

reactions using isotope tracing (DeBerardinis et al., Proc Natl Acad Sci U S A 104, 19345 (2007)).

SUMMARY

As demonstrated herein, mitochondrial glycine (and one-carbon, or 1-C, metabolism) is important in cancer cell metabolism. Glycine consumption is altered in some cancers, and is a unique predictor of antifolate sensitivity as well as of cancer cell proliferation. Glycine starvation can reduce the proliferation of sensitive cancers.

In addition, MTHFD2, which is a part of the mitochondrial 1-C pathway, is one of the strongest differentially expressed genes and, in the present analysis, is the most differentially expressed metabolic enzyme. MTHFD2 appears to be an embryonic enzyme that is resurrected in cancer; MTHFD2 is highly upregulated across many cancers relative to normal tissues. Genetic silencing of MTHFD2 and inhibition with small molecules slows proliferation across a number of cancer cell lines.

In one aspect, there is provided use of an antifolate drug in the manufacture of a medicament for treating a cancer in a subject, wherein the subject is one who has a level of glycine consumption above a reference level, the subject selected using a method comprising: determining the level of one or more of glycine consumption in a sample comprising tumor cells that has been obtained from the subject; and comparing the level of glycine consumption in the sample to a reference level of glycine consumption. Disclosed are~~Thus, in a first aspect the invention provides~~ methods for treating a cancer in a subject. The methods include obtaining a sample comprising tumor cells from the subject; determining a level of one or more of glycine consumption in the sample; comparing the level of glycine consumption in the sample to a reference level of glycine consumption; selecting a subject who has a level of glycine consumption above the reference level; and treating the subject by administering a therapeutically effective amount of an antifolate drug.

In some embodiments, the antifolate drug is methotrexate.

In some embodiments, the antifolate drug is linked covalently to a mitochondrial targeting moiety.

In some embodiments, the level of glycine consumption is determined by imaging a tumor in a living subject using a labeled substrate, e.g., ^{11}C -glycine, e.g., imaging a tumor in a living subject using PET.

In another aspect, there is provided use of one or both of an antifolate drug and an agent that inhibits a mitochondrial 1-carbon (1-C) pathway enzyme, e.g., an agent that inhibits SHMT2 or MTHFD2, in the manufacture of a medicament for treating a cancer in a subject, wherein the subject is one who has a level of SHMT2, MTHFD2, and/or MTHFD1L protein, mRNA, or activity above the reference level, the subject selected using a method comprising: determining the level of one or more of SHMT2, MTHFD2, and/or MTHFD1L protein, mRNA, or activity in a sample comprising tumor cells that has been obtained from the subject; and comparing the level of SHMT2, MTHFD2, and/or MTHFD1L protein, mRNA, or activity in the sample to a reference level of SHMT2, MTHFD2, and/or MTHFD1L protein, mRNA, or activity. Disclosed are~~the invention provides~~ methods for treating a cancer in a subject. The methods include obtaining a sample comprising tumor cells from the subject; determining a level of one or more of SHMT2, MTHFD2, and/or MTHFD1L protein, mRNA, or activity in the sample; comparing the level of SHMT2, MTHFD2, and/or MTHFD1L protein, mRNA, or activity in the sample to a reference level of SHMT2, MTHFD2, and/or MTHFD1L protein, mRNA, or activity; selecting a subject

who has a level of SHMT2, MTHFD2, and/or MTHFD1L protein, mRNA, or activity above the reference level; and treating the subject by administering a therapeutically effective amount of one or both of an antifolate drug and an agent that inhibits a mitochondrial 1-carbon (1-C) pathway enzyme, e.g., an agent that inhibits SHMT2 or MTHFD2.

In some embodiments, the antifolate drug is methotrexate.

In some embodiments, the agent that inhibits MTHFD2 is 6-hydroxy-DL DOPA, calmidazolium chloride, CDOO, ebselen, celestrol, GW5074, iodoacetamide, para-benzoquinone, or protoporphyrin IX disodium.

In some embodiments, the agent that inhibits a mitochondrial 1-carbon (1-C) pathway enzyme is an inhibitory nucleic acid that inhibits SHMT2 or MTHFD2, preferably SHMT2.

In some embodiments, the inhibitory nucleic acid is an siRNA, shRNA, or antisense oligonucleotide.

In another aspect, there is provided use of a composition comprising an active agent that inhibits MTHFD2, or a composition thereof, in the manufacture of a medicament for treating a cancer in a subject. Disclosed are the invention provides methods for treating a cancer in a subject. The methods include administering to the subject a therapeutically effective amount of a composition comprising an active agent that inhibits MTHFD2, or a composition thereof.

In some embodiments, the active agent is selected from the group consisting of 6-hydroxy-DL DOPA, calmidazolium chloride, CDOO, ebselen, celestrol, GW5074, iodoacetamide, para-benzoquinone, protoporphyrin IX disodium, methotrexate, pemetrexed, or 5-fluorouracil, optionally.

In some embodiments, the active agent is ebselen.

In some embodiments, the inhibitor covalently modifies MTHFD2.

In some embodiments, the inhibitor covalently or non-covalently modifies one or both of Cys145 or Cys166 of MTHFD2, preferably Cys145.

In some embodiments, the active agent is linked covalently to a mitochondrial-targeting moiety, e.g., tetraphenylphosphonium.

In some embodiments, the method or use include identifying the cancer in the subject as having a level of SHMT2, MTHFD2, and/or MTHFD1L protein, mRNA, or activity above a reference level of SHMT2, MTHFD2, and/or MTHFD1L protein, mRNA, or activity.

In another aspect, there is provided a method of predicting likelihood of survival in a subject who has cancer, the method comprising: determining a level of SHMT2, MTHFD2, and/or MTHFD1L protein, mRNA, or activity in a sample comprising tumor cells that has been obtained from the subject; comparing the level of SHMT2, MTHFD2, and/or MTHFD1L protein, mRNA, or activity in the sample to a reference level of SHMT2, MTHFD2, and/or MTHFD1L protein, mRNA, or activity; assigning a high predicted likelihood of survival to a subject who has a level of SHMT2, MTHFD2, and/or MTHFD1L above the reference level, or assigning a low predicted likelihood of survival to a subject who has a level of SHMT2, MTHFD2, and/or MTHFD1L below the reference level. Disclosed ~~are the invention provides~~ methods for predicting likelihood of survival in a subject who has cancer. The methods include obtaining a sample comprising tumor cells from the subject; determining a level of SHMT2, MTHFD2, and/or MTHFD1L protein, mRNA, or activity in the sample; comparing the level of SHMT2, MTHFD2, and/or MTHFD1L protein, mRNA, or activity in the sample to a reference level of SHMT2, MTHFD2, and/or MTHFD1L protein, mRNA, or activity; assigning a high predicted likelihood of survival to a subject who has a level of SHMT2, MTHFD2, and/or MTHFD1L above the reference level, or assigning a low predicted likelihood of survival to a subject who has a level of SHMT2, MTHFD2, and/or MTHFD1L below the reference level.

In another aspect, there is provided a method of diagnosing cancer in a subject, the method comprising: determining a level of SHMT2, MTHFD2, and/or MTHFD1L protein, mRNA, or activity in a sample suspected of comprising tumor cells that has been obtained from the subject; comparing the level of SHMT2, MTHFD2, and/or MTHFD1L protein, mRNA, or activity in the sample to a reference level of SHMT2, MTHFD2, and/or MTHFD1L protein, mRNA, or activity; and diagnosing a subject who has a level of SHMT2, MTHFD2, and/or MTHFD1L above the reference level as having cancer. Disclosed ~~are the invention provides~~ methods for diagnosing cancer in a subject. The methods include obtaining a sample suspected of comprising tumor cells from the subject; determining a level of SHMT2, MTHFD2, and/or MTHFD1L protein, mRNA, or activity in the sample; comparing the level of SHMT2, MTHFD2, and/or MTHFD1L protein, mRNA, or activity in the sample to a reference level of SHMT2, MTHFD2, and/or MTHFD1L protein, mRNA, or activity; and diagnosing a subject who has a level of SHMT2, MTHFD2, and/or MTHFD1L above the reference level as having cancer.

In some embodiments of the methods or uses described herein, the MTHFD2 activity is NAD-dependent methylenetetrahydrofolate dehydrogenase / cyclohydrolase activity.

In another aspect, there is provided a method of identifying a candidate compound for the treatment of cancer, the method comprising: providing a sample comprising a cell, e.g., a tumor cell, expressing MTHFD2; contacting the sample with a test compound; determining a level of NAD-dependent methylenetetrahydrofolate dehydrogenase / cyclohydrolase activity in the sample in the presence of the test compound in the absence of reducing agents, e.g., DTT or mercaptoethanol; comparing the level of activity in the presence of the test compound to a reference level of activity; and selecting a compound that is associated with reduced activity as a candidate compound. Disclosed ~~are the invention provides~~ methods for identifying a candidate compound for the treatment of cancer. The methods include providing a sample comprising a cell, e.g., a tumor cell, expressing MTHFD2; contacting the sample with a test compound; determining a level of NAD-dependent methylenetetrahydrofolate dehydrogenase / cyclohydrolase activity in the sample in the presence of the test compound in the absence of reducing agents, e.g., DTT or mercaptoethanol; comparing the level of activity in the presence of the test compound to a reference level of activity; and selecting a compound that is associated with reduced activity as a candidate compound.

In some embodiments, the reference level is a level of NAD-dependent methylenetetrahydrofolate dehydrogenase / cyclohydrolase activity in the absence of the test compound, e.g., in a control sample.

In some embodiments, the test compound is a cysteine modifying agent.

In some embodiments, the method include contacting a cancer cell with the candidate compound; determining a rate of proliferation or viability in the presence of the candidate compound; comparing the rate of proliferation or viability in the presence of the candidate compound to a reference rate of proliferation or viability; and selecting as a candidate therapeutic compound a candidate compound that decreases the rate of proliferation or viability.

In some embodiments, the reference rate of proliferation or viability is a level of rate of proliferation or viability in the absence of the candidate compound, e.g., in a control sample.

In some embodiments, the method include administering the candidate therapeutic compound to an animal model of cancer, e.g., a xenograft model; determining an effect of the candidate therapeutic compound on a parameter of the cancer in the animal model; and selecting as a therapeutic compound a candidate therapeutic compound that improves one or more parameters of cancer in the animal model. In some embodiments, the parameter is tumor size, tumor growth, time to tumor development (or average age of tumor development), or metastasis, and an improvement is a reduction in tumor size, tumor growth rate, or metastasis, or an increase in time to tumor development (or average age of tumor development).

~~Disclosed are~~In another aspect, the invention provides methods for treating or identifying a subject for treatment with a low-glycine diet and/or administration of sodium benzoate. The methods include determining levels of glycine uptake in a sample comprising cancer cells from the subject, comparing the levels of glycine uptake to reference levels of glycine uptake, selecting a subject who has levels of levels of glycine uptake above the reference levels for treatment with a low-glycine diet or administration of sodium benzoate, and optionally administering the treatment to the subject. In one aspect, there is provided use of a low-glycine diet and/or sodium benzoate in the manufacture of a medicament for treating a subject, wherein the subject is one who has levels of glycine uptake above the reference levels, the subject selected using a method comprising: determining levels of glycine uptake in a sample comprising cancer cells from the subject, and comparing the levels of glycine uptake to reference levels of glycine uptake. In another aspect, there is provided a method of identifying a subject for treatment with a low-glycine diet and/or administration of sodium benzoate, the method comprising: determining levels of glycine uptake in a sample comprising cancer cells from the subject, comparing the levels of glycine uptake to reference levels of glycine uptake, selecting a subject who has levels of levels of glycine uptake above the reference levels for treatment with a low-glycine diet or administration of sodium benzoate.

In another aspect, there is invention provideds a method of predicting aggressiveness or growth rate of a tumor in a subject, the method comprising: determining a level of glycine uptake in a sample comprising cells from the tumor; comparing the level of glycine uptake in the sample to a reference level of glycine uptake; assigning a high likelihood of aggressiveness and high growth rate to a subject who has a tumor with a level of glycine uptake above the reference level, or assigning a low likelihood of aggressiveness and high growth rate to a subject who has a tumor with a level of glycine uptake below the reference level. Disclosed are methods for predicting aggressiveness or growth rate of a tumor in a subject. The methods include determining a level of glycine uptake in a sample comprising cells from the tumor; comparing the level of glycine uptake in the sample to a reference level of glycine uptake; assigning a high likelihood of aggressiveness and high growth rate to a subject who has a tumor with a level of glycine uptake above the reference level, or

assigning a low likelihood of aggressiveness and high growth rate to a subject who has a tumor with a level of glycine uptake below the reference level.

In another aspect, there is ~~invention~~ provided a method of predicting aggressiveness or growth rate of a tumor in a subject, the method comprising: determining a level of one or more of SHMT2, MTHFD2, and/or MTHFD1L mRNA, protein, or activity in a sample comprising cells from the tumor; comparing the level of SHMT2, MTHFD2, and/or MTHFD1L protein, mRNA, or activity in the sample to a reference level of SHMT2, MTHFD2, and/or MTHFD1L protein, mRNA, or activity; assigning a high likelihood of aggressiveness and high growth rate to a subject who has a tumor with a level of SHMT2, MTHFD2, and/or MTHFD1L above the reference level, or assigning a low likelihood of aggressiveness and high growth rate to a subject who has a tumor with a level of SHMT2, MTHFD2, and/or MTHFD1L below the reference level. Disclosed are methods for predicting aggressiveness or growth rate of a tumor in a subject. The methods include determining a level of one or more of SHMT2, MTHFD2, and/or MTHFD1L mRNA, protein, or activity in a sample comprising cells from the tumor; comparing the level of SHMT2, MTHFD2, and/or MTHFD1L protein, mRNA, or activity in the sample to a reference level of SHMT2, MTHFD2, and/or MTHFD1L protein, mRNA, or activity; assigning a high likelihood of aggressiveness and high growth rate to a subject who has a tumor with a level of SHMT2, MTHFD2, and/or MTHFD1L above the reference level, or assigning a low likelihood of aggressiveness and high growth rate to a subject who has a tumor with a level of SHMT2, MTHFD2, and/or MTHFD1L below the reference level.

In some embodiments, the present ~~disclosure~~ invention provides a method for treating a cancer, e.g., a carcinoma, in a patient, comprising administering to the patient an inhibitor of MTHFD2, or pharmaceutically acceptable composition thereof.

In some embodiments of the uses or methods described herein, the cancer is a carcinoma, e.g., glioma, glioblastoma, breast, ovarian, renal cell, lung; melanoma, cervical, adrenal, brain, esophagus, gastric, germ cell, head / neck, prostate, melanoma, liver, pancreas, testicular, or colon cancer.

In some embodiments, the carcinoma is not breast or bladder cancer.

In another aspect, there is provided an *in vitro* method of inhibiting proliferation of glycine consuming cells, the method comprising contacting the cells with an antifolate agent. Disclosed is ~~In another aspect, the invention provides a methods for~~ of inhibiting proliferation of glycine consuming cells, the method comprising contacting the cells with an antifolate agent.

~~Disclosed are~~ In another aspect, the invention provides methods for treating cancer by inhibiting proliferation of glycine consuming cells.

In certain embodiments, the present ~~disclosure~~ invention provides a method of inhibiting MTHFD2 in a biological sample, comprising contacting said sample with a compound that inhibits MTHFD2.

In certain embodiments, the present ~~disclosure~~ invention provides a method of inhibiting MTHFD2 in a patient, comprising administering to the patient a compound that inhibits MTHFD2, or a pharmaceutically acceptable composition thereof.

~~Disclosed are~~In another aspect, the invention provides methods for inhibiting MTHFD2 in a patient or a biological sample comprising administering to the patient, or contacting the biological sample with, an inhibitor of MTHFD2.

~~Disclosed are~~In another aspect, the invention provides methods for inhibiting glycine metabolism or NAD-dependent methylenetetrahydrofolate dehydrogenase activity in a cell, comprising contacting the cell with an antifolate agent.

~~In another aspect, there is provided use of an antifolate agent in the manufacture of a medicament for inhibiting proliferation of glycine consuming cells.~~

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this ~~disclosure~~~~invention~~ belongs. Methods, uses and materials are described herein for use in the present ~~disclosure~~~~invention~~; other, suitable methods, uses and materials known in the art can also be used. The materials, methods, uses and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

Other features and advantages of the ~~disclosure~~~~invention~~ will be apparent from the following detailed description and figures, and from the claims.

DESCRIPTION OF DRAWINGS

FIGs. 1A-G: Glycine consumption and synthesis are correlated with rapid cancer cell proliferation. (1A) Distribution of Spearman correlations between 111 metabolite CORE profiles and proliferation rate across 60 cancer cell lines. Only metabolites highlighted in red are significant at $P < 0.05$, Bonferroni-corrected. (1B) Glycine CORE versus proliferation rate across 60 cancer cell lines (left) and selected solid tumor types (right). Cell lines selected for follow-up experiments are highlighted in red. Joined data points represent replicate cultures. P values are Bonferroni-corrected for 111 tested metabolites. (1C) Distribution of Spearman correlations between gene expression of 1425 metabolic enzymes and proliferation rates across 60 cancer cell lines. Highlighted are mitochondrial (MTHFD2, SHMT2, MTHFD1L) and cytosolic (MTHFD1, SHMT1) glycine metabolism enzymes. (1D) Schematic of cytosolic and mitochondrial glycine metabolism. (1E) Abundance of unlabeled (0) and labeled (+1) intracellular glycine and serine in LOX IMVI cells grown on 100% extracellular (13C)glycine. (1F) Growth of A498 and LOXIMVI cells expressing shRNAs targeting SHMT2 (sh1-4) or control shRNA (shCtrl), cultured in the absence

WHAT IS CLAIMED IS:

1. Use of an antifolate drug in the manufacture of a medicament for~~A method of treating~~
a cancer in a subject, wherein the subject is one who has a level of glycine
consumption above a reference level, the subject selected using a method comprising:
5 obtaining a sample comprising tumor cells from the subject;
determining a~~the~~ level of one or more of glycine consumption in the a sample
comprising tumor cells that has been obtained from the subject; and
comparing the level of glycine consumption in the sample to a reference level of
glycine consumption;
10 selecting a subject who has a level of glycine consumption above the reference level;
and
treating the subject by administering a therapeutically effective amount of an
antifolate drug.
2. The ~~use method~~ of claim 1, wherein the antifolate drug is methotrexate.
- 15 3. The ~~use method~~ of claim 1, wherein the antifolate drug is linked covalently to a
mitochondrial targeting moiety.
4. The ~~use method~~ of claim 1, wherein the level of glycine consumption is
determined by imaging a tumor in a living subject using a labeled substrate,
e.g., ^{11}C -glycine.
- 20 5. The ~~use method~~ of claim 4, comprising imaging a tumor in a living subject
using PET.
6. Use of one or both of an antifolate drug and an agent that inhibits a
mitochondrial 1-carbon (1-C) pathway enzyme, e.g., an agent that inhibits
SHMT2 or MTHFD2, in the manufacture of a medicament for~~A method of~~
25 treating a cancer in a subject, wherein the subject is one who has a level of
SHMT2, MTHFD2, and/or MTHFD1L protein, mRNA, or activity above the
reference level, the subject selected using a method comprising:
obtaining a sample comprising tumor cells from the subject;
determining a~~the~~ level of one or more of SHMT2, MTHFD2, and/or
30 MTHFD1L protein, mRNA, or activity in the a sample comprising tumor cells
that has been obtained from the subject; and
comparing the level of SHMT2, MTHFD2, and/or MTHFD1L protein,
mRNA, or activity in the sample to a reference level of SHMT2, MTHFD2,
and/or MTHFD1L protein, mRNA, or activity;
35 selecting a subject who has a level of SHMT2, MTHFD2, and/or MTHFD1L
protein, mRNA, or activity above the reference level; and
treating the subject by administering a therapeutically effective amount of one
or both of an antifolate drug and an agent that inhibits a mitochondrial 1-

carbon (1-C) pathway enzyme, e.g., an agent that inhibits SHMT2 or MTHFD2..

7. The ~~use~~method of claim 6, wherein the antifolate drug is methotrexate.
8. The ~~use~~method of claim 6, wherein the agent that inhibits MTHFD2 is 6-hydroxy-DL DOPA, calmidazolium chloride, CDOO, ebselen, celestrol, GW5074, iodoacetamide, para-benzoquinone, or protoporphyrin IX disodium.
9. The ~~use~~method of claim 6, wherein the agent that inhibits a mitochondrial 1-carbon (1-C) pathway enzyme is an inhibitory nucleic acid that inhibits SHMT2 or MTHFD2, preferably SHMT2.
10. The ~~use~~method of claim 6, wherein the inhibitory nucleic acid is an siRNA, shRNA, or antisense oligonucleotide.
11. ~~A method of treating a cancer in a subject, the method comprising administering to the subject a therapeutically effective amount~~Use of a composition comprising an active agent that inhibits MTHFD2, or a composition thereof, in the manufacture of a medicament for treating a cancer in a subject.
12. The ~~use~~method of claim 11, wherein the active agent is selected from the group consisting of 6-hydroxy-DL DOPA, calmidazolium chloride, CDOO, ebselen, celestrol, GW5074, iodoacetamide, para-benzoquinone, protoporphyrin IX disodium, methotrexate, pemetrexed, or 5-fluorouracil, optionally.
13. The ~~use~~method of claim 12, wherein the active agent is ebselen.
14. The ~~use~~method of claim 12, wherein the inhibitor covalently or non-covalently modifies MTHFD2.
15. The ~~use~~method of claim 14, wherein the inhibitor covalently or non-covalently modifies one or both of Cys145 or Cys166 of MTHFD2, preferably Cys145.
16. The ~~use~~method of any of claims 3 or 12-15, wherein the active agent is linked covalently to a mitochondrial-targeting moiety.

17. The ~~use~~method of claim 16, wherein the mitochondrial-targeting moiety is tetraphenylphosphonium.
18. The ~~use~~method of claim 12, further comprising identifying the cancer in the subject as having a level of SHMT2, MTHFD2, and/or MTHFD1L protein, mRNA, or activity above a reference level of SHMT2, MTHFD2, and/or MTHFD1L protein, mRNA, or activity.
19. A method of predicting likelihood of survival in a subject who has cancer, the method comprising:
 - ~~obtaining a sample comprising tumor cells from the subject;~~
 - determining a level of SHMT2, MTHFD2, and/or MTHFD1L protein, mRNA, or activity in ~~the~~ a sample comprising tumor cells that has been obtained from the subject;
 - comparing the level of SHMT2, MTHFD2, and/or MTHFD1L protein, mRNA, or activity in the sample to a reference level of SHMT2, MTHFD2, and/or MTHFD1L protein, mRNA, or activity;
 - assigning a high predicted likelihood of survival to a subject who has a level of SHMT2, MTHFD2, and/or MTHFD1L above the reference level, or assigning a low predicted likelihood of survival to a subject who has a level of SHMT2, MTHFD2, and/or MTHFD1L below the reference level.
20. A method of diagnosing cancer in a subject, the method comprising:
 - ~~obtaining a sample suspected of comprising tumor cells from the subject;~~
 - determining a level of SHMT2, MTHFD2, and/or MTHFD1L protein, mRNA, or activity in ~~the~~ a sample suspected of comprising tumor cells that has been obtained from the subject;
 - comparing the level of SHMT2, MTHFD2, and/or MTHFD1L protein, mRNA, or activity in the sample to a reference level of SHMT2, MTHFD2, and/or MTHFD1L protein, mRNA, or activity; and
 - diagnosing a subject who has a level of SHMT2, MTHFD2, and/or MTHFD1L above the reference level as having cancer.
21. The ~~method-use~~ of claims 1-20 18, or the method of claim 19 or 20, wherein the MTHFD2 activity is NAD-dependent methylenetetrahydrofolate dehydrogenase / cyclohydrolase activity.
22. A method of identifying a candidate compound for the treatment of cancer, the method comprising:

selecting as a therapeutic compound a candidate therapeutic compound that improves one or more parameters of cancer in the animal model.

28. The method of claim 27, wherein the parameter is tumor size, tumor growth, time to tumor development (or average age of tumor development), or metastasis, and an improvement is a reduction in tumor size, tumor growth rate, or metastasis, or an increase in time to tumor development (or average age of tumor development).
29. ~~A method of treating or identifying a subject for treatment with~~ Use of a low-glycine diet and/or administration of sodium benzoate in the manufacture of a medicament for treating a subject, wherein the subject is one who has levels of glycine uptake above the reference levels, the subject selected using a method comprising:
determining levels of glycine uptake in a sample comprising cancer cells from the subject, and
comparing the levels of glycine uptake to reference levels of glycine uptake;
~~selecting a subject who has levels of levels of glycine uptake above the reference levels for treatment with a low-glycine diet or administration of sodium benzoate, and optionally administering the treatment to the subject.~~
30. A method of identifying a subject for treatment with a low-glycine diet and/or administration of sodium benzoate, the method comprising:
determining levels of glycine uptake in a sample comprising cancer cells from the subject,
comparing the levels of glycine uptake to reference levels of glycine uptake,
selecting a subject who has levels of levels of glycine uptake above the reference levels for treatment with a low-glycine diet or administration of sodium benzoate.
- 30.31. A method of predicting aggressiveness or growth rate of a tumor in a subject, the method comprising:
determining a level of glycine uptake in a sample comprising cells from the tumor;
comparing the level of glycine uptake in the sample to a reference level of glycine uptake;
assigning a high likelihood of aggressiveness and high growth rate to a subject who has a tumor with a level of glycine uptake above the reference level, or assigning a low likelihood of aggressiveness and high growth rate to a subject who has a tumor with a level of glycine uptake below the reference level.
- 31.32. A method of predicting aggressiveness or growth rate of a tumor in a subject, the method comprising:
determining a level of one or more of SHMT2, MTHFD2, and/or MTHFD1L mRNA, protein, or activity in a sample comprising cells from the tumor;
comparing the level of SHMT2, MTHFD2, and/or MTHFD1L protein, mRNA, or activity in the sample to a reference level of SHMT2, MTHFD2,

and/or MTHFD1L protein, mRNA, or activity;
 assigning a high likelihood of aggressiveness and high growth rate to a subject
 who has a tumor with a level of HMT2, MTHFD2, and/or MTHFD1L above
 the reference level, or assigning a low likelihood of aggressiveness and high
 growth rate to a subject who has a tumor with a level of SHMT2, MTHFD2,
 and/or MTHFD1L below the reference level.

~~32.33.~~ The use or method of any of the preceding claims, wherein the cancer
 is a carcinoma.

~~33.34.~~ The use or method of claim ~~32.33~~, wherein the carcinoma is glioma,
 glioblastoma, breast, ovarian, renal cell, lung; melanoma, cervical, adrenal,
 brain, esophagus, gastric, germ cell, head / neck, prostate, melanoma, liver,
 pancreas, testicular, or colon cancer.

~~34.35.~~ The use or method of claim ~~32.33~~, wherein the carcinoma is not breast
 or bladder cancer.

~~35.36.~~ An *in vitro* method of inhibiting proliferation of glycine consuming
 cells, the method comprising contacting the cells with an antifolate agent.

37. Use of an antifolate agent in the manufacture of a medicament for inhibiting
 proliferation of glycine consuming cells.

~~36. A method of treating cancer by inhibiting proliferation of glycine consuming
 cells.~~