

activation) to the cells will occur. An IL2 variant able to selectively activate IL2R $\alpha\beta\gamma$ comprising the corresponding variant α subunit of IL2R on immune effector cells, e.g., T cells such as memory T cells, naïve T cells and effector T cells as well as NK cells, in preference to cells expressing wild type IL2R $\alpha\beta\gamma$ is expected to have an improved therapeutic index over wild type IL2 and a reduced toxicity profile. An IL2 variant with an improved therapeutic index would have a significantly expanded range of use in the treatment of disorders requiring immune system stimulation, for example in the treatment of cancer (as a direct and/or adjunct therapy). In particular, administration of IL2 variant RNA is a promising approach to boost the therapeutic efficacy of multiple T and NK cell-based (cancer) immunotherapies.

Immune effector cells harboring a variant IL2R described herein may be generated *in vitro* and subsequently administered to a subject in need of treatment or may be generated *in vivo* in a subject in need of treatment. Administering to the subject the corresponding IL2 variant, a polynucleotide encoding the corresponding IL2 variant or a host cell genetically modified to express the corresponding IL2 variant allows for the specific stimulation of receptor-engineered immune effector cells. The methods and agents described herein are, in particular, useful for the treatment of diseases characterized by diseased cells expressing an antigen the immune effector cells are directed to. In one example, the immune effector cells carry an antigen receptor such a T cell receptor (TCR) or chimeric antigen receptor (CAR) having a binding specificity for the antigen or a procession product thereof. In one example, the immune effector cells are present in a subject to be treated and are genetically modified *in vivo* in the subject to express a receptor polypeptide described herein. In one example, immune effector cells either from a subject to be treated or from a different subject are administered to the subject to be treated. The administered immune effector cells may be genetically modified *ex vivo* prior to administration or genetically modified *in vivo* in the subject following administration to express a receptor polypeptide described herein. In one example, an antigen receptor is endogenous to the immune effector cells. In one example, the immune effector cells are genetically modified, *ex vivo* or *in vivo*, to express the antigen receptor. Thus, such genetic modification with antigen receptor may be effected *in vitro* (optionally together with genetic modification by the IL2 receptor polypeptides described herein) and subsequently the immune effector cells administered to a subject in need of treatment or may be effected *in vivo* (optionally together with genetic modification by the IL2 receptor polypeptides described herein) in a subject in need of treatment. Thus, in one example, the present disclosure generally embraces the treatment of diseases by targeting cells expressing an antigen such as diseased cells, in particular cancer cells expressing a tumor antigen. The target cells may express the antigen on the cell surface or may present a procession product of the antigen. In one example, the antigen is a tumor-associated antigen and the disease is cancer. Such

treatment provides for the selective eradication of cells that express an antigen, thereby minimizing adverse effects to normal cells not expressing the antigen. In one example, vaccine antigen or polynucleotide coding therefor is administered to provide (optionally following expression of the polynucleotide by appropriate target cells) antigen for stimulation, priming and/or expansion of the immune effector cells, wherein the immune effector cells (optionally genetically modified to express an antigen receptor) are targeted to the antigen or a procession product thereof and the immune response is an immune response to a target cell population or target tissue expressing the antigen. In one example, the polynucleotide encoding the vaccine antigen is RNA. Immune effector cells such as T cells stimulated, primed and/or expanded in the patient are able to recognize cells expressing an antigen resulting in the eradication of diseased cells. The methods and agents described herein are particularly effective if RNA encoding IL2 variants is targeted to the liver for systemic availability. Liver cells can be efficiently transfected and are able to produce large amounts of protein. Antigen-encoding RNA is preferably targeted to secondary lymphoid organs.

Accordingly, one example relates to systems of alpha subunit of interleukin-2 receptor (IL2R α) and interleukin-2 (IL2) variants for specific activation of immune effector cells in the treatment of diseases where such immune effector cells can be effective such as cancer including but not limited to solid tumors. In one example, the present disclosure relates to a strategy of adoptive cell transfer of cells such as T cells transduced to express a CAR. CARs are molecules that combine specificity for a desired antigen (e.g., tumor antigen) which preferably is antibody-based with a T cell receptor-activating intracellular domain to generate a chimeric protein that exhibits a specific cellular immune activity (e.g., a specific anti-tumor cellular immune activity). Preferably, a cell can be genetically modified to stably express a CAR (and an IL2 receptor polypeptide described herein) on its surface, conferring novel antigen specificity that is MHC independent. T cells expressing a CAR are referred to herein as CAR T cells or CAR-modified T cells.

One aspect of the disclosure relates to a system comprising:

- (i) a receptor polypeptide comprising a mutein of the alpha subunit of interleukin-2 receptor (IL2R) or of a functional variant of the alpha subunit of IL2R, wherein the alpha subunit of IL2R or the functional variant thereof is substituted at at least one position,
 - (ii) a ligand polypeptide comprising a mutein of IL2 or of a functional variant of IL2, wherein the IL2 or the functional variant thereof is substituted at at least one position,
- wherein the substitutions are such that

(a) the mutein under (ii) binds to and activates IL2R comprising the mutein under (i) as alpha subunit, and
(b) binding to and/or activation of IL2R comprising the mutein under (i) as alpha subunit by the mutein under (ii) exceeds binding to and/or activation of IL2R comprising the alpha subunit of IL2R or the functional variant thereof as alpha subunit by the mutein under (ii).

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In one example, binding to and/or activation of IL2R comprising the mutein under (i) as alpha subunit by the mutein under (ii) exceeds binding to and/or activation of IL2R comprising the mutein under (i) as alpha subunit by IL2 or the functional variant thereof. In one example, binding to and/or activation of IL2R comprising the alpha subunit of IL2R or the functional variant thereof as alpha subunit by IL2 or the functional variant thereof exceeds binding to and/or activation of IL2R comprising the alpha subunit of IL2R or the functional variant thereof as alpha subunit by the mutein under (ii). In one example, binding to and/or activation of IL2R comprising the alpha subunit of IL2R or the functional variant thereof as alpha subunit by IL2 or the functional variant thereof exceeds binding to and/or activation of IL2R comprising the mutein under (i) as alpha subunit by IL2 or the functional variant thereof.

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In one example, the system of the disclosure comprises

(i) a receptor polypeptide comprising a mutein of the alpha subunit of IL2R or of a functional variant of the alpha subunit of IL2R, wherein the alpha subunit of IL2R or the functional variant thereof is substituted at at least a position having an acidic amino acid residue in wild type alpha subunit of IL2R that contacts a basic amino acid residue in wild type IL2 and/or is substituted at at least a position having a basic amino acid residue in wild type alpha subunit of IL2R that contacts an acidic amino acid residue in wild type IL2, wherein if the amino acid residue is an acidic amino acid residue in wild type alpha subunit of IL2R the substitution is by a basic amino acid residue and if the amino acid residue is a basic amino acid residue in wild type alpha subunit of IL2R the substitution is by an acidic amino acid residue,

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(ii) a ligand polypeptide comprising a mutein of IL2 or of a functional variant of IL2, wherein when the alpha subunit of IL2R or the functional variant thereof is substituted at at least a position having an acidic amino acid residue in wild type alpha subunit of IL2R that contacts a basic amino acid residue in wild type IL2, the IL2 or the functional variant thereof is substituted at at least said basic amino acid residue in wild type IL2 and/or when the alpha subunit of IL2R or the functional variant thereof is substituted at at least a position having a basic amino acid residue in wild type alpha subunit of IL2R that contacts an acidic amino acid residue in wild type IL2, the IL2 or the functional variant thereof is substituted at at least said acidic amino acid residue in wild type IL2, wherein if the amino acid residue is a basic amino acid residue in wild

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type IL2 the substitution is by an acidic amino acid residue and if the amino acid residue is an acidic amino acid residue in wild type IL2 the substitution is by a basic amino acid residue.

In one aspect, the system of the disclosure comprises

- 5 (i) a receptor polypeptide comprising a mutein of the alpha subunit of interleukin-2 receptor (IL2R) or of a functional variant of the alpha subunit of IL2R, wherein the alpha subunit of IL2R or the functional variant thereof is substituted at at least a position having an acidic amino acid residue in wild type alpha subunit of IL2R that contacts a basic amino acid residue in wild type IL2 and/or is substituted at at least a position having a basic amino acid residue in wild type alpha subunit of IL2R that contacts an acidic amino acid residue in wild type IL2, wherein if the amino acid residue is an acidic amino acid residue in wild type alpha subunit of IL2R the substitution is by a basic amino acid residue and if the amino acid residue is a basic amino acid residue in wild type alpha subunit of IL2R the substitution is by an acidic amino acid residue,
- 10 (ii) a ligand polypeptide comprising a mutein of IL2 or of a functional variant of IL2, wherein when the alpha subunit of IL2R or the functional variant thereof is substituted at at least a position having an acidic amino acid residue in wild type alpha subunit of IL2R that contacts a basic amino acid residue in wild type IL2, the IL2 or the functional variant thereof is substituted at at least said basic amino acid residue in wild type IL2 and/or when the alpha subunit of IL2R or the functional variant thereof is substituted at at least a position having a basic amino acid residue in wild type alpha subunit of IL2R that contacts an acidic amino acid residue in wild type IL2, the IL2 or the functional variant thereof is substituted at at least said acidic amino acid residue in wild type IL2, wherein if the amino acid residue is a basic amino acid residue in wild type IL2 the substitution is by an acidic amino acid residue and if the amino acid residue is an acidic amino acid residue in wild type IL2 the substitution is by a basic amino acid residue.
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- 25 In one example of the system of the disclosure the alpha subunit of IL2R is the human alpha subunit of IL2R. In one example of the system of the disclosure the IL2 is human IL2.

In different examples, the human alpha subunit of IL2R or the functional variant thereof and the human IL2 or the functional variant thereof are substituted at at least the following positions (relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and relative to wild type human IL2 and numbered in accordance with wild type human IL2):

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- (i) IL2R or functional variant thereof: position 1 (glutamic acid), and
IL2 or functional variant thereof: position 35 (lysine);

- (ii) IL2R or functional variant thereof: position 29 (glutamic acid), and
IL2 or functional variant thereof: position 43 (lysine);
- (iii) IL2R or functional variant thereof: position 38 (lysine), and
IL2 or functional variant thereof: position 61 (glutamic acid);
- 5 (iv) IL2R or functional variant thereof: position 1 (glutamic acid),
IL2 or functional variant thereof: position 35 (lysine),
IL2R or functional variant thereof: position 29 (glutamic acid), and
IL2 or functional variant thereof: position 43 (lysine);
- (v) IL2R or functional variant thereof: position 1 (glutamic acid),
10 IL2 or functional variant thereof: position 35 (lysine),
IL2R or functional variant thereof: position 38 (lysine), and
IL2 or functional variant thereof: position 61 (glutamic acid);
- (vi) IL2R or functional variant thereof: position 29 (glutamic acid),
IL2 or functional variant thereof: position 43 (lysine),
15 IL2R or functional variant thereof: position 38 (lysine), and
IL2 or functional variant thereof: position 61 (glutamic acid); or
- (vii) IL2R or functional variant thereof: position 1 (glutamic acid),
IL2 or functional variant thereof: position 35 (lysine),
IL2R or functional variant thereof: position 29 (glutamic acid),
20 IL2 or functional variant thereof: position 43 (lysine),
IL2R or functional variant thereof: position 38 (lysine), and
IL2 or functional variant thereof: position 61 (glutamic acid).

IL2R or the functional variant thereof: In one example, position 1 is substituted with lysine. In one example,
25 position 29 is substituted with lysine. In one example, position 38 is substituted with glutamic acid.

IL2 or the functional variant thereof: In one example, position 35 is substituted with glutamic acid. In one
example, position 43 is substituted with glutamic acid. In one example, position 61 is substituted with
lysine.

30 In one example of the system of the disclosure

(i) the alpha subunit of IL2R is the human alpha subunit of IL2R and the alpha subunit of IL2R or the
functional variant thereof is substituted at at least position 1 (glutamic acid) relative to wild type human
alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and

(ii) the IL2 is human IL2 and the IL2 or the functional variant thereof is substituted at at least position 35 (lysine) relative to wild type human IL2 and numbered in accordance with wild type human IL2.

5 In one example, position 1 is substituted with lysine. In one example, position 35 is substituted with glutamic acid.

In one example of the system of the disclosure

10 (i) the alpha subunit of IL2R is the human alpha subunit of IL2R and the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 29 (glutamic acid) relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and
(ii) the IL2 is human IL2 and the IL2 or the functional variant thereof is substituted at at least position 43 (lysine) relative to wild type human IL2 and numbered in accordance with wild type human IL2.

15 In one example, position 29 is substituted with lysine. In one example, position 43 is substituted with glutamic acid.

In one example of the system of the disclosure

20 (i) the alpha subunit of IL2R is the human alpha subunit of IL2R and the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 38 (lysine) relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and
(ii) the IL2 is human IL2 and the IL2 or the functional variant thereof is substituted at at least position 61 (glutamic acid) relative to wild type human IL2 and numbered in accordance with wild type human IL2.

25 In one example, position 38 is substituted with glutamic acid. In one example, position 61 is substituted with lysine.

In one example of the system of the disclosure

30 (i) the alpha subunit of IL2R is the human alpha subunit of IL2R and the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 1 (glutamic acid) by lysine, position 29 (glutamic acid) by lysine and position 38 (lysine) by glutamic acid relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and

(ii) the IL2 is human IL2 and the IL2 or the functional variant thereof is substituted at at least position 35 (lysine) by glutamic acid, position 43 (lysine) by glutamic acid and position 61 (glutamic acid) by lysine relative to wild type human IL2 and numbered in accordance with wild type human IL2.

5 In one example of the system of the disclosure

(i) the alpha subunit of IL2R is the human alpha subunit of IL2R and the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 29 (glutamic acid) by lysine and position 38 (lysine) by glutamic acid relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and

10 (ii) the IL2 is human IL2 and the IL2 or the functional variant thereof is substituted at at least position 43 (lysine) by glutamic acid and position 61 (glutamic acid) by lysine relative to wild type human IL2 and numbered in accordance with wild type human IL2.

In one example of the system of the disclosure

15 (i) the alpha subunit of IL2R is the human alpha subunit of IL2R and the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 38 (lysine) by glutamic acid relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and

20 (ii) the IL2 is human IL2 and the IL2 or the functional variant thereof is substituted at at least position 61 (glutamic acid) by lysine relative to wild type human IL2 and numbered in accordance with wild type human IL2.

In one example of the system of the disclosure

25 (i) the alpha subunit of IL2R is the human alpha subunit of IL2R and the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 29 (glutamic acid) by lysine relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and

30 (ii) the IL2 is human IL2 and the IL2 or the functional variant thereof is substituted at at least position 43 (lysine) by glutamic acid relative to wild type human IL2 and numbered in accordance with wild type human IL2.

In one example of the system of the disclosure

(i) the alpha subunit of IL2R is the human alpha subunit of IL2R and the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 29 (glutamic acid) by lysine and position 38 (lysine) by glutamic acid relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and

- 5 (ii) the IL2 is human IL2 and the IL2 or the functional variant thereof is substituted at at least position 35 (lysine) by glutamic acid, position 43 (lysine) by glutamic acid and position 61 (glutamic acid) by lysine relative to wild type human IL2 and numbered in accordance with wild type human IL2.

In one example of the system of the disclosure

- 10 (i) the alpha subunit of IL2R is the human alpha subunit of IL2R and the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 29 (glutamic acid) by lysine relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and

- (ii) the IL2 is human IL2 and the IL2 or the functional variant thereof is substituted at at least position 35
15 (lysine) by glutamic acid, position 43 (lysine) by glutamic acid and position 61 (glutamic acid) by lysine relative to wild type human IL2 and numbered in accordance with wild type human IL2.

In one example of the system of the disclosure

- (i) the alpha subunit of IL2R is the human alpha subunit of IL2R and the alpha subunit of IL2R or the
20 functional variant thereof is substituted at at least position 29 (glutamic acid) by lysine relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and

- (ii) the IL2 is human IL2 and the IL2 or the functional variant thereof is substituted at at least position 43
25 (lysine) by glutamic acid and position 61 (glutamic acid) by lysine relative to wild type human IL2 and numbered in accordance with wild type human IL2.

In one example of the system of the disclosure

- (i) the alpha subunit of IL2R is the human alpha subunit of IL2R and the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 1 (glutamic acid) by lysine, position 29 (glutamic acid) by lysine and position 38 (lysine) by glutamic acid relative to wild type human alpha subunit of IL2R
30 and numbered in accordance with wild type human alpha subunit of IL2R, and

(ii) the IL2 is human IL2 and the IL2 or the functional variant thereof is substituted at at least position 43 (lysine) by glutamic acid and position 61 (glutamic acid) by lysine relative to wild type human IL2 and numbered in accordance with wild type human IL2.

5 In another aspect, the present disclosure relates to a system comprising:

(i) a receptor polypeptide comprising a mutein of the human alpha subunit of interleukin-2 receptor (IL2R) or of a functional variant of the human alpha subunit of IL2R, wherein the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 1 (glutamic acid) by a basic amino acid residue relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human
10 alpha subunit of IL2R, and

(ii) a ligand polypeptide comprising a mutein of human IL2 or of a functional variant of human IL2, wherein the IL2 or the functional variant thereof is substituted at at least position 35 (lysine) by an acidic amino acid residue relative to wild type human IL2 and numbered in accordance with wild type human IL2, optionally wherein position 1 is substituted with lysine and/or wherein position 35 is substituted with
15 glutamic acid.

A further example of the disclosure relates to a system comprising:

(i) a receptor polypeptide comprising a mutein of the human alpha subunit of interleukin-2 receptor (IL2R) or of a functional variant of the human alpha subunit of IL2R, wherein the alpha subunit of IL2R or the
20 functional variant thereof is substituted at at least position 1 (glutamic acid) by a basic amino acid residue relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and

(ii) a ligand polypeptide comprising a mutein of human IL2 or of a functional variant of human IL2, wherein the IL2 or the functional variant thereof is substituted at at least position 35 (lysine) by an acidic amino
25 acid residue relative to wild type human IL2 and numbered in accordance with wild type human IL2.

In one example, position 1 is substituted with lysine. In one example, position 35 is substituted with glutamic acid.

30 In yet another aspect, the present disclosure relates to a system comprising:

(i) a receptor polypeptide comprising a mutein of the human alpha subunit of interleukin-2 receptor (IL2R) or of a functional variant of the human alpha subunit of IL2R, wherein the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 29 (glutamic acid) by a basic amino acid residue

relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and

- 5 (ii) a ligand polypeptide comprising a mutein of human IL2 or of a functional variant of human IL2, wherein the IL2 or the functional variant thereof is substituted at at least position 43 (lysine) by an acidic amino acid residue relative to wild type human IL2 and numbered in accordance with wild type human IL2, optionally wherein position 29 is substituted with lysine and/or wherein position 43 is substituted with glutamic acid.

A further example of the disclosure relates to a system comprising:

- 10 (i) a receptor polypeptide comprising a mutein of the human alpha subunit of interleukin-2 receptor (IL2R) or of a functional variant of the human alpha subunit of IL2R, wherein the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 29 (glutamic acid) by a basic amino acid residue relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and
- 15 (ii) a ligand polypeptide comprising a mutein of human IL2 or of a functional variant of human IL2, wherein the IL2 or the functional variant thereof is substituted at at least position 43 (lysine) by an acidic amino acid residue relative to wild type human IL2 and numbered in accordance with wild type human IL2.

20 In one example, position 29 is substituted with lysine. In one example, position 43 is substituted with glutamic acid.

In yet another aspect, the present disclosure relates to a system comprising:

- 25 (i) a receptor polypeptide comprising a mutein of the human alpha subunit of interleukin-2 receptor (IL2R) or of a functional variant of the human alpha subunit of IL2R, wherein the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 38 (lysine) by an acidic amino acid residue relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and
- 30 (ii) a ligand polypeptide comprising a mutein of human IL2 or of a functional variant of human IL2, wherein the IL2 or the functional variant thereof is substituted at at least position 61 (glutamic acid) by a basic amino acid residue relative to wild type human IL2 and numbered in accordance with wild type human IL2, optionally wherein position 38 is substituted with glutamic acid and/or wherein position 61 is substituted with lysine.

A further example of the disclosure relates to a system comprising:

(i) a receptor polypeptide comprising a mutein of the human alpha subunit of interleukin-2 receptor (IL2R) or of a functional variant of the human alpha subunit of IL2R, wherein the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 38 (lysine) by an acidic amino acid residue relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and

(ii) a ligand polypeptide comprising a mutein of human IL2 or of a functional variant of human IL2, wherein the IL2 or the functional variant thereof is substituted at at least position 61 (glutamic acid) by a basic amino acid residue relative to wild type human IL2 and numbered in accordance with wild type human IL2.

In one example, position 38 is substituted with glutamic acid. In one example, position 61 is substituted with lysine.

A further aspect of the disclosure relates to a system comprising:

(i) a receptor polypeptide comprising a mutein of the human alpha subunit of interleukin-2 receptor (IL2R) or of a functional variant of the human alpha subunit of IL2R, wherein the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 1 (glutamic acid) by lysine, position 29 (glutamic acid) by lysine and position 38 (lysine) by glutamic acid relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and

(ii) a ligand polypeptide comprising a mutein of human IL2 or of a functional variant of human IL2, wherein the IL2 or the functional variant thereof is substituted at at least position 35 (lysine) by glutamic acid, position 43 (lysine) by glutamic acid and position 61 (glutamic acid) by lysine relative to wild type human IL2 and numbered in accordance with wild type human IL2.

A further aspect of the disclosure relates to a system comprising:

(i) a receptor polypeptide comprising a mutein of the human alpha subunit of interleukin-2 receptor (IL2R) or of a functional variant of the human alpha subunit of IL2R, wherein the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 29 (glutamic acid) by lysine and position 38 (lysine) by glutamic acid relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and

(ii) a ligand polypeptide comprising a mutein of human IL2 or of a functional variant of human IL2, wherein the IL2 or the functional variant thereof is substituted at at least position 43 (lysine) by glutamic acid and

position 61 (glutamic acid) by lysine relative to wild type human IL2 and numbered in accordance with wild type human IL2.

A further aspect of the disclosure relates to a system comprising:

- 5 (i) a receptor polypeptide comprising a mutein of the human alpha subunit of interleukin-2 receptor (IL2R) or of a functional variant of the human alpha subunit of IL2R, wherein the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 38 (lysine) by glutamic acid relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and
- 10 (ii) a ligand polypeptide comprising a mutein of human IL2 or of a functional variant of human IL2, wherein the IL2 or the functional variant thereof is substituted at at least position 61 (glutamic acid) by lysine relative to wild type human IL2 and numbered in accordance with wild type human IL2.

A further aspect of the disclosure relates to a system comprising:

- 15 (i) a receptor polypeptide comprising a mutein of the human alpha subunit of interleukin-2 receptor (IL2R) or of a functional variant of the human alpha subunit of IL2R, wherein the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 29 (glutamic acid) by lysine relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and
- 20 (ii) a ligand polypeptide comprising a mutein of human IL2 or of a functional variant of human IL2, wherein the IL2 or the functional variant thereof is substituted at at least position 43 (lysine) by glutamic acid relative to wild type human IL2 and numbered in accordance with wild type human IL2.

A further aspect of the disclosure relates to a system comprising:

- 25 (i) a receptor polypeptide comprising a mutein of the human alpha subunit of interleukin-2 receptor (IL2R) or of a functional variant of the human alpha subunit of IL2R, wherein the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 29 (glutamic acid) by lysine and position 38 (lysine) by glutamic acid relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and
- 30 (ii) a ligand polypeptide comprising a mutein of human IL2 or of a functional variant of human IL2, wherein the IL2 or the functional variant thereof is substituted at at least position 35 (lysine) by glutamic acid, position 43 (lysine) by glutamic acid and position 61 (glutamic acid) by lysine relative to wild type human IL2 and numbered in accordance with wild type human IL2.

A further example of the disclosure relates to a system comprising:

- (i) a receptor polypeptide comprising a mutein of the human alpha subunit of interleukin-2 receptor (IL2R) or of a functional variant of the human alpha subunit of IL2R, wherein the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 29 (glutamic acid) by lysine relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and
- (ii) a ligand polypeptide comprising a mutein of human IL2 or of a functional variant of human IL2, wherein the IL2 or the functional variant thereof is substituted at at least position 35 (lysine) by glutamic acid, position 43 (lysine) by glutamic acid and position 61 (glutamic acid) by lysine relative to wild type human IL2 and numbered in accordance with wild type human IL2.

A further aspect of the disclosure relates to a system comprising:

- (i) a receptor polypeptide comprising a mutein of the human alpha subunit of interleukin-2 receptor (IL2R) or of a functional variant of the human alpha subunit of IL2R, wherein the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 29 (glutamic acid) by lysine relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and
- (ii) a ligand polypeptide comprising a mutein of human IL2 or of a functional variant of human IL2, wherein the IL2 or the functional variant thereof is substituted at at least position 43 (lysine) by glutamic acid and position 61 (glutamic acid) by lysine relative to wild type human IL2 and numbered in accordance with wild type human IL2.

A further example of the disclosure relates to a system comprising:

- (i) a receptor polypeptide comprising a mutein of the human alpha subunit of interleukin-2 receptor (IL2R) or of a functional variant of the human alpha subunit of IL2R, wherein the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 1 (glutamic acid) by lysine, position 29 (glutamic acid) by lysine and position 38 (lysine) by glutamic acid relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and
- (ii) a ligand polypeptide comprising a mutein of human IL2 or of a functional variant of human IL2, wherein the IL2 or the functional variant thereof is substituted at at least position 43 (lysine) by glutamic acid and position 61 (glutamic acid) by lysine relative to wild type human IL2 and numbered in accordance with wild type human IL2.

In different examples, the alpha subunit of IL2R or functional variant thereof and/or the IL2 or functional variant thereof is each substituted at one or more, such as two or more or three or more, such as 2, 3, 4, 5, 6, 7 or 8 positions, in particular positions having an acidic or basic amino acid residue in wild type alpha subunit of IL2R and/or wild type IL2, wherein the respective positions in wild type alpha subunit of IL2R and/or wild type IL2 are positions forming pairs of acidic/basic amino acids contacting each other. In one example, an acidic amino acid residue in wild type IL2 contacts a basic amino acid residue in the alpha subunit of IL2R. In one example, a basic amino acid residue in wild type IL2 contacts an acidic amino acid residue in the alpha subunit of IL2R.

In one example of the system, the disclosure provides that the alpha subunit of IL2R has the amino acid sequence according to SEQ ID NO: 2.

In one example of the system, the disclosure provides that the IL2 has the amino acid sequence according to SEQ ID NO: 1.

In one example, the mutein under (ii) binds to and activates IL2R comprising the mutein under (i) as alpha subunit. In one example, binding to and/or activation of IL2R comprising the mutein under (i) as alpha subunit by the mutein under (ii) exceeds binding to and/or activation of IL2R comprising the alpha subunit of IL2R or the functional variant thereof as alpha subunit by the mutein under (ii). In one example, binding to and/or activation of IL2R comprising the mutein under (i) as alpha subunit by the mutein under (ii) exceeds binding to and/or activation of IL2R comprising the mutein under (i) as alpha subunit by IL2 or the functional variant thereof. In one example, binding to and/or activation of IL2R comprising the alpha subunit of IL2R or the functional variant thereof as alpha subunit by IL2 or the functional variant thereof exceeds binding to and/or activation of IL2R comprising the alpha subunit of IL2R or the functional variant thereof as alpha subunit by the mutein under (ii). In one example, binding to and/or activation of IL2R comprising the alpha subunit of IL2R or the functional variant thereof as alpha subunit by IL2 or the functional variant thereof exceeds binding to and/or activation of IL2R comprising the mutein under (i) as alpha subunit by IL2 or the functional variant thereof.

In one example of the system, the disclosure provides that the substitution in IL2 or the functional variant thereof reduces the affinity for IL2R comprising the wild type alpha subunit of IL2R as alpha subunit (IL2R $\alpha\beta\gamma$).

In one example of the system, the disclosure provides that the substitution in IL2 or the functional variant thereof reduces the affinity for IL2R comprising the wild type alpha subunit of IL2R as alpha subunit (IL2R $\alpha\beta\gamma$) to a greater extent than for the $\beta\gamma$ IL2 receptor complex (IL2R $\beta\gamma$).

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In one example of the system, the disclosure provides that the mutein under (ii) has a decreased ability to stimulate regulatory T cells compared to wild type IL2.

10 In one example, the substituted IL2 or functional variant thereof (IL2 mutein) described above has an amino acid sequence identical to wild type IL2 at the other, non-substituted residues. In one example, the IL2 mutein described above has amino acid modifications such as amino acid substitutions at one or more sites in or at the other residues of wild type IL2. In one example, such amino acid substitutions result in relatively increased affinity for IL2R $\beta\gamma$ when compared to wild type IL2 (also termed "mut $\beta\gamma$ " mutations herein). Such mutants are potent IL2 signaling agonists. In one example, such amino acid substitutions
15 are at amino acid residues that contact IL2R β and/or IL2R γ .

In one example, the one or more amino acid substitutions which enhance the affinity for IL2R $\beta\gamma$ comprise substitutions at one or more positions of IL2 selected from the group consisting of K9, L12, Q13, E15, H16, D20, Q74, L80, R81, D84, L85, I86, N88, I92, L94, and E95.

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In one example, the one or more amino acid substitutions which enhance the affinity for IL2R $\beta\gamma$ comprise a substitution at at least one of positions 24, 65, 74, 80, 81, 85, 86, 89, 92, and 93 relative to wild type human IL2 and numbered in accordance with wild type human IL2. The substituted amino acid residue(s) can be, but are not necessarily, conservative substitutions. For example, the mutation can be: I24V, P65H,
25 Q74R, Q74H, Q74N, Q74S, L80F, L80V, R81I, R81T, R81D, L85V, I86V, I89V, I92F, V93I.

In one example, the IL2 mutein comprises the following set of amino acid substitutions: 80F/81D/85V/86V/92F. The IL2 mutein may further comprise the amino acid substitution 42A. The IL2 mutein may further comprise one or more of the following amino acid substitutions: 24V, 65H, 74R, 74H,
30 74N, 74S, 89V, 93I.

In some examples, the IL2 mutein comprises a set of amino acid substitutions selected from the group consisting of:

- (i) 74N, 80F, 81D, 85V, 86V, 89V, 92F;
- (ii) 74H, 80F, 81D, 85V, 86V, 92F;
- (iii) 74S, 80F, 81D, 85V, 86V, 92F;
- (iv) 74N, 80F, 81D, 85V, 86V, 92F;
- 5 (v) 80F, 81D, 85V, 86V, 92F;
- (vi) 80F, 81D, 85V, 86V, 89V, 92F, 93I;
- (vii) 18R, 22E, 80F, 81D, 85V, 86V, 89V, 92F, 93I, 126T;
- (viii) 18R, 22E, 74S, 80F, 81T, 85V, 86V, 89V, 92F, 93I, 126T.

10 In one example of the system, the disclosure provides that the mutein under (ii) further comprises one or more amino acid substitutions which enhance the affinity for IL2R $\beta\gamma$. In one example, the one or more amino acid substitutions which enhance the affinity for IL2R $\beta\gamma$ comprise the following set of substitutions: 80F, 81D, 85V, 86V, 92F.

15 The IL2 mutein described herein may be attached to a pharmacokinetic modifying group and, thus, may be an "extended-pharmacokinetic (PK) IL2". In one example, the ligand polypeptide described herein is an extended pharmacokinetic (PK) IL2 further comprising an amino acid sequence which is heterologous to the IL2 or functional variant thereof fused to the IL2 mutein. In one example, the amino acid sequence which is heterologous to the IL2 or functional variant thereof is selected from the group consisting of serum
20 albumin, an immunoglobulin fragment, transferrin, and Fn3, or variants thereof. In one example, the serum albumin comprises mouse serum albumin or human serum albumin. In one example, the immunoglobulin fragment comprises an immunoglobulin Fc domain.

In one example of the system, the disclosure provides that the ligand polypeptide is an extended
25 pharmacokinetic (PK) polypeptide. In one example, the extended-PK polypeptide comprises a fusion protein. In one example, the fusion protein comprises a moiety of the mutein under (ii) and a moiety which is heterologous to IL2 or the functional variant thereof. In one example, the fusion protein comprises a moiety of the mutein under (ii) and a moiety selected from the group consisting of serum albumin, an immunoglobulin fragment, transferrin, Fn3, and variants thereof. In one example, the serum albumin
30 comprises mouse serum albumin or human serum albumin. In one example, the immunoglobulin fragment comprises an immunoglobulin Fc domain.

The above described receptor polypeptides are also termed "IL2R α variant polypeptide", "IL2R variant polypeptide" or simply "IL2R variant" herein. The above described ligand polypeptides are also termed "IL2 variant polypeptide" or simply "IL2 variant" herein.

- 5 In different examples, a system described herein comprises combinations of receptor polypeptides and ligand polypeptides comprising IL2R receptor polypeptides and IL2 ligand polypeptides, respectively, selected from the following:

IL2R receptor polypeptide	IL2 ligand polypeptide
SEQ ID NO: 17 (optionally without N-terminal 21 aa signal peptide)	SEQ ID NO: 5 or SEQ ID NO: 10
SEQ ID NO: 14 (optionally without N-terminal 21 aa signal peptide)	SEQ ID NO: 6 or SEQ ID NO: 11
SEQ ID NO: 15 (optionally without N-terminal 21 aa signal peptide)	SEQ ID NO: 7 or SEQ ID NO: 12
SEQ ID NO: 16 (optionally without N-terminal 21 aa signal peptide)	SEQ ID NO: 8 or SEQ ID NO: 13
SEQ ID NO: 14 (optionally without N-terminal 21 aa signal peptide)	SEQ ID NO: 5 or SEQ ID NO: 10
SEQ ID NO: 16 (optionally without N-terminal 21 aa signal peptide)	SEQ ID NO: 6 or SEQ ID NO: 11
SEQ ID NO: 16 (optionally without N-terminal 21 aa signal peptide)	SEQ ID NO: 5 or SEQ ID NO: 10
SEQ ID NO: 17 (optionally without N-terminal 21 aa signal peptide)	SEQ ID NO: 6 or SEQ ID NO: 11

A further aspect of the disclosure relates to a receptor polypeptide of any of the systems described herein.

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In another aspect, the present disclosure relates to a polynucleotide encoding the receptor polypeptide described herein, optionally wherein the polynucleotide is RNA. A further example of the disclosure relates to a polynucleotide encoding the receptor polypeptide described herein. In one example, the polynucleotide is RNA.

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A further example of the disclosure relates to a host cell comprising the polynucleotide described herein. In another aspect, the present disclosure relates to a host cell genetically modified to express a receptor polypeptide of the system described herein, optionally wherein the cell is an immune effector cell, for example, a T cell. A further example of the disclosure relates to a host cell genetically modified to express a receptor polypeptide of any of the systems described herein. In one example, the host cell is an immune effector cell. In one example, the immune effector cell is a T cell.

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A further aspect of the disclosure relates to a pharmaceutical composition comprising the polynucleotide described herein or the host cell described herein. In one example, the pharmaceutical composition further comprises one or more pharmaceutically acceptable carriers, diluents and/or excipients.

5 In another aspect, the present disclosure relates to the polynucleotide described herein, the host cell described herein, or the pharmaceutical composition described herein for use in therapy. In yet another aspect, the present disclosure relates to a use of the polynucleotide described herein, the host cell described herein, or the pharmaceutical composition described herein in the manufacture of a medicament for treating or preventing cancer in a subject.

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A further example of the disclosure relates to a method of treating a subject comprising administering to the subject the polynucleotide described herein, the host cell described herein, or the pharmaceutical composition described herein. In one example, the method is a method for treating or preventing cancer in a subject.

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In another aspect, the present disclosure relates to a medical preparation comprising:

(i) a polynucleotide encoding a receptor polypeptide of the system described herein or an immune effector cell genetically modified to express a receptor polypeptide of the system described herein, and
(ii) the corresponding ligand polypeptide of the system under (i), a polynucleotide encoding the ligand polypeptide, or a host cell genetically modified to express the ligand polypeptide, optionally wherein the
20 medical preparation is a kit.

A further example of the disclosure relates to a medical preparation comprising:

(i) a polynucleotide encoding a receptor polypeptide of the system described herein or an immune effector
25 cell genetically modified to express a receptor polypeptide of the system described herein, and
(ii) the corresponding ligand polypeptide of the system under (i), a polynucleotide encoding the ligand polypeptide, or a host cell genetically modified to express the ligand polypeptide.

In one example, the medical preparation is a kit. In one example, the medical preparation comprises each
30 component (i) and (ii) in separate containers. In one example, the components are present in a pharmaceutical composition. In one example, the pharmaceutical composition further comprises one or more pharmaceutically acceptable carriers, diluents and/or excipients. In one example, the medical

preparation further comprises instructions for use of the medical preparation for treating or preventing cancer.

5 In yet another aspect, the present disclosure relates to the medical preparation described herein for use in therapy. A further example of the disclosure relates to the medical preparation described herein for pharmaceutical use. In one example, the pharmaceutical use comprises a therapeutic or prophylactic treatment of a disease or disorder.

10 In yet another aspect, the present disclosure relates to a use of the medical preparation described herein in the manufacture of a medicament for treating or preventing cancer in a subject. A further example of the disclosure relates to the medical preparation described herein for use in a method for treating or preventing cancer in a subject.

15 In yet another aspect, the present disclosure relates to a use of immune effector cells genetically modified to express a receptor polypeptide of the system described herein in the manufacture of a medicament for treating cancer in a subject associated with expression or elevated expression of a tumor-associated antigen, comprising that:

(i) the immune effector cells genetically modified to express a receptor polypeptide of the system as described herein are to be provided to the subject, the immune effector cells being targeted to the antigen
20 or cells expressing the antigen and
(ii) the corresponding ligand polypeptide of the system under (i), a polynucleotide encoding the ligand polypeptide, or a host cell genetically modified to express the ligand polypeptide is to be administered to the subject

25 A further example of the disclosure relates to a method for treating a subject comprising:

(i) providing to the subject immune effector cells genetically modified to express a receptor polypeptide of the system described herein, and
(ii) administering to the subject the corresponding ligand polypeptide of the system under (i), a polynucleotide encoding the ligand polypeptide, or a host cell genetically modified to express the ligand
30 polypeptide.

In one example, the subject has cancer.

In one example, the method is a method of inducing an immune response in said subject. In one example, the immune response is a T cell-mediated immune response.

A further example of the disclosure relates to a method for treating a subject having a disease, disorder or condition associated with expression or elevated expression of an antigen comprising:

(i) providing to the subject immune effector cells genetically modified to express a receptor polypeptide of the system described herein, the immune effector cells being targeted to the antigen or cells expressing the antigen and

(ii) administering to the subject the corresponding ligand polypeptide of the system under (i), a polynucleotide encoding the ligand polypeptide, or a host cell genetically modified to express the ligand polypeptide.

In one example, the disease, disorder or condition is cancer and the antigen is a tumor-associated antigen.

In one example, the immune effector cells genetically modified to express a receptor polypeptide are provided to the subject by administering the immune effector cells genetically modified to express a receptor polypeptide or by generating the immune effector cells genetically modified to express a receptor polypeptide in the subject.

In one example, the method described herein is a method for treating or preventing cancer in a subject. In one example, the cancer is selected from the group consisting of melanoma, leukemia, lymphoma, lung cancer, breast cancer, prostate cancer, ovarian cancer, colon cancer, mesothelioma, renal cell carcinoma, and brain cancer.

In one example of a medical preparation or method described herein, the polynucleotide encoding the receptor polypeptide and/or the polynucleotide encoding the ligand polypeptide is RNA.

In one example of the medical preparation, the RNA is present in a form selected from a liquid form, a solid form, or a combination thereof. In one example, the solid form is a frozen form or a dehydrated form. In one example, the dehydrated form is a freeze-dried or spray-dried form.

In one example of a medical preparation or method described herein, the immune effector cells genetically modified to express a receptor polypeptide comprise a polynucleotide encoding the receptor polypeptide.

In one example of a medical preparation or method described herein, the host cell genetically modified to express the ligand polypeptide comprises a polynucleotide encoding the ligand polypeptide.

5 In one example of a medical preparation or method described herein, the polynucleotide encoding the receptor polypeptide and/or the polynucleotide encoding the ligand polypeptide is RNA.

In one example of a medical preparation or method described herein, the immune effector cells are T cells.

10 In one example, the methods described herein further comprise administering to the subject an immune checkpoint inhibitor. In one example, the immune checkpoint inhibitor targets the interaction between (i) PD-1 and PD-L1, or (ii) CTLA-4 and CD80 or CD86. In one example, the immune checkpoint inhibitor is an antibody or antibody fragment. In one example, the antibody or antibody fragment targets PD-1, PD-L1, or CTLA-4.

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Similarly, in one example, the medical preparation described herein further comprises an immune checkpoint inhibitor. In one example, the immune checkpoint inhibitor targets the interaction between (i) PD-1 and PD-L1, or (ii) CTLA-4 and CD80 or CD86. In one example, the immune checkpoint inhibitor is an antibody or antibody fragment. In one example, the antibody or antibody fragment targets PD-1, PD-L1, or CTLA-4.

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In a further example, the disclosure relates to the agents and compositions described herein, e.g., IL2R variants, IL2 variants or IL2R/IL2 variant systems described herein, polynucleotides coding for IL2R variants, IL2 variants or IL2R/IL2 variant systems described herein, cells expressing IL2R variants described herein, for therapeutic use, in particular for use in the methods described herein.

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Other features and advantages of the instant disclosure will be apparent from the following detailed description and claims.

Claims

1. A system comprising:

(i) a receptor polypeptide comprising a mutein of the alpha subunit of interleukin-2 receptor (IL2R) or of a functional variant of the alpha subunit of IL2R, wherein the alpha subunit of IL2R or the functional variant thereof is substituted at at least one position,

(ii) a ligand polypeptide comprising a mutein of IL2 or of a functional variant of IL2, wherein the IL2 or the functional variant thereof is substituted at at least one position,

wherein the substitutions are such that

(a) the mutein under (ii) binds to and activates IL2R comprising the mutein under (i) as alpha subunit, and

(b) binding to and/or activation of IL2R comprising the mutein under (i) as alpha subunit by the mutein under (ii) exceeds binding to and/or activation of IL2R comprising the alpha subunit of IL2R or the functional variant thereof as alpha subunit by the mutein under (ii).

2. The system of claim 1, wherein binding to and/or activation of IL2R comprising the mutein under (i) as alpha subunit by the mutein under (ii) exceeds binding to and/or activation of IL2R comprising the mutein under (i) as alpha subunit by IL2 or the functional variant thereof and/or,

wherein binding to and/or activation of IL2R comprising the alpha subunit of IL2R or the functional variant thereof as alpha subunit by IL2 or the functional variant thereof exceeds binding to and/or activation of IL2R comprising the alpha subunit of IL2R or the functional variant thereof as alpha subunit by the mutein under (ii), and/or

wherein binding to and/or activation of IL2R comprising the alpha subunit of IL2R or the functional variant thereof as alpha subunit by IL2 or the functional variant thereof exceeds binding to and/or activation of IL2R comprising the mutein under (i) as alpha subunit by IL2 or the functional variant thereof.

3. The system of claim 1 or 2, comprising

(i) a receptor polypeptide comprising a mutein of the alpha subunit of IL2R or of a functional variant of the alpha subunit of IL2R, wherein the alpha subunit of IL2R or the functional variant thereof is substituted at at least a position having an acidic amino acid residue in wild type alpha subunit of IL2R that contacts a basic amino acid residue in wild type IL2 and/or is substituted at at least a position having a basic amino acid residue in wild type alpha subunit of IL2R that contacts an acidic amino acid residue in wild type IL2, wherein if the amino acid residue is an acidic amino acid residue in wild type

alpha subunit of IL2R the substitution is by a basic amino acid residue and if the amino acid residue is a basic amino acid residue in wild type alpha subunit of IL2R the substitution is by an acidic amino acid residue,

(ii) a ligand polypeptide comprising a mutein of IL2 or of a functional variant of IL2, wherein when the alpha subunit of IL2R or the functional variant thereof is substituted at at least a position having an acidic amino acid residue in wild type alpha subunit of IL2R that contacts a basic amino acid residue in wild type IL2, the IL2 or the functional variant thereof is substituted at at least said basic amino acid residue in wild type IL2 and/or when the alpha subunit of IL2R or the functional variant thereof is substituted at at least a position having a basic amino acid residue in wild type alpha subunit of IL2R that contacts an acidic amino acid residue in wild type IL2, the IL2 or the functional variant thereof is substituted at at least said acidic amino acid residue in wild type IL2, wherein if the amino acid residue is a basic amino acid residue in wild type IL2 the substitution is by an acidic amino acid residue and if the amino acid residue is an acidic amino acid residue in wild type IL2 the substitution is by a basic amino acid residue.

4. A system comprising:

(i) a receptor polypeptide comprising a mutein of the alpha subunit of interleukin-2 receptor (IL2R) or of a functional variant of the alpha subunit of IL2R, wherein the alpha subunit of IL2R or the functional variant thereof is substituted at at least a position having an acidic amino acid residue in wild type alpha subunit of IL2R that contacts a basic amino acid residue in wild type IL2 and/or is substituted at at least a position having a basic amino acid residue in wild type alpha subunit of IL2R that contacts an acidic amino acid residue in wild type IL2, wherein if the amino acid residue is an acidic amino acid residue in wild type alpha subunit of IL2R the substitution is by a basic amino acid residue and if the amino acid residue is a basic amino acid residue in wild type alpha subunit of IL2R the substitution is by an acidic amino acid residue,

(ii) a ligand polypeptide comprising a mutein of IL2 or of a functional variant of IL2, wherein when the alpha subunit of IL2R or the functional variant thereof is substituted at at least a position having an acidic amino acid residue in wild type alpha subunit of IL2R that contacts a basic amino acid residue in wild type IL2, the IL2 or the functional variant thereof is substituted at at least said basic amino acid residue in wild type IL2 and/or when the alpha subunit of IL2R or the functional variant thereof is substituted at at least a position having a basic amino acid residue in wild type alpha subunit of IL2R that contacts an acidic amino acid residue in wild type IL2, the IL2 or the functional variant thereof is substituted at at least said acidic amino acid residue in wild type IL2, wherein if the amino acid residue is a basic amino acid residue in wild type IL2 the substitution is by an acidic amino acid residue and if

the amino acid residue is an acidic amino acid residue in wild type IL2 the substitution is by a basic amino acid residue.

5. The system of any one of claims 1 to 4, wherein the alpha subunit of IL2R is the human alpha subunit of IL2R, and/or
wherein the IL2 is human IL2.

6. The system of any one of claims 1 to 5, wherein

(i) the alpha subunit of IL2R is the human alpha subunit of IL2R and the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 1 (glutamic acid) relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and
(ii) the IL2 is human IL2 and the IL2 or the functional variant thereof is substituted at at least position 35 (lysine) relative to wild type human IL2 and numbered in accordance with wild type human IL2,
optionally wherein position 1 is substituted with lysine and/or
wherein position 35 is substituted with glutamic acid.

7. The system of any one of claims 1 to 6, wherein

(i) the alpha subunit of IL2R is the human alpha subunit of IL2R and the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 29 (glutamic acid) relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and
(ii) the IL2 is human IL2 and the IL2 or the functional variant thereof is substituted at at least position 43 (lysine) relative to wild type human IL2 and numbered in accordance with wild type human IL2,
optionally wherein position 29 is substituted with lysine and/or
wherein position 43 is substituted with glutamic acid.

8. The system of any one of claims 1 to 7, wherein

(i) the alpha subunit of IL2R is the human alpha subunit of IL2R and the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 38 (lysine) relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and
(ii) the IL2 is human IL2 and the IL2 or the functional variant thereof is substituted at at least position 61 (glutamic acid) relative to wild type human IL2 and numbered in accordance with wild type human IL2,
optionally wherein position 38 is substituted with glutamic acid and/or
wherein position 61 is substituted with lysine.

9. The system of any one of claims 1 to 8,

wherein (i) the alpha subunit of IL2R is the human alpha subunit of IL2R and the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 1 (glutamic acid) by lysine, position 29 (glutamic acid) by lysine and position 38 (lysine) by glutamic acid relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and

(ii) the IL2 is human IL2 and the IL2 or the functional variant thereof is substituted at at least position 35 (lysine) by glutamic acid, position 43 (lysine) by glutamic acid and position 61 (glutamic acid) by lysine relative to wild type human IL2 and numbered in accordance with wild type human IL2, or

wherein (i) the alpha subunit of IL2R is the human alpha subunit of IL2R and the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 29 (glutamic acid) by lysine and position 38 (lysine) by glutamic acid relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and

(ii) the IL2 is human IL2 and the IL2 or the functional variant thereof is substituted at at least position 43 (lysine) by glutamic acid and position 61 (glutamic acid) by lysine relative to wild type human IL2 and numbered in accordance with wild type human IL2, or

wherein (i) the alpha subunit of IL2R is the human alpha subunit of IL2R and the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 38 (lysine) by glutamic acid relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and

(ii) the IL2 is human IL2 and the IL2 or the functional variant thereof is substituted at at least position 61 (glutamic acid) by lysine relative to wild type human IL2 and numbered in accordance with wild type human IL2, or

wherein (i) the alpha subunit of IL2R is the human alpha subunit of IL2R and the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 29 (glutamic acid) by lysine relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and

(ii) the IL2 is human IL2 and the IL2 or the functional variant thereof is substituted at at least position 43 (lysine) by glutamic acid relative to wild type human IL2 and numbered in accordance with wild type human IL2, or

wherein (i) the alpha subunit of IL2R is the human alpha subunit of IL2R and the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 29 (glutamic acid) by lysine and position 38 (lysine) by glutamic acid relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and

(ii) the IL2 is human IL2 and the IL2 or the functional variant thereof is substituted at at least position 35 (lysine) by glutamic acid, position 43 (lysine) by glutamic acid and position 61 (glutamic acid) by lysine relative to wild type human IL2 and numbered in accordance with wild type human IL2, or wherein (i) the alpha subunit of IL2R is the human alpha subunit of IL2R and the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 29 (glutamic acid) by lysine relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and

(ii) the IL2 is human IL2 and the IL2 or the functional variant thereof is substituted at at least position 43 (lysine) by glutamic acid and position 61 (glutamic acid) by lysine relative to wild type human IL2 and numbered in accordance with wild type human IL2.

10. A system comprising:

(i) a receptor polypeptide comprising a mutein of the human alpha subunit of interleukin-2 receptor (IL2R) or of a functional variant of the human alpha subunit of IL2R, wherein the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 1 (glutamic acid) by a basic amino acid residue relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and

(ii) a ligand polypeptide comprising a mutein of human IL2 or of a functional variant of human IL2, wherein the IL2 or the functional variant thereof is substituted at at least position 35 (lysine) by an acidic amino acid residue relative to wild type human IL2 and numbered in accordance with wild type human IL2, optionally wherein position 1 is substituted with lysine and/or wherein position 35 is substituted with glutamic acid.

11. A system comprising:

(i) a receptor polypeptide comprising a mutein of the human alpha subunit of interleukin-2 receptor (IL2R) or of a functional variant of the human alpha subunit of IL2R, wherein the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 29 (glutamic acid) by a basic amino acid residue relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and

(ii) a ligand polypeptide comprising a mutein of human IL2 or of a functional variant of human IL2, wherein the IL2 or the functional variant thereof is substituted at at least position 43 (lysine) by an acidic amino acid residue relative to wild type human IL2 and numbered in accordance with wild type human IL2, optionally wherein position 29 is substituted with lysine and/or wherein position 43 is substituted with glutamic acid.

12. A system comprising:

- (i) a receptor polypeptide comprising a mutein of the human alpha subunit of interleukin-2 receptor (IL2R) or of a functional variant of the human alpha subunit of IL2R, wherein the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 38 (lysine) by an acidic amino acid residue relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and
- (ii) a ligand polypeptide comprising a mutein of human IL2 or of a functional variant of human IL2, wherein the IL2 or the functional variant thereof is substituted at at least position 61 (glutamic acid) by a basic amino acid residue relative to wild type human IL2 and numbered in accordance with wild type human IL2, optionally wherein position 38 is substituted with glutamic acid and/or wherein position 61 is substituted with lysine.

13. A system comprising:

- (i) a receptor polypeptide comprising a mutein of the human alpha subunit of interleukin-2 receptor (IL2R) or of a functional variant of the human alpha subunit of IL2R, wherein the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 1 (glutamic acid) by lysine, position 29 (glutamic acid) by lysine and position 38 (lysine) by glutamic acid relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and
- (ii) a ligand polypeptide comprising a mutein of human IL2 or of a functional variant of human IL2, wherein the IL2 or the functional variant thereof is substituted at at least position 35 (lysine) by glutamic acid, position 43 (lysine) by glutamic acid and position 61 (glutamic acid) by lysine relative to wild type human IL2 and numbered in accordance with wild type human IL2.

14. A system comprising:

- (i) a receptor polypeptide comprising a mutein of the human alpha subunit of interleukin-2 receptor (IL2R) or of a functional variant of the human alpha subunit of IL2R, wherein the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 29 (glutamic acid) by lysine and position 38 (lysine) by glutamic acid relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and
- (ii) a ligand polypeptide comprising a mutein of human IL2 or of a functional variant of human IL2, wherein the IL2 or the functional variant thereof is substituted at at least position 43 (lysine) by glutamic acid and position 61 (glutamic acid) by lysine relative to wild type human IL2 and numbered in accordance with wild type human IL2.

15. A system comprising:

- (i) a receptor polypeptide comprising a mutein of the human alpha subunit of interleukin-2 receptor (IL2R) or of a functional variant of the human alpha subunit of IL2R, wherein the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 38 (lysine) by glutamic acid relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and
- (ii) a ligand polypeptide comprising a mutein of human IL2 or of a functional variant of human IL2, wherein the IL2 or the functional variant thereof is substituted at at least position 61 (glutamic acid) by lysine relative to wild type human IL2 and numbered in accordance with wild type human IL2.

16. A system comprising:

- (i) a receptor polypeptide comprising a mutein of the human alpha subunit of interleukin-2 receptor (IL2R) or of a functional variant of the human alpha subunit of IL2R, wherein the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 29 (glutamic acid) by lysine relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and
- (ii) a ligand polypeptide comprising a mutein of human IL2 or of a functional variant of human IL2, wherein the IL2 or the functional variant thereof is substituted at at least position 43 (lysine) by glutamic acid relative to wild type human IL2 and numbered in accordance with wild type human IL2.

17. A system comprising:

- (i) a receptor polypeptide comprising a mutein of the human alpha subunit of interleukin-2 receptor (IL2R) or of a functional variant of the human alpha subunit of IL2R, wherein the alpha subunit of IL2R or the functional variant thereof is substituted at at least position 29 (glutamic acid) by lysine and position 38 (lysine) by glutamic acid relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and
- (ii) a ligand polypeptide comprising a mutein of human IL2 or of a functional variant of human IL2, wherein the IL2 or the functional variant thereof is substituted at at least position 35 (lysine) by glutamic acid, position 43 (lysine) by glutamic acid and position 61 (glutamic acid) by lysine relative to wild type human IL2 and numbered in accordance with wild type human IL2.

18. A system comprising:

- (i) a receptor polypeptide comprising a mutein of the human alpha subunit of interleukin-2 receptor (IL2R) or of a functional variant of the human alpha subunit of IL2R, wherein the alpha subunit of IL2R

or the functional variant thereof is substituted at at least position 29 (glutamic acid) by lysine relative to wild type human alpha subunit of IL2R and numbered in accordance with wild type human alpha subunit of IL2R, and

(ii) a ligand polypeptide comprising a mutein of human IL2 or of a functional variant of human IL2, wherein the IL2 or the functional variant thereof is substituted at at least position 43 (lysine) by glutamic acid and position 61 (glutamic acid) by lysine relative to wild type human IL2 and numbered in accordance with wild type human IL2.

19. The system of any one of claims 1 to 18, wherein the alpha subunit of IL2R has the amino acid sequence according to SEQ ID NO: 2 and/or wherein the IL2 has the amino acid sequence according to SEQ ID NO: 1.

20. The system of any one of claims 4 to 19, wherein the mutein under (ii) binds to and activates IL2R comprising the mutein under (i) as alpha subunit.

21. The system of claim 20, wherein binding to and/or activation of IL2R comprising the mutein under (i) as alpha subunit by the mutein under (ii) exceeds binding to and/or activation of IL2R comprising the alpha subunit of IL2R or the functional variant thereof as alpha subunit by the mutein under (ii), and/or wherein binding to and/or activation of IL2R comprising the mutein under (i) as alpha subunit by the mutein under (ii) exceeds binding to and/or activation of IL2R comprising the mutein under (i) as alpha subunit by IL2 or the functional variant thereof, and/or

wherein binding to and/or activation of IL2R comprising the alpha subunit of IL2R or the functional variant thereof as alpha subunit by IL2 or the functional variant thereof exceeds binding to and/or activation of IL2R comprising the alpha subunit of IL2R or the functional variant thereof as alpha subunit by the mutein under (ii), and/or

wherein binding to and/or activation of IL2R comprising the alpha subunit of IL2R or the functional variant thereof as alpha subunit by IL2 or the functional variant thereof exceeds binding to and/or activation of IL2R comprising the mutein under (i) as alpha subunit by IL2 or the functional variant thereof, and/or

wherein the substitution in IL2 or the functional variant thereof reduces the affinity for IL2R comprising the wild type alpha subunit of IL2R as alpha subunit (IL2R $\alpha\beta\gamma$), and/or

wherein the substitution in IL2 or the functional variant thereof reduces the affinity for IL2R comprising the wild type alpha subunit of IL2R as alpha subunit (IL2R $\alpha\beta\gamma$) to a greater extent than for the $\beta\gamma$ IL2 receptor complex (IL2R $\beta\gamma$), and/or

wherein the mutein under (ii) has a decreased ability to stimulate regulatory T cells compared to wild type IL2.

22. The system of any one of claims 1 to 21, wherein the mutein under (ii) further comprises one or more amino acid substitutions which enhance the affinity for IL2R $\beta\gamma$, optionally wherein the one or more amino acid substitutions which enhance the affinity for IL2R $\beta\gamma$ comprise the following set of substitutions: 80F, 81D, 85V, 86V, 92F.

23. The system of any one of claims 1 to 22, wherein the ligand polypeptide is an extended pharmacokinetic (PK) polypeptide.

24. The system of claim 23, wherein the extended-PK polypeptide comprises a fusion protein, optionally wherein the fusion protein comprises a moiety of the mutein under (ii) and a moiety which is heterologous to IL2 or the functional variant thereof and/or wherein the fusion protein comprises a moiety of the mutein under (ii) and a moiety selected from the group consisting of serum albumin, an immunoglobulin fragment, transferrin, Fn3, and variants thereof.

25. The system of claim 24, wherein the serum albumin comprises mouse serum albumin or human serum albumin and/or wherein the immunoglobulin fragment comprises an immunoglobulin Fc domain.

26. A receptor polypeptide of the system of any one of claims 1 to 25.

27. A polynucleotide encoding the receptor polypeptide of claim 26, optionally wherein the polynucleotide is RNA.

28. A host cell comprising the polynucleotide of claim 27.

29. A host cell genetically modified to express a receptor polypeptide of the system of any one of claims 1 to 25, optionally wherein the cell is an immune effector cell, for example, a T cell.

30. A pharmaceutical composition comprising the polynucleotide of claim 27 or the host cell of claim 29.

31. The polynucleotide of claim 27, the host cell of claim 29, or the pharmaceutical composition of claim 30 for use in therapy.

32. Use of the polynucleotide of claim 27, the host cell of claim 29, or the pharmaceutical composition of claim 30 in the manufacture of a medicament for treating or preventing cancer in a subject.

33. A medical preparation comprising:

(i) a polynucleotide encoding a receptor polypeptide of the system of any one of claims 1 to 25 or an immune effector cell genetically modified to express a receptor polypeptide of the system of any one of claims 1 to 25, and

(ii) the corresponding ligand polypeptide of the system under (i), a polynucleotide encoding the ligand polypeptide, or a host cell genetically modified to express the ligand polypeptide, optionally wherein the medical preparation is a kit.

34. The medical preparation of claim 33, which comprises each component (i) and (ii) in separate containers.

35. The medical preparation of claim 33 or 34, further comprising instructions for use of the medical preparation for treating or preventing cancer.

36. The medical preparation of claim 33 or 34 for use in therapy.

37. Use of the medical preparation of any one of claims 33 to 36 in the manufacture of a medicament for treating or preventing cancer in a subject.

38. Use of immune effector cells genetically modified to express a receptor polypeptide of the system of any one of claims 1 to 25 in the manufacture of a medicament for treating cancer in a subject associated with expression or elevated expression of a tumor-associated antigen, comprising that:

(i) the immune effector cells genetically modified to express a receptor polypeptide of the system of any one of claims 1 to 25 are to be provided to the subject, the immune effector cells being targeted to the antigen or cells expressing the antigen and

(ii) the corresponding ligand polypeptide of the system under (i), a polynucleotide encoding the ligand polypeptide, or a host cell genetically modified to express the ligand polypeptide is to be administered to the subject.

39. The use of claim 38, wherein the immune effector cells genetically modified to express a receptor polypeptide are to be provided to the subject by administration of the immune effector cells genetically modified to express a receptor polypeptide or by generation of the immune effector cells genetically modified to express a receptor polypeptide in the subject.

40. The medical preparation of any one of claims 33 to 35, or the use of claim 37 or 38, wherein the polynucleotide encoding the receptor polypeptide and/or the polynucleotide encoding the ligand polypeptide is RNA, or
wherein the immune effector cells genetically modified to express a receptor polypeptide comprise a polynucleotide encoding the receptor polypeptide, or
wherein the host cell genetically modified to express the ligand polypeptide comprises a polynucleotide encoding the ligand polypeptide.

41. The medical preparation or the use of claim 40, wherein the polynucleotide encoding the receptor polypeptide and/or the polynucleotide encoding the ligand polypeptide is RNA.

42. The medical preparation or the use of any one of claims 33 to 41, wherein the immune effector cells are T cells.