

BRIEF SUMMARY

[0007] Embodiments of the disclosure encompass methods and compositions for cell therapy. In particular embodiments, the cell therapy comprises modified cells having a particular receptor. In specific embodiments, the cells are immune cells, including immune cells that are T cells that have a particular receptor comprising a particular amino acid sequence therein. In certain aspects, there are compositions having cells with a modified T cell receptor comprising particular alpha and beta chains that provide selective antitumor activity. In specific embodiments, the cells comprise one or both of a beta chain of said receptor comprising SEQ ID NO:1 or a functional fragment or functional derivative thereof; and an alpha chain of said receptor comprising SEQ ID NO:2 or a functional fragment or functional derivative thereof.

[0008] In a first aspect, provided herein are genetically engineered immune cells, *e.g.*, T cells (T lymphocytes), natural killer (NK) cells or NKT cells, that are directed to survivin and are specific for “on target on tumor” selectivity. In a specific embodiment, the genetically engineered immune cells are T cells. In specific embodiments, the genetically engineered immune cells, *e.g.*, T cells, comprise a receptor that is sensitive for level of surviving expression on cancer cells but not for level of expression of survivin on non-cancer cells (or at least at a reduced level for survivin on non-cancer cells compared to cancer cells). In specific embodiments, when the TCR binds to the surviving-MHC complex on the cancer cells, the immune cell kills the cancer cells. In certain embodiments, the immune cell, *e.g.*, T cell, comprises a TCR comprising one or both of SEQ ID NO:1 and SEQ ID NO:2 or functional fragments or derivatives thereof. Cells of the disclosure that may be modified to target cancers expressing survivin include at least T-cells (which may be referred to as cytotoxic T lymphocytes (CTLs)), NK-cells, NKT-cells, or any other cellular elements with the capability of inducing an effector immune response. In particular cases the cells harbor a polynucleotide that encodes the survivin-specific TCR.

[0009] In another aspect, provided herein are any of the survivin-specific TCR polypeptides described herein. Also provided are polynucleotides encoding such TCRs. In embodiments of the invention, there is a polynucleotide comprising sequence that encodes a survivin-specific TCR and particularly one with specific alpha and/or beta chains. In embodiments of the invention, there is a polynucleotide comprising sequence that encodes a survivin-specific TCR.

[0015] Embodiments of the disclosure provide survivin-specific T-cell receptors targeting cancer but not targeting T cells. Disclosed herein are T-cell receptors that provide epitope specificity, antitumor activity for survivin-expressing cancers, and lack of autoreactivity.

[0016] In certain embodiments, there is a composition comprising an immune cell, said cell comprising an engineered survivin-specific T cell receptor, wherein the receptor comprises one or both of the following: a beta chain of said receptor comprising SEQ ID NO:1 or a functional fragment or functional derivative thereof; and an alpha chain of said receptor comprising SEQ ID NO:2 or a functional fragment or functional derivative thereof. In a specific embodiment, the immune cell is a T cell. In some embodiments, the cell comprises an antigen recognition moiety that is not the T cell receptor. In specific embodiments, the antigen recognition moiety is a chimeric antigen receptor, an engager molecule, or another T cell receptor. The antigen recognition moiety recognizes surviving or a tumor antigen other than survivin, in specific embodiments. In some cases, the cell is autologous to an individual, although it may be allogeneic to an individual. In particular embodiments, the functional fragment of SEQ ID NO:1 has a N-terminal truncation of the sequence of SEQ ID NO:1. In some cases, the N-terminal truncation has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more amino acids truncated from the N-terminus of SEQ ID NO:1. In certain cases, the functional fragment of SEQ ID NO:1 has a C-terminal truncation of the sequence of SEQ ID NO:1. In specific embodiments, the C-terminal truncation has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more amino acids truncated from the C-terminus of SEQ ID NO:1. A specific embodiment provides that the functional derivative of SEQ ID NO:1 is 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to SEQ ID NO:1. In certain embodiments, the functional fragment of SEQ ID NO:2 has a N-terminal truncation of the sequence of SEQ ID NO:2. In specific embodiments, the N-terminal truncation has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more amino acids truncated from the N-terminus of SEQ ID NO:2. In particular embodiments, the functional fragment of SEQ ID NO:2 has a C-terminal truncation of the sequence of SEQ ID NO:2. In specific embodiments, the C-terminal truncation has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more amino acids truncated from the C-terminus of SEQ ID NO:2. In particular embodiments, the functional derivative of SEQ ID NO:2 is 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to SEQ ID NO:2. In particular embodiments, the functional fragment of SEQ ID NO:1 has an N-terminal and a C-terminal truncation of the sequence of SEQ ID NO:1. In some cases,

DETAILED DESCRIPTION

[0038] In keeping with long-standing patent law convention, the words “a” and “an” when used in the present specification in concert with the word comprising, including the claims, denote “one or more.” Some embodiments of the invention may consist of or consist essentially of one or more elements, method steps, and/or methods of the invention. It is contemplated that any method or composition described herein can be implemented with respect to any other method or composition described herein embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

[0039] Survivin is a broadly expressed tumor-associated antigen exerting crucial functions in cancer cells. Immunotherapeutic targeting of this antigen using transgenic T-cell receptors (TCRs) has however been impeded by the “fratricide” activity observed in T cells expressing high-avidity survivin-specific TCRs isolated from allogeneic HLA-mismatched TCR repertoires. Herein it is shown that an HLA-A2-restricted survivin-specific TCR with antitumor activity *in vitro* and *in vivo* but lacking fratricidal toxicity can be isolated when starting from autologous TCR repertoires. To understand the mechanistic basis of this selective activity alanine-scanning was performed, revealing that the autologous-derived TCR had a more specific interaction with the surviving peptide as compared to a “fratricide” TCR. Thus, maximal peptide recognition is key for TCR selectivity and may be critical in reducing unwanted off-target toxicities. This strategy may be adapted to identify and select other shared tumor/self-antigen-specific TCRs that will possess selective antitumor activity.

I. Exemplary Survivin-Specific T Cell Receptors

[0040] The present disclosure concerns T cell receptors (TCR) having particular alpha and beta chains, wherein the T cell is specific for the survivin antigen. In particular aspects, the receptor is an engineered receptor by the hand of man. The receptor is recombinantly produced, in particular embodiments, and is expressed on an immune cell, such as a T cell. The receptor is capable of recognizing the survivin antigen on cancer cells and, in specific embodiments, the receptor does not recognize the survivin antigen on non-cancer cells or recognizes it at a reduced level compared to cancer cells.

[0041] In specific embodiments, the TCR comprises a beta chain that comprises SEQ ID NO:1 or a functional fragment or functional derivative thereof. In specific embodiments, the TCR comprises an alpha chain that comprises SEQ ID NO:2 or a functional

fragment or functional derivative thereof. In specific embodiments, the TCR comprises both a beta chain that comprises SEQ ID NO:1 or a functional fragment or functional derivative thereof and an alpha chain that comprises SEQ ID NO:2 or a functional fragment or functional derivative thereof.

[0042] In embodiments of the disclosure, the T cell receptors bind to one or more epitopes on survivin. Although the epitope may be of any kind, in specific embodiments the epitope comprises, consists of, or consists essentially of SEQ ID NO:15 or SEQ ID NO:16 or a fragment thereof, including a functional fragment thereof. In particular embodiments, the epitope that is recognized by the T cell receptor of the disclosure is or is at least 70, 75, 77, 80, 85, 88, 90, 91, 95, 97, or 99% identical to SEQ ID NO:15 or SEQ ID NO:16 or a fragment thereof, including a functional fragment thereof. The epitope may have N-terminal and/or C-terminal extensions or truncation in relation to SEQ ID NO:15 or SEQ ID NO:16. Such N-terminal and/or C-terminal extensions may be 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more amino acids. Such N-terminal and/or C-terminal truncations may be 1, 2, 3, 4, or 5 or more amino acids. The TCRs of the disclosure may be able to bind survivin epitopes that have 1, 2, 3, 4, 5, or more alterations at particular residues compared to SEQ ID NO:15 or SEQ ID NO:16. In specific embodiments, Leu4, Gly5 and/or Phe7 of SEQ ID NO:15 are not altered, although in alternative embodiments one or more of them are altered.

A. Proteinaceous Compositions, Generally

[0043] In certain embodiments, the present invention concerns novel TCR compositions comprising at least one proteinaceous molecule. As used herein, a "proteinaceous molecule," "proteinaceous composition," "proteinaceous compound," "proteinaceous chain" or "proteinaceous material" generally refers, but is not limited to, a protein of greater than about 200 amino acids or the full length endogenous sequence translated from a gene; a polypeptide of greater than about 100 amino acids; and/or a peptide of from about 3 to about 100 amino acids. All the "proteinaceous" terms described above may be used interchangeably herein.

[0044] In certain embodiments the size of the at least one proteinaceous molecule may comprise, but is not limited to, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37,

proteins. As used herein, the term "autologous protein, polypeptide or peptide" refers to a protein, polypeptide or peptide which is derived or obtained from an organism, with a selected animal or human subject being preferred. The "autologous protein, polypeptide or peptide" may then be used as a component of a composition intended for application to the selected animal or human subject. In certain aspects, the autologous proteins or peptides are prepared, for example from whole plasma of the selected donor. The plasma is placed in tubes and placed in a freezer at about -80oC for at least about 12 hours and then centrifuged at about 12,000 times g for about 15 minutes to obtain the precipitate. The precipitate, such as fibrinogen may be stored for up to about one year (Oz, 1990).

B. Biological Functional Equivalents

[0052] As modifications and/or changes may be made in the structure of the TCR polynucleotides and and/or proteins according to the present invention, while obtaining molecules having similar or improved characteristics, such biologically functional equivalents are also encompassed within the present invention.

[0053] The disclosure provides TCR beta and alpha chains that are fragments and/or derivatives of SEQ ID NO:1 and SEQ ID NO:2, respectively. Such fragments and/or derivatives will maintain the activity of SEQ ID NO:1 and SEQ ID NO:2, respectively. In particular embodiments, the fragments and/or derivatives as part of a TCR in its totality will provide selective antitumor activity.

1. Modified Polynucleotides and Polypeptides

[0054] The biological functional equivalent may comprise a polynucleotide that has been engineered to contain distinct sequences while at the same time retaining the capacity to encode the "wild-type" or standard protein. This can be accomplished to the degeneracy of the genetic code, *i.e.*, the presence of multiple codons, which encode for the same amino acids. In one example, one of skill in the art may wish to introduce a restriction enzyme recognition sequence into a polynucleotide while not disturbing the ability of that polynucleotide to encode a protein.

[0055] In another example, a polynucleotide may be (and encode) a biological functional equivalent with more significant changes. Certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies, binding sites on

activity of individual cells, and the like. Therefore, it is expected that for each individual patient, even if there were universal cells which could be administered to the population at large, each patient would be monitored for the proper dosage for the individual, and such practices of monitoring a patient are routine in the art.

[0112] In another aspect, provided herein is a method of treating an individual having a tumor cell, comprising administering to the individual a therapeutically effective amount of cells expressing at least TCR. In a related aspect, provided herein is a method of treating an individual having a tumor cell, comprising administering to the individual a therapeutically effective amount of cells expressing at least the survivin-specific TCR. In a specific embodiment, said administering results in a measurable decrease in the growth of the tumor in the individual. In another specific embodiment, said administering results in a measurable decrease in the size of the tumor in the individual. In various embodiments, the size or growth rate of a tumor may be determinable by, *e.g.*, direct imaging (*e.g.*, CT scan, MRI, PET scan or the like), fluorescent imaging, tissue biopsy, and/or evaluation of relevant physiological markers (*e.g.*, PSA levels for prostate cancer; HCG levels for choriocarcinoma, and the like). In specific embodiments of the invention, the individual has a high level of an antigen that is correlated to poor prognosis. In some embodiments, the individual is provided with an additional cancer therapy, such as surgery, radiation, chemotherapy, hormone therapy, immunotherapy, or a combination thereof.

IV. Kits of the Disclosure

[0113] Embodiments relate to a kit comprising cells as defined herein, TCR constructs as defined herein, a nucleic acid sequence as defined herein, and/or a vector as defined herein. It is also contemplated that the kit of this disclosure comprises a pharmaceutical composition as described herein above, either alone or in combination with further medicaments to be administered to an individual in need of medical treatment or intervention.

[0114] Any of the compositions described herein may be comprised in a kit. In a non-limiting example, cells for cell therapy or one or more reagents to produce the cells may be comprised in a kit. The kits will thus comprise, in suitable container means, cells, vectors, primers, enzymes, buffers, salts, nucleotides, polynucleotides, and so forth may be comprised in a kit. In particular embodiments, the cells have been transduced with a particular vector encoding a TCR specific for survivin, including a TCR comprising one or both of a beta chain

of said receptor comprising SEQ ID NO:1 or a functional fragment or functional derivative thereof; and an alpha chain of said receptor comprising SEQ ID NO:2 or a functional fragment or functional derivative thereof. The kits may also comprise bacteria for further manipulation of the receptor or a polynucleotide encoding same. The kits may comprise untransduced mammalian immune cells, such as immune cells capable of being transduced with a vector encoding part or all of a TCR of the disclosure. The kits may comprise polynucleotides that encode part or all of a TCR of the disclosure, including a vector polynucleotide, such as one that comprise an expression construct.

[0115] The kits may comprise a suitably aliquoted cell compositions of the present disclosure, or suitably aliquoted reagents to generate cells. The components of the kits may be packaged either in aqueous media or in lyophilized form. The container means of the kits may generally include at least one vial, test tube, flask, bottle, syringe or other container means, into which a component may be placed, and preferably, suitably aliquoted. The kits may also comprise a second container means for containing a sterile, pharmaceutically acceptable buffer and/or other diluent. Where there are more than one component in the kit, the kit also will generally contain a second, third or other additional container into which the additional component(s) may be separately placed. However, various combinations of components may be comprised in a vial. The kit may have a single container means, and/or it may have distinct container means for each compound. The kits of the present invention also will typically include a means for containing any container(s) in close confinement for commercial sale. Such containers may include injection or blow-molded plastic containers into which the desired vials are retained.

[0116] Kits of the present disclosure may comprise polynucleotides that encode part or all of a T cell receptor and/or primers to produce such polynucleotides, such as by amplification. Such polynucleotides may encode the T cell receptor beta chain, T cell receptor alpha chain, or another region of the receptor, for example.

[0117] When the components of the kit are provided in one and/or more liquid solutions, the liquid solution may be an aqueous solution, with a sterile aqueous solution being particularly preferred. The compositions may also be formulated into a deliverable composition. In which case, the container means may itself be a syringe, pipette, and/or other such like