

dismal (Stupp et al, 2009, Lancet Oncol, 10(5):459-466; Omuro and DeAngelis, 2013, JAMA, 310(17):1842-1850). Major limitations in the treatment of GBM are the tumor's location within the brain that impedes delivery of cytotoxic agents across the blood-brain barrier (Ashby and Ryken, 2006, Neurosurgical Focus, 20(4):E3), compounded with a strong immunosuppressive environment (Rolle et al., 2012, Adv Exp Med Biol, 746:53-76) and chemo- and radioresistant glioma-initiating cells (Bao et al, 2006, Nature, 444(7120):756-760; Frosina, Mol Canc Res, 2009 7(7):989-999). As a result, novel strategies are continually being tested to improve patient survival, quality of life, and overall outcomes.

[0006] Accordingly, described herein are compositions and methods for more efficacious treatment of cancers in which malignant cells express or over-express IL13R α 2, including brain cancers.

SUMMARY OF THE DISCLOSURE

[0006a] In one aspect, there is provided a chimeric nucleic acid sequence comprising a first nucleic acid sequence encoding an IL13CAR comprising an IL13 ligand domain which binds an IL13 α 2 receptor (SEQ ID No.:44) comprising an amino acid sequence which is at least 90% identical to a sequence selected from the group consisting of SEQ ID NO:26, SEQ ID NO:36, and SEQ ID NO:37, a transmembrane domain, and a cytoplasmic domain comprising a CD3-zeta signaling domain; a second nucleic acid sequence encoding an MGMT polypeptide, selected from the group consisting of SEQ ID NO:33, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, and SEQ ID NO:43; and a third nucleic acid sequence encoding a self-cleaving linker peptide, wherein the third nucleic acid sequence is positioned between the first nucleic acid and second nucleic acid.

[0006b] In another aspect, there is provided a nucleic acid sequence comprising SEQ ID NO:1 (IL13 CAR-P140KMGMT), SEQ ID NO:2 (IL-13(E13Y) CAR-P140KMGMT), SEQ ID NO:3 (IL-13(E13K R109K) CAR-P140KMGMT), or a combination thereof.

[0006c] In another aspect, there is provided a vector comprising the chimeric nucleic acid sequence or the nucleic acid sequence described herein.

[0006d] In another aspect, there is provided a host cell comprising a chimeric nucleic acid sequence or the nucleic acid sequence described herein.

[0006e] In another aspect, there is provided a host cell comprising a first nucleic acid sequence encoding an IL13CAR comprising an IL13 ligand domain which binds an IL13 α 2 receptor (SEQ ID No.:44) comprising an amino acid sequence which is at least 90% identical to a sequence selected from the group consisting of SEQ ID NO:26, SEQ ID NO:36, and SEQ ID NO:37, a transmembrane domain, and a cytoplasmic domain comprising a CD3-zeta

signaling domain; a second nucleic acid sequence encoding an MGMT polypeptide selected from the group consisting of SEQ ID NO:33, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41 and SEQ ID NO:43; and a third nucleic acid sequence encoding a self-cleaving linker peptide, wherein the third nucleic acid sequence is positioned between the first nucleic acid and second nucleic acid.

[0006f] In another aspect, there is provided a pharmaceutical composition comprising the host cell described herein and a carrier.

[0006g] In another aspect, there is provided a pharmaceutical composition comprising the chimeric nucleic acid disclosed herein and a carrier.

[0006h] In another aspect, there is provided a method for producing a mammalian cell which expresses an IL13CAR protein and an MGMT protein comprising: a) introducing into the cell, a first nucleic acid sequence encoding the IL13CAR protein, a second nucleic acid sequence encoding the MGMT protein and a third nucleic acid sequence encoding a self-cleaving linker peptide, wherein the third nucleic acid sequence is positioned between the first nucleic acid and second nucleic acid, wherein the IL13CAR protein comprises an IL13 ligand domain which binds an IL13 α 2 receptor (SEQ ID No.:44) comprising an amino acid sequence which is at least 90% identical to a sequence selected from the group consisting of SEQ ID NO:26, SEQ ID NO:36, and SEQ ID NO:37, a transmembrane domain, and a cytoplasmic domain comprising a CD3-zeta signaling domain; and the MGMT protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO:33, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, and SEQ ID NO:43; and b) maintaining the cell under conditions in which the IL13CAR protein and the MGMT protein are expressed by the cell.

[0006i] In another aspect, there is provided a use of T cells that express proteins encoded by a first nucleic acid sequence encoding an IL13CAR comprising an IL13 ligand domain which binds an IL13 α 2 receptor (SEQ ID No.:44) comprising an amino acid sequence which is at least 90% identical to a sequence selected from the group consisting of SEQ ID NO:26, SEQ ID NO:36, and SEQ ID NO:37, a transmembrane domain, and a cytoplasmic domain comprising a CD3-zeta signaling domain; a second nucleic acid sequence encoding an MGMT polypeptide selected from the group consisting of SEQ ID NO:33, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41 and SEQ ID NO:43 and a third nucleic acid sequence encoding a self-cleaving linker peptide, wherein the third nucleic acid sequence is positioned between the first nucleic acid and second nucleic acid, in the manufacture of a medicament for treating brain cancer, wherein the medicament is to be administered to a subject in need thereof.

[0007] In one embodiment, a chimeric nucleic acid sequence is provided, wherein the chimeric nucleic acid sequence comprises a first nucleic acid which encodes an IL13 chimeric

antigen receptor (IL13CAR) which binds the IL1R α 2 receptor (IL13R α 2) and a second nucleic acid which encodes a drug-resistance polypeptide which is a O⁶-methylguanine DNA methyltransferase (MGMT) protein.

[0008] In one embodiment, the IL13CAR comprises a ligand to the IL13R α 2. In another embodiment, the ligand is IL13. In yet another embodiment, the ligand is a fragment of IL13 which binds the IL13R α 2. In still another embodiment, the ligand is an antibody variable domain or fragment thereof which selectively binds IL13R α 2.

[0009] In one embodiment, the MGMT protein comprises a P140K substitution.

[0010] In one embodiment, the chimeric nucleic acid sequence comprises a first nucleic acid sequence encoding the IL13CAR and a second nucleic acid sequence encoding the MGMT protein.

[0011] In one embodiment, the first nucleic acid sequence encoding the IL13CAR is 5' to the second nucleic acid sequence encoding the MGMT polypeptide. In an alternative embodiment, the first nucleic acid sequence encoding the IL13CAR is 3' to the second nucleic acid sequence encoding the MGMT polypeptide.

[0012] In one embodiment, the first nucleic acid sequence encoding the IL13CAR comprises in a 5' to 3' direction: a nucleic acid sequence encoding an IL13R α 2 ligand domain, a nucleic acid sequence encoding a transmembrane (TM) domain, and a nucleic acid sequence encoding a cytoplasmic domain which comprises a CD3 zeta signaling domain. In another embodiment, the first nucleic acid sequence further comprises a nucleic acid sequence which encodes a hinge region, wherein the hinge region positioned between the

CLAIMS

1. A chimeric nucleic acid sequence comprising
 - a first nucleic acid sequence encoding an IL13CAR comprising
 - an IL13 ligand domain which binds an IL13 α 2 receptor (SEQ ID No.:44);
 - comprising an amino acid sequence which is at least 90% identical to a sequence selected from the group consisting of SEQ ID NO:26, SEQ ID NO:36, and SEQ ID NO:37,
 - a transmembrane domain, and
 - a cytoplasmic domain comprising a CD3-zeta signaling domain;
 - a second nucleic acid sequence encoding an MGMT polypeptide, selected from the group consisting of SEQ ID NO:33, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, and SEQ ID NO:43; and
 - a third nucleic acid sequence encoding a self-cleaving linker peptide, wherein the third nucleic acid sequence is positioned between the first nucleic acid and second nucleic acid.
2. The chimeric nucleic acid sequence according to claim 1, wherein the IL13CAR further comprises a signal peptide.
3. The chimeric nucleic acid sequence according to claim 1 or claim 2, wherein the IL13CAR further comprises a hinge region.
4. The chimeric nucleic acid sequence according to any one of claims 1-3, wherein the cytoplasmic domain further comprises a co-stimulatory domain selected from the group consisting of CD28, 4-1BB (CD137), and OX40 (CD134) .
5. The chimeric nucleic acid sequence according to claim 4, wherein the CD28 co-stimulatory domain is at least 90% identical to SEQ ID NO:29.
6. The chimeric nucleic acid sequence according to any one of claims 1-5, wherein the CD3-zeta signaling domain is at least 90% identical to SEQ ID NO:30.
7. The chimeric nucleic acid sequence according to any one of claims 4-6, wherein the first nucleic acid further comprises a nucleic acid sequence encoding a dipeptide linker which is positioned between the IL13 ligand domain and the hinge domain, between the hinge domain and the transmembrane domain, between the transmembrane domain and the CD28 co-stimulatory domain, or between the CD28 co-stimulatory domain and the CD3-zeta signaling domain.

8. The chimeric nucleic acid sequence according to any one of claims 1-7, further comprising a nucleic acid sequence encoding a linker peptide between the CD3-zeta signaling domain and the self-cleaving peptide.
9. The chimeric nucleic acid sequence according to any one of claims 1-8, selected from the group consisting of SEQ ID NO:1 nucleotides 109 to 1839, SEQ ID NO:2 nucleotides 109 to 1839, and SEQ ID NO:3 nucleotides 109 to 1839.
10. A nucleic acid sequence comprising SEQ ID NO:1 (IL13 CAR-P140KMGMT), SEQ ID NO:2 (IL-13(E13Y) CAR-P140KMGMT), SEQ ID NO:3 (IL-13(E13K R109K) CAR-P140KMGMT), or a combination thereof.
11. A vector comprising the chimeric nucleic acid sequence of any one of claims 1-9 or the nucleic acid sequence of claim 10.
12. The vector according to claim 11, wherein the vector is a viral vector.
13. The vector according to claim 12, wherein the viral vector is a retroviral vector.
14. A host cell comprising a chimeric nucleic acid sequence according to any one of claims 1-9 or the nucleic acid sequence of claim 10.
15. The host cell according to claim 14, wherein the cell is a mammalian cell.
16. The host cell according to claim 14 or 15, wherein the cell is a T cell.
17. The host cell according to any one of claims 14-16, wherein the cell is an autologous cell or a human leukocyte antigen (HLA)- matched cell.
18. The host cell according to any one of claims 14-17, wherein the cell is obtained from one or more individuals with brain cancer.
19. A host cell comprising
 - a first nucleic acid sequence encoding an IL13CAR comprising
 - an IL13 ligand domain which binds an IL13 α 2 receptor (SEQ ID NO:44) comprising an amino acid sequence which is at least 90% identical to a sequence selected from the group consisting of SEQ ID NO:26, SEQ ID NO:36, and SEQ ID NO:37,
 - a transmembrane domain, and
 - a cytoplasmic domain comprising a CD3-zeta signaling domain;

a second nucleic acid sequence encoding an MGMT polypeptide selected from the group consisting of SEQ ID NO:33, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41 and SEQ ID NO:43; and

a third nucleic acid sequence encoding a self-cleaving linker peptide, wherein the third nucleic acid sequence is positioned between the first nucleic acid and second nucleic acid.

20. A pharmaceutical composition comprising the host cell according to any one of claims 14-19 and a carrier.

21. A pharmaceutical composition comprising the chimeric nucleic acid according to any one of claims 1-8 and a carrier.

22. A method for producing a mammalian cell which expresses an IL13CAR protein and an MGMT protein comprising:

a) introducing into the cell,

a first nucleic acid sequence encoding the IL13CAR protein, a second nucleic acid sequence encoding the MGMT protein and a third nucleic acid sequence encoding a self-cleaving linker peptide, wherein the third nucleic acid sequence is positioned between the first nucleic acid and second nucleic acid, wherein

the IL13CAR protein comprises an IL13 ligand domain which binds an IL13 α 2 receptor (SEQ ID NO:44) comprising an amino acid sequence which is at least 90% identical to a sequence selected from the group consisting of SEQ ID NO:26, SEQ ID NO:36, and SEQ ID NO:37, a transmembrane domain, and a cytoplasmic domain comprising a CD3-zeta signaling domain; and

the MGMT protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO:33, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, and SEQ ID NO:43; and

b) maintaining the cell under conditions in which the IL13CAR protein and the MGMT protein are expressed by the cell.

23. The method according to claim 22, wherein the nucleic acid sequence is introduced into the cell using a viral vector.
24. The method of claim 23, wherein the viral vector is selected from the group consisting of a retroviral vector, a lentiviral vector, an adenoviral vector or an adeno-associated viral vector.
25. Use of T cells that express proteins encoded by
- a first nucleic acid sequence encoding an IL13CAR comprising
 - an IL13 ligand domain which binds an IL13 α 2 receptor (SEQ ID NO:44)
 - comprising an amino acid sequence which is at least 90% identical to a sequence selected from the group consisting of SEQ ID NO:26, SEQ ID NO:36, and SEQ ID NO:37,
 - a transmembrane domain, and
 - a cytoplasmic domain comprising a CD3-zeta signaling domain;

a second nucleic acid sequence encoding an MGMT polypeptide selected from the group consisting of SEQ ID NO:33, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41 and SEQ ID NO:43; and

a third nucleic acid sequence encoding a self-cleaving linker peptide, wherein the third nucleic acid sequence is positioned between the first nucleic acid and second nucleic acid, in the manufacture of a medicament for treating brain cancer, wherein the medicament is to be administered to a subject in need thereof.

26. The use according to claim 25, wherein the brain cancer is a high-grade malignant glioma.

27. The use according to claim 25 or 26, wherein the subject is being treated with, has been treated with, or will be treated with a DNA-methylating chemotherapeutic agent.

28. The use according to claim 27, wherein the DNA-methylating chemotherapeutic agent is TMZ.

29. The use according to claim 27 or 28, wherein the DNA-methylating chemotherapeutic agent is to be administered before, concurrently with, or after the medicament.