100, preincubation) blocks SLF-FITC binding. SLF, a mimic of FK506, presents a 10 times lower binding affinity to FKBPFR fusion receptor, compared to the parent ligand FK506. See FIG. **11** and compare with FIG. **10**.

2.7 Binding Curve of FA-Rhodamine in 4M5.3FR Fusion Receptor

[0143] FA-Rhodamine can bind to 4M5.3FR fusion receptor that stably expressed on human T cell with high affinity (Kd=2.25 nM), which is comparable to the affinity of FA-Rhodamine in FR+ KB cell (Kd around 1 nM). Therefore, FR binding property is preserved in 4M5.3FR fusion receptor. See FIG. **12**.

2.8 FITC-AF647 Binding Curve in 4M5.3FR Fusion Receptor

[0144] FITC-AF647 can bind to 4M5.3FR fusion receptor that stably expression on human T cell with high affinity (Kd=8.03 nM). 100× comp indicates free FITC sodium. The binding property of scFv 4M5.3 with FITC is preserved in 4M5.3FR fusion receptor. See FIG. **13**.

3.1. FA-Tubulysin Killing Effect Against FF3+ Human T Cell

[0145] FA-Tubulysin is able to mediate a receptor specific killing effect against FF3+ human T cell. Compensation with FA (100× preincubation with FA) blocks the effect. This implies the successful internalization and release of the free drug Tubulysin through the FF3 fusion receptor system. See FIG. **14**.

3.2 FA-Tubulysin Killing Effect Against hFF3 + Population in a Mixed Human T Cell Culture

[0146] FA-Tubulysin specifically kills the hFF3+ population in a mixed human T cell culture. Percentage of hFF3+ cell decrease as the FA-Tub concentration increases. See FIG. **15**.

3.3 SLF-Tub Specifically Kill the hFF3+ Jurkat Cells With a IC_{50}=138 nM

[0147] 2 h incubation of SLF-Tub with hFF3 Jurkat cells is able to kill the receptor positive cells. This indicates the successful internalization of SLF-Tub by the FKBPFR3GS fusion receptor and the release of the Tubulysin inside the cell. See FIG. **16**.

3.4. FITC-DM4 and FITC-Tub Killing Effects Against 4M5.3FR+ Human T Cells

[0148] Both FITC-DM4 and FITC-Tub can specifically kill the 4M5.3FR+ human T cells, while FITC-Tub has a higher IC_{50}. Compensation of free FITC sodium (100× preincubation) blocks the receptor mediated killing effect. This implies the successful internalization and release of FITC-cytotoxic drug into T cell through 4M5.3FR fusion receptor. See FIG. **17**.

3.5. FITC-Tubulysin Specifically Kill the 4M5.3FR+ Population in a Mixed Human T Cell Culture

[0149] Absolute number of 4M5.3FR+ cell decrease as the FITC-Tub concentration increases, while 4M5.3FR− cells are killed also through released drugs and bystander effect at high concentration. See FIG. **18**.

3.6. FITC-DM4 Specifically Kill the 4M5.3FR+ Population in a Mixed Human T Cell Culture

[0150] Absolute number of 4M5.3FR+ cell decrease as the FITC-DM4 concentration increases, while 4M5.3FR− cells are killed also through released drugs and bystander effect at high concentration. See FIG. **19**.

3.7 Kinase Inhibitors Modulation Effect on Anti CD19 CAR T Cells Against CD19+ Raji

[0151] Dasatinib (Lck inhibitor) and Ibrutinib (ITK inhibitor) at 10 nM concentration decrease the lysis effect of antiCD19 CAR T cell (FMC63 CAR T, Effector) against CD 19+ Raji tumor cell (Target). Two Effector: Target ratio (E:T) have been tested. Normal T cell and antiCD19 CAR T with CD19− K562 cell were used as negative control. See FIG. **20**.

3.8. FITC-Dasatinib Can Decrease the Lysis Effect of FMC63+4MFR+hT Cell Towards Raji Cell

[0152] This implies the successful internalization and release of FITC-Dasatinib into T cell through 4M5.3FR fusion receptor and the release of Dasatinib into T cell. See FIG. **21**.

3.9 TC-PTP Inhibitor at 100 nM Concentration Decrease the Co-Inhibitor Molecule Population in Exhausted antiCD19 CAR T Cell

[0153] Exhausted antiCD19 CAR T cells are generated by 7 times of stimulation with CD19+Raji cells, see detailed procedure in material and methods section). PD-1 positive, LAG3 positive and double positive population decreases upon treatment. See FIG. **22**.

4. Other FK506-Payload to Control the Activity of Cell Therapy

[0154] The technical advantageous feature of this drug payload delivery system is to have multi-functionality. The potential payloads and corresponding effects are listed below (Table 1). The small molecule payloads are selected based on the following parameters: 1. the functionality assay of the free drug, both in vitro and in vivo, has been confirmed by either published literature or work in our lab. 2. The chemical structure of the drug has relatively more accessible free amine for derivatization. 3. Any of the following will be preferable: FDA proved drug; commercially available for reasonable price. The FK506-payload will be tested first for in vitro experiments, T cell activation and stem cell cytokine release will be monitored by multiplex immunoassays. For in vivo disease models, we have well established CAR T therapy and bone fracture mouse

models in our lab, and several potential collaborators for the neurodegenerative mouse models.

Disease Model	Subtype	Cell Type	Cell source	Payload type
stem cell therapy	HSC transplant	BMSC	Murine BM	GSK3b inhibitor

-continued

Disease Model	Subtype	Cell Type	Cell source	Payload type
Tumor	tumor microenvironment	CAR T	hPBMC T cell	GSK3b inhibitor, HDAC inhibitor, MAPK inhibitor

SEQUENCE LISTING

Sequence total quantity: 22

SEQ ID NO: 1 moltype = AA length = 367
FEATURE Location/Qualifiers
REGION 1..367
note = A synthetic amino acid sequence for mouse FKBP-FRa with linker peptide
source 1..367
mol_type = protein
organism = synthetic construct
SEQUENCE: 1
MAHLMTVQLL LLVMWMAECA QSRAGVQVET ISPGDGRTFP KRGQTCVVHY TGMLEDGKKF 60
DSSRDRNKPF KFTLGKQEVI RGWEEGVAQM SVGQRAKLII SSDYAYGATG HPGIIPPHAT 120
LVFDVELLKL ESGGGSTRAR TELLNVCMDA KHHKEKPGPE DNLHDQCSPW KTNSCCSTNT 180
SQEAHKDISY LYRFNWNHCG TMTSECKRHF IQDTCLYECS PNLGPWIQQV DQSWRKERIL 240
DVPLCKEDCQ QWWEDCQSSF TCKSNWHKGW NWSSGHNECP VGASCHPFTF YFPTSAALCE 300
EIWSHSYKLS NYSRGSGRCI QMWFDPAQGN PNEEVARFYA EAMSGAGFHG TWPLLCSLSL 360
VLLWVIS 367

SEQ ID NO: 2 moltype = AA length = 369
FEATURE Location/Qualifiers
REGION 1..369
note = A synthetic amino acid sequence for human FKBP-FRa with linker peptide
source 1..369
mol_type = protein
organism = synthetic construct
SEQUENCE: 2
MAQRMTTQLL LLLVWVAVVG EAQTGVQVET ISPGDGRTFP KRGQTCVVHY TGMLEDGKKF 60
DSSRDRNKPF KFMLGKQEVI RGWEEGVAQM SVGQRAKLTI SPDYAYGATG HPGIIPPHAT 120
LVFDVELLKL ESGGGSRIAW ARTELLNVCM NAKHHKEKPG PEDKLHEQCR PWRKNACCST 180
NTSQEAHKDV SYLYRFNWNH CGEMAPACKR HFIQDTCLYE CSPNLGPWIQ QVDQSWRKER 240
VLNVPLCKED CEQWWEDCRT SYTCKSNWHK GWNWTSGFNK CAVGAACQPF HFYFPTPTVL 300
CNEIWTHSYK VSNYSRGSGR CIQMWFDPAQ GNPNEEVARF YAAAMSGAGP WAAWPFLLSL 360
ALMLLWLLS 369

SEQ ID NO: 3 moltype = AA length = 485
FEATURE Location/Qualifiers
REGION 1..485
note = A synthetic amino acid sequence for mouse antiCD19 CAR T construct
source 1..485
mol_type = protein
organism = synthetic construct
SEQUENCE: 3
MGVPTQLLGL LLLWITDAIC EQKLISEEDL DIQMTQSPAS LSTSLGETVT IQCQASEDIY 60
SGLAWYQQKP GKSPQLLIYG ASDLQDGVPS RFSGSGSGTQ YSLKITSMQT EDEGVYFCQQ 120
GLTYPRTFGG GTKLELKGGG GSGGGGSGGG GSEVQLQQSG AELVRPGTSV KLSCKVSGDT 180
ITFYYMHFVK QRPGQGLEWI GRIDPEDEST KYSEKFKNKA TLTADTSSNT AYLKLSSLTS 240
EDTATYFCIY GGYYFDYWGQ GVMVTVSSIE FMYPPPYLDN ERSNGTIIHI KEKHLCHTQS 300
SPKLFWALVV VAGVLFCYGL LVTVALCVIW TNSRRNRGGQ SDYMNMTPRR PGLTRKPYQP 360
YAPARDFAAY RPRAKFSRSA ETAANLQDPN QLYNELNLGR REEYDVLEKK RARDPEMGGK 420
QQRRRNPQEG VYNALQKDKM AEAYSEIGTK GERRRGKGHD GLYQGLSTAT KDTYDALHMQ 480
TLAPR 485

SEQ ID NO: 4 moltype = AA length = 496
FEATURE Location/Qualifiers
REGION 1..496
note = A synthetic amino acid sequence for human antiCD19 CAR T construct
source 1..496
mol_type = protein
organism = synthetic construct
SEQUENCE: 4

-continued

```
MALPVTALLL PLALLLHAAR PEQKLISEED LDIQMTQTTS SLSASLGDRV TISCRASQDI  60
SKYLNWYQQK PDGTVKLLIY HTSRLHSGVP SRFSGSGSGT DYSLTISNLE QEDIATYFCQ  120
QGNTLPYTFG GGTKLEITGG GGSGGGGSGG GGSEVKLQES GPGLVAPSQS LSVTCTVSGV  180
SLPDYGVSWI RQPPRKGLEW LGVIWGSETT YYNSALKSRL TIIKDNSKSQ VFLKMNSLQT  240
DDTAIYYCAK HYYYGGSYAM DYWGQGTSVT VSSAAAIEVM YPPPYLDNEK SNGTIIHVKG  300
KHLCPSPLFP GPSKPFWVLV VVGGVLACYS LLVTVAFIIF WVRSKRSRLL HSDYMNMTPR  360
RPGPTRKHYQ PYAPPRDFAA YRSRVKFSRS ADAPAYQQGQ NQLYNELNLG RREEYDVLDK  420
RRGRDPEMGG KPQRRKNPQE GLYNELQKDK MAEAYSEIGM KGERRRGKGH DGLYQGLSTA  480
TKDTYDALHM QALPPR                                                  496

SEQ ID NO: 5           moltype = DNA  length = 12182
FEATURE                Location/Qualifiers
misc_feature           1..12182
                       note = A synthetic vector sequence for pWPI for human T
                        cell transduction
source                 1..12182
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 5
ttggaagggc taattcactc ccaaagaaga caagatatcc ttgatctgtg gatctaccac  60
acacaaggct acttccctga ttagcagaac tacacaccag ggccaggggt cagatatcca  120
ctgacctttg gatggtgcta caagctagta ccagttgagc cagataaggt agaagaggcc  180
aataaaggag agaacaccag cttgttacac cctgtgagcc tgcatgggat ggatgacccg  240
gagagagaag tgttagagtg gaggtttgac agccgcctag catttcatca cgtggcccga  300
gagctgcatc cggagtactt caagaactgc tgatatcgag cttgctacaa gggactttcc  360
gctggggact ttccagggag gcgtggcctg ggcgggactg gggagtggcg agccctcaga  420
tcctgcatat aagcagctgc tttttgcctg tactgggtct ctctggttag accagatctg  480
agcctgggag ctctctggct aactagggaa cccactgctt aagcctcaat aaagcttgcc  540
ttgagtgctt caagtagtgt gtgcccgtct gttgtgtgac tctggtaact agagatccct  600
cagacccttt tagtcagtgt ggaaaatctc tagcagtggc gcccgaacag ggacttgaaa  660
gcgaaaggga aaccagagga gctctctcga cgcaggactc ggcttgctga agcgcgcacg  720
gcaagaggcg aggggcggcg actggtgagt acgccaaaaa ttttgactag cggaggctag  780
aaggagagag atgggtgcga gagcgtcagt attaagcggg ggagaattag atcgcgatgg  840
gaaaaaattc ggttaaggcc agggggaaag aaaaaatata aattaaaaca tatagtatgg  900
gcaagcaggg agctagaacg attcgcagtt aatcctggcc tgttagaaac atcagaaggc  960
tgtagacaaa tactgggaca gctacaacca tcccttcaga caggatcaga agaacttaga  1020
tcattatata atacagtagc aaccctctat tgtgtgcatc aaaggataga gataaaagac  1080
accaaggaag ctttagacaa gatagaggaa gagcaaaaca aaagtaagac caccgcacag  1140
caagcggccg ctgatcttca gacctggagg aggagatatg agggacaatt ggagaagtga  1200
attatataaa tataaagtag taaaaattga accattagga gtagcaccca ccaaggcaaa  1260
gagaagagtg gtgcagagag aaaaaagagc agtgggaata ggagctttgt tccttgggtt  1320
cttgggagca gcaggaagca ctatgggcgc agcgtcaatg acgctgacgg tacaggccag  1380
acaattattg tctggtatag tgcagcagca gaacaatttg ctgagggcta ttgaggcgca  1440
acagcatctg ttgcaactca cagtctgggg catcaagcag ctccaggcaa gaatcctggc  1500
tgtggaaaga tacctaaagg atcaacagct cctggggatt tggggttgct ctggaaaact  1560
catttgcacc actgctgtgc cttggaatgc tagttggagt aataaatctc tggaacagat  1620
ttggaatcac acgacctgga tggagtggga cagagaaatt aacaattaca caagcttaat  1680
acactcctta attgaagaat cgcaaaacca gcaagaaaag aatgaacaag aattattgga  1740
attagataaa tgggcaagtt tgtggaattg gtttaacata acaaattggc tgtggtatat  1800
aaaattattc ataatgatag taggaggctt ggtaggttta agaatagttt ttgctgtact  1860
ttctatagtg aatagagtta ggcagggata ttcaccatta tcgtttcaga cccacctccc  1920
aaccccgagg ggacccgaca ggcccgaagg aatagaagaa gaaggtggag agagagacag  1980
agacagatcc attcgattag tgaacggatc tcgacggtat cgatgtcgac gataagcttt  2040
gcaaagatgg ataaagtttt aaacagagag gaatctttgc agctaatgga ccttctaggt  2100
cttgaaagga gtgggaattg gctccggtgc ccgtcagtgg gcagagcgca catcgcccac  2160
agtccccgag aagttggggg gaggggtcgg caattgaacc ggtgcctaga gaaggtggcg  2220
cggggtaaac tgggaaagtg atgtcgtgta ctggctccgc ctttttcccg agggtggggg  2280
agaaccgtat ataagtgcag tagtcgccgt gaacgttctt tttcgcaacg ggtttgccgc  2340
cagaacacag gtaagtgccg tgtgtggttc ccgcgggcct ggcctcttta cgggttatgg  2400
cccttgcgtg ccttgaatta cttccactgg ctgcagtacg tgattcttga tcccgagctt  2460
cgggttggaa gtgggtggga gagttcgagg ccttgcgctt aaggagcccc ttcgcctcgt  2520
gcttgagttg aggcctggcc tgggcgctgg ggccgccgcg tgcgaatctg gtggcacctt  2580
cgcgcctgtc tcgctgcttt cgataagtct ctagccattt aaaatttttg atgacctgct  2640
gcgacgcttt ttttctggca agatagtctt gtaaatgcgg gccaagatct gcacactggt  2700
atttcggttt ttggggccgc gggcggcgac ggggcccgtg cgtcccagcg cacatgttcg  2760
gcgaggcggg gcctgcgagc gcggccaccg agaatcggac gggggtagtc tcaagctggc  2820
cggcctgctc tggtgcctgg cctcgcgccg ccgtgtatcg ccccgccctg ggcggcaagg  2880
ctggccccgt cggcaccagt tgcgtgagcg gaaagatggc cgcttcccgg ccctgctgca  2940
gggagctcaa aatggaggac gcggcgctcg ggagagcggg cgggtgagtc acccacacaa  3000
aggaaaaggg cctttccgtc ctcagccgtc gcttcatgtg actccacgga gtaccgggcg  3060
ccgtccaggc acctcgatta gttctcgagc ttttggagta cgtcgtcttt aggttggggg  3120
gaggggtttt atgcgatgga gtttccccac actgagtggg tggagactga agttaggcca  3180
gcttggcact tgatgtaatt ctccttggaa tttgcccttt ttgagtttgg atcttggttc  3240
attctcaagc ctcagacagt ggttcaaagt ttttttcttc catttcaggt gtcgtgagga  3300
atttcgacat ttaaatttaa ttaatctcga cggtatcggt taacttttaa aagaaaaggg  3360
gggattgggg ggtacagtgc aggggaaaga atagtagaca taatagcaac agacatacaa  3420
```

-continued

```
actaaagaat tacaaaaaca aattacaaaa attcaaaatt ttatcgatca cgagactagc  3480
ctcgaggttt atggctcacc tgatgactgt gcagttgttg ctcctggtga tgtggatggc  3540
cgaatgtgct cagtccagag ctggagtgca ggtggagacc atctctcctg gagacgggcg  3600
caccttccca aagcgcggcc agacctgcgt ggtgcactac acggggatgc ttgaagatgg  3660
aaagaaattt gattcctctc gggacagaaa caagcctttt aagtttacac taggcaagca  3720
ggaggtgatc cgaggctggg aggaaggggt agcccagatg agtgtgggtc agagagccaa  3780
actgataatc tcctcagact atgcctatgg agccaccggg cacccaggca tcatcccacc  3840
acatgccact cttgtttttg atgtggagct tctaaaactg gaaagcggcg gcggcagcac  3900
tcgggccagg actgaacttc tcaatgtctg catggatgcc aaacaccaca aagaaaaacc  3960
gggccctgag gacaatttac acgaccagtg cagcccctgg aagacgaatt cctgctgttc  4020
cacgaacaca agccaggaag cacataagga catttcctac ctgtaccggt tcaactggaa  4080
ccactgcgga actatgacat cggaatgcaa acggcacttt atccaagaca cctgcctcta  4140
tgagtgttcc ccgaacttgg gaccctggat ccagcaggtg gaccagagct ggcgcaaaga  4200
gcggatcctt gatgttcccc tgtgcaaaga ggactgtcag cagtggtggg aggactgcca  4260
gagctctttt acctgcaaga gcaattggca caagggatgg aactggtcct cggggcataa  4320
cgagtgtcct gtgggagcct cctgccatcc cttcaccttc tacttcccca catctgctgc  4380
tctgtgtgag gaaatctgga gtcactccta caagctcagc aactacagtc gagggagcgg  4440
ccgctgcatt cagatgtggt tcgacccagc ccagggcaac cccaacgagg aagtggcgag  4500
gttctatgcc gaggccatga gtggagctgg gtttcatggg acctggccac tcttgtgcag  4560
cctgtcctta gtgctgctct gggtgatcag ctgaaaacta cgggctgcag gaattccgcc  4620
ccccccccc taacgttact ggccgaagcc gcttggaata aggccggtgt gcgtttgtct  4680
atatgttatt ttccaccata ttgccgtctt ttggcaatgt gagggcccgg aaacctggcc  4740
ctgtcttctt gacgagcatt cctaggggtc tttcccctct cgccaaagga atgcaaggtc  4800
tgttgaatgt cgtgaaggaa gcagttcctc tggaagcttc ttgaagacaa acaacgtctg  4860
tagcgaccct ttgcaggcag cggaaccccc cacctggcga caggtgcctc tgcggccaaa  4920
agccacgtgt ataagataca cctgcaaagg cggcacaacc ccagtgccac gttgtgagtt  4980
ggatagttgt ggaaagagtc aaatggctct cctcaagcgt attcaacaag gggctgaagg  5040
atgcccagaa ggtaccccat tgtatgggat ctgatctggg gcctcggtgc acatgcttta  5100
catgtgttta gtcgaggtta aaaaacgtct aggccccccg aaccacgggg acgtggtttt  5160
cctttgaaaa acacgatgat aataccatgg tgagcaaggg cgaggagctg ttcaccgggg  5220
tggtgcccat cctggtcgag ctggacggcg acgtaaacgg ccacaagttc agcgtgtccg  5280
gcgagggcga gggcgatgcc acctacggca agctgaccct gaagttcatc tgcaccaccg  5340
gcaagctgcc cgtgccctgg cccaccctcg tgaccaccct gacctacggc gtgcagtgct  5400
tcagccgcta ccccgaccac atgaagcagc acgacttctt caagtccgcc atgcccgaag  5460
gctacgtcca ggagcgcacc atcttcttca aggacgacgg caactacaag acccgcgccg  5520
aggtgaagtt cgagggcgac accctggtga accgcatcga gctgaagggc atcgacttca  5580
aggaggacgg caacatcctg gggcacaagc tggagtacaa ctacaacagc cacaacgtct  5640
atatcatggc cgacaagcag aagaacggca tcaaggtgaa cttcaagatc cgccacaaca  5700
tcgaggacgg cagcgtgcag ctcgccgacc actaccagca gaacaccccc atcggcgacg  5760
gccccgtgct gctgcccgac aaccactacc tgagcaccca gtccgccctg agcaaagacc  5820
ccaacgagaa gcgcgatcac atggtcctgc tggagttcgt gaccgccgcc gggatcactc  5880
tcggcatgga cgagctgtac aagtccggac tcagatctcg actagctagt agctagctag  5940
ctagtcgagc tcaagcttcg aattcgatat caagcttatc gcgataccgt cgacctcgag  6000
ggaattccga taatcaacct ctggattaca aaatttgtga aagattgact ggtattctta  6060
actatgttgc tccttttacg ctatgtggat acgctgcttt aatgcctttg tatcatgcta  6120
ttgcttcccg tatggctttc attttctcct ccttgtataa atcctggttg ctgtctcttt  6180
atgaggagtt gtggcccgtt gtcaggcaac gtggcgtggt gtgcactgtg tttgctgacg  6240
caaccccac tggttggggc attgccacca cctgtcagct cctttccggg actttcgctt  6300
tccccctccc tattgccacg gcggaactca tcgccgcctg ccttgcccgc tgctggacag  6360
gggctcggct gttgggcact gacaattccg tggtgttgtc ggggaagctg acgtcctttc  6420
catggctgct cgcctgtgtt gccacctgga ttctgcgcgg gacgtccttc tgctacgtcc  6480
cttcggccct caatccagcg gaccttcctt cccgcggcct gctgccggct ctgcggcctc  6540
ttccgcgtct tcgccttcgc cctcagacga gtcggatctc cctttgggcc gcctccccgc  6600
atcgggaatt cgagctcggt acctttaaga ccaatgactt acaaggcagc tgtagatctt  6660
agccactttt taaaagaaaa ggggggactg gaagggctaa ttcactccca acgaagacaa  6720
gatgggatca attcaccatg ggaataactt cgtatagcat acattatacg aagttatgct  6780
gcttttgct tgtactgggt ctctctggtt agaccagatc tgagcctggg agctctctgg  6840
ctaactaggg aacccactgc ttaagcctca ataaagcttg ccttgagtgc ttcaagtagt  6900
gtgtgcccgt ctgttgtgtg actctggtaa ctagagatcc ctcagaccct tttagtcagt  6960
gtggaaaatc tctagcagca tctagaatta attccgtgta ttctatagtg tcacctaaat  7020
cgtatgtgta tgatacataa ggttatgtat taattgtagc cgcgttctaa cgacaatatg  7080
tacaagccta attgtgtagc atctggctta ctgaagcaga ccctatcatc tctctcgtaa  7140
actgccgtca gagtcggttt ggttggacga accttctgag tttctggtaa cgccgtcccg  7200
cacccggaaa tggtcagcga accaatcagc agggtcatcg ctagccagat cctctacgcc  7260
ggacgcatcg tggccggcat caccggcgcc acaggtgcgg ttgctggcgc ctatatcgcc  7320
gacatcaccg atggggaaga tcgggctcgc cacttcgggc tcatgagcgc ttgtttcggc  7380
gtgggtatgg tggcaggccc cgtggccggg ggactgttgg gcgccatctc cttgcatgca  7440
ccattccttg cggcggcggt gctcaacggc ctcaacctac tactgggctg cttcctaatg  7500
caggagtcgc ataagggaga gcgtcgaatg gtgcactctc agtacaatct gctctgatgc  7560
cgcatagtta agccagcccc gacacccgcc aacacccgct gacgcgccct gacgggcttg  7620
tctgctcccg gcatccgctt acagacaagc tgtgaccgtc tccgggagct gcatgtgtca  7680
gaggttttca ccgtcatcac cgaaacgcgc gagacgaaag ggcctcgtga tacgcctatt  7740
tttataggtt aatgtcatga taataatggt ttcttagacg tcaggtggca cttttcgggg  7800
aaatgtgcgc ggaaccccta tttgtttatt tttctaaata cattcaaata tgtatccgct  7860
catgagacaa taaccctgat aaatgcttca ataatattga aaaaggaaga gtatgagtat  7920
tcaacatttc cgtgtcgccc ttattccctt ttttgcggca ttttgccttc ctgtttttgc  7980
```

-continued

```
tcacccagaa acgctggtga aagtaaaaga tgctgaagat cagttgggtg cacgagtggg  8040
ttacatcgaa ctggatctca acagcggtaa gatccttgag agttttcgcc ccgaagaacg  8100
ttttccaatg atgagcactt ttaaagttct gctatgtggc gcggtattat cccgtattga  8160
cgccgggcaa gagcaactcg gtcgccgcat acactattct cagaatgact tggttgagta  8220
ctcaccagtc acagaaaagc atcttacgga tggcatgaca gtaagagaat tatgcagtgc  8280
tgccataacc atgagtgata acactgcggc caacttactt ctgacaacga tcggaggacc  8340
gaaggagcta accgcttttt tgcacaacat gggggatcat gtaactcgcc ttgatcgttg  8400
ggaaccggag ctgaatgaag ccataccaaa cgacgagcgt gacaccacga tgcctgtagc  8460
aatggcaaca acgttgcgca aactattaac tggcgaacta cttactctag cttcccggca  8520
acaattaata gactggatgg aggcggataa agttgcagga ccacttctgc gctcggccct  8580
tccggctggc tggtttattg ctgataaatc tggagccggt gagcgtgggt ctcgcggtat  8640
cattgcagca ctggggccag atggtaagcc ctcccgtatc gtagttatct acacgacggg  8700
gagtcaggca actatggatg aacgaaatag acagatcgct gagataggtg cctcactgat  8760
taagcattgg taactgtcag accaagttta ctcatatata ctttagattg atttaaaact  8820
tcatttttaa tttaaaagga tctaggtgaa gatccttttt gataatctca tgaccaaaat  8880
cccttaacgt gagttttcgt tccactgagc gtcagacccc gtagaaaaga tcaaaggatc  8940
ttcttgagat cctttttttc tgcgcgtaat ctgctgcttg caaacaaaaa aaccaccgct  9000
accagcggtg gtttgtttgc cggatcaaga gctaccaact ctttttccga aggtaactgg  9060
cttcagcaga gcgcagatac caaatactgt ccttctagtg tagccgtagt taggccacca  9120
cttcaagaac tctgtagcac cgcctacata cctcgctctg ctaatcctgt taccagtggc  9180
tgctgccagt ggcgataagt cgtgtcttac cgggttggac tcaagacgat agttaccgga  9240
taaggcgcag cggtcgggct gaacgggggg ttcgtgcaca cagcccagct tggagcgaac  9300
gacctacacc gaactgagat acctacagcg tgagcattga gaaagcgcca cgcttcccga  9360
agggagaaag gcggacaggt atccggtaag cggcagggtc ggaacaggag agcgcacgag  9420
ggagcttcca gggggaaacg cctggtatct ttatagtcct gtcgggtttc gccacctctg  9480
acttgagcgt cgatttttgt gatgctcgtc agggggggcgg agcctatgga aaaacgccag  9540
caacgcggcc tttttacggt tcctggcctt ttgctggcct tttgctcaca tgttctttcc  9600
tgcgttatcc cctgattctg tggataaccg tattaccgcc tttgagtgag ctgataccgc  9660
tcgccgcagc cgaacgaccg agcgcagcga gtcagtgagc gaggaagcgg aagagcgccc  9720
aatacgcaaa ccgcctctcc ccgcgcgttg gccgattcat taatgcagct gtggaatgtg  9780
tgtcagttag ggtgtggaaa gtccccaggc tccccagcag gcagaagtat gcaaagcatg  9840
catctcaatt agtcagcaac caggtgtgga aagtccccag gctccccagc aggcagaagt  9900
atgcaaagca tgcatctcaa ttagtcagca accatagtcc cgcccctaac tccgcccatc  9960
ccgcccctaa ctccgcccag ttccgcccat tctccgcccc atggctgact aatttttttt  10020
atttatgcag aggccgaggc cgcctcggcc tctgagctat tccagaagta gtgaggaggc  10080
ttttttggag gcctaggctt ttgcaaaaag cttggacaca agacaggctt gcgagatatg  10140
tttgagaata ccactttatc ccgcgtcagg gagaggcagt gcgtaaaaag acgcggactc  10200
atgtgaaata ctggtttttа gtgcgccaga tctctataat ctcgcgcaac ctattttccc  10260
ctcgaacact ttttaagccg tagataaaca ggctgggaca cttcacatga gcgaaaaata  10320
catcgtcacc tgggacatgt tgcagatcca tgcacgtaaa ctcgcaagcc gactgatgcc  10380
ttctgaacaa tggaaaggca ttattgccgt aagccgtggc ggtctgtacc gggtgcgtta  10440
ctggcgcgtg aactgggtat tcgtcatgtc gataccgttt gtatttccag ctacgatcac  10500
gacaaccagc gcgagcttaa agtgctgaaa cgcgcagaag gcgatggcga aggcttcatc  10560
gttattgatg acctggtgga taccggtggt actgcggttg cgattcgtga aatgtatcca  10620
aaagcgcact ttgtcaccat cttcgcaaaa ccggctggtc gtccgctggt tgatgactat  10680
gttgttgata tcccgcaaga tacctggatt gaacagccgt gggatatggg cgtcgtattc  10740
gtcccgccaa tctccggtcg ctaatctttt caacgcctgg cactgccggg cgttgttctt  10800
tttaacttca ggcgggttac aatagtttcc agtaagtatt ctggaggctg catccatgac  10860
acaggcaaac ctgagcgaaa ccctgttcaa accccgcttt aaacatcctg aaacctcgac  10920
gctagtccgc cgctttaatc acggcgcaca accgcctgtg cagtcggccc ttgatggtaa  10980
aaccatccct cactggtatc gcatgattaa ccgtctgatg tggatctggc gcggcattga  11040
cccacgcgaa atcctcgacg tccaggcacg tattgtgatg agcgatgccg aacgtaccga  11100
cgatgattta tacgatacgg tgattggcta ccgtggcggc aactggattt atgagtgggc  11160
cccggatctt tgtgaaggaa ccttacttct gtggtgtgac ataattggac aaactaccta  11220
cagagattta aagctctaag gtaaatataa aatttttaag tgtataatgt gttaaactac  11280
tgattctaat tgtttgtgta ttttagattc caacctatgg aactgatgaa tgggagcagt  11340
ggtggaatgc ctttaatgag gaaaacctgt tttgctcaga agaaatgcca tctagtgatg  11400
atgaggctac tgctgactct caacattcta ctcctccaaa aaagaagaga aaggtagaag  11460
accccaagga ctttccttca gaattgctaa gttttttgag tcatgctgtg tttagtaata  11520
gaactcttgc ttgctttgct atttacacca caaaggaaaa agctgcactg ctatacaaga  11580
aaattatgga aaaatattct gtaaccttta taagtaggca taacagttat aatcataaca  11640
tactgttttt tcttactcca cacaggcata gagtgtctgc tattaataac tatgctcaaa  11700
aattgtgtac ctttagcttt ttaatttgta aaggggttaa taaggaatat ttgatgtata  11760
gtgccttgac tagagatcat aatcagccat accacatttg tagaggtttt acttgcttta  11820
aaaaacctcc cacacctccc cctgaacctg aaacataaaa tgaatgcaat tgttgttgtt  11880
aacttgttta ttgcagctta taatggttac aaataaagca atagcatcac aaatttcaca  11940
aataaagcat ttttttcact gcattctagt tgtggtttgt ccaaactcat caatgtatct  12000
tatcatgtct ggatcaactg gataactcaa gctaaccaaa atcatcccaa acttcccacc  12060
ccataccta ttaccactgc caattaccta gtggtttcat ttactctaaa cctgtgattc  12120
ctctgaatta ttttcatttt aaagaaattg tatttgttaa atatgtacta caaacttagt  12180
ag                                                                12182

SEQ ID NO: 6           moltype = DNA  length = 6939
FEATURE                Location/Qualifiers
misc_feature           1..6939
                       note = A synthetic pMP71 gb NotIEcoRI mouse antiCD19 for
```

-continued

	mouse T cell transduction
source	1..6939
	mol_type = other DNA
	organism = synthetic construct

SEQUENCE: 6

```
tcaaggttag gaacagagag acaggagaat atgggccaaa caggatatct gtggtaagca  60
gttcctgccc cggctcaggg ccaagaacag ttggaacagc agaatatggg ccaaacagga  120
tatctgtggt aagcagttcc tgccccggct cagggccaag aacagatggt ccccagatgc  180
ggtcccgccc tcagcagttt ctagagaacc atcagatgtt tccagggtgc cccaaggacc  240
tgaaatgacc ctgtgcctta tttgaactaa ccaatcagtt cgcttctcgc ttctgttcgc  300
gcgcttctgc tccccgagct caataaaaga gcccacaacc cctcactcgg cgcgccagtc  360
ctccgattga ctgcgtcgcc cgggtacccg tattcccaat aaagcctctt gctgtttgca  420
tccgaatcgt ggactcgctg atccttggga gggtctcctc agattgattg actgcccacc  480
tcgggggtct ttcatttgga ggttccaccg agatttggag acccctgccc agggaccacc  540
gacccccccg ccgggaggta agctggccag cggtcgtttc gtgtctgtct ctgtctttgg  600
gcgtgtttgt gccggcatct aatgtttgcg cctgcgtctg tactagttgg ctaactagat  660
ctgtatctgg cggtcccgcg gaagaactga cgagttcgta ttcccggccg cagcccctgg  720
gagacgtccc agcggcctcg ggggcccgtt ttgtggccca ttctgtatca gttaacctac  780
ccgagtcgga ctttttggag ctccgccact gtccgagggg tacgtggctt tgttgggggа  840
cgagagacag agacacttcc cgcccccgtc tgaatttttg ctttcggttt tacgccgaaa  900
ccgcgccgcg cgtcttgtct gctgcagcat cgttctgtgt tgtctctgtc tgactgtgtt  960
tctgtatttg tctgaaaatt agctcgacaa agttaagtaa tagtccctct ctccaagctc  1020
acttacaggc atgggggttc ccacccagct gttggggttg ctgctcctct ggatcactga  1080
tgccatctgc gaacagaagc tcatttctga agaagatctg gacatacaga tgacccagag  1140
tcctgccagc ttgtccacat ccctcggaga gaccgtgaca attcaatgcc aggcctctga  1200
ggacatatat tctggattgg cttggtacca gcaaaagcca ggtaaaagtc ctcagttgtt  1260
gatatacggc gcttctgatt tgcaagacgg ggtgccctca cgatttagcg ggtctggaag  1320
tggcactcag tacagtctga agattacttc aatgcaaaca gaagacgagg gtgtgtattt  1380
ttgccaacag gggctgacct acccaaggac attcgggggc ggtacaaagc ttgaacttaa  1440
gggcggcggt gggtctggag gtggtggatc tggcggaggg ggaagtgagg tacagctgca  1500
acagtccggc gccgaactcg ttcgccctgg aacctcagtc aaattgtcat gcaaggtgag  1560
tggcgacaca ataacctttt actatatgca cttcgttaaa cagaggccag gtcaaggtct  1620
ggaatggata ggcagaattg atccagaaga tgagtccacc aaatactcag aaaagttcaa  1680
aaacaaagcc actcttactg ccgacacctc aagcaacaca gcatatctta agctcagttc  1740
acttaccagc gaagacaccg ccacctattt ttgtatttat ggtggctatt actttgacta  1800
ctgggggcaa ggtgttatgg taacagtttc ttccattgaa ttcatgtatc caccccccta  1860
tttggataat gaacgatcta atgggactat aatacatatc aaggaaaagc atctgtgtca  1920
tacccaaagt tcccctaagc ttttctgggc cctcgtcgtt gtggcaggag tgctttttg  1980
ctatggattg ttggttactg tggctctctg cgtcatttgg acaaatagta ggaggaatcg  2040
ggggggacaa tctgattaca tgaacatgac accacggagg ccaggcctta ccagaaagcc  2100
ctaccaacct tatgcaccag cacgagactt cgccgcatac aggccaaggg ctaagttttc  2160
ccgcagcgcc gaaaccgcag ccaacctcca agatcctaat cagctctata acgaattgaa  2220
tcttggccgc agagaggagt acgacgtact tgagaaaaag agagctaggg accctgaaat  2280
gggtgggaag caacagcgaa gaaggaaccc acaggaaggg gtgtataatg cccttcaaaa  2340
ggataaaatg gcagaggcat acagtgaaat cggaaccaag ggggagagac gcagagggaa  2400
aggccatgac ggcctttatc agggtttgtc aactgctact aaagacactt atgatgcctt  2460
gcatatgcaa actctcgcac ccagatgacg agcatcttac cgccatttat tcccatattt  2520
gttctgtttt tcttgatttg ggtatacatt taaatgttaa taaaacaaaa tggtggggca  2580
atcatttaca ttttatggga tatgtaatta ctagttcagg tgtattgcca caagacaaac  2640
atgttaagaa actttcccgt tatttacgct ctgttcctgt taatcaacct ctggattaca  2700
aaatttgtga aagattgact gatattctta actatgttgc tccttttacg ctgtgtggat  2760
atgctgcttt aatgcctctg tatcatgcta ttgcttcccg tacggctttc gttttctcct  2820
ccttgtataa atcctggttg ctgtctcttt atgaggagtt gtggcccgtt gtccgtcaac  2880
gtggcgtggt gtgctctgtg tttgctgacg caacccccac tggctggggc attgccacca  2940
cctgtcaact cctttctggg actttcgctt tcccccctcc gatcgccacg gcagaactca  3000
tcgccgcctg ccttgcccgc tgctggacag gggctaggtt gctgggcact gataattccg  3060
tggtgttgtc ggggaagctg acgtcctttc catggctgct cgcctgtgtt gccaactgga  3120
tcctgcgcgg gacgtccttc tgctacgtcc cttcggctct caatccagcg gacctccctt  3180
cccgaggcct tctgccggtt ctgcggcctc tcccgcgtct tcgctttcgg cctccgacga  3240
gtcggatctc cctttgggcc gcctccccgc ctgtttcgcc tcggcgtccg gtccgtgttg  3300
cttggtcgtc acctgtgcag aattgcgaac catggattcc accgtgaact ttgtctcctg  3360
gcatgcaaat cgtcaacttg gcatgccaag aattaattcg gatccaagct taggcctgct  3420
cgctttcttg ctgtcccatt tctattaaag gttcctttgt tccctaagtc caactactaa  3480
actgggggat attatgaagg gccttgagca tctggattct gcctagcgct aagcttccta  3540
acacgagcca tagatagaat aaaagatttt atttagtctc cagaaaaagg ggggaatgaa  3600
agaccccacc tgtaggtttg gcaagctagc ttaagtaagc cattttgcaa ggcatggaaa  3660
aatacataac tgagaataga gaagttcaga tcaaggttag gaacagagag acaggagaat  3720
atgggccaaa caggatatct gtggtaagca gttcctgccc cggctcaggg ccaagaacag  3780
ttggaacagc agaatatggg ccaaacagga tatctgtggt aagcagttcc tgccccggct  3840
cagggccaag aacagatggt ccccagatgc ggtcccgccc tcagcagttt ctagagaacc  3900
atcagatgtt tccagggtgc cccaaggacc tgaaatgacc ctgtgcctta tttgaactaa  3960
ccaatcagtt cgcttctcgc ttctgttcgc gcgcttctgc tccccgagct caataaaaga  4020
gcccacaacc cctcactcgg cgcgccagtc ctccgataga ctgcgtcgcc cggggtaccc  4080
gtattcccaa taaagcctct tgctgtttgc atccgaatcg tggactcgct gatccttggg  4140
agggtctcct cagattgatt gactgcccac ctcgggggtc tttcattctc gagagctttg  4200
gcgtaatcat ggtcatagct gtttcctgtg tgaaattgtt atccgctcac aattccacac  4260
```

-continued

```
aacatacgag ccggaagcat aaagtgtaaa gcctggggtg cctaatgagt gagctaactc  4320
acattaattg cgttgcgctc actgcccgct ttccagtcgg gaaacctgtc gtgccagctg  4380
cattaatgaa tcggccaacg cgcggggaga ggcggtttgc gtattgggcg ctcttccgct  4440
tcctcgctca ctgactcgct gcgctcggtc gttcggctgc ggcgagcggt atcagctcac  4500
tcaaaggcgg taatacggtt atccacagaa tcaggggata acgcaggaaa gaacatgtga  4560
gcaaaaggcc agcaaaaggc caggaaccgt aaaaaggccg cgttgctggc gtttttccat  4620
aggctccgcc cccctgacga gcatcacaaa aatcgacgct caagtcagag gtggcgaaac  4680
ccgacaggac tataaagata ccaggcgttt cccccctggaa gctccctcgt gcgctctcct  4740
gttccgaccc tgccgcttac cggatacctg tccgcctttc tcccttcggg aagcgtggcg  4800
ctttctcaat gctcacgctg taggtatctc agttcggtgt aggtcgttcg ctccaagctg  4860
ggctgtgtgc acgaaccccc cgttcagccc gaccgctgcg ccttatccgg taactatcgt  4920
cttgagtcca acccggtaag acacgactta tcgccactgg cagcagccac tggtaacagg  4980
attagcagag cgaggtatgt aggcggtgct acagagttct tgaagtggtg gcctaactac  5040
ggctacacta gaaggacagt atttggtatc tgcgctctgc tgaagccagt taccttcgga  5100
aaaagagttg gtagctcttg atccggcaaa caaaccaccg ctggtagcgg tggttttttt  5160
gtttgcaagc agcagattac gcgcagaaaa aaaggatctc aagaagatcc tttgatcttt  5220
tctacggggt ctgacgctca gtggaacgaa aactcacgtt aagggatttt ggtcatgaga  5280
ttatcaaaaa ggatcttcac ctagatcctt ttaaattaaa aatgaagttt taaatcaatc  5340
taaagtatat atgagtaaac ttggtctgac agttaccaat gcttaatcag tgaggcacct  5400
atctcagcga tctgtctatt tcgttcatcc atagttgcct gactccccgt cgtgtagata  5460
actacgatac gggagggctt accatctggc cccagtgctg caatgatacc gcgagaccca  5520
cgctcaccgg ctccagattt atcagcaata aaccagccag ccggaagggc cgagcgcaga  5580
agtggtcctg caactttatc cgcctccatc cagtctatta attgttgccg ggaagctaga  5640
gtaagtagtt cgccagttaa tagtttgcgc aacgttgttg ccattgctgc tggcatcgtg  5700
gtgtcacgct cgtcgtttgg tatggcttca ttcagctccg gttcccaacg atcaaggcga  5760
gttacatgat cccccatgtt gtgcaaaaaa gcggttagct ccttcggtcc tccgatcgtt  5820
gtcagaagta agttggccgc agtgttatca ctcatggtta tggcagcact gcataattct  5880
cttactgtca tgccatccgt aagatgcttt tctgtgactg gtgagtactc aaccaagtca  5940
ttctgagaat agtgtatgcg gcgaccgagt tgctcttgcc cggcgtcaat acgggataat  6000
accgcgccac atagcagaac tttaaaagtg ctcatcattg gaaaacgttc ttcggggcga  6060
aaactctcaa ggatcttacc gctgttgaga tccagttcga tgtaacccac tcgtgcaccc  6120
aactgatctt cagcatcttt tactttcacc agcgtttctg ggtgagcaaa aacaggaagg  6180
caaaatgccg caaaaaaggg aataagggcg acacggaaat gttgaatact catactcttc  6240
ctttttcaat attattgaag catttatcag ggttattgtc tcatgagcgg atacatattt  6300
gaatgtattt agaaaaataa acaaatagggg gttccgcgca catttccccg aaaagtgcca  6360
cctgacgtct aagaaaccat tattatcatg acattaacct ataaaaatag gcgtatcacg  6420
aggccctttc gtcttcaagc tgcctcgcgc gtttcggtga tgacggtgaa aacctctgac  6480
acatgcagct cccggagacg gtcacagctt gtctgtaagc ggatgccggg agcagacaag  6540
cccgtcaggg cgcgtcagcg ggtgttggcg ggtgtcgggg cgcagccatg acccagtcac  6600
gtagcgatag ttactatgcg gcatcagagc agattgtact gagagtgcac catatgcggt  6660
gtgaaatacc gcacagatgc gtaaggagaa aataccgcat caggcgccat tcgccattca  6720
ggctgcgcaa ctgttgggaa gggcgatcgg tgcgggcctc ttcgctatta cgccagctgg  6780
cgaaaggggg atgtgctgca aggcgattaa gttgggtaac gccagggttt tcccagtcac  6840
gacgttgtaa aacgacggcc agtgaattag tactctagct taagtaagcc attttgcaag  6900
gcatggaaaa atacataact gagaatagag aagttcaga                          6939

SEQ ID NO: 7           moltype = DNA  length = 12188
FEATURE                Location/Qualifiers
misc_feature           1..12188
                       note = A synthetic pWPI-FRa 1-24 FKBP FRa
source                 1..12188
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 7
ttggaagggc taattcactc ccaaagaaga caagatatcc ttgatctgtg gatctaccac  60
acacaaggct acttccctga ttagcagaac tacacaccag ggccaggggt cagatatcca  120
ctgacctttg gatggtgcta caagctagta ccagttgagc cagataaggt agaagaggcc  180
aataaaggag agaacaccag cttgttacac cctgtgagcc tgcatgggat ggatgacccg  240
gagagagaag tgttagagtg gaggtttgac agccgcctag catttcatca cgtggcccga  300
gagctgcatc cggagtactt caagaactgc tgatatcgag cttgctacaa gggactttcc  360
gctggggact ttccagggag gcgtggcctg ggcgggactg gggagtggcg agccctcaga  420
tcctgcatat aagcagctgc tttttgcctg tactgggtct ctctggttag accagatctg  480
agcctgggag ctctctggct aactagggaa cccactgctt aagcctcaat aaagcttgcc  540
ttgagtgctt caagtagtgt gtgcccgtct gttgtgtgac tctggtaact agagatccct  600
cagacccttt tagtcagtgt ggaaaatctc tagcagtggc gcccgaacag ggacttgaaa  660
gcgaaaggga aaccagagga gctctctcga cgcaggactc ggcttgctga agcgcgcacg  720
gcaagaggcg aggggcggcg actggtgagt acgccaaaaa ttttgactag cggaggctag  780
aaggagagag atgggtgcga gagcgtcagt attaagcggg ggagaattag atcgcgatgg  840
gaaaaaattc ggttaaggcc agggggaaag aaaaaatata aattaaaaca tatagtatgg  900
gcaagcaggg agctagaacg attcgcagtt aatcctggcc tgttagaaac atcagaaggc  960
tgtagacaaa tactgggaca gctacaacca tcccttcaga caggatcaga agaacttaga  1020
tcattatata atacagtagc aaccctctat tgtgtgcatc aaaggataga gataaaagac  1080
accaaggaag ctttagacaa gatagaggaa gagcaaaaca aaagtaagac caccgcacag  1140
caagcggccg ctgatcttca gacctggagg aggagatatg agggacaatt ggagaagtga  1200
attatataaa tataaagtag taaaaattga accattagga gtagcaccca ccaaggcaaa  1260
gagaagagtg gtgcagagag aaaaaagagc agtgggaata ggagctttgt tccttgggtt  1320
```

-continued

```
cttgggagca gcaggaagca ctatgggcgc agcgtcaatg acgctgacgg tacaggccag  1380
acaattattg tctggtatag tgcagcagca gaacaatttg ctgagggcta ttgaggcgca  1440
acagcatctg ttgcaactca cagtctgggg catcaagcag ctccaggcaa gaatcctggc  1500
tgtggaaaga tacctaaagg atcaacagct cctggggatt tggggttgct ctggaaaact  1560
catttgcacc actgctgtgc cttggaatgc tagttggagt aataaatctc tggaacagat  1620
ttggaatcac acgacctgga tggagtggga cagagaaatt aacaattaca caagcttaat  1680
acactcctta attgaagaat cgcaaaacca gcaagaaaag aatgaacaag aattattgga  1740
attagataaa tgggcaagtt tgtggaattg gtttaacata acaaattggc tgtggtatat  1800
aaaattattc ataatgatag taggaggctt ggtaggttta agaatagttt ttgctgtact  1860
ttctatagtg aatagagtta ggcagggata ttcaccatta tcgtttcaga cccacctccc  1920
aaccccgagg ggacccgaca ggcccgaagg aatagaagaa gaaggtggag agagagacag  1980
agacagatcc attcgattag tgaacggatc tcgacggtat cgatgtcgac gataagcttt  2040
gcaaagatgg ataaagtttt aaacagagag gaatctttgc agctaatgga ccttctaggt  2100
cttgaaagga gtgggaattg gctccggtgc ccgtcagtgg gcagagcgca catcgcccac  2160
agtccccgag aagttggggg gaggggtcgg caattgaacc ggtgcctaga gaaggtggcg  2220
cggggtaaac tgggaaagtg atgtcgtgta ctggctccgc ctttttcccg agggtggggg  2280
agaaccgtat ataagtgcag tagtcgccgt gaacgttctt tttcgcaacg ggtttgccgc  2340
cagaacacag gtaagtgccg tgtgtggttc ccgcgggcct ggcctcttta cgggttatgg  2400
cccttgcgtg ccttgaatta cttccactgg ctgcagtacg tgattcttga tcccgagctt  2460
cgggttggaa gtgggtggga gagttcgagg ccttgcgctt aaggagcccc ttcgcctcgt  2520
gcttgagttg aggcctggcc tgggcgctgg ggccgccgcg tgcgaatctg gtggcacctt  2580
cgcgcctgtc tcgctgcttt cgataagtct ctagccattt aaaatttttg atgacctgct  2640
gcgacgcttt ttttctggca agatagtctt gtaaatgcgg gccaagatct gcacactggt  2700
atttcggttt ttggggccgc gggcggcgac ggggcccgtg cgtcccagcg cacatgttcg  2760
gcgaggcggg gcctgcgagc gcggccaccg agaatcggac gggggtagtc tcaagctggc  2820
cggcctgctc tggtgcctgg cctcgcgccg ccgtgtatcg ccccgccctg ggcggcaagg  2880
ctggcccggt cggcaccagt tgcgtgagcg gaaagatggc cgcttcccgg ccctgctgca  2940
gggagctcaa aatggaggac gcggcgctcg ggagagcggg cgggtgagtc acccacacaa  3000
aggaaaaggg cctttccgtc ctcagccgtc gcttcatgtg actccacgga gtaccgggcg  3060
ccgtccaggc acctcgatta gttctcgagc ttttggagta cgtcgtcttt aggttggggg  3120
gaggggtttt atgcgatgga gtttccccac actgagtggg tggagactga agttaggcca  3180
gcttggcact tgatgtaatt ctccttggaa tttgcccttt ttgagtttgg atcttggttc  3240
attctcaagc ctcagacagt ggttcaaagt ttttttcttc catttcaggt gtcgtgagga  3300
atttcgacat ttaaatttaa ttaatctcga cggtatcggt taacttttaa aagaaaaggg  3360
gggattgggg ggtacagtgc aggggaaaga atagtagaca taatagcaac agacatacaa  3420
actaaagaat tacaaaaaca aattacaaaa attcaaaatt ttatcgatca cgagactagc  3480
ctcgaggttt atggctcagc ggatgacaac acagctgctg ctccttctag tgtgggtggc  3540
tgtagtaggg gaggctcaga caggagtgca ggtggagact atctccccag gagacgggcg  3600
caccttcccc aagcgcggcc agacctgcgt ggtgcactac accgggatgc ttgaagatgg  3660
aaagaaattt gattcctccc gggacagaaa caagcccttt aagtttatgc taggcaagca  3720
ggaggtgatc cgaggctggg aagaaggggt tgcccagatg agtgtgggtc agagagccaa  3780
actgactata tctccagatt atgcctatgg tgccactggg cacccaggca tcatcccacc  3840
acatgccact ctcgtcttcg atgtggagct tctaaaactg gaatctggcg gtggatccag  3900
gattgcatgg gccaggactg agcttctcaa tgtctgcatg aacgccaagc accacaagga  3960
aaagccaggc cccgaggaca agttgcatga gcagtgtcga ccctggagga agaatgcctg  4020
ctgttctacc aacaccagcc aggaagccca taaggatgtt tcctacctat atagattcaa  4080
ctggaaccac tgtggagaga tggcacctgc ctgcaaacgg catttcatcc aggacacctg  4140
cctctacgag tgctccccca acttggggcc ctggatccag caggtggatc agagctggcg  4200
caaagagcgg gtactgaacg tgcccctgtg caaagaggac tgtgagcaat ggtgggaaga  4260
ttgtcgcacc tcctacacct gcaagagcaa ctggcacaag ggctggaact ggacttcagg  4320
gtttaacaag tgcgcagtgg gagctgcctg ccaacctttc catttctact tccccacacc  4380
cactgttctg tgcaatgaaa tctggactca ctcctacaag gtcagcaact acagccgagg  4440
gagtggccgc tgcatccaga tgtggttcga cccagcccag ggcaacccca atgaggaggt  4500
ggcgaggttc tatgctgcag ccatgagtgg ggctgggccc tgggcagcct ggcctttcct  4560
gcttagcctg gccctaatgc tgctgtggct gctcagctga aaactacggg ctgcaggaat  4620
tccgcccccc ccccctaac gttactggcc gaagccgctt ggaataaggc cggtgtgcgt  4680
ttgtctatat gttattttcc accatattgc cgtcttttgg caatgtgagg gcccggaaac  4740
ctggccctgt cttcttgacg agcattccta ggggtctttc ccctctcgcc aaaggaatgc  4800
aaggtctgtt gaatgtcgtg aaggaagcag ttcctctgga agcttcttga agacaaacaa  4860
cgtctgtagc gacccttttgc aggcagcgga acccccccacc tggcgacagg tgcctctgcg  4920
gccaaaagcc acgtgtataa gatacacctg caaaggcggc acaaccccag tgccacgttg  4980
tgagttggat agttgtggaa agagtcaaat ggctctcctc aagcgtattc aacaaggggc  5040
tgaaggatgc ccagaaggta ccccattgta tgggatctga tctggggcct cggtgcacat  5100
gctttacatg tgtttagtcg aggttaaaaa acgtctaggc cccccgaacc acggggacgt  5160
ggttttcctt tgaaaaacac gatgataata ccatggtgag caagggcgag gagctgttca  5220
ccggggtggt gcccatcctg gtcgagctgg acggcgacgt aaacggccac aagttcagcg  5280
tgtccggcga gggcgagggc gatgccacct acggcaagct gaccctgaag ttcatctgca  5340
ccaccggcaa gctgcccgtg ccctggccca ccctcgtgac caccctgacc tacggcgtgc  5400
agtgcttcag ccgctacccc gaccacatga agcagcacga cttcttcaag tccgccatgc  5460
ccgaaggcta cgtccaggag cgcaccatct tcttcaagga cgacggcaac tacaagaccc  5520
gcgccgaggt gaagttcgag ggcgacaccc tggtgaaccg catcgagctg aagggcatcg  5580
acttcaagga ggacggcaac atcctggggc acaagctgga gtacaactac aacagccaca  5640
acgtctatat catggccgac aagcagaaga acggcatcaa ggtgaacttc aagatccgcc  5700
acaacatcga ggacggcagc gtgcagctcg ccgaccacta ccagcagaac accccatcg  5760
gcgacggccc cgtgctgctg cccgacaacc actacctgag cacccagtcc gccctgagca  5820
aagaccccaa cgagaagcgc gatcacatgg tcctgctgga gttcgtgacc gccgccggga  5880
```

-continued

```
tcactctcgg catggacgag ctgtacaagt ccggactcag atctcgacta gctagtagct  5940
agctagctag tcgagctcaa gcttcgaatt cgatatcaag cttatcgcga taccgtcgac  6000
ctcgagggaa ttccgataat caacctctgg attacaaaat ttgtgaaaga ttgactggta  6060
ttcttaacta tgttgctcct tttacgctat gtggatacgc tgctttaatg cctttgtatc  6120
atgctattgc ttcccgtatg gctttcattt tctcctcctt gtataaatcc tggttgctgt  6180
ctctttatga ggagttgtgg cccgttgtca ggcaacgtgg cgtggtgtgc actgtgtttg  6240
ctgacgcaac ccccactggt tggggcattg ccaccacctg tcagctcctt tccgggactt  6300
tcgctttccc cctccctatt gccacggcgg aactcatcgc cgcctgcctt gcccgctgct  6360
ggacaggggc tcggctgttg ggcactgaca attccgtggt gttgtcgggg aagctgacgt  6420
cctttccatg gctgctcgcc tgtgttgcca cctggattct gcgcgggacg tccttctgct  6480
acgtcccttc ggccctcaat ccagcggacc ttccttcccg cggcctgctg ccggctctgc  6540
ggcctcttcc gcgtcttcgc cttcgccctc agacgagtcg gatctccctt tgggccgcct  6600
ccccgcatcg ggaattcgag ctcggtacct ttaagaccaa tgacttacaa ggcagctgta  6660
gatcttagcc acttttttaaa agaaaagggg ggactggaag ggctaattca ctcccaacga  6720
agacaagatg ggatcaattc accatgggaa taacttcgta tagcatacat tatacgaagt  6780
tatgctgctt tttgcttgta ctgggtctct ctggttagac cagatctgag cctgggagct  6840
ctctggctaa ctagggaacc cactgcttaa gcctcaataa agcttgcctt gagtgcttca  6900
agtagtgtgt gcccgtctgt tgtgtgactc tggtaactag agatccctca gacccttta  6960
gtcagtgtgg aaaatctcta gcagcatcta gaattaattc cgtgtattct atagtgtcac  7020
ctaaatcgta tgtgtatgat acataaggtt atgtattaat tgtagccgcg ttctaacgac  7080
aatatgtaca agcctaattg tgtagcatct ggcttactga agcagaccct atcatctctc  7140
tcgtaaactg ccgtcagagt cggtttggtt ggacgaacct tctgagtttc tggtaacgcc  7200
gtcccgcacc cggaaatggt cagcgaacca atcagcaggg tcatcgctag ccagatcctc  7260
tacgccggac gcatcgtggc cggcatcacc ggcgccacag gtgcggttgc tggcgcctat  7320
atcgccgaca tcaccgatgg ggaagatcgg gctcgccact tcgggctcat gagcgcttgt  7380
ttcggcgtgg gtatggtggc aggccccgtg gccgggggac tgttgggcgc catctccttg  7440
catgcaccat tccttgcggc ggcggtgctc aacggcctca acctactact gggctgcttc  7500
ctaatgcagg agtcgcataa gggagagcgt cgaatggtgc actctcagta caatctgctc  7560
tgatgccgca tagttaagcc agccccgaca cccgccaaca cccgctgacg cgccctgacg  7620
ggcttgtctg ctcccggcat ccgcttacag acaagctgtg accgtctccg ggagctgcat  7680
gtgtcagagg ttttcaccgt catcaccgaa acgcgcgaga cgaaagggcc tcgtgatacg  7740
cctatttta taggttaatg tcatgataat aatggtttct tagacgtcag gtggcacttt  7800
tcggggaaat gtgcgcggaa cccctatttg tttatttttc taaatacatt caaatatgta  7860
tccgctcatg agacaataac cctgataaat gcttcaataa tattgaaaaa ggaagagtat  7920
gagtattcaa catttccgtg tcgcccttat tccctttttt gcggcatttt gccttcctgt  7980
ttttgctcac ccagaaacgc tggtgaaagt aaaagatgct gaagatcagt tgggtgcacg  8040
agtgggttac atcgaactgg atctcaacag cggtaagatc cttgagagtt ttcgccccga  8100
agaacgtttt ccaatgatga gcacttttaa agttctgcta tgtggcgcgg tattatcccg  8160
tattgacgcc gggcaagagc aactcggtcg ccgcatacac tattctcaga atgacttggt  8220
tgagtactca ccagtcacag aaaagcatct tacggatggc atgacagtaa gagaattatg  8280
cagtgctgcc ataaccatga gtgataacac tgcggccaac ttacttctga caacgatcgg  8340
aggaccgaag gagctaaccg ctttttgca caacatgggg gatcatgtaa ctcgccttga  8400
tcgttgggaa ccggagctga atgaagccat accaaacgac gagcgtgaca ccacgatgcc  8460
tgtagcaatg gcaacaacgt tgcgcaaact attaactggc gaactactta ctctagcttc  8520
ccggcaacaa ttaatagact ggatggaggc ggataaagtt gcaggaccac ttctgcgctc  8580
ggcccttccg gctggctggt ttattgctga taaatctgga gccggtgagc gtgggtctcg  8640
cggtatcatt gcagcactgg ggccagatgg taagccctcc cgtatcgtag ttatctacac  8700
gacggggagt caggcaacta tggatgaacg aaatagacag atcgctgaga taggtgcctc  8760
actgattaag cattggtaac tgtcagacca agtttactca tatatacttt agattgattt  8820
aaaacttcat ttttaattta aaaggatcta ggtgaagatc ctttttgata atctcatgac  8880
caaaatccct taacgtgagt tttcgttcca ctgagcgtca gaccccgtag aaaagatcaa  8940
aggatcttct tgagatcctt ttttctgcg cgtaatctgc tgcttgcaaa caaaaaaacc  9000
accgctacca gcggtggttt gtttgccgga tcaagagcta ccaactcttt ttccgaaggt  9060
aactggcttc agcagagcgc agataccaaa tactgtcctt ctagtgtagc cgtagttagg  9120
ccaccacttc aagaactctg tagcaccgcc tacatacctc gctctgctaa tcctgttacc  9180
agtggctgct gccagtggcg ataagtcgtg tcttaccggg ttggactcaa gacgatagtt  9240
accggataag gcgcagcggt cgggctgaac gggggttcg tgcacacagc ccagcttgga  9300
gcgaacgacc tacaccgaac tgagatacct acagcgtgag cattgagaaa gcgccacgct  9360
tcccgaaggg agaaaggcgg acaggtatcc ggtaagcggc agggtcggaa caggagagcg  9420
cacgagggag cttccagggg gaaacgcctg gtatctttat agtcctgtcg ggtttcgcca  9480
cctctgactt gagcgtcgat ttttgtgatg ctcgtcaggg gggcggagcc tatggaaaaa  9540
cgccagcaac gcggcctttt tacggttcct ggccttttgc tggccttttg ctcacatgtt  9600
ctttcctgcg ttatcccctg attctgtgga taaccgtatt accgcctttg agtgagctga  9660
taccgctcgc cgcagccgaa cgaccgagcg cagcgagtca gtgagcgagg aagcggaaga  9720
gcgcccaata cgcaaaccgc ctctccccgc gcgttggccg attcattaat gcagctgtgg  9780
aatgtgtgtc agttagggtg tggaaagtcc ccaggctccc cagcaggcag aagtatgcaa  9840
agcatgcatc tcaattagtc agcaaccagg tgtggaaagt ccccaggctc cccagcaggc  9900
agaagtatgc aaagcatgca tctcaattag tcagcaacca tagtcccgcc cctaactccg  9960
cccatcccgc ccctaactcc gcccagttcc gcccattctc cgccccatgg ctgactaatt 10020
ttttttattt atgcagaggc cgaggccgcc tcggcctctg agctattcca gaagtagtga 10080
ggaggctttt ttggaggcct aggcttttgc aaaaagcttg gacacaagac aggcttgcga 10140
gatatgtttg agaataccac tttatcccgc gtcagggaga ggcagtgcgt aaaaagacgc 10200
ggactcatgt gaaatactgg tttttagtgc gccagatctc tataatctcg cgcaacctat 10260
tttcccctcg aacacttttt aagccgtaga taaacaggct gggacacttc acatgagcga 10320
aaaatacatc gtcacctggg acatgttgca gatccatgca cgtaaactcg caagccgact 10380
gatgccttct gaacaatgga aaggcattat tgccgtaagc cgtggcggtc tgtaccgggt 10440
```

-continued

```
gcgttactgg cgcgtgaact gggtattcgt catgtcgata ccgtttgtat ttccagctac  10500
gatcacgaca accagcgcga gcttaaagtg ctgaaacgcg cagaaggcga tggcgaaggc  10560
ttcatcgtta ttgatgacct ggtggatacc ggtggtactg cggttgcgat tcgtgaaatg  10620
tatccaaaag cgcactttgt caccatcttc gcaaaaccgg ctggtcgtcc gctggttgat  10680
gactatgttg ttgatatccc gcaagatacc tggattgaac agccgtggga tatgggcgtc  10740
gtattcgtcc cgccaatctc cggtcgctaa tcttttcaac gcctggcact gccgggcgtt  10800
gttcttttta acttcaggcg ggttacaata gtttccagta agtattctgg aggctgcatc  10860
catgacacag gcaaacctga gcgaaaccct gttcaaaccc cgctttaaac atcctgaaac  10920
ctcgacgcta gtccgccgct ttaatcacgg cgcacaaccg cctgtgcagt cggcccttga  10980
tggtaaaacc atccctcact ggtatcgcat gattaaccgt ctgatgtgga tctggcgcgg  11040
cattgaccca cgcgaaatcc tcgacgtcca ggcacgtatt gtgatgagcg atgccgaacg  11100
taccgacgat gatttatacg atacggtgat tggctaccgt ggcggcaact ggatttatga  11160
gtgggccccg gatctttgtg aaggaacctt acttctgtgg tgtgacataa ttggacaaac  11220
tacctacaga gatttaaagc tctaaggtaa atataaaatt tttaagtgta taatgtgtta  11280
aactactgat tctaattgtt tgtgtatttt agattccaac ctatggaact gatgaatggg  11340
agcagtggtg gaatgccttt aatgaggaaa acctgttttg ctcagaagaa atgccatcta  11400
gtgatgatga ggctactgct gactctcaac attctactcc tccaaaaaag aagagaaagg  11460
tagaagaccc caaggacttt ccttcagaat tgctaagttt tttgagtcat gctgtgttta  11520
gtaatagaac tcttgcttgc tttgctattt acaccacaaa ggaaaaagct gcactgctat  11580
acaagaaaat tatggaaaaa tattctgtaa ccttttataag taggcataac agttataatc  11640
ataacatact gttttttctt actccacaca ggcatagagt gtctgctatt aataactatg  11700
ctcaaaaatt gtgtaccttt agctttttaa tttgtaaagg ggttaataag gaatatttga  11760
tgtatagtgc cttgactaga gatcataatc agccatacca catttgtaga ggttttactt  11820
gctttaaaaa acctcccaca cctccccctg aacctgaaac ataaaatgaa tgcaattgtt  11880
gttgttaact tgtttattgc agcttataat ggttacaaat aaagcaatag catcacaaat  11940
ttcacaaata aagcattttt ttcactgcat tctagttgtg gtttgtccaa actcatcaat  12000
gtatcttatc atgtctggat caactggata actcaagcta accaaaatca tcccaaactt  12060
cccaccccat accctattac cactgccaat tacctagtgg tttcatttac tctaaacctg  12120
tgattcctct gaattatttt cattttaaag aaattgtatt tgttaaatat gtactacaaa  12180
cttagtag                                                          12188

SEQ ID NO: 8           moltype = DNA  length = 12182
FEATURE                Location/Qualifiers
misc_feature           1..12182
                       note = A synthetic pWPI mFKBP-mFRa SGGGS
source                 1..12182
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 8
ttggaagggc taattcactc ccaaagaaga caagatatcc ttgatctgtg gatctaccac  60
acacaaggct acttccctga ttagcagaac tacacaccag ggccaggggt cagatatcca  120
ctgacctttg gatggtgcta caagctagta ccagttgagc cagataaggt agaagaggcc  180
aataaaggag agaacaccag cttgttacac cctgtgagcc tgcatgggat ggatgacccg  240
gagagagaag tgttagagtg gaggtttgac agccgcctag catttcatca cgtggcccga  300
gagctgcatc cggagtactt caagaactgc tgatatcgag cttgctacaa gggactttcc  360
gctggggact ttccagggag gcgtggcctg ggcgggactg gggagtggcg agccctcaga  420
tcctgcatat aagcagctgc tttttgcctg tactgggtct ctctggttag accagatctg  480
agcctgggag ctctctggct aactagggaa cccactgctt aagcctcaat aaagcttgcc  540
ttgagtgctt caagtagtgt gtgcccgtct gttgtgtgac tctggtaact agagatccct  600
cagacccttt tagtcagtgt ggaaaatctc tagcagtggc gcccgaacag ggacttgaaa  660
gcgaaaggga aaccagagga gctctctcga cgcaggactc ggcttgctga agcgcgcacg  720
gcaagaggcg aggggcggcg actggtgagt acgccaaaaa ttttgactag cggaggctag  780
aaggagagag atgggtgcga gagcgtcagt attaagcggg ggagaattag atcgcgatgg  840
gaaaaaattc ggttaaggcc aggggggaaag aaaaaatata aattaaaaca tatagtatgg  900
gcaagcaggg agctagaacg attcgcagtt aatcctggcc tgttagaaac atcagaaggc  960
tgtagacaaa tactgggaca gctacaacca tcccttcaga caggatcaga agaacttaga  1020
tcattatata atacagtagc aaccctctat tgtgtgcatc aaaggataga gataaaagac  1080
accaaggaag ctttagacaa gatagaggaa gagcaaaaca aaagtaagac caccgcacag  1140
caagcggccg ctgatcttca gacctggagg aggagatatg agggacaatt ggagaagtga  1200
attatataaa tataaagtag taaaaattga accattagga gtagcaccca ccaaggcaaa  1260
gagaagagtg gtgcagagag aaaaaagagc agtgggaata ggagctttgt tccttgggtt  1320
cttgggagca gcaggaagca ctatgggcgc agcgtcaatg acgctgacgg tacaggccag  1380
acaattattg tctggtatag tgcagcagca gaacaatttg ctgagggcta ttgaggcgca  1440
acagcatctg ttgcaactca cagtctgggg catcaagcag ctccaggcaa gaatcctggc  1500
tgtggaaaga tacctaaagg atcaacagct cctggggatt tggggttgct ctggaaaact  1560
catttgcacc actgctgtgc cttggaatgc tagttggagt aataaatctc tggaacagat  1620
ttggaatcac acgacctgga tggagtggga cagagaaatt aacaattaca caagcttaat  1680
acactcctta attgaagaat cgcaaaacca gcaagaaaag aatgaacaag aattattgga  1740
attagataaa tgggcaagtt tgtggaattg gtttaacata acaaattggc tgtggtatat  1800
aaaattattc ataatgatag taggaggctt ggtaggttta agaatagttt ttgctgtact  1860
ttctatagtg aatagagtta ggcagggata ttcaccatta tcgtttcaga cccacctccc  1920
aaccccgagg ggacccgaca ggcccgaagg aatagaagaa gaaggtggag agagagacag  1980
agacagatcc attcgattag tgaacggatc tcgacggtat cgatgtcgac gataagcttt  2040
gcaaagatgg ataaagtttt aaacagagag gaatctttgc agctaatgga ccttctaggt  2100
cttgaaagga gtgggaattg gctccggtgc ccgtcagtgg gcagagcgca catcgcccac  2160
agtccccgag aagttggggg gaggggtcgg caattgaacc ggtgcctaga gaaggtggcg  2220
```

-continued

```
cggggtaaac tgggaaagtg atgtcgtgta ctggctccgc ctttttcccg agggtggggg  2280
agaaccgtat ataagtgcag tagtcgccgt gaacgttctt tttcgcaacg ggtttgccgc  2340
cagaacacag gtaagtgccg tgtgtggttc ccgcgggcct ggcctcttta cgggttatgg  2400
cccttgcgtg ccttgaatta cttccactgg ctgcagtacg tgattcttga tcccgagctt  2460
cgggttggaa gtgggtggga gagttcgagg ccttgcgctt aaggagcccc ttcgcctcgt  2520
gcttgagttg aggcctggcc tgggcgctgg ggccgccgcg tgcgaatctg gtggcacctt  2580
cgcgcctgtc tcgctgcttt cgataagtct ctagccattt aaaatttttg atgacctgct  2640
gcgacgcttt ttttctggca agatagtctt gtaaatgcgg gccaagatct gcacactggt  2700
atttcggttt ttggggccgc gggcggcgac ggggcccgtg cgtcccagcg cacatgttcg  2760
gcgaggcggg gcctgcgagc gcggccaccg agaatcggac gggggtagtc tcaagctggc  2820
cggcctgctc tggtgcctgg cctcgcgccg ccgtgtatcg ccccgccctg ggcggcaagg  2880
ctggcccggt cggcaccagt tgcgtgagcg gaaagatggc cgcttcccgg ccctgctgca  2940
gggagctcaa aatggaggac gcggcgctcg ggagagcggg cgggtgagtc acccacacaa  3000
aggaaaaggg cctttccgtc ctcagccgtc gcttcatgtg actccacgga gtaccgggcg  3060
ccgtccaggc acctcgatta gttctcgagc ttttggagta cgtcgtcttt aggttggggg  3120
gaggggtttt atgcgatgga gtttccccac actgagtggg tggagactga agttaggcca  3180
gcttggcact tgatgtaatt ctccttggaa tttgcccttt ttgagtttgg atcttggttc  3240
attctcaagc ctcagacagt ggttcaaagt ttttttcttc catttcaggt gtcgtgagga  3300
atttcgacat ttaaatttaa ttaatctcga cggtatcggt taacttttaa aagaaaaggg  3360
gggattgggg ggtacagtgc aggggaaaga atagtagaca taatagcaac agacatacaa  3420
actaaagaat tacaaaaaca aattacaaaa attcaaaatt ttatcgatca cgagactagc  3480
ctcgaggttt atggctcacc tgatgactgt gcagttgttg ctcctggtga tgtggatggc  3540
cgaatgtgct cagtccagag ctggagtgca ggtggagacc atctctcctg gagacgggcg  3600
caccttccca aagcgcggcc agacctgcgt ggtgcactac acggggatgc ttgaagatgg  3660
aaagaaattt gattcctctc gggacagaaa caagcctttt aagtttacac taggcaagca  3720
ggaggtgatc cgaggctggg aggaaggggt agcccagatg agtgtgggtc agagagccaa  3780
actgataatc tcctcagact atgcctatgg agccaccggg cacccaggca tcatcccacc  3840
acatgccact cttgtttttg atgtggagct tctaaaactg gaaagcggcg gcggcagcac  3900
tcgggccagg actgaacttc tcaatgtctg catggatgcc aaacaccaca aagaaaaacc  3960
gggccctgag gacaatttac acgaccagtg cagcccctgg aagacgaatt cctgctgttc  4020
cacgaacaca agccaggaag cacataagga catttcctac ctgtaccggt tcaactggaa  4080
ccactgcgga actatgacat cggaatgcaa acggcacttt atccaagaca cctgcctcta  4140
tgagtgttcc ccgaacttgg gaccctggat ccagcaggtg gaccagagct ggcgcaaaga  4200
gcggatcctt gatgttcccc tgtgcaaaga ggactgtcag cagtggtggg aggactgcca  4260
gagctctttt acctgcaaga gcaattggca caagggatgg aactggtcct cggggcataa  4320
cgagtgtcct gtgggagcct cctgccatcc cttcaccttc tacttcccca catctgctgc  4380
tctgtgtgag gaaatctgga gtcactccta caagctcagc aactacagtc gagggagcgg  4440
ccgctgcatt cagatgtggt tcgacccagc ccagggcaac cccaacgagg aagtggcgag  4500
gttctatgcc gaggccatga gtggagctgg gtttcatggg acctggccac tcttgtgcag  4560
cctgtcctta gtgctgctct gggtgatcag ctgaaaacta cgggctgcag gaattccgcc  4620
ccccccccc taacgttact ggccgaagcc gcttggaata aggccggtgt gcgtttgtct  4680
atatgttatt ttccaccata ttgccgtctt ttggcaatgt gagggcccgg aaacctggcc  4740
ctgtcttctt gacgagcatt cctaggggtc tttcccctct cgccaaagga atgcaaggtc  4800
tgttgaatgt cgtgaaggaa gcagttcctc tggaagcttc ttgaagacaa acaacgtctg  4860
tagcgaccct ttgcaggcag cggaaccccc cacctggcga caggtgcctc tgcggccaaa  4920
agccacgtgt ataagataca cctgcaaagg cggcacaacc ccagtgccac gttgtgagtt  4980
ggatagttgt ggaaagagtc aaatggctct cctcaagcgt attcaacaag gggctgaagg  5040
atgcccagaa ggtaccccat tgtatgggat ctgatctggg gcctcggtgc acatgcttta  5100
catgtgttta gtcgaggtta aaaaacgtct aggccccccg aaccacgggg acgtggtttt  5160
cctttgaaaa acacgatgat aataccatgg tgagcaaggg cgaggagctg ttcaccgggg  5220
tggtgcccat cctggtcgag ctggacggcg acgtaaacgg ccacaagttc agcgtgtccg  5280
gcgagggcga gggcgatgcc acctacggca agctgaccct gaagttcatc tgcaccaccg  5340
gcaagctgcc cgtgccctgg cccaccctcg tgaccaccct gacctacggc gtgcagtgct  5400
tcagccgcta ccccgaccac atgaagcagc acgacttctt caagtccgcc atgcccgaag  5460
gctacgtcca ggagcgcacc atcttcttca aggacgacgg caactacaag acccgcgccg  5520
aggtgaagtt cgagggcgac accctggtga accgcatcga gctgaagggc atcgacttca  5580
aggaggacgg caacatcctg gggcacaagc tggagtacaa ctacaacagc cacaacgtct  5640
atatcatggc cgacaagcag aagaacggca tcaaggtgaa cttcaagatc cgccacaaca  5700
tcgaggacgg cagcgtgcag ctcgccgacc actaccagca gaacaccccc atcggcgacg  5760
gccccgtgct gctgcccgac aaccactacc tgagcaccca gtccgccctg agcaaagacc  5820
ccaacgagaa gcgcgatcac atggtcctgc tggagttcgt gaccgccgcc gggatcactc  5880
tcggcatgga cgagctgtac aagtccggac tcagatctcg actagctagt agctagctag  5940
ctagtcgagc tcaagcttcg aattcgatat caagcttatc gcgataccgt cgacctcgag  6000
ggaattccga taatcaacct ctggattaca aaatttgtga aagattgact ggtattctta  6060
actatgttgc tccttttacg ctatgtggat acgctgcttt aatgcctttg tatcatgcta  6120
ttgcttcccg tatggctttc attttctcct ccttgtataa atcctggttg ctgtctcttt  6180
atgaggagtt gtggcccgtt gtcaggcaac gtggcgtggt gtgcactgtg tttgctgacg  6240
caaccccac tggttggggc attgccacca cctgtcagct cctttccggg actttcgctt  6300
tccccctccc tattgccacg gcggaactca tcgccgcctg ccttgcccgc tgctggacag  6360
gggctcggct gttgggcact gacaattccg tggtgttgtc ggggaagctg acgtcctttc  6420
catggctgct cgcctgtgtt gccacctgga ttctgcgcgg gacgtccttc tgctacgtcc  6480
cttcggccct caatccagcg gaccttcctt cccgcggcct gctgccggct ctgcggcctc  6540
ttccgcgtct tcgccttcgc cctcagacga gtcggatctc cctttgggcc gcctccccgc  6600
atcgggaatt cgagctcggt acctttaaga ccaatgactt acaaggcagc tgtagatctt  6660
agccactttt taaaagaaaa gggggggactg gaagggctaa ttcactccca acgaagacaa  6720
gatgggatca attcaccatg ggaataacttt cgtatagcat acattatacg aagttatgct  6780
```

-continued

```
gctttttgct tgtactgggt ctctctggtt agaccagatc tgagcctggg agctctctgg  6840
ctaactaggg aacccactgc ttaagcctca ataaagcttg ccttgagtgc ttcaagtagt  6900
gtgtgcccgt ctgttgtgtg actctggtaa ctagagatcc ctcagaccct tttagtcagt  6960
gtggaaaatc tctagcagca tctagaatta attccgtgta ttctatagtg tcacctaaat  7020
cgtatgtgta tgatacataa ggttatgtat taattgtagc cgcgttctaa cgacaatatg  7080
tacaagccta attgtgtagc atctggctta ctgaagcaga ccctatcatc tctctcgtaa  7140
actgccgtca gagtcggttt ggttggacga accttctgag tttctggtaa cgccgtcccg  7200
cacccggaaa tggtcagcga accaatcagc agggtcatcg ctagccagat cctctacgcc  7260
ggacgcatcg tggccggcat caccggcgcc acaggtgcgg ttgctggcgc ctatatcgcc  7320
gacatcaccg atggggaaga tcgggctcgc cacttcgggc tcatgagcgc ttgtttcggc  7380
gtgggtatgg tggcaggccc cgtggccggg ggactgttgg gcgccatctc cttgcatgca  7440
ccattccttg cggcggcggt gctcaacggc ctcaacctac tactgggctg cttcctaatg  7500
caggagtcgc ataagggaga gcgtcgaatg gtgcactctc agtacaatct gctctgatgc  7560
cgcatagtta agccagcccc gacacccgcc aacacccgct gacgcgccct gacgggcttg  7620
tctgctcccg gcatccgctt acagacaagc tgtgaccgtc tccgggagct gcatgtgtca  7680
gaggttttca ccgtcatcac cgaaacgcgc gagacgaaag ggcctcgtga tacgcctatt  7740
tttataggtt aatgtcatga taataatggt ttcttagacg tcaggtggca cttttcgggg  7800
aaatgtgcgc ggaaccccta tttgtttatt tttctaaata cattcaaata tgtatccgct  7860
catgagacaa taaccctgat aaatgcttca ataatattga aaaaggaaga gtatgagtat  7920
tcaacatttc cgtgtcgccc ttattccctt ttttgcggca ttttgccttc ctgtttttgc  7980
tcacccagaa acgctggtga aagtaaaaga tgctgaagat cagttgggtg cacgagtggg  8040
ttacatcgaa ctggatctca acagcggtaa gatccttgag agttttcgcc ccgaagaacg  8100
ttttccaatg atgagcactt ttaaagttct gctatgtggc gcggtattat cccgtattga  8160
cgccgggcaa gagcaactcg gtcgccgcat acactattct cagaatgact tggttgagta  8220
ctcaccagtc acagaaaagc atcttacgga tggcatgaca gtaagagaat tatgcagtgc  8280
tgccataacc atgagtgata acactgcggc caacttactt ctgacaacga tcggaggacc  8340
gaaggagcta accgcttttt tgcacaacat gggggatcat gtaactcgcc ttgatcgttg  8400
ggaaccggag ctgaatgaag ccataccaaa cgacgagcgt gacaccacga tgcctgtagc  8460
aatggcaaca acgttgcgca aactattaac tggcgaacta cttactctag cttcccggca  8520
acaattaata gactggatgg aggcggataa agttgcagga ccacttctgc gctcggccct  8580
tccggctggc tggtttattg ctgataaatc tggagccggt gagcgtgggt ctcgcggtat  8640
cattgcagca ctggggccag atggtaagcc ctcccgtatc gtagttatct acacgacggg  8700
gagtcaggca actatggatg aacgaaatag acagatcgct gagataggtg cctcactgat  8760
taagcattgg taactgtcag accaagttta ctcatatata ctttagattg atttaaaact  8820
tcatttttaa tttaaaagga tctaggtgaa gatccttttt gataatctca tgaccaaaat  8880
cccttaacgt gagttttcgt tccactgagc gtcagacccc gtagaaaaga tcaaaggatc  8940
ttcttgagat cctttttttc tgcgcgtaat ctgctgcttg caaacaaaaa aaccaccgct  9000
accagcggtg gtttgtttgc cggatcaaga gctaccaact ctttttccga aggtaactgg  9060
cttcagcaga gcgcagatac caaatactgt ccttctagtg tagccgtagt taggccacca  9120
cttcaagaac tctgtagcac cgcctacata cctcgctctg ctaatcctgt taccagtggc  9180
tgctgccagt ggcgataagt cgtgtcttac cgggttggac tcaagacgat agttaccgga  9240
taaggcgcag cggtcgggct gaacgggggg ttcgtgcaca cagcccagct tggagcgaac  9300
gacctacacc gaactgagat acctacagcg tgagcattga gaaagcgcca cgcttcccga  9360
agggagaaag gcggacaggt atccggtaag cggcagggtc ggaacaggag agcgcacgag  9420
ggagcttcca gggggaaacg cctggtatct ttatagtcct gtcgggtttc gccacctctg  9480
acttgagcgt cgatttttgt gatgctcgtc agggggggcgg agcctatgga aaaacgccag  9540
caacgcggcc tttttacggt tcctggcctt ttgctggcct tttgctcaca tgttctttcc  9600
tgcgttatcc cctgattctg tggataaccg tattaccgcc tttgagtgag ctgataccgc  9660
tcgccgcagc cgaacgaccg agcgcagcga gtcagtgagc gaggaagcgg aagagcgccc  9720
aatacgcaaa ccgcctctcc ccgcgcgttg gccgattcat taatgcagct gtggaatgtg  9780
tgtcagttag ggtgtggaaa gtccccaggc tccccagcag gcagaagtat gcaaagcatg  9840
catctcaatt agtcagcaac caggtgtgga aagtccccag gctcccccagc aggcagaagt  9900
atgcaaagca tgcatctcaa ttagtcagca accatagtcc cgcccctaac tccgcccatc  9960
ccgcccctaa ctccgcccag ttccgcccat tctccgcccc atggctgact aatttttttt 10020
atttatgcag aggccgaggc cgcctcggcc tctgagctat tccagaagta gtgaggaggc 10080
ttttttggag gcctaggctt ttgcaaaaag cttggacaca agacaggctt gcgagatatg 10140
tttgagaata ccactttatc ccgcgtcagg gagaggcagt gcgtaaaaag acgcggactc 10200
atgtgaaata ctggtttttа gtgcgccaga tctctataat ctcgcgcaac ctattttccc 10260
ctcgaacact ttttaagccg tagataaaca ggctgggaca cttcacatga gcgaaaaata 10320
catcgtcacc tgggacatgt tgcagatcca tgcacgtaaa ctcgcaagcc gactgatgcc 10380
ttctgaacaa tggaaaggca ttattgccgt aagccgtggc ggtctgtacc gggtgcgtta 10440
ctggcgcgtg aactgggtat tcgtcatgtc gataccgttt gtatttccag ctacgatcac 10500
gacaaccagc gcgagcttaa agtgctgaaa cgcgcagaag gcgatggcga aggcttcatc 10560
gttattgatg acctggtgga taccggtggt actgcggttg cgattcgtga aatgtatcca 10620
aaagcgcact ttgtcaccat cttcgcaaaa ccggctggtc gtccgctggt tgatgactat 10680
gttgttgata tcccgcaaga tacctggatt gaacagccgt gggatatggg cgtcgtattc 10740
gtcccgccaa tctccggtcg ctaatctttt caacgcctgg cactgccggg cgttgttctt 10800
tttaacttca ggcgggttac aatagtttcc agtaagtatt ctggaggctg catccatgac 10860
acaggcaaac ctgagcgaaa ccctgttcaa accccgcttt aaacatcctg aaacctcgac 10920
gctagtccgc cgctttaatc acggcgcaca accgcctgtg cagtcggccc ttgatggtaa 10980
aaccatccct cactggtatc gcatgattaa ccgtctgatg tggatctggc gcggcattga 11040
cccacgcgaa atcctcgacg tccaggcacg tattgtgatg agcgatgccg aacgtaccga 11100
cgatgattta tacgatacgg tgattggcta ccgtggcggc aactggattt atgagtgggc 11160
cccggatctt tgtgaaggaa ccttacttct gtggtgtgac ataattggac aaactaccta 11220
cagagattta aagctctaag gtaaatataa aatttttaag tgtataatgt gttaaactac 11280
tgattctaat tgtttgtgta ttttagattc caacctatgg aactgatgaa tgggagcagt 11340
```

-continued

```
ggtggaatgc ctttaatgag gaaaacctgt tttgctcaga agaaatgcca tctagtgatg  11400
atgaggctac tgctgactct caacattcta ctcctccaaa aaagaagaga aaggtagaag  11460
accccaagga ctttccttca gaattgctaa gttttttgag tcatgctgtg tttagtaata  11520
gaactcttgc ttgctttgct atttacacca caaaggaaaa agctgcactg ctatacaaga  11580
aaattatgga aaaatattct gtaaccttta taagtaggca taacagttat aatcataaca  11640
tactgttttt tcttactcca cacaggcata gagtgtctgc tattaataac tatgctcaaa  11700
aattgtgtac ctttagcttt ttaatttgta aaggggttaa taaggaatat ttgatgtata  11760
gtgccttgac tagagatcat aatcagccat accacatttg tagaggtttt acttgcttta  11820
aaaaacctcc cacacctccc cctgaacctg aaacataaaa tgaatgcaat tgttgttgtt  11880
aacttgttta ttgcagctta taatggttac aaataaagca atagcatcac aaatttcaca  11940
aataaagcat ttttttcact gcattctagt tgtggtttgt ccaaactcat caatgtatct  12000
tatcatgtct ggatcaactg gataactcaa gctaaccaaa atcatcccaa acttcccacc  12060
ccataccta ttaccactgc caattaccta gtggtttcat ttactctaaa cctgtgattc  12120
ctctgaatta ttttcatttt aaagaaattg tatttgttaa atatgtacta caaacttagt  12180
ag                                                                12182

SEQ ID NO: 9           moltype = DNA  length = 10447
FEATURE                Location/Qualifiers
misc_feature           1..10447
                       note = A synthetic pHR EcorI hAnti cd19 1D3 myc hinge cd28
                        cd3zeta
source                 1..10447
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 9
cgataccgtc gaccaaggca gctgtagatc ttagccactt tttaaaagaa aaggggggac  60
tggaagggct aattcactcc caacgaagac aagatctgct ttttgcttgt actgggtctc  120
tctggttaga ccagatctga gcctgggagc tctctggcta actagggaac ccactgctta  180
agcctcaata aagcttgcct tgagtgcttc aagtagtgtg tgcccgtctg ttgtgtgact  240
ctggtaacta gagatccctc agacccttttt agtcagtgtg gaaaatctct agcagcatct  300
agaattaatt ccgtgtattc tatagtgtca cctaaatcgt atgtgtatga tacataaggt  360
tatgtattaa ttgtagccgc gttctaacga caatatgtac aagcctaatt gtgtagcatc  420
tggcttactg aagcagaccc tatcatctct ctcgtaaact gccgtcagag tcggtttggt  480
tggacgaacc ttctgagttt ctggtaacgc cgtcccgcac ccggaaatgg tcagcgaacc  540
aatcagcagg gtcatcgcta gccagatcct ctacgccgga cgcatcgtgg ccggcatcac  600
cggcgccaca ggtgcggttg ctggcgccta tatcgccgac atcaccgatg gggaagatcg  660
ggctcgccac ttcgggctca tgagcgcttg tttcggcgtg ggtatggtgg caggccccgt  720
ggccgggggа ctgttgggcg ccatctcctt gcatgcacca ttccttgcgg cggcggtgct  780
caacggcctc aacctactac tgggctgctt cctaatgcag gagtcgcata agggagagcg  840
tcgaatggtg cactctcagt acaatctagc tctgatgccg catagttaag ccagccccga  900
cacccgccaa cacccgctga cgcgccctga cgggcttgtc tgctcccggc atccgcttac  960
agacaagctg tgaccgtctc cgggagctgc atgtgtcaga ggttttcacc gtcatcaccg  1020
aaacgcgcga gacgaaaggg cctcgtgata cgcctatttt tataggttaa tgtcatgata  1080
ataatggttt cttagacgtc aggtggcact tttcggggaa atgtgcgcgg aacccctatt  1140
tgtttatttt tctaaataca ttcaaatatg tatccgctca tgagacaata accctgataa  1200
atgcttcaat aatattgaaa aaggaagagt atgagtattc aacatttccg tgtcgccctt  1260
attccctttt ttgcggcatt ttgccttcct gtttttgctc acccagaaac gctggtgaaa  1320
gtaaaagatg ctgaagatca gttgggtgca cgagtgggtt acatcgaact ggatctcaac  1380
agcggtaaga tccttgagag ttttcgcccc gaagaacgtt ttccaatgat gagcactttt  1440
aaagttctgc tatgtggcgc ggtattatcc cgtattgacg ccgggcaaga gcaactcggt  1500
cgccgcatac actattctca gaatgacttg gttgagtact caccagtcac agaaaagcat  1560
cttacggatg gcatgacagt aagagaatta tgcagtgctg ccataaccat gagtgataac  1620
actgcggcca acttacttct gacaacgatc ggaggaccga aggagctaac cgcttttttg  1680
cacaacatgg gggatcatgt aactcgcctt gatcgttggg aaccggagct gaatgaagcc  1740
ataccaaacg acgagcgtga caccacgatg cctgtagcaa tggcaacaac gttgcgcaaa  1800
ctattaactg gcgaactact tactctagct tcccggcaac aattaataga ctggatggag  1860
gcggataaag ttgcaggacc acttctgcgc tcggcccttc cggctggctg gtttattgct  1920
gataaatctg gagccggtga gcgtgggtct cgcggtatca ttgcagcact ggggccagat  1980
ggtaagccct cccgtatcgt agttatctac acgacgggga gtcaggcaac tatggatgaa  2040
cgaaatagac agatcgctga gataggtgcc tcactgatta agcattggta actgtcagac  2100
caagtttact catatatact ttagattgat ttaaaacttc attttaatt taaaaggatc  2160
taggtgaaga tccttttttga taatctcatg accaaaatcc cttaacgtga gttttcgttc  2220
cactgagcgt cagaccccgt agaaaagatc aaaggatctt cttgagatcc ttttttttctg  2280
cgcgtaatct gctgcttgca aacaaaaaaa ccaccgctac cagcggtggt ttgtttgccg  2340
gatcaagagc taccaactct ttttccgaag gtaactggct tcagcagagc gcagatacca  2400
aatactgtcc ttctagtgta gccgtagtta ggccaccact tcaagaactc tgtagcaccg  2460
cctacatacc tcgctctgct aatcctgtta ccagtggctg ctgccagtgg cgataagtcg  2520
tgtcttaccg ggttggactc aagacgatag ttaccggata aggcgcagcg gtcgggctga  2580
acgggggggtt cgtgcacaca gcccagcttg gagcgaacga cctacaccga actgagatac  2640
ctacagcgtg agctatgaga aagcgccacg cttcccgaag ggagaaaggc ggacaggtat  2700
ccggtaagcg gcagggtcgg aacaggagag cgcacgaggg agcttccagg gggaaacgcc  2760
tggtatcttt atagtcctgt cgggtttcgc cacctctgac ttgagcgtcg atttttgtga  2820
tgctcgtcag gggggcggag cctatggaaa aacgccagca acgcggcctt tttacggttc  2880
ctggcctttt gctggccttt tgctcacatg ttctttcctg cgttatcccc tgattctgtg  2940
gataaccgta ttaccgcctt tgagtgagct gataccgctc gccgcagccg aacgaccgag  3000
cgcagcgagt cagtgagcga ggaagcggaa gagcgcccaa tacgcaaacc gcctctcccc  3060
```

-continued

gcgcgttggc cgattcatta atgcagctgt ggaatgtgtg tcagttaggg tgtggaaagt 3120
ccccaggctc cccagcaggc agaagtatgc aaagcatgca tctcaattag tcagcaacca 3180
ggtgtggaaa gtccccaggc tccccagcag gcagaagtat gcaaagcatg catctcaatt 3240
agtcagcaac catagtcccg cccctaactc cgcccatccc gcccctaact ccgcccagtt 3300
ccgcccattc tccgccccat ggctgactaa ttttttttat ttatgcagag gccgaggccg 3360
cctcggcctc tgagctattc cagaagtagt gaggaggctt ttttggaggc ctaggctttt 3420
gcaaaaagct tggacacaag acaggcttgc gagatatgtt tgagaatacc actttatccc 3480
gcgtcaggga gaggcagtgc gtaaaaagac gcggactcat gtgaaatact ggtttttagt 3540
gcgccagatc tctataatct cgcgcaacct attttcccct cgaacacttt ttaagccgta 3600
gataaacagg ctgggacact tcacatgagc gaaaaataca tcgtcacctg ggacatgttg 3660
cagatccatg cacgtaaact cgcaagccga ctgatgcctt ctgaacaatg gaaaggcatt 3720
attgccgtaa gccgtggcgg tctgtaccgg gtgcgttact ggcgcgtgaa ctgggtattc 3780
gtcatgtcga taccgtttgt atttccagct acgatcacga caaccagcgc gagcttaaag 3840
tgctgaaacg cgcagaaggc gatggcgaag gcttcatcgt tattgatgac ctggtggata 3900
ccggtggtac tgcggttgcg attcgtgaaa tgtatccaaa agcgcacttt gtcaccatct 3960
tcgcaaaacc ggctggtcgt ccgctggttg atgactatgt tgttgatatc ccgcaagata 4020
cctggattga acagccgtgg gatatgggcg tcgtattcgt cccgccaatc tccggtcgct 4080
aatcttttca acgcctggca ctgccgggcg ttgttctttt taacttcagg cgggttacaa 4140
tagtttccag taagtattct ggaggctgca tccatgacac aggcaaacct gagcgaaacc 4200
ctgttcaaac cccgctttaa acatcctgaa acctcgacgc tagtccgccg ctttaatcac 4260
ggcgcacaac cgcctgtgca gtcggccctt gatggtaaaa ccatccctca ctggtatcgc 4320
atgattaacc gtctgatgtg gatctggcgc ggcattgacc cacgcgaaat cctcgacgtc 4380
caggcacgta ttgtgatgag cgatgccgaa cgtaccgacg atgatttata cgatacggtg 4440
attggctacc gtggcggcaa ctggatttat gagtgggccc cggatctttg tgaaggaacc 4500
ttacttctgt ggtgtgacat aattggacaa actacctaca gagatttaaa gctctaaggt 4560
aaatataaaa tttttaagtg tataatgtgt taaactactg attctaattg tttgtgtatt 4620
ttagattcca acctatggaa ctgatgaatg ggagcagtgg tggaatgcct ttaatgagga 4680
aaacctgttt tgctcagaag aaatgccatc tagtgatgat gaggctactg ctgactctca 4740
acattctact cctccaaaaa agaagagaaa ggtagaagac cccaaggact ttccttcaga 4800
attgctaagt tttttgagtc atgctgtgtt tagtaataga actcttgctt gctttgctat 4860
ttacaccaca aaggaaaaag ctgcactgct atacaagaaa attatggaaa aatattctgt 4920
aacctttata agtaggcata acagttataa tcataacata ctgtttttttc ttactccaca 4980
caggcataga gtgtctgcta ttaataacta tgctcaaaaa ttgtgtacct ttagcttttt 5040
aatttgtaaa ggggttaata aggaatattt gatgtatagt gccttgacta gagatcataa 5100
tcagccatac cacatttgta gaggttttac ttgctttaaa aaacctccca cacctccccc 5160
tgaacctgaa acataaaatg aatgcaattg ttgttgttaa cttgtttatt gcagcttata 5220
atggttacaa ataaagcaat agcatcacaa atttcacaaa taaagcattt ttttcactgc 5280
attctagttg tggtttgtcc aaactcatca atgtatctta tcatgtctgg atcaactgga 5340
taactcaagc taaccaaaat catcccaaac ttcccacccc ataccctatt accactgcca 5400
attacctagt ggtttcattt actctaaacc tgtgattcct ctgaattatt ttcattttaa 5460
agaaattgta tttgttaaat atgtactaca aacttagtag ttggaagggc taattcactc 5520
ccaaagaaga caagatatcc ttgatctgtg gatctaccac acacaaggct acttccctga 5580
ttagcagaac tacacaccag ggccaggggt cagatatcca ctgacctttg gatggtgcta 5640
caagctagta ccagttgagc cagataaggt agaagaggcc aataaaggag agaacaccag 5700
cttgttacac cctgtgagcc tgcatgggat ggatgacccg gagagagaag tgttagagtg 5760
gaggtttgac agccgcctag catttcatca cgtggcccga gagctgcatc cggagtactt 5820
caagaactgc tgatatcgag cttgctacaa gggactttcc gctggggact ttccagggag 5880
gcgtggcctg ggcgggactg gggagtggcg agccctcaga tcctgcatat aagcagctgc 5940
tttttgcctg tactgggtct ctctggttag accagatctg agcctgggag ctctctggct 6000
aactagggaa cccactgctt aagcctcaat aaagcttgcc ttgagtgctt caagtagtgt 6060
gtgcccgtct gttgtgtgac tctggtaact agagatccct cagacccttt tagtcagtgt 6120
ggaaaatctc tagcagtggc gcccgaacag ggacttgaaa gcgaaaggga aaccagagga 6180
gctctctcga cgcaggactc ggcttgctga agcgcgcacg gcaagaggcg aggggcggcg 6240
actggtgagt acgccaaaaa ttttgactag cggaggctag aaggagagag atgggtgcga 6300
gagcgtcagt attaagcggg ggagaattag atcgcgatgg gaaaaaattc ggttaaggcc 6360
aggggaaaag aaaaaatata aattaaaaca tatagtatgg gcaagcaggg agctagaacg 6420
attcgcagtt aatcctggcc tgttagaaac atcagaaggc tgtagacaaa tactgggaca 6480
gctacaacca tcccttcaga caggatcaga agaacttaga tcattatata atacagtagc 6540
aaccctctat tgtgtgcatc aaaggataga gataaaagac accaaggaag ctttagacaa 6600
gatagaggaa gagcaaaaca aaagtaagac caccgcacag caagcggccg gtgatcttca 6660
gacctggacg atatatatga gggacaattg gagaagtgaa ttatataaat ataaagtagt 6720
aaaaattgaa ccattaggag tagcacccac caaggcaaag agaagagtgg tgcagagaga 6780
aaaaagagca gtgggaatag gagctttgtt ccttgggttc ttgggagcag caggaagcac 6840
tatgggcgca gcgtcaatga cgctgacggt acaggccaga caattattgt ctggtatagt 6900
gcagcagcag aacaatttgc tgagggctat tgaggcgcaa cagcatctgt tgcaactcac 6960
agtctggggc atcaagcagc tccaggcaag aatcctggct gtggaaagat acctaaagga 7020
tcaacagctc ctggggattt ggggttgctc tggaaaactc atttgcacca ctgctgtgcc 7080
ttggaatgct agttggagta ataaatctct ggaacagatt tggaatcaca cgacctggat 7140
ggagtgggac agagaaatta acaattacac aagcttaata cactccttaa ttgaagaatc 7200
gcaaaaccag caagaaaaga atgaacaaga attattggaa ttagataaat gggcaagttt 7260
gtggaattgg tttaacataa caaattggct gtggtatata aaattattca taatgatagt 7320
aggaggcttg gtaggtttaa gaatagtttt tgctgtactt tctatagtga atagagttag 7380
gcagggatat tcaccattat cgtttcagac ccacctccca accccgaggg gacccgacag 7440
gcccgaagga atagaagaag aaggtggaga gagagacaga gacagatcca ttcgattagt 7500
gaacggatct cgacggtcgc caaatggcag tattcatcca caattttaaa agaaaagggg 7560
ggattggggg gtacagtgca ggggaaagaa tagtagacat aatagcaaca gacatacaaa 7620

-continued

```
ctaaagaatt acaaaaacaa attacaaaaa ttcaaaattt tcgggtttat tacagggaca  7680
gcagagatcc agtttggatc gataagcttg atatcgaatt gggtagggga ggcgcttttc  7740
ccaaggcagt ctggagcatg cgctttagca gccccgctgg gcacttggcg ctacacaagt  7800
ggcctctggc ctcgcacaca ttccacatcc accggtaggc gccaaccggc tccgttcttt  7860
ggtggcccct tcgcgccacc ttctactcct cccctagtca ggaagttccc ccccgccccg  7920
cagctcgcgt cgtgcaggac gtgacaaatg gaagtagcac gtctcactag tctcgtgcag  7980
atggacagca ccgctgagca atggaagcgg gtaggccttt ggggcagcgg ccaatagcag  8040
ctttgctcct tcgctttctg ggctcagagg ctgggaaggg gtgggtccgg gggcgggctc  8100
aggggcgggc tcaggggcgg ggcgggcgcc cgaaggtcct ccggaggccc ggcattctgc  8160
acgcttcaaa agcgcacgtc tgccgcgctg ttctcctctt cctcatctcc gggcctttcg  8220
atggctcttc cagtgactgc tcttctcctc ccgcttgcgc tgctgttgca tgcggctcgg  8280
ccggagcaaa agctgatttc agaagaggac ttggatatcc agatgacaca gaccacttca  8340
tctctttctg ctagcctggg ggatcgggtc acaataagct gtcgcgcatc ccaagacata  8400
agcaaatatc tgaattggta tcaacagaaa cccgatggaa ctgtgaaact tctcatctac  8460
catacgagca gactgcattc tggggttcct agccgctttt cagggtctgg atctggaacg  8520
gactattcac ttaccatatc taatttggaa caagaagaca tcgcgaccta tttctgtcag  8580
caaggcaata cgctccctta tacttttgga gggggaacga agttggagat cacaggtgga  8640
ggcggcagtg gcgggggagg atctggtgga ggtggttctg aggtcaagct gcaggagagt  8700
ggtcccgggc tggtagcccc gagccagagt ctgtctgtta cttgcactgt gtcaggcgtg  8760
agtctcccag actatggtgt atcatggatt cgacagccgc cccggaaagg acttgagtgg  8820
ctcggagtga tctggggatc cgaaacgacg tactacaata gcgcgctcaa aagccggctg  8880
accatcatta aggataactc taaaagccag gtgttcttga aaatgaattc cttgcagaca  8940
gatgatacgg cgatctatta ctgtgccaag cactactact atggaggcag ctatgccatg  9000
gattattggg gtcaaggcac ttctgtgaca gtgagcagtg ccgcagctat tgaagtgatg  9060
tacccgcctc cgtatcttga taatgagaaa tctaacggaa ccataataca cgtgaaaggc  9120
aaacatttgt gtccgtctcc tctgttcccc gggcctagta aaccgttttg ggtactggtg  9180
gtggtaggcg gagtacttgc atgttactca ctcctcgtta ccgtcgcatt cattatcttc  9240
tgggtacgga gcaagagatc tcggctgctt catagcgatt atatgaatat gacacccaga  9300
cgcccaggtc ctactaggaa acattaccag ccgtatgcgc cgccgaggga cttcgccgca  9360
tacagatctc gcgtgaagtt ctcaagatct gccgatgcac cggcttatca gcagggacaa  9420
aaccaactgt ataatgagct gaacctgggg cggagggagg agtatgatgt cctggacaag  9480
cgaagagggc gagaccccga aatgggaggc aagcctcaac ggcggaagaa cccacaagag  9540
ggcctgtata acgagcttca aaaggacaaa atggcggaag cgtatagcga gatcggaatg  9600
aagggcgaac gaaggagagg gaaaggtcac gatgggctct accaggggct cagcacggca  9660
acaaaagaca catatgacgc attgcatatg caggcgctgc caccgagatg actcacgcgt  9720
caagtggagc aaggcaggtg gacagtggat ccttgacttg cggccgcaac tcccacctgc  9780
aacatgcgtg actgactgag gccgcgactc tagagtcgac ctgcaggcat gcaagcttga  9840
tatcaagctt atcgataatc aacctctgga ttacaaaatt tgtgaaagat tgactggtat  9900
tcttaactat gttgctcctt ttacgctatg tggatacgct gctttaatgc ctttgtatca  9960
tgctattgct tcccgtatgg ctttcatttt ctcctccttg tataaatcct ggttgctgtc  10020
tctttatgag gagttgtggc ccgttgtcag gcaacgtggc gtggtgtgca ctgtgtttgc  10080
tgacgcaacc cccactggtt ggggcattgc caccacctgt cagctccttt ccgggacttt  10140
cgctttcccc ctccctattg ccacggcgga actcatcgcc gcctgccttg cccgctgctg  10200
gacaggggct cggctgttgg gcactgacaa ttccgtggtg ttgtcgggga aatcatcgtc  10260
ctttccttgg ctgctcgcct gtgttgccac ctggattctg cgcgggacgt ccttctgcta  10320
cgtcccttcg gccctcaatc cagcggacct tccttcccgc ggcctgctgc cggctctgcg  10380
gcctcttccg cgtcttcgcc ttcgccctca gacgagtcgg atctcccttt gggccgcctc  10440
cccgcat                                                           10447
```

```
SEQ ID NO: 10          moltype = DNA  length = 12536
FEATURE                Location/Qualifiers
misc_feature           1..12536
                       note = A synthetic pWPI pmei mAnti cd19 1D3 myc hinge cd28
                        cd3zeta
source                 1..12536
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 10
ttggaagggc taattcactc ccaaagaaga caagatatcc ttgatctgtg gatctaccac  60
acacaaggct acttccctga ttagcagaac tacacaccag ggccaggggt cagatatcca  120
ctgacctttg gatggtgcta caagctagta ccagttgagc cagataaggt agaagaggcc  180
aataaaggag agaacaccag cttgttacac cctgtgagcc tgcatgggat ggatgacccg  240
gagagagaag tgttagagtg gaggtttgac agccgcctag catttcatca cgtggcccga  300
gagctgcatc cggagtactt caagaactgc tgatatcgag cttgctacaa gggactttcc  360
gctggggact ttccagggag gcgtggcctg ggcgggactg gggagtggcg agccctcaga  420
tcctgcatat aagcagctgc tttttgcctg tactgggtct ctctggttag accagatctg  480
agcctgggag ctctctggct aactagggaa cccactgctt aagcctcaat aaagcttgcc  540
ttgagtgctt caagtagtgt gtgcccgtct gttgtgtgac tctggtaact agagatccct  600
cagacccttt tagtcagtgt ggaaaatctc tagcagtggc gcccgaacag ggacttgaaa  660
gcgaaaggga aaccagagga gctctctcga cgcaggactc ggcttgctga agcgcgcacg  720
gcaagaggcg aggggcggcg actggtgagt acgccaaaaa ttttgactag cggaggctag  780
aaggagagag atgggtgcga gagcgtcagt attaagcggg ggagaattag atcgcgatgg  840
gaaaaaattc ggttaaggcc aggggggaaag aaaaaatata aattaaaaca tatagtatgg  900
gcaagcaggg agctagaacg attcgcagtt aatcctggcc tgttagaaac atcagaaggc  960
tgtagacaaa tactgggaca gctacaacca tcccttcaga caggatcaga agaacttaga  1020
tcattatata atacagtagc aaccctctat tgtgtgcatc aaaggataga gataaaagac  1080
```

-continued

```
accaaggaag ctttagacaa gatagaggaa gagcaaaaca aaagtaagac caccgcacag  1140
caagcggccg ctgatcttca gacctggagg aggagatatg agggacaatt ggagaagtga  1200
attatataaa tataaagtag taaaaattga accattagga gtagcaccca ccaaggcaaa  1260
gagaagagtg gtgcagagag aaaaaagagc agtgggaata ggagctttgt tccttgggtt  1320
cttgggagca gcaggaagca ctatgggcgc agcgtcaatg acgctgacgg tacaggccag  1380
acaattattg tctggtatag tgcagcagca gaacaatttg ctgagggcta ttgaggcgca  1440
acagcatctg ttgcaactca cagtctgggg catcaagcag ctccaggcaa gaatcctggc  1500
tgtggaaaga tacctaaagg atcaacagct cctggggatt tggggttgct ctggaaaact  1560
catttgcacc actgctgtgc cttggaatgc tagttggagt aataaatctc tggaacagat  1620
ttggaatcac acgacctgga tggagtggga cagagaaatt aacaattaca caagcttaat  1680
acactcctta attgaagaat cgcaaaacca gcaagaaaag aatgaacaag aattattgga  1740
attagataaa tgggcaagtt tgtggaattg gtttaacata acaaattggc tgtggtatat  1800
aaaattattc ataatgatag taggaggctt ggtaggttta agaatagttt ttgctgtact  1860
ttctatagtg aatagagtta ggcagggata ttcaccatta tcgtttcaga cccacctccc  1920
aaccccgagg ggacccgaca ggcccgaagg aatagaagaa gaaggtggag agagagacag  1980
agacagatcc attcgattag tgaacggatc tcgacggtat cgatgtcgac gataagcttt  2040
gcaaagatgg ataaagtttt aaacagagag gaatctttgc agctaatgga ccttctaggt  2100
cttgaaagga gtgggaattg gctccggtgc ccgtcagtgg gcagagcgca catcgcccac  2160
agtccccgag aagttggggg gaggggtcgg caattgaacc ggtgcctaga gaaggtggcg  2220
cggggtaaac tgggaaagtg atgtcgtgta ctggctccgc ctttttcccg agggtggggg  2280
agaaccgtat ataagtgcag tagtcgccgt gaacgttctt tttcgcaacg ggtttgccgc  2340
cagaacacag gtaagtgccg tgtgtggttc ccgcgggcct ggcctcttta cgggttatgg  2400
cccttgcgtg ccttgaatta cttccactgg ctgcagtacg tgattcttga tcccgagctt  2460
cgggttggaa gtgggtggga gagttcgagg ccttgcgctt aaggagcccc ttcgcctcgt  2520
gcttgagttg aggcctggcc tgggcgctgg ggccgccgcg tgcgaatctg gtggcacctt  2580
cgcgcctgtc tcgctgcttt cgataagtct ctagccattt aaaatttttg atgacctgct  2640
gcgacgcttt ttttctggca agatagtctt gtaaatgcgg gccaagatct gcacactggt  2700
atttcggttt ttggggccgc gggcggcgac ggggcccgtg cgtcccagcg cacatgttcg  2760
gcgaggcggg gcctgcgagc gcggccaccg agaatcggac gggggtagtc tcaagctggc  2820
cggcctgctc tggtgcctgg cctcgcgccg ccgtgtatcg ccccgccctg ggcggcaagg  2880
ctggcccggt cggcaccagt tgcgtgagcg gaaagatggc cgcttcccgg ccctgctgca  2940
gggagctcaa aatggaggac gcggcgctcg ggagagcggg cgggtgagtc acccacacaa  3000
aggaaaaggg cctttccgtc ctcagccgtc gcttcatgtg actccacgga gtaccgggcg  3060
ccgtccaggc acctcgatta gttctcgagc ttttggagta cgtcgtcttt aggttggggg  3120
gaggggtttt atgcgatgga gtttccccac actgagtggg tggagactga agttaggcca  3180
gcttggcact tgatgtaatt ctccttggaa tttgcccttt ttgagtttgg atcttggttc  3240
attctcaagc ctcagacagt ggttcaaagt ttttttcttc catttcaggt gtcgtgagga  3300
atttcgacat ttaaatttaa ttaatctcga cggtatcggt taacttttaa aagaaaaggg  3360
gggattgggg ggtacagtgc aggggaaaga atagtagaca taatagcaac agacatacaa  3420
actaaagaat tacaaaaaca aattacaaaa attcaaaatt ttatcgatca cgagactagc  3480
ctcgaggttt atgggggttc ccacccagct gttggggttg ctgctcctct ggatcactga  3540
tgccatctgc gaacagaagc tcatttctga agaagatctg gacatacaga tgacccagag  3600
tcctgccagc ttgtccacat ccctcggaga gaccgtgaca attcaatgcc aggcctctga  3660
ggacatatat tctggattgg cttggtacca gcaaaagcca ggtaaaagtc ctcagttgtt  3720
gatatacggc gcttctgatt tgcaagacgg ggtgccctca cgatttagcg ggtctggaag  3780
tggcactcag tacagtctga agattacttc aatgcaaaca gaagacgagg gtgtgtattt  3840
ttgccaacag gggctgacct acccaaggac attcgggggc ggtacaaagc ttgaacttaa  3900
gggcggcggt gggtctggag gtggtggatc tggcggaggg ggaagtgagg tacagctgca  3960
acagtccggc gccgaactcg ttcgccctgg aacctcagtc aaattgtcat gcaaggtgag  4020
tggcgacaca ataacctttt actatatgca cttcgttaaa cagaggccag gtcaaggtct  4080
ggaatggata ggcagaattg atccagaaga tgagtccacc aaatactcag aaaagttcaa  4140
aaacaaagcc actcttactg ccgacacctc aagcaacaca gcatatctta agctcagttc  4200
acttaccagc gaagacaccg ccacctattt ttgtatttat ggtggctatt actttgacta  4260
ctgggggcaa ggtgttatgg taacagtttc ttccattgaa ttcatgtatc caccccccta  4320
tttggataat gaacgatcta atgggactat aatacatatc aaggaaaagc atctgtgtca  4380
tacccaaagt tcccctaagc ttttctgggc cctcgtcgtt gtggcaggag tgctttttg  4440
ctatggattg ttggttactg tggctctctg cgtcatttgg acaaatagta ggaggaatcg  4500
gggggacaa tctgattaca tgaacatgac accacggagg ccaggcctta ccagaaagcc  4560
ctaccaacct tatgcaccag cacgagactt cgccgcatac aggccaaggg ctaagttttc  4620
ccgcagcgcc gaaaccgcag ccaacctcca agatcctaat cagctctata acgaattgaa  4680
tcttggccgc agagaggagt acgacgtact tgagaaaaag agagctaggg accctgaaat  4740
gggtgggaag caacagcgaa gaaggaaccc acaggaaggg gtgtataatg cccttcaaaa  4800
ggataaaatg gcagaggcat acagtgaaat cggaaccaag ggggagagac gcagagggaa  4860
aggccatgac ggcctttatc agggtttgtc aactgctact aaagacactt atgatgcctt  4920
gcatatgcaa actctcgcac ccagatgaaa actacgggct gcaggaattc cgccccccc  4980
cccctaacgt tactggccga agccgcttgg aataaggccg gtgtgcgttt gtctatatgt  5040
tattttccac catattgccg tcttttggca atgtgagggc ccggaaacct ggccctgtct  5100
tcttgacgag cattcctagg ggtctttccc ctctcgccaa aggaatgcaa ggtctgttga  5160
atgtcgtgaa ggaagcagtt cctctggaag cttcttgaag acaaacaacg tctgtagcga  5220
ccctttgcag gcagcggaac ccccacctg gcgacaggtg cctctgcggc caaaagccac  5280
gtgtataaga tacacctgca aaggcggcac aaccccagtg ccacgttgtg agttggatag  5340
ttgtggaaag agtcaaatgg ctctcctcaa gcgtattcaa caaggggctg aaggatgccc  5400
agaaggtacc ccattgtatg ggatctgatc tggggcctcg gtgcacatgc tttacatgtg  5460
tttagtcgag gttaaaaaac gtctaggccc cccgaaccac ggggacgtgg ttttcctttg  5520
aaaaacacga tgataatacc atggtgagca agggcgagga gctgttcacc ggggtggtgc  5580
ccatcctggt cgagctggac ggcgacgtaa acggccacaa gttcagcgtg tccggcgagg  5640
```

-continued

```
gcgagggcga tgccacctac ggcaagctga ccctgaagtt catctgcacc accggcaagc  5700
tgcccgtgcc ctggcccacc ctcgtgacca ccctgaccta cggcgtgcag tgcttcagcc  5760
gctaccccga ccacatgaag cagcacgact tcttcaagtc cgccatgccc gaaggctacg  5820
tccaggagcg caccatcttc ttcaaggacg acggcaacta caagacccgc gccgaggtga  5880
agttcgaggg cgacaccctg gtgaaccgca tcgagctgaa gggcatcgac ttcaaggagg  5940
acggcaacat cctggggcac aagctggagt acaactacaa cagccacaac gtctatatca  6000
tggccgacaa gcagaagaac ggcatcaagg tgaacttcaa gatccgccac aacatcgagg  6060
acggcagcgt gcagctcgcc gaccactacc agcagaacac ccccatcggc gacggccccg  6120
tgctgctgcc cgacaaccac tacctgagca cccagtccgc cctgagcaaa gaccccaacg  6180
agaagcgcga tcacatggtc ctgctggagt tcgtgaccgc cgccgggatc actctcggca  6240
tggacgagct gtacaagtcc ggactcagat ctcgactagc tagtagctag ctagctagtc  6300
gagctcaagc ttcgaattcg atatcaagct tatcgcgata ccgtcgacct cgagggaatt  6360
ccgataatca acctctggat tacaaaattt gtgaaagatt gactggtatt cttaactatg  6420
ttgctccttt tacgctatgt ggatacgctg ctttaatgcc tttgtatcat gctattgctt  6480
cccgtatggc tttcattttc tcctccttgt ataaatcctg gttgctgtct ctttatgagg  6540
agttgtggcc cgttgtcagg caacgtggcg tggtgtgcac tgtgtttgct gacgcaaccc  6600
ccactggttg gggcattgcc accacctgtc agctcctttc cgggactttc gctttccccc  6660
tccctattgc cacggcggaa ctcatcgccg cctgccttgc ccgctgctgg acaggggctc  6720
ggctgttggg cactgacaat tccgtggtgt tgtcggggaa gctgacgtcc tttccatggc  6780
tgctcgcctg tgttgccacc tggattctgc gcgggacgtc cttctgctac gtcccttcgg  6840
ccctcaatcc agcggacctt ccttcccgcg gcctgctgcc ggctctgcgg cctcttccgc  6900
gtcttcgcct tcgccctcag acgagtcgga tctcccttttg ggccgcctcc ccgcatcggg  6960
aattcgagct cggtaccttt aagaccaatg acttacaagg cagctgtaga tcttagccac  7020
tttttaaaag aaaaggggggg actggaaggg ctaattcact cccaacgaag acaagatggg  7080
atcaattcac catgggaata acttcgtata gcatacatta tacgaagtta tgctgctttt  7140
tgcttgtact gggtctctct ggttagacca gatctgagcc tgggagctct ctggctaact  7200
agggaaccca ctgcttaagc ctcaataaag cttgccttga gtgcttcaag tagtgtgtgc  7260
ccgtctgttg tgtgactctg gtaactagag atccctcaga cccttttagt cagtgtggaa  7320
aatctctagc agcatctaga attaattccg tgtattctat agtgtcacct aaatcgtatg  7380
tgtatgatac ataaggttat gtattaattg tagccgcgtt ctaacgacaa tatgtacaag  7440
cctaattgtg tagcatctgg cttactgaag cagaccctat catctctctc gtaaactgcc  7500
gtcagagtcg gtttggttgg acgaaccttc tgagtttctg gtaacgccgt cccgcacccg  7560
gaaatggtca gcgaaccaat cagcagggtc atcgctagcc agatcctcta cgccggacgc  7620
atcgtggccg gcatcaccgg cgccacaggt gcggttgctg gcgcctatat cgccgacatc  7680
accgatgggg aagatcgggc tcgccacttc gggctcatga gcgcttgttt cggcgtgggt  7740
atggtggcag gccccgtggc cgggggactg ttgggcgcca tctccttgca tgcaccattc  7800
cttgcggcgg cggtgctcaa cggcctcaac ctactactgg gctgcttcct aatgcaggag  7860
tcgcataagg gagagcgtcg aatggtgcac tctcagtaca atctgctctg atgccgcata  7920
gttaagccag ccccgacacc cgccaacacc cgctgacgcg ccctgacggg cttgtctgct  7980
cccggcatcc gcttacagac aagctgtgac cgtctccggg agctgcatgt gtcagaggtt  8040
ttcaccgtca tcaccgaaac gcgcgagacg aaagggcctc gtgatacgcc tatttttata  8100
ggttaatgtc atgataataa tggtttctta gacgtcaggt ggcacttttc ggggaaatgt  8160
gcgcggaacc cctatttgtt tatttttcta aatacattca aatatgtatc cgctcatgag  8220
acaataaccc tgataaatgc ttcaataata ttgaaaaagg aagagtatga gtattcaaca  8280
tttccgtgtc gcccttattc ccttttttgc ggcattttgc cttcctgttt ttgctcaccc  8340
agaaacgctg gtgaaagtaa aagatgctga agatcagttg ggtgcacgag tgggttacat  8400
cgaactggat ctcaacagcg gtaagatcct tgagagtttt cgccccgaag aacgttttcc  8460
aatgatgagc acttttaaag ttctgctatg tggcgcggta ttatcccgta ttgacgccgg  8520
gcaagagcaa ctcggtcgcc gcatacacta ttctcagaat gacttggttg agtactcacc  8580
agtcacagaa aagcatctta cggatggcat gacagtaaga gaattatgca gtgctgccat  8640
aaccatgagt gataacactg cggccaactt acttctgaca acgatcggag gaccgaagga  8700
gctaaccgct tttttgcaca acatgggggа tcatgtaact cgccttgatc gttgggaacc  8760
ggagctgaat gaagccatac caaacgacga gcgtgacacc acgatgcctg tagcaatggc  8820
aacaacgttg cgcaaactat taactggcga actacttact ctagcttccc ggcaacaatt  8880
aatagactgg atggaggcgg ataaagttgc aggaccactt ctgcgctcgg cccttccggc  8940
tggctggttt attgctgata aatctggagc cggtgagcgt gggtctcgcg gtatcattgc  9000
agcactgggg ccagatggta agccctcccg tatcgtagtt atctacacga cggggagtca  9060
ggcaactatg gatgaacgaa atagacagat cgctgagata ggtgcctcac tgattaagca  9120
ttggtaactg tcagaccaag tttactcata tatactttag attgatttaa aacttcattt  9180
ttaatttaaa aggatctagg tgaagatcct ttttgataat ctcatgacca aaatccctta  9240
acgtgagttt tcgttccact gagcgtcaga ccccgtagaa agatcaaag gatcttcttg  9300
agatcctttt tttctgcgcg taatctgctg cttgcaaaca aaaaaaccac cgctaccagc  9360
ggtggtttgt ttgccggatc aagagctacc aactcttttt ccgaaggtaa ctggcttcag  9420
cagagcgcag ataccaaata ctgtccttct agtgtagccg tagttaggcc accacttcaa  9480
gaactctgta gcaccgccta catacctcgc tctgctaatc ctgttaccag tggctgctgc  9540
cagtggcgat aagtcgtgtc ttaccgggtt ggactcaaga cgatagttac cggataaggc  9600
gcagcggtcg ggctgaacgg ggggttcgtg cacacagccc agcttggagc gaacgaccta  9660
caccgaactg agatacctac agcgtgagca ttgagaaagc gccacgcttc ccgaagggag  9720
aaaggcggac aggtatccgg taagcggcag ggtcggaaca ggagagcgca cgagggagct  9780
tccagggggа aacgcctggt atctttatag tcctgtcggg tttcgccacc tctgacttga  9840
gcgtcgattt ttgtgatgct cgtcaggggg gcggagccta tggaaaaacg ccagcaacgc  9900
ggcctttttа cggttcctgg ccttttgctg gccttttgct cacatgttct ttcctgcgtt  9960
atcccctgat tctgtggata accgtattac cgcctttgag tgagctgata ccgctcgccg  10020
cagccgaacg accgagcgca gcgagtcagt gagcgaggaa gcggaagagc gcccaatacg  10080
caaaccgcct ctccccgcgc gttggccgat tcattaatgc agctgtggaa tgtgtgtcag  10140
ttagggtgtg gaaagtcccc aggctcccca gcaggcagaa gtatgcaaag catgcatctc  10200
```

-continued

```
aattagtcag caaccaggtg tggaaagtcc ccaggctccc cagcaggcag aagtatgcaa  10260
agcatgcatc tcaattagtc agcaaccata gtcccgcccc taactccgcc catcccgccc  10320
ctaactccgc ccagttccgc ccattctccg ccccatggct gactaatttt ttttatttat  10380
gcagaggccg aggccgcctc ggcctctgag ctattccaga agtagtgagg aggcttttt  10440
ggaggcctag gcttttgcaa aaagcttgga cacaagacag gcttgcgaga tatgtttgag  10500
aataccactt tatcccgcgt cagggagagg cagtgcgtaa aaagacgcgg actcatgtga  10560
aatactggtt tttagtgcgc cagatctcta taatctcgcg caacctattt tcccctcgaa  10620
cacttttaa gccgtagata aacaggctgg gacacttcac atgagcgaaa aatacatcgt  10680
cacctgggac atgttgcaga tccatgcacg taaactcgca agccgactga tgccttctga  10740
acaatggaaa ggcattattg ccgtaagccg tggcggtctg taccgggtgc gttactggcg  10800
cgtgaactgg gtattcgtca tgtcgatacc gtttgtattt ccagctacga tcacgacaac  10860
cagcgcgagc ttaaagtgct gaaacgcgca gaaggcgatg gcgaaggctt catcgttatt  10920
gatgacctgg tggataccgg tggtactgcg gttgcgattc gtgaaatgta tccaaaagcg  10980
cactttgtca ccatcttcgc aaaaccggct ggtcgtccgc tggttgatga ctatgttgtt  11040
gatatcccgc aagatacctg gattgaacag ccgtgggata tgggcgtcgt attcgtcccg  11100
ccaatctccg gtcgctaatc ttttcaacgc ctggcactgc cgggcgttgt tcttttaac  11160
ttcaggcggg ttacaatagt ttccagtaag tattctggag gctgcatcca tgacacaggc  11220
aaacctgagc gaaaccctgt tcaaaccccg ctttaaacat cctgaaacct cgacgctagt  11280
ccgccgcttt aatcacggcg cacaaccgcc tgtgcagtcg gcccttgatg gtaaaaccat  11340
ccctcactgg tatcgcatga ttaaccgtct gatgtggatc tggcgcggca ttgacccacg  11400
cgaaatcctc gacgtccagg cacgtattgt gatgagcgat gccgaacgta ccgacgatga  11460
tttatacgat acggtgattg gctaccgtgg cggcaactgg atttatgagt gggccccgga  11520
tctttgtgaa ggaaccttac ttctgtggtg tgacataatt ggacaaacta cctacagaga  11580
tttaaagctc taaggtaaat ataaaatttt taagtgtata atgtgttaaa ctactgattc  11640
taattgtttg tgtattttag attccaacct atggaactga tgaatgggag cagtggtgga  11700
atgcctttaa tgaggaaaac ctgttttgct cagaagaaat gccatctagt gatgatgagg  11760
ctactgctga ctctcaacat tctactcctc caaaaaagaa gagaaaggta gaagacccca  11820
aggactttcc ttcagaattg ctaagttttt tgagtcatgc tgtgtttagt aatagaactc  11880
ttgcttgctt tgctatttac accacaaagg aaaaagctgc actgctatac aagaaaatta  11940
tggaaaaata ttctgtaacc tttataagta ggcataacag ttataatcat aacatactgt  12000
tttttcttac tccacacagg catagagtgt ctgctattaa taactatgct caaaaattgt  12060
gtacctttag ctttttaatt tgtaaagggg ttaataagga atatttgatg tatagtgcct  12120
tgactagaga tcataatcag ccataccaca tttgtagagg ttttacttgc tttaaaaaac  12180
ctcccacacc tccccctgaa cctgaaacat aaaatgaatg caattgttgt tgttaacttg  12240
tttattgcag cttataatgg ttacaaataa agcaatagca tcacaaattt cacaaataaa  12300
gcatttttttt cactgcattc tagttgtggt ttgtccaaac tcatcaatgt atcttatcat  12360
gtctggatca actggataac tcaagctaac caaaatcatc ccaaacttcc caccccatac  12420
cctattacca ctgccaatta cctagtggtt tcatttactc taaacctgtg attcctctga  12480
attattttca ttttaaagaa attgtatttg ttaaatatgt actacaaact tagtag      12536

SEQ ID NO: 11          moltype = AA  length = 365
FEATURE                Location/Qualifiers
REGION                 1..365
                       note = A synthetic FKBP-1SG-FR with GPI anchor amino acid
                        sequence
source                 1..365
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 11
MTTQLLLLLV WVAVVGEAQT GVQVETISPG DGRTFPKRGQ TCVVHYTGML EDGKKFDSSR  60
DRNKPFKFML GKQEVIRGWE EGVAQMSVGQ RAKLTISPDY AYGATGHPGI IPPHATLVFD  120
VELLKLESGG GSRIAWARTE LLNVCMNAKH HKEKPGPEDK LHEQCRPWRK NACCSTNTSQ  180
EAHKDVSYLY RFNWNHCGEM APACKRHFIQ DTCLYECSPN LGPWIQQVDQ SWRKERVLNV  240
PLCKEDCEQW WEDCRTSYTC KSNWHKGWNW TSGFNKCAVG AACQPFHFYF PTPTVLCNEI  300
WTHSYKVSNY SRGSGRCIQM WFDPAQGNPN EEVARFYAAA MSGAGPWAAW PFLLSLALML  360
LWLLS                                                              365

SEQ ID NO: 12          moltype = AA  length = 375
FEATURE                Location/Qualifiers
REGION                 1..375
                       note = A synthetic FKBP-3SG-FR with GPI anchor amino acid
                        sequence
source                 1..375
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 12
MTTQLLLLLV WVAVVGEAQT GVQVETISPG DGRTFPKRGQ TCVVHYTGML EDGKKFDSSR  60
DRNKPFKFML GKQEVIRGWE EGVAQMSVGQ RAKLTISPDY AYGATGHPGI IPPHATLVFD  120
VELLKLEGGG GSGGGGSGGG GSRIAWARTE LLNVCMNAKH HKEKPGPEDK LHEQCRPWRK  180
NACCSTNTSQ EAHKDVSYLY RFNWNHCGEM APACKRHFIQ DTCLYECSPN LGPWIQQVDQ  240
SWRKERVLNV PLCKEDCEQW WEDCRTSYTC KSNWHKGWNW TSGFNKCAVG AACQPFHFYF  300
PTPTVLCNEI WTHSYKVSNY SRGSGRCIQM WFDPAQGNPN EEVARFYAAA MSGAGPWAAW  360
PFLLSLALML LWLLS                                                   375

SEQ ID NO: 13          moltype = AA  length = 513
FEATURE                Location/Qualifiers
```

-continued

```
REGION                  1..513
                        note = A synthetic 4M5.3-FR with GPI anchor amino acid
                         sequence
source                  1..513
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 13
MALPVTALLL PLALLLHAAR PDVVMTQTPL SLPVSLGDQA SISCRSSQSL VHSNGNTYLR  60
WYLQKPGQSP KVLIYKVSNR VSGVPDRFSG SGSGTDFTLK INRVEAEDLG VYFCSQSTHV  120
PWTFGGGTKL EIKSSADDAK KDAAKKDDAK KDDAKKDGGV KLDETGGGLV QPGGAMKLSC  180
VTSGFTFGHY WMNWVRQSPE KGLEWVAQFR NKPYNYETYY SDSVKGRFTI SRDDSKSSVY  240
LQMNNLRVED TGIYYCTGAS YGMEYLGQGT SVTVSSGGGS RIAWARTELL NVCMNAKHHK  300
EKPGPEDKLH EQCRPWRKNA CCSTNTSQEA HKDVSYLYRF NWNHCGEMAP ACKRHFIQDT  360
CLYECSPNLG PWIQQVDQSW RKERVLNVPL CKEDCEQWWE DCRTSYTCKS NWHKGWNWTS  420
GFNKCAVGAA CQPFHFYFPT PTVLCNEIWT HSYKVSNYSR GSGRCIQMWF DPAQGNPNEE  480
VARFYAAAMS GAGPWAAWPF LLSLALMLLW LLS                               513

SEQ ID NO: 14           moltype = AA  length = 895
FEATURE                 Location/Qualifiers
REGION                  1..895
                        note = A synthetic FMC63-T2A-FKBP3SGFR
source                  1..895
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 14
MALPVTALLL PLALLLHAAR PEQKLISEED LDIQMTQTTS SLSASLGDRV TISCRASQDI  60
SKYLNWYQQK PDGTVKLLIY HTSRLHSGVP SRFSGSGSGT DYSLTISNLE QEDIATYFCQ  120
QGNTLPYTFG GGTKLEITGG GGSGGGGSGG GGSEVKLQES GPGLVAPSQS LSVTCTVSGV  180
SLPDYGVSWI RQPPRKGLEW LGVIWGSETT YYNSALKSRL TIIKDNSKSQ VFLKMNSLQT  240
DDTAIYYCAK HYYYGGSYAM DYWGQGTSVT VSSAAAIEVM YPPPYLDNEK SNGTIIHVKG  300
KHLCPSPLFP GPSKPFWVLV VVGGVLACYS LLVTVAFIIF WVRSKRSRLL HSDYMNMTPR  360
RPGPTRKHYQ PYAPPRDFAA YRSRVKFSRS ADAPAYQQGQ NQLYNELNLG RREEYDVLDK  420
RRGRDPEMGG KPRRKNPQEG LYNELQKDKM AEAYSEIGMK GERRRGKGHD GLYQGLSTAT  480
KDTYDALHMQ ALPPRGSGEG RGSLLTCGDV EENPGPMAQR MTTQLLLLLV WVAVVGEAQT  540
GVQVETISPG DGRTFPKRGQ TCVVHYTGML EDGKKFDSSR DRNKPFKFML GKQEVIRGWE  600
EGVAQMSVGQ RAKLTISPDY AYGATGHPGI IPPHATLVFD VELLKLEGGG GSGGGGSGGG  660
GSRIAWARTE LLNVCMNAKH HKEKPGPEDK LHEQCRPWRK NACCSTNTSQ EAHKDVSYLY  720
RFNWNHCGEM APACKRHFIQ DTCLYECSPN LGPWIQQVDQ SWRKERVLNV PLCKEDCEQW  780
WEDCRTSYTC KSNWHKGWNW TSGFNKCAVG AACQPFHFYF PTPTVLCNEI WTHSYKVSNY  840
SRGSGRCIQM WFDPAQGNPN EEVARFYAAA MSGAGPWAAW PFLLSLALML LWLLS       895

SEQ ID NO: 15           moltype = AA  length = 1029
FEATURE                 Location/Qualifiers
REGION                  1..1029
                        note = A synthetic FMC63-T2A-4M5.3SGFR
source                  1..1029
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 15
MALPVTALLL PLALLLHAAR PEQKLISEED LDIQMTQTTS SLSASLGDRV TISCRASQDI  60
SKYLNWYQQK PDGTVKLLIY HTSRLHSGVP SRFSGSGSGT DYSLTISNLE QEDIATYFCQ  120
QGNTLPYTFG GGTKLEITGG GGSGGGGSGG GGSEVKLQES GPGLVAPSQS LSVTCTVSGV  180
SLPDYGVSWI RQPPRKGLEW LGVIWGSETT YYNSALKSRL TIIKDNSKSQ VFLKMNSLQT  240
DDTAIYYCAK HYYYGGSYAM DYWGQGTSVT VSSAAAIEVM YPPPYLDNEK SNGTIIHVKG  300
KHLCPSPLFP GPSKPFWVLV VVGGVLACYS LLVTVAFIIF WVRSKRSRLL HSDYMNMTPR  360
RPGPTRKHYQ PYAPPRDFAA YRSRVKFSRS ADAPAYQQGQ NQLYNELNLG RREEYDVLDK  420
RRGRDPEMGG KPRRKNPQEG LYNELQKDKM AEAYSEIGMK GERRRGKGHD GLYQGLSTAT  480
KDTYDALHMQ ALPPRGSGEG RGSLLTCGDV EENPGPMALP VTALLLPLAL LLHAARPDVV  540
MTQTPLSLPV SLGDQASISC RSSQSLVHSN GNTYLRWYLQ KPGQSPKVLI YKVSNRVSGV  600
PDRFSGSGSG TDFTLKINRV EAEDLGVYFC SQSTHVPWTF GGGTKLEIKS SADDAKKDAA  660
KKDDAKKDDA KKDGGVKLDE TGGGLVQPGG AMKLSCVTSG FTFGHYWMNW VRQSPEKGLE  720
WVAQFRNKPY NYETYYSDSV KGRFTISRDD SKSSVYLQMN NLRVEDTGIY YCTGASYGME  780
YLGQGTSVTV SSGGGSRIAW ARTELLNVCM NAKHHKEKPG PEDKLHEQCR PWRKNACCST  840
NTSQEAHKDV SYLYRFNWNH CGEMAPACKR HFIQDTCLYE CSPNLGPWIQ QVDQSWRKER  900
VLNVPLCKED CEQWWEDCRT SYTCKSNWHK GWNWTSGFNK CAVGAACQPF HFYFPTPTVL  960
CNEIWTHSYK VSNYSRGSGR CIQMWFDPAQ GNPNEEVARF YAAAMSGAGP WAAWPFLLSL  1020
ALMLLWLLS                                                          1029

SEQ ID NO: 16           moltype = AA  length = 255
FEATURE                 Location/Qualifiers
REGION                  1..255
                        note = A synthetic FRb with signal peptide
source                  1..255
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 16
```

-continued

```
MVWKWMPLLL LLVCVATMCS AQDRTDLLNV CMDAKHHKTK PGPEDKLHDQ CSPWKKNACC  60
TASTSQELHK DTSRLYNFNW DHCGKMEPAC KRHFIQDTCL YECSPNLGPW IQQVNQSWRK  120
ERFLDVPLCK EDCQRWWEDC HTSHTCKSNW HRGWDWTSGV NKCPAGALCR TFESYFPTPA  180
ALCEGLWSHS YKVSNYSRGS GRCIQMWFDS AQGNPNEEVA RFYAAAMHVN AGEMLHGTGG  240
LLLSLALMLQ LWLLG                                                   255

SEQ ID NO: 17          moltype = AA  length = 335
FEATURE                Location/Qualifiers
REGION                 1..335
                       note = A synthetic uPAR with signal peptide
source                 1..335
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 17
MGHPPLLPLL LLLHTCVPAS WGLRCMQCKT NGDCRVEECA LGQDLCRTTI VRLWEEGEEL  60
ELVEKSCTHS EKTNRTLSYR TGLKITSLTE VVCGLDLCNQ GNSGRAVTYS RSRYLECISC  120
GSSDMSCERG RHQSLQCRSP EEQCLDVVTH WIQEGEEGRP KDDRHLRGCG YLPGCPGSNG  180
FHNNDTFHFL KCCNTTKCNE GPILELENLP QNGRQCYSCK GNSTHGCSSE ETFLIDCRGP  240
MNQCLVATGT HEPKNQSYMV RGCATASMCQ HAHLGDAFSM NHIDVSCCTK SGCNHPDLDV  300
QYRSGAAPQP GPAHLSLTIT LLMTARLWGG TLLWT                             335

SEQ ID NO: 18          moltype = AA  length = 187
FEATURE                Location/Qualifiers
REGION                 1..187
                       note = A synthetic DHFR
source                 1..187
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 18
MVGSLNCIVA VSQNMGIGKN GDLPWPPLRN EFRYFQRMTT TSSVEGKQNL VIMGKKTWFS  60
IPEKNRPLKG RINLVLSREL KEPPQGAHFL SRSLDDALKL TEQPELANKV DMVWIVGGSS  120
VYKEAMNHPG HLKLFVTRIM QDFESDTFFP EIDLEKYKLL PEYPGVLSDV QEEKGIKYKF  180
EVYEKND                                                            187

SEQ ID NO: 19          moltype = AA  length = 255
FEATURE                Location/Qualifiers
REGION                 1..255
                       note = A synthetic scFv against FITC: 4M5.3(Kd = 200pM)
source                 1..255
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 19
DVVMTQTPLS LPVSLGDQAS ISCRSSQSLV HSNGNTYLRW YLQKPGQSPK VLIYKVSNRV  60
SGVPDRFSGS GSGTDFTLKI NRVEAEDLGV YFCSQSTHVP WTFGGGTKLE IKSSADDAKK  120
DAAKKDDAKK DDAKKDGGVK LDETGGGLVQ PGGAMKLSCV TSGFTFGHYW MNWVRQSPEK  180
GLEWVAQFRN KPYNYETYYS DSVKGRFTIS RDDSKSSVYL QMNNLRVEDT GIYYCTGASY  240
GMEYLGQGTS VTVSS                                                   255

SEQ ID NO: 20          moltype = AA  length = 244
FEATURE                Location/Qualifiers
REGION                 1..244
                       note = A synthetic scFv against FITC 4D5Flu (Kd=10nM)
source                 1..244
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 20
DIQMTQSPSS LSASVGDRVT ITCRASQDVN TAVAWYQQKP GKAPKLLIYS ASFLYSGVPS  60
RFSGSRSGTD FTLTISSLQP EDFATYYCQQ HYTTPPTFGQ GTKVEIKRTG GGGSGGGGSG  120
GGGSEVQLVE SGGGLVQPGG SLRLSCAASG FNIKDTYIHW VRQAPGKGLE WVARIYPTNG  180
YTRYADSVKG RFTISADTSK NTAYLQMNSL RAEDTAVYYC SRWGGDGFYA MDYWGQGTLV  240
TVSS                                                               244

SEQ ID NO: 21          moltype = AA  length = 244
FEATURE                Location/Qualifiers
REGION                 1..244
                       note = A synthetic scFv against DNP SPE7
source                 1..244
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 21
QAVVTQESAL TTSPGETVTL TCRSSTGAVT TSNYANWVQE KPDHLFTGLI GGTNNRAPGV  60
PARFSGSLIG DKAALTITGA QTEDEAIYFC ALWYSNHLVF GGGTKLTVLG GGGSGGGGSG  120
GGGSEVQLQQ PGAELVKPGA SVKLSCKASG YTFTSYWMHW VKQRPGRGLE WIGRIDPNGG  180
GTKYNEKFKS KATLTVDKPS STAYMQLSSL TSEDSAVYYC ARMWYYGTYY FDYWGQGTTL  240
TVSS                                                               244
```

-continued

SEQ ID NO: 22	moltype = AA length = 4
FEATURE	Location/Qualifiers
REGION	1..4
	note = A synthetic sequence
source	1..4
	mol_type = protein
	organism = synthetic construct
SEQUENCE: 22	
DDRD	4

1. A method to modulate cell therapy, comprising:

a. identifying a target cell for transplant, wherein the target cell has a cell therapy function;

b. providing an engineered protein on the surface of the target cell, wherein:

the engineered protein comprises a first component and a second component, the first component and the second component are connected by a peptide linker, the first component is a non-membrane protein, and the second component is a membrane anchored peptide or protein;

c. providing a conjugate comprising a payload drug to the target cell, wherein the payload drug is conjugated to a small molecule ligand through a linker, wherein the small molecule ligand binds to at least one component of the engineered protein with high affinity and is internalized by the target cell together with the payload drug; and

d. releasing the payload drug within the target cell to modulate the target cell therapy function.

2. The method according to claim **1**, wherein the cell therapy function is to provide optically guided surgery to a subject.

3. The method according to claim **1**, wherein the cell therapy function is to control the target cell proliferation.

4. The method according to claim **1**, wherein the cell therapy function is to execute cytotoxicity to the target cell engaged cancer cell.

5. The method according to claim **1**, wherein the target cell for transplant is an immune cell, a chimeric antigen receptor (CAR) T cell, a stem cell, a progenitor cell, or a transplanted cell designed to synthesize a biochemical that is deficient in a patient.

6. The method according to claim **1**, wherein the payload drug is an imaging agent or a radioisotope imaging agent.

7. The method according to claim **6**, wherein the imaging agent is selected from the group consisting of rhodamine, fluorescein, S0456, radioisotope chelating imaging moieties, EC 20 chelating head, NOTA, and DOTA.

8. The method according to claim **1**, wherein the payload drug is a cytotoxic drug or a modulator of gene expression.

9. The method according to claim **8**, wherein the payload drug is selected from an HDAC inhibitor, a kinase inhibitor, a metabolic inhibitor, tubulysin, DM1, DM4, an auristatin, a Dasatinib, a MEK1/2 inhibitor, a PI3 Kinase inhibitor, a GSK3 beta inhibitor, a MAO-B inhibitor, a Cdk5 inhibitor, an RORγt agonist, and an siRNA.

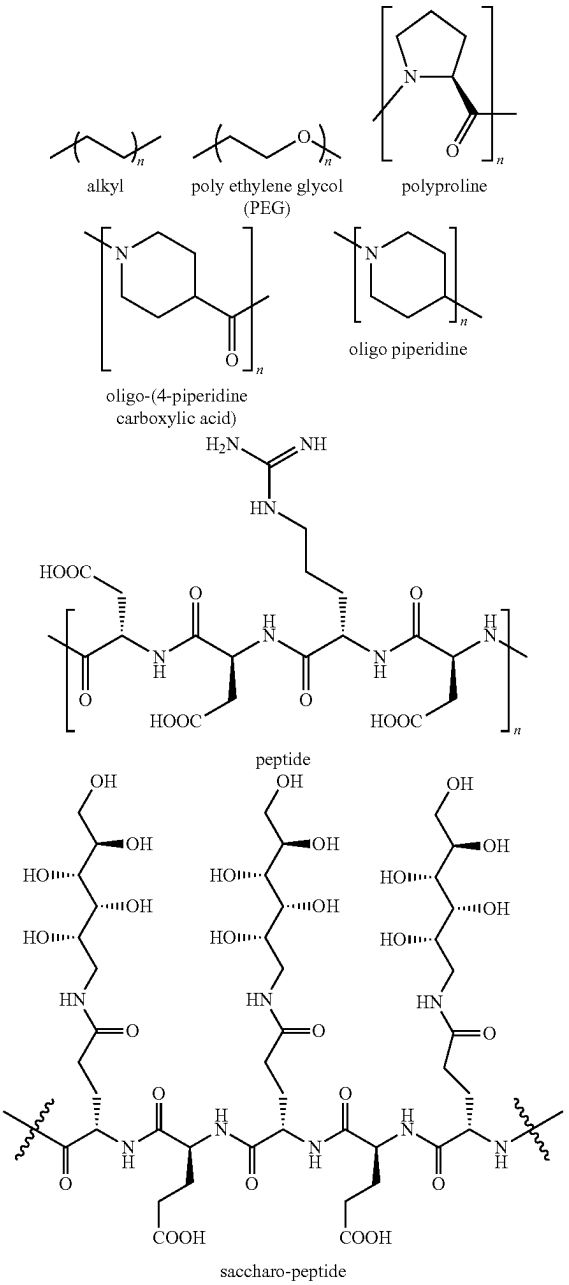
10. The method according to claim **1**, wherein the target cell for transplant comprises a fusion protein comprising the sequence of amino acids set forth in any one of SEQ ID NOS: 1, 2, or 12-15.

11. The method according to claim **1**, wherein the first component and/or the second component are independently selected from the group consisting of FRa, FRb, uPAR, FKBP, DHFR, scFv against FITC, and scFv against DNP.

12. The method according to claim **1**, wherein the small molecule ligand is selected from the group consisting of FK506, FK506 derivatives, synthetic ligand of FKBP (SLF), SLF derivatives, folic acid (FA), and FA derivatives.

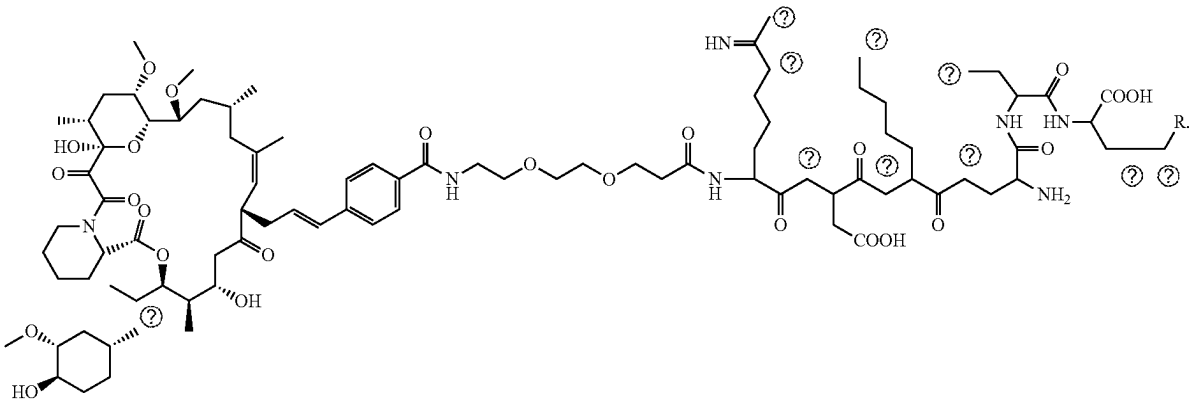
13. The drug delivery platform according to claim **12**, wherein the small molecule ligand is FK506 or its derivative.

14. The method according to claim **1**, wherein the linker to connect the small molecule ligand and the payload drug is selected from the group consisting of:



15. The method according to claim **1**, wherein the target cell for transplant is a CAR T cell.

16. The method according to claim **1**, wherein the conjugate comprises a FK506-releasable linker of formula I:



? indicates text missing or illegible when filed

17. The method according to claim **1**, wherein the target cell for transplant is a CAR T cell and the conjugate is designed to control a cytokine storm induced by the CAR T cell, or contains a modulator designed to control unwanted T cell proliferation.

18. The method according to claim **1**, wherein the target cell for transplant is a NK cell and the conjugate is a RORγt agonist to control Th17 cell mediated immune responses.

19. The method according to claim **1**, wherein the payload drug is a phosphatase inhibitor.

20. The method according to claim **19**, wherein the phosphatase inhibitor is an inhibitor against SHP1/2 or TC-PTP.

* * * * *