types including CD8+ and CD4+ T effector cells, NK cells and T regulatory cells. Macrophages have been shown to be a main immune infiltrate in malignant tumors and have been shown to have a broad immunosuppressive influence on effector immune infiltration and function.

SUMMARY

[0004a] In one aspect, provided herein is use of a composition in the manufacture of a medicament for treating cancer, wherein said composition comprises a therapeutically effective dose of an engineered RNA encapsulated in a lipid nanoparticle, wherein the engineered RNA comprises a sequence encoding a myeloid cell-specific chimeric antigen receptor, the chimeric antigen receptor comprising: (i) an extracellular domain comprising an anti-TROP2 binding domain and (ii) a CD89 transmembrane domain; wherein the therapeutically effective dose is an amount of from 0.001 mg of the nucleic acid/kg body weight of the subject to 0.2 mg of the nucleic acid/kg body weight of the subject (mg/kg).

[0005] The diverse functionality of myeloid cells makes them an ideal cell therapy candidate that can be engineered to have numerous therapeutic effects. The present disclosure is related to immunotherapy using myeloid cells (e.g., CD14+ cells) of the immune system, particularly phagocytic cells. A number of therapeutic indications could be contemplated using myeloid cells. For example, myeloid cell immunotherapy could be exceedingly important in treating cancer, autoimmunity, fibrotic diseases and infections. The present disclosure is related to immunotherapy using myeloid cells, including phagocytic cells of the immune system, particularly monocytes. It is an object of the invention disclosed herein to harness one or more of these functions of myeloid cells for therapeutic uses. For example, it is an object of the invention disclosed herein to harness the phagocytic activity of myeloid cells, including engineered myeloid cells, for therapeutic uses. For example, it is an object of the invention disclosed herein to harness the ability of myeloid cells, including engineered myeloid cells, to promote T cell activation. For example, it is an object of the invention disclosed herein to harness the ability of myeloid cells, including engineered myeloid cells, to promote secretion of tumoricidal molecules. For example, it is an object of the invention disclosed herein to harness the ability of myeloid cells, including engineered myeloid cells, to promote recruitment and trafficking of immune cells and molecules. In one aspect the disclosure provides new and useful chimeric constructs that, when expressed in a myeloid cell, the myeloid cell can drive targeted attack and phagocytosis of the molecule, molecular assembly, object or a cell that comprises the target, e.g., a target antigen on its surface. One of the many facets of the present disclosure is to (i) enhance the phagocytic ability of the myeloid cells (e.g., the engineered myeloid cells expressing the new and improved chimeric constructs); help initiate a coordinated and sustained immune response against the target (e.g., target antigen). 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. The resultant cells may be termed therapeutically effective engineered myeloid cells, or effector myeloid cells. One strategy for improvement described herein is to induce an inflammatory phenotype of the myeloid cells to develop effector myeloid cells. One strategy is to generate effector myeloid cells capable of mounting an inflammatory phenotype upon engagement with the target.

[0006] Provided herein is a composition comprising a recombinant polynucleotide, wherein the recombinant polynucleotide comprises a sequence encoding a chimeric fusion protein (CFP), the CFP comprising: (i) an extracellular domain comprising a TROP2 antigen binding domain, (ii) a

[0022] In some embodiments, the recombinant polynucleotide is an mRNA.

[0023] In some embodiments, the recombinant polynucleotide is associated with one or more lipids.

[0024] In some embodiments, the recombinant polynucleotide is encapsulated in a liposome.

[0025] In some embodiments, the liposome is a lipid nanoparticle.

[0026] Provided herein is a pharmaceutical product comprising a recombinant mRNA encoding a chimeric antigen receptor comprising: an anti-TROP2 binding scFv extracellular domain; and a CD89 transmembrane domain; wherein the pharmaceutical product is formulated in an aqueous formulation for delivery systemically. In some embodiments, the pharmaceutical composition comprises an scFv comprising a heavy chain and a light chain, the heavy chain comprising a CDR3 having a sequence GGFGSSYWYFDV (SEQ ID NO: 42), and the light chain comprising a CDR3 having a sequence QQHYITPLT (SEQ ID NO: 43). In some embodiments, the heavy chain further comprises a CDR1 sequence of NYGMN (SEQ ID NO: 44), and a CDR2 sequence of WINTYTGEPTYTDDFKG (SEQ ID NO: 45); and the light chain further comprises a CDR1 sequence of KASQDVSIAVA (SEQ ID NO: 46), and a CDR2 sequence of SASYRYT (SEQ ID NO: 47). The pharmaceutical composition described above comprises a CD89 transmembrane domain having a sequence LIRMAVAGLVLVALLAILV (SEQ ID NO: 11).

[0027] In some embodiments, the recombinant polynucleotide comprises a vector.

[0028] Also provided herein is a composition comprising a nanoparticle delivery vehicle and a recombinant polynucleotide described herein, wherein the recombinant polynucleotide is associated with or within the nanoparticle delivery vehicle.

[0029] In some embodiments, the transmembrane domain is a transmembrane domain from a protein that dimerizes with endogenous FcR-gamma receptors in myeloid cells.

[0030] In some embodiments, the transmembrane domain comprises a transmembrane domain from CD16a, CD64, CD68 or CD89.

[0031] In some embodiments, the recombinant polynucleotide is associated with or within a nanoparticle delivery vehicle, wherein the nanoparticle delivery vehicle comprises a lipid nanoparticle or a polymeric nanoparticle.

[0032] In some embodiments, the recombinant polynucleotide is an mRNA and the nanoparticle encapsulates the mRNA.

[0033] Also provided herein is a composition comprising a cell comprising a recombinant polynucleotide described herein.

[0034] In some embodiments, the cell is an immune cell.

[0035] In some embodiments, the cell is a myeloid cell, a lymphoid cell, a precursor cell, a stem cell or an induced pluripotent cell.

[0036] In some embodiments, the cell is CD14+CD16- cell.

[0037] Also provided herein is a pharmaceutical composition comprising a composition described herein; and a pharmaceutically acceptable excipient.

[0038] Also provided herein is a method of treating a cancer in a subject comprising: administering to the subject a pharmaceutical composition described herein.

[0039] In one aspect, provided herein is a pharmaceutical composition comprising of a polynucleotide encoding a chimeric antigen receptor comprising: an anti-TROP2 binding scFv extracellular domain; and a CD89 transmembrane domain formulated in an aqueous formulation for delivery systemically, at a therapeutically effective amount and time interval suitable for treating a cancer in a subject. In some embodiments, the subject is a human subject. In some embodiments, the polynucleotide is an engineered RNA. In some embodiments, the polynucleotide is an engineered mRNA. In some embodiments, the anti-TROP2 binding scFv comprises a heavy chain, and a light chain, wherein the heavy chain comprises a CDR3 having a sequence GGFGSSYWYFDV (SEQ ID NO: 42), and the light chain comprises a CDR3 having a sequence QQHYITPLT (SEQ ID NO: 43). In some embodiments, the heavy chain further comprises a CDR1 sequence of NYGMN (SEQ ID NO: 44), and a CDR2 sequence of WINTYTGEPTYTDDFKG (SEQ ID NO: 45); and the light chain further comprises a CDR1 sequence of KASQDVSIAVA (SEQ ID NO: 46), and a CDR2 sequence of SASYRYT (SEQ ID NO: 47).

[0040] In some embodiments, the CD89 transmembrane domain comprises a sequence LIRMAVAGLVLVALLAILV (SEQ ID NO: 11).

[0041] In some embodiments, the pharmaceutical composition further comprises a lipid nanoparticle delivery vehicle. In some embodiments, the lipid nanoparticle delivery vehicle comprises a cationic lipid, a non-cationic lipid, a neutral lipid, a PEGylated lipid, or a combination thereof. In some embodiments, the subject is a human subject. In some embodiments, the cancer is an epithelial cancer. In some embodiments, the subject has an epithelial cancer. In some embodiments, the cancer is selected from Urothelial, Cervical, Ovarian epithelial, Triple-negative breast, HR+/HER2- breast, Pancreatic ductal adenocarcinoma, Gastric adenocarcinoma, Esophageal carcinoma, Non-small cell lung and Colorectal cancer.

[0042] In some embodiments, the cancer is metastatic.

[0043] In some embodiments, the pharmaceutical composition is formulated for systemic delivery.

[0044] In some embodiments, the pharmaceutical composition is formulated for intravenous delivery.

[0045] In some embodiments, the pharmaceutical composition is formulated for an intravenous injection or an infusion.

[0046] In some embodiments, the therapeutically effective amount of a dose comprises 1-5000 microgram/microliter of the engineered RNA.

about 0.035, at least about 0.04, at least about 0.045, at least about 0.05, at least about 0.055, at least about 0.06, at least about 0.065, at least about 0.07, at least about 0.075, at least about 0.08, at least about 0.085, at least about 0.09, at least about 0.095, or at least about 0.1 mg/kg, or at least about 0.2 mg/kg, or at least about 0.3 mg/kg, or at least about 0.4 mg/kg, or at least about 0.5 mg/kg, or at least about 0.6 mg/kg, or at least about 0.7 mg/kg, or at least about 0.8 mg/kg, or at least about 0.9 mg/kg, or at least about 1 mg/kg of the engineered RNA per dose of the pharmaceutical composition.

[0054] In some embodiments, the pharmaceutical composition comprises a concentration of the engineered RNA is between about 0.5 to about 1.5 mg/mL or between about 0.7 to about 1.3 mg/mL when stored in a container.

[0055] In some embodiments, the container is a single-use or multi-use vial.

[0056] In some embodiments, a total volume of the aqueous formulation is from about 1.0 to about 3.0 mL, from about 1.5 to about 2.5 mL, or about 2.0 mL.

[0057] In some embodiments, the dosing interval is 14 days for 3 doses, followed an interval of 28 days for three doses.

[0058] In one aspect, provided herein is a method of treating a cancer in a subject comprising: administering to the subject the pharmaceutical composition of any one of embodiments described above.

[0059] In some embodiments, the subject is a subject who is over 18 years of age.

[0060] In some embodiments, the subject exhibited progressive disease at baseline, or a refractory disease or a relapse in response to standard of care.

[0061] In some embodiments, the subject is not pregnant or donating sperm.

[0062] In some embodiments, the subject does not have CNS metastasis or carcinomatous meningitis.

[0063] In some embodiments, the pharmaceutical composition or the method of treating described herein comprises, reducing a tumor by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9% or at least 10% after the treatment.

[0064] In some embodiments, the pharmaceutical composition or the method of treating described herein comprises alleviating at least one of the symptoms associated with the cancer.

[0065] In some embodiments, the pharmaceutical composition comprises, an engineered RNA encapsulated in a lipid nanoparticle, wherein the engineered RNA comprises a sequence encoding a chimeric antigenic receptor (CAR) having an extracellular antigen binding domain comprising an scFv that binds to TROP2, having a heavy chain that comprises a CDR1 sequence of NYGMN (SEQ ID NO: 44), a CDR2 sequence of WINTYTGEPTYTDDFKG (SEQ ID NO: 45) and a CDR3 sequence of GGFGSSYWYFDV (SEQ ID NO: 42); and the light chain further comprises a CDR1 sequence of KASQDVSIAVA (SEQ ID NO: 46), a CDR2 sequence of SASYRYT (SEQ ID NO: 47), and a CDR3 sequence of QQHYITPLT (SEQ ID NO: 43), a transmembrane domain and an intracellular domain from CD89, having a sequence

DSIHQDYTTQN*LIRMAVAGLVLVALLAILV*ENWHSHTALNKEASADVAEPSWSQQMCQP GLTFARTPSVCK (SEQ ID NO: 48), wherein the pharmaceutical composition has no dose limiting toxicities (DLT) at a dose of at least up to 0.03 mg/kg in a human subject in need thereof, when administered at a dosing regimen that is at least twice weekly for about 18 weeks.

[0066] In some embodiments, the extracellular antigen binding domain comprises a sequence that is at least 90%, identical to the sequence QVQLQQSGSELKKPGASVKVSCKASGYTFTNYGMNWVKQAPGQGLKWMGWINTYTGEP TYTDDFKGRFAFSLDTSVSTAYLQISSLKADDTAVYFCARGGFGSSYWYFDVWGQGSLVT VSSGGGGSGGGGSGGGGSDIQLTQSPSSLSASVGDRVSITCKASQDVSIAVAWYQQKPGKA PKLLIYSASYRYTGVPDRFSGSGSGTDFTLTISSLQPEDFAVYYCQQHYITPLTFGAGTKVEIK R (SEQ ID NO: 3).

[0067] In some embodiments, the engineered CAR comprises a sequence that is at least 95% identical to the sequence in SEQ ID NO: 3.

[0068] In some embodiments, the engineered CAR comprises a sequence that is at least 95% identical to the sequence in SEQ ID NO: 3.

[0069] In some embodiments, the engineered CAR comprises a sequence that is at least 99% identical to the sequence in SEQ ID NO: 3.

[0070] In some embodiments, the engineered CAR comprises a sequence that is at least 90% identical to any one of the sequences of SEQ ID NO: 26 and SEQ ID NO: 41.

[0071] In some embodiments, the engineered CAR comprises a sequence that is at least 90% identical to the sequences of SEQ ID NO: 27 and SEQ ID NO: 34.

[0072] In some embodiments, the engineered CAR comprises a sequence that is at least 90% identical to the sequences of SEQ ID NO: 28 and SEQ ID NO: 35.

[0073] In some embodiments, the engineered CAR comprises a sequence that is at least 90% identical to the sequences of SEQ ID NO: 29 and SEQ ID NO: 36.

[0074] In some embodiments, the engineered CAR comprises a sequence that is at least 90% identical to the sequences of SEQ ID NO: 30 and SEQ ID NO: 37.

[0075] In some embodiments, the engineered CAR comprises a sequence that is at least 90% identical to the sequences of SEQ ID NO: 31 and SEQ ID NO: 38.

[0076] In some embodiments, the engineered CAR comprises a sequence that is at least 90% identical to the sequences of SEQ ID NO: 32 and SEQ ID NO: 39.

[0077] In some embodiments, the engineered CAR comprises a sequence that is at least 90% identical to the sequences of SEQ ID NO: 33 and SEQ ID NO: 40.

[0078] In some embodiments, the engineered CAR comprises a sequence that is at least 95% identical to any one of the sequences of SEQ ID NOs: 26-41.

[00169] TROP2 binding chimeric receptors for phagocytic cell activation

[00170] Extracellular antigen binding domain

[00171] Table 1A shows exemplary sequences of chimeric fusion protein domains and/or fragments thereof that are meant to be non-limiting for the disclosure. Underlines denote the CDR sequences in sequential order of CDR1, CDR2 and CDR3 for the respective heavy and light chains in accordance to the Kabat numbering system.

TABLE 1A. Exemplary Chimeric Fusion Protein Antigen Binding Domains

SEQ ID NO	Antigen Binding Domain	Sequence
1	Anti-TROP2 VH domain	QVQLQQSGSELKKPGASVKVSCKASGYTFTNYGMNWVKQAPGQGLKWMG WINTYTGEPTYTDDFKGRFAFSLDTSVSTAYLQISSLKADDTAVYFCARGGFG SSYWYFDVWGQGSLVTVSS HCDR1 sequence: NYGMN (SEQ ID NO: 44) (Kabat) GYTFTNY (SEQ ID NO: 49) (Chothia), GYTFTNYG (SEQ ID NO: 50) (IMGT) HCDR2 sequence: WINTYTGEPTYTDDFKG (SEQ ID NO: 45) (Kabat), NTYTGE (SEQ ID NO: 51) (Chothia), INTYTGEP (SEQ ID NO: 52) (IMGT) HCDR3 sequence: GGFGSSYWYFDV (SEQ ID NO: 42) (Kabat), GGFGSSYWYFDV (SEQ ID NO: 42) (Chothia) ARGGFGSSYWYFDV (SEQ ID NO: 53) IMGT)
2	Anti-TROP2 VL domain	DIQLTQSPSSLSASVGDRVSITCKASQDVSIAVAWYQQKPGKAPKLLIYSASYR YTGVPDRFSGSGSGTDFTLTISSLQPEDFAVYYCQQHYITPLTFGAGTKVEIKR LCDR1 sequence: KASQDVSIAVA (SEQ ID NO: 46) (Kabat), KASQDVSIAVA (SEQ ID NO: 46) (Chothia), QDVSIA (SEQ ID NO: 54) (IMGT) LCDR2 sequence: SASYRYT (SEQ ID NO: 47) (Kabat), SASYRYT (SEQ ID NO: 47) (Chothia), SAS (IMGT) LCDR3 sequence: QQHYITPLT (SEQ ID NO: 43) (Kabat), QQHYITPLT (SEQ ID NO: 43) (Chothia), QQHYITPLT (SEQ ID NO: 43) (IMGT)
3	Anti-TROP2 binding domain (scFv)	QVQLQQSGSELKKPGASVKVSCKASGYTFTNYGMNWVKQAPGQGLKWMG WINTYTGEPTYTDDFKGRFAFSLDTSVSTAYLQISSLKADDTAVYFCARGGFG SSYWYFDVWGQGSLVTVSSGGGGSGGGGSGGGGSDIQLTQSPSSLSASVGDR VSITCKASQDVSIAVAWYQQKPGKAPKLLIYSASYRYTGVPDRFSGSGSGTDF TLTISSLQPEDFAVYYCQQHYITPLTFGAGTKVEIKR

[00172] Transmembrane domains

[00173] In some embodiments, the transmembrane domain and the antigen binding domain are operatively linked through a linker. In some embodiments, the transmembrane domain and the antigen binding domain are operatively linked through a linker such as a hinge region of CD8α, IgG1 or IgG4.

[00174] In some embodiments, the extracellular domain comprises a multimerization scaffold.

does not express a chimeric protein. In some embodiments, such effectiveness is referred to as the enhanced NF-kappa B activation, or interferon activation in the cell expressing the chimeric protein, compared to an identical cell that does not express a chimeric protein.

[00190] Delivery vehicles - nanoparticles

[00191] In some embodiments, the myeloid cells are specifically targeted for delivery. Myeloid cells can be targeted using specialized biodegradable polymers, such as PLGA (poly(lactic-co-glycolic) acid and/or polyvinyl alcohol (PVA). In some embodiments, one or more compounds can be selectively incorporated in such polymeric structures to affect the myeloid cell function. In some embodiments, the targeting structures are multilayered, e.g., of one or more PLGA and one or more PVA layers. In some embodiments, the targeting structures are assembled in an order for a layered activity. In some embodiments, the targeted polymeric structures are organized in specific shaped components, such as labile structures that can adhere to a myeloid cell surface and deliver one or more components such as growth factors and cytokines, such as to maintain the myeloid cell in a microenvironment that endows a specific polarization. In some embodiments, the polymeric structures are such that they are not phagocytosed by the myeloid cell, but they can remain adhered on the surface. In some embodiments the one or more growth factors may be M1 polarization factors, such as a cytokine. In some embodiments the one or more growth factors may be an M2 polarization factor, such as a cytokine. In some embodiments, the one or more growth factors may be a macrophage activating cytokine, such as IFNγ. In some embodiments the polymeric structures are capable of sustained release of the one or more growth factors in an in vivo environment, such as in a solid tumor.

[00192] In some embodiments, the recombinant nucleic acid comprises a sequence encoding a homeostatic regulator of inflammation. In some embodiments, the homeostatic regulator of inflammation is a sequence in an untranslated region (UTR) of an mRNA. In some embodiments, the sequence in the UTR is a sequence that binds to an RNA binding protein. In some embodiments, translation is inhibited or prevented upon binding of the RNA binding protein to the sequence in an untranslated region (UTR). In some embodiments the RNA has a poly A tail. In some embodiments, the RNA (mRNA) has a poly A tail that has 200-1000 A (adenosine) residues (SEQ ID NO: 55). In some embodiments the mRNA has a poly A tail of about 200-800 A residues. In some embodiments the mRNA has a poly A tail of about 800-1200 A residues. In some embodiments the mRNA has a poly A tail of about, or at the most 1500 A residues.

[00193] 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,

[00199] Table 5 – Exemplary TROP2 binder sequences (CDR sequences are underlined)

Structure	Domain	Sequence
Anti-TROP2-CD89	MWLQSLLLLGTVACSISQVQLQQSGSELKKPGASVKVSCKASGYTFTNYGMNWVKQAPGQGLKWMGWINTYTGEPTYTDDFKGRFAFSLDTSVSTAYLQISSLKADDTAVYFCARGGFGSSYWYFDVWGQGSLVTVSSGGGGSGGGGSGGGGSDIQLTQSPSSLSASVGDRVSITCKASQDVSIAVAWYQQKPGKAPKLLIYSASYRYTGVPDRFSGSGSGTDFTLTISSLQPEDFAVYYCQQHYITPLTFGAGTKVEIKRSGGGGAAADYKDDDDKGSDSIHQDYTTQNLIRMAVAGLVLVALLAILVENWHSHTALNKEASADVAEPSWSQQMCQPGLTFARTPSVCK (SEQ ID NO: 34)		
	Signal peptide	MWLQSLLLLGTVACSIS (SEQ ID NO: 7)
	Anti-TROP2 domain	QVQLQQSGSELKKPGASVKVSCKASGYTFTNYGMNWVKQAPGQGLKWMGWINTYTGEPTYTDDFKGRFAFSLDTSVSTAYLQISSLKADDTAVYFCARGGFGSSYWYFDVWGQGSLVTVSSGGGGSGGGGSGGGGSDIQLTQSPSSLSASVGDRVSITCKASQDVSIAVAWYQQKPGKAPKLLIYSASYRYTGVPDRFSGSGSGTDFTLTISSLQPEDFAVYYCQQHYITPLTFGAGTKVEIKR (SEQ ID NO: 3)
	linker	SGGGGAAADYKDDDDKGS (SEQ ID NO: 9)
	CD89 TMD	LIRMAVAGLVLVALLAILV (SEQ ID NO: 11)
	CD89 TMD and intracellular domain	DSIHQDYTTQNLIRMAVAGLVLVALLAILVENWHSHTALNKEASADVAEPSWSQQMCQPGLTFARTPSVCK (SEQ ID NO: 48)
Anti-TROP2-CD89-FcR	MWLQSLLLLGTVACSISQVQLQQSGSELKKPGASVKVSCKASGYTFTNYGMNWVKQAPGQGLKWMGWINTYTGEPTYTDDFKGRFAFSLDTSVSTAYLQISSLKADDTAVYFCARGGFGSSYWYFDVWGQGSLVTVSSGGGGSGGGGSGGGGSDIQLTQSPSSLSASVGDRVSITCKASQDVSIAVAWYQQKPGKAPKLLIYSASYRYTGVPDRFSGSGSGTDFTLTISSLQPEDFAVYYCQQHYITPLTFGAGTKVEIKRSGGGGAAADYKDDDDKGSDSIHQDYTTQNLIRMAVAGLVLVALLAILVRLKIQVRKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPPQ (SEQ ID NO: 35)		
	Signal peptide	MWLQSLLLLGTVACSIS (SEQ ID NO: 7)
	Anti-TROP2 domain	QVQLQQSGSELKKPGASVKVSCKASGYTFTNYGMNWVKQAPGQGLKWMGWINTYTGEPTYTDDFKGRFAFSLDTSVSTAYLQISSLKADDTAVYFCARGGFGSSYWYFDVWGQGSLVTVSSGGGGSGGGGSGGGGSDIQLTQSPSSLSASVGDRVSITCKASQDVSIAV

		AWYQQKPGKAPKLLIYSASYRYTGVPDRFSGSGSGTDFTLTISS LQPEDFAVYYCQQHYITPLTFGAGTKVEIKR (SEQ ID NO: 3)
	linker	SGGGGAAADYKDDDDKGS (SEQ ID NO: 9)
	CD89 TMD	DSIHQDYTTQNLIRMAVAGLVLVALLAILV (SEQ ID NO: 56)
	FcR intracellular domain (ICD)	RLKIQVRKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPPQ (SEQ ID NO: 15)
Anti-TROP2-CD89-FcR-PI3K	MWLQSLLLLGTVACSISQVQLQQSGSELKKPGASVKVSCKASGYTFTNYGMN WVKQAPGQGLKWMGWINTYTGEPTYTDDFKGRFAFSLDTSVSTAYLQISSLK ADDTAVYFCARGGFGSSYWYFDVWGQGSLVTVSSGGGGSGGGGSGGGGSDI QLTQSPSSLSASVGDRVSITCKASQDVSIAVAWYQQKPGKAPKLLIYSASYRY TGVPDRFSGSGSGTDFTLTISSLQPEDFAVYYCQQHYITPLTFGAGTKVEIKRS GGGGAAADYKDDDDKGSDSIHQDYTTQNLIRMAVAGLVLVALLAILVRLKIQ VRKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPPQGSYEDMRGILYAAPQL RSIRGQPGPNHEEDADSYENM (SEQ ID NO: 36)	
	Signal peptide	MWLQSLLLLGTVACSIS (SEQ ID NO: 7)
	Anti-TROP2 domain	QVQLQQSGSELKKPGASVKVSCKASGYTFTNYGMNWVKQAP GQGLKWMGWINTYTGEPTYTDDFKGRFAFSLDTSVSTAYLQIS SLKADDTAVYFCARGGFGSSYWYFDVWGQGSLVTVSSGGGG SGGGGSGGGGSDIQLTQSPSSLSASVGDRVSITCKASQDVSIAV AWYQQKPGKAPKLLIYSASYRYTGVPDRFSGSGSGTDFTLTISS LQPEDFAVYYCQQHYITPLTFGAGTKVEIKR (SEQ ID NO: 3)
	Linker	SGGGGAAADYKDDDDKGS (SEQ ID NO: 9)
	CD89 TMD	DSIHQDYTTQNLIRMAVAGLVLVALLAILV (SEQ ID NO: 56)
	FcR intracellular domain (ICD)	RLKIQVRKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPPQ (SEQ ID NO: 15)
	Linker	GS
	PI3K recruitment domain (PI3K) ICD	YEDMRGILYAAPQLRSIRGQPGPNHEEDADSYENM (SEQ ID NO: 17)
Anti-TROP2-	MWLQSLLLLGTVACSISQVQLQQSGSELKKPGASVKVSCKASGYTFTNYGMN WVKQAPGQGLKWMGWINTYTGEPTYTDDFKGRFAFSLDTSVSTAYLQISSLK ADDTAVYFCARGGFGSSYWYFDVWGQGSLVTVSSGGGGSGGGGSGGGGSDI	

CD89-CD40	QLTQSPSSLSASVGDRVSITCKASQDVSIAVAWYQQKPGKAPKLLIYSASYRYTGVPDRFSGSGSGTDFTLTISSLQPEDFAVYYCQQHYITPLTFGAGTKVEIKRSGGGGAAADYKDDDDKGSDSIHQDYTTQNLIRMAVAGLVLVALLAILVKKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQERQ (SEQ ID NO: 37)	
	Leader	MWLQSLLLLGTVACSIS (SEQ ID NO: 7)
	Anti-TROP2 domain	QVQLQQSGSELKKPGASVKVSCKASGYTFTNYGMNWVKQAPGQGLKWMGWINTYTGEPTYTDDFKGRFAFSLDTSVSTAYLQISSLKADDTAVYFCARGGFGSSYWYFDVWGQGSLVTVSSGGGGSGGGGSGGGGSDIQLTQSPSSLSASVGDRVSITCKASQDVSIAVAWYQQKPGKAPKLLIYSASYRYTGVPDRFSGSGSGTDFTLTISSLQPEDFAVYYCQQHYITPLTFGAGTKVEIKR (SEQ ID NO: 3)
	Linker	SGGGGAAADYKDDDDKGS (SEQ ID NO: 9)
	CD89 TMD	DSIHQDYTTQNLIRMAVAGLVLVALLAILV (SEQ ID NO: 56)
	CD40 ICD	KKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQERQ (SEQ ID NO: 18)
Anti-TROP2-CD89-CD40-FcR	MWLQSLLLLGTVACSISQVQLQQSGSELKKPGASVKVSCKASGYTFTNYGMNWVKQAPGQGLKWMGWINTYTGEPTYTDDFKGRFAFSLDTSVSTAYLQISSLKADDTAVYFCARGGFGSSYWYFDVWGQGSLVTVSSGGGGSGGGGSGGGGSDIQLTQSPSSLSASVGDRVSITCKASQDVSIAVAWYQQKPGKAPKLLIYSASYRYTGVPDRFSGSGSGTDFTLTISSLQPEDFAVYYCQQHYITPLTFGAGTKVEIKRSGGGGAAADYKDDDDKGSDSIHQDYTTQNLIRMAVAGLVLVALLAILVKKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQERQGSRLKIQVRKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPPQ (SEQ ID NO: 38)	
	Signal peptide	MWLQSLLLLGTVACSIS (SEQ ID NO: 7)
	Anti-TROP2 domain	QVQLQQSGSELKKPGASVKVSCKASGYTFTNYGMNWVKQAPGQGLKWMGWINTYTGEPTYTDDFKGRFAFSLDTSVSTAYLQISSLKADDTAVYFCARGGFGSSYWYFDVWGQGSLVTVSSGGGGSGGGGSGGGGSDIQLTQSPSSLSASVGDRVSITCKASQDVSIAVAWYQQKPGKAPKLLIYSASYRYTGVPDRFSGSGSGTDFTLTISSLQPEDFAVYYCQQHYITPLTFGAGTKVEIKR (SEQ ID NO: 3)
	Linker	SGGGGAAADYKDDDDKGS (SEQ ID NO: 9)
	CD89 TMD	DSIHQDYTTQNLIRMAVAGLVLVALLAILV (SEQ ID NO: 56)

	CD40 ICD	KKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGC QPVTQEDGKESRISVQERQ (SEQ ID NO: 18)
	Linker	GS
	FcR ICD	RLKIQVRKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPPQ (SEQ ID NO: 15)
Anti-TROP2-CD89-TRIF	MWLQSLLLLGTVACSISQVQLQQSGSELKKPGASVKVSCKASGYTFTNYGMN WVKQAPGQGLKWMGWINTYTGEPTYTDDFKGRFAFSLDTSVSTAYLQISSLK ADDTAVYFCARGGFGSSYWYFDVWGQGSLVTVSSGGGGSGGGGSGGGGSDI QLTQSPSSLSASVGDRVSITCKASQDVSIAVAWYQQKPGKAPKLLIYSASYRY TGVPDRFSGSGSGTDFTLTISSLQPEDFAVYYCQQHYITPLTFGAGTKVEIKRS GGGGAAADYKDDDDKGSDSIHQDYTTQNLIRMAVAGLVLVALLAILVRGSIR TLQSNLGCLPPSSALPSGTRSLPRPIDGVSDWSQGCSLRSTGSPASLASNLEISQ SPTMPFLSLHRSPHGPSKLCDDPQASLVPEPVPGGCQEPEEMSWPPSGEIASPP ELPSSPPPGLPEVAPDATSTGLPDTPAAPETSTNYPVECTEGSAGPQSLPLPILEP VKNPCSVKDQTPLQLSVEDTTSPNTKPCPPTPTTPETSPPPPPPPSSTPCSAHLT PSSLFPSSLE (SEQ ID NO: 39)	
	Signal peptide	MWLQSLLLLGTVACSIS (SEQ ID NO: 7)
	Anti-TROP2 domain	QVQLQQSGSELKKPGASVKVSCKASGYTFTNYGMNWVKQAP GQGLKWMGWINTYTGEPTYTDDFKGRFAFSLDTSVSTAYLQIS SLKADDTAVYFCARGGFGSSYWYFDVWGQGSLVTVSSGGGG SGGGGSGGGGSDIQLTQSPSSLSASVGDRVSITCKASQDVSIAV AWYQQKPGKAPKLLIYSASYRYTGVPDRFSGSGSGTDFTLTISS LQPEDFAVYYCQQHYITPLTFGAGTKVEIKR (SEQ ID NO: 3)
	Linker	SGGGGAAADYKDDDDKGS (SEQ ID NO: 9)
	CD89 TMD	DSIHQDYTTQNLIRMAVAGLVLVALLAILV (SEQ ID NO: 56)
	TRIF-ICD	RGSIRTLQSNLGCLPPSSALPSGTRSLPRPIDGVSDWSQGCSLRS TGSPASLASNLEISQSPTMPFLSLHRSPHGPSKLCDDPQASLVPE PVPGGCQEPEEMSWPPSGEIASPPELPSSPPPGLPEVAPDATSTG LPDTPAAPETSTNYPVECTEGSAGPQSLPLPILEPVKNPCSVKD QTPLQLSVEDTTSPNTKPCPPTPTTPETSPPPPPPPSSTPCSAHL TPSSLFPSSLE (SEQ ID NO: 21)
Anti-TROP2-CD89-	MWLQSLLLLGTVACSISQVQLQQSGSELKKPGASVKVSCKASGYTFTNYGMNWVKQ APGQGLKWMGWINTYTGEPTYTDDFKGRFAFSLDTSVSTAYLQISSLKADDTAVYFC ARGGFGSSYWYFDVWGQGSLVTVSSGGGGSGGGGSGGGGSDIQLTQSPSSLSASVGD RVSITCKASQDVSIAVAWYQQKPGKAPKLLIYSASYRY	

FcR-TRIF	TGVPDRFSGSGSGTDFTLTISSLQPEDFAVYYCQQHYITPLTFGAGTKVEIKRSGGGGAAADYKDDDDKGSDSIHQDYTTQNLIRMAVAGLVLVALLAILVRLKIQVRKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPPQGSGSIRTLQSNLGCLPPSSALPSGTRSLPRPIDGVSDWSQGCSLRSTGSPASLASNLEISQSPTMPFLSLHRSPHGPSKLCDDPQASLVPEPVPGGCQEPEEMSWPPSGEIASPPELPSSPPPGLPEVAPDATSTGLPDTPAAPETSTNYPVECTEGSAGPQSLPLPILEPVKNPCSVKDQTPLQLSVEDTTSPNTKPCPPTPTTPETSPPPPPPPSSTPCSAHLTPSSLFPSSLE (SEQ ID NO: 40)	
	Signal peptide	MWLQSLLLLGTVACSIS (SEQ ID NO: 7)
	Anti-TROP2 domain	QVQLQQSGSELKKPGASVKVSCKASGYTFTNYGMNWVKQAPGQGLKWMGWINTYTGEPTYTDDFKGRFAFSLDTSVSTAYLQISSLKADDTAVYFCARGGFGSSYWYFDVWGQGSLVTVSSGGGGSGGGGSGGGGSDIQLTQSPSSLSASVGDRVSITCKASQDVSIAVAWYQQKPGKAPKLLIYSASYRYTGVPDRFSGSGSGTDFTLTISSLQPEDFAVYYCQQHYITPLTFGAGTKVEIKR (SEQ ID NO: 3)
	Linker	SGGGGAAADYKDDDDKGS (SEQ ID NO: 9)
	CD89 TMD	DSIHQDYTTQNLIRMAVAGLVLVALLAILV (SEQ ID NO: 56)
	FcR ICD	RLKIQVRKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPPQ (SEQ ID NO: 15)
	Linker	GS
	TRIF-ICD	GSIRTLQSNLGCLPPSSALPSGTRSLPRPIDGVSDWSQGCSLRSTGSPASLASNLEISQSPTMPFLSLHRSPHGPSKLCDDPQASLVPEPVPGGCQEPEEMSWPPSGEIASPPELPSSPPPGLPEVAPDATSTGLPDTPAAPETSTNYPVECTEGSAGPQSLPLPILEPVKNPCSVKDQTPLQLSVEDTTSPNTKPCPPTPTTPETSPPPPPPPSSTPCSAHLTPSSLFPSSLE (SEQ ID NO: 20)
Anti-TROP2-CD89	MWLQSLLLLGTVACSISQVQLQQSGSELKKPGASVKVSCKASGYTFTNYGMNWVKQAPGQGLKWMGWINTYTGEPTYTDDFKGRFAFSLDTSVSTAYLQISSLKADDTAVYFCARGGFGSSYWYFDVWGQGSLVTVSSGGGGSGGGGSGGGGSDIQLTQSPSSLSASVGDRVSITCKASQDVSIAVAWYQQKPGKAPKLLIYSASYRYTGVPDRFSGSGSGTDFTLTISSLQPEDFAVYYCQQHYITPLTFGAGTKVEIKRGSGGSDSIHQDYTTQN*LIRMAVAGLVLVALLAILV*ENWHSHTALNKEASADVAEPSWSQQMCQPGLTFARTPSVCK (SEQ ID NO: 41)	
	Signal peptide	MWLQSLLLLGTVACSIS (SEQ ID NO: 7)

	Anti-TROP2 scFv VH domain	QVQLQQSGSELKKPGASVKVSCKASGYTFTNYGMNWVKQAPGQGLKWMGWINTYTGEPTYTDDFKGRFAFSLDTSVSTAYLQISSLKADDTAVYFCARGGFGSSYWYFDVWGQGSLVTVSS (SEQ ID NO: 1)
	Linker 1	GGGGSGGGGSGGGGS (SEQ ID NO: 57)
	Anti-TROP2 scFv VL domain	DIQLTQSPSSLSASVGDRVSITCKASQDVSIAVAWYQQKPGKAPKLLIYSASYRYTGVPDRFSGSGSGTDFTLTISSLQPEDFAVYYCQQHYITPLTFGAGTKVEIKR (SEQ ID NO: 2)
	Linker 2	GSGGS (SEQ ID NO: 58)
	CD89 TM and ICD (italicized section denotes the transmembrane domain [TM] sequence)	DSIHQDYTTQN*LIRMAVAGLVLVALLAILV*ENWHSHTALNKEASADVAEPSWSQQMCQPGLTFARTPSVCK (SEQ ID NO: 48)

[00200] 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: an extracellular domain comprising an antigen binding domain specific to an antigen of a target cell; a transmembrane domain; and an intracellular domain comprising an intracellular signaling domain; and wherein the transmembrane domain and the extracellular domain are operatively linked; and wherein upon binding of the CFP to the antigen on the target cell, the killing or phagocytosis activity of the myeloid cell, such as a neutrophil, monocyte, myeloid dendritic cell (mDC), mast cell or macrophage cell expressing the CFP is increased by at least 1.1-fold, 1.5-fold, 2-fold, 2.5-fold, 3-fold, 3.5-fold, 4-fold, 4.5-fold, 5-fold, 5.5-fold, 6-fold, 6.5-fold, 7-fold, 7.5-fold, 8-fold, 8.5-fold, 9-fold, 9.5-fold, 10-fold, 11-fold, 12-fold, 13-fold, 14-fold, 15-fold, 16-fold,-fold, 17-fold, 18-fold, 19-fold, 20-fold, 25-fold, 30-fold, 40-fold, 50-fold, 75-fold, or 100-fold compared to a cell not expressing the CFP.

[00201] Provided herein is a recombinant nucleic acid sequence encoding a CFP as described in the immediate above paragraph, wherein the intracellular domain comprises at least one innate immune activating intracellular domain, e.g., a pattern recognition receptor intracellular signaling domain, a TLR intracellular signaling domain, an FcR intracellular signaling domain, an intracellular adapter protein signaling domain, or fragments thereof, that is capable of activating innate immune response of the myeloid cell, activating its phagocytic potential, activating inflammatory cytokine and chemokine response, antigen presentation and T cell activation of the myeloid cell that expresses the

intracellular domain of a CFP comprises an intracellular signaling domain derived from an TRIF intracellular signaling domain having at least 90% sequence identity to any one of SEQ ID NOs: 19-22; wherein the CFP comprises and extracellular binding domain capable of binding a TROP2 molecule on a target cell, a CD89 transmembrane domain, and/or one or more additional intracellular signaling domains.

[00208] In some embodiments, an exemplary anti-TROP2-binding CFP described herein comprises an extracellular antigen binding domain having a sequence of any one of SEQ ID NOs: 1-3, or a heavy chain variable domain comprising a CDR3 sequence of GGFGSSYWYFDV (SEQ ID NO: 42), and/or a light chain variable domain comprising a CDR3 sequence of QQHYITPLT (SEQ ID NO: 43) and further comprises an intracellular domain of any one of the sequences in Table 2 or Table 3. In some embodiments, an exemplary anti-TROP2-binding CFP described herein comprises a sequence with at least 80-100% sequence identity to any one of SEQ ID NOs: 26-33.

[00209] In some embodiments, provided herein is a pharmaceutical product comprising a recombinant mRNA encoding a chimeric antigen receptor comprising: an anti-TROP2 binding scFv extracellular domain; and a CD89 transmembrane domain; wherein the pharmaceutical product is formulated in an aqueous formulation for delivery systemically. In some embodiments, the pharmaceutical composition comprises an scFv comprising a heavy chain and a light chain, the heavy chain comprising a CDR3 having a sequence GGFGSSYWYFDV (SEQ ID NO: 42), and the light chain comprising a CDR3 having a sequence QQHYITPLT (SEQ ID NO: 43). In some embodiments, the heavy chain further comprises a CDR1 sequence of NYGMN (SEQ ID NO: 44), and a CDR2 sequence of WINTYTGEPTYTDDFKG (SEQ ID NO: 45); and the light chain further comprises a CDR1 sequence of KASQDVSIAVA (SEQ ID NO: 46), and a CDR2 sequence of SASYRYT (SEQ ID NO: 47). The pharmaceutical composition described above comprises a CD89 transmembrane domain having a sequence LIRMAVAGLVLVALLAILV (SEQ ID NO: 11).

[00210] An exemplary anti-TROP2 second generation chimeric fusion protein (e.g., a drug product) comprising an anti-TROP2 scFv comprising a heavy chain variable domain having a sequence (CDRs highlighted according to Kabat naming convention) QVQLQQSGSELKKPGASVKVSCKASGYTFTNYGMNWVKQAPGQGLKWMGWINTYTGEPTYTDDFKGRFAFSLDTSVSTAYLQISSLKADDTAVYFCARGGFGSSYWYFDVWGQGSLVTVSSG (SEQ ID NO: 59). An exemplary TROP2 ScFv complete construct has the following sequence, **MWLQSLLLLGTVACSIS**QVQLQQSGSELKKPGASVKVSCKASGYTFTNYGMNWVKQAPGQGLKWMGWINTYTGEPTYTDDFKGRFAFSLDTSVSTAYLQISSLKADDTAVYFCARGGFGSSYWYFDVWGQGSLVTVSSGGGGSGGGGSGGGGSDIQLTQSPSSLSASVGDRVSITCKASQDVSIAVAWYQQKPGKAPKLLIYSASYRYTGVPDRFSGSGSGTDFTLTISSLQPEDFAVYYCQQHYITPLTFGAGTKVEIKR*GSGGS*DSIHQDYTTQN*LIRMAVAGLVLVALLAILV*ENWHSHTALNKEASADVAEPSWSQQMCQPGLTFARTPSVCK. (SEQ ID NO: 41) The bold letters represent the signal peptide sequence. A mature protein may lack this sequence, and

surgical resection will be followed through Week 48. If patient develops progressive disease, they may resume dosing on the dose and dose schedule when they discontinued.

[00342] All patients should be closely monitored over the course of their treatment. NCI CTCAE version 5.0 will be used to grade all adverse event and to provide dose reduction, delay, or cessation guidelines in the event of treatment-related toxicity. All patients will also have CT/MRI scans throughout the study to assess for progression of disease as well as response to study drug

[00343] The development of any Grade 2 or 3 treatment-related toxicity at the time of a scheduled treatment day will delay treatment by one week. If toxicity has resolved to Grade 1 or lower at that time, treatment may continue with the dose reduced by 25%. If the toxicity reoccurs, the treatment can continue with a 50% reduction from initial dose, and if the toxicity worsened treatment will be permanently discontinued. The decision to continue or discontinue treatment is based solely on physician discretion. The development of any Grade 4 treatment related toxicities, the patient should be discontinued permanently.

[00344] In Part A of the study a Continuous Reassessment Method (CRM) will be used to determine the recommended dose for Part B. Primary assessment of safety and tolerability will be at Day 28. Primary assessment of efficacy will be at 12 weeks. Subjects may enroll in a long-term extension study at 48 weeks if they have stable disease (SD), partial response (PR), or complete response (CR).

[00345] In Part B the maximum acceptable dose (MAD) from Part A will be assessed for safety, tolerability, and efficacy in patients with CRLM. Primary assessment of efficacy will be objective response rate (ORR) at 12 weeks.

[00346] In the event of treatment termination due to unacceptable toxicity the patient will continue in the study until the occurrence of progression of disease, at which time an end-of-study evaluation will be performed, with additional follow-up required until resolution or stabilization of any treatment-related toxicity. All patients will be followed for survival.

[00347] To ensure the safety of study subjects, the first two subjects in each dose cohort will be staggered by an interval of 14 days. If there are no safety concerns, then the remaining subjects may enroll at that dose level simultaneously.

[00348] The pharmaceutical composition comprises an scFv comprising a heavy chain and a light chain, the heavy chain comprising a CDR3 having a sequence GGFGSSYWYFDV (SEQ ID NO: 42), and the light chain comprising a CDR3 having a sequence QQHYITPLT (SEQ ID NO: 43). In some embodiments, the heavy chain further comprises a CDR1 sequence of NYGMN (SEQ ID NO: 44), and a CDR2 sequence of WINTYTGEPTYTDDFKG (SEQ ID NO: 45); and the light chain further comprises a CDR1 sequence of

KASQDVSIAVA (SEQ ID NO: 46), and a CDR2 sequence of SASYRYT (SEQ ID NO: 47). The pharmaceutical composition described above comprises a CD89 transmembrane domain having a sequence LIRMAVAGLVLVALLAILV (SEQ ID NO: 11).

[00349] An exemplary anti-TROP2 second generation chimeric fusion protein (e.g., a drug product) comprising an anti-TROP2 scFv comprising a heavy chain variable domain having a sequence (CDRs highlighted according to Kabat naming convention) QVQLQQSGSELKKPGASVKVSCKASGYTFTNYGMNWVKQAPGQGLKWMGWINTYTG EPTYTDDFKGRFAFSLDTSVSTAYLQISSLKADDTAVYFCARGGFGSSYWYFDVWGQG SLVTVSSG (SEQ ID NO: 59).

[00350] An exemplary anti-TROP2 chimeric fusion protein comprising an antiTROP2 scFv comprises a light chain variable domain having a sequence DIQLTQSPSSLSASVGDRVSITCKASQDVSIAVAWYQQKPGKAPKLLIYSASYRYTGVPD RFSGSGSGTDFTLTISSLQPEDFAVYYCQQHYITPLTFGAGTKVEIKR (SEQ ID NO: 2)

[00351] An exemplary TROP2 scFv construct has the following sequence,

[00352] **MWLQSLLLLGTVACSIS**QVQLQQSGSELKKPGASVKVSCKASGYTFTNYGMNWVKQ APGQGLKWMGWINTYTGEPTYTDDFKGRFAFSLDTSVSTAYLQISSLKADDTAVYFCARGGFG SSYWYFDVWGQGSLVTVSSGGGGSGGGGSGGGGSDIQLTQSPSSLSASVGDRVSITCKASQDVS IAVAWYQQKPGKAPKLLIYSASYRYTGVPDRFSGSGSGTDFTLTISSLQPEDFAVYYCQQHYITP LTFGAGTKVEIKRGSGGSDSIHQDYTTQN*LIRMAVAGLVLVALLAILV*ENWHSHTALNKEASADVA EPSWSQQMCQPGLTFARTPSVCK (SEQ ID NO: 41)

The bold letters represent the signal peptide sequence. A mature protein may lack this sequence, and one of skill in the art can interpret the protein sequence as expressed without the signal peptide sequence. The underlined regions are the CDR sequence of the scFV that binds to TROP2 in sequence, CDR1, CDR2, CDR3 for the heavy and light chains. The italicize alphabets represent the amino acid sequence of the CD89 TMD.

[00353] An exemplary TROP2 scFv product used for in vivo study can comprise a sequence QVQLQQSGSELKKPGASVKVSCKASGYTFTNYGMNWVKQAPGQGLKWMGWINTYTGEPTYT DDFKGRFAFSLDTSVSTAYLQISSLKADDTAVYFCARGGFGSSYWYFDVWGQGSLVTVSSGGG GSGGGGSGGGGSDIQLTQSPSSLSASVGDRVSITCKASQDVSIAVAWYQQKPGKAPKLLIYSASY RYTGVPDRFSGSGSGTDFTLTISSLQPEDFAVYYCQQHYITPLTFGAGTKVEIKRSGGGGAAADY KDDDDKGSDSIHQDYTTQNLIRMAVAGLVLVALLAILVENWHSHTALNKEASADVAEPSWSQQ MCQPGLTFARTPSVCK (SEQ ID NO: 27)

[00354] An exemplary TROP2 scFv product used for in vivo study can comprise a sequence

[00355] QVQLQQSGSELKKPGASVKVSCKASGYTFTNYGMNWVKQAPGQGLKWMGWINTYTG EPTYTDDFKGRFAFSLDTSVSTAYLQISSLKADDTAVYFCARGGFGSSYWYFDVWGQGSLVTVS SGGGGSGGGGSGGGGSDIQLTQSPSSLSASVGDRVSITCKASQDVSIAVAWYQQKPGKAPKLLIY SASYRYTGVPDRFSGSGSGTDFTLTISSLQPEDFAVYYCQQHYITPLTFGAG

TKVEIKRGSGGSDSIHQDYTTQN*LIRMAVAGLVLVALLAILV*ENWHSHTALNKEASADVAEPSWSQ QMCQPGLTFARTPSVCK (SEQ ID NO: 26)

[00356] This approach takes advantage of the natural propensity for intravenous (i.v) delivery.

[00357] The proprietary mRNA construct encodes for a receptor consisting of a TROP2-targeted scFv (derived from the complementarity-determining regions (CDR) of sacituzumab), as well as the transmembrane domain and cytoplasmic tail of CD89. Upon administration of the anti-TROP2 binder, the LNP is taken up by numerous cell types, however, a functional CAR can only be expressed on the surface of cells that also express the Fc receptor common gamma chain, predominately myeloid cells. The common gamma chain includes the immunoreceptor tyrosine-based activation motif (ITAM) domains necessary for cellular signaling. Upon scFV recognition of TROP2, the intracellular signaling domains become activated, resulting in tumor cell phagocytosis, inflammatory cytokine production and presentation of tumor antigens to T cells.

[00358] The primary objective of this study is to evaluate the safety and tolerability of anti-TROP2 second generation chimeric fusion protein in subjects with $TROP2^{+}$ metastatic colorectal cancer, and to establish the maximum acceptable dose (MAD) and recommended Phase 2 dose (RP2D), based on observed adverse events (AEs) including all potential dose limiting toxicities (DLTs).

[00359] The secondary objectives of the this study are to determine: (i) Pharmacokinetics (ii) Objective response of anti-TROP2 second generation chimeric fusion protein [Time Frame: Up to 12 months] (iii) Duration of response.

[00360] Secondary objectives further include:

- Area under the curve [Time frame: Up to 12 months]
- Maximum plasma concentration [Time Frame: Up to 12 months]
- Time of maximum plasma concentration [Time Frame: Up to 12 months]
- Half-life [Time Frame: Up to 12 months]
- Objective response of anti-TROP2 second generation chimeric fusion PROTEIN [Time Frame: Up to 12 months]
- Duration of response

[00361] The exploratory objectives of this study are to determine (a) Preliminary efficacy including Progression Free Survival (PFS) and Overall Survival (OS) (b) Conversion to surgical candidate (c) Correlative biomarker studies.

[00362] In general, the exploratory objective include:

- Progression Free Survival (PFS)
- Overall Survival (OS)
- Conversion to surgical candidate

[00379] Table 6. The schedule of events

Table 6. Schedule of Events																		
	Screening & Prep		Cycle 1												Cycle 2,3	Biopsy	Cycles 4-12	End of Study
			Dose 1			Dose 2			Dose 3			Dose 4			Dose 5-12		Dose 13-30	
Study Day	S1	Baseline	D1	D2	D3	D 8	D9	D 10	D15	D16	D17	D22	D23	D24	D29-D90	D91	D91-D248	
Study Week/ Month			W1			W2			W3			W4			W5-W12	W12	W13-W48	
Window	**-28**	**-5**		**0**	**0**	**1**	**0**	**0**	**1**	**0**	**0**	**1**	**0**	**0**	**±3**	**±3**	**+/-7 days**	
Informed Consent	X																	
Confirm eligibility	X																	
Medical History	X																	
Complete Physical Exam	X																	
Targeted Physical Exam			X	X	X	X	X	X	X	X	X	X	X	X	X		X	X
Vital Signs	X		X	X	X	X	X	X	X	X	X	X	X	X	X		X	X
ECOG Performance Score	X																	
12 Lead ECG	X																	
ECHO																		
MRI/CT	X											X			X		X	X
Pulse Oximetry	X		X			X			X									
Laboratory:																		
Serum Chemistry	X	X	X			X			X			X			X		X	X
CEA	X		X			X			X			X			X		X	X
CBC w/ Differential, Platelets	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X
Uninalysis	X		X															X
INR and aPTT	X		X			X			X			X			X		X	X

[00380] Example 2. A Multicenter, open-label, Phase 1 first-in-human study to assess the safety, tolerability in advanced epithelial cancer.

[00381] The pharmaceutical composition comprises an scFv comprising a heavy chain and a light chain, the heavy chain comprising a CDR3 having a sequence GGFGSSYWYFDV (SEQ ID NO: 42), and the light chain comprising a CDR3 having a sequence QQHYITPLT (SEQ ID NO: 43). In some embodiments, the heavy chain further comprises a CDR1 sequence of NYGMN (SEQ ID NO: 44), and a CDR2 sequence of WINTYTGEPTYTDDFKG (SEQ ID NO: 45); and

the light chain further comprises a CDR1 sequence of KASQDVSIAVA (SEQ ID NO: 46), and a CDR2 sequence of SASYRYT (SEQ ID NO: 47). The pharmaceutical composition described above comprises a CD89 transmembrane domain having a sequence LIRMAVAGLVLVALLAILV (SEQ ID NO: 11).

[00382] The study has 4 Cohorts. Each Cohort has 4 Cycles. For Cohorts 1-3, the dosing regimen will be every 14 days for 3 doses, followed by administration once every 28 days for three doses. For Cohort 4, the dosing regimen will be modified. Participants will receive one dose of anti-TROP2-chimeric antigen receptor investigational drug every week for 3 doses, followed by administration once every 28 days for three additional doses.

[00383] The conditions treated in this clinical study include malignant epithelial tumors.

[00384] The study type is interventional, Phase 1.

[00385] The study model is a Single Group Assignment. Number of arm, 1.

[00386] The study anticipates to enroll 48 patients.

[00387] **Primary Outcome Measure:**

[00388] 1. To evaluate the safety and tolerability of the anti-TROP2-chimeric antigen receptor investigational drug through incidence of Adverse Events. Adverse Events will be graded according to the NCI-CTCAE, version 5.0 [Time Frame: Up to Week 20].

[00389] 2. To establish the maximum tolerated dose (MTD) based on dose limiting toxicities (DLTs) [Time Frame: Up to Week 20]

[00390] 3. to establish the maximum tolerated dose (MTD) based on recommended Phase 2 dose (RP2D) [Time Frame: Up to Week 20]

[00391] **Secondary Outcome Measure:**

[00392] 4. To further characterize the safety of the anti-trop2-chimeric antigen receptor investigational drug through incidence of Adverse Events. Adverse Events will be graded according to the NCI-CTCAE, version 5.0 [Time Frame: Up to Week 20].

[00393] 5. To determine the pharmacokinetics (PK) of the anti-trop2-chimeric antigen receptor investigational drug PK Parameter: Plasma concentrations [Time Frame: Up to Week 20]

[00394] 6. To determine the pharmacokinetics (PK) of the anti-trop2-chimeric antigen receptor investigational drug PK Parameter: Area under the curve [Time Frame: Up to Week 20]

[00395] 7. To determine the pharmacokinetics (PK) of the anti-trop2-chimeric antigen receptor investigational drug PK Parameter: Maximum observed plasma concentration [Time Frame: Up to Week 20]

[00396] 8. To determine the pharmacokinetics (PK) of the anti-trop2-chimeric antigen receptor investigational drug PK Parameter: Time of maximum observed plasma concentration [Time Frame: Up to Week 20]

[00397] 9. To determine the pharmacokinetics (PK) of the anti-trop2-chimeric antigen receptor investigational drug PK Parameter: Apparent terminal Half-life [Time Frame: Up to Week 20]