CLAIMS

1. A construct for engineering a cell to obtain a genomically edited cell, comprising: (i) a left homology arm and a right homology arm targeting CD38 at a selected position in CD38 locus (LHA/CD38, RHA/CD38) in the cell genome, operatively linked to (ii) one or more nucleic acid sequences encoding one or more exogenous proteins;

in which the genomically edited cell is an induced pluripotent cell (iPSC), a clonal iPSC, an iPS cell line cell, a derivative effector cell differentiated from an iPSC, or an immune cell; and

in which the genomically edited cell comprises a targeted integration of the one or more nucleic acid sequences encoding one or more exogenous protein at the selected position in CD38 locus, and CD38 knockout.

2. A construct according to Claim 1, in which the one or more nucleic acid sequences encoding one or more exogenous proteins are operatively linked to, and are driven by, an endogenous promoter of CD38 upon integration, or in which the selected position in CD38 locus is in an exon of CD38.

3. A construct according to Claim 1, in which the one or more exogenous proteins comprise at least one or both of: (i) a CD16 or a variant thereof; and (ii) a cell surface expressed exogenous IL15/IL15 receptor fusion protein (IL15RF) or a variant thereof; and in which the genomically edited cell and the derived effector cell therefrom comprise the one or more nucleic acid sequences encoding CD16 and IL15RF, or variants thereof.

4. A construct according to Claim 3,

(i) in which the CD16 or a variant thereof comprises at least one of:

(a) F176V and S197P in ectodomain domain of CD16;

(b) a full or partial ectodomain originated from CD64;

(c) a non-native (or non-CD16) transmembrane domain;

(d) a non-native (or non-CD16) intracellular domain;

(e) a non-native (or non-CD16) signaling domain;

(f) a non-native stimulatory domain; and

(g) transmembrane, signaling, and stimulatory domains that are not originated from CD16, and are originated from a same or different polypeptide;

or

(ii) in which the IL15RF or a variant thereof comprises at least one of:

(a) co-expressed IL15 and IL15Rα by using a self-cleaving peptide;

(b) a fusion protein of IL15 and IL15Rα;

(c) an IL15/IL15Rα fusion protein with intracellular domain of IL15Rα truncated;

(d) a fusion protein of IL15 and membrane bound Sushi domain of IL15Rα;

(e) a fusion protein of IL15 and IL15Rβ;

(f) a fusion protein of IL15 and common receptor γC, in which the common receptor γC is native or modified;

(g) a homodimer of IL15Rβ; and

(h) a polynucleotide encoding an IL15RF comprising an amino acid sequence of at least 75%, 80%, 85%, 90%, 95% or 99% identity to SEQ ID NOs: 17, 19 or 21.

5. A construct according to Claim 4, in which the CD16 or a variant thereof comprises one or more of:

(i) a non-native transmembrane domain derived from CD3D, CD3E, CD3G, CD3ζ, CD4, CD8, CD8a, CD8b, CD27, CD28, CD40, CD84, CD166, 4-1BB, OX40, ICOS, ICAM-1, CTLA-4, PD-1, LAG-3, 2B4, BTLA, CD16, IL7, IL12, IL15, KIR2DL4, KIR2DS1, NKp30, NKp44, NKp46, NKG2C, NKG2D, or T cell receptor (TCR) polypeptide;

(ii) a non-native stimulatory domain derived from CD27, CD28, 4-1BB, OX40, ICOS, PD-1, LAG-3, 2B4, BTLA, DAP10, DAP12, CTLA-4, or NKG2D polypeptide;

(iii) a non-native signaling domain derived from CD3ζ, 2B4, DAP10, DAP12, DNAM1, CD137 (41BB), IL21, IL7, IL12, IL15, NKp30, NKp44, NKp46, NKG2C, or NKG2D polypeptide; or

(iv) a non-native transmembrane domain is derived from NKG2D, the non-native stimulatory domain is derived from 2B4, and the non-native signaling domain is derived from CD3ζ.

6. A construct according to Claim 1, in which the genomically edited cell further comprises a chimeric antigen receptor (CAR), in which the CAR has at least one following characteristics:

(i) T cell specific or NK cell specific;

(ii) bi-specific antigen binding CAR;

(iii) a switchable CAR;

(iv) a dimerized CAR;

(v) a split CAR;

(vi) a multi-chain CAR;

(vii) an inducible CAR;

(viii) co-expressed with another CAR;

(ix) co-expressed with a partial or full peptide of a cell surface expressed exogenous cytokine or a receptor thereof;

(xi) co-expressed with a checkpoint inhibitor;

(xii) encoded by a nucleic acid further comprised in the construct, in which the CAR is inserted at the selected position in CD38 locus;

(xiii) inserted at a TCR locus, and/or is driven by an endogenous promoter of TCR, and/or the TCR is knocked out by the CAR insertion;

(xiii) specific to CD19 or BCMA; and/or

(xiv) specific to any one of ADGRE2, carbonic anhydrase IX (CAIX), CCR1, CCR4, carcinoembryonic antigen (CEA), CD3, CD5, CD7, CD8, CD10, CD20, CD22, CD30,

CD33, CD34, CD38, CD41, CD44, CD44V6, CD49f, CD56, CD70, CD74, CD99, CD123, CD133, CD138, CDS, CLEC12A, an antigen of a cytomegalovirus (CMV) infected cell, epithelial glycoprotein2 (EGP 2), epithelial glycoprotein-40 (EGP-40), epithelial cell adhesion molecule (EpCAM), EGFRvIII, receptor tyrosine-protein kinases erb- B2,3,4, EGFIR, EGFR-VIII, ERBB folate-binding protein (FBP), fetal acetylcholine receptor (AChR), folate receptor-a, Ganglioside G2 (GD2), Ganglioside G3 (GD3), human Epidermal Growth Factor Receptor 2 (HER-2), human telomerase reverse transcriptase (hTERT), ICAM-1, Integrin B7, Interleukin-13 receptor subunit alpha-2 (IL-13Rα2), κ-light chain, kinase insert domain receptor (KDR), Lewis A (CA19.9), Lewis Y (LeY), L1 cell adhesion molecule (L1-CAM), LILRB2, melanoma antigen family A 1 (MAGE-A1), MICA/B, Mucin 1 (Muc-1), Mucin 16 (Muc-16), Mesothelin (MSLN), NKCSI, NKG2D ligands, c-Met, cancer-testis antigen NY-ESO-1, oncofetal antigen (h5T4), PRAME, prostate stem cell antigen (PSCA), PRAME prostate-specific membrane antigen (PSMA), tumor- associated glycoprotein 72 (TAG-72), TIM-3, TRBCI, TRBC2, vascular endothelial growth factor R2 (VEGF- R2), Wilms tumor protein (WT-1), and a pathogen antigen.

7. A construct according to Claim 1, further comprising one or more of:

(i) an exogenous promoter that drives expression of the one or more nucleic acid sequences encoding one or more exogenous proteins, in which the exogenous promoter is constitutive, inducible, temporal-, tissue-, and/or cell type- specific;

(ii) a linker sequence inserted between two nucleic acid sequences encoding exogenous proteins;

(iii) an insulator at the 3’ end of LHA/CD38 and an insulator at the 5’ end of RHA/CD38; and

(iv) a polyA signal.

8. A construct according to Claim 7, in which

(i) the exogenous promoter is CMV, EF1α, PGK, CAG, or UBC;

(ii) the linker sequence comprises a 2A sequence encoding a self-cleaving 2A peptide; and/or

(iii) the linker sequence comprises an Internal Ribosome Entry Sequence (IRES).

9. A construct according to Claim 1, in which the genomically edited cell comprises one or more following phenotypes:

(i) comprising longer telomeres in comparison to its native counterpart cell obtained from peripheral blood, umbilical cord blood, or any other donor tissues;

(ii) $CD38^{-/-}$ CD16;

(iiii) $CD38^{-/-}$ IL15; and/or

(iv) $CD38^{-/-}$ CD16 IL15.

10. A construct according to Claim 1, in which the genomically edited cell that is an iPSC or the iPSC derived effector cell further comprises one or more of:

(i) HLA-I deficiency;

(ii) HLA-II deficiency;

(iii) introduced expression of HLA-G or non-cleavable HLA-G;

(iv) deletion or reduced expression in at least one of B2M, TAP1, TAP2, Tapasin, NLRC5, PD1, LAG3, TIM3, RFXANK, CIITA, RFX5, RFXAP, and any gene in the chromosome 6p21 region; and

(v) introduced or increased expression in at least one of HLA-E, 41BBL, CD4, CD8, CD16, CD47, CD113, CD131, CD137, CD80, PDL1, $A_{2A}R$, CAR, Fc receptor, an engager, and surface triggering receptor for coupling with bi- or multi- specific or universal engagers.

11. A construct according to Claim 1, in which the iPSC derived effector cell comprises derivative CD34 cell, derivative hematopoietic stem and progenitor cell, derivative hematopoietic multipotent progenitor cell, derivative T cell progenitor, derivative NK cell progenitor, derivative T cell, derivative NKT cell, derivative NK cell, or derivative B cell; or in which the iPSC derived effector cell comprises an immune cell having at least one of the following characteristics comprising:

(i) improved persistency and/or survival;

(ii) increased resistance to native immune cells;

(iii) increased cytotoxicity;

(iv) improved tumor penetration;

(v) enhanced or acquired ADCC;

(vi) enhanced ability in migrating, and/or activating or recruiting bystander immune cells, to tumor sites;

(vii) enhanced ability to reduce tumor immunosuppression;

(viii) improved ability in rescuing tumor antigen escape; and

(ix) reduced fratricide in the presence of anti-CD38 antibodies or CD38 CAR,

in comparison to its native counterpart cell obtained from peripheral blood, umbilical cord blood, or other donor tissues.

12. A construct according to Claim 11, in which the anti-CD38 antibody is daratumumab, isatuximab, or MOR202, or any of the humanized or Fc modified variants or fragments, functional equivalents and biosimilars thereof.

13. An expression vector that comprises a construct according to any preceding claim.

14. A host cell comprising a construct according to any of Claims 1 to 12, or an expression vector according to Claim 13.

15. A composition that comprises a construct according to any of Claims 1 to 12, or an expression vector according to Claim 13, for manufacturing a genomically edited iPSC or derived effector cell differentiated therefrom.

16. A kit comprising a construct according to any of Claims 1 to 12, an expression vector according to Claim 13, or a host cell according to Claim 14, and one or more medium for iPSC culturing or differentiation.

17. A construct according to any of Claims 1 to 12, an expression vector according to Claim 13, or a host cell according to Claim 14, for use of a medicament.

18. A method of producing a genomically edited iPSC or effector cell derived therefrom using a construct according to any of Claims 1 to 12 comprising:

(i) introducing the construct to an iPSC,

(ii) incubating the iPSC for a sufficient period of time to enable targeted integration of one or more nucleic acid sequence encoding one or more exogenous protein at CD38 locus and consequently knocking out the expression of CD38 of the iPSC, thereby obtaining a genomically edited iPSC.

19. A method according to Claim 18, further comprising:

(iii) directing differentiation of the genomically edited iPSC from step (ii) to obtain the effector cell comprising the same targeted integration of one or more nucleic acid sequence encoding one or more exogenous protein at CD38 locus and CD38 knockout as the genomically edited iPSC.

20. A method according to Claim 18, in which the genomically edited iPSC has improved genomic stability than iPSC undergoing integration and knocking out in separate and step-wise editing events.

21. A method according to Claim 18, in which the introducing step further comprises introducing a CRISPR-cas/gNA (guide nucleic acid), a ZFN, a TALEN, a homing nuclease, or any other functional variation thereof.

CLAIMS

1. A construct for engineering a cell to obtain a genomically edited cell, comprising: (i) a left homology arm and a right homology arm targeting CD38 at a selected position in CD38 locus (LHA/CD38, RHA/CD38) in the cell genome, operatively linked to (ii) one or more nucleic acid sequences encoding one or more exogenous proteins;

in which the genomically edited cell is an induced pluripotent cell (iPSC), a clonal iPSC, an iPS cell line cell, a derivative effector cell differentiated from an iPSC, or an immune cell; and

in which the genomically edited cell comprises a targeted integration of the one or more nucleic acid sequences encoding one or more exogenous protein at the selected position in CD38 locus, and CD38 knockout.

2. A construct according to Claim 1, in which the one or more nucleic acid sequences encoding one or more exogenous proteins are operatively linked to, and are driven by, an endogenous promoter of CD38 upon integration; or in which the selected position in CD38 locus is in an exon of CD38.

3. A construct according to Claim 1, in which the one or more exogenous proteins comprise at least one or both of: (i) a CD16 or a functional variant thereof; and (ii) a cell surface expressed exogenous IL15/IL15 receptor fusion protein (IL15RF) or a functional variant thereof; and in which the genomically edited cell and the derived effector cell therefrom comprise the one or more nucleic acid sequences encoding CD16 and IL15RF, or functional variants thereof.

4. A construct according to Claim 3,

(i) in which the CD16 or a functional variant thereof comprises at least one of:

(a) F176V and S197P in ectodomain domain of CD16;

(b) a functional full or partial ectodomain of a CD64;

(c) a non-native (or non-CD16) transmembrane domain;

(d) a non-native (or non-CD16) intracellular domain;

(e) a non-native (or non-CD16) signaling domain;

(f) a non-native stimulatory domain; and

(g) transmembrane, signaling, and stimulatory domains that are domains of a protein other than CD16, and are domains of a same or different polypeptide;

or

(ii) in which the IL15RF or a variant thereof comprises at least one of:

(a) co-expressed IL15 and IL15Rα by using a self-cleaving peptide;

(b) a fusion protein of IL15 and IL15Rα;

(c) an IL15/IL15Rα fusion protein with intracellular domain of IL15Rα truncated;

(d) a fusion protein of IL15 and membrane bound Sushi domain of IL15Rα;

(e) a fusion protein of IL15 and IL15Rβ;

(f) a fusion protein of IL15 and common receptor γC;

(g) a homodimer of IL15Rβ; and

(h) a polynucleotide encoding an IL15RF comprising an amino acid sequence of SEQ ID NOs: 17, 19 or 21.

5. A construct according to Claim 4, in which the CD16 or a functional variant thereof comprises one or more of:

(i) a non-native transmembrane domain derived from CD3D, CD3E, CD3G, CD3ζ, CD4, CD8, CD8a, CD8b, CD27, CD28, CD40, CD84, CD166, 4-1BB, OX40, ICOS, ICAM-1, CTLA-4, PD-1, LAG-3, 2B4, BTLA, CD16, IL7, IL12, IL15, KIR2DL4, KIR2DS1, NKp30, NKp44, NKp46, NKG2C, NKG2D, or T cell receptor (TCR) polypeptide;

(ii) a non-native stimulatory domain derived from CD27, CD28, 4-1BB, OX40, ICOS, PD-1, LAG-3, 2B4, BTLA, DAP10, DAP12, CTLA-4, or NKG2D polypeptide;

(iii) a non-native signaling domain derived from CD3ζ, 2B4, DAP10, DAP12, DNAM1, CD137 (41BB), IL21, IL7, IL12, IL15, NKp30, NKp44, NKp46, NKG2C, or NKG2D polypeptide; or

(iv) a non-native transmembrane domain is derived from NKG2D, the non-native stimulatory domain is derived from 2B4, and the non-native signaling domain is derived from CD3ζ.

6. A genomically edited cell that is edited with a construct according to Claim 1, in which the genomically edited cell further comprises a chimeric antigen receptor (CAR), in which the CAR has at least one following characteristics:

(i) T cell specific or NK cell specific;

(ii) bi-specific antigen binding CAR;

(iii) a switchable CAR;

(iv) a dimerized CAR;

(v) a split CAR;

(vi) a multi-chain CAR;

(vii) an inducible CAR;

(viii) co-expressed with another CAR;

(ix) co-expressed with a partial or full peptide of a cell surface expressed exogenous cytokine or a receptor thereof;

(x) co-expressed with a checkpoint inhibitor;

(xi) encoded by a nucleic acid further comprised in the construct, in which the CAR is inserted at the selected position in CD38 locus;

(xii) inserted at a TCR locus, and/or is driven by an endogenous promoter of TCR, and/or the TCR is knocked out by the CAR insertion;

(xiii) specific to CD19 or BCMA; and/or

(xiv) specific to any one of ADGRE2, carbonic anhydrase IX (CAIX), CCR1, CCR4, carcinoembryonic antigen (CEA), CD3, CD5, CD7, CD8, CD10, CD20, CD22, CD30,

CD33, CD34, CD38, CD41, CD44, CD44V6, CD49f, CD56, CD70, CD74, CD99, CD123, CD133, CD138, CDS, CLEC12A, an antigen of a cytomegalovirus (CMV) infected cell, epithelial glycoprotein2 (EGP 2), epithelial glycoprotein-40 (EGP-40), epithelial cell adhesion molecule (EpCAM), EGFRvIII, receptor tyrosine-protein kinases erb- B2,3,4, EGFIR, EGFR-VIII, ERBB folate-binding protein (FBP), fetal acetylcholine receptor (AChR), folate receptor-a, Ganglioside G2 (GD2), Ganglioside G3 (GD3), human Epidermal Growth Factor Receptor 2 (HER2), human telomerase reverse transcriptase (hTERT), ICAM-1, Integrin B7, Interleukin-13 receptor subunit alpha-2 (IL-13Rα2), κ-light chain, kinase insert domain receptor (KDR), Lewis A (CA19.9), Lewis Y (LeY), L1 cell adhesion molecule (L1-CAM), LILRB2, melanoma antigen family A 1 (MAGE-A1), MICA/B, Mucin 1 (Muc-1), Mucin 16 (Muc-16), Mesothelin (MSLN), NKCSI, NKG2D ligands, c-Met, cancer-testis antigen NY-ESO-1, oncofetal antigen (h5T4), PRAME, prostate stem cell antigen (PSCA), PRAME prostate-specific membrane antigen (PSMA), tumor- associated glycoprotein 72 (TAG-72), TIM-3, TRBCI, TRBC2, vascular endothelial growth factor R2 (VEGF- R2), Wilms tumor protein (WT-1), and a pathogen antigen.

7. A construct according to Claim 1, further comprising one or more of:

(i) an exogenous promoter that drives expression of the one or more nucleic acid sequences encoding one or more exogenous proteins, in which the exogenous promoter is constitutive, inducible, temporal-, tissue-, and/or cell type- specific;

(ii) a linker sequence inserted between two nucleic acid sequences encoding exogenous proteins;

(iii) an insulator at the 3’ end of LHA/CD38 and an insulator at the 5’ end of RHA/CD38; and

(iv) a polyA signal.

8. A construct according to Claim 7, in which

(i) the exogenous promoter is CMV, EF1α, PGK, CAG, or UBC;

(ii) the linker sequence comprises a 2A sequence encoding a self-cleaving 2A peptide; and/or

(iii) the linker sequence comprises an Internal Ribosome Entry Sequence (IRES).

9. A genomically edited cell that is edited with a construct according to Claim 1, in which the genomically edited cell comprises one or more following phenotypes:

(i) comprising longer telomeres in comparison to its native counterpart cell obtained from peripheral blood, umbilical cord blood, or any other donor tissues;

(ii) $CD38^{-/-}$ CD16;

(iii) $CD38^{-/-}$ IL15; and/or

(iv) $CD38^{-/-}$ CD16 IL15.

10. A genomically edited cell that is edited with a construct according to Claim 1, in which the genomically edited cell that is an iPSC or the iPSC derived effector cell further comprises one or more of:

(i) HLA-I deficiency;

(ii) HLA-II deficiency;

(iii) introduced expression of HLA-G or non-cleavable HLA-G;

(iv) deletion or reduced expression in at least one of B2M, TAP1, TAP2, Tapasin, NLRC5, PD1, LAG3, TIM3, RFXANK, CIITA, RFX5, RFXAP, and any gene in the chromosome 6p21 region; and

(v) introduced or increased expression in at least one of HLA-E, 41BBL, CD4, CD8, CD16, CD47, CD113, CD131, CD137, CD80, PDL1, $A_{2A}R$, CAR, Fc receptor, an engager, and surface triggering receptor for coupling with bi- or multi- specific or universal engagers.

11. A genomically edited cell that is edited with a construct according to Claim 1, in which the iPSC derived effector cell comprises a derivative CD34 cell, a derivative hematopoietic stem and progenitor cell, a derivative hematopoietic multipotent progenitor cell, a derivative T cell progenitor, a derivative NK cell progenitor, a derivative T cell, derivative NKT cell, a derivative NK cell, or a derivative B cell; or in which the iPSC derived effector cell comprises an immune cell having at least one of the following characteristics comprising:

(i) improved persistency and/or survival;

(ii) increased resistance to native immune cells;

(iii) increased cytotoxicity;

(iv) improved tumor penetration;

(v) enhanced or acquired ADCC;

(vi) enhanced ability in migrating, and/or activating or recruiting bystander immune cells, to tumor sites;

(vii) enhanced ability to reduce tumor immunosuppression;

(viii) improved ability in rescuing tumor antigen escape; and

(ix) reduced fratricide in the presence of anti-CD38 antibodies or CD38 CAR,

in comparison to its native counterpart cell obtained from peripheral blood, umbilical cord blood, or other donor tissues.

12. A genomically edited cell according to Claim 11, in which the anti-CD38 antibody is daratumumab, isatuximab, or MOR202, or any of the humanized or Fc modified variants or fragments, functional equivalents and biosimilars thereof.

13. An expression vector that comprises a construct according to any of Claims 1 to 5, 7 or 8.

14. A host cell comprising a construct according to any of Claims 1 to 5, 7 or 8, or an expression vector according to Claim 13.

15. A composition that comprises a construct according to any of Claims 1 to 5, 7 or 8, or an expression vector according to Claim 13, for manufacturing a genomically edited iPSC or derived effector cell differentiated therefrom.

16. A kit comprising a construct according to any of Claims 1 to 5, 7 or 8, an expression vector according to Claim 13, or a host cell according to Claim 14, and one or more medium for iPSC culturing or differentiation.

17. A construct according to any of Claims 1 to 5, 7 or 8, an expression vector according to Claim 13, or a host cell according to Claim 14, for use in the manufacture of a medicament.

18. A method of producing a genomically edited iPSC or effector cell derived therefrom using a construct according to any of Claims 1 to 5, 7 or 8 comprising:

(i) introducing the construct to an iPSC,

(ii) incubating the iPSC for a sufficient period of time to enable targeted integration of one or more nucleic acid sequence encoding one or more exogenous protein at CD38 locus and consequently knocking out the expression of CD38 of the iPSC, thereby obtaining a genomically edited iPSC.

19. A method according to Claim 18, further comprising:

(iii) directing differentiation of the genomically edited iPSC from step (ii) to obtain the effector cell comprising the same targeted integration of one or more nucleic acid sequence encoding one or more exogenous protein at CD38 locus and CD38 knockout as the genomically edited iPSC.

20. A method according to Claim 18, in which the genomically edited iPSC has improved genomic stability than iPSC undergoing integration and knocking out in separate and step-wise editing events.

21. A method according to Claim 18, in which the introducing step further comprises introducing a CRISPR-cas/gNA (guide nucleic acid), a ZFN, a TALEN, or a homing nuclease.