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(54) **Title:** SIGLEC-BASED CHIMERIC POLYPEPTIDES AND USES THEREOF

(57) **Abstract:** Provided are Siglec-based chimeric polypeptides. Accordingly there is provided a chimeric receptor comprising: (a) an extracellular domain comprising an amino acid sequence of a Siglec-7 or a Siglec-9 receptor capable of binding a Siglec-7 and/or a Siglec-9 ligand; and (b) an intracellular domain comprising an amino acid sequence capable of transmitting a co- stimulatory signal in an immune cell expressing the chimeric receptor upon binding of said extracellular domain to said ligand. Also provided are polynucleotides encoding same, cells expressing same and methods of use thereof.

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SIGLEC-BASED CHIMERIC POLYPEPTIDES AND USES THEREOF

RELATED APPLICATION

This application claims priority from US Patent Application No. 62/834,995 filed on April 17, 2019, the contents of which are incorporated herein by reference in their entirety.

SEQUENCE LISTING STATEMENT

The ASCII file, entitled 82417_ST25, created on 15 April 2020, comprising 11,052 bytes, submitted concurrently with the filing of this application is incorporated herein by reference.

FIELD AND BACKGROUND OF THE INVENTION

The present invention, in some embodiments thereof, relates to Siglec-based chimeric polypeptides and uses thereof.

Siglecs (Sialic acid-binding immunoglobulin-type lectins) are cell surface proteins that bind sialic acid, and more specifically sialic acid–containing carbohydrates (sialoglycans). There are 14 different mammalian Siglecs, providing an array of different functions based on cell surface receptor-ligand interactions. These receptor-glycan interactions can mediate, among other things, cell adhesion and cell signaling. Although sialic acid is ubiquitously expressed, typically at the terminal position of glycoproteins and lipids, only very specific, distinct sialoglycan structures are recognized by individual Siglecs, depending on identity and linkage to sub-terminal carbohydrate moieties.

Siglecs are Type I transmembrane proteins that contain an extracellular N-terminal V-type immunoglobulin-like domain (Ig-like domain) that acts as the binding receptor for sialic acid. All Siglecs extend from the cell surface by means of intervening C2-type Ig-like domains which have no binding activity. The cytoplasmic domains of most Siglecs contain immunoreceptor tyrosine-based inhibitory motifs (ITIMs), which recruit the tyrosine phosphatases, SHP1 and/or SHP2 thereby inhibit immune cell activation.

Upon malignant transformation, sialylation of cell surface glycoconjugates is frequently altered in tumor cells, resulting in the expression of sialylated tumor-associated carbohydrate antigens that are specific markers for the disease. As sialoglycans are involved in many biological processes, their expression by tumor cells has been shown to influence many crucial steps in tumor biology, including immune-editing, tumor growth and proliferation, invasion, metastasis, and angiogenesis (Kannagi R, et al. *Cancer Sci.* 2010; 101: 586-93).

Over the years it has been observed that expression of sialic acids on cancer cells serves as a mechanism for evading the immune response elicited against the cancer. By this strategy, tumor cells might counter, for example, NK cell activation by interacting with the ITIM-containing Siglec receptors expressed on the NK cell (Jandus C, et al. *J. Clin. Invest* 2014; 124: 1810-20).

Consequently, direct targeting of cancer cells expressing siglecs or inhibiting siglecs' activity has been suggested for the treatment of cancer (e.g. Angata et al. (2015), TRENDS IN PHARMACOLOGICAL SCIENCES, 36, 10, 645-660; and International Patent Application Publication Nos: WO2018002640 and WO2017123745).

Additional background art includes International Patent Application Publication No: WO2016178996.

SUMMARY OF THE INVENTION

According to an aspect of some embodiments of the present invention there is provided a chimeric receptor comprising:

(a) an extracellular domain comprising an amino acid sequence of a Siglec-7 or a Siglec-9 receptor capable of binding a Siglec-7 and/or a Siglec-9 ligand and an Ig-like C2-type domain; and

(b) an intracellular domain comprising an amino acid sequence capable of transmitting an activating signal and/or a co-stimulatory signal in an immune cell expressing the chimeric receptor upon binding of the extracellular domain to the ligand.

According to an aspect of some embodiments of the present invention there is provided a chimeric receptor comprising:

(a) an extracellular domain comprising an amino acid sequence of a Siglec-7 or a Siglec-9 receptor capable of binding a Siglec-7 and/or a Siglec-9 ligand; and

(b) an intracellular domain comprising an amino acid sequence capable of transmitting a co-stimulatory signal in an immune cell expressing the chimeric receptor upon binding of the extracellular domain to the ligand, the intracellular domain being devoid of an amino acid sequence capable of transmitting an activating signal.

According to an aspect of some embodiments of the present invention there is provided a chimeric receptor comprising:

(a) an extracellular domain comprising an amino acid sequence of a Siglec-7 or a Siglec-9 receptor capable of binding a Siglec-7 and/or a Siglec-9 ligand;

(b) an intracellular domain comprising an amino acid sequence capable of transmitting an activating signal and/or a co-stimulatory signal in an immune cell expressing the chimeric receptor upon binding of the extracellular domain to the ligand; and

(c) a transmembrane domain of CD28 or CD8a.

According to an aspect of some embodiments of the present invention there is provided a chimeric receptor comprising:

(a) an extracellular domain comprising an amino acid sequence of a Siglec-7 or a Siglec-9 receptor capable of binding a Siglec-7 and/or a Siglec-9 ligand;

(b) an intracellular domain comprising an amino acid sequence capable of transmitting an activating signal and/or a co-stimulatory signal in an immune cell expressing the chimeric receptor upon binding of the extracellular domain to the ligand; and

(c) a transmembrane domain of CD28.

According to some embodiments of the invention, the extracellular domain comprises an Ig-like C2-type domain.

According to some embodiments of the invention, the Ig-like C2-type domain is of the Siglec-7 or the Siglec-9 receptor.

According to some embodiments of the invention, the Ig-like C2-type domain is located C-terminally to the amino acid sequence capable of binding the Siglec-7 and/or the Siglec-9 ligand.

According to some embodiments of the invention, the Ig-like C2-type domain is a single Ig-like C2-type domain.

According to some embodiments of the invention, the Ig-like C2-type domain is the third Ig-like domain located N to C in a full length Siglec-7 or Siglec-9 receptor.

According to some embodiments of the invention, an amino acid sequence of the Ig-like C2-type domain comprises an amino acid sequence having at least 80 % identity to:
EGTASTALGNSSSLSVLEGQSLRLVCAVDSNPPARLSWTWRSLTLYPSQPSNPLVLELQV HLGDEGEFTCRAQNSLGSQHVSLNLSLQQEY (SEQ ID NO: 4); or
DGTVSTVLGNGSSLSLPEGQSLRLVCAVDAVDSNPPARLSLSWRGLTLCPSQPSNPGVLE LPWVHLRDAAEFTCRAQNPLGSQQVYLNVSLQSKA (SEQ ID NO: 8).

According to some embodiments of the invention, the amino acid sequence of the Ig-like C2-type domain comprises:

EGTASTALGNSSSLSVLEGQSLRLVCAVDSNPPARLSWTWRSLTLYPSQPSNPLVLELQV HLGDEGEFTCRAQNSLGSQHVSLNLSLQQEY (SEQ ID NO: 4); or DGTVSTVLGNGSSLSLPEGQSLRLVCAVDAVDSNPPARLSLSWRGLTLCPSQPSNPGVLE LPWVHLRDAAEFTCRAQNPLGSQQVYLNVSLQSKA (SEQ ID NO: 8).

According to some embodiments of the invention, the extracellular domain is devoid of an Ig-like C2-type domain which is the second Ig-like domain located N to C in a full length Siglec-7 or Siglec-9 receptor.

According to some embodiments of the invention, the extracellular domain is devoid of an Ig-like C2-type domain comprising an amino acid sequence of:

HRPNILIPGTLESGCFQNLTCSVPWACEQGTPPMISWMGTSVSPLHPSTTRSSVLT LIPQPQHHGTSLTCQVTLPGAGVTTNRTIQLNVS (SEQ ID NO: 3); or HRPNILIPGTLESGCPQNLTCSVPWACEQGTPPMISWIGTSVSPLDPSTTRSSVLTLIPQPQ DHGTSLTCQVTFPGASVTTNKTVHLNVS (SEQ ID NO: 7).

According to some embodiments of the invention, the intracellular domain is devoid of an amino acid sequence capable of transmitting an activating signal.

According to some embodiments of the invention, the amino acid sequence capable of transmitting an activating signal comprises an amino acid sequence of CD3zeta and/or FcR gamma.

According to some embodiments of the invention, the amino acid sequence capable of transmitting a co-stimulatory signal comprises an amino acid sequence of 4-1BB, OX40, CD28, CD27, ICOS, CD40L, GITR, LIGHT, HVEM and/or CD30.

According to some embodiments of the invention, the chimeric receptor comprising a transmembrane domain of CD28.

According to some embodiments of the invention, the chimeric receptor comprising a transmembrane domain of CD8a.

According to some embodiments of the invention, the chimeric receptor comprising a transmembrane domain of a Siglec-7 or a Siglec-9 receptor.

According to some embodiments of the invention, the amino acid sequence capable of binding the Siglec-7 and/or the Siglec-9 ligand comprises an amino acid sequence having at least 80 % identity to:

QKSNRKDYSLTMQSSVTVQEGMCVHVRCSFSYPVDSQTDSDPVHGYWFRAGNDISWK APVATNNPAWAVQEETRDRFHLLGDPQTKNCTLSIRDARMSDAGRYFFRMEKGNIKW NYKYDQLSVNVTAL (SEQ ID NO: 2); or GQTSKLLTMQSSVTVQEGLCVHVPCSFSYPSHGWIYPGPVVHGYWFREGANTDQDAPV

ATNNPARAVWEETRDRFHLLGDPHTKNCTLSIRDARRSDAGRYFFRMEKGSIKWNYKH HRLSVNVTAL (SEQ ID NO: 6).

According to some embodiments of the invention, the amino acid sequence capable of binding the Siglec-7 and/or the Siglec-9 ligand comprises:

QKSNRKDYSLTMQSSVTVQEGMCVHVRCSFSYPVDSQTDSDPVHGYWFRAGNDISWK APVATNNPAWAVQEETRDRFHLLGDPQTKNCTLSIRDARMSDAGRYFFRMEKGNIKW NYKYDQLSVNVTAL (SEQ ID NO: 2); or

GQTSKLLTMQSSVTVQEGLCVHVPCSFSYPSHGWIYPGPVVHGYWFREGANTDQDAPV ATNNPARAVWEETRDRFHLLGDPHTKNCTLSIRDARRSDAGRYFFRMEKGSIKWNYKH HRLSVNVTAL (SEQ ID NO: 6).

According to an aspect of some embodiments of the present invention there is provided a polynucleotide encoding the chimeric receptor.

According to an aspect of some embodiments of the present invention there is provided a host cell expressing the chimeric receptor.

According to some embodiments of the invention, the host cell being an immune cell.

According to some embodiments of the invention, the immune cell is a T cell.

According to some embodiments of the invention, the immune cell is an NK cell.

According to an aspect of some embodiments of the present invention there is provided an NK cell expressing a chimeric receptor comprising:

(a) an extracellular domain comprising an amino acid sequence of a Siglec-7 or a Siglec-9 receptor capable of binding a Siglec-7 and/or a Siglec-9 ligand; and

(b) an intracellular domain comprising an amino acid sequence capable of transmitting an activating signal and/or a co-stimulatory signal in an immune cell expressing the chimeric receptor upon binding of the extracellular domain to the ligand.

According to some embodiments of the invention, the chimeric receptor is the chimeric receptor disclosed herein.

According to an aspect of some embodiments of the present invention there is provided a method of treating a disease associated with cells expressing a Siglec-7 and/or a Siglec-9 ligand in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the chimeric receptor, the polynucleotide or the cells, thereby treating the disease in the subject.

According to an aspect of some embodiments of the present invention there is provided the chimeric receptor, the polynucleotide or the cell, for use in the treatment of a disease associated with cells expressing the Siglec-7 and/or the Siglec-9 ligand.

According to some embodiments of the invention, the disease is cancer or an infection.

According to some embodiments of the invention, the cancer is selected form the group consisting of melanoma, hepatocellular cancer, pancreatic cancer, colon adenocarcinoma, cervical cancer, breast cancer, lung cancer lymphoma and leukaemia.

According to some embodiments of the invention, the cancer is selected form the group consisting of ovarian carcinoma, cervical cancer, pancreatic adenocarcinoma, leukaemia, melanoma, colon carcinoma, breast carcinoma and lung adenocarcinoma.

According to some embodiments of the invention, the infection is an HIV infection.

Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

Some embodiments of the invention are herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of embodiments of the invention. In this regard, the description taken with the drawings makes apparent to those skilled in the art how embodiments of the invention may be practiced.

In the drawings:

FIG. 1 is a schematic representation of the Siglec-7/9 chimeric constructs. The numbering relates to the amino acids coordinates in the original protein (SIGLEC 7: Q9Y286 (SIGL7_HUMAN); SIGLEC 9: Q9Y336 (SIGL9_HUMAN); CD28: P10747 (CD28_HUMAN); and P20963 (CD3Z_HUMAN). EC: extracellular domain, TM: transmembrane domain, IC: intracellular domain.

FIG. 2 shows flow cytometry analysis plots demonstrating electroporation efficiency of lymphocytes with the indicated Siglec-7/9 chimeric constructs. The percentage of positive cells and MFI (Mean Fluorescent Intensity) are indicated. The anti-fab staining of the non-electroporated control is shown as a white peak in each histogram. The results are representatives of 3 independent experiments performed with 3 different donors.

FIG. 3 shows flow cytometry analysis plots demonstrating expression of the Siglec-7/9 chimeric S7TM28 and S9TM28 constructs in transduced OKT3-stimulated human primary PBLs. The mock-transduced control is shown as a dotted line peak. The percentage of positive cells and the MFI are indicated.

FIG. 4 demonstrates expression of Siglec-7/9 ligands in the indicated cell lines, as determined by flow cytometry. Results are shown as the MFI ratio of stained sample out of background staining.

FIG. 5 shows graphs of IFNγ secretion by lymphocytes electroporated with the indicated Siglec-7/9 chimeric constructs following co-culture with the indicated cell lines, as determined by ELISA. The cell line A549 served as a negative control.

FIG. 6 shows graphs of IFNγ secretion by OKT3-stimulated human primary PBLs transduced with the indicated Siglec-7/9 chimeric constructs following co-culture with the indicated cell lines, as determined by ELISA. A truncated CD34 construct was used to transduce T-cells as a negative control. The results are representatives of 3 independent experiments.

FIG. 7 demonstrates the *in-vivo* anti-tumor effect of T cells transduced with Siglec-7/9 chimeric constructs. NSG-mice were inoculated 3 times with $5x10^6$ T-cells transduced with S7TM28 and S9TM28 constructs and $2X10^6$ K562 tumor cells in the upper flank (4 mice per group) in 200 µl Matrigel. Tumor growth was calculated as Dd^2*Pi/2.

FIG. 8 is a schematic representation of Siglec-7/9 chimeric constructs encompassing different Siglec-7/9 domains in the extracellular part of the receptor. The numbering relates to the amino acids coordinates in the original protein (SIGLEC 7: Q9Y286 (SIGL7_HUMAN); SIGLEC 9: Q9Y336 (SIGL9_HUMAN); CD28: P10747 (CD28_HUMAN); and P20963 (CD3Z_HUMAN). EC: extracellular domain, TM: transmembrane domain, IC: intracellular domain.

FIG. 9 shows flow cytometry analysis plots demonstrating expression of the indicated Siglec-7/9 chimeric constructs in transduced OKT3-stimulated human primary PBLs. The mock-transduced control is shown as a dotted line peak. The percentage of positive cells and the MFI (in brackets) are indicated.

FIG. 10 is a graph of IFNγ secretion by OKT3-stimulated human primary PBLs transduced with the indicated Siglec-7/9 chimeric constructs following co-culture with K562 cells, as determined by ELISA.

FIG. 11 is a schematic representation of different Siglec-7/9 chimeric Co-stimulatory switch receptors (CSR). The numbering relates to the amino acids coordinates in the original protein [SIGLEC 7: Q9Y286 (SIGL7_HUMAN); SIGLEC 9: Q9Y336 (SIGL9_HUMAN);

CD28: P10747 (CD28_HUMAN); CD8a: P01732 (CD8A_HUMAN); 41BB Q07011 (TNR9_HUMAN); and P20963 (CD3Z_HUMAN)]. EC: extracellular domain, TM: transmembrane domain, IC: intracellular domain.

FIG. 12 shows flow cytometry analysis plots demonstrating expression of the indicated Siglec-7/9 CSR constructs in transduced OKT3-stimulated human primary PBLs. The mock-transduced control is shown as a black line peak. The percentage of positive cells and the MFI are indicated.

FIG. 13 is a graph of TNFα secretion by OKT3-stimulated human primary PBLs transduced with the indicated Siglec-7/9 CSR + CD19-specific 4-1BB CAR constructs following co-culture with K562/cd19 or Raji cells, as determined by ELISA. Co-culture with K562 cells served as a negative control.

FIG. 14 is a graph of TNFα secretion by OKT3-stimulated human primary PBLs transduced with the indicated Siglec-9 CSR + NYESO1-specific 1G4 TCR constructs following co-culture with A375 and 624.38 cells, as determined by ELISA. Co-culture with 888A2 cells served as a negative control. The results were normalized to that obtained in the positive control - A375 with TCR 1G4 only.

FIG. 15 is a graph of TNFα secretion by OKT3-stimulated human primary PBLs transduced with the indicated Siglec-9 CSRs + MART1-specific F4 TCR constructs following co-culture with 888A2 and SKMEL23 cells, as determined by ELISA. Co-culture with A375 cells served as a negative control.

FIG. 16 is a graph of IFNγ secretion by OKT3-stimulated human primary PBLs transduced with the indicated Siglec-9 CSRs + MART1-specific F4 TCR constructs following co-culture with 888A2, 624.38 and SKMEL23 cells, as determined by ELISA. Co-culture with A375 cells served as a negative control.

DESCRIPTION OF SPECIFIC EMBODIMENTS OF THE INVENTION

The present invention, in some embodiments thereof, relates to Siglec-based chimeric polypeptides and uses thereof.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not necessarily limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways.

Siglecs (Sialic acid-binding immunoglobulin-type lectins) are cell surface Type I transmembrane proteins that bind sialic acid, and more specifically sialic acid–containing

carbohydrates (sialoglycans). Siglecs contain an extracellular N-terminal V-type immunoglobulin-like domain (Ig-like domain) that acts as the binding receptor for sialic acid. All Siglecs extend from the cell surface by means of intervening C2-type Ig-like domains which have no binding activity. The cytoplasmic domains of most Siglecs contain immunoreceptor tyrosine-based inhibitory motifs (ITIMs), which recruit the tyrosine phosphatases, SHP1 and/or SHP2 thereby inhibit immune cell activation.

Upon malignant transformation, sialylation of cell surface glycoconjugates is frequently altered in tumor cells, resulting in the expression of sialylated tumor-associated carbohydrate antigens that are specific markers for the disease. As sialoglycans are involved in many biological processes, their expression by tumor cells has been shown to influence many crucial steps in tumor biology. Over the years it has been observed that expression of sialic acids on cancer cells serves as a mechanism for evading the immune response elicited against the cancer. By this strategy, tumor cells might counter, for example, NK cell activation by interacting with the ITIM-containing Siglec receptors expressed on the NK cell (Jandus C, et al. *J. Clin. Invest* 2014; 124: 1810-20).

Whilst reducing specific embodiments of the present invention to practice, the present inventors designed and expressed several chimeric receptors comprising an extracellular domain of a Siglec-7 or a Siglec-9 receptor capable of binding a Siglec-7 and/or a Siglec-9 ligand and a signaling amino acid sequence capable of transmitting an activating signal (e.g. CD3 zeta) and/or a co-stimulatory signal (e.g. CD28, 4-1BB) (Examples 1-3 of the Examples section which follows). As is illustrated hereinunder and in the examples section, which follows, the present inventors further show that the generated chimeric receptors can be expressed in immune cells and induce *in-vitro* and *in-vivo* anti-tumor effects.

Consequently, specific embodiments of the present teachings suggest immune cells (e.g. T cells, NK cells) genetically engineered to express the chimeric receptor; and methods of using these immune cells to treat diseases associated with cells expressing a Siglec-7 and/or a Siglec-9 ligand (e.g. cancer).

Thus, according to an aspect of the present invention, there is provided a chimeric receptor comprising:

(a) an extracellular domain comprising an amino acid sequence of a Siglec-7 or a Siglec-9 receptor capable of binding a Siglec-7 and/or a Siglec-9 ligand; and

(b) an intracellular domain comprising an amino acid sequence capable of transmitting a co-stimulatory signal in an immune cell expressing the chimeric receptor upon

binding of said extracellular domain to said ligand, said intracellular domain being devoid of an amino acid sequence capable of transmitting an activating signal.

According to an additional or an alternative aspect of the present invention, there is provided a chimeric receptor comprising:

(a) an extracellular domain comprising an amino acid sequence of a Siglec-7 or a Siglec-9 receptor capable of binding a Siglec-7 and/or a Siglec-9 ligand and an Ig-like C2-type domain; and

(b) an intracellular domain comprising an amino acid sequence capable of transmitting an activating signal and/or a co-stimulatory signal in an immune cell expressing the chimeric receptor upon binding of said extracellular domain to said ligand.

According to an additional or an alternative aspect of the present invention, there is provided a chimeric receptor comprising:

(a) an extracellular domain comprising an amino acid sequence of a Siglec-7 or a Siglec-9 receptor capable of binding a Siglec-7 and/or a Siglec-9 ligand;

(b) an intracellular domain comprising an amino acid sequence capable of transmitting an activating signal and/or a co-stimulatory signal in an immune cell expressing the chimeric receptor upon binding of said extracellular domain to said ligand; and

(c) a transmembrane domain of CD28 or CD8a.

According to an additional or an alternative aspect of the present invention, there is provided a chimeric receptor comprising:

(a) an extracellular domain comprising an amino acid sequence of a Siglec-7 or a Siglec-9 receptor capable of binding a Siglec-7 and/or a Siglec-9 ligand;

(b) an intracellular domain comprising an amino acid sequence capable of transmitting an activating signal and/or a co-stimulatory signal in an immune cell expressing the chimeric receptor upon binding of said extracellular domain to said ligand; and

(c) a transmembrane domain of CD28.

The term “receptor”, hereinafter also referred to as “a polypeptide”, comprises an extracellular binding domain, a transmembrane domain and an intracellular signalling domain.

As used herein, the terms "polypeptide" and “peptide”, which are interchangeably used herein, encompass native peptides (either degradation products, synthetically synthesized peptides or recombinant peptides) and peptidomimetics (typically, synthetically synthesized peptides), as well as peptoids and semipeptoids which are peptide analogs, which may have, for example, modifications rendering the peptides more stable while in a body or more capable of penetrating into cells. Such modifications include, but are not limited to N terminus

modification, C terminus modification, peptide bond modification, backbone modifications, and residue modification. Methods for preparing peptidomimetic compounds are well known in the art and are specified, for example, in Quantitative Drug Design, C.A. Ramsden Gd., Chapter 17.2, F. Choplin Pergamon Press (1992), which is incorporated by reference as if fully set forth herein.

As used herein, the term "chimeric receptor" or "chimeric polypeptide", also known as fusion receptor, refers to a receptor comprising an amino acid sequence having two or more parts which are not found together as a dingle protein expression product in nature.

The chimeric receptor disclosed herein comprises at least an extracellular domain comprising an amino acid sequence capable of binding a Siglec-7 and/or a Siglec-9 ligand and a heterologous intracellular signaling amino acid sequence.

As used herein, the term "heterologous" refers to an amino acid sequence which is not native to the recited amino acid sequence (e.g. an amino acid sequence capable of binding a Siglec-7 and/or a Siglec-9 ligand) at least in localization or is completely absent from the native sequence of the recited amino acid sequence.

The term "amino acid" or "amino acids" is understood to include the 20 naturally occurring amino acids; those amino acids often modified post-translationally in vivo, including, for example, hydroxyproline, phosphoserine and phosphothreonine; and other unusual amino acids including, but not limited to, 2-aminoadipic acid, hydroxylysine, isodesmosine, nor-valine, nor-leucine and ornithine. Furthermore, the term "amino acid" includes both D- and L-amino acids.

A used herein, the phrase "an amino acid sequence of a Siglec-7 or a Siglec-9 receptor capable of binding a Siglec-7 and/or a Siglec-9 ligand" refers to a fragment of a Siglec-7 of a Siglec-9 receptor which comprises an extracellular domain of Siglec-7 of a Siglec-9 receptor capable of binding a Siglec-7 and/or a Siglec-9 ligand. Assays for testing binding are well known in the art and include, but not limited to flow cytometry, BiaCore, bio-layer interferometry Blitz® assay, HPLC, surface plasmon resonance.

According to specific embodiments, the Siglec-7 or a Siglec-9 receptor capable of binding a Siglec-7 and/or a Siglec-9 ligand binds the Siglec-7 and/or a Siglec-9 ligand with a Kd $\geq 10^{-5}$, 10^{-4} or 10^{-3}.

According to specific embodiments, the Siglec-7 or a Siglec-9 receptor capable of binding a Siglec-7 and/or a Siglec-9 ligand binds the Siglec-7 and/or a Siglec-9 ligand with a Kd of $10^{-3} - 10^{-4}$ M.

As used herein, the term "Siglec-7 receptor", also known as CD328, refers to the polypeptide expression product of the SIGLEC7 gene (Gene ID 27036). According to specific embodiments, Siglec-7 is the human Siglec-7, such as provided in the following Accession Nos. NP_055200, NP_001264130, NP_057627.

Siglec-7 ligands include, but are not limited to, alpha-2,8-, alpha-2,6 and alpha-2,3-linked sialic acid, disialogangliosides (disialogalactosyl globoside, disialyl lactotetraosylceramide, disialyl GalNAc lactotetraoslylceramide), disialyl Lewis-a and sialyl 6-sulfo Lewis-x.

The term "Siglec-7" also encompasses functional homologues (naturally occurring or synthetically/recombinantly produced), which exhibit the desired activity (*i.e.*, binding a Siglec-7 ligand). Such homologues can be, for example, at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identical or homologous to the polypeptide provided in Accession No. NP_055200 (SEQ ID NO: 1), NP_001264130, or NP_057627; or at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identical to the polynucleotide sequence encoding same.

Sequence identity or homology can be determined using any protein or nucleic acid sequence alignment algorithm such as Blast, ClustalW, and MUSCLE.

The homolog may also refer to an ortholog, a deletion, insertion, or substitution variant, including a conservative and non-conservative amino acid substitution, as further described hereinbelow.

The term "conservative substitution" as used herein, refers to the replacement of an amino acid present in the native sequence in the peptide with a naturally or non-naturally occurring amino or a peptidomimetics having similar steric properties. Where the side-chain of the native amino acid to be replaced is either polar or hydrophobic, the conservative substitution should be with a naturally occurring amino acid, a non-naturally occurring amino acid or with a peptidomimetic moiety which is also polar or hydrophobic (in addition to having the same steric properties as the side-chain of the replaced amino acid).

As naturally occurring amino acids are typically grouped according to their properties, conservative substitutions by naturally occurring amino acids can be easily determined bearing

in mind the fact that in accordance with the invention replacement of charged amino acids by sterically similar non-charged amino acids are considered as conservative substitutions.

For producing conservative substitutions by non-naturally occurring amino acids it is also possible to use amino acid analogs (synthetic amino acids) well known in the art. A peptidomimetic of the naturally occurring amino acid is well documented in the literature known to the skilled practitioner.

When affecting conservative substitutions, the substituting amino acid should have the same or a similar functional group in the side chain as the original amino acid.

The phrase "non-conservative substitutions" as used herein refers to replacement of the amino acid as present in the parent sequence by another naturally or non-naturally occurring amino acid, having different electrochemical and/or steric properties. Thus, the side chain of the substituting amino acid can be significantly larger (or smaller) than the side chain of the native amino acid being substituted and/or can have functional groups with significantly different electronic properties than the amino acid being substituted. Examples of non-conservative substitutions of this type include the substitution of phenylalanine or cycohexylmethyl glycine for alanine, isoleucine for glycine, or -NH-CH[(-CH_2)$_5$-COOH]-CO- for aspartic acid. Those non-conservative substitutions which fall under the scope of the present invention are those which still constitute an amino acid sequence capable of binding an anti-CD3 antibody.

According to specific embodiments, the amino acid sequence of Siglec-7 receptor capable of binding a Siglec-7 and/or a Siglec-9 ligand comprises an Ig-like V-type domain (InterPro database entry IPR013106, Pfam database entry PF07686).

According to specific embodiments, the amino acid sequence of Siglec-7 receptor capable of binding a Siglec-7 and/or a Siglec-9 ligand comprises an amino acid sequence having at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to QKSNRKDYSLTMQSSVTVQEGMCVHVRCSFSYPVDSQTDSDPVHGYWFRAGNDISWK APVATNNPAWAVQEETRDRFHLLGDPQTKNCTLSIRDARMSDAGRYFFRMEKGNIKW NYKYDQLSVNVTAL (SEQ ID NO: 2)

According to specific embodiments, the amino acid sequence of Siglec-7 receptor capable of binding a Siglec-7 and/or a Siglec-9 ligand comprises an amino acid sequence having at least 80 % identity to SEQ ID NO: 2.

According to specific embodiments, the amino acid sequence of Siglec-7 receptor capable of binding a Siglec-7 and/or a Siglec-9 ligand comprises SEQ ID NO: 2.

According to specific embodiments, the amino acid sequence of Siglec-7 receptor capable of binding a Siglec-7 and/or a Siglec-9 ligand consists of SEQ ID NO: 2.

As used herein, the term "Siglec-9 receptor", also known as CD329, refers to the polypeptide expression product of the SIGLEC9 gene (Gene ID 27180). According to specific embodiments, Siglec-7 is the human Siglec-7, such as provided in the following Accession Nos. NP_001185487, NP_055256.

Siglec-9 ligands include, but are not limited to, sialic acid in either the alpha-2,3- or alpha-2,6- linked sialic acid, MUC16, vascular adhesion protein 1 (VAP-1), MUC1.

The term "Siglec-9" also encompasses functional homologues (naturally occurring or synthetically/recombinantly produced), which exhibit the desired activity (*i.e.*, binding a Siglec-7 ligand). Such homologues can be, for example, at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identical or homologous to the polypeptide provided in Accession No. NP_001185487 or NP_055256; or at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identical to the polynucleotide sequence encoding same.

According to specific embodiments, the amino acid sequence of Siglec-9 receptor capable of binding a Siglec-7 and/or a Siglec-9 ligand comprises an Ig-like V-type domain (InterPro database entry IPR013106, Pfam database entry PF07686).

According to specific embodiments, the amino acid sequence of Siglec-9 receptor capable of binding a Siglec-7 and/or a Siglec-9 ligand comprises an amino acid sequence having at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to GQTSKLLTMQSSVTVQEGLCVHVPCSFSYPSHGWIYPGPVVHGYWFREGANTDQDAPV ATNNPARAVWEETRDRFHLLGDPHTKNCTLSIRDARRSDAGRYFFRMEKGSIKWNYKH HRLSVNVTAL (SEQ ID NO: 6).

According to specific embodiments, the amino acid sequence of Siglec-9 receptor capable of binding a Siglec-7 and/or a Siglec-9 ligand comprises an amino acid sequence having at least 80 % identity to SEQ ID NO: 6.

According to specific embodiments, the amino acid sequence of Siglec-9 receptor capable of binding a Siglec-7 and/or a Siglec-9 ligand comprises SEQ ID NO: 6.

According to specific embodiments, the amino acid sequence of Siglec-9 receptor capable of binding a Siglec-7 and/or a Siglec-9 ligand consists of SEQ ID NO: 6.

According to specific embodiments, the extracellular domain comprises an Ig-like C2-type domain.

The Ig-like C2-type domain may be located C-terminally or N-terminally to the amino acid sequence capable of binding the Siglec-7 and/or the Siglec-9 ligand.

According to specific embodiments, the Ig-like C2-type domain is located C-terminally to the amino acid sequence capable of binding the Siglec-7 and/or the Siglec-9 ligand.

As used herein the phrase "Ig-like C2-type domain" refers to an amino acid sequence which comprises a protein motif as set forth in InterPro database entry IPR008424or Pfam database entry PF05790.

According to specific embodiments, the Ig-like C2-type domain is of a Siglec-7 or a Siglec-9 receptor.

According to specific embodiments, the Ig-like C2-type domain has at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to the Ig-like C2-type domain of a Siglec-7 or a Siglec-9 receptor.

As Siglec 7 and Siglec 9 receptors comprises two Ig-like C2-type domains, the extracellular domain may comprise an amino acid sequence of one of the Ig-like C2-type domains or of the two Ig-like C2-type domains.

According to specific embodiments, the extracellular domain comprises an amino acid sequence of only one of the Siglec 7 or Siglec 9 receptor Ig-like C2-type domains.

According to specific embodiments, the extracellular domain of the chimeric receptor comprises a single Ig-like C2-type domain.

According to specific embodiments, the Ig-like C2-type domain is the third Ig-like domain located N to C in a full length Siglec-7 or Siglec-9 receptor.

According to specific embodiments, the Ig-like C2-type domain has at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at

least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to the the third Ig-like domain located N to C in a full length Siglec-7 or Siglec-9 receptor.

According to specific embodiments, the Ig-like C2-type domain corresponds to amino acids coordinates 251-336 of NP_055200 (SEQ ID NO: 1).

According to specific embodiments, the Ig-like C2-type domain corresponds to amino acids coordinates 247-341 of NP_001185487 (SEQ ID NO: 5).

According to specific embodiments, the amino acid sequence of the Ig-like C2-type domain comprises an amino acid sequence having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity identity to SEQ ID NO: 4 or 8.

According to specific embodiments, the amino acid sequence of the Ig-like C2-type domain comprises an amino acid sequence having at least 80 % identity identity to SEQ ID NO: 4 or 8.

According to specific embodiments, the amino acid sequence of the Ig-like C2-type domain comprises SEQ ID NO: 4 or 8.

According to specific embodiments, the amino acid sequence of the Ig-like C2-type domain consists of SEQ ID NO: 4 or 8.

According to specific embodiments, the amino acid sequence of the Ig-like C2-type domain is at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90 or at least 91 amino acids in length, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the Ig-like C2-type domain is not the Ig-like C2-type domain which is the second Ig-like domain located N to C in a full length Siglec-7 or Siglec-9 receptor.

According to specific embodiments, the Ig-like C2-type domain is not the Ig-like C2-type domain corresponding to amino acid coordinates 144-233 of NP_055200 (SEQ ID NO: 1) or amino acid coordinates 144-233 of NP_001185487 (SEQ ID NO: 5).

According to specific embodiments, the Ig-like C2-type domain is not the Ig-like C2-type domain comprising SEQ ID NO: 3 or 7.

According to specific embodiments, the extracellular domain of the chimeric receptor is devoid of an Ig-like C2-type domain which is the second Ig-like domain located N to C in a full length Siglec-7 or Siglec-9 receptor.

According to specific embodiments, the extracellular domain of the chimeric receptor is devoid of an Ig-like C2-type domain corresponding to amino acid coordinates 144-233 of NP_055200 (SEQ ID NO: 1) or amino acid coordinates 144-233 of NP_001185487 (SEQ ID NO: 5).

According to specific embodiments, the extracellular domain of the chimeric receptor is devoid of an Ig-like C2-type domain comprising an amino acid sequence of SEQ ID NO: 3 or 7.

As noted, the chimeric receptor disclosed herein comprises an intracellular domain comprising an amino acid sequence capable of transmitting an activating and/or a co-stimulatory signal upon binding of the extracellular domain to the ligand.

According to specific embodiments, the chimeric receptor comprises a co-stimulatory signal amino acid sequence and is devoid of an activating amino acid sequence.

As used herein the phrase "activating signal" refers to an amino acid sequence capable of transmitting a primary stimulatory signal resulting in cellular proliferation, maturation, cytokine production and/or induction of regulatory or effector functions. Typically, an activating signal domain comprises an ITAM domain.

Any known activating signal domain can be used with specific embodiments of the present invention. Non-limiting examples of activating signaling domains include the signaling domains of CD3zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, and CD66d.

According to specific embodiments, the amino acid sequence capable of transmitting an activating signal comprises an amino acid sequence of CD3zeta.

As used herein the term "CD3zeta" also known as TCRzeta or CD247 refers to the polypeptide expression product of the *CD247* gene (Gene ID 919). According to a specific embodiment, the CD3zeta protein refers to the human protein, such as provided in the following GenBank Numbers NP_000725 and/or NP_932170.

According to specific embodiments, the amino acid sequence of CD3zeta comprises SEQ ID NO: 24.

According to specific embodiments, the amino acid sequence capable of transmitting an activating signal comprises an amino acid sequence of FcR gamma.

As used herein the phrase "FcR gamma" refers to the polypeptide expression product of the FCER1G gene (Gene ID 2207). According to specific embodiments, FcR gamma protein refers to the human protein, such as provided in the following GenBank Number NP_004097.

Methods of determining signaling of a stimulatory signal are well known in the art, and include, but are not limited to, enzymatic activity assays such as kinase activity assays, and expression of molecules involved in the signaling cascade using e.g. PCR, Western blot, immunoprecipitation and immunohistochemistry. Additionally or alternatively, determining transmission of an activating signal can be effected by evaluating T cell activation or function. Methods of evaluating T cell activation or function are well known in the art and include, but are not limited to, proliferation assays such as CFSE staining, MTT, Alamar blue, BRDU and thymidine incorporation, cytotoxicity assays such as CFSE staining, chromium release, Calcin AM, cytokine secretion assays such as intracellular cytokine staining, ELISPOT and ELISA, expression of activation markers such as CD25, CD69, CD137, CD107a, PD1, and CD62L using flow cytometry.

According to a specific embodiment, the intracellular domain of the chimeric receptor is devoid of an amino acid sequence capable of transmitting an activating signal.

According to a specific embodiment, the intracellular domain of the chimeric receptor is devoid of an an ITAM domain.

According to a specific embodiment, the intracellular domain of the chimeric receptor is devoid of CD3zeta signalling domain.

As used herein, the phrase "co-stimulatory signal" refers to an amino acid sequence of a co-stimulatory molecule capable of transmitting a secondary stimulatory signal resulting in activation of the T cell. Typically, a co-stimulatory signal domain does not comprise an ITAM domain. Non-limiting examples of co-stimulatory signal domains include YXXM (SEQ ID NO: 51) e.g. YMNM (SEQ ID NO: 52, PRRP (SEQ ID NO: 53) / PYAP (SEQ ID NO: 54) / YRS, YVN, QEED (SEQ ID NO: 55) / EEEE (SEQ ID NO: 56), TPIQEE (SEQ ID NO: 57) / EEGKE (SEQ ID NO: 58), VTTVEVEET (SEQ ID NO: 59), PIQED (SEQ ID NO: 60).

Any known co-stimulatory signal domain can be used with specific embodiments of the present invention. Non-limiting examples of co-stimulatory signal domains include the signaling domains of 4-1BB, CD28, OX40, ICOS, CD27, GITR, HVEM, TIM1, LFA1(CD11a), CD2, CD40L, LIGHT, CD30, DNAM.

According to specific embodiments, the amino acid sequence capable of transmitting a co-stimulatory signal comprises an amino acid sequence of 4-1BB, OX40, CD28, CD27, ICOS, CD40L, GITR, LIGHT, HVEM and/or CD30.

According to specific embodiments, the amino acid sequence capable of transmitting a co-stimulatory signal comprises an amino acid sequence of CD28.As used herein the term "CD28" refers to the polypeptide of the *CD28* gene (Gene ID 940). According to a specific embodiment, the CD28 protein refers to the human protein, such as provided in the following GenBank Number NP_001230006, NP_001230007, NP_006130.

According to specific embodiments, the co-stimulatory signal amino acid sequence of CD28 comprises SEQ ID NO: 25.

According to specific embodiments, the amino acid sequence capable of transmitting a co-stimulatory signal comprises an amino acid sequence of 4-1BB.

As used herein the term "4-1BB (also known as CD137 and TNFRSF9)" refers to the polypeptide of the *TNFRSF9* gene (Gene ID 3604). According to a specific embodiment, the 4-1BB protein refers to the human protein, such as provided in the following GenBank Number NP_001552.

According to specific embodiments, the co-stimulatory signal amino acid sequence of 4-1BB comprises SEQ ID NO: 26.

The chimeric receptor disclosed herein further comprises a hinge domain and/or a transmembrane domain.

The hinge domain and/or the transmembrane domain of any transmembrane receptor may be used with specific embodiments of the invention. According to specific embodiments, the chimeric receptor comprises a hinge domain and/or a transmembrane domain of a protein selected from the group consisting of Siglec 7, Siglec 9, CD28 and CD8.

According to a specific embodiment, the chimeric receptor comprises a hinge domain and/or a transmembrane domain of CD28.

According to specific embodiments, the transmembrane domain of CD28 comprises SEQ ID NO: 27.

According to specific embodiments, the hinge domain of CD28 comprises SEQ ID NO: 28.

According to a specific embodiment, the chimeric receptor comprises a hinge domain and/or a transmembrane domain of Siglec-7.

According to specific embodiments, the transmembrane domain of Siglec-7 comprises SEQ ID NO: 29.

According to specific embodiments, the hinge domain of Siglec-7 comprises SEQ ID NO: 30 or 31.

According to a specific embodiment, the chimeric receptor comprises a hinge domain and/or a transmembrane domain of Siglec-9.

According to specific embodiments, the transmembrane domain of Siglec-9 comprises SEQ ID NO: 32.

According to specific embodiments, the hinge domain of Siglec-9 comprises SEQ ID NO: 33.

According to specific embodiments, the chimeric receptor comprises a hinge domain and/or a transmembrane domain of a CD8.

As used herein the term "CD8" refers to the polypeptide of the *CD8A* or *CD8B* gene (Gene ID 925 or 926). According to a specific embodiment, the CD8 protein refers to the human CD8a protein, such as provided in the following GenBank Number NP_001139345, NP_001759, NP_741969. According to a specific embodiment, the CD8 protein refers to the human CD8b protein, such as provided in the following GenBank Number NP_742099, NP_004922, NP_757362, NP_001171571, NP_742100.

According to specific embodiments, the chimeric receptor comprises a hinge domain and/or a transmembrane domain of a CD8a.

According to specific embodiments, the transmembrane domain of CD8a comprises SEQ ID NO: 35

According to specific embodiments, the hinge domain of CD8a comprises SEQ ID NO: 34.

The components of the chimeric receptor described herein may be linked to each other directly or via a linker. Any linker known in the art can be used with specific embodiments of the invention. According to specific embodiments, the linker may be derived from naturally-occurring multi-domain proteins or is an empirical linker as described, for example, in Chichili et al., (2013), Protein Sci. 22(2): 153-167, Chen et al, (2013), Adv Drug Deliv Rev. 65(10): 1357-1369, the entire contents of which are hereby incorporated by reference. In some embodiments, the linker may be designed using linker designing databases and computer programs such as those described in Chen et al., (2013), Adv Drug Deliv Rev. 65(10): 1357-1369 and Crasto et al., (2000), Protein Eng. 13(5):309-312, the entire contents of which are hereby incorporated by reference.

According to specific embodiments, the linker is a synthetic linker.

According to specific embodiments, the linker is a polypeptide.

Non-limiting examples of linkers that can be used include AS, GS, $(GGGGS)_n$ (n=1-4) (SEQ ID NO: 62), GGGGSGGGG (SEQ ID NO: 32), $(Gly)_8$, $(Gly)_6$, $(EAAAK)_n$ (n=1-3) (SEQ ID NO: 63), PAPAP (SEQ ID NO: 64).

According to a specific embodiment, the components of the chimeric receptor described herein are directly linked to each other without any linker.

According to a specific embodiment, the components of the chimeric receptor described herein are linked to each via a short linker such as AS or GS.

According to specific embodiments, the chimeric receptor comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 9-23, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the chimeric receptor comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 7 and 12.

According to specific embodiments, the chimeric receptor comprises SEQ ID NO: 17.

According to specific embodiments, the chimeric receptor comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 18-23, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the chimeric receptor may further comprise a detectable tag. Non-limiting examples of detectable tags include chitin binding protein (CBP)-tag, maltose binding protein (MBP)-tag, glutathione-S-transferase (GST)-tag, poly(His)-tag, FLAG tag, Epitope tags, such as, V5-tag, c-myc-tag, and HA-tag, and fluorescence tags such as green fluorescent protein (GFP), red fluorescent protein (RFP), yellow fluorescent protein (YFP), blue fluorescent protein (BFP), and cyan fluorescent protein (CFP); as well as derivatives of these tags, or any tag known in the art.

The receptors of some embodiments of the invention may be synthesized and purified by any techniques that are known to those skilled in the art of peptide synthesis, such as, but not limited to, solid phase and recombinant techniques.

According to specific embodiments, production of the polypeptide involves solid phase synthesis.

For solid phase peptide synthesis, a summary of the many techniques may be found in J. M. Stewart and J. D. Young, Solid Phase Peptide Synthesis, W. H. Freeman Co. (San Francisco), 1963 and J. Meienhofer, Hormonal Proteins and Peptides, vol. 2, p. 46, Academic Press (New York), 1973. For classical solution synthesis see G. Schroder and K. Lupke, The Peptides, vol. 1, Academic Press (New York), 1965.

In general, these methods comprise the sequential addition of one or more amino acids or suitably protected amino acids to a growing peptide chain. Normally, either the amino or carboxyl group of the first amino acid is protected by a suitable protecting group. The protected or derivatized amino acid can then either be attached to an inert solid support or utilized in solution by adding the next amino acid in the sequence having the complimentary (amino or carboxyl) group suitably protected, under conditions suitable for forming the amide linkage. The protecting group is then removed from this newly added amino acid residue and the next amino acid (suitably protected) is then added, and so forth. After all the desired amino acids have been linked in the proper sequence, any remaining protecting groups (and any solid support) are removed sequentially or concurrently, to afford the final peptide compound. By simple modification of this general procedure, it is possible to add more than one amino acid at a time to a growing chain, for example, by coupling (under conditions which do not racemize chiral centers) a protected tripeptide with a properly protected dipeptide to form, after deprotection, a pentapeptide and so forth. Further description of peptide synthesis is disclosed in U.S. Pat. No. 6,472,505.

According to specific embodiments, the polypeptide is produced by recombinant DNA technology.

Thus, according to an aspect of the present invention, there is provided a polynucleotide encoding the chimeric receptor.

As used herein the term “polynucleotide” refers to a single or double stranded nucleic acid sequence which is isolated and provided in the form of an RNA sequence, a complementary polynucleotide sequence (cDNA), a genomic polynucleotide sequence and/or a composite polynucleotide sequences (e.g., a combination of the above).

A non-limiting example of a polynucleotide encoding the chimeric receptor of some embodiments of the invention is provided in SEQ ID NO: 36-50, each possibility represents a separate embodiment of the present invention.

To express any of the disclosed polypeptides in a cell, a polynucleotide sequence encoding the polypeptide is preferably ligated into a nucleic acid construct suitable for cell expression. Such a nucleic acid construct includes a promoter sequence for directing transcription of the polynucleotide sequence in the cell in a constitutive or inducible manner.

The nucleic acid construct (also referred to herein as an "expression vector") of some embodiments of the invention includes additional sequences which render this vector suitable for replication and integration (e.g., shuttle vectors). In addition, a typical cloning vector may also contain a transcription and translation initiation sequence, transcription and translation

terminator and a polyadenylation signal. By way of example, such constructs will typically include a 5' LTR, a tRNA binding site, a packaging signal, an origin of second-strand DNA synthesis, and a 3' LTR or a portion thereof.

The nucleic acid construct of some embodiments of the invention typically includes or encodes a signal sequence for targeting the polypeptide to the cell surface. According to a specific embodiment, the signal sequence for this purpose is a mammalian signal sequence or the signal sequence of the polypeptide variants of some embodiments of the invention.

Eukaryotic promoters typically contain two types of recognition sequences, the TATA box and upstream promoter elements. The TATA box, located 25-30 base pairs upstream of the transcription initiation site, is thought to be involved in directing RNA polymerase to begin RNA synthesis. The other upstream promoter elements determine the rate at which transcription is initiated.

Preferably, the promoter utilized by the nucleic acid construct of some embodiments of the invention is active in the specific cell population transformed, i.e. T cells. Examples of T cell specific promoters include lymphoid specific promoters [Calame et al., (1988) Adv. Immunol. 43:235-275]; in particular promoters of T-cell receptors [Winoto et al., (1989) EMBO J. 8:729-733].

Enhancer elements can stimulate transcription up to 1,000 fold from linked homologous or heterologous promoters. Enhancers are active when placed downstream or upstream from the transcription initiation site. Many enhancer elements derived from viruses have a broad host range and are active in a variety of tissues. For example, the SV40 early gene enhancer is suitable for many cell types. Other enhancer/promoter combinations that are suitable for some embodiments of the invention include those derived from polyoma virus, human or murine cytomegalovirus (CMV), the long-term repeat from various retroviruses such as murine leukemia virus, murine or Rous sarcoma virus and HIV. See, Enhancers and Eukaryotic Expression, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. 1983, which is incorporated herein by reference.

In the construction of the expression vector, the promoter is preferably positioned approximately the same distance from the heterologous transcription start site as it is from the transcription start site in its natural setting. As is known in the art, however, some variation in this distance can be accommodated without loss of promoter function.

Polyadenylation sequences can also be added to the expression vector in order to increase the efficiency of mRNA translation. Two distinct sequence elements are required for accurate and efficient polyadenylation: GU or U rich sequences located downstream from the

polyadenylation site and a highly conserved sequence of six nucleotides, AAUAAA, located 11-30 nucleotides upstream. Termination and polyadenylation signals that are suitable for some embodiments of the invention include those derived from SV40.

In addition to the elements already described, the expression vector of some embodiments of the invention may typically contain other specialized elements intended to increase the level of expression of cloned nucleic acids or to facilitate the identification of cells that carry the recombinant DNA. For example, a number of animal viruses contain DNA sequences that promote the extra chromosomal replication of the viral genome in permissive cell types. Plasmids bearing these viral replicons are replicated episomally as long as the appropriate factors are provided by genes either carried on the plasmid or with the genome of the host cell.

The vector may or may not include a eukaryotic replicon. If a eukaryotic replicon is present, then the vector is amplifiable in eukaryotic cells using the appropriate selectable marker. If the vector does not comprise a eukaryotic replicon, no episomal amplification is possible. Instead, the recombinant DNA integrates into the genome of the engineered cell, where the promoter directs expression of the desired nucleic acid.

The expression vector of some embodiments of the invention can further include additional polynucleotide sequences that allow, for example, the translation of several proteins from a single mRNA such as an internal ribosome entry site (IRES) or a self-cleavable peptide; and sequences for genomic integration of the promoter-chimeric polypeptide.

It will be appreciated that the individual elements comprised in the expression vector can be arranged in a variety of configurations. For example, enhancer elements, promoters and the like, and even the polynucleotide sequence(s) encoding the polypeptide can be arranged in a "head-to-tail" configuration, may be present as an inverted complement, or in a complementary configuration, as an anti-parallel strand. While such variety of configuration is more likely to occur with non-coding elements of the expression vector, alternative configurations of the coding sequence within the expression vector are also envisioned.

Examples for mammalian expression vectors include, but are not limited to, pcDNA3, pcDNA3.1(+/-), pGL3, pZeoSV2(+/-), pSecTag2, pDisplay, pEF/myc/cyto, pCMV/myc/cyto, pCR3.1, pSinRep5, DH26S, DHBB, pNMT1, pNMT41, pNMT81, which are available from Invitrogen, pCI which is available from Promega, pMbac, pPbac, pBK-RSV and pBK-CMV which are available from Strategene, pTRES which is available from Clontech, and their derivatives.

Expression vectors containing regulatory elements from eukaryotic viruses such as retroviruses can be also used. SV40 vectors include pSVT7 and pMT2. Vectors derived from

bovine papilloma virus include pBV-1MTHA, and vectors derived from Epstein Bar virus include pHEBO, and p2O5. Other exemplary vectors include pMSG, pAV009/A$^+$, pMTO10/A$^+$, pMAMneo-5, baculovirus pDSVE, and any other vector allowing expression of proteins under the direction of the SV-40 early promoter, SV-40 later promoter, metallothionein promoter, murine mammary tumor virus promoter, Rous sarcoma virus promoter, polyhedrin promoter, or other promoters shown effective for expression in eukaryotic cells.

As described above, viruses are very specialized infectious agents that have evolved, in many cases, to elude host defense mechanisms. Typically, viruses infect and propagate in specific cell types. The targeting specificity of viral vectors utilizes its natural specificity to specifically target predetermined cell types and thereby introduce a recombinant gene into the infected cell. The ability to select suitable vectors for transforming immune cells (e.g. T cells, NK cells) is well within the capabilities of the ordinary skilled artisan and as such no general description of selection consideration is provided herein.

Recombinant viral vectors are useful for *in vivo* expression of the polypeptides since they offer advantages such as lateral infection and targeting specificity. Lateral infection is inherent in the life cycle of, for example, retrovirus and is the process by which a single infected cell produces many progeny virions that bud off and infect neighboring cells. The result is that a large area becomes rapidly infected, most of which was not initially infected by the original viral particles. This is in contrast to vertical-type of infection in which the infectious agent spreads only through daughter progeny. Viral vectors can also be produced that are unable to spread laterally. This characteristic can be useful if the desired purpose is to introduce a specified gene into only a localized number of targeted cells.

Various methods can be used to introduce the expression vector of some embodiments of the invention into cells. Such methods are generally described in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Harbor Laboratory, New York (1989, 1992), in Ausubel et al., Current Protocols in Molecular Biology, John Wiley and Sons, Baltimore, Md. (1989), Chang et al., Somatic Gene Therapy, CRC Press, Ann Arbor, Mich. (1995), Vega et al., Gene Targeting, CRC Press, Ann Arbor Mich. (1995), Vectors: A Survey of Molecular Cloning Vectors and Their Uses, Butterworths, Boston Mass. (1988) and Gilboa et at. [Biotechniques 4 (6): 504-512, 1986] and include, for example, stable or transient transfection, lipofection, electroporation and infection with recombinant viral vectors. In addition, see U.S. Pat. Nos. 5,464,764 and 5,487,992 for positive-negative selection methods.

Introduction of nucleic acids by viral infection offers several advantages over other methods such as lipofection and electroporation, since higher transfection efficiency can be obtained due to the infectious nature of viruses.

Currently preferred *in vivo* nucleic acid transfer techniques include transfection with viral or non-viral constructs, such as adenovirus, lentivirus, Herpes simplex I virus, or adeno-associated virus (AAV) and lipid-based systems. Useful lipids for lipid-mediated transfer of the gene are, for example, DOTMA, DOPE, and DC-Chol [Tonkinson et al., Cancer Investigation, 14(1): 54-65 (1996)]. The most preferred constructs for use in gene therapy are viruses, most preferably adenoviruses, AAV, lentiviruses, or retroviruses. A viral construct such as a retroviral construct includes at least one transcriptional promoter/enhancer or locus-defining element(s), or other elements that control gene expression by other means such as alternate splicing, nuclear RNA export, or post-translational modification of messenger. Such vector constructs also include a packaging signal, long terminal repeats (LTRs) or portions thereof, and positive and negative strand primer binding sites appropriate to the virus used, unless it is already present in the viral construct. In addition, such a construct typically includes a signal sequence for targeting the polypeptide to the desired site in a cell. Optionally, the construct may also include a signal that directs polyadenylation, as well as one or more restriction sites and a translation termination sequence. By way of example, such constructs will typically include a 5' LTR, a tRNA binding site, a packaging signal, an origin of second-strand DNA synthesis, and a 3' LTR or a portion thereof. Other vectors can be used that are non-viral, such as cationic lipids, polylysine, and dendrimers.

Specific embodiments of the present invention also contemplates cells comprising the chimeric polypeptide described herein and method of generating same.

Thus, according to an aspect of the present invention, there is provided a host cell expressing the polypeptide or the polynucleotide encoding same.

According to an additional or an alternative aspect of the present invention, there is provided a method of expressing a chimeric receptor in a cell, the method comprising introducing into a cell the polynucleotide encoding the chimeric receptor, under conditions which allow expression of the chimeric receptor.

Such conditions may be for example an appropriate temperature (e.g., 37 ºC), atmosphere (e.g., air plus 5 % CO_2), pH, light, medium, supplements and the like.

According to other specific embodiments, the introducing is effected *in-vivo*.

According to specific embodiments, the introducing is effected *in-vitro* or *ex-vivo*.

According to specific embodiments, the cell is a human cell.

According to specific embodiments, the cell is of a healthy subject.

According to specific embodiments, the cell is of a subject suffering from a pathology (e.g. cancer).

According to specific embodiments, the cell is an immune cell.

Non-limiting examples of immune cells that can be used with specific embodiments of the invention include T cells, NK cells, NKT cells, B cells, macrophages, dendritic cells (DCs) and granulocytes.

Methods of obtaining immune cells are well known in the art. Thus, for examples, PBMCs can be isolated by drawing whole blood from a subject and collection in a container containing an anti-coagulant (e.g. heparin or citrate); and apheresis. According to other specific embodiments, the immune cells are obtained from a tissue comprising cells associated with a pathology. Methods for obtaining a tissue sample from a subject are well known in the art and include e.g. biopsy, surgery or necropsy and preparing a single cell suspension thereof. Following, according to specific embodiments, at least one type of an immune cell is purified from the peripheral blood or from the single cell suspension. There are several methods and reagents known to those skilled in the art for purifying immune cells such as leukapheresis, sedimentation, density gradient centrifugation (e.g. ficoll), centrifugal elutriation, fractionation, chemical lysis of e.g. red blood cells (e.g. by ACK), selection of specific cell types using cell surface markers (using e.g. FACS sorter or magnetic cell separation techniques such as are commercially available e.g. from Invitrogen, Stemcell Technologies, Cellpro, Advanced Magnetics, or Miltenyi Biotec.), and depletion of specific cell types by methods such as eradication (e.g. killing) with specific antibodies or by affinity based purification based on negative selection (using e.g. magnetic cell separation techniques, FACS sorter and/or capture ELISA labeling). Such methods are described for example in THE HANDBOOK OF EXPERIMENTAL IMMUNOLOGY, Volumes 1 to 4, (D.N. Weir, editor) and FLOW CYTOMETRY AND CELL SORTING (A. Radbruch, editor, Springer Verlag, 2000).

According to specific embodiments, the immune cell is a T cell.

As used herein, the term "T cell" refers to a differentiated lymphocyte with a CD3+, T cell receptor (TCR)+ having either CD4+ or CD8+ phenotype.

According to specific embodiments, the T cell is an effector cell.

As used herein, the term "effector T cell" refers to a T cell that activates or directs other immune cells e.g. by producing cytokines or has a cytotoxic activity e.g., CD4+, Th1/Th2, CD8+ cytotoxic T lymphocyte.

According to other specific embodiments, the T cell is a regulatory cell.

As used herein, the term "regulatory T cell" or "Treg" refers to a T cell that negatively regulates the activation of other T cells, including effector T cells, as well as innate immune system cells. Treg cells are characterized by sustained suppression of effector T cell responses. According to a specific embodiment, the Treg is a CD4+CD25+Foxp3+ T cell.

According to specific embodiments, the T cell is a CD4+ T cell.

According to other specific embodiments, the T cell is a CD8+ T cell.

According to specific embodiments, the T cell is a naïve T cell.

According to specific embodiments, the T cell is a memory T cell. Non-limiting examples of memory T cells include effector memory CD4+ T cells with a CD3+/CD4+/CD45RA-/CCR7- phenotype, central memory CD4+ T cells with a CD3+/CD4+/CD45RA-/CCR7+ phenotype, effector memory CD8+ T cells with a CD3+/CD8+ CD45RA-/CCR7-phenotype and central memory CD8+ T cells with a CD3+/CD8+ CD45RA-/CCR7+ phenotype.

According to specific embodiments, the T cell is expressing a T cell receptor specific for a pathologic (diseased, e.g. cancerous) cell, i.e. recognizes an antigen presented in the context of MHC which is overexpressed or solely expressed by a pathologic cell as compared to a non-pathologic cell.

According to specific embodiments, the pathologic cell is expressing a Siglec-7 and/or a Siglec-9 ligand.

According to specific embodiments, the T cell is endogenously expressing a T cell receptor specific for a pathologic cell (e.g. cancerous cell).

According to specific embodiments, the T cell is an engineered T cells transduced with a T cell receptor (TCR).

As used herein the phrase "transduced with a TCR" or "genetically engineered to express a TCR" refers to cloning of variable α- and β-chains from T cells with specificity against a desired antigen presented in the context of MHC. Methods of transducing with a TCR are known in the art and are disclosed e.g. in Nicholson et al. Adv Hematol. 2012; 2012:404081; Wang and Rivière Cancer Gene Ther. 2015 Mar;22(2):85-94); and Lamers et al, Cancer Gene Therapy (2002) 9, 613–623. According to specific embodiments, the TCR is specific for a pathologic cell.

According to specific embodiments, the method further comprises transducing the T cell with a TCR.

According to specific embodiments, the T cell is an engineered T cells transduced with a chimeric antigen receptor (CAR).

As used herein, the phrase "transduced with a CAR" or "genetically engineered to express a CAR" refers to cloning of a nucleic acid sequence encoding a chimeric antigen receptor (CAR), wherein the CAR comprises an antigen recognition moiety and a T-cell activation moiety. A chimeric antigen receptor (CAR) is an artificially constructed hybrid protein or polypeptide containing an antigen binding domain of an antibody (e.g., a single chain variable fragment (scFv)) linked to T-cell signaling or T-cell activation domains. Method of transducing with a CAR are known in the art and are disclosed e.g. in Davila et al. Oncoimmunology. 2012 Dec 1;1(9):1577-1583; Wang and Rivière Cancer Gene Ther. 2015 Mar;22(2):85-94); Maus et al. Blood. 2014 Apr 24;123(17):2625-35; Porter DL The New England journal of medicine. 2011, 365(8):725-733; Jackson HJ, Nat Rev Clin Oncol. 2016;13(6):370-383; and Globerson-Levin et al. Mol Ther. 2014;22(5):1029-1038.

According to specific embodiments, the antigen recognition moiety is specific for a pathologic (diseased, e.g. cancerous) cell, i.e. recognizes an antigen which is overexpressed or solely expressed by a pathologic cell as compared to a non-pathologic cell.

According to other specific embodiments, the T cell is not transduced (i.e. does not express) a CAR.

According to specific embodiments, the method further comprises transducing the T cell with a CAR.

According to specific embodiments, the immune cells comprise NK cells.

Thus, according to an aspect of the present invention, there is provided an NK cell expressing a chimeric receptor comprising:

(a) an extracellular domain comprising an amino acid sequence of a Siglec-7 or a Siglec-9 receptor capable of binding a Siglec-7 and/or a Siglec-9 ligand; and

(b) an intracellular domain comprising an amino acid sequence capable of transmitting an activating signal and/or a co-stimulatory signal in an immune cell expressing the chimeric receptor upon binding of said extracellular domain to said ligand.

As used herein the term "NK cells" refers to differentiated lymphocytes with a CD16+ CD56+ and/or CD57+ TCR- phenotype. NK are characterized by their ability to bind to and kill cells that fail to express "self" MHC/HLA antigens by the activation of specific cytolytic enzymes, the ability to kill tumor cells or other diseased cells that express a ligand for NK activating receptors, and the ability to release protein molecules called cytokines that stimulate or inhibit the immune response.

According to specific embodiments, the immune cells comprise NKT cells.

As used herein the term "NKT cells" refers to a specialized population of T cells that express a semi-invariant αβ T-cell receptor, but also express a variety of molecular markers that are typically associated with NK cells, such as NK1.1. NKT cells include NK1.1+ and NK1.1−, as well as CD4+, CD4−, CD8+ and CD8− cells. The TCR on NKT cells is unique in that it recognizes glycolipid antigens presented by the MHC I-like molecule CD1d. NKT cells can have either protective or deleterious effects due to their abilities to produce cytokines that promote either inflammation or immune tolerance.

According to specific embodiments, the immune cells comprise B cells.

As used herein the term "B cells" refers to a lymphocyte with a B cell receptor (BCR)+, CD19+ and or B220+ phenotype. B cells are characterized by their ability to bind a specific antigen and elicit a humoral response.

According to specific embodiments, the immune cells comprise phagocytic cells.

As used herein, the term "phagocytic cells" refer to a cell that is capable of phagocytosis and include both professional and non-professional phagocytic cells. Methods of analyzing phagocytosis are well known in the art and include for examples killing assays, flow cytometry and/or microscopic evaluation (live cell imaging, fluorescence microscopy, confocal microscopy, electron microscopy). According to specific embodiments, the phagocytic cells are selected from the group consisting of monocytes, dendritic cells (DCs) and granulocytes.

According to specific embodiments, the immune cells comprise monocytes.

According to specific embodiments, the term "monocytes" refers to both circulating monocytes and to macrophages (also referred to as mononuclear phagocytes) present in a tissue.

According to specific embodiments, the monocytes comprise macrophages. Typically, cell surface phenotype of macrophages include CD14, CD40, CD11b, CD64, F4/80 (mice)/EMR1 (human), lysozyme M, MAC-1/MAC-3 and CD68.

According to specific embodiments, the monocytes comprise circulating monocytes. Typically, cell surface phenotypes of circulating monocytes include CD14 and CD16 (e.g. CD14++ CD16-, CD14+CD16++, CD14++CD16+).

According to specific embodiments, the immune cells comprise DCs.

As used herein the term "dendritic cells (DCs)" refers to any member of a diverse population of morphologically similar cell types found in lymphoid or non-lymphoid tissues. DCs are a class of professional antigen presenting cells, and have a high capacity for sensitizing HLA-restricted T cells. DCs include, for example, plasmacytoid dendritic cells, myeloid dendritic cells (including immature and mature dendritic cells), Langerhans cells, interdigitating cells, follicular dendritic cells. Dendritic cells may be recognized by function, or by phenotype,

particularly by cell surface phenotype. These cells are characterized by their distinctive morphology having veil-like projections on the cell surface, intermediate to high levels of surface HLA-class II expression and ability to present antigen to T cells, particularly to naive T cells (See Steinman R, et al., Ann. Rev. Immunol. 1991; 9:271-196.). Typically, cell surface phenotype of DCs include CDla+, CD4+, CD86+, or HLA-DR. The term DCs encompasses both immature and mature DCs.

According to specific embodiments, the immune cells comprise granulocytes.

As used herein, the tern "granulocytes" refer to polymorphonuclear leukocytes characterized by the presence of granules in their cytoplasm.

According to specific embodiments, the granulocytes comprise neutrophils.

According to specific embodiments, the granulocytes comprise mast-cells.

According to specific embodiments, when the chimeric receptor comprises a co-stimulatory signal amino acid sequence and is devoid of an activating amino acid sequence, the cells (e.g. T cells) endogenously or exogenously express an activating receptor (e.g. TCR, CAR) specific for a pathologic (e.g. cancerous) cell.

According to specific embodiments, the cells can be freshly isolated, stored e.g., cryopreserved (i.e. frozen) at e.g. liquid nitrogen temperature at any stage for long periods of time (e.g., months, years) for future use; and cell lines.

Methods of cryopreservation are commonly known by one of ordinary skill in the art and are disclosed e.g. in International Patent Application Publication Nos. WO2007054160 and WO 2001039594 and US Patent Application Publication No. US20120149108.

According to specific embodiments, the cells can be stored in a cell bank or a depository or storage facility.

Consequently, the present teachings further suggest the use of the cells and the methods disclosed herein as, but not limited to, a source for adoptive cells therapies.

Thus, according to an aspect of the present invention, the cells (e.g. immune cells) disclosed herein are for use in adoptive cell therapy.

The cells used according to specific embodiments of the present invention may be autologous or non-autologous; they can be syngeneic or non-syngeneic: allogeneic or xenogeneic to the subject; each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the cells are autologous to the subject.

According to specific embodiments, the cells are non-autologous to the subject.

According to specific embodiments, the cells described herein are cultured, expanded and/or activated ex-vivo prior to administration to the subject.

Methods of culturing, expanding and activating cells such as immune cells are well known to the skilled in the art. For example, T cells may be activated *ex-vivo* in the presence of one or more molecule such as, but not limited to, an anti-CD3 antibody, an anti-CD28 antibody, anti-CD3 and anti-CD28 coated beads (such as the CD3CD28 MACSiBeads obtained from Miltenyi Biotec), IL-2, phytohemoagglutinin, an antigen-loaded antigen presenting cell [APC, e.g. dendritic cell], a peptide loaded recombinant MHC.

Since the cells of specific embodiments of the present invention are activated upon binding of the binding domain of the chimeric receptor to a Siglec-7 and/or Siglec-9 ligand, they may be used for, but not limited to, treating diseases associated with cells expressing a Siglec-7 and/or a Siglec-9 ligand.

Thus, according to an aspect of the present invention, there is provided a method of treating a disease associated with cells expressing a Siglec-7 and/or a Siglec-9 ligand in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the chimeric receptor, the polynucleotide or the cells of disclosed herein, thereby treating the disease in the subject.

According to an additional or an alternative aspect of the present invention, there is provided the chimeric receptor, the polynucleotide or the cell disclosed herein, for use in the treatment of a disease associated with cells expressing said Siglec-7 and/or said Siglec-9 ligand.

As used herein, the term "subject" or "subject in need thereof" includes mammals, preferably human beings at any age or gender. The subject may be healthy or showing preliminary signs of a pathology, e.g. cancer. This term also encompasses individuals who are at risk to develop the pathology.

As used herein the term "treating" refers to curing, reversing, attenuating, alleviating, minimizing, suppressing or halting the deleterious effects of a disease or disorder (e.g. cancer). Those of skill in the art will understand that various methodologies and assays can be used to assess the development of a pathology, and similarly, various methodologies and assays may be used to assess the reduction, remission or regression of a pathology (e.g. a malignancy), as discussed below.

As used herein the phrase, "a disease associated with cells expressing a Siglec-7 and/or a Siglec-9 ligand" means that cells expressing a Siglec-7 and/or a Siglec-9 ligand drive onset and/or progression of the disease.

According to specific embodiments, the expression of Siglec-7 and/or Siglec-9 ligand on the cells is at least 2 fold, at least 2.5 fold or at least 3 fold higher compared to background (i.e. non-specific staining), as determined by flow cytometry [e.g. using mean fluorescence intensity (MFI)].

According to specific embodiments, the disease can benefit from activating the immune cells of the subject.

As used herein the phrase "a disease that can benefit from activating immune cells" refers to diseases in which the subject's immune response activity may be sufficient to at least ameliorate symptoms of the disease or delay onset of symptoms, however for any reason the activity of the subject's immune response in doing so is less than optimal.

Non-limiting examples of diseases treated by some embodiments of the invention include cancer, infections, inflammatory diseases and autoimmune diseases.

According to specific embodiments, the disease comprises an infection.

As used herein, the term "infection" or "infectious disease" refers to a disease induced by a pathogen. Specific examples of pathogens include, viral pathogens, bacterial pathogens e.g., intracellular mycobacterial pathogens (such as, for example, Mycobacterium tuberculosis), intracellular bacterial pathogens (such as, for example, Listeria monocytogenes), or intracellular protozoan pathogens (such as, for example, Leishmania and Trypanosoma).

Specific types of viral pathogens causing infectious diseases include, but are not limited to, retroviruses, circoviruses, parvoviruses, papovaviruses, adenoviruses, herpesviruses, iridoviruses, poxviruses, hepadnaviruses, picornaviruses, caliciviruses, togaviruses, flaviviruses, reoviruses, orthomyxoviruses, paramyxoviruses, rhabdoviruses, bunyaviruses, coronaviruses, arenaviruses, and filoviruses.

Specific examples of viral infections which may be treated according to specific embodiments of the present invention include, but are not limited to, human immunodeficiency virus (HIV)-induced acquired immunodeficiency syndrome (AIDS), Group B Strep Infection, influenza, rhinoviral infection, viral meningitis, Epstein-Barr virus (EBV) infection, hepatitis A, B or C virus infection, measles, papilloma virus infection/warts, cytomegalovirus (CMV) infection, Herpes simplex virus infection, yellow fever, Ebola virus infection, rabies, etc.

According to a specific embodiment, the infection is an HIV infection.

According to specific embodiments, the disease is cancer.

Cancers which may be treated by some embodiments of the invention can be any solid or non-solid tumor, cancer metastasis and/or a pre-cancer.

According to specific embodiments, the cancer is a malignant cancer.

Examples of cancer include but are not limited to, carcinoma, blastoma, sarcoma and lymphoma. More particular examples of such cancers include, but are not limited to, tumors of the gastrointestinal tract (colon carcinoma, rectal carcinoma, colorectal carcinoma, colorectal cancer, colorectal adenoma, hereditary nonpolyposis type 1, hereditary nonpolyposis type 2, hereditary nonpolyposis type 3, hereditary nonpolyposis type 6; colorectal cancer, hereditary nonpolyposis type 7, small and/or large bowel carcinoma, esophageal carcinoma, tylosis with esophageal cancer, stomach carcinoma, pancreatic carcinoma, pancreatic endocrine tumors), endometrial carcinoma, dermatofibrosarcoma protuberans, gallbladder carcinoma, Biliary tract tumors, prostate cancer, prostate adenocarcinoma, renal cancer (e.g., Wilms' tumor type 2 or type 1), liver cancer (e.g., hepatoblastoma, hepatocellular carcinoma, hepatocellular cancer), bladder cancer, embryonal rhabdomyosarcoma, germ cell tumor, trophoblastic tumor, testicular germ cells tumor, immature teratoma of ovary, uterine, epithelial ovarian, sacrococcygeal tumor, choriocarcinoma, placental site trophoblastic tumor, epithelial adult tumor, ovarian carcinoma, serous ovarian cancer, ovarian sex cord tumors, cervical carcinoma, uterine cervix carcinoma, small-cell and non-small cell lung carcinoma, nasopharyngeal, breast carcinoma (e.g., ductal breast cancer, invasive intraductal breast cancer, sporadic ; breast cancer, susceptibility to breast cancer, type 4 breast cancer, breast cancer-1, breast cancer-3; breast-ovarian cancer), squamous cell carcinoma (e.g., in head and neck), neurogenic tumor, astrocytoma, ganglioblastoma, neuroblastoma, lymphomas (e.g., Hodgkin's disease, non-Hodgkin's lymphoma, B cell, Burkitt, cutaneous T cell, histiocytic, lymphoblastic, T cell, thymic), gliomas, adenocarcinoma, adrenal tumor, hereditary adrenocortical carcinoma, brain malignancy (tumor), various other carcinomas (e.g., bronchogenic large cell, ductal, Ehrlich-Lettre ascites, epidermoid, large cell, Lewis lung, medullary, mucoepidermoid, oat cell, small cell, spindle cell, spinocellular, transitional cell, undifferentiated, carcinosarcoma, choriocarcinoma, cystadenocarcinoma), ependimoblastoma, epithelioma, erythroleukemia (e.g., Friend, lymphoblast), fibrosarcoma, giant cell tumor, glial tumor, glioblastoma (e.g., multiforme, astrocytoma), glioma hepatoma, heterohybridoma, heteromyeloma, histiocytoma, hybridoma (e.g., B cell), hypernephroma, insulinoma, islet tumor, keratoma, leiomyoblastoma, leiomyosarcoma, leukemia (e.g., acute lymphatic, acute lymphoblastic, acute lymphoblastic pre-B cell, acute lymphoblastic T cell leukemia, acute - megakaryoblastic, monocytic, acute myelogenous, acute myeloid, acute myeloid with eosinophilia, B cell, basophilic, chronic myeloid, chronic, B cell, eosinophilic, Friend, granulocytic or myelocytic, hairy cell, lymphocytic, megakaryoblastic, monocytic, monocytic-macrophage, myeloblastic, myeloid, myelomonocytic, plasma cell, pre-B cell, promyelocytic, subacute, T cell, lymphoid neoplasm, predisposition to myeloid malignancy, acute

nonlymphocytic leukemia), lymphosarcoma, melanoma, mammary tumor, mastocytoma, medulloblastoma, mesothelioma, metastatic tumor, monocyte tumor, multiple myeloma, myelodysplastic syndrome, myeloma, nephroblastoma, nervous tissue glial tumor, nervous tissue neuronal tumor, neurinoma, neuroblastoma, oligodendroglioma, osteochondroma, osteomyeloma, osteosarcoma (e.g., Ewing's), papilloma, transitional cell, pheochromocytoma, pituitary tumor (invasive), plasmacytoma, retinoblastoma, rhabdomyosarcoma, sarcoma (e.g., Ewing's, histiocytic cell, Jensen, osteogenic, reticulum cell), schwannoma, subcutaneous tumor, teratocarcinoma (e.g., pluripotent), teratoma, testicular tumor, thymoma and trichoepithelioma, gastric cancer, fibrosarcoma, glioblastoma multiforme; multiple glomus tumors, Li-Fraumeni syndrome, liposarcoma, lynch cancer family syndrome II, male germ cell tumor, mast cell leukemia, medullary thyroid, multiple meningioma, endocrine neoplasia myxosarcoma, paraganglioma, familial nonchromaffin, pilomatricoma, papillary, familial and sporadic, rhabdoid predisposition syndrome, familial, rhabdoid tumors, soft tissue sarcoma, and Turcot syndrome with glioblastoma.

According to specific embodiments, the cancer is a pre-malignant cancer.

Pre-cancers are well characterized and known in the art (refer, for example, to Berman JJ. and Henson DE., 2003. Classifying the pre-cancers: a metadata approach. BMC Med Inform Decis Mak. 3:8). Examples of pre-cancers include, but are not limited to, acquired small pre-cancers, acquired large lesions with nuclear atypia, precursor lesions occurring with inherited hyperplastic syndromes that progress to cancer, and acquired diffuse hyperplasias and diffuse metaplasias. Non-limiting examples of small pre-cancers include HGSIL (High grade squamous intraepithelial lesion of uterine cervix), AIN (anal intraepithelial neoplasia), dysplasia of vocal cord, aberrant crypts (of colon), PIN (prostatic intraepithelial neoplasia).

Non-limiting examples of acquired large lesions with nuclear atypia include tubular adenoma, AILD (angioimmunoblastic lymphadenopathy with dysproteinemia), atypical meningioma, gastric polyp, large plaque parapsoriasis, myelodysplasia, papillary transitional cell carcinoma *in-situ*, refractory anemia with excess blasts, and Schneiderian papilloma. Non-limiting examples of precursor lesions occurring with inherited hyperplastic syndromes that progress to cancer include atypical mole syndrome, C cell adenomatosis and MEA. Non-limiting examples of acquired diffuse hyperplasias and diffuse metaplasias include Paget's disease of bone and ulcerative colitis.

According to specific embodiments, the cancer is selected form the group consisting of melanoma, hepatocellular cancer, pancreatic cancer, colon adenocarcinoma, cervical cancer, breast cancer, lung cancer lymphoma and leukaemia.

According to specific embodiments, the cancer is selected form the group consisting of ovarian carcinoma, cervical cancer, pancreatic adenocarcinoma, leukaemia, melanoma, colon carcinoma, breast carcinoma and lung adenocarcinoma.

According to specific embodiments, the cancer is melanoma.

According to specific embodiments, the disease is an inflammatory disease. Non-limiting examples of inflammatory diseases that can be treated according to specific embodiments of the present invention include liver fibrosis, airway inflammatory diseases e.g. chronic rhinosinusitis.

According to specific embodiments, chimeric receptor, the polynucleotide or the cell disclosed herein can be administered to a subject in combination with other established or experimental therapeutic regimen to treat a disease associated with with cells expressing a Siglec-7 and/or a Siglec-9 ligand (e.g. cancer) including, but not limited to analgesics, chemotherapeutic agents, radiotherapeutic agents, cytotoxic therapies (conditioning), hormonal therapy and other treatment regimens (e.g., surgery) which are well known in the art.

The the chimeric receptor, the polynucleotide or the cell disclosed herein can be administered to the subject *per se*, or in a pharmaceutical composition where it is mixed with suitable carriers or excipients.

As used herein a "pharmaceutical composition" refers to a preparation of one or more of the active ingredients described herein with other chemical components such as physiologically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

Herein the term "active ingredient" refers to the chimeric receptor, the polynucleotide or the cell accountable for the biological effect.

Thus, according to specific embodiments, the chimeric receptor, the polynucleotide or the cell disclosed herein is the active ingredient in the formulation.

According to specific embodiments, the chimeric receptor, the polynucleotide or the cell disclosed herein is the only active ingredient in the formulation.

Hereinafter, the phrases "physiologically acceptable carrier" and "pharmaceutically acceptable carrier" which may be interchangeably used refer to a carrier or a diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound. An adjuvant is included under these phrases.

Herein the term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

Techniques for formulation and administration of drugs may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition, which is incorporated herein by reference.

Suitable routes of administration may, for example, include oral, rectal, transmucosal, especially transnasal, intestinal or parenteral delivery, including intramuscular, intradermal, subcutaneous and intramedullary injections as well as intrathecal, direct intraventricular, intracardiac, e.g., into the right or left ventricular cavity, into the common coronary artery, intravenous, intraperitoneal, intranasal, or intraocular injections.

Conventional approaches for drug delivery to the central nervous system (CNS) include: neurosurgical strategies (e.g., intracerebral injection or intracerebroventricular infusion); molecular manipulation of the agent (e.g., production of a chimeric fusion protein that comprises a transport peptide that has an affinity for an endothelial cell surface molecule in combination with an agent that is itself incapable of crossing the BBB) in an attempt to exploit one of the endogenous transport pathways of the BBB; pharmacological strategies designed to increase the lipid solubility of an agent (e.g., conjugation of water-soluble agents to lipid or cholesterol carriers); and the transitory disruption of the integrity of the BBB by hyperosmotic disruption (resulting from the infusion of a mannitol solution into the carotid artery or the use of a biologically active agent such as an angiotensin peptide). However, each of these strategies has limitations, such as the inherent risks associated with an invasive surgical procedure, a size limitation imposed by a limitation inherent in the endogenous transport systems, potentially undesirable biological side effects associated with the systemic administration of a chimeric molecule comprised of a carrier motif that could be active outside of the CNS, and the possible risk of brain damage within regions of the brain where the BBB is disrupted, which renders it a suboptimal delivery method.

Alternately, one may administer the pharmaceutical composition in a local rather than systemic manner, for example, via injection of the pharmaceutical composition directly into a tissue region of a patient.

Pharmaceutical compositions of some embodiments of the invention may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with some embodiments of the invention thus may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active

ingredients into preparations which, can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the active ingredients of the pharmaceutical composition may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological salt buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the pharmaceutical composition can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the pharmaceutical composition to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for oral ingestion by a patient. Pharmacological preparations for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carbomethylcellulose; and/or physiologically acceptable polymers such as polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical compositions which can be used orally, include push-fit capsules made of gelatin as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules may contain the active ingredients in admixture with filler such as lactose, binders such as starches, lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active ingredients may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for the chosen route of administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by nasal inhalation, the active ingredients for use according to some embodiments of the invention are conveniently delivered in the form of an aerosol spray presentation from a pressurized pack or a nebulizer with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichloro-tetrafluoroethane or carbon dioxide. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in a dispenser may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The pharmaceutical composition described herein may be formulated for parenteral administration, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multidose containers with optionally, an added preservative. The compositions may be suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical compositions for parenteral administration include aqueous solutions of the active preparation in water-soluble form. Additionally, suspensions of the active ingredients may be prepared as appropriate oily or water based injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acids esters such as ethyl oleate, triglycerides or liposomes. Aqueous injection suspensions may contain substances, which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the active ingredients to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water based solution, before use.

The pharmaceutical composition of some embodiments of the invention may also be formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides.

Alternative embodiments include depots providing sustained release or prolonged duration of activity of the active ingredient in the subject, as are well known in the art.

Pharmaceutical compositions suitable for use in context of some embodiments of the invention include compositions wherein the active ingredients are contained in an amount

effective to achieve the intended purpose. More specifically, a therapeutically effective amount means an amount of active ingredients effective to prevent, alleviate or ameliorate symptoms of a disorder (e.g., cancer) or prolong the survival of the subject being treated.

Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

For any preparation used in the methods of the invention, the therapeutically effective amount or dose can be estimated initially from in vitro and cell culture assays. For example, a dose can be formulated in animal models to achieve a desired concentration or titer. Such information can be used to more accurately determine useful doses in humans.

Toxicity and therapeutic efficacy of the active ingredients described herein can be determined by standard pharmaceutical procedures in vitro, in cell cultures or experimental animals. The data obtained from these in vitro and cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g., Fingl, et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1).

Dosage amount and interval may be adjusted individually to provide levels of the active ingredient are sufficient to induce or suppress the biological effect (minimal effective concentration, MEC). The MEC will vary for each preparation, but can be estimated from in vitro data. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. Detection assays can be used to determine plasma concentrations.

Depending on the severity and responsiveness of the condition to be treated, dosing can be of a single or a plurality of administrations, with course of treatment lasting from several days to several weeks or until cure is effected or diminution of the disease state is achieved.

The amount of a composition to be administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

Compositions of some embodiments of the invention may, if desired, be presented in a pack or dispenser device, such as an FDA approved kit, which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser may also be accommodated by a notice associated with the container in a form prescribed by a governmental agency regulating the

manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions or human or veterinary administration. Such notice, for example, may be of labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert. Compositions comprising a preparation of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition, as is further detailed above.

As used herein the term "about" refers to ± 10 %

The terms "comprises", "comprising", "includes", "including", "having" and their conjugates mean "including but not limited to".

The term "consisting of" means "including and limited to".

The term "consisting essentially of" means that the composition, method or structure may include additional ingredients, steps and/or parts, but only if the additional ingredients, steps and/or parts do not materially alter the basic and novel characteristics of the claimed composition, method or structure.

As used herein, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or "at least one compound" may include a plurality of compounds, including mixtures thereof.

Throughout this application, various embodiments of this invention may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases "ranging/ranges between" a first indicate number and a second indicate number and "ranging/ranges from" a first indicate number "to" a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals therebetween.

As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques

and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

When reference is made to particular sequence listings, such reference is to be understood to also encompass sequences that substantially correspond to its complementary sequence as including minor sequence variations, resulting from, e.g., sequencing errors, cloning errors, or other alterations resulting in base substitution, base deletion or base addition, provided that the frequency of such variations is less than 1 in 50 nucleotides, alternatively, less than 1 in 100 nucleotides, alternatively, less than 1 in 200 nucleotides, alternatively, less than 1 in 500 nucleotides, alternatively, less than 1 in 1000 nucleotides, alternatively, less than 1 in 5,000 nucleotides, alternatively, less than 1 in 10,000 nucleotides.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions illustrate some embodiments of the invention in a non limiting fashion.

Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E.,

ed. (1994); "Culture of Animal Cells - A Manual of Basic Technique" by Freshney, Wiley-Liss, N. Y. (1994), Third Edition; "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

MATERIALS AND METHODS

PBMcs and cell lines - PBLs were obtained from healthy donors from the Israeli Blood Bank (Tel-Hashomer, Israel) after obtaining an informed consent. The following cells and cell lines were used: DU-145 (ATCC/HTB-81), a prostate cancer line derived from metastatic site (brain); HeLa (ATCC/CCL-2), a cervix adenocarcinoma line; K562 (ATCC/CCL-243), a chronic myelogenous leukemia (CML) line; A375 (ATCC/CRL-1619), an epithelial skin melanoma line; 624.38 (CVCL_C588)), a melanoma cell line, HLA*A0201^{+}, MART1^{+}, NYESO-1^{+}; 888A2 (CVCL_4632) HLA*A0201^{+}, MART1^{+}, NYESO-1^{-}, a melanoma cell line; SK-MEL23 is a HLA-A2^{+} melanoma cell line (CVCL_6027) HLA*A0201^{+}, MART1^{+}.; Jurkat T-3.5 (ATCC/TIB-153), a T-cell leukemia line; PANC-1 (ATCC/CRL-1469), a pancreatic epitheloid carcinoma; A549 (ATCC/CCL-185), a lung epithelial carcinoma line; MCF7 (ATCC/ HTB-22), an adenocarcinoma breast cancer of the mammary gland, derived from metastatic site; OVCAR8 a High grade ovarian serous adenocarcinoma cell line; M#3 and M#14, melanoma primary tumors (previously described in Eisenberg et al., Frontiers in immunology, 2017); packaging line

293GP (expressing GAG and POL, previously described in Eisenberg et al.). Tumor cells were cultured in RPMI (Invitrogen, Carlsbad, CA, USA) or in DMEM (Invitrogen, Carlsbad, CA, USA), both supplemented with 10 % FBS (Biological Industries, Beth Haemek, Israel). Lymphocytes were cultured in BioTarget medium (Biological Industries, Beth Haemek, Israel), 10 % FBS, and 300 IU / ml IL-2. Cells were maintained at 37 °C and 5 % CO2.

SIGLEC 7/9 chimeras constructs - The different chimeras (Figures 2, 8 and 11, SEQ ID NOs: 9-23, 36-50) were created by overlapping PCR with the DNA plasmid encoding the human SIGLEC 7/9 receptors. These chimeras were cloned into a retroviral vector backbone.

T-cell receptors (TCRs) and chimeric antigen receptor (CARs) constructs - The α and β chains from previously characterized TCRs specific for MART-1_{26-35} / HLA*0201 termed F4 were subcloned into the MSGV1 vector as described previously (Haga-Friedman A, Horovitz-Fried M, Cohen CJ. J Immunol 2012;188:5538-46).

Similarly, the chains encoding a codon-optimized version of the 1G4 TCR which is specific for the NYESO-$1_{157-165}$/HLA*A0201 epitope, were cloned in this retroviral vector. Similarly, an anti-CD19 CAR, that is composed of a scFv specific for the lymphoma CD19 (FMC63), fused to a 41BB and CD3z signaling molecules (aCD19BBz CAR), was synthesized and cloned into this vector.

Electroporation of PBLs – Effected as described in Haga-Friedman et al, JI, (2012). Briefly, in-vitro-transcribed mRNA for SIGLEC-7/9 different chimeric receptors were electroporated into OKT3-stimulated PBLs at 400V/500μs using an ElectroSquare Porator ECM 830 (BTX, San Diego, CA). The amount of in-vitro-transcribed mRNA was 2 μg per 1×10^6 PBMCs.

Transduction of PBLs - For virus production, transfection of 2×10^6 293GP cells with 9 μg DNA of MSGV1-based retroviral construct and 4.5 μg envelop plasmid (VSV-G) was performed using JetPrime transfection reagent (Polyplus, France). Retroviral supernatant was collected 36 hours following DNA transfection. Freshly isolated human PBLs were stimulated for 48 hours in the presence of 50 ng / ml OKT3 (eBioscience, San Diego, CA, USA) prior to transduction. Following stimulation, lymphocytes were transduced with retroviral vectors by transfer to non-treated tissue culture dishes (Nunc, Rochester, NY, USA) that had been pre-coated with RetroNectin (Takara, Japan) and retroviral vectors as previously described (Eisenberg et al, Front. Immun., 2017).

Flow cytometry analysis and antibodies - Fluorophore-labeled anti-human CD8, CD137, SIGLEC-7 and SIGLEC-9 were purchased from BioLegend (San Diego, CA, USA). For ligand expression tests, a recombinant Human Siglec-7/9 Fc Chimera Protein and as a secondary

antibody- Human IgG Fc PE-conjugated Antibody (R&D Systems, Minneapolis, MN, USA) were used. Immunofluorescence, analyzed as the relative log fluorescence of live cells, was measured using a CyAn-ADP flow cytometer (Beckman Coulter, Brea, CA, USA). Approximately 1×10^4–1×10^5 cells were analyzed. Cells were stained in a FACS buffer made of PBS, 0.5 % BSA, and 0.02 % sodium azide.

Cytokine release assays - Lymphocyte cultures were tested for reactivity in cytokine release assays using commercially available ELISA kits for IFNγ and TNFα (R&D Systems, Minneapolis, MN, USA). For these assays, 2×10^5 responder cells (T-cells) and 1×10^5 stimulator cells (tumor cells) were incubated in a 0.2-ml culture volume in individual wells of 96-wells plates. Stimulator cells and responder cells were co-cultured for 18 hours. Cytokine secretion was measured in culture supernatants diluted to be in the linear range of the assay.

Cell-Mediated cytotoxicity assay - For cytotoxicity assay the CytoTox-ONE™ Homogeneous Membrane Integrity Assay was used, according to the manufacturer's instructions (Promega). Cytotoxicity was analyzed as follows: Percent Cytotoxicity = 100 × (Experimental – Culture Medium Background)\(Maximum LDH Release – Culture Medium Background). Cells were incubated in 37 °C for 4 hours, at different effector : target (E : T) ratios (as indicated in the figure legend).

Animal Models and in Vivo Experiments - 1 - 2×10^6 tumor cells were injected subcutaneously into the back flank area of 6 to 8 weeks year-old NOD-SCID-IL2R-mice (Harlan, Jerusalem, Israel). Mice received three IV injections of 5×10^6 transduced lymphocytes, at a volume of 200 µL in HBSS medium four days following SC injection and thereafter two injections every three days. Tumor size was measured (every 2 days) in a blinded fashion using a caliper and calculated using the following formula: [D × d2] × Π/6, where D is the largest tumor diameter and d its perpendicular one. The study was conducted in compliance with the protocols approved by the Animal Care and Use Committees of Bar Ilan University, Ramat Gan, Israel.

EXAMPLE 1

EXPRESSION OF FUNCTIONAL SIGLEC-7 AND SIGLEC-9 BASED CHIMERIC RECEPTORS IN LYMPOCYTES

Several chimeric receptors based on the extracellular domain of Siglec-7 or Siglec-9 fused to activating and/or co-stimulatory signaling moieties were generated (Figure 1). These constructs were expressed in primary T-cells either by electroporation of by retroviral

transduction and their expression was assessed by flow cytometry following staining with an anti-Siglec-7 or anti-Siglec-9 fluorescent antibody (Figures 2 and 3).

Following, the expression of Siglec-7 and Siglec-9 ligands on multiple tumor lines was assessed (Figure 4), indicating most of the tested cell lines express significant levels of Siglec-7 and/or Siglec-9 ligands. Hence, the function of the Siglec-7/9 based chimeric receptors expressed in T-cells was assessed in-vitro in co-cultures with several tumor lines by determining secretion of IFNγ as an immunological activity marker (Figures 5 and 6).

Overall, all the Siglec-7/9 chimeric receptors were functionally expressed, while the Siglec-7/9 chimeric receptors comprising a CD28 transmembrane region were the best constructs in both terms of expression and function.

To further evaluate the therapeutic potential of these generated Siglec-7/9 based chimeric receptors, T-cells engineered to express the Siglec-7/9 chimeric receptors comprising a CD28 transmembrane region were adoptively transferred to NSG mice that were previously inoculated with 2e6 M#3 melanoma cells. As shown in Figure 7, T cells expressing Siglec-7 or Siglec-9 based chimeric receptors significantly inhibited tumor growth compared to control injections (CD34), thus demonstrating the utility of this approach for targeting tumors *in-vivo*.

EXAMPLE 2

THE IG-LIKE V-TYPE DOMAIN AND THE IG-LIKE C2-TYPE DOMAIN OF SIGLEC-7/9 MEDIATE RECOGNITION AND FUNCTION OF THE SIGLEC-7/9 BASED CHIMERIC RECEPTORS

In order to determine which of the 3 Ig-like domains of Siglec-7/9 receptor are essential for the recognition and function of the Siglec-7/9 based chimeric receptors, constructs encoding chimeric receptors wherein the extracellular Siglec-based moiety comprises either the N terminal Ig-like V-type domain (domain 1), the N-terminal Ig-like V-type domain and the intermediate Ig-like C2-type domain (domains 1+2) or the N-terminal Ig-like V-type domain and the C-teminal Ig-like C2-type domain (domains 1+3) were generated (Figure 8)

These constructs were expressed in primary T-cells and their expression was analyzed by flow cytometry (Figure 9). Subsequently, their function was also assessed in co-cultures with the K562 cells as target cells, by determining secretion of IFNγ (Figure 10).

As shown in Figures 9-10, inclusion of either domain 2 or 3 in the construct increased both expression and function of the chimeric receptor.

EXAMPLE 3

EXPRESSION OF FUNCTIONAL SIGLEC-7 AND SIGLEC-9 BASED CHIMERIC CO-STIMULATORY SWITCH RECEPTORS IN LYMPHOCYTES

An additional possibility to harness the presence of Siglec-7/9 ligands on the surface tumor cells in the context of T-cell engineering is to use chimeric co-stimulatory switch receptors (CSR) based on the extracellular domain of Siglec-7 or Siglec-9 fused to a co-stimulatory signaling moiety without a an activating signaling moiety (Figure 11). Such co-stimulatory signaling moiety can be the intracellular domain of CD28 or any other co-stimulatory molecule (e.g. 41BB, OX40, ICOS).

To demonstrate the potential of Siglec-7/9 based CSR, the generated CSR construct S728 or S929 was transduced along with a CD19 specific 4-1BB based CAR into primary human T-cells and expression was analyzed by flow cytometry (Figure 12). Subsequently, the function of the transduced cells was also assessed in co-cultures with K562 or Raji target cells, by determining secretion of TNFα (Figure 13).

As shown in Figures 12-13, Siglec-7/9 based CSRs enhanced the anti-tumor function of CAR-T cells.

In the next step, the potential of Siglec-7/9 based CSR, was assessed in the context of T-cells engineered to express an exogenous TCR directed against a defined epitope.

To demonstrate the potential of Siglec-based CSR, S928 (Figure 11) was transduced along with an NYESO-1 specific TCR (termed 1G4) into primary human T-cells. Subsequently, the function of the transduced cells was assessed in co-cultures with A375 and 624.38 target cells, by determining secretion of TNFα (Figure 14). T-cells expressing the 1G4 TCR and S928 secreted significantly more cytokine compared to T-cells that expressed only the TCR.

Following, the enhancing effect mediated by SIGLEC-based CSRs was determined with another TCR, namely a melanoma specific TCR (termed F4) that recognize a MART-1 derived specific epitope. To this end, primary human T-cells were transduced with the F4 TCR and different Siglec9-based CSR (Figure 11) and their function was assessed in co-cultures with 888A2, SK-mel23 and 624.38 target cells, by determining secretion of TNFα or IFNγ. As shown in Figures 15-16, Siglec-based CSRs enhanced the anti-tumor function of the TCR transduced-T cells.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention. To the extent that section headings are used, they should not be construed as necessarily limiting.

In addition, any priority document(s) of this application is/are hereby incorporated herein by reference in its/their entirety.

WHAT IS CLAIMED IS:

1. A chimeric receptor comprising:

(a) an extracellular domain comprising an amino acid sequence of a Siglec-7 or a Siglec-9 receptor capable of binding a Siglec-7 and/or a Siglec-9 ligand; and

(b) an intracellular domain comprising an amino acid sequence capable of transmitting a co-stimulatory signal in an immune cell expressing the chimeric receptor upon binding of said extracellular domain to said ligand, said intracellular domain being devoid of an amino acid sequence capable of transmitting an activating signal.

2. A chimeric receptor comprising:

(a) an extracellular domain comprising an amino acid sequence of a Siglec-7 or a Siglec-9 receptor capable of binding a Siglec-7 and/or a Siglec-9 ligand and an Ig-like C2-type domain; and

(b) an intracellular domain comprising an amino acid sequence capable of transmitting an activating signal and/or a co-stimulatory signal in an immune cell expressing the chimeric receptor upon binding of said extracellular domain to said ligand.

3. A chimeric receptor comprising:

(a) an extracellular domain comprising an amino acid sequence of a Siglec-7 or a Siglec-9 receptor capable of binding a Siglec-7 and/or a Siglec-9 ligand;

(b) an intracellular domain comprising an amino acid sequence capable of transmitting an activating signal and/or a co-stimulatory signal in an immune cell expressing the chimeric receptor upon binding of said extracellular domain to said ligand; and

(c) a transmembrane domain of CD28 or CD8a.

4. A chimeric receptor comprising:

(a) an extracellular domain comprising an amino acid sequence of a Siglec-7 or a Siglec-9 receptor capable of binding a Siglec-7 and/or a Siglec-9 ligand;

(b) an intracellular domain comprising an amino acid sequence capable of transmitting an activating signal and/or a co-stimulatory signal in an immune cell expressing the chimeric receptor upon binding of said extracellular domain to said ligand; and

(c) a transmembrane domain of CD28.

5. The chimeric receptor of any one of claims 1 and 3-4, wherein said extracellular domain comprises an Ig-like C2-type domain.

6. The chimeric receptor of any one of claims 2 and 5, wherein said Ig-like C2-type domain is of said Siglec-7 or said Siglec-9 receptor.

7. The chimeric receptor of any one of claims 2 and 5-6, wherein said Ig-like C2-type domain is located C-terminally to said amino acid sequence capable of binding said Siglec-7 and/or said Siglec-9 ligand.

8. The chimeric receptor of any one of claims 2 and 5-7, wherein said Ig-like C2-type domain is a single Ig-like C2-type domain.

9. The chimeric receptor of any one of claims 6-8 wherein said Ig-like C2-type domain is the third Ig-like domain located N to C in a full length Siglec-7 or Siglec-9 receptor.

10. The chimeric receptor of any one of claims 2 and 5-9, wherein an amino acid sequence of said Ig-like C2-type domain comprises an amino acid sequence having at least 80 % identity to SEQ ID NO: 4 or 8.

11. The chimeric receptor of any one of claims 2 and 5-9, wherein said amino acid sequence of said Ig-like C2-type domain comprises SEQ ID NO: 4 or 8.

12. The chimeric receptor of any one of claims 2 and 5-11, wherein said extracellular domain is devoid of an Ig-like C2-type domain which is the second Ig-like domain located N to C in a full length Siglec-7 or Siglec-9 receptor.

13. The chimeric receptor of any one of claims 2 and 5-12, wherein said extracellular domain is devoid of an Ig-like C2-type domain comprising an amino acid sequence of SEQ ID NO: 3 or 7.

14. The chimeric receptor of any one of claims 2-13, wherein said intracellular domain is devoid of an amino acid sequence capable of transmitting an activating signal.

15. The chimeric receptor of any one of claims 1-14, wherein said amino acid sequence capable of transmitting an activating signal comprises an amino acid sequence of CD3zeta and/or FcR gamma.

16. The chimeric receptor of any one of claims 1-15, wherein said amino acid sequence capable of transmitting a co-stimulatory signal comprises an amino acid sequence of 4-1BB, OX40, CD28, CD27, ICOS, CD40L, GITR, LIGHT, HVEM and/or CD30.

17. The chimeric receptor of any one of claims 1-2 and 5-16, comprising a transmembrane domain of CD28.

18. The chimeric receptor of any one of claims 1-2 and 5-16, comprising a transmembrane domain of CD8a.

19. The chimeric receptor of any one of claims 1-2 and 5-16, comprising a transmembrane domain of a Siglec-7 or a Siglec-9 receptor.

20. The chimeric receptor of any one of claims 1-19, wherein said amino acid sequence capable of binding said Siglec-7 and/or said Siglec-9 ligand comprises an amino acid sequence having at least 80 % identity to SEQ ID NO: 2 or 6.

21. The chimeric receptor of any one of claims 1-19, wherein said amino acid sequence capable of binding said Siglec-7 and/or said Siglec-9 ligand comprises SEQ ID NO: 2 or 6.

22. A polynucleotide encoding the chimeric receptor of any one of claims 1-21.

23. A host cell expressing the chimeric receptor of any one of claims 1-21.

24. The host cell of claim 23, being an immune cell.

25. The chimeric receptor of any one of claims 1-21, the polynucleotide of claim 22 of the host cell of claim 24, wherein said immune cell is a T cell.

26. The chimeric receptor of any one of claims 1-21, the polynucleotide of claim 22 of the host cell of claim 24, wherein said immune cell is an NK cell.

27. An NK cell expressing a chimeric receptor comprising:

(a) an extracellular domain comprising an amino acid sequence of a Siglec-7 or a Siglec-9 receptor capable of binding a Siglec-7 and/or a Siglec-9 ligand; and

(b) an intracellular domain comprising an amino acid sequence capable of transmitting an activating signal and/or a co-stimulatory signal in an immune cell expressing the chimeric receptor upon binding of said extracellular domain to said ligand.

28. The NK cell of claim 27, wherein said chimeric receptor is the chimeric receptor of any one of claims 1-21.

29. A method of treating a disease associated with cells expressing a Siglec-7 and/or a Siglec-9 ligand in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the chimeric receptor, the polynucleotide or the cells of any one of claims 1-22 and 24 – 28, thereby treating the disease in the subject.

30. The chimeric receptor, the polynucleotide or the cell of any one of claims 1-22 and 24 – 28, for use in the treatment of a disease associated with cells expressing said Siglec-7 and/or said Siglec-9 ligand.

31. The method of claim 29 or the chimeric receptor, the polynucleotide or the cell for use of claim 30, wherein said disease is cancer or an infection.

32. The method of the chimeric receptor, the polynucleotide or the cell for use of claim 31, wherein said cancer is selected form the group consisting of melanoma, hepatocellular cancer, pancreatic cancer, colon adenocarcinoma, cervical cancer, breast cancer, lung cancer lymphoma and leukaemia.

33. The method or the chimeric receptor, the polynucleotide or the cell for use of claim 31, wherein said cancer is selected form the group consisting of ovarian carcinoma, cervical cancer, pancreatic adenocarcinoma, leukaemia, melanoma, colon carcinoma, breast carcinoma and lung adenocarcinoma.

34. The method or the chimeric receptor, the polynucleotide or the cell for use of any one of claims 31-33, wherein said infection is an HIV infection.

FIG. 1

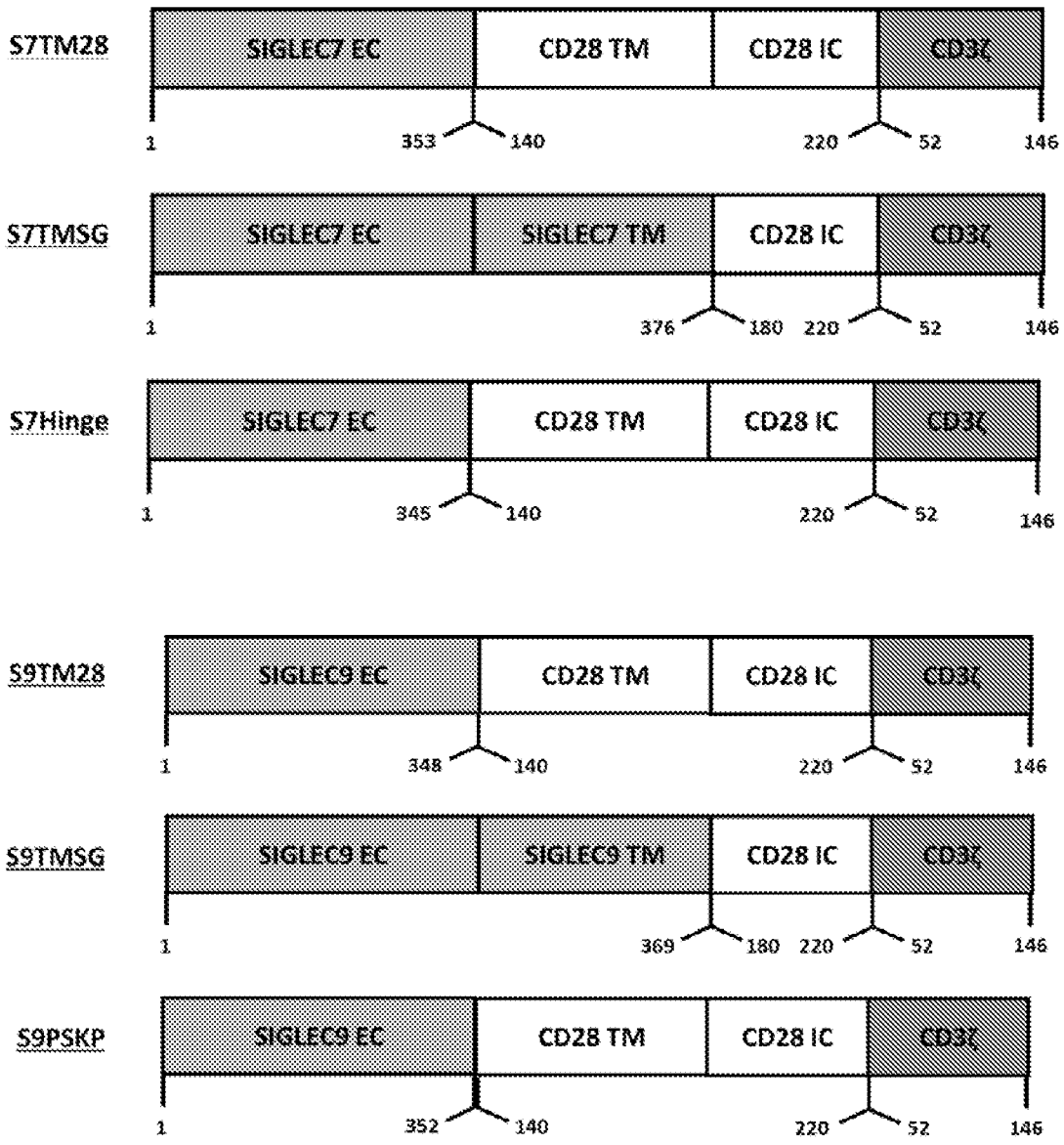

S7TM28
SIGLEC7 EC
CD28 TM
CD28 IC
CD3ζ
1
353
140
220
52
146
S7TMSG
SIGLEC7 EC
SIGLEC7 TM
CD28 IC
CD3ζ
1
376
180
220
52
146
S7Hinge
SIGLEC7 EC
CD28 TM
CD28 IC
CD3ζ
1
345
140
220
52
146
S9TM28
SIGLEC9 EC
CD28 TM
CD28 IC
CD3ζ
1
348
140
220
52
146
S9TMSG
SIGLEC9 EC
SIGLEC9 TM
CD28 IC
CD3ζ
1
369
180
220
52
146
S9PSKP
SIGLEC9 EC
CD28 TM
CD28 IC
CD3ζ
1
352
140
220
52
146

FIG. 2

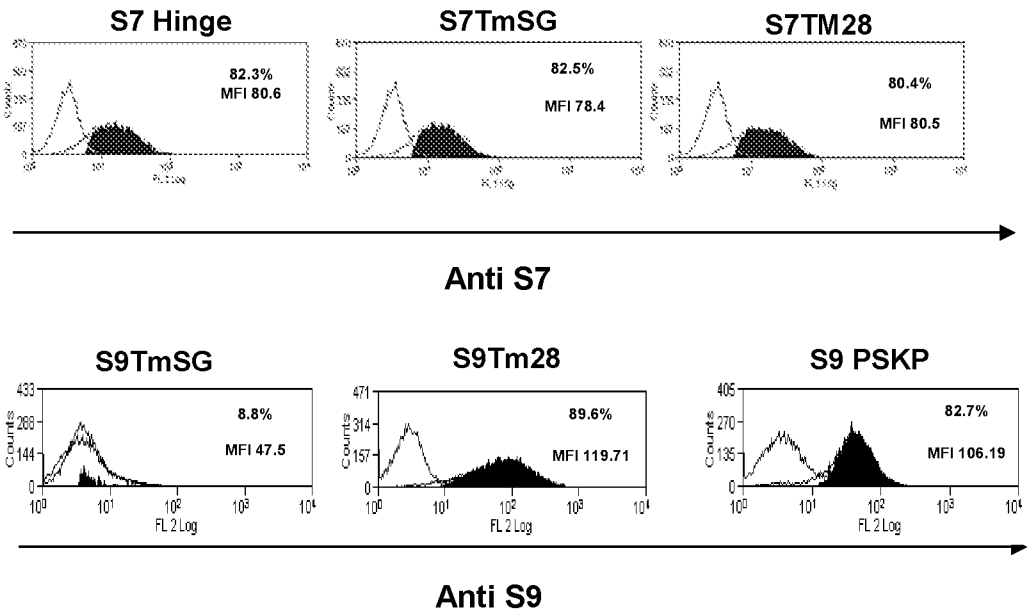

S7 Hinge
82.3%
MFI 80.6
S7TmSG
82.5%
MFI 78.4
S7TM28
80.4%
MFI 80.5
Anti S7
S9TmSG
8.8%
MFI 47.5
S9Tm28
89.6%
MFI 119.71
S9 PSKP
82.7%
MFI 106.19
FL 2 Log
Anti S9

FIG. 3

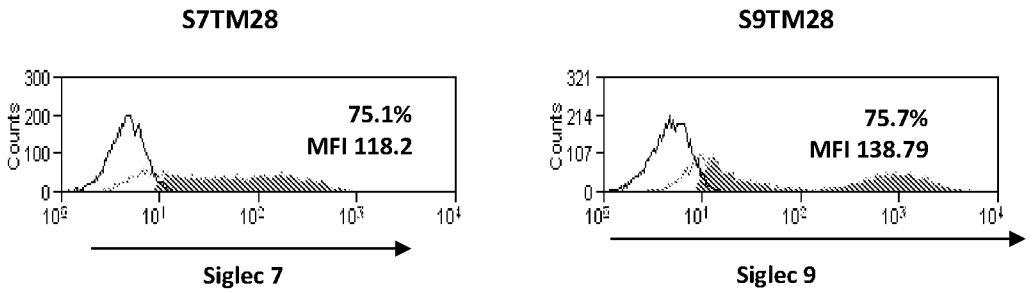

S7TM28
75.1%
MFI 118.2
Siglec 7
S9TM28
75.7%
MFI 138.79
Siglec 9
Counts

FIG. 4

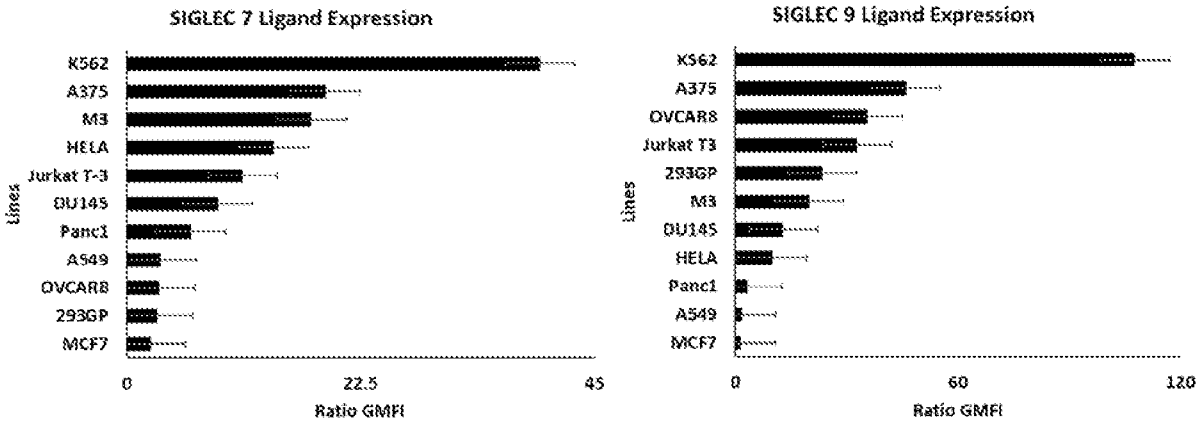

SIGLEC 7 Ligand Expression
K562
A375
M3
HELA
Jurkat T-3
OU145
Panc1
A549
OVCAR8
293GP
MCF7
Lines
0
22.5
45
Ratio GMFI
SIGLEC 9 Ligand Expression
K562
A375
OVCAR8
Jurkat T3
293GP
M3
OU145
HELA
Panc1
A549
MCF7
Lines
0
60
120
Ratio GMFI

FIG. 5

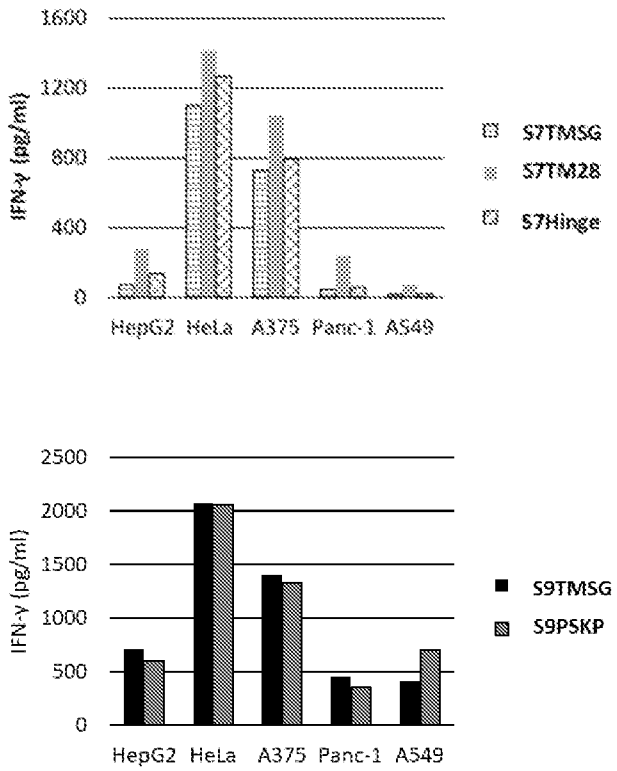

1600
1200
800
400
0
IFN-γ (pg/ml)
HepG2 HeLa A375 Panc-1 A549
S7TMSG
S7TM28
S7Hinge
2500
2000
1500
1000
500
0
IFN-γ (pg/ml)
HepG2 HeLa A375 Panc-1 A549
S9TMSG
S9PSKP

FIG. 6

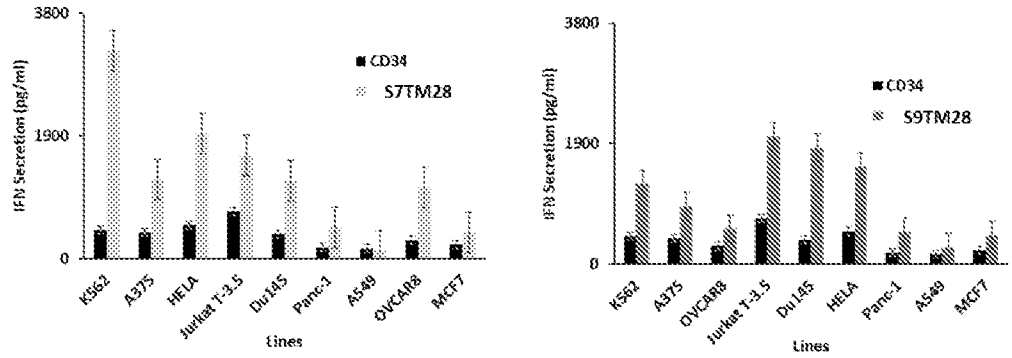

3800
1900
0
IFN Secretion (pg/ml)
CD34
S7TM28
K562
A375
HELA
Jurkat T-3.5
Du145
Panc-1
A549
OVCAR8
MCF7
Lines
3800
1900
0
IFN Secretion (pg/ml)
CD34
S9TM28
K562
A375
OVCAR8
Jurkat T-3.5
Du145
HELA
Panc-1
A549
MCF7
Lines

FIG. 7

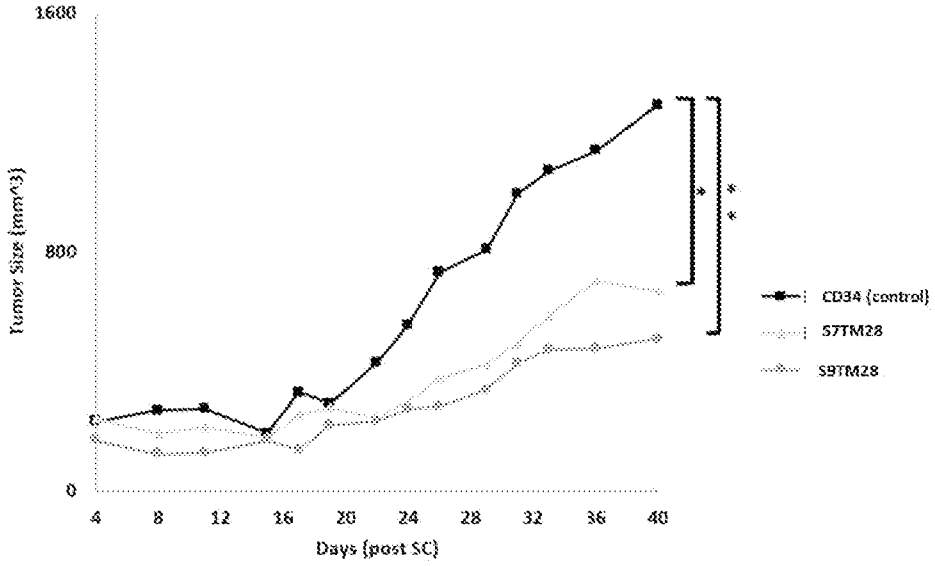

1600
800
0
Tumor Size (mm^3)
4
8
12
16
20
24
28
32
36
40
Days (post SC)
*
*
CD34 (control)
S7TM28
S9TM28

FIG. 8

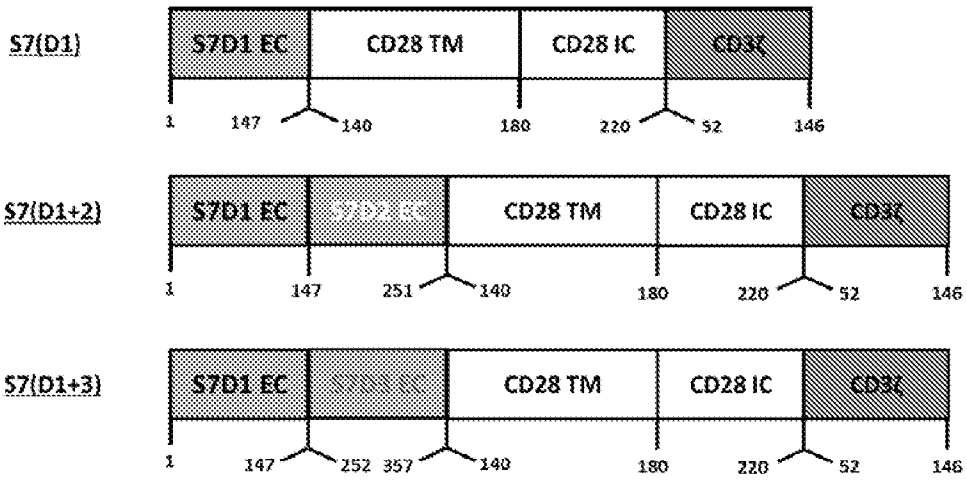

S7(D1)
S7D1 EC
CD28 TM
CD28 IC
CD3ζ
1
147
140
180
220
52
146
S7(D1+2)
S7D1 EC
S7D2 EC
CD28 TM
CD28 IC
CD3ζ
1
147
251
140
180
220
52
146
S7(D1+3)
S7D1 EC
S7D3 EC
CD28 TM
CD28 IC
CD3ζ
1
147
252
357
140
180
220
52
146

FIG. 9

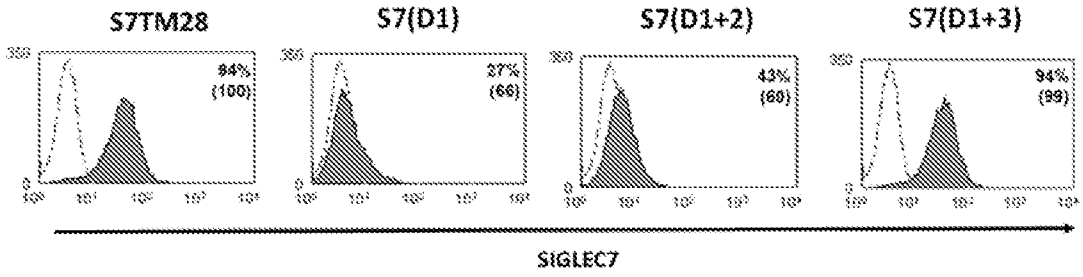

S7TM28
S7(D1)
S7(D1+2)
S7(D1+3)
94%
(100)
27%
(66)
43%
(60)
94%
(99)
SIGLEC7

FIG. 10

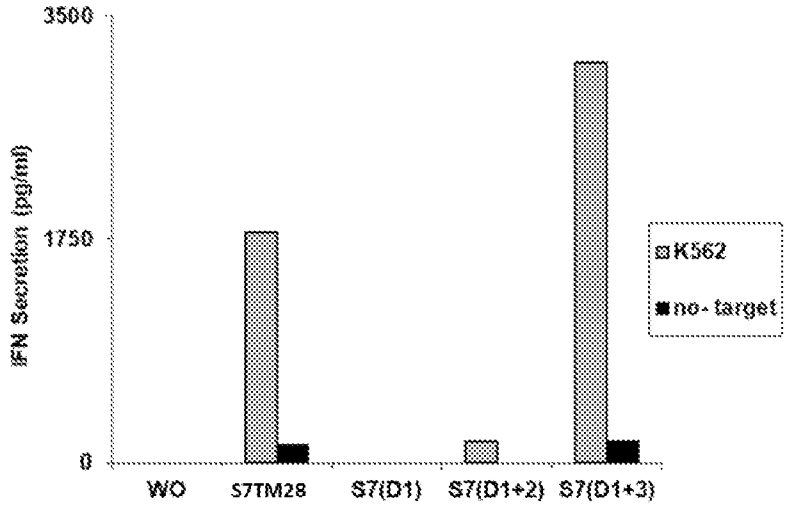

3500
1750
0
IFN Secretion (pg/ml)
WO
S7TM28
S7(D1)
S7(D1+2)
S7(D1+3)
K562
no-target

FIG. 11

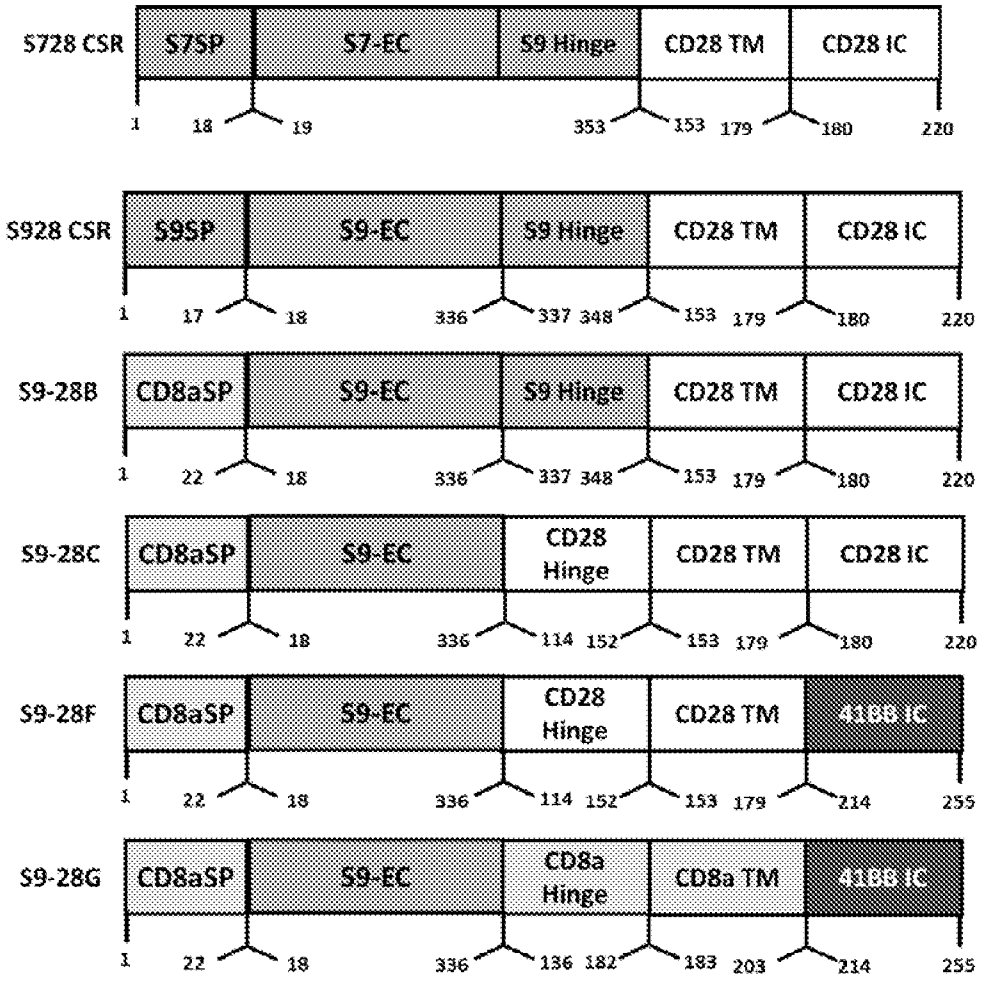

S728 CSR
S7SP
S7-EC
S9 Hinge
CD28 TM
CD28 IC
1
18
19
353
153
179
180
220
S928 CSR
S9SP
S9-EC
S9 Hinge
CD28 TM
CD28 IC
1
17
18
336
337
348
153
179
180
220
S9-28B
CD8aSP
S9-EC
S9 Hinge
CD28 TM
CD28 IC
1
22
18
336
337
348
153
179
180
220
S9-28C
CD8aSP
S9-EC
CD28 Hinge
CD28 TM
CD28 IC
1
22
18
336
114
152
153
179
180
220
S9-28F
CD8aSP
S9-EC
CD28 Hinge
CD28 TM
4-1BB IC
1
22
18
336
114
152
153
179
214
255
S9-28G
CD8aSP
S9-EC
CD8a Hinge
CD8a TM
4-1BB IC
1
22
18
336
136
182
183
203
214
255

FIG. 12

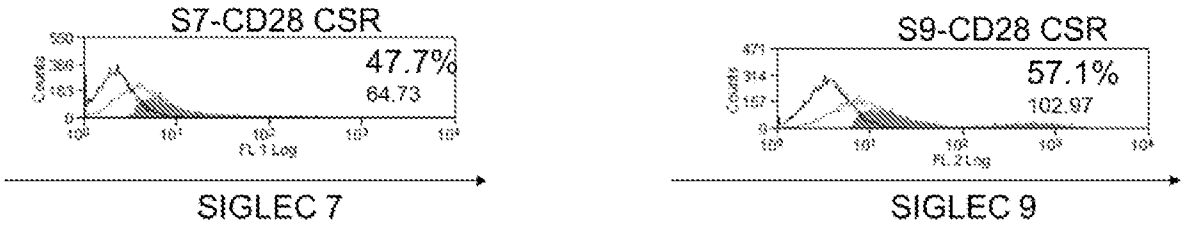

S7-CD28 CSR
47.7%
64.73
SIGLEC 7
S9-CD28 CSR
57.1%
102.97
SIGLEC 9

FIG. 13

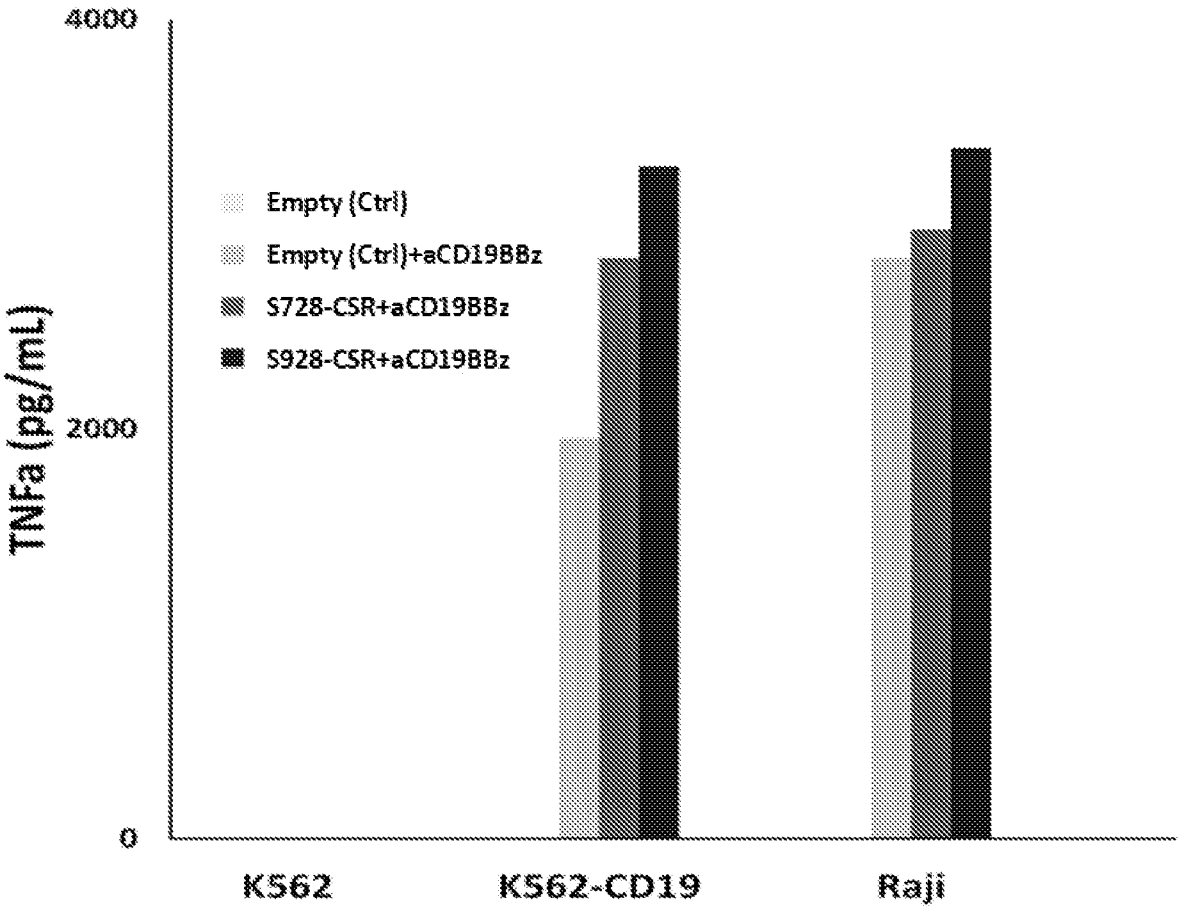

4000
2000
0
TNFa (pg/mL)
Empty (Ctrl)
Empty (Ctrl)+aCD19BBz
S728-CSR+aCD19BBz
S928-CSR+aCD19BBz
K562
K562-CD19
Raji

FIG. 14

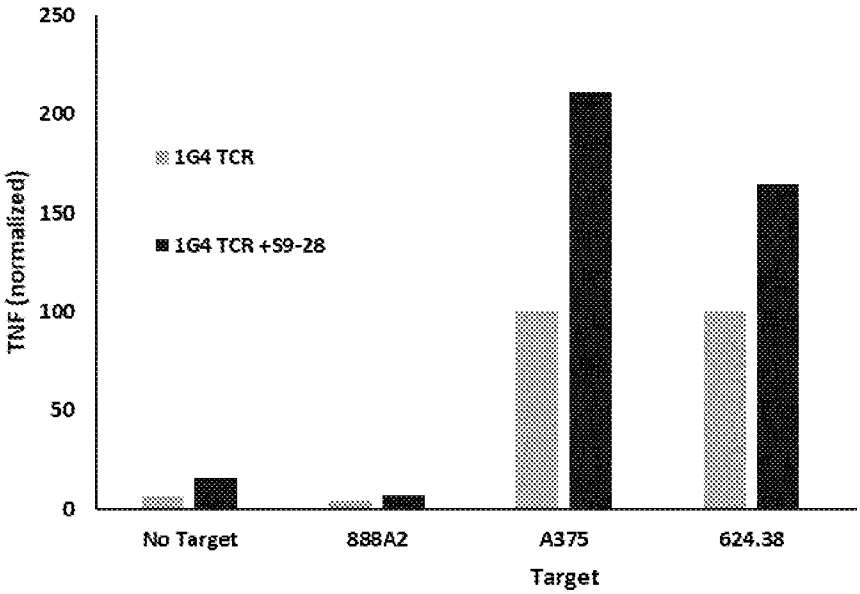

250
200
150
100
50
0
TNF (normalized)
1G4 TCR
1G4 TCR +S9-28
No Target
888A2
A375
624.38
Target

FIG. 15

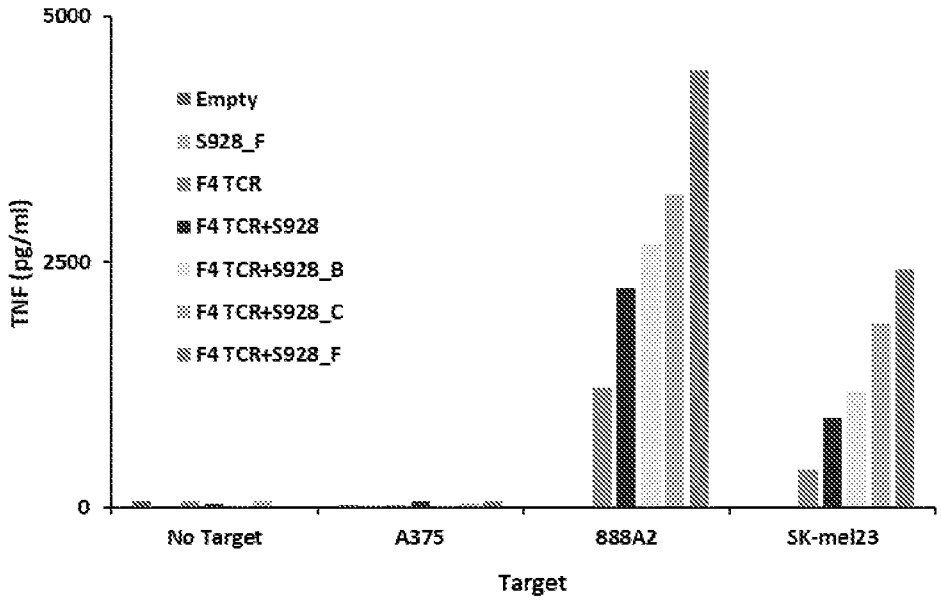
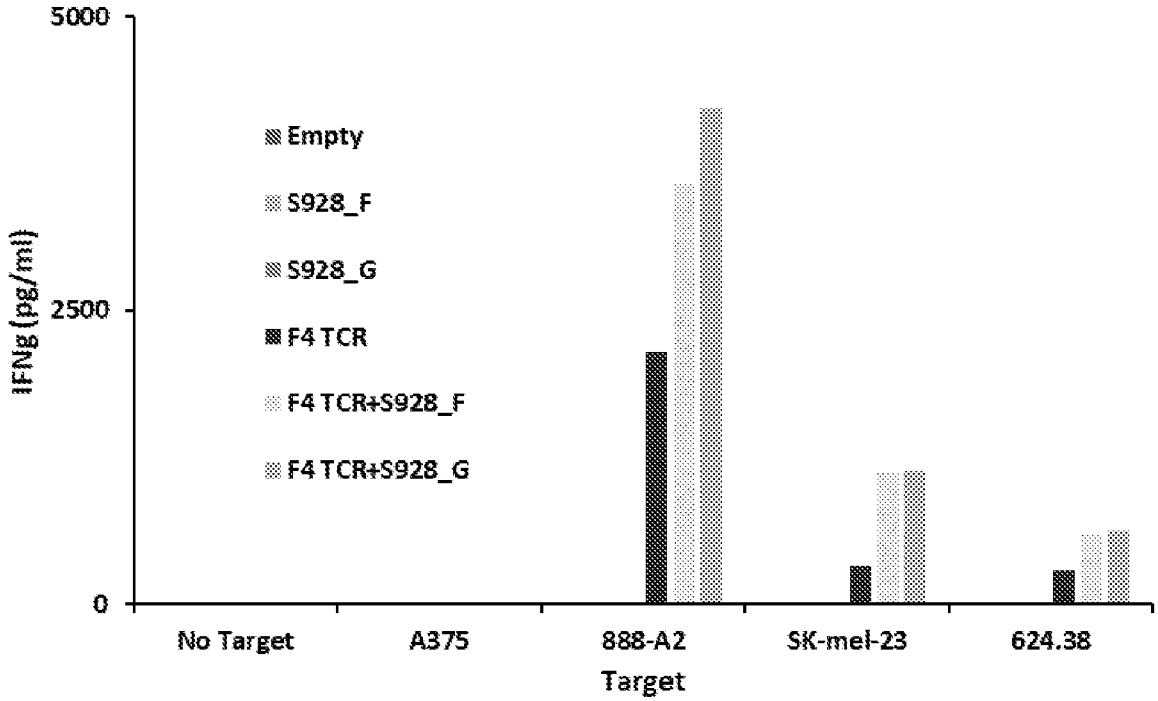

5000
2500
0
TNF (pg/ml)
Empty
S928_F
F4 TCR
F4 TCR+S928
F4 TCR+S928_B
F4 TCR+S928_C
F4 TCR+S928_F
No Target
A375
888A2
SK-mel23
Target

FIG. 16


5000
2500
0
IFNg (pg/ml)
Empty
S928_F
S928_G
F4 TCR
F4 TCR+S928_F
F4 TCR+S928_G
No Target
A375
888-A2
SK-mel-23
624.38
Target

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL2020/050445

A. CLASSIFICATION OF SUBJECT MATTER
IPC (20200101) A61K 39/00, C12N 5/07, C07K 14/47, A61K 35/17
CPC (20180801) A61K 39/001102, C12N 5/06, C07K 14/4726, A61K 35/17
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC (20200101) A61K 39/00, C12N 5/07, C07K 14/47, A61K 35/17
CPC (20180801) A61K 39/001102, C12N 5/06, C07K 14/4726, A61K 35/17

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Databases consulted: PATENTSCOPE, THOMSON INNOVATION, Esp@cenet, Google Patents, Google Scholar
Search terms used: siglec "chimeric receptor"

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Walter, Roland B., et al. "ITIM-dependent endocytosis of CD33-related Siglecs: role of intracellular domain, tyrosine phosphorylation, and the tyrosine phosphatases, Shp1 and Shp2." Journal of leukocyte biology 83.1 (2008): 200-211. Walter, Roland B., et al. 26 Sep 2007 (2007/09/26) Whole document	1-34

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 Jun 2020	28 Jun 2020

Name and mailing address of the ISA:	Authorized officer
Israel Patent Office Technology Park, Bldg.5, Malcha, Jerusalem, 9695101, Israel Email address: pctoffice@justice.gov.il	POUNY Yehonathan Telephone No. 972-73-3927124