CLAIMS

1. An enucleated erythroid cell engineered to stimulate an immune killer cell, wherein the enucleated erythroid cell comprises:

a first exogenous stimulatory polypeptide, wherein the first exogenous stimulatory polypeptide is a fusion polypeptide comprising a heterologous transmembrane domain from a polypeptide selected from the group consisting of: glycophorin A, glycophorin B, cluster of differentiation (CD) 147, CD44, CD58, intercellular adhesion molecule 4, basal cell adhesion molecule, complement receptor 1, CD99, erythroblast membrane-associated protein (ERMAP), junctional adhesion molecule A, neuroplastin, amphoterin-induced protein 2, Down Syndrome cell adhesion molecule-like 1, small integral membrane protein 1, transferrin receptor, Fas ligand, and Kell; and

a second exogenous stimulatory polypeptide,

wherein the first and second exogenous stimulatory polypeptides are sufficient to stimulate the immune killer cell.

2. The enucleated erythroid cell of claim 1, wherein the immune killer cell is a natural killer (NK) cell or a $CD8^+$ T cell.

3. The enucleated erythroid cell of claim 1, wherein the enucleated erythroid cell further comprises a third exogenous stimulatory polypeptide.

4. The enucleated erythroid cell of claim 1, wherein one or both of the first and second exogenous stimulatory polypeptides comprises a polypeptide selected from the group consisting of: interleukin (IL)-1 (IL-1), IL-2, IL-12, IL-15, IL-15/IL-15 receptor alpha (IL-15/IL-15RA) fusion, IL-18, IL-21, interferon alpha (IFNα), 4-1BBL, poliovirus receptor (PVR), CD48, human leukocyte antigen (HLA)-A, HLA-C, HLA-G, heparan sulfate, HLA-E, immunoglobulin G (IgG), a MHC class I chain-related (MIC) protein, B7-H6, NK cell activating receptor 44 ligand, Nectin2, NK-T-B antigen, activation-induced C-type lectin (AICL), and insulin-like growth factor 1.

5. The enucleated erythroid cell of claim 4, wherein the MIC protein is selected from the group consisting of: MICA, MICB, and UL16-binding proteins (ULBP).

6. The enucleated erythroid cell of claim 1, wherein the first exogenous stimulatory polypeptide comprises IL-15/IL-15RA fusion and the second exogenous stimulatory polypeptide

comprises a polypeptide selected from the group consisting of: IL-1, IL-2, IL-12, IL-18, IL-21, 4-1BBL, IFNα, MICA, MICB, PVR, and CD48.

7. The enucleated erythroid cell of claim 1, wherein the first exogenous stimulatory polypeptide and the second exogenous stimulatory polypeptide are selected from the group consisting of: IL-18 and IL-12, IL-18 and IL-21, IL-12 and 4-1BBL, IL-12 and IL-15/IL-15RA fusion, and 4-1BBL and IL-15/IL-15RA fusion.

8. The enucleated erythroid cell of claim 1, wherein the second exogenous stimulatory polypeptide is a fusion polypeptide.

9. The enucleated erythroid cell of claim 3, wherein the second and the third exogenous stimulatory polypeptides are present as fusion polypeptides.

10. An engineered enucleated erythroid cell comprising a first exogenous stimulatory polypeptide, wherein the first exogenous stimulatory polypeptide is a fusion polypeptide comprising (i) an interleukin-15 (IL-15) polypeptide, or a fragment thereof, (ii) an extracellular portion of an interleukin-15 receptor alpha (IL-15RA) polypeptide, or a fragment thereof, and (iii) a heterologous transmembrane domain from a polypeptide selected from the group consisting of: glycophorin A, glycophorin B, CD147, CD44, CD58, intercellular adhesion molecule 4, basal cell adhesion molecule, complement receptor 1, CD99, ERMAP, junctional adhesion molecule A, neuroplastin, amphoterin-induced protein 2, Down Syndrome cell adhesion molecule-like 1, small integral membrane protein 1, transferrin receptor, Fas ligand, and Kell.

11. The engineered enucleated erythroid cell of claim 10, wherein the IL-15 polypeptide and the extracellular portion of an IL-15RA polypeptide are present as a complex.

12. The engineered enucleated erythroid cell of claim 10, wherein the fusion polypeptide comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 1.

13. The engineered enucleated erythroid cell of claim 10, wherein the fusion polypeptide comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 29 or SEQ ID NO: 37.

14. The engineered enucleated erythroid cell of claim 10, wherein the fusion polypeptide comprises an IL-15 polypeptide, or a fragment thereof, and an IL-15 receptor alpha sushi domain.

15. The engineered enucleated erythroid cell of claim 14, wherein the fusion polypeptide comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 2 or SEQ ID NO: 31.

16. The engineered enucleated erythroid cell of claim 10, further comprising a second exogenous stimulatory polypeptide, wherein the second exogenous stimulatory polypeptide comprises 4-1BBL.

17. The engineered enucleated erythroid cell of claim 16, wherein the second exogenous stimulatory polypeptide comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 41 or SEQ ID NO: 43.

18. The engineered enucleated erythroid cell of claim 10, further comprising a second exogenous stimulatory polypeptide, wherein the second exogenous stimulatory polypeptide comprises IL-12.

19. The engineered enucleated erythroid cell of claim 18, wherein the second exogenous stimulatory polypeptide comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 37 or SEQ ID NO: 55.

20. An engineered enucleated erythroid cell comprising at least a first exogenous stimulatory polypeptide comprising a fusion polypeptide comprising (i) a polypeptide selected from the group consisting of: MICA, MICB, and IGF-1, and (ii) a heterologous transmembrane domain from a polypeptide selected from the group consisting of: glycophorin A, glycophorin B, CD147, CD44, CD58, intercellular adhesion molecule 4, basal cell adhesion molecule, complement receptor 1, CD99, ERMAP, junctional adhesion molecule A, neuroplastin, amphoterin-induced protein 2, Down Syndrome cell adhesion molecule-like 1, small integral membrane protein 1, transferrin receptor, Fas ligand, and Kell.

21. A method of stimulating an immune killer cell, comprising contacting the immune killer cell with the enucleated erythroid cell of claim 1 or the engineered enucleated erythroid cell of claim 10, in an amount effective to stimulate the immune killer cell.

22. The method of claim 21, wherein the enucleated erythroid cell or the engineered enucleated erythroid cell is to be administered to a subject in need of immune killer cell activation.

23. The method of claim 22, wherein the subject has cancer or an infectious disease.

24. The method of claim 23, wherein the cancer is selected from the group consisting of: acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), anal cancer, bile duct cancer, bladder cancer, bone cancer, bowel cancer, brain tumor, breast cancer, cancer of unknown primary, cancer spread to bone, cancer spread to brain, cancer spread to liver, cancer spread to lung, carcinoid, cervical cancer, choriocarcinoma, chronic lymphocytic leukemia (CLL), chronic myeloid leukemia (CML), colon cancer, colorectal cancer, endometrial cancer, eye cancer, gallbladder cancer, gastric cancer, gestational trophoblastic tumor (GTT), hairy cell leukemia, head and neck cancer, Hodgkin's lymphoma, kidney cancer, laryngeal cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma skin cancer, mesothelioma, men's cancer, molar pregnancy, mouth and oropharyngeal cancer, myeloma, nasal and sinus cancers, nasopharyngeal cancer, non-Hodgkin's lymphoma (NHL), oesophageal cancer, ovarian cancer, pancreatic cancer, penile cancer, prostate cancer, rare cancers, rectal cancer, salivary gland cancer, secondary cancers, skin cancer (non-melanoma), soft tissue sarcoma, stomach cancer, testicular cancer, thyroid cancer, unknown primary cancer, uterine cancer, vaginal cancer, and vulvar cancer.

25. Use of an enucleated erythroid cell of claim 1 or an engineered enucleated erythroid cell of claim 10, in the manufacture of a medicament for the treatment of a cancer in a subject, wherein the enucleated erythroid cell or the engineered enucleated erythroid cell is to be administered in an amount effective to treat the cancer in the subject.

26. The use of claim 25, wherein the cancer is selected from the group consisting of: acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), anal cancer, bile duct cancer, bladder cancer, bone cancer, bowel cancer, brain tumor, breast cancer, cancer of unknown primary, cancer spread to bone, cancer spread to brain, cancer spread to liver, cancer spread to lung, carcinoid, cervical cancer, choriocarcinoma, chronic lymphocytic leukemia (CLL), chronic myeloid leukemia (CML), colon cancer, colorectal cancer, endometrial cancer, eye cancer, gallbladder cancer, gastric cancer, gestational trophoblastic tumor (GTT), hairy cell leukemia, head and neck cancer, Hodgkin's lymphoma, kidney cancer, laryngeal cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma skin cancer, mesothelioma, men's cancer, molar pregnancy, mouth and oropharyngeal cancer, myeloma, nasal and sinus cancers, nasopharyngeal cancer, non-Hodgkin's lymphoma (NHL), oesophageal cancer, ovarian cancer, pancreatic cancer, penile cancer, prostate cancer, rare cancers, rectal cancer, salivary gland cancer, secondary cancers, skin cancer (non-melanoma), soft tissue sarcoma, stomach cancer, testicular cancer, thyroid cancer, unknown primary cancer, uterine cancer, vaginal cancer, and vulvar cancer.

27. Use of an enucleated erythroid cell of claim 1 or an engineered enucleated erythroid cell of claim 10, in the manufacture of a medicament for the treatment of an infectious disease in a subject, wherein the enucleated erythroid cell or the engineered enucleated erythroid cell is to be administered in an amount effective to treat the infectious disease in the subject.

28. A method of making the enucleated erythroid cell of claim 1, the method comprising: introducing at least one exogenous nucleic acid into a nucleated erythroid cell precursor, wherein the at least one exogenous nucleic acid encodes the first and second exogenous stimulatory polypeptides; and culturing the nucleated erythroid cell precursor under conditions suitable for enucleation of the nucleated erythroid cell precursor and for production of the first and second exogenous stimulatory polypeptides, thereby making the enucleated erythroid cell.

29. An engineered enucleated erythroid cell, comprising:

i. a first exogenous stimulatory polypeptide, wherein the first exogenous stimulatory polypeptide is a fusion polypeptide comprising an IL-15/IL-15RA fusion comprising an IL-15 polypeptide, or a fragment thereof, linked to the extracellular portion of an IL-15RA polypeptide, or a fragment thereof, by a linker; and

ii. a second exogenous stimulatory polypeptide, wherein the second exogenous stimulatory polypeptide comprises 4-1BBL, or a functional fragment thereof,

wherein one or both of the first exogenous stimulatory polypeptide and the second exogenous stimulatory polypeptide comprise a heterologous transmembrane domain from a polypeptide selected from the group consisting of: glycophorin A, glycophorin B, CD147, CD44, CD58, intercellular adhesion molecule 4, basal cell adhesion molecule, complement receptor 1, CD99, ERMAP, junctional adhesion molecule A, neuroplastin, amphoterin-induced protein 2, Down Syndrome cell adhesion molecule-like 1, small integral membrane protein 1, transferrin receptor, Fas ligand, and Kell.

30. An engineered enucleated erythroid cell, comprising:

i. a first exogenous stimulatory polypeptide, wherein the first exogenous stimulatory polypeptide comprises 4-1BBL, or a fragment thereof; and

ii. a second exogenous stimulatory polypeptide, wherein the second exogenous stimulatory polypeptide comprises IL-12, or a fragment thereof,

wherein one or both of the first exogenous stimulatory polypeptide and the second exogenous stimulatory polypeptide is a fusion polypeptide further comprising a heterologous transmembrane domain from a polypeptide selected from the group consisting of: glycophorin A, glycophorin B,

CD147, CD44, CD58, intercellular adhesion molecule 4, basal cell adhesion molecule, complement receptor 1, CD99, ERMAP, junctional adhesion molecule A, neuroplastin, amphoterin-induced protein 2, Down Syndrome cell adhesion molecule-like 1, small integral membrane protein 1, transferrin receptor, Fas ligand, and Kell.

31. An enucleated erythroid cell of claim 1 or an engineered enucleated erythroid cell of claim 10, for use in therapy.