Summary

The disclosure relates to agents and methods for targeted delivery of immune effector cells to target cells, e.g., diseased cells. Targeted delivery of the immune effector cells to target cells may be achieved using a docking compound binding to the immune effector cells via their antigen receptor, said docking compound comprising a targeting moiety for targeting target cells. The docking compound binds to the antigen receptor via a tag/binder system comprising an ALFA-tag and a single-domain antibody described herein.

In some examples, the disclosure involves immune effector cells genetically modified to express a chimeric antigen receptor (CAR) and a compound (docking compound) comprising a binding moiety for an antigen on target cells and a further binding moiety for a CAR. Providing to a subject the immune effector cells and the docking compound may deliver the immune effector cells to target cells. The CAR and the binding moiety for a CAR bind to each other by means of an ALFA-tag and a single-domain antibody binding to the ALFA-tag. In some examples, the moiety of the CAR binding to the binding moiety for a CAR comprises an ALFA-tag and the binding moiety for a CAR comprises the single-domain antibody. In some examples, the moiety of the CAR binding to the binding moiety for a CAR comprises the single-domain antibody and the binding moiety for a CAR comprises an ALFA-tag.

The immune effector cells may be genetically modified *in vitro/ex vivo* or *in vivo* to express the antigen receptor. Genetic modification may be achieved using a nucleic acid encoding an antigen receptor for transfection of immune effector cells.

In some examples, the immune effector cells genetically modified to express a CAR are provided to a subject by administering the immune effector cells. In some examples, the immune effector cells genetically modified to express a CAR are provided to a subject by generating the immune effector cells in the subject, e.g., by administering nucleic acid, e.g., DNA or RNA, encoding the CAR for transfection of immune effector cells.

The docking compound may be provided by adding it to target cells, e.g., the docking compound may be administered to a subject, or by adding nucleic acid, e.g., RNA, encoding the docking compound, e.g., RNA encoding the docking compound may be administered to a subject for expression of the docking compound.

In some examples, the docking compound is provided to a subject by administering nucleic acid, e.g., RNA, encoding the docking compound. In some examples, the docking compound is provided to a subject by administering the docking compound. In some examples, a preformed complex wherein the docking compound is bound to the immune effector cells genetically modified to express a CAR is provided to a subject by administration.

The binding moiety for an antigen on target cells may bind to a target antigen such as a cancer antigen on cancer cells and the immune effector cells may target the binding moiety for a CAR to thereby precisely deliver the immune effector cells to the target cells such as cancer cells.

Accordingly, the present disclosure relates to an approach wherein a docking compound that labels target cells, e.g., by binding to a cell surface antigen, is used. The docking compound comprises a moiety which will be targeted by immune effector cells, equipped with an antigen receptor targeting the moiety.

The approach described herein may deliver immune effector cells to target cells *in vitro/ex vivo* as well as *in vivo*. Immune effector cells genetically modified to express an antigen receptor described herein are useful in the treatment of diseases wherein targeting cells such as diseased cells expressing an antigen such as a tumor antigen is beneficial. The target cells may express the antigen on the cell surface for recognition by a docking compound. The treatments described herein may provide for the selective eradication of such cells expressing an antigen, thereby minimizing adverse effects to normal cells not expressing the antigen. The methods and agents described herein are, in particular, useful for the treatment of diseases characterized by diseased cells expressing an antigen the docking compound is directed to.

The concept described herein allows to use a single type of immune effector cells for targeting a wide range of target cells, i.e., by using a single type of immune effector cells in combination with different docking compounds targeting different antigen targets and comprising an identical CAR binding moiety.

In one aspect the disclosure provides a system comprising:

(i) an immune effector cell genetically modified to express a chimeric antigen receptor (CAR), or a nucleic acid encoding a CAR useful for genetically modifying an immune effector cell to express the CAR; and

(ii) a compound comprising a binding moiety for the CAR and a binding moiety for an antigen on target cells, or a nucleic acid encoding the compound,

wherein the CAR and the binding moiety for the CAR comprise a binding system comprising an ALFA-tag and a single-domain antibody binding to the ALFA-tag,

wherein the antibody comprises the CDR1 sequence as shown in SEQ ID NO: 94 or a sequence having 1 or 2 mutations relative to said sequence, the CDR2 sequence as shown in SEQ ID NO: 97 or a sequence having 1 or 2 mutations relative to said sequence, and the CDR3 sequence as shown in SEQ ID NO: 98 or a sequence having 1 or 2 mutations relative to said sequence;

wherein the antibody further comprises at positions corresponding to sequence position 47, 48, and 50 of SEQ ID NO: 1 the following amino acid residues: Arg at position 47, Arg at position 48, Met at position 50;

and wherein the antibody comprises a sequence having at least 90% sequence identity to any one of SEQ ID NOs: 118-125.

In some examples, the antibody comprises the CDR1 sequence as shown in SEQ ID NO: 94, the CDR2 sequence as shown in SEQ ID NO: 97, and the CDR3 sequence as shown in SEQ ID NO: 98.

In some examples, the immune effector cell comprises a T cell.

In some examples, the immune effector cell expresses the CAR on its cell surface.

In some examples, the CAR comprises a moiety binding to the binding moiety for the CAR.

In some examples, the moiety of the CAR binding to the binding moiety for the CAR is comprised by an extracellular moiety of the CAR.

In some examples, the moiety of the CAR binding to the binding moiety for the CAR comprises an ALFA-tag and the binding moiety for the CAR comprises the antibody.

In some examples, the moiety of the CAR binding to the binding moiety for the CAR comprises the antibody and the binding moiety for the CAR comprises an ALFA-tag.

In some examples, the antibody specifically binds to an epitope tag comprising the sequence SRLEEELRRRLTE (SEQ ID NO: 2).

In some examples, the antibody is humanized and/or wherein the antibody comprises a sequence that has at least about 70% V segment identity to the closest human germline sequence.

In some examples, the antibody has a higher Tm as compared to the antibody of SEQ ID NO: 1.

In some examples, the antibody comprises a His residue at the position corresponding to sequence position 99 of SEQ ID NO: 1, a Val residue at the position corresponding to sequence position 100 of SEQ ID NO: 1, and/or a Tyr residue at the position corresponding to sequence position 40 of SEQ ID NO: 1.

In some examples, the antibody comprises FR1, FR2, FR3, and FR4 sequences, that have at least 80%, at least 85%, at least 90%, or at least 95% sequence identity or are identical to the sequences set forth in SEQ ID NOs: 99, 102, 108, and 109, respectively.

In some examples, the antibody comprises a protein A binding site.

In some examples, the antibody comprises a sequence having at least 95% sequence identity to any one of SEQ ID NOs: 118-125, or wherein the antibody comprises a sequence set forth in any one of SEQ ID NOs: 118-125.

In some examples, the ALFA-tag comprises the amino acid sequence of X1-X2-L-E-X5-E-X7-R-R-R-L-X12-X13 (SEQ ID NO: 3), wherein X1 is G or S or P or T, X2 is R or G or P, X5 is E or Q, X7 is L or I, X12 is S or T or P or A or D or E, and wherein X13 is P or A or S or K or D or E or no amino acid.

In some examples, the binding moiety for the CAR and the binding moiety for an antigen on target cells are connected through a linking moiety.

In some examples, the linking moiety comprises a polymer.

In some examples, the linking moiety comprises a non-peptidic moiety.

In some examples, the linking moiety comprises a polymer selected from the group consisting of poly(ethylene glycol) (PEG), polysarcosine (pSar) (poly(N-methylglycine)), polyoxazoline (POX), polyoxazine (POZ), and poly-2-(2-(2-aminoethoxy)ethoxy)acetic acid (pAEEA), derivatives and combinations thereof.

In some examples, the linking moiety comprises a peptidic moiety.

In some examples, the binding moiety for an antigen on target cells comprises a moiety selected from the group consisting of an antibody binding to the antigen, an antibody derivative binding to the antigen and a ligand of the antigen.

In some examples, the antibody derivative binding to the antigen comprises an antibody fragment.

In some examples, the binding moiety for an antigen on target cells comprises a peptidic moiety.

In some examples, the compound comprises a fusion polypeptide comprising the binding moiety for the CAR and the binding moiety for an antigen on target cells.

In some examples, the ligand of the antigen comprises DUPA or FAPI.

In some examples, the target cells are diseased cells.

In some examples, the target cells are cancer cells.

In some examples, the antigen on target cells comprises a cell surface antigen.

In some examples, the antigen on target cells comprises a tumor antigen.

In some examples, the nucleic acid encoding a CAR comprises DNA.

In some examples, the nucleic acid encoding the compound comprises RNA.

In some examples, binding of a complex comprising:

(i) the immune effector cell genetically modified to express a chimeric antigen receptor (CAR); and

(ii) the compound comprising a binding moiety for the CAR and a binding moiety for an antigen on target cells

to cells expressing the antigen results in killing of the cells expressing the antigen.

In a further example the disclosure provides a method for treating a subject having a disease, disorder or condition characterized by cells expressing an antigen, comprising:

(i) providing to the subject immune effector cells genetically modified to express a chimeric antigen receptor (CAR); and

(ii) providing to the subject a compound comprising a binding moiety for the CAR and a binding moiety for the antigen,

wherein the CAR and the binding moiety for the CAR comprise a binding system comprising an ALFA-tag and a single-domain antibody binding to the ALFA-tag,

wherein the antibody comprises the CDR1 sequence as shown in SEQ ID NO: 94 or a sequence having 1 or 2 mutations relative to said sequence, the CDR2 sequence as shown in SEQ ID NO:

97 or a sequence having 1 or 2 mutations relative to said sequence, and the CDR3 sequence as shown in SEQ ID NO: 98 or a sequence having 1 or 2 mutations relative to said sequence;
wherein the antibody further comprises at positions corresponding to sequence position 47, 48, and 50 of SEQ ID NO: 1 the following amino acid residues: Arg at position 47, Arg at position 48, Met at position 50;
and wherein the antibody comprises a sequence having at least 90% sequence identity to any one of SEQ ID NOs: 118-125.

In some examples, the antibody comprises the CDR1 sequence as shown in SEQ ID NO: 94, the CDR2 sequence as shown in SEQ ID NO: 97, and the CDR3 sequence as shown in SEQ ID NO: 98.

In some examples, the immune effector cells comprise T cells.

In some examples, the immune effector cells express the CAR on their cell surface.

In some examples, the CAR comprises a moiety binding to the binding moiety for the CAR.

In some examples, the moiety of the CAR binding to the binding moiety for the CAR is comprised by an extracellular moiety of the CAR.

In some examples, the moiety of the CAR binding to the binding moiety for the CAR comprises an ALFA-tag and the binding moiety for the CAR comprises the antibody.

In some examples, the moiety of the CAR binding to the binding moiety for the CAR comprises the antibody and the binding moiety for the CAR comprises an ALFA-tag.

In some examples, the antibody specifically binds to an epitope tag comprising the sequence SRLEEELRRRLTE (SEQ ID NO: 2).

In some examples, the antibody is humanized and/or wherein the antibody comprises a sequence that has at least about 70% V segment identity to the closest human germline sequence.

In some examples, the antibody has a higher Tm as compared to the antibody of SEQ ID NO: 1.

In some examples, the antibody comprises a His residue at the position corresponding to sequence position 99 of SEQ ID NO: 1, a Val residue at the position corresponding to sequence position 100 of SEQ ID NO: 1, and/or a Tyr residue at the position corresponding to sequence position 40 of SEQ ID NO: 1.

In some examples, the antibody comprises FR1, FR2, FR3, and FR4 sequences, that have at least 80%, at least 85%, at least 90%, or at least 95% sequence identity or are identical to the sequences set forth in SEQ ID NOs: 99, 102, 108, and 109, respectively.

In some examples, the antibody comprises a protein A binding site.

In some examples, the antibody comprises a sequence having at least 95% sequence identity to any one of SEQ ID NOs: 118-125, or wherein the antibody comprises a sequence set forth in any one of SEQ ID NOs: 118-125.

In some examples, the ALFA-tag comprises the amino acid sequence of X1-X2-L-E-X5-E-X7-R-R-R-L-X12-X13 (SEQ ID NO: 3), wherein X1 is G or S or P or T, X2 is R or G or P, X5 is E or Q, X7 is L or I, X12 is S or T or P or A or D or E, and wherein X13 is P or A or S or K or D or E or no amino acid.

In some examples, the binding moiety for the CAR and the binding moiety for an antigen on target cells are connected through a linking moiety.

In some examples, the linking moiety comprises a polymer.

In some examples, the linking moiety comprises a non-peptidic moiety.

In some examples, the linking moiety comprises a polymer selected from the group consisting of poly(ethylene glycol) (PEG), polysarcosine (pSar) (poly(N-methylglycine)), polyoxazoline (POX), polyoxazine (POZ), and poly-2-(2-(2-aminoethoxy)ethoxy)acetic acid (pAEEA), derivatives and combinations thereof.

In some examples, the linking moiety comprises a peptidic moiety.

In some examples, the binding moiety for the antigen comprises a moiety selected from the group consisting of an antibody binding to the antigen, an antibody derivative binding to the antigen and a ligand of the antigen.

In some examples, the antibody derivative binding to the antigen comprises an antibody fragment.

In some examples, the binding moiety for the antigen comprises a peptidic moiety.

In some examples, the compound comprises a fusion polypeptide comprising the binding moiety for the CAR and the binding moiety for the antigen.

In some examples, the ligand of the antigen comprises DUPA or FAPI.

In some examples, the cells expressing an antigen are diseased cells.

In some examples, the cells expressing an antigen are cancer cells.

In some examples, the antigen comprises a cell surface antigen.

In some examples, the antigen comprises a tumor antigen.

In some examples, binding of a complex comprising:

(i) the immune effector cells genetically modified to express a chimeric antigen receptor (CAR); and

(ii) the compound comprising a binding moiety for the CAR and a binding moiety for an antigen

to cells expressing the antigen results in killing of the cells expressing the antigen.

In some examples, the method comprises administering the immune effector cells genetically modified to express a CAR to the subject.

In some examples, the method comprises generating the immune effector cells genetically modified to express a CAR in the subject.

In some examples, the method comprises administering nucleic acid encoding the CAR to the subject.

In some examples, the nucleic acid encoding the CAR comprises DNA.

In some examples, the method comprises administering the compound to the subject.

In some examples, the method comprises generating the compound in the subject.

In some examples, the method comprises administering nucleic acid encoding the compound to the subject.

In some examples, the nucleic acid encoding the compound comprises RNA.

In some examples, the method comprises allowing expression of the compound by cells in the subject such that the compound becomes associated with cells expressing the antigen and the binding moiety for the CAR is available for binding by the immune effector cells.

In some examples, the cells expressing the compound are transfected with the nucleic acid encoding the compound.

In some examples, the cells expressing the compound secrete the compound.

In some examples, the cells expressing the compound express the compound such that it is released into the bloodstream.

In some examples, the nucleic acid encoding the CAR and/or the nucleic acid encoding the compound is administered as particulate formulation such as formulated as lipid nanoparticles.

In some examples, the disease, disorder or condition is cancer.

In a further aspect the disclosure provides a chimeric antigen receptor (CAR) comprising a single-domain antibody binding to an ALFA-tag,

wherein the antibody comprises the CDR1 sequence as shown in SEQ ID NO: 94 or a sequence having 1 or 2 mutations relative to said sequence, the CDR2 sequence as shown in SEQ ID NO: 97 or a sequence having 1 or 2 mutations relative to said sequence, and the CDR3 sequence as shown in SEQ ID NO: 98 or a sequence having 1 or 2 mutations relative to said sequence;

wherein the antibody further comprises at positions corresponding to sequence position 47, 48, and 50 of SEQ ID NO: 1 the following amino acid residues: Arg at position 47, Arg at position 48, Met at position 50;

and wherein the antibody comprises a sequence having at least 90% sequence identity to any one of SEQ ID NOs: 118-125.

In some examples, the antibody comprises the CDR1 sequence as shown in SEQ ID NO: 94, the CDR2 sequence as shown in SEQ ID NO: 97, and the CDR3 sequence as shown in SEQ ID NO: 98.

In some examples, the antibody specifically binds to an epitope tag comprising the sequence SRLEEELRRRLTE (SEQ ID NO: 2).

In some examples, the antibody is humanized and/or wherein the antibody comprises a sequence that has at least about 70% V segment identity to the closest human germline sequence.

In some examples, the antibody has a higher Tm as compared to the antibody of SEQ ID NO: 1.

In some examples, the antibody comprises a His residue at the position corresponding to sequence position 99 of SEQ ID NO: 1, a Val residue at the position corresponding to sequence position 100 of SEQ ID NO: 1, and/or a Tyr residue at the position corresponding to sequence position 40 of SEQ ID NO: 1.

In some examples, the antibody comprises FR1, FR2, FR3, and FR4 sequences, that have at least 80%, at least 85%, at least 90%, or at least 95% sequence identity or are identical to the sequences set forth in SEQ ID NOs: 99, 102, 108, and 109, respectively.
In some examples, the antibody comprises a protein A binding site.
In some examples, the antibody comprises a sequence having at least 95% sequence identity to any one of SEQ ID NOs: 118-125, or wherein the antibody comprises a sequence set forth in any one of SEQ ID NOs: 118-125.
In some examples, the antibody is comprised by an extracellular domain of the CAR.

In a further example the disclosure provides a nucleic acid encoding the CAR described herein.
In some examples, the nucleic acid is DNA.

In a further example the disclosure provides a host cell comprising the nucleic acid encoding the CAR described herein and/or expressing the CAR described herein.
In some examples, the host cell comprises an immune effector cell.
In some examples, the immune effector cell comprises a T cell.
In some examples, the host cell is genetically modified to comprise the nucleic acid and/or expresses the CAR.
In some examples, the host cell expresses the CAR on its cell surface.

In a further aspect the disclosure provides a compound comprising a single-domain antibody binding to an ALFA-tag and a binding moiety for an antigen on target cells,
wherein the antibody comprises the CDR1 sequence as shown in SEQ ID NO: 94 or a sequence having 1 or 2 mutations relative to said sequence, the CDR2 sequence as shown in SEQ ID NO: 97 or a sequence having 1 or 2 mutations relative to said sequence, and the CDR3 sequence as shown in SEQ ID NO: 98 or a sequence having 1 or 2 mutations relative to said sequence;
wherein the antibody further comprises at positions corresponding to sequence position 47, 48, and 50 of SEQ ID NO: 1 the following amino acid residues: Arg at position 47, Arg at position 48, Met at position 50;

and wherein the antibody comprises a sequence having at least 90% sequence identity to any one of SEQ ID NOs: 118-125.

In some examples, the antibody comprises the CDR1 sequence as shown in SEQ ID NO: 94, the CDR2 sequence as shown in SEQ ID NO: 97, and the CDR3 sequence as shown in SEQ ID NO: 98.

In some examples, the antibody specifically binds to an epitope tag comprising the sequence SRLEEELRRRLTE (SEQ ID NO: 2).

In some examples, the antibody is humanized and/or wherein the antibody comprises a sequence that has at least about 70% V segment identity to the closest human germline sequence.

In some examples, the antibody has a higher Tm as compared to the antibody of SEQ ID NO: 1.

In some examples, the antibody comprises a His residue at the position corresponding to sequence position 99 of SEQ ID NO: 1, a Val residue at the position corresponding to sequence position 100 of SEQ ID NO: 1, and/or a Tyr residue at the position corresponding to sequence position 40 of SEQ ID NO: 1.

In some examples, the antibody comprises FR1, FR2, FR3, and FR4 sequences, that have at least 80%, at least 85%, at least 90%, or at least 95% sequence identity or are identical to the sequences set forth in SEQ ID NOs: 99, 102, 108, and 109, respectively.

In some examples, the antibody comprises a protein A binding site.

In some examples, the antibody comprises a sequence having at least 95% sequence identity to any one of SEQ ID NOs: 118-125, or wherein the antibody comprises a sequence set forth in any one of SEQ ID NOs: 118-125.

In some examples, the antibody and the binding moiety for an antigen on target cells are connected through a linking moiety.

In some examples, the linking moiety comprises a polymer.

In some examples, the linking moiety comprises a non-peptidic moiety.

In some examples, the linking moiety comprises a polymer selected from the group consisting of poly(ethylene glycol) (PEG), polysarcosine (pSar) (poly(N-methylglycine)),

polyoxazoline (POX), polyoxazine (POZ), and poly-2-(2-(2-aminoethoxy)ethoxy)acetic acid (pAEEA), derivatives and combinations thereof.

In some examples, the linking moiety comprises a peptidic moiety.

In some examples, the binding moiety for an antigen on target cells comprises a moiety selected from the group consisting of an antibody binding to the antigen, an antibody derivative binding to the antigen and a ligand of the antigen.

In some examples, the antibody derivative binding to the antigen comprises an antibody fragment.

In some examples, the binding moiety for an antigen on target cells comprises a peptidic moiety.

In some examples, the compound comprises a fusion polypeptide comprising the antibody and the binding moiety for an antigen on target cells.

In some examples, the ligand of the antigen comprises DUPA or FAPI.

In some examples, the target cells are diseased cells.

In some examples, the target cells are cancer cells.

In some examples, the antigen on target cells comprises a cell surface antigen.

In some examples, the antigen on target cells comprises a tumor antigen.

In a further example the disclosure provides a nucleic acid encoding the compound comprising a single-domain antibody binding to an ALFA-tag and a binding moiety for an antigen on target cells described herein.

In some examples, the nucleic acid is RNA.

In a further example the disclosure provides a host cell comprising the nucleic acid encoding the compound comprising a single-domain antibody binding to an ALFA-tag and a binding moiety for an antigen on target cells described herein and/or expressing the compound comprising a single-domain antibody binding to an ALFA-tag and a binding moiety for an antigen on target cells described herein.

In a further example the disclosure provides the agents, compositions and systems described herein for use in the methods described herein.

In a further aspect, the present disclosure provides a use of immune effector cells genetically modified to express a chimeric antigen receptor (CAR) and a compound comprising a binding moiety for the CAR and a binding moiety for the antigen in the manufacture of a medicament for treating a disease, disorder or condition characterized by cells expressing an antigen in a subject in need thereof, wherein the CAR and the binding moiety for the CAR comprise a binding system comprising an ALFA-tag and a single-domain antibody binding to the ALFA-tag, wherein the antibody comprises the CDR1 sequence as shown in SEQ ID NO: 94 or a sequence having 1 or 2 mutations relative to said sequence, the CDR2 sequence as shown in SEQ ID NO: 97 or a sequence having 1 or 2 mutations relative to said sequence, and the CDR3 sequence as shown in SEQ ID NO: 98 or a sequence having 1 or 2 mutations relative to said sequence; wherein the antibody further comprises at positions corresponding to sequence position 47, 48, and 50 of SEQ ID NO: 1 the following amino acid residues: Arg at position 47, Arg at position 48, Met at position 50; and wherein the antibody comprises a sequence having at least 90% sequence identity to any one of SEQ ID NOs: 118-125.

In a further aspect, the present disclosure provides a nucleic acid encoding the CAR described herein, wherein preferably the nucleic acid is DNA.

In a further aspect, the present disclosure provides a host cell comprising the nucleic acid described herein and/or expressing the CAR described herein, wherein preferably (a) the host cell comprises an immune effector cell, wherein preferably the immune effector cell comprises a T cell; (b) the host cell is genetically modified to comprise the nucleic acid and/or expresses the CAR; and/or (c) the host cell expresses the CAR on its cell surface.

In a further aspect, the present disclosure provides a nucleic acid encoding the compound described herein, wherein preferably the nucleic acid is RNA.

In a further aspect, the present disclosure provides a host cell comprising the nucleic acid described herein and/or expressing the compound described herein.

Claims

1. A system comprising:

(i) an immune effector cell genetically modified to express a chimeric antigen receptor (CAR), or a nucleic acid encoding a CAR useful for genetically modifying an immune effector cell to express the CAR; and

(ii) a compound comprising a binding moiety for the CAR and a binding moiety for an antigen on target cells, or a nucleic acid encoding the compound,

wherein the CAR and the binding moiety for the CAR comprise a binding system comprising an ALFA-tag and a single-domain antibody binding to the ALFA-tag,

wherein the antibody comprises the CDR1 sequence as shown in SEQ ID NO: 94 or a sequence having 1 or 2 mutations relative to said sequence, the CDR2 sequence as shown in SEQ ID NO: 97 or a sequence having 1 or 2 mutations relative to said sequence, and the CDR3 sequence as shown in SEQ ID NO: 98 or a sequence having 1 or 2 mutations relative to said sequence;

wherein the antibody further comprises at positions corresponding to sequence position 47, 48, and 50 of SEQ ID NO: 1 the following amino acid residues: Arg at position 47, Arg at position 48, Met at position 50;

and wherein the antibody comprises a sequence having at least 90% sequence identity to any one of SEQ ID NOs: 118-125.

2. The system of claim 1, wherein

(a) the immune effector cell comprises a T cell;

(b) the immune effector cell expresses the CAR on its cell surface;

(c) the CAR comprises a moiety binding to the binding moiety for the CAR, wherein preferably (i) the moiety of the CAR binding to the binding moiety for the CAR is comprised by an extracellular moiety of the CAR; and/or (ii) the moiety of the CAR binding to the binding moiety for the CAR comprises an ALFA-tag and the binding moiety for the CAR comprises the antibody or the moiety of the CAR binding to the binding moiety for the CAR comprises the antibody and the binding moiety for the CAR comprises an ALFA-tag;

(d) the antibody specifically binds to an epitope tag comprising the sequence SRLEEELRRRLTE (SEQ ID NO: 2);

(e) the antibody is humanized and/or wherein the antibody comprises a sequence that has at least about 70% V segment identity to the closest human germline sequence;

(f) the antibody has a higher Tm as compared to the antibody of SEQ ID NO: 1;

(g) the antibody comprises a His residue at the position corresponding to sequence position 99 of SEQ ID NO: 1, a Val residue at the position corresponding to sequence position 100 of SEQ ID NO: 1, and/or a Tyr residue at the position corresponding to sequence position 40 of SEQ ID NO: 1;

(h) the antibody comprises FR1, FR2, FR3, and FR4 sequences, that have at least 80%, at least 85%, at least 90%, or at least 95% sequence identity or are identical to the sequences set forth in SEQ ID NOs: 99, 102, 108, and 109, respectively;

(i) the antibody comprises a protein A binding site;

(j) the antibody comprises a sequence having at least 95% sequence identity to any one of SEQ ID NOs: 118-125, or wherein the antibody comprises a sequence set forth in any one of SEQ ID NOs: 118-125;

(k) the ALFA-tag comprises the amino acid sequence of X1-X2-L-E-X5-E-X7-R-R-R-L-X12-X13 (SEQ ID NO: 3), wherein X1 is G or S or P or T, X2 is R or G or P, X5 is E or Q, X7 is L or I, X12 is S or T or P or A or D or E, and wherein X13 is P or A or S or K or D or E or no amino acid;

(l) the binding moiety for the CAR and the binding moiety for an antigen on target cells are connected through a linking moiety, wherein preferably the linking moiety comprises (i) a polymer, and/or (ii) a non-peptidic moiety, and/or (iii) a polymer selected from the group consisting of poly(ethylene glycol) (PEG), polysarcosine (pSar) (poly(N-methylglycine)), polyoxazoline (POX), polyoxazine (POZ), and poly-2-(2-(2-aminoethoxy)ethoxy)acetic acid (pAEEA), derivatives and combinations thereof, and/or (iv) a peptidic moiety;

(m) the binding moiety for an antigen on target cells comprises a moiety selected from the group consisting of an antibody binding to the antigen, an antibody derivative binding to the antigen and a ligand of the antigen, wherein preferably the antibody derivative binding to the antigen comprises an antibody fragment or the ligand of the antigen comprises DUPA or FAPI;

(n) the binding moiety for an antigen on target cells comprises a peptidic moiety;

(o) the compound comprises a fusion polypeptide comprising the binding moiety for the CAR and the binding moiety for an antigen on target cells;

(p) the target cells are diseased cells and/or the target cells are cancer cells;

(q) the antigen on target cells comprises a cell surface antigen and/or a tumor antigen;

(r) the nucleic acid encoding a CAR comprises DNA and/or the nucleic acid encoding the compound comprises RNA; and/or

(s) binding of a complex comprising:

(i) the immune effector cell genetically modified to express a chimeric antigen receptor (CAR); and

(ii) the compound comprising a binding moiety for the CAR and a binding moiety for an antigen on target cells

to cells expressing the antigen results in killing of the cells expressing the antigen.

3. Use of immune effector cells genetically modified to express a chimeric antigen receptor (CAR) and a compound comprising a binding moiety for the CAR and a binding moiety for the antigen in the manufacture of a medicament for treating a disease, disorder or condition characterized by cells expressing an antigen in a subject in need thereof,

wherein the CAR and the binding moiety for the CAR comprise a binding system comprising an ALFA-tag and a single-domain antibody binding to the ALFA-tag,

wherein the antibody comprises the CDR1 sequence as shown in SEQ ID NO: 94 or a sequence having 1 or 2 mutations relative to said sequence, the CDR2 sequence as shown in SEQ ID NO: 97 or a sequence having 1 or 2 mutations relative to said sequence, and the CDR3 sequence as shown in SEQ ID NO: 98 or a sequence having 1 or 2 mutations relative to said sequence;

wherein the antibody further comprises at positions corresponding to sequence position 47, 48, and 50 of SEQ ID NO: 1 the following amino acid residues: Arg at position 47, Arg at position 48, Met at position 50;

and wherein the antibody comprises a sequence having at least 90% sequence identity to any one of SEQ ID NOs: 118-125.

4. The use of claim 3, wherein

(a) the immune effector cells comprise T cells;

(b) the immune effector cells express the CAR on their cell surface;

(c) the CAR comprises a moiety binding to the binding moiety for the CAR, wherein preferably (i) the moiety of the CAR binding to the binding moiety for the CAR is comprised by an extracellular moiety of the CAR; and/or (ii) the moiety of the CAR binding to the binding moiety for the CAR comprises an ALFA-tag and the binding moiety for the CAR comprises the

antibody, or the moiety of the CAR binding to the binding moiety for the CAR comprises the antibody and the binding moiety for the CAR comprises an ALFA-tag;

(d) the antibody specifically binds to an epitope tag comprising the sequence SRLEEELRRRLTE (SEQ ID NO: 2);

(e) the antibody is humanized and/or wherein the antibody comprises a sequence that has at least about 70% V segment identity to the closest human germline sequence;

(f) the antibody has a higher Tm as compared to the antibody of SEQ ID NO: 1;

(g) the antibody comprises a His residue at the position corresponding to sequence position 99 of SEQ ID NO: 1, a Val residue at the position corresponding to sequence position 100 of SEQ ID NO: 1, and/or a Tyr residue at the position corresponding to sequence position 40 of SEQ ID NO: 1;

(h) the antibody comprises FR1, FR2, FR3, and FR4 sequences, that have at least 80%, at least 85%, at least 90%, or at least 95% sequence identity or are identical to the sequences set forth in SEQ ID NOs: 99, 102, 108, and 109, respectively;

(i) the antibody comprises a protein A binding site;

(j) the antibody comprises a sequence having at least 95% sequence identity to any one of SEQ ID NOs: 118-125, or wherein the antibody comprises a sequence set forth in any one of SEQ ID NOs: 118-125;

(k) the ALFA-tag comprises the amino acid sequence of X1-X2-L-E-X5-E-X7-R-R-R-L-X12-X13 (SEQ ID NO: 3), wherein X1 is G or S or P or T, X2 is R or G or P, X5 is E or Q, X7 is L or I, X12 is S or T or P or A or D or E, and wherein X13 is P or A or S or K or D or E or no amino acid;

(l) the binding moiety for the CAR and the binding moiety for an antigen on target cells are connected through a linking moiety, wherein preferably the linking moiety comprises (i) a polymer, and/or (ii) a non-peptidic moiety, and/or (iii) a polymer selected from the group consisting of poly(ethylene glycol) (PEG), polysarcosine (pSar) (poly(N-methylglycine)), polyoxazoline (POX), polyoxazine (POZ), and poly-2-(2-(2-aminoethoxy)ethoxy)acetic acid (pAEEA), derivatives and combinations thereof, and/or (iv) a peptidic moiety;

(m) the binding moiety for the antigen comprises a moiety selected from the group consisting of an antibody binding to the antigen, an antibody derivative binding to the antigen and a ligand of the antigen, wherein preferably the antibody derivative binding to the antigen comprises an antibody fragment or the ligand of the antigen comprises DUPA or FAPI;

(n) the binding moiety for the antigen comprises a peptidic moiety;

(o) the compound comprises a fusion polypeptide comprising the binding moiety for the CAR and the binding moiety for the antigen;

(p) the cells expressing an antigen are diseased cells and/or cancer cells;

(q) the antigen comprises a cell surface antigen and/or a tumor antigen; and/or

(r) binding of a complex comprising:

(i) the immune effector cells genetically modified to express a chimeric antigen receptor (CAR); and

(ii) the compound comprising a binding moiety for the CAR and a binding moiety for an antigen

to cells expressing the antigen results in killing of the cells expressing the antigen.

5. The use of claim 3 or 4, wherein the use comprises

(a) the immune effector cells genetically modified to express a CAR to be administered to the subject;

(b) the immune effector cells genetically modified to express a CAR to be generated in the subject;

(c) nucleic acid encoding the CAR to be administered to the subject, wherein preferably the nucleic acid encoding the CAR comprises DNA;

(d) the compound to be administered to the subject; and/or

(e) the compound to be generated in the subject; and/or

(f) nucleic acid encoding the compound to be administered to the subject, wherein preferably the nucleic acid encoding the compound comprises RNA; and/or the compound is to be expressed by cells in the subject such that the compound becomes associated with cells expressing the antigen and the binding moiety for the CAR is available for binding by the immune effector cells, wherein preferably the cells expressing the compound are to be transfected with the nucleic acid encoding the compound, secrete the compound, and/or express the compound such that it is released into the bloodstream.

6. The use of claim 5, wherein the nucleic acid encoding the CAR and/or the nucleic acid encoding the compound is to be administered as particulate formulation such as formulated as lipid nanoparticles.

7. The use of any one of claims 3 to 6, wherein the disease, disorder or condition is cancer.

8. A chimeric antigen receptor (CAR) comprising a single-domain antibody binding to an ALFA-tag,
wherein the antibody comprises the CDR1 sequence as shown in SEQ ID NO: 94 or a sequence having 1 or 2 mutations relative to said sequence, the CDR2 sequence as shown in SEQ ID NO: 97 or a sequence having 1 or 2 mutations relative to said sequence, and the CDR3 sequence as shown in SEQ ID NO: 98 or a sequence having 1 or 2 mutations relative to said sequence;
wherein the antibody further comprises at positions corresponding to sequence position 47, 48, and 50 of SEQ ID NO: 1 the following amino acid residues: Arg at position 47, Arg at position 48, Met at position 50;
and wherein the antibody comprises a sequence having at least 90% sequence identity to any one of SEQ ID NOs: 118-125.

9. The CAR of claim 8, wherein
(a) the antibody specifically binds to an epitope tag comprising the sequence SRLEEELRRRLTE (SEQ ID NO: 2);
(b) the antibody is humanized and/or wherein the antibody comprises a sequence that has at least about 70% V segment identity to the closest human germline sequence;
(c) the antibody has a higher Tm as compared to the antibody of SEQ ID NO: 1;
(d) the antibody comprises a His residue at the position corresponding to sequence position 99 of SEQ ID NO: 1, a Val residue at the position corresponding to sequence position 100 of SEQ ID NO: 1, and/or a Tyr residue at the position corresponding to sequence position 40 of SEQ ID NO: 1;
(e) the antibody comprises FR1, FR2, FR3, and FR4 sequences, that have at least 80%, at least 85%, at least 90%, or at least 95% sequence identity or are identical to the sequences set forth in SEQ ID NOs: 99, 102, 108, and 109, respectively;
(f) the antibody comprises a protein A binding site;
(g) the antibody comprises a sequence having at least 95% sequence identity to any one of SEQ ID NOs: 118-125, or wherein the antibody comprises a sequence set forth in any one of SEQ ID NOs: 118-125; and/or
(h) the antibody is comprised by an extracellular domain of the CAR.

10. A nucleic acid encoding the CAR of claim 8 or 9, wherein preferably the nucleic acid is DNA.

11. A host cell comprising the nucleic acid of claim 10 and/or expressing the CAR of claim 8 or 9, wherein preferably

(a) the host cell comprises an immune effector cell, wherein preferably the immune effector cell comprises a T cell;

(b) the host cell is genetically modified to comprise the nucleic acid and/or expresses the CAR; and/or

(c) the host cell expresses the CAR on its cell surface.

12. A compound comprising a single-domain antibody binding to an ALFA-tag and a binding moiety for an antigen on target cells,

wherein the antibody comprises the CDR1 sequence as shown in SEQ ID NO: 94 or a sequence having 1 or 2 mutations relative to said sequence, the CDR2 sequence as shown in SEQ ID NO: 97 or a sequence having 1 or 2 mutations relative to said sequence, and the CDR3 sequence as shown in SEQ ID NO: 98 or a sequence having 1 or 2 mutations relative to said sequence;

wherein the antibody further comprises at positions corresponding to sequence position 47, 48, and 50 of SEQ ID NO: 1 the following amino acid residues: Arg at position 47, Arg at position 48, Met at position 50;

and wherein the antibody comprises a sequence having at least 90% sequence identity to any one of SEQ ID NOs: 118-125.

13. The compound of claim 12, wherein

(a) the antibody specifically binds to an epitope tag comprising the sequence SRLEEELRRRLTE (SEQ ID NO: 2);

(b) the antibody is humanized and/or wherein the antibody comprises a sequence that has at least about 70% V segment identity to the closest human germline sequence;

(c) the antibody has a higher Tm as compared to the antibody of SEQ ID NO: 1;

(d) the antibody comprises a His residue at the position corresponding to sequence position 99 of SEQ ID NO: 1, a Val residue at the position corresponding to sequence position 100 of

SEQ ID NO: 1, and/or a Tyr residue at the position corresponding to sequence position 40 of SEQ ID NO: 1;

(e) the antibody comprises FR1, FR2, FR3, and FR4 sequences, that have at least 80%, at least 85%, at least 90%, or at least 95% sequence identity or are identical to the sequences set forth in SEQ ID NOs: 99, 102, 108, and 109, respectively;

(f) the antibody comprises a protein A binding site;

(g) the antibody comprises a sequence having at least 95% sequence identity to any one of SEQ ID NOs: 118-125, or wherein the antibody comprises a sequence set forth in any one of SEQ ID NOs: 118-125;

(h) the antibody and the binding moiety for an antigen on target cells are connected through a linking moiety, wherein preferably the linking moiety comprises (i) a polymer, and/or (ii) a non-peptidic moiety, and/or (iii) a polymer selected from the group consisting of poly(ethylene glycol) (PEG), polysarcosine (pSar) (poly(N-methylglycine)), polyoxazoline (POX), polyoxazine (POZ), and poly-2-(2-(2-aminoethoxy)ethoxy)acetic acid (pAEEA), derivatives and combinations thereof, and/or

(iv) a peptidic moiety;

(i) the binding moiety for an antigen on target cells comprises a moiety selected from the group consisting of an antibody binding to the antigen, an antibody derivative binding to the antigen and a ligand of the antigen, wherein preferably the antibody derivative binding to the antigen comprises an antibody fragment or the ligand of the antigen comprises DUPA or FAPI;

(j) the binding moiety for an antigen on target cells comprises a peptidic moiety;

(k) the compound comprises a fusion polypeptide comprising the antibody and the binding moiety for an antigen on target cells;

(l) the target cells are diseased cells;

(m) the target cells are cancer cells; and/or

(n) the antigen on target cells comprises a cell surface antigen and/or a tumor antigen.

14. A nucleic acid encoding the compound of claim 12 or 13, wherein preferably the nucleic acid is RNA.

15. A host cell comprising the nucleic acid of claim 14 and/or expressing the compound of claim 12 or 13.