

CLAIMS

What is claimed is:

1. A genetically modified anti-B7-H4 CAR NK cell, comprising:
 - a recombinantly expressed cytokine;
 - a recombinantly expressed CD16; and
 - a membrane bound recombinantly expressed anti-B7-H4 chimeric antigen receptor (CAR) that comprises in a single polypeptide chain an extracellular binding domain, a hinge domain, a transmembrane domain, and a Fc ϵ RI γ signaling domain and/or a CD3zeta signaling domain.
2. The genetically modified NK cell of claim 1 wherein the NK cell is an NK-92 cell.
3. The genetically modified NK cell of any one of the preceding claims wherein the recombinantly expressed cytokine comprises IL-2 or IL-15.
4. The genetically modified NK cell of any one of the preceding claims wherein the recombinantly expressed cytokine comprises an endoplasmic retention sequence.
5. The genetically modified NK cell of any one of the preceding claims wherein the recombinantly expressed CD16 is a high-affinity CD16 variant having a 158V mutation.
6. The genetically modified NK cell of any one of the preceding claims wherein the extracellular binding domain comprises a scFv.
7. The genetically modified NK cell of any one of the preceding claims wherein the anti-B7-H4 CAR has an amino acid sequence of SEQ ID NO:1.
8. The genetically modified NK cell of any one of the preceding claims wherein recombinantly expressed cytokine, the recombinantly expressed CD16, and the recombinantly expressed CAR are expressed from a tricistronic recombinant nucleic acid.
9. The genetically modified NK cell of any one of the preceding claims wherein the recombinantly expressed cytokine and/or the recombinantly expressed CD16 is expressed from a recombinant nucleic acid that is integrated into the genome of the NK cell.

10. A recombinant nucleic acid, comprising:
 - a first sequence portion encoding a cytokine;
 - a second sequence portion encoding a CD16;
 - a third sequence portion encoding an anti-B7-H4 CAR that comprises in a single polypeptide chain an extracellular binding domain, a hinge domain, a transmembrane domain, and a Fc ϵ RI γ signaling domain and/or a CD3zeta signaling domain; andwherein the first, the second, and the third sequence portions are on the same nucleic acid.
11. The recombinant nucleic acid of claim 10 wherein the nucleic acid is a tricistronic RNA.
12. The recombinant nucleic acid of claim 10 wherein the nucleic acid is a tricistronic DNA.
13. The recombinant nucleic acid of any one of claims 10-12 wherein the cytokine is IL-2 or IL15.
14. The recombinant nucleic acid of any one of claims 10-13 wherein the cytokine comprises an endoplasmic retention sequence.
15. The recombinant nucleic acid of any one of claims 10-14 wherein the CD16 is a high-affinity CD16 variant having a 158V mutation.
16. The recombinant nucleic acid of any one of claims 10-15 wherein the extracellular binding domain comprises a scFv.
17. The recombinant nucleic acid of any one of claims 10-16 wherein the hinge domain and/or the transmembrane domain comprise a CD8 hinge domain and/or a CD28 transmembrane domain.
18. The recombinant nucleic acid of any one of claims 10-17 wherein the anti-B7-H4 CAR has an amino acid sequence of SEQ ID NO:1.
19. A recombinant cell comprising the recombinant nucleic acid of any one of claims 10-18.
20. The recombinant cell of claim 19 wherein the cell is a bacterial cell.
21. The recombinant cell of claim 19 wherein the cell is an autologous NK cell.

22. The recombinant cell of claim 21 wherein the NK cell is an NK-92 cell that is optionally genetically modified.
23. A method of treating cancer in a patient in need thereof, comprising administering to the patient a therapeutically effective amount of any one of the genetically modified NK cells of claims 1-9, thereby treating the cancer.
24. The method of claim 23 further comprising a step of administering at least one additional therapeutic entity selected from the group consisting of a viral cancer vaccine, a bacterial cancer vaccine, a yeast cancer vaccine, N-803, a bi-specific engager, an antibody, a stem cell transplant, a primary NK cell, and a tumor targeted cytokine.
25. The method of claim 23 or 24, wherein the cancer is selected from leukemia, acute lymphocytic leukemia, acute myelocytic leukemia, chronic leukemias, chronic myelocytic (granulocytic) leukemia, chronic lymphocytic leukemia, polycythemia vera, lymphomas, Hodgkin's disease, non-Hodgkin's disease, multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyo sarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma and retinoblastoma.
26. Use of a genetically modified NK cell of any one of claims 1-9 in the treatment of cancer.

27. A method of administering NK cells to an individual, comprising administering a first composition comprising NK cells that express a B7-H4 CAR and a second composition comprising primary NK cells.