overexpression of LCK led to increased basal CAR-CD3ζ phosphorylation and intracellular calcium mobilization after antigenic stimulation, and these LCK -BBz-CAR-T cells showed improved expansion capacities and tumor control *in vivo* as compared to conventional BBz-CAR-T cell lacking LCK overexpression (Sun et al., (2020) Cancer Cell, (2) 216-225; WO2021087183). However, in that study it was not shown whether overexpression of LCK improved the sensitivity of BBz-CAR-T cells against antigen-low tumor cells, and if increased LCK -mediated basal CAR-CD3ζ phosphorylation eventually caused tonic CAR signalling resulting in antigen-independent T cell exhaustion and T cell differentiation, which is a common problem seen with 28z-CAR constructs (Long et al., (2015) Nat Med, (6) 581-590).

Yet another study described the expression of a chimeric molecule called T cell antigen coupler (TAC), a chimeric receptor comprising an antigen-recognition domain, a TCR-recruitment domain (an anti-CD3ε scFv), and a coreceptor domain (hinge, transmembrane and cytosolic regions from CD4 or CD8α) (Helsen et al., (2018) Nat Commun, (1) 3049; WO2015117229). Rather than modifying current CAR designs, the TAC receptor was designed to engage both the coreceptor and endogenously expressed TCRs in response to binding to a single antigen, aiming to recapitulate the architecture of a TCR-coreceptor complex to activate natural signalling pathways and T cell responses.

SUMMARY

The present disclosure relates generally to the development of immunotherapeutics, including recombinant cells, containing polypeptides or polynucleotides encoding such polypeptides, such as a chimeric antigen receptor (CAR) containing an antigen-recognition domain fused to hinge, transmembrane and T cell activating domains, and a chimeric coreceptor containing an antigen-recognition domain fused to an extracellular domain, a hinge domain, a transmembrane domain and a cytosolic domain (a) capable of binding LCK and/or (b) comprising LCK (CoCAR), as well as pharmaceutical compositions containing the same for use in enhancing immune therapies in e.g. treating various conditions, such as diseases (e.g. cancer). As described in greater detail below, different CAR and CoCAR constructs have been prepared and found to have dramatic effects to enhance activation and increase the potency of immune cells (e.g. T cells) co-expressing a 4-1BB-based second-generation CAR and a CoCAR in response to tumor cells, even when the antigen is expressed at a very low density.

In one aspect, the present disclosure provides a coreceptor-CAR (CoCAR), comprising (i) a first extracellular antigen-recognition domain, (ii) a first hinge domain, (iii) a first transmembrane domain, and, (iv) at least one cytosolic domain capable of binding LCK; wherein the CoCAR does not comprise a CD3zeta domain. According to a first example, the present disclosure provides a coreceptor-CAR (CoCAR), comprising (i) a first extracellular antigen-recognition domain, (ii) a first hinge domain, (iii) a first transmembrane domain, and (iv) at least one cytosolic domain (a) capable of binding LCK, and/or (b) comprising LCK, or a variant or fragment thereof.

In particular, (i) the first extracellular antigen-recognition domain, (ii) the first hinge domain, (iii) the first transmembrane domain, and (iv) the at least one cytosolic domain are arranged from N to C terminal.

In particular, the coreceptor-CAR does not comprise a CD3zeta domain.

According to an example, the first antigen-recognition domain is a scFv.

According to an example, the first hinge domain is derived from a CD4, CD8, CD28 or IgG-Fc hinge domain.

According to another example the first transmembrane domain is derived from a CD4, CD8, CD28, or CD3zeta transmembrane domains.

According to yet another example the at least one cytosolic domain (a) capable of binding LCK is (1) an intracellular signaling domain derived from a CD4, CD8α, CD3ε, CD28, CD44, or CD146 intracellular signaling domain, or a variant or fragment thereof, and/or (2) comprises one or more motifs capable of binding LCK.

In particular, the intracellular signaling domain (1) is derived from a CD4, CD8α, CD3ε, CD44, or CD146 intracellular signaling domain, or a variant or fragment thereof.

In particular, the intracellular signaling domain (1) is derived from a CD4, CD8α, CD28, CD44, or CD146 intracellular signaling domain, or a variant or fragment thereof.

In particular, the intracellular domain (1) is derived from a CD4, CD8α, CD44, or CD146 intracellular signaling domain, or a variant or fragment thereof.

In particular, the intracellular domain (1) is derived from a CD4 or CD8α intracellular signaling domain, or a variant or fragment thereof,

In particular, the motif is derived from a CD4, CD8α, CD28, CD3ε, CD44, or CD146 intracellular signaling domain.

In particular, the motif capable of binding LCK is derived from a CD4, CD8α, CD28, CD3ε, CD44, or CD146 intracellular signaling domain.

In particular, the motif capable of binding LCK is derived from a CD4, CD8α, CD3ε, CD44, or CD146 intracellular signaling domain, or a variant or fragment thereof.

In particular, the motif capable of binding LCK is derived from a CD4, CD8α, CD28, CD44, or CD146 intracellular signaling domain, or a variant or fragment thereof.

In particular, the motif capable of binding LCK is derived from a CD4, CD8α, CD44, or CD146 intracellular signaling domain, or a variant or fragment thereof.

In particular, the motif capable of binding LCK is derived from a CD28, CD3ε, CD44, or CD146 intracellular signaling domain.In particular, the motif capable of binding LCK is derived from a CD28, CD44, or CD146 intracellular signaling domain.

In particular, the motif capable of binding LCK is derived from a CD4 or CD8α intracellular signaling domain, or a variant or fragment thereof,

According to yet another example, the CoCAR is capable of specifically binding to an antigen on a target cell.

According to a second aspect, the present disclosure provides a combination comprising (a) the CoCAR of the present disclosure, and (b) a chimeric antigen receptor (CAR).

According to an example, the CAR (b) comprises (i) a second extracellular antigen-recognition domain, (ii) a second hinge region, (iii) a second transmembrane domain, (iv) a 4-1BB domain and/or a CD28 domain, and (v) a CD3zeta domain.

In particular, in the CAR, (i) the second extracellular antigen-recognition domain, (ii) the second hinge region, (iii) the second transmembrane domain, (iv) the 4-1BB domain and/or a CD28 domain, and (v) the CD3zeta domain are arranged from N to C terminal.

In a third aspect, the present disclosure provides a nucleic acid or a combination of a first and second nucleic acid, wherein (a) the nucleic acid encodes the CoCAR as disclosed herein or the combination as disclosed herein, or (b) the combination of a first and second nucleic acid encodes the combination as disclosed herein, wherein the first nucleic acid encodes the CoCAR (a), and the second nucleic acid encodes the CAR (b). In a further aspect, the present disclosure provides a nucleic acid construct or a combination of a first and second nucleic acid construct, wherein (a) the nucleic acid construct comprises the nucleic acid or the combination of a first and second nucleic acid as disclosed herein, wherein the nucleic acid and/or the first and second nucleic acids are functionally linked to at least one expression control sequence, and (b) in the combination of the first and second nucleic acid construct, the first nucleic construct comprises the first nucleic acid as disclosed herein, and the second nucleic construct comprises the second nucleic acid as disclosed herein, wherein the first and second nucleic acids independently are functionally linked to at least one expression control sequence. According to a third example, the present disclosure provides a nucleic acid, nucleic acids, a nucleic acid construct and nucleic acid constructs encoding the CoCAR of the present disclosure or the combination of the present disclosure.

In a fourth aspect, the present disclosure provides a vector or a combination of a first and second vector, wherein (a) the vector comprises the nucleic acid or a combination of a first and second nucleic acid as disclosed herein and/or the nucleic acid construct or combination of nucleic acid constructs as disclosed herein, and/or (b) in the combination, the first vector comprises the first nucleic acid and/or the first nucleic acid construct, and the second vector comprises the second nucleic acid and/or the second nucleic acid construct as disclosed herein. According to a fourth example, the present disclosure provides a vector or a combination of vectors comprising the nucleic acid, nucleic acids, the nucleic acid construct and/or nucleic acid constructs of the present disclosure.

According to a fifth aspect, the present disclosure provides a recombinant cell, comprising the nucleic acid construct of the present disclosure

According to an example, the cell of the present disclosure is an immune effector cell.

According to an example, the cell of the present disclosure expresses the CoCAR of the present disclosure on the cell surface.

According to an example, the cell of the present disclosure expresses the combination of the present disclosure on the cell surface.

According to yet another example, in the cell of the present disclosure, (i) the CAR and (ii) the CoCAR recognize different epitopes located in the same antigen expressed on the surface of a target cell.

According to yet another example, in the cell of the present disclosure, (i) the CAR and (ii) the CoCAR recognize different antigens expressed on the surface of a target cell.

In a sixth aspect, the present disclosure provides a pharmaceutical composition comprising (i) the CoCAR as disclosed herein, (ii) the combination as disclosed herein, (iii) the nucleic acid or the combination of nucleic acids as disclosed herein, the nucleic acid construct or the combination of nucleic constructs as disclosed herein, the vector or the combination of vectors as disclosed herein, and/or (iv) the recombinant cell as disclosed herein, and a pharmaceutically acceptable carrier. According to a sixth example, the present disclosure provides a pharmaceutical composition comprising (i) the CoCAR of the present disclosure, (ii) the combination of the present disclosure, (iii) the nucleic acid construct of the present disclosure, and/or (iv) the recombinant cell of the present disclosure, and a pharmaceutically acceptable carrier.

In a seventh aspect, the present disclosure provides the CoCAR as disclosed herein, the combination as disclosed herein, nucleic acid or the combination of nucleic acids as disclosed herein, the nucleic acid construct or the combination of nucleic constructs as disclosed herein, the vector or the combination of vectors as disclosed herein, the recombinant cell as disclosed herein, and/or the pharmaceutical composition as disclosed herein, for use in therapy. According to a seventh example, the present disclosure provides the CoCAR of the present disclosure, the combination of the present disclosure, the nucleic acid construct according of the present disclosure, the recombinant cell of the present disclosure, or the pharmaceutical composition of the present disclosure, for use in medicine.

In an eighth aspect, the present disclosure provides a use of (i) the CoCAR as disclosed herein, (ii) the combination as disclosed herein, (iii) the nucleic acid, or the combination of nucleic acids as disclosed herein, the nucleic acid construct, or the combination of nucleic constructs as disclosed herein, the vector, or the combination of vectors as disclosed herein, and/or (iv) the recombinant cell as disclosed herein in the manufacture of a medicament for treating cancer in a subject in need thereof. According to an eighth example, the present disclosure provides the CoCAR of the present disclosure, the combination of the present disclosure, the nucleic acid construct according of the present disclosure, the recombinant cell of the present disclosure, or the pharmaceutical composition of the present disclosure, for use in treating cancer.

According to an example, the CoCAR, the combination, the nucleic acid construct, the recombinant cell, or the pharmaceutical composition for use of the present disclosure, in treating a malignancy with cell-surface expression is preferably selected from the group consisting of B7-H3

(CD276), BCMA, CD3, CD5, CD19, CD20, CD22, CD23, CD30, CD33, CD38, CD44, CD44v6, CD52, CD70, CD79A, CD79B, CD123, CD138, CD171, CEA, Claudin-6, Claudin-18.2, CLL1, CXCR5, EGFR, EGFRvIII, EPH-receptor A2, IGLV3-21, GP-2, GP-40, HER2, ErbB3, ErbB4, FBP, AchR, Fr-a, GD2, GD3, HMW-MAA, IL13Ra2, Kappa-LC, IGLV3-21-R110, Lewis Y, Mesothelin, MUC1, MUC16, NKG2D Ligands, NCAM, NY-ESO1, Oncofetal antigen h5T4, PSCA, PSMA, ROR1, TAG-72, VEGFR, GOLPH2, and SLAMF7.

According to a ninth example, the present disclosure provides the CoCAR, the combination, the nucleic acid construct, the recombinant cell, and/or the pharmaceutical composition of the disclosure, for use in treating a B cell malignancy.

According to a tenth example, the present disclosure provides a method of treating cancer in a subject in need thereof, comprising the step of administering to the subject an effective amount of (i) the CoCAR of the present disclosure, (ii) the combination of the present disclosure, (iii) the nucleic acid construct of the present disclosure, and/or (iv) the recombinant cell of the present disclosure.

According to an example, the cancer is a malignancy positive for at least one tumor-associated antigen preferably selected from the group consisting of B7-H3 (CD276), BCMA, CD3, CD5, CD19, CD20, CD22, CD23, CD30, CD33, CD38, CD44, CD44v6, CD52, CD70, CD79A, CD79B, CD123, CD138, CD171, CEA, Claudin-6, Claudin-18.2, CLL1, CXCR5, EGFR, EGFRvIII, EPH-receptor A2, IGLV3-21, GP-2, GP-40, HER2, ErbB3, ErbB4, FBP, AchR, Fr-a, GD2, GD3, HMW-MAA, IL13Ra2, Kappa-LC, IGLV3-21-R110, Lewis Y, Mesothelin, MUC1, MUC16, NKG2D Ligands, NCAM, NY-ESO1, Oncofetal antigen h5T4, PSCA, PSMA, ROR1, TAG-72, VEGFR, GOLPH2, and SLAMF7.

According to another example, the cancer is a B cell malignancy.

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

FIGURES

Figure 1 shows the properties of untransduced (UTD) T cells, or T cells expressing an anti-CD22 BBz-CAR with or without overexpression of LCK. (**A**) Co-expressing LCK with a BBz-CAR may increases basal CAR-CD3ζ phosphorylation, thereby enhancing the activity of CAR-T cells. (**B**) The phenotype of T cells was analyzed by flow cytometry on day 14 after initial T cell activation, and T cell subsets are defined according to expression of CAR, CD4, CD8, CCR7 (CD197) and CD45R0. CCR7+/CD45R0- cells represent naïve-like T cells, CCR7+/CD45R0+ cells represent central-memory T cells, CCR7-/CD45R0+ cells represent effector-memory T cells, and CCR7-/CD45R0- cells represent effector T cells. These data show that overexpression of LCK together with a BBz-CAR leads to T cell differentiation towards effector-memory and effector T cells. (**C**) shows the ex vivo expansion of T cells expressing the indicated constructs. While untransduced and anti-CD22 BBz-CAR-transduced T cells efficiently proliferate in the first 14 days after T cell activation by CD3/CD28 agonistic agents on day 0, CAR-T cells overexpressing LCK show only poor expansion capacities.

Figure 2 illustrates the concept of the present invention of co-expressing a BBz-CAR and a CoCAR. The CoCAR comprises an antigen-recognition domain fused to a coreceptor, in this example to