cells, such as monocytes/macrophages, comprise many potent degradative enzymes that can disrupt nucleic acid integrity and make gene transfer into these cells an inefficient process. This is especially true of activated macrophages which undergo a dramatic change in their physiology following exposure to immune or inflammatory stimuli. Viral transduction of these cells has been hampered because macrophages are end-stage cells that generally do not divide; therefore, some of the vectors that depend on integration into a replicative genome have met with limited success. Furthermore, macrophages are quite responsive to "danger signals," and therefore several of the original viral vectors that were used for gene transfer induced potent anti-viral responses in these cells making these vectors inappropriate for gene delivery. The present disclosure provides innovative methods and compositions that can successfully transfect or transduce a myeloid cell, or otherwise induce a genetic modification in a myeloid cell, with the purpose of augmenting a functional aspect of a myeloid cell, additionally, without compromising the cell's differentiation capability, maturation potential, and/or its plasticity.

[0012a] In one aspect, the present disclosure refers to a composition comprising a recombinant polynucleic acid comprising a sequence encoding a chimeric fusion protein (CFP) comprising: (a) an extracellular domain comprising an anti-tumor-associated calcium signal transducer-2 (anti-TROP2) binding domain, and (b) a transmembrane domain operatively linked to the extracellular domain; wherein the transmembrane domain is a transmembrane domain from a protein that dimerizes with endogenous FcR-gamma receptors expressed in myeloid cells, monocytes, or macrophages; wherein the CFP is expressed on the surface of myeloid cells, monocytes, or macrophages that express endogenous FcR-gamma receptors.

[0012b] In another aspect, the present disclosure refers to a cell comprising the composition as described herein, wherein the cell is a CD14+ cell.

[0012c] In another aspect, the present disclosure refers to a pharmaceutical composition comprising (i) a lipid nanoparticle, encapsulating the recombinant nucleic acid as described herein.

[0012d] In another aspect, the present disclosure refers to the use of the composition as described herein, the cell as described herein, or the pharmaceutical composition as described herein, in the manufacture of a medicament for treating a cancer in a subject, wherein the cancer is selected from a group consisting of gastric cancer, ovarian cancer, renal cancer, breast cancer, prostate cancer, liver cancer, brain cancer, lymphoma, leukemia, skin cancer, pancreatic cancer, colorectal cancer, glioblastoma and lung cancer.

[0012e] In another aspect, the present disclosure refers to a composition as described herein, a cell as described herein, or a pharmaceutical composition as described herein for use in therapy.

[0013] Provided herein are therapeutic agents directed to bind to certain antigens that are expressed on a diseased cell, for example, a cancer cell, and binding of the therapeutic agents (e.g., targeted "binders") to a target antigen on a target diseased cells initiates the process of destruction of the target cell. A therapeutic agent described herein may be a recombinant nucleic acid that can be expressed in

a suitable cell, such as a mammalian cell, such as a human cell, wherein the suitable cell may be a myeloid cell. In some embodiments, a therapeutic agent described herein may be a recombinant protein that can bind to the target antigen on a target cell. In some a therapeutic agent described herein may be a cell, for example, a myeloid cell, wherein the myeloid cell comprises a recombinant nucleic acid described herein, and/or expresses a recombinant protein described herein, such that the myeloid cell can be targeted to a diseased cell, expressing the target antigen on the surface of the cell; and the myeloid cell lyses or phagocytizes the diseased cell.

[0014] In some embodiments, the therapeutic agent is a myeloid cell such as described herein. In some embodiments, the myeloid cell is a myeloid precursor cell. In some embodiments, the myeloid cell is an undifferentiated and/or unpolarized myeloid cell. In some embodiments, the myeloid cell is a phagocytic cell. In some embodiments, the myeloid cell is a CD14+/CD16- cell.

[0015] In some embodiments, the therapeutic agent is a recombinant or engineered nucleic acid. In some embodiments, the nucleic acid is RNA.

[0016] In some embodiments, the engineered nucleic acid is an mRNA. In some embodiments, the therapeutic agent that is a recombinant or engineered nucleic acid encodes a chimeric fusion receptor protein (CFP).

[00185] FIG. 28 shows flow cytometry data showing expression of a CFP construct in THP-1 cells electroporated with mRNA encoding the CFP.

[00186] FIG. 29 shows a schematic demonstrating generation of mouse anti-GP75 (Trp-1) CFP ATAK cells. The anti-GP75 CFP is efficiently expressed in the mouse monocytes via electroporation of mRNA encoding the anti-GP75 CFP and cells expressing the anti-GP75 CFP have phagocytic activity.

[00187] FIG. 30 shows data demonstrating anti-GP75 ATAK monocytes suppress tumors in syngeneic B16 murine model. The data shows a graph of tumor volume in a mouse tumor model refractory to CAR-T and checkpoint inhibitors over time post treatment with mouse monocytes expressing an anti-GP75 CFP. Mice were administered 8 infusions of 2 x 10^6 cells vs vehicle. Infusions daily x4, 3 days rest, daily x4 +/- SD (Mann-Whitney test).

[00188] FIG. 31 shows graphs demonstrating that after infusion into a mouse tumor model, mouse monocytes expressing an anti-GP75 CFP penetrate into tumors, sustain expression of the CFP and differentiate into effector cells, such as inflammatory cell, dendritic cells and macrophages. Left graph shows that about ~40% of the mouse monocytes expressing the anti-GP75 CFP maintained expression of the CFP 5 days after infusion. Top right graph shows ATAK Cells (CFP expressing cells) penetrate tumor and spleen and become dendritic cells. Bottom right graph shows ATAK Cells penetrate tumor and spleen and become macrophage cells.

[00189] FIG. 32 depicts data of cytokine production of cells from naive mice or a mouse tumor model treated with vehicle or myeloid cells expressing an anti-GP75 CFP construct. Graphs of production of the indicated cytokines/chemokines are shown. Treatment responses correlated with anti-tumor serum cytokine profile. Analysis includes responder animals in treatment group at study termination.

[00190] FIG. 33 depicts data showing cross-presentation of tumor antigen by mouse monocytes expressing an anti-GP75 CFP. The mouse monocytes expressing an anti-GP75 CFP are able to phagocytize, process and present a surrogate tumor neoantigen, in this case derived from the OVA protein, to T cells that bear a cognate TCR for a class I restricted peptide derived from OVA (SIINFEKL (SEQ ID NO: 87)), and T cell activation is determined by IL2 (left) or Interferon (right) generation; while monocytes from the same mouse that do not express the ATAK receptor cannot. This proves that mouse monocytes expressing an anti-GP75 CFP can process and cross-present antigen to adaptive immune cells such as CD8 T cells. ATAK-GP75 (aka TRP-1 ATAK) construct.

[00191] FIG. 34 depicts data showing that human monocytes expressing a CFP harness innate immunity and stimulate adaptive anti-tumor immune responses. The data demonstrates that human monocytes expressing a CFP penetrate tumors, accumulate in tumors and recognize tumors (upper left). The data demonstrates that human monocytes expressing a CFP result in myeloid cell

the sequence in an untranslated region (UTR). In some embodiments, the sequence in the UTR comprises a consensus sequence of WWWU(AUUUA)UUUW, wherein W is A or U (SEQ ID NO: 85). In some embodiments, the recombinant nucleic acid is expressed on a bicistronic vector.

[00334] In some embodiments, the target cell is a mammalian cell. In some embodiments, the target cell is a human cell. In some embodiments, the target cell comprises a cell infected with a pathogen. In some embodiments, the target cell is a cancer cell. In some embodiments, the target cell is a cancer cell that is a lymphocyte. In some embodiments, the target cell is a cancer cell that is an ovarian cancer cell. In some embodiments, the target cell is a cancer cell that is a breast cell. In some embodiments, the target cell is a cancer cell that is a pancreatic cell. In some embodiments, the target cell is a cancer cell that is a glioblastoma cell.

[00335] In some embodiments, the recombinant nucleic acid is DNA. In some embodiments, the recombinant nucleic acid is RNA. In some embodiments, the recombinant nucleic acid is mRNA. In some embodiments, the recombinant nucleic acid is an unmodified mRNA. In some embodiments, the recombinant nucleic acid is a modified mRNA. In some embodiments, the recombinant nucleic acid is a circRNA. In some embodiments, the recombinant nucleic acid is a tRNA. In some embodiments, the recombinant nucleic acid is a microRNA. In some embodiments, the recombinant nucleic acid is a self-replicating RNA.

[00336] Also provided herein is a vector comprising a recombinant nucleic acid sequence encoding a CFP described herein. In some embodiments, the vector is viral vector. In some embodiments, the viral vector is retroviral vector or a lentiviral vector. In some embodiments, the vector further comprises a promoter operably linked to at least one nucleic acid sequence encoding one or more polypeptides. In some embodiments, the vector is polycistronic. In some embodiments, each of the at least one nucleic acid sequence is operably linked to a separate promoter. In some embodiments, the vector further comprises one or more internal ribosome entry sites (IRESs). In some embodiments, the vector further comprises a 5'UTR and/or a 3'UTR flanking the at least one nucleic acid sequence encoding one or more polypeptides. In some embodiments, the vector further comprises one or more regulatory regions.

[00337] Also provided herein is a polypeptide encoded by the recombinant nucleic acid of a composition described herein.

[00338] Provided herein is a composition comprising a recombinant nucleic acid sequence encoding a CFP comprising a phagocytic or tethering receptor (PR) subunit (e.g., a phagocytic receptor fusion protein (PFP)) comprising: a PR subunit comprising: a transmembrane domain, and an intracellular domain comprising an intracellular signaling domain; and an extracellular domain comprising an antigen binding domain specific to an antigen of a target cell; wherein the transmembrane domain and the extracellular domain are operatively linked; and wherein upon

NO: 33, or a sequence that has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to SEQ ID NO: 33.

[00396] In some embodiments, the CFP comprises an extracellular domain comprising a sequence as set forth in SEQ ID NO: 33, or a sequence having at least 80% identity to SEQ ID NO: 33; operably linked to a TM domain comprising a sequence of CD16 transmembrane domain (e.g. comprising a short hinge domain) as set forth in Table 4. In some embodiments, the CFP comprises an extracellular domain comprising a sequence as set forth in SEQ ID NO: 33, or a sequence having at least 80% identity to SEQ ID NO: 33; operably linked to a TM domain comprising a CD64 transmembrane domain sequence. In some embodiments, the CFP comprises an extracellular domain comprising a sequence as set forth in SEQ ID NO: 33; operably linked to a TM domain comprising a sequence of a CD89 transmembrane domain (e.g. comprising a short hinge domain) as set forth in Table 4. In some embodiments, the CFP comprises an ECD with a sequence set forth in SEQ ID NO: 33, or a sequence having at least 80% identity to SEQ ID NO: 33; a TM domain comprising a sequence from the TM domain of a CD16 protein, or the TMD of a CD64 protein or a TMD of a CD89 protein, and a corresponding intracellular domain (ICD), or a portion thereof, e.g., from a CD16 protein, a CD64 protein or a CD89 protein respectively, optionally, in addition to other distinct ICDs described herein. Exemplary CD16 and CD89 ICD sequences are provided in Table 4.

[00397] In one embodiment, the extracellular binding domain comprises a scFv that is specific to human claudin 3.0. In some embodiments, the extracellular binding domain comprises a claudin 3 binding domain comprising a sequence as shown in Table 2. Table 2 shows exemplary sequences of human claudin 3.0 binding domains and/or fragments thereof that are meant to be non-limiting for the disclosure.

TABLE 2

Domain	Sequence	SEQ ID NO:	Domain	Sequence	SEQ ID NO:
1HC-CDR1	GYTMN	38	1LC-CDR1	KSSQSLLYGSN QKNYL	57
1HC- CDR2	LINPYNGGTSYN QKFKD	39	1LC- CDR2	WASTRES	58

1HC-CDR3	GSYGSSYFDY	40	1LC-CDR3	QQYYNFPYT	59
Heavy chain-1	ELVKPGASMKISCKASG YSFTGYTMNWMKQGHG KNLEWIGLINPYNGGTS YNQKFKDKATLTLDKSS SSAYMELLSLTSEDSAV YYCARGSYGSSYFDYW GQGTTLTVSS	41	Light Chain-1	DIVMSQSPSSLAVSV GEKVTMSCKSSQSLL YGSNQKNYLAWYQQ KPGQSPKLLIYWAST RESGVPDRFTGSGSG TDFTLTISSVKAEDLA VYYCQQYYNFPYTF GGGTKLEIKR	60
2HC-CDR1	DYYMN	42	2LC-CDR1	RASESVDSYGNSFMH	61
2HC- CDR2	RVNPSNGGTSYNQKFK	43	2LC- CDR2	RASNLES	62
2HC-CDR3	GLAYYSNSFVY	44	2LC-CDR3	QQNNEDPWT	63
Heavy chain-2	EVQLQQSGPELVKPGAS VKMSCKASGYTFTDYY MNWVKQSHGKSLEWIG RVNPSNGGTSYNQKFKG KATLTVDKSLSTAYMQL NSLTSEDSAVYYCARGL AYYSNSFVYWGQGTLV TVSA	45	Light Chain-2	KIVLTQSPASLAVSLR QRATISCRASESVDSY GNSFMHWYQQKPGQ PPKLLIYRASNLESGV PARFSGSGSRTDFTLT IDPVEADDAATYYCQ QNNEDPWTFGGGTK LEIKR	64
3HC-CDR1	GYFMN	46	3LC-CDR1	RASKSVSTSSYSYMH	65
3HC- CDR2	RINPYNGDTFYNQKFKG	47	3LC- CDR2	FASYLES	66
3HC-CDR3	SGDWYFDV	48	3LC-CDR3	PVEEEFPRT	67

Heavy chain-3	EVQLQQSGPELVKPGASVKISCKASGYSFTGYFMNWVKQSHGKSLEWIGRINPYNGDTFYNQKFKGKATLTVDKSSSTAHMELRSLTSEDSAVYYCARSGDWYFDVWGAGTTVTVSS	49	Light Chain-3	DIVLTQSPASLAVSLGQRATISCRASKSVSTSSYSYMHWYQQKPGQPPKLLIKFASYLESGVPARFSGSGSGTDFTLNIHPVEEEDAATYYCQHSREFPRTFGGGTKLEIKR	68
4HC-CDR1	GYFMN	46	4LC-CDR1	KASENVVSYVS	69
4HC- CDR2	RINPYNGDTFYNQKFKG	47	4LC- CDR2	GASNRYT	70
4HC-CDR3	GDGYYVTSLAY	50	4LC-CDR3	GQSYSYPLT	71
Heavy chain-4	EVQLQQSGPELVKPGASVKISCKASGYSFTGYFMNWVKQSHGKSLEWLGRINPYNGDTFYNQKFKGKATLTVDKSSNTAHMELRSLTSEDSAVYYCARGDGYYVTSLAYWGQGTLVTVSA	51	Light Chain-4	NIVMTQSPKSMSMSVGERVTLSCKASENVVSYVSWFQQKPEQSPKLLIYGASNRYTGVPDRFTGSGSATDFTLTISSVQAEDLADYYCGQSYSYPLTFGAGTKLELKR	72
5HC-CDR1	DYYMN	42	5LC-CDR1	RASESVDSYGNSFMH	61
5HC- CDR2	RVNPSNGGTSYNQKFKG	52	5LC- CDR2	RASNLES	62
5HC-CDR3	GLAYYSNSFTY	53	5LC-CDR3	QQNNEDPWT	63
Heavy chain-5	EVQLQQSGPELVKPGASVKMSCKASGYTFTDYYMNWVKQSHGKSLEWIGRVNPSNGGTSY	54	Light Chain-5	KIVLTQSPASLAVSLRQRATISCRASESVDSYGNSFMHWYQQKPGQPPKLLIYRASNLES	64

	NQKFKGKATLTVDKSLSTAYMQLNSLTSEDSAVYYCARGLAYYSNSFTYWGQGTLVTVSA			GVPARFSGSGSRTDFTLTIDPVEADDAATYYCQQNNEDPWTFGGGTKLEIKR	
6HC-CDR1	GYFMN	46	6LC-CDR1	RASESVEYYGTSLMQ	73
6HC- CDR2	RINPYNGDTFYNQKFKG	47	6LC- CDR2	GASNVES	74
6HC-CDR3	SGNYVMDY	55	6LC-CDR3	QQSRKVPWT	75
Heavy chain-6	EVQLQQSGPELVKPGASVKMSCKASGYSFTGYFMNWVKQSHGKSLEWIGRINPYNGDTFYNQKFKGKATLTVDKSSSTAHMELRSLTSEDSALYYCARSGNYVMDYWGQGTSVTVSS	56	Light Chain-6	DIVLTQSPASLAVSLGQSVTISCRASESVEYYGTSLMQWYQQKPGQPPKLLIYGASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYFCQQSRKVPWTFGGGTKLEIKR	76

[00398] A claudin 3 binding domain can comprises one or more of the sequences from Table 2. In some embodiments the claudin 3 binding domain comprises an ScFv, comprising a sequence of Table 2. In some embodiments, the binding domain comprises an antigen-binding fragment (Fab), a single-chain variable fragment (scFv), a nanobody, a VH domain, a VL domain, a single domain antibody (sdAb), a VNAR domain, and a VHH domain, a bispecific antibody, a diabody, or a functional fragment of any thereof, comprising a sequence of Table 2. In some embodiments, the antigen-binding fragment (Fab), a single-chain variable fragment (scFv), a nanobody, a VH domain, a VL domain, a single domain antibody (sdAb), a VNAR domain, and a VHH domain, a bispecific antibody, a diabody, or a functional fragment of any thereof specifically bind to one or more antigens.

[00399] In some embodiments the chimeric fusion protein is a receptor, comprising an extracellular binding domain, such as an antigen binding domain; wherein the extracellular binding domain may comprises a sequence that can bind to claudin 18.2 protein as described herein; or can bind a claudin 3 protein as described herein; and may further comprise a transmembrane domain with or without a hinge domain in between the extracellular antigen binding domain and an intracellular domain. In some embodiments, the extracellular domain

Receptor	**Expressed in (cell type)**	**Oligomerization or association characteristic at the cell membrane**
FcγRIIB (CD32b)	B cells, DCs, mast cell	no
FcγRIIC (CD32c)	NK cells /Monocytes/ Macrophages /neutrophils	no
FcγRIIIA (CD16)	NK cells /Monocytes/ Macrophages	FcR γ–chain dimer
FcγRIIIB (CD16b)	Neutrophil, Eosinophil, Basophil	no
FcαR1 (CD89)	Monocyte/Macrophage/Neutrophil/DCs/Kupfer cells	FcR γ–chain dimer
FcμR	Lymphocytes	no
FcεR1	Basophils, Mast cells	FcR γ–chain dimer and β-chain

[00485] Exemplary FcR domain sequences used in constructing chimeric fusion protein receptors disclosed herein are provided in Table 4.

[00486] Table 4

1a	**Human CD16 TM sequence incorporated in a CFP (includes an extracellular hinge domain)**	
	GLAVSTISSFFPPGYQVSFCLVMVLLFAVDTGLYFSV (SEQ ID NO: 77)	
	Extracellular hinge domain	GLAVSTISSFFPPGYQV (SEQ ID NO: 78)
	TM domain	SFCLVMVLLFAVDTGLYFSV (SEQ ID NO: 79)
1b	**Human CD16 Cytoplasmic domain**	
	KTNIRSSTRDWKDHKFKWRKDPQDK (SEQ ID NO: 80)	
2a	**Human CD89 TM sequence incorporated in a CFP (includes an extracellular hinge domain)**	
	IHQDYTTQNLIRMAVAGLVLVALLAILV (SEQ ID NO: 81)	
	Extracellular hinge domain	IHQDYTTQN (SEQ ID NO: 82)

	TM domain	LIRMAVAGLVLVALLAILV (SEQ ID NO: 83)
2b	**Human CD89 cytoplasmic domain**	
	ENWHSHTALNKEASADVAEPSWSQQMCQPGLTFARTPSVCK (SEQ ID NO: 84)	

[00487] In some embodiments, the CFP is designed to comprise a transmembrane domain derived from the transmembrane domain of protein selected from FcγR1 (CD64). In some embodiments, the CFP is designed to comprise a transmembrane domain derived from the transmembrane domain of protein selected from FcγRIIIA (CD16). In some embodiments, the CFP is designed to comprise a transmembrane domain derived from the transmembrane domain of protein selected from FcγRIIA (CD32a). In some embodiments, the CFP is designed to comprise a transmembrane domain derived from the transmembrane domain of protein selected from FcαR1 (CD89). Any of the domains in the CFP described herein can be from a suitable mammalian origin, either depending on or irrespective of the origin of any other adjoining domains. Non-human mammalian transmembrane domains are contemplated within the scope of the instant disclosure.

[00488] In some embodiments, the CFP can comprise 2, 3, 4, 5, 6, 7, 8 9, 10, 11, 12, 13, 14, 15, 16, 17 18, 19 or 20 amino acid residues from the protein from which the TMD is derived, in the extracellular domain. In some embodiments, the CFP can comprise 2, 3, 4, 5, 6, 7, 8 9, 10, 11, 12, 13, 14, 15, 16, 17 18, 19 or 20 or more amino acid residues from the protein from which the TMD is derived, in the intracellular domain.

[00489] In some embodiments, the CFP comprises an intracellular domain from the protein FcγR1 (CD64), FcγRIIIA (CD16), or FcγRIIA (CD32a) from which the TMD is derived. In some embodiments, the TMD has a sequence that is identical to the sequence of the TMD of the protein FcγR1 (CD64), FcγRIIIA (CD16), or FcγRIIA (CD32a).

[00490] Myeloid cell-specific CFP constructs are designed to comprise a transmembrane domain that dimerizes with an endogenous Fc-gamma receptor that is specifically expressed in a myeloid cell, thereby becoming functionally active as a result of dimerization.

[00491] In some embodiments, myeloid cell-specific CFP constructs comprises an FcαR1 (CD89) transmembrane domain. In some embodiments, myeloid cell-specific CFP constructs comprises a human FcαR1 (CD89) transmembrane domain. In some embodiments, myeloid cell-specific CFP constructs comprises a mouse FcαR1 (CD89) transmembrane domain. In some embodiments, myeloid cell-specific CFP constructs comprises a rodent FcαR1 (CD89) transmembrane domain. In some embodiments, myeloid cell-specific CFP constructs comprises a equine FcαR1 (CD89) transmembrane domain. In some embodiments,

plasminogen activator; plasmin; thrombin; BMP-1 (procollagen C-peptidase); ADAM 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11; and, granzymes A, B, C, D, E, F, G, and H.

[00539] In some embodiments the protease is Hepatitis C virus (HCV) nonstructural protein 3 protease NS3. In some embodiments the NS3 cleavable sequence is EDVVCC (SEQ ID NO: 36). In some embodiments, the NS3 cleavable sequence is DEMEEC (SEQ ID NO: 37).

[00540] In some embodiments, the transcription activator comprises a DNA binding domain. In some embodiments, the DNA binding domain is GAL4 domain. In some embodiments, the DNA binding domain is that of ZFHD1 or tetR. In some embodiments the transcriptional activator comprises the VP64 transactivation domain (a tetrameric repeat of the minimal activation domain (amino acids 437-447) of the herpes simplex protein VP16).

[00541] In some embodiments the degron is a HIF-degron.

[00542] In some embodiments, provided herein are one or more recombinant nucleic acid comprising: (A) a first nucleic acid sequence encoding an exogenous polypeptide; (B) a second nucleic acid sequence encoding a chimeric antigen receptor (CFP), wherein the CFP comprises: (a) an intracellular signaling subunit comprising an intracellular signaling domain having tyrosine residues that are phosphorylated upon activation of the CFP; (b) a transmembrane domain, and (c) an extracellular binding domain having binding specificity for a component on the surface of a target cell, wherein the extracellular binding domain is operably linked to the transmembrane domain and the intracellular signaling subunit; and (d) a transcription activator domain operably linked to the intracellular signaling subunit by a protease cleavage sequence, wherein the transcription activator domain promotes transcription of the first nucleic acid sequence encoding the exogenous polypeptide; and (C) a third nucleic acid sequence encoding (i) a protease that cleaves the protease cleavage sequence that operably links the transcription activator domain to the intracellular signaling subunit; (ii) a domain that binds to the tyrosine residues that are phosphorylated upon activation of the CFP; wherein the protease that cleaves the protease cleavage sequence and the domain that binds to the tyrosine residues are operably linked.

[00543] In some embodiments, provided herein is a recombinant nucleic acid for a modular polypeptide that can be expressed in a myeloid cell for use in immunotherapy. The recombinant nucleic acid encodes a chimeric protein comprising: (a) a cytotoxic polypeptide; (b) a protease cleavage sequence; and (c) an inhibitory polypeptide domain, wherein the inhibitory polypeptide domain inhibits the cytotoxic polypeptide; wherein the cytotoxic polypeptide, protease cleavage sequence and the inhibitory polypeptide domain are operably linked. In some embodiments, the cytotoxic polypeptide is a human eosinophil major basic protein cytotoxic domain. In some embodiments, the cytotoxic polypeptide is a human eosinophil major basic protein acidic domain.

tissues that indicate high myeloid cell activity necessary for tumor killing. **FIG. 20C** demonstrates that the engineered monocytes recruit T cells in an in vitro assay. **FIG. 21** and **FIGs. 41** and **42** show increased inflammatory cytokine secretion by cells isolated from mice with tumor, those of which were administered the CFP constructs. Such inflammatory cytokines turn potentially turn a cold tumor into a hot TME.

[00600] **FIG. 22** shows data to confirm tumor reduction in mice that received the LNP comprising CFP injection. A syngeneic mouse model was generated with myeloid cells expressing GP75 binding CFP. Generation of Mouse anti-GP75 (Trp-1) ATAK Cells is shown in **FIG. 29**. The ATAK-GP75 receptor is efficiently expressed in the mouse monocytes via electroporation of mRNA as demonstrated in the flow cytometry plot at the lower left panel. These cells were able to efficiently phagocytose GP75 expressing tumor cells (FIG. 29 lower, right graph). Similar data is corroborated by the data in **FIG. 30**, and **36 and 37** depicting tumor reduction and survival advantage in mice expressing the respective CFP. **FIG. 30** represents the first in vivo illustration of the power of ATAK myeloid cells to contain tumor growth in a tumor model that is *refractory to CAR T & Checkpoint inhibitors*. 8 infusions of $2x10^6$ ATAK cells vs Vehicle. Infusions daily x4, 3 days rest, daily x4 +/- SD (Mann-Whitney test). When GP-75 ATAK monocytes are injected i.v. into immunocompetent C57Bl/6 mice bearing s.c. implanted GP75+ B16 melanoma tumors (these mice are tolerant to the B16 syngeneic tumor), the mice were then able to control the growth of the tumors and experience significantly improved survival compared with mice administered vehicle or control monocytes. In addition, when tumors were removed from animals and examined for immune cell content, the animals treated with the GP75-ATAK monocytes showed significantly higher levels of infiltration of the tumor by inflammatory monocyte/macrophages and neutrophils as well as increases in dendritic cells vs. MDSC's. This proved that ATAK monocytes traffic to tumor sites, suppress tumor growth, and recruit other immune cell populations to the TME.

[00601] **FIG. 21** specifically shows that the treatment of mice with myeloid cells expressing the CFP was associated with broad T cell activity in spleen re-stimulated cultures, including spread of CD4 epitopes. In this experiment splenocyte recall cultures were undertaken, in which spleen cells were stimulated for 6 hours with $OVA_{323*329}$SINFECKEL ("SINFECKEL" disclosed as SEQ ID NO: 86) or PMA/ionomycin. The cytokines were detected by flow cytometry. GP75-ATAK cell treatment was associated with increased non-antigen specific activity, which is indicative of cross-presentation.

[00602] Positive CFP expression was identified in myeloid cells and some CD3+ cells (**FIG. 23 and FIG. 24**). Increased phagocytosis, and cytokine secretion is also evident in data shown in **FIGs 31**, **32**, **33 and 35**. As shown in **FIG. 31**, the monocytes further became DCs and mature monocytes inside the tumor and spleen. Serum cytokine levels shown in **FIG. 32** illustrate broad

myeloid activity in the responders. Treatment Responses Correlated With Anti-Tumor Serum Cytokine Profile. The other significant observation was the Cross-Presentation of Tumor Antigen by ATAK monocytes (**FIG. 33**). ATAK myeloid cells can phagocytose, liberate & present neoantigen to T cells, resulting in adaptive T cell immune response. The ATAK-GP75 (aka TRP-1 ATAK) monocytes are able to phagocytize, process and present a surrogate tumor neoantigen, in this case derived from the OVA protein, to T cells that bear a cognate TCR for a class I restricted peptide derived from OVA (SIINFEKL (SEQ ID NO: 87)) while monocytes from the same mouse that do not express the ATAK receptor cannot. This proves that ATAK monocytes can process and cross-present antigen to adaptive immune cells such as CD8 T cells. This further indicates that potential novel subsets of T cells could be stimulated via function of these myeloid cells in vivo, thereby demonstrating wider immunological coverage as opposed to treatment by other immune cell types, such as T cells.

[00603] Preferential expression of the constructs in myeloid cells in vivo was corroborated in the data depicted in **FIG. 38**. LNP-FcRα chain receptor was expressed by inflammatory monocytes within the TME, and greater than 15% of tumor myeloid cells express GP-75-FcaR construct.

Example 11. Microenvironment modification by CFP expressing modification in vivo

[00604] In this example, inflammatory gene expression was profiled from in vivo tumor samples which indicate that the CFP constructs turn the tumor microenvironment into an active inflammatory site, with expression of proinflammatory cytokines and chemokines (**FIG. 26**), (heating of the TME) and **FIG. 34** (top right). **FIG. 27** and **FIG. 34** show data indicating that the CFP injection in vivo was capable of T cell induction. **FIG. 34** demonstrates that the human ATAK monocytes as disclosed herein were able to harness innate immunity and stimulate adaptive anti-tumor immune response. The key summary points indicating that the engineered human cell products were confirmed to have key functional mechanism are enumerated below:

[00605] Tumor Penetration & Recognition

- Penetration
- Accumulation
- Tumor recognition

[00606] Signaling Dynamics

- Myeloid cell activation
- Cytokine production
- Chemokine production
- Inflammatory polarization

[00607] Direct Tumor Kill

exemplary construct is an HCV non-structural protease (NS3) fused to a PTB-HIF degron; the PTB-HIF-NS3 sequence is T2A cleavable. The cleavable sequence that is the substrate of a phosphotyrosine binding protein (PTB) dependent protease is EDVVCC (SEQ ID NO: 36) or DEMEEC (SEQ ID NO: 37). A second vector construct is used to co-express a sequence encoding a target gene, the transcription of which is activated by the transcription activation factor encoded upstream. The target gene is driven by CMV minipromoter, with an upstream GAL4 binding and activation domain. The nucleic acid sequence encoding an extracellular cancer cell binding moiety, encodes an scfv antibody, which in the exemplary construct, is a CD19-binding scfv, but could be an scfv specific for another protein that is specifically expressed on a cancer cell, or a target cell. The transcription binding and activation factor comprises a 5X GAL4 sequence fused to a VP64 sequence, which can bind via the GAL-4 DNA binding region upstream of a CMV promoter, thereby activating transcription of the target gene. The PTB-HIF and the adjoining protease sequences are flanked by T2A cleavable sequence and are auto-cleavable by T2A protease, thereby releasing the protease following translation.

[00616] The vector design encoding the various parts shown in the example can be variously designed otherwise. For example, a polycistronic design or multiple vector design is equally contemplated herein.

[00617] The modular vector design with the co-expression of the components described above makes way for a flexible incorporation of a target gene downstream of CMV promoter that can lead to activation and potentiation of macrophage mediated phagocytosis and killing of the target cell. Exemplary target genes are inflammatory genes, inflammasome activation genes, cytokines, chemokines, REDOX genes.

[00618] The basic structural layout of the extracellular and intracellular domains and the cleavable units are demonstrated in **FIG. 61A**. The construct as described above is expressed in a macrophage cell. Following expression, the expected mode of action after translation and release of the mature proteins is shown in **FIG. 61B**. The HIF-degron, when binds to a phosphotyrosine binding element on the intracellular domain via a PTB and ITAM interaction is deactivated, and allows proper function of the protease, thereby allowing cleavage and release of the transcription factor. In absence of the association with the ITAM, the degron is responsible for the degradation of the HIF-protease element, as shown in FIG. 2B. Therefore this function allows for a cancer cell-specific activation of the transcription factor and in turn the specific target gene transcription driven by the CMV promoter, since the ITAM is activated and recruits PTB only when it receives signal from the extracellular domain, e.g. the scfv –bound to its CD19 target on a cancer cell.

Example 15. Generation of a tumor activated eosinophil lytic protein