Treatment of Myxoid/Round Cell Liposarcoma Patients

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of the filing dates of U.S. Provisional Application No. 63/279,797, filed November 16, 2021, the entire contents of which are incorporated by reference herein.

BACKGROUND OF DISCLOSURE

Sarcomas, including soft tissue sarcoma (STS), are rare group of malignancies of mesenchymal origin accounting for about 20% of all pediatric and about 1% of adult solid tumors (Abaricia & Hirbe, 2018; Hui, 2016). Liposarcoma (LPS), the most common type of STS, arises from adipose tissue and is a malignant neoplasm affecting fat differentiation. Myxoid/round cell liposarcoma (MRCLS) accounts for approximately 30% of LPS and tends to occur in slightly younger age group with peak incidence in the fourth decade of life.

Standard treatment for MRCLS consists of surgical resection for localized, primary disease. MRCLS is known for its sensitivity to radiation therapy and cytotoxic chemotherapy in comparison to the other LPS subtypes. However, despite the appropriate local treatment, about 40% of patients do relapse. Still, treatment options for patients with MRCLS continue to be poor and new therapy options are required for this high-medical need indication.

SUMMARY

The present disclosure is based, at least in part, on the unexpected discovery that Glypican 3 (GPC3) can serve as a diagnostic biomarker and treatment target for MRCLS. Upon surveying the expression profile of GPC3 in solid tumor biopsies from cancer patients to better understand GPC3 positive tumor prevalence and for indication prioritization for an anti-GPC3 therapy, a sub-group of LPS, namely MRCLS, is identified as a relatively high GPC3 expressing patient population, utilizing new scoring rules to quantify the level of GPC3 expression accurately and reliably across multiple tissue samples and pathologists doing the scoring. This finding is unexpected given the complexity of staining patterns observed in various tumor tissue sections stained with an anti-GPC3 antibody (GC33) and suggests MRCLS to be prioritized and/or selected for treating with an anti-GPC3 therapeutic agent.

Accordingly, the present disclosure features, in some aspects, use of an anti-Glypican-3 (GPC3) therapeutic agent in the manufacture of a medicament for treating a patient diagnosed with myxoid/round cell liposarcoma. In some examples, the present disclosure refers to a method of treating a patient diagnosed with myxoid/round cell liposarcoma, the method comprising administering an anti-Glypican-3 (GPC3) therapeutic agent to the patient. In some examples, the patient is selected for treatment by diagnosing myxoid/round cell liposarcoma. In some examples, the myxoid/round cell liposarcoma expresses GPC3.

In some examples, the patient diagnosed with the myxoid/round cell liposarcoma is selected by immunostaining, for example, by immunohistochemistry (IHC) staining. In some examples, the patient diagnosed with the myxoid/round cell liposarcoma is selected if the patient has a cytoplasmic/membranous H-score of greater than 30.

In some examples, the patient may be diagnosed by a process comprising:

(a) obtaining a tissue section from a tumor biopsy sample, the section having a thickness between 3 µm and 15 µm,

(b) immunostaining preferably by IHC with an antibody that specifically binds to GPC3, more specifically using the antibody GC33,

(c) determining the cytoplasmic/membranous H-score, and

(d) selecting patients with an H-score of greater than 30 for the treatment.

In some examples, the selection may comprise immunostaining, for example, immunohistochemical staining of GPC3 in a tumor sample from the patient. The GPC3 expression level may be determined and compared to a predetermined threshold level of GPC3 expression. The patient is selected for treatment in case the patient has a GPC3 expression level equal or higher to the predetermined threshold level.

In some examples, the therapeutic agent comprises an anti-GPC3 binding domain, for example, an anti-GPC3 antibody, e.g., a full-length antibody or functional fragment thereof retaining binding to GPC3. In some examples, the therapeutic agent may comprise an anti-GPC3 antibody, an anti-GPC3 antibody-drug conjugate, an anti-GPC3 antibody-radionuclide conjugate, or a fusion protein of an anti-GPC3 antibody or antibody derivative binding to GPC3 with an anti-CD3 binding domain or an immunostimulatory polypeptide. In some examples, the therapeutic agent comprises genetically engineered hematopoietic cells expressing an anti-GPC3 chimeric receptor polypeptide (CAR), which may comprise:

(a) an extracellular binding domain binding to GPC3;

(b) a transmembrane domain; and

(c) a cytoplasmic signaling domain.

In some examples, the hematopoietic cell may further exogenously expresses a gene that improves viability and/or functionality of the hematopoietic cell in the solid tumor microenvironment. In some examples, the hematopoietic cells may have an improved glucose uptake activity as relative to a wild-type hematopoietic cell of the same type, whereas the hematopoietic cell exogenously expresses a glucose importation polypeptide. In some examples, the glucose importation polypeptide is a glucose transporter (GLUT) or a sodium-glucose cotransporter (SGLT). Examples include, but are not limited to, GLUT1, GLUT3, GLUT1 S226D, SGLT1, SGLT2, GLUT8, GLUT8 L12A L13A, GLUT11, GLUT7, and GLUT4.

In some examples, the hematopoietic cells may have a modulated Krebs cycle as relative to a wild-type hematopoietic cell of the same type, whereas the hematopoietic cell exogenously expresses a Krebs cycle modulating polypeptide. In some examples, the Krebs cycle modulating factor is an enzyme that catalyzes a reaction in the Krebs cycle. Examples include, but are not limited to, isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), or phosphoglycerate dehydrogenase (PHGDH). In some examples, the Krebs cycle modulating factor is an enzyme that uses a Krebs cycle metabolite as a substrate, for example, glutamic-oxaloacetic transaminase (GOT) or phosphoenolpyruvate carboxykinase 1 (PCK1). Alternatively, the Krebs cycle modulating factor may be an enzyme that converts a precursor to a Krebs cycle metabolite. Examples include, but are not limited to, phosphoserine aminotransferase (PSAT1), glutamate dehydrogenase (GDH1), glutamate-pyruvate transaminase 1 (GPT1), or glutaminase (GLS).

In some examples, the hematopoietic cells may have enhanced intracellular lactate concentrations relative to a wild-type hematopoietic cell of the same type, whereas the hematopoietic cell exogenously expresses a lactate-modulating polypeptide. In some examples, the lactate modulation polypeptide is monocarboxylate transporter (MCT), for example, MCT1, MCT2, or MCT4. In other examples, the lactate modulating polypeptide is an enzyme involved in lactate synthesis, *e.g.*, lactate dehydrogenase A (LDHA). Alternatively, the lactate modulating polypeptide is a polypeptide that inhibits a pathway that competes for lactate-synthesis substrates, for example, pyruvate dehydrogenase kinase 1 (PDK1).

In any of the methods disclosed herein, the extracellular antigen binding domain is a single chain antibody fragment (scFv) that binds to a GPC3. In some examples, the scFv is derived from the GC33 antibody. In one example, the scFv may comprise (e.g., consists of) the

sequence of SEQ ID NO: 2. In some examples, the anti-GPC3 CAR polypeptide may comprise a CD28 co-stimulatory domain, in combination with a CD28 transmembrane domain, a CD28 hinge domain, or a combination thereof (e.g., SEQ ID NO: 4). Alternatively, the anti-GPC3 CAR polypeptide may comprise a 4-1BB co-stimulatory domain (e.g., SEQ ID NO: 5), in combination with a CD8 transmembrane domain, a CD8 hinge domain, or a combination thereof (e.g., SEQ ID NO: 3). Alternatively or in addition, the anti-GPC3 CAR polypeptide may comprise a cytoplasmic signaling domain of (c), which may be a cytoplasmic domain of CD3ζ, preferably SEQ ID NO: 7, or FcεR1γ. In specific examples, the anti-GPC3 CAR may comprise the amino acid sequence of SEQ ID NO: 8 or SEQ ID NO: 9.

Any of the hematopoietic cells disclosed herein may be natural killer (NK) cells, macrophages, neutrophils, eosinophils, or T cells. In some examples, the hematopoietic cells are T cells. In some examples, the expression of an endogenous T cell receptor, an endogenous major histocompatibility complex, an endogenous beta-2-microglobulin, or a combination thereof has been inhibited or eliminated in such T cells. In some examples, the hematopoietic cells may be derived from peripheral blood mononuclear cells (PBMC), hematopoietic stem cells (HSCs), or inducible pluripotent stem cells (iPSCs). In some examples, the hematopoietic cells are autologous to the patient. In other examples, the hematopoietic cells are allogeneic to the patient.

In some examples, the hematopoietic cells may comprise a nucleic acid or a set of nucleic acids, for example, a DNA molecule or a set of DNA molecules, which collectively comprises: (a) a first nucleotide sequence encoding the glucose importation polypeptide, the Krebs cycle modulating polypeptide and/or the lactate-modulating polypeptide; and (b) a second nucleotide sequence encoding the chimeric antigen receptor polypeptide. In some examples, the hematopoietic cells may comprise the nucleic acid, which comprises both the first nucleotide sequence and the second nucleotide sequence. In some examples, the nucleic acid may comprise a third nucleotide sequence located between the first nucleotide sequence and the second nucleotide sequence. The third nucleotide sequence may encode a ribosomal skipping site, an internal ribosome entry site (IRES), or a second promoter. In some examples, the third nucleotide sequence encodes a ribosomal skipping site, for example, a P2A peptide. In some examples, the nucleic acid or the nucleic acid set may be comprised within a vector or a set of vectors, for example, an expression vector or a set of expression vectors. In specific

examples, the vector or set of vectors comprises one or more viral vectors, e.g., a lentiviral vector or retroviral vector.

In some examples, at least about 5 x 10^4 anti-GPC3- CAR T cells per kg are administered to the patient. In some examples, about 5 x 10^4 to about 1 x 10^{12} anti-GPC3-CAR T cells/kg are administered to the patient.

In some examples, the therapeutic agent may comprise an anti-GPC3 targeted polypeptide or polypeptide fusion, for example, an anti-GPC3 antibody, an anti-GPC3 bi- or multiple specific protein or an anti-GPC3 antibody-drug-conjugate.

In some examples, the administration of the anti-GPC3 therapeutic agent is effective in achieving stable disease according to RECIST (*e.g.*, RECIST 1.1) as measured by computerized tomography (CT) scan. Alternatively, or in addition, the administration of the anti-GPC3 therapeutic agent is achieving an objective response according to RECIST (*e.g.*, RECIST 1.1) as measured by computerized tomography (CT) scan.

Any of the methods disclosed herein may further comprise administering at least one immunomodulatory agent to the patient in parallel or sequential to the therapeutic agent. In some examples, the immunomodulatory agent can be an immune checkpoint inhibitor or an immunostimulatory cytokine. Alternatively, or in addition, the method may further comprise subjecting the patient to a lymphocyte reduction treatment, which may comprise cyclophosphamide, fludarabine, or a combination thereof.

Also within the scope of the present disclosure are, as an example, anti-GPC3 therapeutic agents (e.g., those disclosed herein) for use in treating a patient diagnosed with myxoid/round cell liposarcoma, as well as use of any of the anti-GPC3 therapeutic agents disclosed herein for manufacturing a medicament for use in treating a patient diagnosed with myxoid/round cell liposarcoma.

Further, the present disclosure also describes, as an example, a method for diagnosing a patient having myxoid/round cell liposarcoma or for selecting a patient for treatment of the disease. The method may comprise:

(a) obtaining a tissue section from a tumor biopsy sample obtained from a patient candidate, the section having a thickness between 3 µm and 15 µm,

(b) immunostaining preferably by IHC with an antibody that specifically binds to GPC3, more specifically using the antibody GC33,

(c) determining the cytoplasmic/membranous H-score, and

(d) diagnosing the patient candidate as having or suspected of having myxoid/round cell liposarcoma based on the H-score.

In some examples, an H-score of greater than 30 is indicative of disease occurrence. In some examples, a patient having an H-score greater than 30 can be selected for treatment.

The details of one or more examples of the invention are set forth in the description below. Other features or advantages of the present invention will be apparent from the following drawings and detailed description of several examples, and also from the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1. shows images of an FFPE tissue section of Myxoid/round cell liposarcoma stained by IHC with the antibody GC33, with the box indicating a particular field viewed at A. 1x, B. 4x, and C. 20x magnification. Cells within each field of view were scored as 1+ for weak staining, 2+ for moderate staining and 3+ for strong staining; examples of 1+, 2+ and 3+ are boxed in C.

Fig. 2. shows images of IHC staining by the antibody GC33 in healthy FFPE tissue sections of A. Breast), B. heart, C. stomach and, D. kidney at 20x magnification.

Fig. 3. shows images of IHC staining by the antibody GC33 in various cancer types namely in FFPE tissue sections of A. smooth muscle (negative control; H-score - 0), B. hepatocellular carcinoma (H-score – 280), C. non-small cell lung carcinoma (H-score – 260), D. Merkel cell carcinoma (H-score – 130), and E. liposarcoma (H-score – 120) at 20x magnification. All staining fields show both membranous and cytoplasmic GPC3 staining.

Fig. 4. shows images of IHC staining by the antibody GC33 in liposarcoma subtypes namely in FFPE tissue sections of A. myxoid/round cell liposarcoma (H-score – 140), B. pleiomorphic liposarcoma (H-score – 0), C. well differentiated liposarcoma (H-score – 0), and D. mixed liposarcoma (H-score – 0), at 20x magnification.

DETAILED DESCRIPTION OF DISCLOSURE

ABBREVIATIONS

Throughout the detailed description and examples of the invention the following abbreviations are used:

CAR	Chimeric antigen receptor

WHAT IS CLAIMED IS:

1. Use of an anti-Glypican-3 (GPC3) therapeutic agent in the manufacture of a medicament for treating a patient diagnosed with myxoid/round cell liposarcoma.

2. The use of claim 1, wherein the patient had been selected for treatment by diagnosing myxoid/round cell liposarcoma.

3. The use of claim 1 or claim 2, wherein the myxoid/round cell liposarcoma expresses GPC3.

4. The use of any one of claims 1 to 3, wherein the patient diagnosed with the myxoid/round cell liposarcoma had been selected by immunostaining, optionally by immunohistochemistry (IHC) staining, preferably with a cytoplasmic/membranous H-score of greater than 30.

5. The use of any one of claims 1 to 4, wherein the patient had been diagnosed by a process comprising:

(a) immunostaining, preferably by IHC with an antibody that specifically binds to GPC3, more specifically using the antibody GC33, a tissue section previously obtained from a tumor biopsy sample, the section having a thickness between 3 µm and 15 µm ,

(b) determining the cytoplasmic/membranous H-score, and

(c) selecting patients with an H-score of greater than 30 for the treatment.

6. The use of any one of claims 1 to 5, wherein the patient diagnosed with the myxoid/round cell liposarcoma had been selected by immunostaining, preferably immunohistochemical staining of GPC3 in a tumor sample from the patient, wherein the GPC3 expression level is determined and compared to a predetermined threshold level of GPC3 expression, and wherein the patient is selected for treatment in case the patient has a GPC3 expression level equal or higher to the predetermined threshold level.

7. The use of any one of claims 1 to 6, wherein the anti-GPC3 therapeutic agent comprises an anti-GPC3 binding domain, preferably an anti-GPC3 antibody or functional fragment thereof retaining binding to GPC3, preferably wherein the therapeutic agent comprises an anti-GPC3 antibody, an anti-GPC3 antibody-drug conjugate, an anti-GPC3 antibody-radionuclide conjugate, or a fusion protein of an anti-GPC3 antibody or antibody derivative binding to GPC3 with an anti-CD3 binding domain or an immunostimulatory polypeptide.

8. The use of any one of claims 1 to 7, wherein the anti-GPC3 therapeutic agent comprises genetically engineered hematopoietic cells expressing an anti-GPC3 chimeric receptor polypeptide (CAR), wherein the CAR polypeptide comprises:

(a) an extracellular binding domain binding to GPC3;

(b) a transmembrane domain; and

(c) a cytoplasmic signaling domain,

and optionally wherein the hematopoietic cell exogenously expresses a gene that improves viability and/or functionality of the hematopoietic cell in the solid tumor microenvironment.

9. The use of claim 8, wherein the hematopoietic cells have

i. an improved glucose uptake activity as relative to a wild-type hematopoietic cell of the same type, whereas the hematopoietic cell exogenously expresses a glucose importation polypeptide, preferably wherein the glucose importation polypeptide is a glucose transporter (GLUT) or a sodium-glucose cotransporter (SGLT), preferably wherein the glucose importation polypeptide is selected from the group consisting of: GLUT1, GLUT3, GLUT1 S226D, SGLT1, SGLT2, GLUT8, GLUT8 L12A L13A, GLUT11, GLUT7, and GLUT4;

ii. a modulated Krebs cycle as relative to a wild-type hematopoietic cell of the same type, whereas the hematopoietic cell exogenously expresses a Krebs cycle modulating polypeptide,

preferably wherein the Krebs cycle modulating factor is

a. an enzyme that catalyzes a reaction in the Krebs cycle, preferably isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), or phosphoglycerate dehydrogenase (PHGDH),

b. an enzyme that uses a Krebs cycle metabolite as a substrate, preferably glutamic-oxaloacetic transaminase (GOT) or phosphoenolpyruvate carboxykinase 1 (PCK1), or

c. an enzyme that converts a precursor to a Krebs cycle metabolite, preferably phosphoserine aminotransferase (PSAT1), glutamate dehydrogenase (GDH1), glutamate-pyruvate transaminase 1 (GPT1), or glutaminase (GLS); and/or

iii. enhanced intracellular lactate concentrations relative to a wild-type hematopoietic cell of the same type, whereas the hematopoietic cell exogenously expresses a lactate-modulating polypeptide,

preferably wherein the lactate modulation polypeptide is

a. monocarboxylate transporter (MCT), preferably MCT1, MCT2, or MCT4,

b. an enzyme involved in lactate synthesis, preferably lactate dehydrogenase A (LDHA), or

c. a polypeptide that inhibits a pathway that competes for lactate-synthesis substrates, preferably pyruvate dehydrogenase kinase 1 (PDK1).

10. The use of claim 8 or claim 9, wherein the extracellular antigen binding domain is a single chain antibody fragment (scFv) that binds to a GPC3, preferably wherein the scFv is derived from the GC33 antibody, optionally wherein the scFv has the sequence of SEQ ID NO: 2.

11. The use of any one of claims 8 to 10, wherein the CAR polypeptide comprises

(i) a CD28 co-stimulatory domain, in combination with a CD28 transmembrane domain, preferably SEQ ID NO: 6, a CD28 hinge domain, or a combination thereof, preferably SEQ ID NO: 4 or

(ii) a 4-1BB co-stimulatory domain, preferably SEQ ID NO: 5, in combination with a CD8 transmembrane domain, a CD8 hinge domain, or a combination thereof (SEQ ID NO: 3);

more preferably wherein the CAR polypeptide comprises the amino acid sequence of SEQ ID NO: 8 or SEQ ID NO: 9.

12. The use of any one of claims 8 to 11, wherein the cytoplasmic signaling domain of (c) is a cytoplasmic domain of CD3ζ, preferably SEQ ID NO: 7, or FcεR1γ.

13. The use of any one of claims 8 to 12, wherein the hematopoietic cells are natural killer (NK) cells, macrophages, neutrophils, eosinophils, or T cells, preferably wherein the hematopoietic cells are T cells, in which the expression of an endogenous T cell receptor, an endogenous major histocompatibility complex, an endogenous beta-2-microglobulin, or a combination thereof has been inhibited or eliminated; and/or

wherein the hematopoietic cells are derived from peripheral blood mononuclear cells (PBMC), hematopoietic stem cells (HSCs), or inducible pluripotent stem cells (iPSCs),

preferably wherein the hematopoietic cells are autologous to the patient.

14. The use of any one of claims 8 to 13, wherein the hematopoietic cells comprise a nucleic acid or a set of nucleic acids, preferably a DNA molecule or a set of DNA molecules, which collectively comprises:

(a) a first nucleotide sequence encoding the glucose importation polypeptide, the Krebs cycle modulating polypeptide and/or the lactate-modulating polypeptide; and

(b) a second nucleotide sequence encoding the chimeric antigen receptor polypeptide;

preferably wherein the hematopoietic cells comprise the nucleic acid, which comprises both the first nucleotide sequence and the second nucleotide sequence; and

(c) optionally a third nucleotide sequence located between the first nucleotide sequence and the second nucleotide sequence, wherein the third nucleotide sequence encodes a ribosomal skipping site, an internal ribosome entry site (IRES), or a second promoter, preferably wherein the third nucleotide sequence encodes a ribosomal skipping site, which is a P2A peptide;

preferably wherein:

(i) the nucleic acid or the nucleic acid set is comprised within a vector or a set of vectors, which preferably is an expression vector or a set of expression vectors; and/or

(ii) the vector or set of vectors comprises one or more viral vectors, which more preferably is a lentiviral vector or retroviral vector.

15. The use of any one of claims 8 to 14, wherein the anti-GPC3 therapeutic agent contains at least about 5 x 10^4 anti-GPC3- CAR T cells per kg of the patient, preferably from about 5 x 10^4 to about 1 x 10^{12} anti-GPC3-CAR T cells/kg of the patient.

16. The use of any one of claims 1 to 15, wherein the therapeutic agent comprises an anti-GPC3 targeted polypeptide or polypeptide fusion, preferably an anti-GPC3 antibody, an anti-GPC3 bi- or multiple specific protein or an anti-GPC3 antibody-drug-conjugate.

17. The use of any one of claims 1 to 16, wherein the anti-GPC3 therapeutic agent is effective in achieving stable disease according to RECIST as measured by computerized tomography (CT) scan.

18. The use of any one of claims 1 to 17, wherein the anti-GPC3 therapeutic agent is achieving an objective response according to RECIST as measured by computerized tomography (CT) scan.

19. The use of any one of claims 1 to 18, wherein the medicament is to be administered in combination with at least one immunomodulatory agent, wherein the immunomodulatory agent is to be administered to the patient in parallel or sequential to the medicament, preferably wherein the immunomodulatory agent is an immune checkpoint inhibitor or an immunostimulatory cytokine.

20. The use of any one of claims 1 to 15 and claims 17 to 19, wherein the medicament is to be administered in combination with a lymphocyte reduction treatment, preferably wherein the lymphocyte reduction treatment comprises cyclophosphamide and/or fludarabine.