

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 in an IL13CAR comprising an IL13 ligand domain which binds the IL13 α 2 receptor (SEQ ID NO:44), a transmembrane domain, a cytoplasmic domain comprising a CD3-zeta signaling domain; and 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.

[0006b] In another aspect, there is provided a nucleic acid sequence comprising SEQ ID NO:1 (IL13 CAR-P140KMGM), SEQ ID NO:2 (IL-13(E13Y) CAR-P140KMGM), SEQ ID NO:3 (IL-13(E13K R109K) CAR-P140KMGM), or a combination thereof.

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

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

[0006e] In another aspect, there is provided a host cell comprising a first nucleic acid sequence encoding in an IL13CAR comprising an IL13 ligand domain which binds the IL13 α 2 receptor (SEQ ID NO:44), a transmembrane domain, a cytoplasmic domain comprising a CD3-zeta signaling domain; and 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.

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

[0006g] In another aspect, there is provided a pharmaceutical composition comprising a first nucleic acid sequence encoding in an IL13CAR comprising an IL13 ligand domain which binds the IL13 α 2 receptor (SEQ ID NO:44), a transmembrane domain, a cytoplasmic domain comprising a CD3-zeta signaling domain; and 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.

[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 nucleic acid sequence encoding the IL13CAR protein and the MGMT protein, wherein the IL13CAR protein comprises an IL13 ligand domain which binds the IL13 α 2 receptor (SEQ ID NO:44), a transmembrane domain, and a cytoplasmic domain comprising a CD3-zeta signaling domain; 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 one or more immune cells that express proteins encoded by a first nucleic acid sequence encoding in an N-terminal to C-terminal direction an IL13CAR comprising an IL13 ligand domain which binds the IL13 α 2 receptor (SEQ ID NO:44), a transmembrane domain, and a cytoplasmic domain comprising a CD3-zeta signaling domain; and 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 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 IL13 α 2 receptor (IL13 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 IL13 α 2. In another embodiment, the ligand is IL13. In yet another embodiment, the ligand is a fragment of IL13 which binds the IL13 α 2. In still another embodiment, the ligand is an antibody variable domain or fragment thereof which selectively binds IL13 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

[0031] In one embodiment, the chimeric nucleic acid sequence comprises a nucleotide sequence which encodes the protein of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:45, SEQ ID NO:46 or SEQ ID NO:47.

[0032] In one embodiment, the IL13R α 2 receptor ligand is a variant of IL-13 or fragment thereof which binds the IL13R α 2 with about 5-fold, 10-fold, or 100-fold less affinity than wild-type IL13 (SEQ ID NO:26). In an alternative embodiment, the IL13R α 2 receptor ligand is a variant of IL13 or fragment thereof which binds the IL13R α 2 with about 5-fold, 10-fold, or 100-fold higher affinity than wild-type IL13 (SEQ ID NO:26).

[0033] In one embodiment, the IL13R α 2 receptor ligand is not identical to wild-type IL-13. In another embodiment, IL13R α 2 receptor ligand is not identical to SEQ ID NO:26.

[0034] In one embodiment, P140KMGMT protein is effective in increasing in vitro and/or in vivo viability of a cell expressing the drug-resistance polypeptide when a cell transfected with an IL13-CAR-T is treated with a chemotherapeutic agent as compared to the cell treated with the chemotherapeutic but not expressing the drug-resistance polypeptide.

[0035] In one embodiment, the IL13R α 2 receptor ligand is not identical to wild-type IL-13. In another embodiment, IL13R α 2 receptor ligand is not identical to SEQ ID NO:26.

[0036] In another embodiment, a vector comprising a nucleic acid sequence which encodes the IL13CAR and MGMT protein as described herein is provided.

[0037] In one embodiment, the vector comprises a monocistronic nucleic acid sequence which encodes an IL13CAR, a self-cleaving peptide, and a MGMT protein as described herein. In another embodiment, the self-cleaving peptide comprises a 2A peptide.

[0038] In an alternative embodiment, the vector comprises a polycistronic chimeric nucleic acid sequence which encodes an IL13CAR and an MGMT protein. In another embodiment the polycistronic chimeric nucleic acid sequence which encodes the IL13CAR and the MGMT protein further comprises an internal ribosome entry site (IRES) positioned between the nucleic acid sequence encoding the IL13CAR and the nucleic acid sequence encoding the MGMT protein. In still another embodiment, the polycistronic chimeric nucleic acid sequence which encodes the IL13CAR and the MGMT protein further comprises a promoter positioned between the nucleic acid sequence encoding the IL13CAR and the nucleic acid sequence encoding the MGMT protein.

[0039] In one embodiment, the vector is a bacterial plasmid vector. In another embodiment, the vector is an expression vector.

[0040] In one embodiment, the vector is a viral vector. In another embodiment, the viral vector is selected from the group consisting of a retroviral vector, a lentiviral vector, an adenoviral vector and an adeno-associated viral vector.

[0041] In another embodiment, a cell transfected with a vector comprising a chimeric nucleic acid sequence which encodes a chimeric antigen receptor (CAR) and a drug-resistance polypeptide as described herein is provided.

[0042] In one embodiment, the cell is selected from the group consisting of a T-cell, an NK-cell, and NKT-cell.

[0043] In another, a recombinant polypeptide is provided comprising in an N-terminal to C-terminal direction, a ligand which binds to a tumor antigen, a transmembrane domain, and a cytoplasmic signaling domain as described herein.

[0044] In one embodiment, the recombinant polypeptide comprising, in an N-terminal to C-terminal direction, a ligand which binds to a tumor antigen, a transmembrane domain, and a cytoplasmic signaling domain, further comprises a self-cleaving peptide positioned between the CAR and drug resistance polypeptide. In another embodiment, the drug resistance polypeptide is N-terminal to the CAR. In still another embodiment, the drug resistance polypeptide is C-terminal to the CAR.

[0045] In another embodiment, a recombinant polypeptide is provided comprising a modified MGMT polypeptide which increases viability of a cell exposed to TMZ, wherein the cell is genetically modified to express a CAR as described herein and wherein the cell is administered to a patient diagnosed with a brain cancer.

[0046] In another embodiment, a composition comprising a first nucleic acid which encodes a CAR as described herein and a second nucleic acid which encodes an MGMT protein as described herein is provided.

[0047] In one embodiment, the first nucleic acid encodes a CAR protein which comprises an IL13 ligand domain as described herein, a TM domain as described herein, and a cytoplasmic domain comprising a CD3-zeta signaling domain as described herein. In another embodiment, the first nucleic acid further encodes a signal sequence which is upstream of the IL13 ligand binding domain of the CAR protein. In still another embodiment, the first nucleic acid further encodes a hinge region as described herein wherein the hinge region is positioned between the IL13 ligand domain and the TM domain of the CAR protein. In yet another embodiment, the first nucleic acid further encodes a CD28 co-stimulatory domain which is positioned between the TM domain and the CD3-zeta signaling domain. In still another embodiment, the first nucleic acid further encodes an additional co-stimulatory domain. In another embodiment, the first nucleic acid further comprises a Kozak sequence upstream of the nucleic acid encoding the CAR protein.

[0048] In one embodiment, the second nucleic acid encodes an MGMT protein which has 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.

[0049] In one embodiment, the MGMT protein does not comprise SEQ ID NO:49.

[0050] In one embodiment, the composition comprises a chimeric nucleic acid which comprises the first nucleic acid and the second nucleic acid.

[0051] In one embodiment, the chimeric nucleic acid further comprises a nucleic acid which encodes a self-cleaving linker peptide as described herein, wherein the nucleic acid encoding the self-cleaving linker peptide is positioned between the first nucleic acid and the second nucleic acid.

[0052] In one embodiment, the chimeric nucleic acid further comprises an internal ribosome entry site (IRES) as described herein, wherein the IRES is positioned between the first nucleic acid and the second nucleic acid.

[0053] In one embodiment, the chimeric nucleic acid is a bicistronic construct which comprises a first promoter upstream of the first nucleic acid encoding the CAR protein and a second promoter upstream of the second nucleic acid encoding the MGMT protein.

[0054] In one embodiment, the composition comprises a first vector which comprises the first nucleic acid which encodes the CAR protein and a second vector which comprises the second nucleic acid which encodes the MGMT protein. In another embodiment, the first and second vectors are each a plasmid or expression vector. In yet another embodiment, the first and second vectors are each a retroviral particle.

[0055] In another embodiment, a host cell comprising a first nucleic acid which encodes a CAR as described herein and a second nucleic acid which encodes an MGMT protein as described herein. In one embodiment, the first nucleic acid encodes a CAR protein which comprises an IL13 ligand domain as described herein, a TM domain as described herein, and a cytoplasmic domain comprising a CD3-zeta signaling domain as described herein. In another embodiment, the first nucleic acid further encodes a signal sequence which is upstream of the IL13 ligand binding domain of the CAR protein. In still another embodiment, the first nucleic acid further encodes a hinge region as described herein wherein the hinge region is positioned between the IL13 ligand domain and the TM domain of the CAR protein. In yet another embodiment, the first nucleic acid further encodes a CD28 co-stimulatory domain which is positioned between the TM domain and the CD3-zeta signaling domain. In still another embodiment, the first nucleic acid further encodes an additional co-stimulatory domain. In another embodiment, the first nucleic acid further comprises a Kozak sequence upstream of the nucleic acid encoding the CAR protein.

[0056] In one embodiment, the second nucleic acid encodes an MGMT protein which has 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. In another embodiment, the MGMT protein is not SEQ ID NO:49.

[0057] In one embodiment, the host cell comprises a chimeric nucleic acid which comprises the first nucleic acid and the second nucleic acid.

[0058] In one embodiment, the chimeric nucleic acid further comprises a nucleic acid which encodes a self-cleaving linker peptide as described herein, wherein the nucleic acid encoding the self-cleaving linker peptide is positioned between the first nucleic acid and the second nucleic acid.

[0059] In one embodiment, the chimeric nucleic acid further comprises an internal ribosome entry site (IRES) as described herein, wherein the IRES is positioned between the first nucleic acid and the second nucleic acid.

[0060] In one embodiment, the chimeric nucleic acid is a dicistronic construct which comprises a first promoter upstream of the first nucleic acid encoding the CAR protein and a second promoter upstream of the second nucleic acid encoding the MGMT protein.

[0061] In one embodiment, the host cell comprises a first vector which comprises the first nucleic acid which encodes the CAR protein and a second vector which comprises the second nucleic acid which encodes the MGMT protein. In another embodiment, the first and second vectors are each a plasmid or expression vector as described herein. In yet another embodiment, the first and second vectors are each a retroviral particle as described herein.

[0062] In another embodiment, a composition comprising a recombinant polypeptide is provided, wherein the recombinant polypeptide comprises in an N-terminal to C-terminal direction, a signal sequence as described herein, an IL13 ligand as described herein, a transmembrane domain as described herein, a cytoplasmic signaling domain as described herein, an MGMT protein as described herein and a pharmaceutically acceptable excipient. In another embodiment, the recombinant polypeptide further comprises a hinge domain as described herein wherein the hinge domain is positioned between the IL13 ligand domain and the transmembrane domain. In still another embodiment, the recombinant polypeptide further comprises a self-cleaving peptide as described herein wherein the self-cleaving peptide is positioned between the cytoplasmic signaling domain and the MGMT protein.

[0063] In one embodiment, the composition is a pharmaceutical composition.

[0064] In another embodiment, a method for treating a subject diagnosed with a cancer is provided.

[0065] In one embodiment, the method comprises obtaining a cell from the subject, transducing the cell with one or more nucleic acids which encode an IL13CAR as described herein and an MGMT protein as described herein, maintaining the cell under conditions in which the nucleic acids are expressed by the cell, and administering to the patient a therapeutically effective number of the cells expressing the IL13CAR and MGMT proteins.

[0066] In one embodiment, the introducing into the cell comprises using a first vector comprising a nucleic acid encoding the IL13CAR and a second vector comprising a nucleic acid encoding the MGMT protein. In another embodiment, the introducing into the cell comprises using a vector that comprises a nucleic acid encoding the IL13CAR and the MGMT protein.

[0067] In one embodiment, the cell is transduced with a vector comprising an IL13CAR-P140KMGMT chimeric construct as described herein.

[0068] In one embodiment the subject is a mammal. In another embodiment, the mammal is a primate, a human, or a mouse.

[0069] In one embodiment, the one or more nucleic acids are introduced into the cell using a viral vector selected from the group consisting of a retroviral vector, a lentiviral vector, an adenoviral vector or a combination thereof.

[0070] In one embodiment, the cell is a T cell.

[0071] In one embodiment, the T cells are obtained using plasmapheresis.

[0072] In one embodiment, the subject has been diagnosed with a cancer selected from the group consisting of brain, breast, pancreatic, head and neck, ovarian and colorectal. In another embodiment, the cancer has metastasized.

[0073] In one embodiment, the subject has been diagnosed with a high-grade malignant glioma. In another embodiment, the subject has been diagnosed with glioblastoma multiforme (GMB), an anaplastic astrocytoma or a pediatric glioma.

[0074] In one embodiment, the brain cancer is a glioblastoma. In another embodiment, the brain cancer is a high-grade astrocytoma.

[0075] In one embodiment, the breast cancer is a basal-like breast cancer.

[0076] In one embodiment, the method further comprises treating the subject with one or more chemotherapeutic agents. In another embodiment, the method comprises treating the subject with temozolomide (TMZ).

[0077] In one embodiment, the one or more chemotherapeutic agents is administered to the subject before, during, and/or after administration of a dose of the modified cells.

[0078] In one embodiment, the administering is intracranial, intramedullary, intradermally, subcutaneously, topically, or intravenously.

[0079] In another embodiment, a method for producing a cell which expresses an IL13CAR-P140KMGMT construct as described herein is provided comprising introducing into the cell a nucleic acid sequence encoding the IL13CAR-P140KMGMT chimeric protein, maintaining the cell under conditions in which the IL13CAR-P140KMGMT chimeric protein is expressed by the cell.

[0080] In one embodiment the cell is a mammalian cell. In another embodiment, the mammalian cell is a human cell, a primate cell or a mouse cell.

[0081] In one embodiment, the cell is a T cell. In another embodiment, the cell is an autologous cell or a human leukocyte antigen (HLA)-matched cell.

[0082] In one embodiment, the cell is obtained from one or more subjects diagnosed with a brain cancer or malignancy.

[0083] In another embodiment, a population of cells comprising the IL13CAR-P140KMGMT construct is provided. In another embodiment, at least about 50%, 60%, 70%, 80%, 90% or 95% of cells in the population of cells express the IL13CAR-P140KMGMT construct is provided.

[0084] In one embodiment, the disclosure is directed to an (one or more) isolated nucleic acid sequence encoding (having; comprising; consisting essentially of; consisting of) a chimeric antigen receptor (CAR) comprising (consisting essentially of; consisting of) a T cell receptor that expresses one or more ligands (e.g., an antibody) to one or tumor antigens of a brain cancer. In some embodiments, the CAR further expresses one or more additional agents useful for treating a brain cancer.

[0085] In another embodiment, the disclosure is directed to an expression construct comprising (consisting essentially of; consisting of) one or more nucleic acid sequences encoding a CAR comprising a T cell receptor that expresses one or more ligands (e.g., an antibody) of one or tumor antigens of a brain cancer (e.g., a cancer antigen-binding domain). In some embodiments, the CAR further expresses one or more additional agents useful for treating a brain cancer.

[0086] In another embodiment, the disclosure is directed to a host cell comprising (consisting essentially of; consisting of) an expression construct comprising one or more nucleic acid sequences encoding a CAR comprising a T cell receptor that expresses one or more ligands (e.g., an antibody) of one or tumor antigens of a brain cancer. In some embodiments, the CAR further expresses one or more additional agents useful for treating a brain cancer.

[0087] In another embodiment, the disclosure is directed to a method of producing a cell which expresses a CAR comprising (consisting essentially of; consisting of) a T cell receptor comprising one or more ligands (e.g., an antibody) of one or tumor antigens of a brain cancer. In a particular embodiment, the CAR further comprises one or more additional agents useful for treating a brain cancer.

[0088] In another embodiment, the disclosure is directed to a CAR polypeptide comprising (having; consisting essentially of; consisting of) a T cell receptor comprising one or more ligands (e.g., an antibody) of one or tumor antigens of a brain cancer. In a particular embodiment, the CAR polypeptide further comprises one or more additional agents useful for treating a brain cancer.

[0089] In another embodiment, the disclosure is directed to a method of treating brain cancer in an individual in need thereof comprising (consisting essentially of; consisting of) administering one or more T cells that express a CAR comprising a T cell receptor that expresses one or more ligands (e.g., an antibody) of one or tumor antigens of a brain cancer. In some embodiments, the CAR further expresses one or more additional agents useful for treating a brain cancer.

[0090] The disclosure is also directed to pharmaceutical compositions comprising compositions provided herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0091] The foregoing will be apparent from the following more particular description of example embodiments of the invention, as illustrated in the accompanying drawings in which like reference characters refer to the same parts throughout the different views. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating embodiments of the present invention.

[0092] FIG. 1A provides a schematic of an IL13E13K.R109K CAR nucleic acid construct.

[0093] FIG. 1B provides a plasmid map of a pMFG host plasmid comprising an IL13E13K.R109K CAR.

[0094] FIG. 2A provides a schematic of an IL13.E13KR109K CAR-2A-P140KMGMT.

[0095] FIG. 2B: provides a plasmid map of a pMFG host plasmid comprising an IL-13-CAR-2A-P140KMGMT.

[0096] FIGS. 3A-3C illustrate FACS analysis of PG13 cells transduced with a virus containing an IL13CAR construct and a IL13CAR-2A-P140KMGMT construct, prior to enrichment (FIG. 3A) and after enrichment (FIG. 3B). FIG. 3C illustrates western blot analysis of cell lysates.

[0097] FIG. 4 provides a graph showing viability of T cells transduced with a retrovirus comprising an IL13CAR-2A-P140KMGMT construct and exposed to TMZ.

[0098] FIGS. 5A and 5B illustrate secretion of IL2 (FIG. 5A) and IFN γ (FIG. 5B) in cells transfected with an IL13CAR-2A-MGMT construct as described herein.

[0099] FIG. 6 illustrates viability of mice harboring a tumor and administered chimeric constructs and/or a chemotherapeutic agent as described herein.

[0100] FIGS. 7A-7B provide the chimeric nucleic acid sequence (FIG. 7A; SEQ ID NO:1) and amino acid sequence (FIG. 7B; SEQ ID NO:4) of an IL13CAR-P140KMGMT construct.

[0101] FIGS. 8A-8B provide the chimeric nucleic acid sequence (FIG. 8A; SEQ ID NO:2) and amino acid sequence (FIG. 8B; SEQ ID NO:5) of an IL13(E13Y)CAR-P140KMGMT construct.

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.

23. A pharmaceutical composition comprising the host cell according to any one of claims 17-21.

24. A pharmaceutical composition comprising

a first nucleic acid sequence encoding in an IL13CAR comprising

an IL13 ligand domain which binds the IL13 α 2 receptor (SEQ ID NO:44),

a transmembrane domain,

a cytoplasmic domain comprising a CD3-zeta signaling domain; and

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.

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

a) introducing into the cell a nucleic acid sequence encoding the IL13CAR protein and the MGMT protein, wherein the IL13CAR protein comprises an IL13 ligand domain which binds the IL13 α 2 receptor (SEQ ID NO:44), a transmembrane domain, and a cytoplasmic domain comprising a CD3-zeta signaling domain; and

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

26. The method according to claim 25, wherein the nucleic acid sequence is introduced into the cell using a viral vector.

27. The method of claim 26, 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.

28. Use of one or more immune cells that express proteins encoded by

a first nucleic acid sequence encoding in an N-terminal to C-terminal direction an IL13CAR comprising

an IL13 ligand domain which binds the IL13 α 2 receptor (SEQ ID NO:44),

a transmembrane domain, and

a cytoplasmic domain comprising a CD3-zeta signaling domain; and

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 in the manufacture of a medicament for treating brain cancer, wherein the medicament is to be administered to a subject in need thereof.

29. The use according to claim 28, wherein the brain cancer is a high-grade malignant glioma.

30. The use according to claim 28 or 29, wherein the subject is being treated with, has been treated with, or will be treated with a DNA-methylating chemotherapeutic agent.

31. The use according to claim 30, wherein the DNA-methylating chemotherapeutic agent is TMZ.

32. The use according to claim 30 or 31, wherein the DNA-methylating chemotherapeutic agent is to be administered before, concurrently with, or after the medicament.