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(54) **Title:** ENGINEERED HUMAN SERINE DEHYDRATASE ENZYMES AND METHODS FOR TREATING CANCER

(57) **Abstract:** Disclosed herein are compositions related to polypeptides with serine and/or threonine-degrading enzyme activity. Also disclosed are nucleic acids, expression vectors, host cells, and pharmaceutical formulations related to the polypeptides. Further disclosed are methods of using the pharmaceutical formulations comprising above to treat cancer.

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DESCRIPTION

ENGINEERED HUMAN SERINE DEHYDRATASE ENZYMES AND METHODS FOR TREATING CANCER

REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the priority benefit of United States provisional application number 63/357,282, filed June 30, 2022, the entire contents of which are incorporated herein by reference.

REFERENCE TO A SEQUENCE LISTING

[0002] This application contains a Sequence Listing XML, which has been submitted electronically and is hereby incorporated by reference in its entirety. Said Sequence Listing XML, created on June 28, 2023, is named UTFBP1304WO_ST26.xml and is 29,731 bytes in size.

BACKGROUND

1. Field

[0003] The invention relates to compositions and methods for the treatment of cancer with enzymes that deplete both L-serine and L-threonine amino acids systemically. More particularly, it concerns the engineering of human serine dehydratase enzymes with greatly improved serine/threonine degrading activity and kinetic stability that is suitable for human cancer therapy.

2. Description of Related Art

[0004] Cancer is one of the leading causes of death in the USA claiming more than half a million people's lives every year despite tremendous effort in the cancer research field[1]. Current chemotherapeutic strategies lack tumor specificity and commonly result in severe toxicities in patients[2]. Targeting altered cancer metabolism is one approach that may have more tumor specificity with fewer off target effects. The conventional cancer metabolism field has focused on central carbon metabolism, including glycolysis and the tricarboxylic acid cycle[3]. However, numerous studies published over the last decade have helped us to understand the importance of amino acids in cancer metabolism, especially their contribution to the mass accumulation of proliferating cells[4]. Since many cancer cells cannot

synthesize adequate amounts of certain non-essential amino acids and/or have a much higher demand for essential amino acids than normal cells, they can rewire metabolic pathways to fulfill their demand[5].

[0005] It is known that under metabolic reprogramming, non-essential amino acids are also a limiting factor for optimal tumor growth[6]. Therefore, many cancer cells are now characterized as 'addicted' or 'auxotrophic' to certain amino acids[7,8]. Serine is one of the most extensively consumed non-essential amino acids (third most consumed nutrient after glucose and glutamine) and is located at a central node for many biosynthetic reactions, including biosynthesis of glutathione, purines, one-carbon units, phospholipid head groups, sphingosine, cysteine, and glycine [9,10].

[0006] While some cancers upregulate the serine biosynthesis pathway, many other tumor cells remain highly dependent on exogenous serine to support maximal proliferation, suggesting that *de novo* serine synthesis alone does not fulfill the requirements[11]. For instance, a human colon cancer model *in vivo* showed a 50% reduction in tumor volume when mice were fed with serine-free diet compared to animals on a normal-diet[12]. In another example, genetically engineered intestinal cancer and lymphoma mouse models experience increased survival rates with dietary restriction of serine[13]. In another study, it is found a metabolic limitation factor when cancer cells upregulate *de novo* serine synthesis and explored why they also depend on extracellular serine for maximal growth. In the study, cancer cells with increased serine biosynthesis levels were shown to rely on a large amount of exogenous serine uptake for maximal proliferation, and removal of serine from media decreased their proliferation[14]. While serine biosynthesis consumes NAD^+, serine uptake from the environment does not require NAD^+. Since there is high demand for the regeneration of NAD^+ to build biomass and support oxidation reactions, this cofactor availability was found to be a limiting factor for optimal proliferation of cancer cells favoring serine biosynthesis upregulation. These cancer cells needed exogenous serine to maintain purine synthesis and could not reach maximal growth by consuming limited NAD^+ via directing serine biosynthesis from glucose. Overall, they suggested that when serine biosynthesis is upregulated, the availability of NAD^+ can be limiting for the biosynthesis reactions[14]. Thus, cancer cells require the uptake of extracellular serine for rapid growth, and when extracellular serine is absent, they become more sensitive to starvation.

[0007] *De novo* serine synthesis occurs in three steps starting with the glycolytic intermediate 3-phosphoglycerate. Phosphoglycerate dehydrogenase (PHGDH) catalyzes the oxidation of 3-phosphoglycerate to 3-phosphohydroxypyruvate as the first step of serine biosynthesis. Then, phosphoserine aminotransferase-1 (PSAT1) converts 3-phosphohydroxypyruvate to 3-phosphoserine, and at the final step, phosphoserine phosphatase (PSPH) removes the phosphate of 3-phosphoserine to produce serine. Thus any cancers with mutations, deletions, or repression of the serine synthesis pathway genes PHGDH, PSAT1 or PSPH would be extremely dependent upon extracellular serine for survival (auxotrophic). A notable example of serine auxotrophy are luminal breast cancers wherein the PSAT1 gene is hypermethylated causing profound downregulation and rendering extreme sensitivity to serine deprivation (Choi et al., 2022).

[0008] Another amino acid that is important for cancer growth is threonine, an essential amino acid. Several studies focused on threonine metabolism in mouse leukemia, adenocarcinoma, and specifically mouse embryonic stem cells in recent years. One of the very early studies on the threonine requirement in cancer cells was performed in the late 70's[15]. In the study, purified sheep threonine deaminase enzyme was added into the media of mouse leukemia cells which were quickly killed by lowering threonine amounts to an undetectable level and affecting DNA, RNA, and protein synthesis. On the other hand, normal mouse and human fibroblast cell lines showed little or no cell death for 3-5 days after the enzyme addition. Another study showed that a threonine-free diet significantly suppressed tumor growth in mice bearing adenocarcinoma, but unfortunately also resulted in weight loss[16]. mESCs overexpress threonine dehydrogenase (TDG) enzyme, which generates glycine and acetyl-coenzyme A from threonine[17]. When each of the 20 amino acids were removed from the media individually, threonine depletion caused a unique event that halted mESC growth compared to depletion of other amino acids[17]. Moreover, in another study, it was found that the critical demand for threonine in mESC is due to the contribution of threonine to S-Adenosyl methionine (SAM) synthesis by providing glycine and acetyl-CoA via threonine dehydrogenase activity[18]. In addition, depletion of threonine from the media or partial depletion of threonine dehydrogenase by RNA interference caused lower SAM accumulation and histone methylation in mESC, and therefore slowed growth and increased differentiation[18].

[0009] In summary, many cancer cells exhibit a much higher requirement for serine due to their rapid proliferation and abnormal demand for several biosynthesis pathways, including one-carbon metabolism, glutathione synthesis, and nucleotide synthesis[10,12,19]. Also, for some cancer cells, as an essential amino acid, threonine is found in higher demand compared to normal cells and characterized as a vital amino acid for mESC survival[15,17]. Although there were several attempts to inhibit the serine biosynthesis pathway by small-molecule inhibitors[20,21], many cancers *in vivo* could survive when their endogenous serine biosynthesis was inhibited, as they can acquire the amino acid from the extracellular environment[13,14,22,23]. In addition, some studies showed a synergistic effect on tumor growth by using both serine biosynthesis inhibition and a serine-free diet[20,23]. Therefore, limiting exogenous serine and threonine availability could help to target cancer cells selectively as they have a higher demand for these amino acids than normal cells do[15,24].

[0010] A promising therapeutic approach for treating cancer patients would be the use of a pharmacologically optimized human enzyme that converts serine/threonine into non-toxic and naturally metabolizable products and systemically depletes the serum pool. A human enzyme, human serine dehydratase (hSDH), degrades both serine and threonine, however, it does not display sufficient catalytic properties to be relevant for clinical applications.

SUMMARY

[0011] Provided herein are compositions comprising and use of engineered hSDH variants with improved catalytic, stability, and manufacturing properties to systemically deplete serine and threonine in serum.

[0012] In one embodiment, provided herein are engineered human serine dehydratase enzymes comprising at least one substitution relative to a native human serine dehydratase enzyme according to SEQ ID NO: 1, wherein the modified enzyme has greater serine and/or threonine degrading activity than the native human serine dehydratase enzyme. The enzymes may have amino acid sequences at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 1. The enzymes have may a kcat/Km for degrading serine that is greater than 0.6 mM-1*s-1. The enzymes may have a kcat/Km for degrading threonine that is greater than 1.1 mM-1*s-1.

[0013] In some aspects, the enzymes comprise at least one substitution at a position corresponding to a position of SEQ ID NO: 1 selected from the group consisting of: I13, S26, R51, S63, M70, L94, I96, L112, D114, G129, A204, K221, A222, W157, D261, and a combination thereof. In some aspects, the enzymes comprise at least one substitution selected from the group consisting of: I13T, S26N, R51H, S63P, M70I, L94P, I96T, L112S, D114E, G129D, A204S, K221F, K221R, A222G, A222S, W157R, D261L, and a combination thereof, wherein the positions are relative to SEQ ID NO: 1.

[0014] In some aspects, the enzymes comprise a set of substitutions at positions corresponding to positions of SEQ ID NO: 1, wherein the set of substitutions is selected from the group consisting of:

(a) K221 and A222;
(b) S26, R51, S63, M70, L94, G129, A204, K221, and A222;
(c) S26, R51, S63, M70, L94, G129, A204, K221, A222, and L112; and
(d) I13, S26, R51, I96, D114, G129, K221, A222, W157, D261, and L112.

[0015] In some aspects, the enzymes comprise a set of substitutions selected from the group consisting of:

(a) K221F and A222S (variant 1);
(b) S26N, R51H, S63P, M70I, L94P, G129D, A204S, K221R, and A222S (variant 2);
(c) S26N, R51H, S63P, M70I, L94P, G129D, A204S, K221R, A222S, and L112S (variant 3);
(d) I13T, S26N, R51H, I96T, D114E, G129D, K221R, A222S, W157R, D261L, and L112S (variant 4); and
(e) I13T, S26N, R51H, I96T, D114E, G129D, K221R, A222G, W157R, D261L, and L112S (variant 5).

[0016] In some aspects, the enzymes comprise the sequence of any one of SEQ ID NOs: 2-6, optionally excluding the initial methionine. In some aspects, the enzymes comprise the initial methionine. In some aspects, the enzymes do not comprise the initial methionine. In cases where the enzymes do not comprise an initial methionine, the first amino acid of the polypeptide may still be a methionine, since the second position of each of SEQ ID NOs: 1-6 is also methionine.

[0017] In some aspects, the enzymes are coupled to at least one polyethylene glycol (PEG). The enzyme may be coupled to the at least one PEG via one or more lysine residues. The at least one PEG may be PEG-5K. The PEG may have an average molecular weight of about 5000 kDa. The PEG may have an average molecular weight of about 500 kDa to about 1000 kDa, about 800 kDa to about 1600 kDa, about 1500 kDa to about 3000 kDa, about 2000 kDa to about 4000 kDa, about 2500 kDa to about 5000 kDa, about 3000 kDa to about 6000 kDa, about 4,000 kDa to about 8,000 kDa, about 6,000 kDa to about 12,000 kDa, about 10,000 kDa to about 20,000 kDa, or about 15,000 kDa to about 30,000 kDa. The enzymes may be couples to at least a second PEG. The at least a second PEG may be conjugated to a second lysine of the enzymes.

[0018] In one embodiment, provided herein are nucleic acids comprising a nucleotide sequence encoding any one of the enzymes provided herein. The nucleic acids may be codon optimized for expression in bacteria, fungus, insects, or mammals. The bacteria may be *E. coli*.

[0019] In one embodiment, provided herein are expression vectors comprising any one of the nucleic acids provided herein.

[0020] In one embodiment, provided herein are host cells comprising any one of the nucleic acids or expression vectors provided herein. The host cells may be bacterial cells, fungal cells, insect cells, or mammalian cells.

[0021] In one embodiment, provided herein are pharmaceutical formulations comprising any one of the enzymes or nucleic acids provided herein, in a pharmaceutically acceptable carrier. The pharmaceutical formulations may be for use in the treatment of a subject having a cancer.

[0022] In one embodiment, provided herein are methods of lowering the blood/plasma serine levels in a subject in need thereof, the methods comprising administering to the subject a therapeutically effective amount of any one of the enzymes, nucleic acids, or formulations provided herein. The methods may reduce the concentration of serine/threonine in the subject's serum by at least 50% within 24 hours of administration.

[0023] In one embodiment, provided herein are methods of treating a subject having cancer, the methods comprising administering to the subject a therapeutically effective

amount of a formulation of any one of the enzymes, nucleic acids, or formulations provided herein.

[0024] A subject may be maintained on a serine restricted diet. A subject may be maintained on a normal diet. A subject may be a human patient.

[0025] A formulation may be formulated for administration or administered intratumorally, intravenously, intradermally, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostaticaly, intrapleurally, intratracheally, intraocularly, intranasally, intravitreally, intravaginally, intrarectally, intramuscularly, subcutaneously, subconjunctival, intravesicularlly, mucosally, intrapericardially, intraumbilically, orally, by inhalation, by injection, by infusion, by continuous infusion, by localized perfusion bathing target cells directly, via a catheter, or via a lavage. A formulation may be formulated for administration or administered to a nutrient medium of a cancer. The nutrient medium may be blood, lymphatic fluid, or spinal fluid.

[0026] A formulation may further comprise or a subject may be further administered at least a second anti-cancer therapy. The second anti-cancer therapy may be a surgical therapy, chemotherapy, radiation therapy, cryotherapy, hormone therapy, immunotherapy or cytokine therapy. The second anti-cancer therapy may comprise a serine synthesis inhibitor.

[0027] The level of serine and/or threonine in the subject's plasma and/or serum may be reduced by any one of the methods or uses provided herein. The tumor growth in a subject may be suppressed and/or delayed by any one of the methods or uses provided herein.

[0028] The subject's cancer may be characterized as addicted to extracellular serine. A subject may be selected for treatment if the subject's cancer is characterized as addicted to extracellular serine.

[0029] The subject's cancer may be characterized as dependent upon external sources of serine. A subject may be selected for treatment if the subject's cancer is characterized as dependent upon external sources of serine.

[0030] The subject's cancer may have been determined to express a decreased level of *PGDGH*, *PSAT1*, and/or *PSPH* compared to a reference level. The subject may be selected for treatment by (a) determining or having determined an expression level of *PGDGH*,

PSAT1, and/or *PSPH* in the subject's cancer; and (b) selecting or having selected the subject for treatment when the cancer expresses a decreased level of *PGDGH*, *PSAT1*, and/or *PSPH* compared to a reference level. Step (a) may comprise (i) obtaining or having obtained a biological sample from the cancer; and (ii) performing or having performed an assay on the biological sample to determine an expression level of *PGDGH*, *PSAT1*, and/or *PSPH*. Determining the expression level of *PGDGH*, *PSAT1*, and/or *PSPH* in the cancer may comprise detecting a PGDGH, PSAT1, and/or PSPH protein in the cancer. The protein may be detected by mass spectrometry, western blot, immunohistochemistry, ELISA, or RIA. Determining the expression level of *PGDGH*, *PSAT1*, and/or *PSPH* in the cancer may comprise detecting a PGDGH, PSAT1, and/or PSPH mRNA in the cancer. The mRNA may be detected by microarray, RT-PCR, qRT-PCR, nanostring assay, or in situ hybridization. The sample may be a formalin-fixed, paraffin-embedded sample. The sample may be a fresh frozen sample. The subject's cancer may be a luminal breast cancer.

[0031] The subject's cancer may have been determined to have a mutation in the *PGDGH* gene, *PSAT1* gene, and/or *PSPH* gene. The mutation may impair the function of PGDGH, PSAT1, and/or PSPH protein. The subject's cancer may be a luminal breast cancer.

[0032] A promoter, untranslated region, and/or gene body of the *PGDGH* gene, *PSAT1* gene, and/or *PSPH* gene may be methylated in the subject's cancer. The subject may be selected for treatment by (a) determining or having determined whether the promoter, untranslated region, and/or gene body of *PGDGH*, *PSAT1*, and/or *PSPH* is methylated in the subject's cancer; and (b) selecting or having selected the subject for treatment when the promoter, untranslated region, and/or gene body of *PGDGH*, *PSAT1*, and/or *PSPH* is methylated in the cancer. Step (a) may comprise (i) obtaining or having obtained a biological sample from the cancer; and (ii) performing or having performed an assay on the biological sample to determine whether the promoter, untranslated region, and/or gene body of *PGDGH*, *PSAT1*, and/or *PSPH* is methylated. The sample may be a formalin-fixed, paraffin-embedded sample. The sample may be a fresh frozen sample. The subject's cancer may be a luminal breast cancer.

[0033] A cancer may be a non-small cell lung cancer, a pancreatic adenocarcinoma, a breast cancer, a prostate cancer, a melanoma, a colon cancer, a leukemia, a lymphoma, a glioblastoma, an osteosarcoma, an oral cavity cancer, an ovarian cancer, a uterine cancer, a bone cancer, a brain cancer, a prostate cancer, a kidney cancer, a stomach cancer, an

esophageal cancer, a rectal cancer, a bladder cancer, a testicular cancer, or a liver cancer. A cancer may be a breast cancer or a colon cancer. A cancer may be a luminal breast cancer.

[0034] In one embodiment, provided herein is the use of any one of the enzymes or nucleic acids provided herein, or a composition comprising any one of the enzymes or nucleic acids provided herein, in the manufacture of a medicament for therapeutic application to a subject having cancer.

[0035] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0036] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0037] FIG. 1. Enzymatic activity graphs of wt hSDH and its engineered variants. Variant 1: K221F and A222S; Variant 2: S26N, R51H, S63P, M70I, L94P, G129D, A204S, K221R, and A222S; Variant 3: S26N, R51H, S63P, M70I, L94P, G129D, A204S, K221R, A222S, and L112S; Variant 4: I13T, S26N, R51H, I96T, D114E, G129D, K221R, A222S, W157R, D261L, and L112S; and Variant 5: I13T, S26N, R51H, I96T, D114E, G129D, K221R, A222G, W157R, D261L, and L112S. Calculated kcat/Km values are shown in Table 2.

[0038] FIG. 2. The effect of active (a, c, d) and heat-inactivated (d) variant 3, and active wt hSDH (b, c) on MCF7 cancer cell viability.

[0039] FIG. 3. Serine/threonine levels in mice serum with the administration of the variant 3. *$p<0.05$, **$p<0.01$. For each group of four bars, they represent, from left to right, "control", "4h", "24h", and "72h".

[0040] FIG. 4. Antitumoral response of the administration of engineered serine/threonine degrading enzymes in MC38 colon cancer bearing mice. *p<0.05, **p<0.01. In the top left panel, the five lines that are the most left on the graph are "control" and the six lines that are most right on the graph are "Variant 3 (50 mg/kg)". In the top right panel, the line that meets the X-axis at 20 days is "control" and the other line is "variant 3 (25 days)".

DETAILED DESCRIPTION

[0041] There is significant interest in therapeutically targeting tumor L-serine metabolism as it is the second most-consumed amino acid by cancer cells after glutamine due to its contributions to diverse anabolic reactions including biosynthesis of purines and thymidine, glutathione, phospholipid head groups, and sphingosine. Certain cancer types have been characterized as 'addicted' to extracellular serine due to increased metabolic demand or in some cases described as 'auxotrophic' for serine as they have lost the ability to produce it. Dietary serine restriction, serine synthesis inhibitors or combinations thereof despite only partially lowering plasma serine levels have been shown to suppress tumor growth in multiple *in vivo* cancer models and there are several companies currently developing these approaches for human cancer therapy.

[0042] Systemic application of therapeutic enzymes is a superior means of depleting key amino acids in comparison to dietary restriction as 1) the depth of depletion, i.e., reduction in systemic concentration of the amino acid can be far more significant, 2) can be controlled/titrated, and 3) circumvents significant issues with patient non-compliance to dietary regimens. As the growth of numerous tumors depends upon external sources of serine, a catalytically relevant human enzyme that degrades L-serine would provide a novel therapeutic agent for treating serine-dependent cancers. Human serine dehydratase (hSDH) is one such enzyme that degrades serine to pyruvate and ammonia.

[0043] Provided herein are compositions of matter comprising, and methods of using, engineered human SDH enzymes that efficiently deplete serine in serum and tumor tissues, and significantly suppress tumor growth in several *in vivo* tumor models including breast and colon cancers. As these enzymes are comprised from human sequence, they are not likely to cause undesired immunogenicity problems in patients. As wild-type human SDH has very poor catalytic activity/stability it was necessary to engineer variants with enhanced activity and stability for therapeutic applications, such as treating cancer. The engineered human SDH

enzymes provided herein are vastly superior in their ability to lower blood/plasma serine levels compared to technologies that are either based around dietary serine limitation or small molecule inhibitors of serine synthesis pathway enzymes. As such, these compositions provide a superior method for lowering serine levels that can be controlled by scheduled dosing, and circumvent significant issues with patient non-compliance to dietary regimens.

I. Definitions

[0044] The term "protein", "peptide", "polypeptide", and "enzyme" are used interchangeably and in their broadest sense to refer to a compound of two or more subunit amino acids, amino acid analogs or peptidomimetics. For example, the terms may refer to compounds comprising amino acids joined *via* peptide bonds. The terms also encompass an amino acid polymer that has been modified; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation, such as conjugation with a labeling component.

[0045] As used herein, the term "fusion protein" refers to a chimeric protein containing proteins or protein fragments operably linked in a non-naturally occurring way. A fusion protein may comprise a protein comprised of domains from more than one naturally occurring or recombinantly produced protein, where generally each domain serves a different function. In this regard, the term "linker" refers to a protein fragment that is used to link these domains together – optionally to preserve the conformation of the fused protein domains and/or prevent unfavorable interactions between the fused protein domains which may compromise their respective functions.

[0046] As used herein, the term "conjugate" refers to at least two molecules or molecular moieties being linked together by a covalent bond. A molecule or molecular moiety is considered to be "conjugated" to another molecule or molecular moiety once they are linked by the bond. Hence, "unconjugated" molecules or molecular moieties are not linked by a covalent bond.

[0047] The term "linker" is meant to refer to a compound or moiety that acts as a molecular bridge to operably link two different molecules, wherein one portion of the linker is operably linked to a first molecule, and wherein another portion of the linker is operably linked to a second molecule.

[0048] The term "PEGylation" refers to the process of covalent attachment of a polyethylene glycol (PEG) molecule(s) to another molecule including but not limited to a protein or a drug. PEG can be coupled (*e.g.*, covalently linked) to active agents through the hydroxy groups at the end of the PEG chain *via* chemical methods. A protein that has undergone PEGylation can be referred to as being "PEGylated". A PEGylated polypeptide may have properties that are qualitatively or quantitively different from a comparable polypeptide without a PEGylation or a polypeptide not PEGylated.

[0049] As used herein, the term "half-life" (½-life) refers to the time that would be required for the concentration of a polypeptide to fall by half *in vitro* (i.e., as measured in the cell culture media) or *in vivo* (i.e., as measured in serum), for example, after injection in a mammal.

[0050] The term "K_M" as used herein refers to the Michaelis-Menten constant for an enzyme and is defined as the concentration of the specific substrate at which a given enzyme yields one-half its maximum velocity in an enzyme catalyzed reaction. K_M may be used to measure the substrate binding affinity of an enzyme.

[0051] The term "k_{cat}" as used herein refers to the turnover number or the number of substrate molecules each enzyme site converts to product per unit time, and in which the enzyme is working at maximum efficiency. k_{cat} may be used to measure the catalytic rate of an enzyme.

[0052] The term "k_{cat}/K_M" as used herein is the specificity constant, which is a measure of how efficiently an enzyme converts a substrate into product. k_{cat}/K_M may be used to measure the catalytic efficiency of an enzyme.

[0053] The term "V_{max}" as used herein refers to the maximal rate of an enzymatic reaction. An enzymatic reaction may reach maximum velocity when the enzyme is saturated with substrates. V_{max} may be a product of k_{cat} and enzyme concentration. V_{max} may be used to measure the catalytic rate of an enzyme.

[0054] The term "catalytic activity" as used herein refers to any qualitative or quantitative properties of the enzymatic kinetics of substrate conversion or production of an enzyme. Catalytic activity may be measured using K_M, k_{cat}, V_{max}, or any derivatives herein and thereof. Other enzymatic kinetics measure may also be used.

[0055] The terms "in operable combination," "in operable order," and "operably linked" refer to a linkage wherein the components so described are in a relationship permitting them to function in their intended manner, for example, a linkage of nucleic acid sequences in such a manner that a nucleic acid molecule capable of directing the transcription of a given gene and/or the synthesis of desired protein molecule, or a linkage of amino acid sequences in such a manner so that a fusion protein is produced.

[0056] The term "gene" refers to a DNA sequence that comprises control and coding sequences necessary for the production of a polypeptide or precursor thereof. The polypeptide can be encoded by a full-length coding sequence or by any portion of the coding sequence so as the desired enzymatic activity is retained.

[0057] The term "native" refers to the typical or wild-type form of a gene, a gene product, or a characteristic of that gene or gene product when isolated from a naturally occurring source. In contrast, the term "engineered," "modified," "variant," "mutein," or "mutant" refers to a gene or gene product that displays modification in sequence and functional properties (*i.e.*, altered characteristics) when compared to the native gene or gene product, wherein the modified gene or gene product is genetically engineered and not naturally present or occurring.

[0058] "Canonical amino acids" refer to those 20 amino acids found naturally in the human body. Substitution, mutation, or replacement variants typically contain the exchange of one amino acid for another at one or more sites within the protein and may be designed to modulate one or more properties of the polypeptide, particularly its effector functions and/or bioavailability.

[0059] Substitutions may or may not be conservative, that is, one amino acid is replaced with one of similar shape and/or charge. Conservative substitutions are well known in the art and include, for example, the changes of: alanine to serine; arginine to lysine; asparagine to glutamine or histidine; aspartate to glutamate; cysteine to serine; glutamine to asparagine; glutamate to aspartate; glycine to proline; histidine to asparagine or glutamine; isoleucine to leucine or valine; leucine to valine or isoleucine; lysine to arginine; methionine to leucine or isoleucine; phenylalanine to tyrosine, leucine, methionine, or tryptophan; serine to threonine; threonine to serine; tryptophan to tyrosine; tyrosine to leucine, methionine, tryptophan, or phenylalanine; and valine to isoleucine or leucine.

[0060] The terms "polynucleotide", "oligonucleotide", or "nucleic acid" are used interchangeably and refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides or analogs thereof. Polynucleotides can have any three-dimensional structure and may perform any function, known or unknown. The following are non-limiting examples of polynucleotides: a gene or gene fragment (for example, a probe, primer, EST or SAGE tag), an exon, an intron, intergenic DNA (including, without limitation, heterochromatic DNA), messenger RNA (mRNA), cDNA, a recombinant polynucleotide, a branched polynucleotide, a plasmid, a vector, isolated DNA of a sequence, isolated RNA of a sequence. A polynucleotide can comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure can be imparted before or after assembly of the polynucleotide. The sequence of nucleotides can be interrupted by non-nucleotide components. A polynucleotide can be further modified after polymerization, such as by conjugation with a labeling component. The term also refers to both double and single stranded molecules. Nucleic acids, including e.g., nucleic acids with a phosphothioate backbone, can include one or more reactive moieties. As used herein, the term reactive moiety includes any group capable of reacting with another molecule, e.g., a nucleic acid or polypeptide through covalent, non-covalent or other interactions. By way of example, the nucleic acid can include an amino acid reactive moiety that reacts with an amino acid on a protein or polypeptide through a covalent, non-covalent, or other interaction. Unless otherwise specified or required, any embodiment of this disclosure that is a polynucleotide encompasses both the double stranded form and each of two complementary single stranded forms known or predicted to make up the double stranded form.

[0061] Polynucleotides useful in the methods of the disclosure can comprise natural nucleic acid sequences and variants thereof, artificial nucleic acid sequences, or a combination of such sequences. In some embodiments, polynucleotides of the disclosure refer to a DNA sequence. In some embodiments, the DNA sequence is interchangeable with a similar RNA sequence. In some embodiments, polynucleotides of the disclosure refer to an RNA sequence. In some embodiments, the RNA sequence is interchangeable with a similar DNA sequence. In some embodiments, Us and Ts of a polynucleotide may be interchanged in a sequence provided herein.

[0062] The term "vector" is used to refer to a carrier nucleic acid molecule into which a nucleic acid sequence can be inserted for introduction into a cell where it can be replicated. A nucleic acid sequence can be "exogenous," which means that it is foreign to the cell into which the vector is being introduced or that the sequence is homologous to a sequence in the cell but in a position within the host cell nucleic acid in which the sequence is ordinarily not found. Vectors include plasmids, cosmids, viruses (bacteriophage, animal viruses, and plant viruses), and artificial chromosomes (*e.g.*, YACs). One of skill in the art would be well equipped to construct a vector through standard recombinant techniques (*see*, for example, Maniatis *et al.*, 1988 and Ausubel *et al.*, 1994, both incorporated herein by reference).

[0063] The term "expression vector" refers to any type of genetic construct comprising a nucleic acid coding for an RNA capable of being transcribed. In some cases, RNA molecules are then translated into a protein, polypeptide, or peptide. In other cases, these sequences are not translated, for example, in the production of antisense molecules or ribozymes. Expression vectors can contain a variety of "control sequences," which refer to nucleic acid sequences necessary for the transcription and possibly translation of an operably linked coding sequence in a particular host cell. In addition to control sequences that govern transcription and translation, vectors and expression vectors may contain nucleic acid sequences that serve other functions as well.

[0064] "Homology" or "identity" or "similarity" can refer to sequence similarity between two peptides or between two nucleic acid molecules. Homology can be determined by comparing a position in each sequence which can be aligned for purposes of comparison. When a position in the compared sequence can be occupied by the same base or amino acid, then the molecules can be homologous at that position. A degree of homology between sequences can be a function of the number of matching or homologous positions shared by the sequences. An "unrelated" or "non-homologous" sequence shares less than 40% identity, or alternatively less than 25% identity, with one of the sequences of the disclosure. Sequence homology can refer to a % identity of a sequence to a reference sequence. As a practical matter, whether any particular sequence can be at least 50%, 60%, 70%, 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to any sequence described herein (which can correspond with a particular nucleic acid sequence described herein), such particular polypeptide sequence can be determined conventionally using known computer programs such the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix,

Genetics Computer Group, University Research Park, 575 Science Drive, Madison, Wis. 53711). When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence, the parameters can be set such that the percentage of identity can be calculated over the full length of the reference sequence and that gaps in sequence homology of up to 5% of the total reference sequence can be allowed.

[0065] The term "biologically functional equivalent" is well understood in the art and is further defined in detail herein. Accordingly, hSDH enzyme sequences that have about 90% or more sequence identity to any of SEQ ID NOs: 1-6, or even between about 91% and about 99% of amino acids (including 92%, 93%, 94%, 95%, 96%, 97%, 98%, and 99%) that are identical to or conservative substitutions of the amino acids of an engineered hSDH enzyme disclosed herein are included, provided the biological activity of the enzyme is maintained such that a measureable biological activity parameter (e.g., conversion of serine to pyruvate) is within about 20%, about 15%, about 10%, or about 5% of an engineered hSDH enzyme disclosed herein.

[0066] The term "effective amount" is an amount sufficient to effect beneficial or desired clinical results. An effective amount can be administered in one or more administrations. For purposes of this application, an effective amount of an antibody or polypeptide is an amount that is sufficient to diagnose, palliate, ameliorate, stabilize, reverse, slow or delay the progression of the disease state.

[0067] The term "unit dose" when used in reference to a therapeutic composition refers to physically discrete units suitable as unitary dosage for the subject, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required diluent, *i.e.*, carrier, or vehicle.

[0068] "Treatment" and "treating" refer to administration or application of a therapeutic agent to a subject or performance of a procedure or modality on a subject to obtain a therapeutic benefit of a disease or health-related condition. For example, treatment includes administration of a therapeutically effective amount of an engineered hSDH enzyme in order to reduce serum serine and/or threonine levels.

[0069] The terms "contacted" and "exposed," when applied to a cell, are used herein to describe the process by which a therapeutic construct is delivered to a target organ or are placed in direct juxtaposition with the target cell.

[0070] The terms "subject," "host," "patient," and "individual" are used interchangeably herein to refer to any mammalian subject for whom diagnosis or therapy is desired, particularly humans. Other subjects may include cattle, dogs, cats, guinea pigs, rabbits, rats, mice, horses, and so on.

[0071] The terms "cell," and "cells," and "cell population," used interchangeably, intend one or more mammalian cells. The term includes progeny of a cell or cell population. Those skilled in the art will recognize that "cells" include progeny of a single cell, and there are variations between the progeny and its original parent cell due to natural, accidental, or deliberate mutation or change.

[0072] The term "cancer cell" as used herein refers to a cell exhibiting a neoplastic cellular phenotype, which may be characterized by one or more of, for example, abnormal cell growth, abnormal cellular proliferation, loss of density dependent growth inhibition, anchorage-independent growth potential, ability to promote tumor growth or development in an immunocompromised non-human animal model, or any appropriate indicator of cellular transformation. "Cancer cell" may be used interchangeably herein with "tumor cell" or "cancerous cell" and encompasses cancer cells of a solid tumor and a liquid tumor. "Cancer" may be used interchangeably herein with "tumor".

[0073] The term "solid tumor" or "solid cancer" as used herein refers to tumors that usually do not contain cysts or liquid areas. Solid tumors can include brain and other central nervous system tumors (including but not limited to tumors of the meninges, brain, spinal cord, cranial nerves and other parts of central nervous system, e.g. glioblastomas or medulla blastomas); head or neck cancer; breast tumors; circulatory system tumors (including but not limited to heart, mediastinum and pleura, and other intrathoracic organs, vascular tumors and tumor-associated vascular tissue); excretory system tumors (including but not limited to tumors of kidney, renal pelvis, ureter, bladder, other and unspecified urinary organs); gastrointestinal tract tumors (including but not limited to tumors of oesophagus, stomach, small intestine, colon, colorectal, rectosigmoid junction, rectum, anus and anal canal, tumors involving the liver and intrahepatic bile ducts, gall bladder, other and unspecified parts of

biliary tract, pancreas, other and digestive organs); oral cavity tumors (including but not limited to tumors of lip, tongue, gum, floor of mouth, palate, and other parts of mouth, parotid gland, and other parts of the salivary glands, tonsil, oropharynx, nasopharynx, pyriform sinus, hypopharynx, and other sites in the lip, oral cavity and pharynx); reproductive system tumors (including but not limited to tumors of vulva, vagina, Cervix uteri, Corpus uteri, uterus, ovary, and other sites associated with female genital organs, placenta, penis, prostate, testis, and other sites associated with male genital organs); respiratory tract tumors (including but not limited to tumors of nasal cavity and middle ear, accessory sinuses, larynx, trachea, bronchus and lung, e.g. small cell lung cancer or non-small cell lung cancer); skeletal system tumors (including but not limited to tumors of bone and articular cartilage of limbs, bone articular cartilage and other sites); skin tumors (including but not limited to malignant melanoma of the skin, non-melanoma skin cancer, basal cell carcinoma of skin, squamous cell carcinoma of skin, mesothelioma, Kaposi's sarcoma); and tumors involving other tissues including peripheral nerves and autonomic nervous system, connective and soft tissue, retroperitoneum and peritoneum, eye and adnexa, thyroid, adrenal gland and other endocrine glands and related structures, secondary and unspecified malignant neoplasm of lymph nodes, secondary malignant neoplasm of respiratory and digestive systems and secondary malignant neoplasm of other sites.

[0074] The term "liquid cancer" or "liquid tumor" as used herein refers to cancer cells that are present in body fluids, such as blood, lymph and bone marrow. Liquid cancers include leukemia, myeloma, myelodysplastic syndrome (MDS), and liquid lymphomas. Liquid lymphomas include lymphomas that contain cysts or liquid areas. Liquid cancers as used herein do not include solid tumors, such as sarcomas and carcinomas or solid lymphomas that do not contain cysts or liquid areas.

[0075] As used herein, "essentially free," in terms of a specified component, is used herein to mean that none of the specified component has been purposefully formulated into a composition and/or is present only as a contaminant or in trace amounts. The total amount of the specified component resulting from any unintended contamination of a composition is therefore well below 0.05%, preferably below 0.01%. Most preferred is a composition in which no amount of the specified component can be detected with standard analytical methods.

[0076] As used herein the specification, "a" or "an" may mean one or more. As used herein in the claim(s), when used in conjunction with the word "comprising," the words "a" or "an" may mean one or more than one.

[0077] The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or." As used herein "another" may mean at least a second or more.

[0078] Throughout this application, the term "about" is used to indicate that a value includes the inherent variation of error for the device, the inherent variation in the method being employed to determine the value, the variation that exists among the study subjects, or a value that is within 10% of a stated value.

II. Enzymes for Serine and/or Threonine Degradation

[0079] Described herein are engineered hSDH enzymes that exhibit at least one functional activity that is improved relative to the unmodified hSDH enzyme. Engineered hSDH enzymes include, for example, a protein that possesses the additional advantage of having improved serine and/or threonine degrading activity, compared to the unmodified hSDH enzyme. An engineered hSDH, in some instances, can function to degrade both serine and threonine with therapeutically relevant kinetics. The unmodified protein or polypeptide may be native human serine dehydratase (SEQ ID NO: 1). An engineered hSDH enzyme, in some instances, can be used to treat a disease. In some instances, a disease can comprise a cancer. This method removes serine and/or threonine from circulation, which has been shown clinically to reduce or delay tumor growth in cancer patients.

[0080] An engineered hSDH enzyme may be identified based on its increase in serine and/or threonine degrading activity. For example, substrate recognition sites of the unmodified polypeptide may be identified. This identification may be based on structural analysis or homology analysis. A population of mutants involving modifications of such substrate recognitions sites may be generated. Mutants with increased serine and/or threonine degrading activity may be selected from the mutant population. Selection of desired mutants may include methods for the detection of byproducts or products from serine and/or threonine degradation.

[0081] Determination of activity may be achieved using assays familiar to those of skill in the art, particularly with respect to the protein's activity, and may include for comparison purposes, for example, the use of native and/or recombinant versions of either the engineered or unmodified enzymes. For example, wild-type human SDH catalyzes the irreversible deamination reactions of L-serine to pyruvate and L-threonine to α-ketobutyrate with a shared second product: ammonia (k_{cat}/K_M ~ 0.6 s^{-1} mM^{-1} and ~1.1 s^{-1} mM^{-1}, respectively). Thus, the serum degrading activity may be determined by any assay to detect the production of any substrates resulting from the degradation of serine and/or threonine, such as the detection of the reaction product pyruvate following derivatization with the agent 3-methyl-2-benzothiazolinone hydrazone (MBTH).

[0082] Engineered hSDH enzymes may possess deletions and/or substitutions of amino acids; thus, an enzyme with a deletion, an enzyme with a substitution, and an enzyme with a deletion and a substitution are engineered hSDH enzymes. These engineered hSDH enzymes may further include insertions or added amino acids, such as with fusion proteins or proteins with linkers, for example. An "engineered deleted hSDH enzyme" lacks one or more residues of the native enzyme (e.g., the initial methionine), but may possess the specificity and/or activity of the native enzyme. An engineered deleted hSDH enzyme may also have reduced immunogenicity or antigenicity. An example of an engineered deleted hSDH enzyme is one that has an amino acid residue deleted from at least one antigenic region, that is, a region of the enzyme determined to be antigenic in a particular organism, such as the type of organism that may be administered the engineered hSDH enzyme.

[0083] Substitution or replacement hSDH enzyme variants may contain the exchange of one amino acid for another at one or more sites within the protein and may be designed to modulate one or more properties of the polypeptide, particularly its effector functions and/or bioavailability. Substitutions may or may not be conservative, that is, one amino acid is replaced with one of similar size and charge. Conservative substitutions are well known in the art and include, for example, the changes of: alanine to serine; arginine to lysine; asparagine to glutamine or histidine; aspartate to glutamate; cysteine to serine; glutamine to asparagine; glutamate to aspartate; glycine to proline; histidine to asparagine or glutamine; isoleucine to leucine or valine; leucine to valine or isoleucine; lysine to arginine; methionine to leucine or isoleucine; phenylalanine to tyrosine, leucine, or methionine; serine to

threonine; threonine to serine; tryptophan to tyrosine; tyrosine to tryptophan or phenylalanine; and valine to isoleucine or leucine.

[0084] In some embodiments, a naturally occurring hSDH enzyme may be modified by one or more other modifications, such as chemical modifications, substitutions, insertions, deletions, fusion, or truncations. In some embodiments, a naturally occurring hSDH enzyme can be modified by substitutions. In some embodiments, a naturally occurring hSDH enzyme can be modified by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more substitutions. In some embodiments, a naturally occurring hSDH enzyme can be modified by a conservative substitution, a non-conservative substitution, a deletion or an insertion at a site that is outside of its active site and still maintain its catalytic activity. In other cases, a naturally occurring hSDH enzyme can be modified in a location that can modulate the protein binding activity, protein stability, protein localization, non-substrate binding activity, chemical modification activity, protein ability to be chemically modified, protein folding, protein transport, any derivatives herein and thereof, or any combination herein and thereof. A modulation described herein and thereof can comprise an increase or decrease.

[0085] In some embodiments, a naturally occurring hSDH enzyme can comprise the sequence or a portion thereof of SEQ ID NO: 1.

[0086] In some cases, an engineered hSDH enzyme with serine and/or threonine degrading activity can comprise sequence having about, at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity (or any range derivable therein) to SEQ ID NO: 1 or a portion thereof. In some embodiments, an engineered hSDH enzyme with serine and/or threonine degrading activity can comprise at least or up to about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, or 327 residues of SEQ ID NO: 1. In some cases, an engineered hSDH enzyme with serine and/or threonine degrading activity can comprise 1-10, 5-15, 10-20, 15-25, 20-30, 25-35, 30-40, 35-45, 40-50, 45-55, 50-60, 55-65, 60-70, 65-75, 70-80, 75-85, 80-90, 85-95, 90-100, 95-105, 100-110, 105-115, 110-120, 115-125, 120-130, 125-135, 130-140, 135-145, 140-150, 145-155, 150-160, 155-165, 160-170, 165-175, 170-180, 175-185, 180-190, 185-195, 190-200, 195-205, 200-210, 205-215, 210-220, 215-225, 220-230, 225-235, 230-240, 235-245, 240-250, 245-255, 250-260, 255-265, 260-270, 265-

275, 270-280, 275-285, 280-290, 285-295, 290-300, 295-305, 300-310, 305-315, 310-320, 315-325 or 320-327 residues of SEQ ID NO: 1. In some cases, an engineered hSDH enzyme with serine and/or threonine degrading activity can comprise a sequence having about at least 80% identity to at least 200 amino acids of SEQ ID NO: 1.

[0087] In some embodiments, an engineered hSDH enzyme can comprise the sequence or a portion thereof of any of SEQ ID NOs: 2-6.

[0088] In some cases, an engineered hSDH enzyme with serine and/or threonine degrading activity can comprise sequence having about, at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity (or any range derivable therein) to any of SEQ ID NOs: 2-6 or a portion thereof. In some embodiments, an engineered hSDH enzyme with serine and/or threonine degrading activity can comprise at least or up to about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, or 327 residues of any of SEQ ID NOs: 2-6. In some cases, an engineered hSDH enzyme with serine and/or threonine degrading activity can comprise 1-10, 5-15, 10-20, 15-25, 20-30, 25-35, 30-40, 35-45, 40-50, 45-55, 50-60, 55-65, 60-70, 65-75, 70-80, 75-85, 80-90, 85-95, 90-100, 95-105, 100-110, 105-115, 110-120, 115-125, 120-130, 125-135, 130-140, 135-145, 140-150, 145-155, 150-160, 155-165, 160-170, 165-175, 170-180, 175-185, 180-190, 185-195, 190-200, 195-205, 200-210, 205-215, 210-220, 215-225, 220-230, 225-235, 230-240, 235-245, 240-250, 245-255, 250-260, 255-265, 260-270, 265-275, 270-280, 275-285, 280-290, 285-295, 290-300, 295-305, 300-310, 305-315, 310-320, 315-325 or 320-327 residues of any of SEQ ID NOs: 2-6. In some cases, an engineered hSDH enzyme with serine and/or threonine degrading activity can comprise a sequence having about at least 80% identity to at least 200 amino acids of any of SEQ ID NOs: 2-6. In some cases, an engineered hSDH enzyme can comprise sequence having about at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 2 and Phe221 and Ser222 of SEQ ID NO: 2. In some cases, an engineered hSDH enzyme can comprise sequence having about at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 2 and Asn26, His51, Pro63, Ile70, Pro94, Asp129, Ser204, Arg221, and Ser222 of SEQ ID NO: 3. In some cases, an engineered hSDH enzyme can comprise sequence having about at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 4 and Asn26, His51, Pro63, Ile70, Pro94, Asp129, Ser204, Arg221, Ser222, and Ser112 of SEQ ID NO: 4. In some cases, an engineered hSDH enzyme

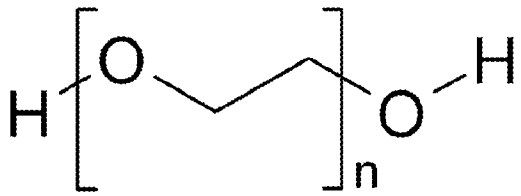
can comprise sequence having about at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 5 and Thr13, Asn26, His51, Thr96, Glu114, Asp129, Arg221, Ser222, Arg157, Leu261, and Ser112 of SEQ ID NO: 5. In some cases, an engineered hSDH enzyme can comprise sequence having about at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 6 and Thr13, Asn26, His51, Thr96, Glu114, Asp129, Arg221, Gly222, Arg157, Leu261, and Ser112 of SEQ ID NO: 6.

[0089] In some instances, a measurement of the serine and/or threonine degrading activity of any engineered hSDH enzyme described herein and thereof can comprise measuring the V_0, V_{max}, K_M, k_{cat}, or a combination thereof of the engineered hSDH enzyme. In some cases, the measurement can comprise an in vitro reaction. In other cases, the measurement can comprise an in vivo reaction.

A. PEGylation

1. Polyethylene Glycol (PEG)

[0090] In some instances, a PEG molecule can comprise a polymer. In some cases, a PEG molecule can comprise homopolymer of ethylene glycol or ethylene oxide. In some cases, a PEG molecule can comprise polyethylene oxide (PEO) or polyoxyethylene (POE). In some cases, a PEG molecule can comprise Formula I:



(I)

[0091] Wherein n is the number of units. In some cases, a PEG molecule can have a molecular weight 44.05n + 18.02 g/mol, where n is the number of units as in Formula I. In some instances, the molecular weight of a PEG molecule species can be used to calculate the number of units, such as that of Formula I or derivatives herein and thereof, and vice versa. In some cases, a PEG molecule can also have a formula H–(O–CH2–CH2)n–OH or $C2_nH_{4n+2}O_{n+1}$, where n is the number of unit.

[0092] In some cases, a PEG molecule can be represented as PEG-N, where N can comprise the number of units, such as n in Formula I. In some cases, a PEG molecule can

also have a formula H–(O–CH2–CH2)n–OH or $C2_nH_{4n+2}O_{n+1}$, where n is the number of units. In some cases, a PEG molecule with different number of units can have different properties. In some cases, a PEG molecule with low molecular weight can be viscous or colorless liquids. In other cases, a PEG molecule with high molecular weight can be crystallized with high melting points. A high melting point can be higher than 70 °C.

[0093] In some cases, a PEG molecule can have an average molecular weight of about 100 kDa, 150 kDa, 200 kDa, 250 kDa, 300 kDa, 350 kDa, 400 kDa, 450 kDa, 500 kDa, 550 kDa, 600 kDa, 650 kDa, 700 kDa, 750 kDa, 800 kDa, 850 kDa, 900 kDa, 950 kDa, 1000 kDa, 1050 kDa, 1100 kDa, 1150 kDa, 1200 kDa, 1250 kDa, 1300 kDa, 1350 kDa, 1400 kDa, 1450 kDa, 1500 kDa, 1550 kDa, 1600 kDa, 1650 kDa, 1700 kDa, 1750 kDa, 1800 kDa, 1850 kDa, 1900 kDa, 1950 kDa, 2000 kDa, 2050 kDa, 2100 kDa, 2150 kDa, 2200 kDa, 2250 kDa, 2300 kDa, 2350 kDa, 2400 kDa, 2450 kDa, 2500 kDa, 2550 kDa, 2600 kDa, 2650 kDa, 2700 kDa, 2750 kDa, 2800 kDa, 2850 kDa, 2900 kDa, 2950 kDa, 3000 kDa, 3050 kDa, 3100 kDa, 3150 kDa, 3200 kDa, 3250 kDa, 3300 kDa, 3350 kDa, 3400 kDa, 3450 kDa, 3500 kDa, 4000 kDa, 5000 kDa, 6000 kDa, 7000 kDa, 8000 kDa, 9000 kDa, 10000 kDa, 11000 kDa, 12000 kDa, 13000 kDa, 14000 kDa, 15000 kDa, 16000 kDa, 17000 kDa, 18000 kDa, 19000 kDa, 20000 kDa, 21000 kDa, 22000 kDa, 23000 kDa, 24000 kDa, 25000 kDa, 26000 kDa, 27000 kDa, 28000 kDa, 29000 kDa, 30000 kDa, 31000 kDa, 32000 kDa, 33000 kDa, 34000 kDa, 35000 kDa or more than 35000 kDa. In some cases, a PEG molecule can have an average molecular weight from about 100 to about 1000 kDa, from about 500 to about 1500 kDa, from about 1000 to about 2000 kDa, from about 1500 to about 2500 kDa, from about 2000 to about 3000 kDa, from about 2500 to about 3500 kDa, from about 3000 to about 4000 kDa, from about 3500 to about 4500 kDa, from about 4000 to about 5000 kDa, from about 4500 to about 5500 kDa, from about 5000 to about 6000 kDa, from about 5500 to about 6500 kDa, from about 6000 to about 7000 kDa, from about 6500 to about 7500 kDa, from about 7000 to about 8000 kDa, from about 7500 to about 8500 kDa, from about 8000 to about 9000 kDa, from about 8500 to about 9500 kDa, from about 9000 to about 10000 kDa, from about 9500 to about 10500 kDa, from about 10000 to about 11000 kDa, from about 10500 to about 11500 kDa, from about 11000 to about 12000 kDa, from about 11500 to about 12500 kDa, from about 12000 to about 13000 kDa, from about 12500 to about 13500 kDa, from about 13000 to about 14000 kDa, from about 13500 to about 14500 kDa, from about 14000 to about 15000 kDa, from about 14500 to about 15500 kDa, from about 15000 to about 16000 kDa, from about 15500 to about 16500 kDa, from about 16000 to about 17000 kDa, from

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[0094] In some instances, a PEG molecule can comprise a glass transition temperature (T_g) from -40 °C to -70 °C. In some cases, a PEG molecule can be dissolved in polar or nonpolar solvents. In some instances, a PEG molecule can be dissolved in hydrophilic solvents. In some instances, a PEG molecule can dissolve in organic solvents. One such organic solvent can comprise alcohol, methylene chloride, acetone, toluene, acetonitrile, benzene, dichloromethane, chloroform, derivatives herein and thereof, or any combination herein and thereof. In other cases, a PEG molecule can also be amphiphilic.

[0095] In some instances, a PEG molecule can have a branched, star, linear, comb-like, structure; derivatives herein and thereof; or any combinations herein and thereof. In some instances, a PEG molecule can comprise a terminal hydroxyl group. In other cases, a

PEG molecule can convert a terminal hydroxyl group into a symmetric or asymmetric functional group. In some cases, a PEG molecule or any manufactures comprising a PEG molecule can be bioinert. Being bioinert may comprise a resistance to biological reaction. One such biological reaction can comprise degradation of a PEG molecule or any manufactures comprising PEG. In other cases, being bioinert can mean having a minimal intrinsic biological activity. In some cases, a PEG molecule can have low immunogenicity. In some cases, a PEG molecule may not have immunogenicity. Immunogenicity of an entity can comprise the ability of an entity to elicit or activate an immune response against the entity when the entity is presented or administered to or detected by an immune system. In some cases, a PEG molecule may not elicit an immune response directed to the PEG molecule when administered or presented to an immune system. In other cases, a PEG molecule may not be toxic. In some instances, a PEG molecule may create a high osmotic pressure. In some cases, a PEG molecule can be crosslinked into a hydrogel. In other cases, a PEG molecule may not carry a charge.

[0096] In some cases, PEO or POE can have a molecular weight of at least about 1x10^5 g/mol, 2x10^5 g/mol, 3x10^5 g/mol, 4x10^5 g/mol, 5x10^5 g/mol, 6x10^5 g/mol, 7x10^5 g, 8x10^5 g/mol, 9x10^5 g/mol, 1x10^6 g/mol, 2x10^6 g/mol, 3x10^6 g/mol, 4x10^6 g/mol, 5x10^6 g/mol, 6x10^6 g/mol, 7x10^6 g/mol, 8x10^6 g/mol, 9x10^6 g/mol, 1x10^7 g/mol, or more.

2. Methods of PEGylation

[0097] In some instances, PEGylation of an engineered hSDH enzyme can be achieved by incubation of a reactive derivative of PEG with the engineered hSDH enzyme. In some cases, PEGylation can be covalent. In other cases, PEGylation can be non-covalent. In some instances, PEGylation of an engineered hSDH enzyme can mean an engineered hSDH enzyme being conjugated to PEG. In other cases, PEGylation can comprise linking a PEG molecule to a recombinant hSDH enzyme, fragment herein and thereof, or portion herein and thereof.

[0098] In some instances, a PEG molecule can be attached, conjugate, or linked to an engineered hSDH enzyme at a lysine, serine, histidine, tyrosine, cysteine, N-terminal amine, phenylalanine, C-terminal cysteine, arginine, aspartic acid, glutamic acid, serine, threonine, asparagine, N-terminus, C-terminus, or any combination herein and thereof of the engineered hSDH enzyme. In some cases, a PEG molecule can be attached, conjugate, or linked to an

engineered hSDH enzyme at a lysine or cysteine of the engineered hSDH enzyme. In some instances, the C-terminal carboxylic acid can also be used in PEGylation of an engineered hSDH enzyme.

[0099] In some instances, a PEG molecule can be activated at each terminus with the same reactive moiety, i.e., the PEG molecule is "homobifunctional". In other cases, a PEG molecule can be activated at each terminus a different reactive moiety, i.e., then the PEG molecule is "heterobifunctional" or "heterofunctional". In some cases, a chemically active or activated derivatives of the PEG molecule can be prepared to attach the PEG molecule to an engineered hSDH enzyme.

[00100] In some instances, a PEG molecule can be attached, conjugate, or linked to an engineered hSDH enzyme at succinimidyl ester, aldehyde, maleimide, *p*-nitrophenyl carbonate ester, any derivative herein and thereof, or any combinations herein and thereof. In some cases, the generation of a PEG molecule for PEGylation of an engineered hSDH enzyme can comprise reacting the PEG molecule with a group that is reactive with hydroxyl groups comprising anhydrides, acid chlorides, chloroformates, or carbonates. In other cases, the generation of a PEG molecule for PEGylation of an engineered hSDH enzyme can comprise reacting the PEG molecule with a group that is reactive with functional groups comprising aldehyde, esters, or amides. In some cases, a methoxy PEG molecule can be used for PEGylation of an engineered hSDH enzyme. In some instances, a polyethylene glycol (PEG diol) can also be used for PEGylation of an engineered hSDH enzyme. In some cases, the diol group can be modified at both ends in order to make a hetero- or homo-dimeric PEGylated engineered hSDH enzyme.

[00101] In some cases, an engineered hSDH enzyme can be PEGylated at nucleophilic sites. In other cases, a nucleophilic site can comprise an unprotonated thiol group or an amino or amine group. In some instances, an amino or amine group on the side chain of a lysine or the N-terminus of a protein can be used for PEGylation of an engineered hSDH enzyme. In some cases, a protein can be conjugated to a PEG molecule through the alkylation of an amine or amino group of on the side chain of a lysine or the N-terminus of the protein. In some cases, a PEG-aldehyde, NHS-PEG, PEG tresylate, PEG isothiocyanate or succinimide a PEG molecule can be used to conjugate the amino group on the side chain of a lysine on an engineered hSDH enzyme. In other cases, succinimidyl carbonate (PEG-SC), benzotriazole carbonate (PEG-BTC), phenyl carbonate, carbonylimidazole, or

thiazolidine-2-thione can also be used for PEGylation of an engineered hSDH enzyme. In some instances, a cyanuric chloride activated a PEG molecule can be used to react with the primary amine groups of a lysine of an engineered hSDH enzyme. In some instances, a PEG aldehyde derivative (e.g., Methoxy PEG propionaldehyde or mPEG-propionaldehyde) can be used to forma stable secondary amine linkage with an amino group of lysine of an engineered hSDH enzyme through reductive alkylation using sodium cyanoborohydride. In some cases, a reaction with a low pH can be used to direct PEGylation to specific groups of amino groups. Such specific groups of amino groups can comprise an amino group with low pKa.

[00102] In some instances, a unprotonated thiol group can comprise a side chain of a cysteine. In some cases, PEG maleimide, PEG iodoacetate, PEG thiols, or PEG vinylsulfone can be used to PEGylate an engineered hSDH enzyme at a cysteine. In some cases, a native cysteine can be used for PEGylation. In other cases, a cysteine residue can be engineered into an engineered hSDH enzyme for PEGylation. Such engineering can comprise adding a cysteine to an engineered hSDH enzyme. In other cases, a cysteine can replace any amino acid residue of an engineered hSDH enzyme. In some instances, a cysteine based PEGylation can comprise reacting a maleimide group attached to PEG with a free cysteine. A free cysteine, in some cases, may not be involved in a disulfide bond.

[00103] In some instances, PEG can be conjugated to an engineered hSDH enzyme at a disulfide bond of the engineered hSDH enzyme. In some cases, disulfide bond based PEGylation can comprise reducing a disulfide under mild conditions and labeling the cysteines involved in the disulfide with a bis(thiol)-specific reagent. In such cases, dithiomaleimide can be used to create the disulfide bond based PEGylation.

[00104] In some cases, PEG can be conjugated to an engineered hSDH enzyme at a tyrosine residue. In some instances, a tyrosine of an engineered hSDH enzyme can be reacted with a 4-phenyl-3H-1,2,4-triazoline-3,5(4H)-dione (PTAD) to create a covalent bond between the hSDH and PEG. In some cases, a PEG-PTAD conjugate can be created first and reacted with a tyrosine of an engineered hSDH enzyme.

[00105] In some cases, a PEGylated can be prepared by direct chemical synthesis of an engineered hSDH enzyme, wherein the engineered hSDH enzyme is attached to a solid phase peptide synthesis (SPPS) and PEG is incorporated in one of the coupling steps or through direct chemical attachment of a native chemical feature of the engineered

hSDH enzyme. In some cases, a native chemical feature can comprise a chemical feature present in the PEGylation conjugation site described herein and thereof. In some cases, a native chemical feature can also comprise an alkylating-containing residue. In other cases, an azide-PEG conjugate can be attached to an alkylating-containing residue with the Huisgen 1,3-dipolar cycloaddition. In other cases, PEG can be conjugated to an engineered hSDH enzyme using an Fmoc-asparagine, where the Fmoc-asparagine is incorporated to the engineered hSDH enzyme during SPPS.

[00106] In some cases, for amine-based PEGylation of an engineered hSDH enzyme, the parameters to be optimized in the PEGylation reaction can comprise the polypeptide concentration, PEG-to-polypeptide ratio on a molar basis, temperature, or pH, reaction time. In some cases, for thiol-based PEGylation of an engineered hSDH enzyme, the parameters to be optimized in the PEGylation reaction can comprise the polypeptide concentration, PEG-to-polypeptide ratio on a molar basis, temperature, pH, reaction time, or exclusion of oxygen. In some instances, the PEGylation reaction can affect the stability of an engineered hSDH enzyme. the reactivity of a PEG molecule should be known before starting a PEGylation reaction. In some cases, for example, if a PEG molecule is only 70% active in a PEGylation reaction of an engineered hSDH enzyme, the amount of the PEG molecule used should ensure that only active PEG molecules can be counted in the hSHD-to-PEG reaction stoichiometry.

3. PEGylated Enzymes

[00107] In some instances, a PEGylated engineered hSDH enzyme can have a modified, eliminated, or added property compared to an engineered hSDH enzyme that is not PEGylated. In some instances, a PEGylated engineered hSDH enzyme can have a modulated property compared to an engineered hSDH enzyme that is not PEGylated. In some cases, a PEGylated engineered hSDH enzyme can a modulated size, solubility, accessibility for proteolytic enzyme, immunogenicity and antigenicity, body-residence, stability, or any combination herein and thereof. In some cases, a PEGylated engineered hSDH enzyme can have an increase in size, increase in solubility, decreased accessibility for proteolytic enzyme, decrease in immunogenicity and antigenicity, increase in body-residence time and stability, or any combination herein and thereof.

[00108] In some cases, an engineered hSDH enzyme can be PEGylated at a lysine residue. In some instances, an engineered hSDH enzyme can be engineered to remove

a lysine residue to prevent the lysine residue from being PEGylated. In some instances, an engineered hSDH enzyme can be engineered to replace a lysine residue with another amino acid to prevent the lysine residue from being PEGylated. In some cases, the lysine residue that is substituted with another amino acid in an engineered hSDH enzyme can be located close to an active site or a binding site.

[00109] In some cases, an engineered hSDH enzyme can be PEGylated at a cysteine residue. In some instances, an engineered hSDH enzyme can be engineered to remove a cysteine residue to prevent the cysteine residue from being PEGylated. In some instances, an engineered hSDH enzyme can be engineered to replace a cysteine residue with another amino acid to prevent the cysteine residue from being PEGylated. In some cases, the cysteine being replaced with another amino acid in an engineered hSDH enzyme can be located close to an active site or a binding site.

[00110] In some instances, a PEGylated or non-PEGylated engineered hSDH enzyme can have a k_{cat}/K_M for serine and/or threonine of at least about 1x10^3 $M^{-1}s^{-1}$, 1.1x10^3 $M^{-1}s^{-1}$, 1.2x10^3 $M^{-1}s^{-1}$, 1.3x10^3 $M^{-1}s^{-1}$, 1.4x10^3 $M^{-1}s^{-1}$, 1.5x10^3 $M^{-1}s^{-1}$, 1.6x10^3 $M^{-1}s^{-1}$, 1.7x10^3 $M^{-1}s^{-1}$, 1.8x10^3 $M^{-1}s^{-1}$, 1.9x10^3 $M^{-1}s^{-1}$, 2x10^3 $M^{-1}s^{-1}$, 2.1x10^3 $M^{-1}s^{-1}$, 2.2x10^3 $M^{-1}s^{-1}$, 2.3x10^3 $M^{-1}s^{-1}$, 2.4x10^3 $M^{-1}s^{-1}$, 2.5x10^3 $M^{-1}s^{-1}$, 2.6x10^3 $M^{-1}s^{-1}$, 2.7x10^3 $M^{-1}s^{-1}$, 2.8x10^3 $M^{-1}s^{-1}$, 2.9x10^3 $M^{-1}s^{-1}$, 3x10^3 $M^{-1}s^{-1}$, 3.1x10^3 $M^{-1}s^{-1}$, 3.2x10^3 $M^{-1}s^{-1}$, 3.3x10^3 $M^{-1}s^{-1}$, 3.4x10^3 $M^{-1}s^{-1}$, 3.5x10^3 $M^{-1}s^{-1}$, 3.6x10^3 $M^{-1}s^{-1}$, 3.7x10^3 $M^{-1}s^{-1}$, 3.8x10^3 $M^{-1}s^{-1}$, 3.9x10^3 $M^{-1}s^{-1}$, 4x10^3 $M^{-1}s^{-1}$, 4.1x10^3 $M^{-1}s^{-1}$, 4.2x10^3 $M^{-1}s^{-1}$, 4.3x10^3 $M^{-1}s^{-1}$, 4.4x10^3 $M^{-1}s^{-1}$, 4.5x10^3 $M^{-1}s^{-1}$, 4.6x10^3 $M^{-1}s^{-1}$, 4.7x10^3 $M^{-1}s^{-1}$, 4.8x10^3 $M^{-1}s^{-1}$, 4.9x10^3 $M^{-1}s^{-1}$, 5x10^3 $M^{-1}s^{-1}$, 5.1x10^3 $M^{-1}s^{-1}$, 5.2x10^3 $M^{-1}s^{-1}$, 5.3x10^3 $M^{-1}s^{-1}$, 5.4x10^3 $M^{-1}s^{-1}$, 5.5x10^3 $M^{-1}s^{-1}$, 5.6x10^3 $M^{-1}s^{-1}$, 5.7x10^3 $M^{-1}s^{-1}$, 5.8x10^3 $M^{-1}s^{-1}$, 5.9x10^3 $M^{-1}s^{-1}$, 6x10^3 $M^{-1}s^{-1}$, 6.1x10^3 $M^{-1}s^{-1}$, 6.2x10^3 $M^{-1}s^{-1}$, 6.3x10^3 $M^{-1}s^{-1}$, 6.4x10^3 $M^{-1}s^{-1}$, 6.5x10^3 $M^{-1}s^{-1}$, 6.6x10^3 $M^{-1}s^{-1}$, 6.7x10^3 $M^{-1}s^{-1}$, 6.8x10^3 $M^{-1}s^{-1}$, 6.9x10^3 $M^{-1}s^{-1}$, 7x10^3 $M^{-1}s^{-1}$, 7.1x10^3 $M^{-1}s^{-1}$, 7.2x10^3 $M^{-1}s^{-1}$, 7.3x10^3 $M^{-1}s^{-1}$, 7.4x10^3 $M^{-1}s^{-1}$, 7.5x10^3 $M^{-1}s^{-1}$, 7.6x10^3 $M^{-1}s^{-1}$, 7.7x10^3 $M^{-1}s^{-1}$, 7.8x10^3 $M^{-1}s^{-1}$, 7.9x10^3 $M^{-1}s^{-1}$, 8x10^3 $M^{-1}s^{-1}$, 8.1x10^3 $M^{-1}s^{-1}$, 8.2x10^3 $M^{-1}s^{-1}$, 8.3x10^3 $M^{-1}s^{-1}$, 8.4x10^3 $M^{-1}s^{-1}$, 8.5x10^3 $M^{-1}s^{-1}$, 8.6x10^3 $M^{-1}s^{-1}$, 8.7x10^3 $M^{-1}s^{-1}$, 8.8x10^3 $M^{-1}s^{-1}$, 8.9x10^3 $M^{-1}s^{-1}$, 9x10^3 $M^{-1}s^{-1}$, 9.1x10^3 $M^{-1}s^{-1}$, 9.2x10^3 $M^{-1}s^{-1}$, 9.3x10^3 $M^{-1}s^{-1}$, 9.4x10^3 $M^{-1}s^{-1}$, 9.5x10^3 $M^{-1}s^{-1}$, 9.6x10^3 $M^{-1}s^{-1}$, 9.7x10^3 $M^{-1}s^{-1}$, 9.8x10^3 $M^{-1}s^{-1}$, 9.9x10^3 $M^{-1}s^{-1}$, 1x10^4 $M^{-1}s^{-1}$, 2x10^4 $M^{-1}s^{-1}$, 3x10^4 $M^{-1}s^{-1}$, 4x10^4 $M^{-1}s^{-1}$, or 5x10^4 $M^{-1}s^{-1}$.

[00111] In some cases, a PEGylated or non-PEGylated engineered hSDH enzyme can have a k_{cat}/K_M for serine and/or threonine from 9x10^2 to 1.1x10^3 $M^{-1}s^{-1}$, from 1x10^3 to 1.2x10^3 $M^{-1}s^{-1}$, from 1.1x10^3 to 1.3x10^3 $M^{-1}s^{-1}$, from 1.2x10^3 to 1.4x10^3 $M^{-1}s^{-1}$, from 1.3x10^3 to 1.5x10^3 $M^{-1}s^{-1}$, from 1.4x10^3 to 1.6x10^3 $M^{-1}s^{-1}$, from 1.5x10^3 to 1.7x10^3 $M^{-1}s^{-1}$, from 1.6x10^3 to 1.8x10^3 $M^{-1}s^{-1}$, from 1.7x10^3 to 1.9x10^3 $M^{-1}s^{-1}$, from 1.8x10^3 to 2x10^3 $M^{-1}s^{-1}$, from 1.9x10^3 to 2.1x10^3 $M^{-1}s^{-1}$, from 2x10^3 to 2.2x10^3 $M^{-1}s^{-1}$, from 2.1x10^3 to 2.3x10^3 $M^{-1}s^{-1}$, from 2.2x10^3 to 2.4x10^3 $M^{-1}s^{-1}$, from 2.3x10^3 to 2.5x10^3 $M^{-1}s^{-1}$, from 2.4x10^3 to 2.6x10^3 $M^{-1}s^{-1}$, from 2.5x10^3 to 2.7x10^3 $M^{-1}s^{-1}$, from 2.6x10^3 to 2.8x10^3 $M^{-1}s^{-1}$, from 2.7x10^3 to 2.9x10^3 $M^{-1}s^{-1}$, from 2.8x10^3 to 3x10^3 $M^{-1}s^{-1}$, from 2.9x10^3 to 3.1x10^3 $M^{-1}s^{-1}$, from 3x10^3 to 3.2x10^3 $M^{-1}s^{-1}$, from 3.1x10^3 to 3.3x10^3 $M^{-1}s^{-1}$, from 3.2x10^3 to 3.4x10^3 $M^{-1}s^{-1}$, from 3.3x10^3 to 3.5x10^3 $M^{-1}s^{-1}$, from 3.4x10^3 to 3.6x10^3 $M^{-1}s^{-1}$, from 3.5x10^3 to 3.7x10^3 $M^{-1}s^{-1}$, from 3.6x10^3 to 3.8x10^3 $M^{-1}s^{-1}$, from 3.7x10^3 to 3.9x10^3 $M^{-1}s^{-1}$, from 3.8x10^3 to 4x10^3 $M^{-1}s^{-1}$, from 3.9x10^3 to 4.1x10^3 $M^{-1}s^{-1}$, from 4x10^3 to 4.2x10^3 $M^{-1}s^{-1}$, from 4.1x10^3 to 4.3x10^3 $M^{-1}s^{-1}$, from 4.2x10^3 to 4.4x10^3 $M^{-1}s^{-1}$, from 4.3x10^3 to 4.5x10^3 $M^{-1}s^{-1}$, from 4.4x10^3 to 4.6x10^3 $M^{-1}s^{-1}$, from 4.5x10^3 to 4.7x10^3 $M^{-1}s^{-1}$, from 4.6x10^3 to 4.8x10^3 $M^{-1}s^{-1}$, from 4.7x10^3 to 4.9x10^3 $M^{-1}s^{-1}$, from 4.8x10^3 to 5x10^3 $M^{-1}s^{-1}$, from 4.9x10^3 to 5.1x10^3 $M^{-1}s^{-1}$, from 5x10^3 to 5.2x10^3 $M^{-1}s^{-1}$, from 5.1x10^3 to 5.3x10^3 $M^{-1}s^{-1}$, from 5.2x10^3 to 5.4x10^3 $M^{-1}s^{-1}$, from 5.3x10^3 to 5.5x10^3 $M^{-1}s^{-1}$, from 5.4x10^3 to 5.6x10^3 $M^{-1}s^{-1}$, from 5.5x10^3 to 5.7x10^3 $M^{-1}s^{-1}$, from 5.6x10^3 to 5.8x10^3 $M^{-1}s^{-1}$, from 5.7x10^3 to 5.9x10^3 $M^{-1}s^{-1}$, from 5.8x10^3 to 6x10^3 $M^{-1}s^{-1}$, from 5.9x10^3 to 6.1x10^3 $M^{-1}s^{-1}$, from 6x10^3 to 6.2x10^3 $M^{-1}s^{-1}$, from 6.1x10^3 to 6.3x10^3 $M^{-1}s^{-1}$, from 6.2x10^3 to 6.4x10^3 $M^{-1}s^{-1}$, from 6.3x10^3 to 6.5x10^3 $M^{-1}s^{-1}$, from 6.4x10^3 to 6.6x10^3 $M^{-1}s^{-1}$, from 6.5x10^3 to 6.7x10^3 $M^{-1}s^{-1}$, from 6.6x10^3 to 6.8x10^3 $M^{-1}s^{-1}$, from 6.7x10^3 to 6.9x10^3 $M^{-1}s^{-1}$, from 6.8x10^3 to 7x10^3 $M^{-1}s^{-1}$, from 6.9x10^3 to 7.1x10^3 $M^{-1}s^{-1}$, from 7x10^3 to 7.2x10^3 $M^{-1}s^{-1}$, from 7.1x10^3 to 7.3x10^3 $M^{-1}s^{-1}$, from 7.2x10^3 to 7.4x10^3 $M^{-1}s^{-1}$, from 7.3x10^3 to 7.5x10^3 $M^{-1}s^{-1}$, from 7.4x10^3 to 7.6x10^3 $M^{-1}s^{-1}$, from 7.5x10^3 to 7.7x10^3 $M^{-1}s^{-1}$, from 7.6x10^3 to 7.8x10^3 $M^{-1}s^{-1}$, from 7.7x10^3 to 7.9x10^3 $M^{-1}s^{-1}$, from 7.8x10^3 to 8x10^3 $M^{-1}s^{-1}$, from 7.9x10^3 to 8.1x10^3 $M^{-1}s^{-1}$, from 8x10^3 to 8.2x10^3 $M^{-1}s^{-1}$, from 8.1x10^3 to 8.3x10^3 $M^{-1}s^{-1}$, from 8.2x10^3 to 8.4x10^3 $M^{-1}s^{-1}$, from 8.3x10^3 to 8.5x10^3 $M^{-1}s^{-1}$, from 8.4x10^3 to 8.6x10^3 $M^{-1}s^{-1}$, from 8.5x10^3 to 8.7x10^3 $M^{-1}s^{-1}$, from 8.6x10^3 to 8.8x10^3 $M^{-1}s^{-1}$, from 8.7x10^3 to 8.9x10^3 $M^{-1}s^{-1}$, from 8.8x10^3 to 9x10^3 $M^{-1}s^{-1}$, from 8.9x10^3 to 9.1x10^3 $M^{-1}s^{-1}$, from 9x10^3 to 9.2x10^3 $M^{-1}s^{-1}$, from 9.1x10^3 to 9.3x10^3 $M^{-1}s^{-1}$, from 9.2x10^3 to 9.4x10^3 $M^{-1}s^{-1}$, from 9.3x10^3 to 9.5x10^3 $M^{-1}s^{-1}$, from 9.4x10^3 to 9.6x10^3 $M^{-1}s^{-1}$, from 9.5x10^3 to

9.7x10^3 $M^{-1}s^{-1}$, from 9.6x10^3 to 9.8x10^3 $M^{-1}s^{-1}$, from 9.7x10^3 to 9.9x10^3 $M^{-1}s^{-1}$, from 9.8x10^3 to 1x10^4 $M^{-1}s^{-1}$.

[00112] In some instances, a PEGylated or non-PEGylated engineered hSDH enzyme can have a k_{cat}/K_M for serine and/or threonine from about 2.7x10^3 to about 4.7x10^3 $M^{-1}s^{-1}$. In some cases, a PEGylated or non-PEGylated engineered hSDH enzyme can have a k_{cat}/K_M for serine of about 2.7x10^3 $M^{-1}s^{-1}$, 3.0x10^3 $M^{-1}s^{-1}$, or 4.7x10^3 $M^{-1}s^{-1}$. In some cases, a PEGylated or non-PEGylated engineered hSDH enzyme can have a k_{cat}/K_M for threonine of about 2.0x10^3 $M^{-1}s^{-1}$, 2.8x10^3 $M^{-1}s^{-1}$, 3.8x10^3 $M^{-1}s^{-1}$, or 6.8x10^3 $M^{-1}s^{-1}$. In some cases, a PEGylated or non-PEGylated engineered hSDH enzyme can have a k_{cat}/K_M for serine of about 3.0x10^3 $M^{-1}s^{-1}$ and a k_{cat}/K_M for threonine of about 3.8x10^3 $M^{-1}s^{-1}$. In some cases, a PEGylated or non-PEGylated engineered hSDH enzyme can have a k_{cat}/K_M for serine of about 2.7x10^3 $M^{-1}s^{-1}$ and a k_{cat}/K_M for threonine of about 2.0x10^3 $M^{-1}s^{-1}$. In some cases, a PEGylated or non-PEGylated engineered hSDH enzyme can have a k_{cat}/K_M for serine of about 3.0x10^3 $M^{-1}s^{-1}$ and a k_{cat}/K_M for threonine of about 2.8x10^3 $M^{-1}s^{-1}$. In some cases, a PEGylated or non-PEGylated engineered hSDH enzyme can have a k_{cat}/K_M for serine of about 4.7x10^3 $M^{-1}s^{-1}$ and a k_{cat}/K_M for threonine of about 6.8x10^3 $M^{-1}s^{-1}$.

[00113] In some instances, the serine and/or threonine degrading activity of a PEGylated or non-PEGylated engineered hSDH enzyme can have a V_{max} of at least 30 %, 40 %, 50 %, 60 %, 70 %, 80 %, 90 %, or 100 % of that of a PEGylated or non-PEGylated hSDH enzyme comprising SEQ ID NO: 1. In some instances, the serine and/or threonine degrading activity of a PEGylated or non-PEGylated engineered hSDH enzyme can have a V_{max} from 30 to 50 %, from 40 to 60 %, from 50 to 70 %, from 60 to 80 %, from 70 to 90 %, or from 80 to 100 % of that of a PEGylated or non-PEGylated hSDH enzyme comprising SEQ ID NO: 1. In some instances, the serine and/or threonine degrading activity of a PEGylated or non-PEGylated engineered hSDH enzyme can have a V_{max} at least 50 % of that of a PEGylated or non-PEGylated hSDH enzyme comprising SEQ ID NO: 1.

[00114] In some instances, the serine and/or threonine degrading activity of a PEGylated or non-PEGylated engineered hSDH enzyme can have a k_{cat}/K_M of at least 30 %, 35 %, 40 %, 45 %, 50 %, 55 %, 60 %, 65 %, 70 %, 75 %, 80 %, 85 %, 90 %, 95 %, 100 %, 105 %, 110 %, 115 %, 120 %, 125 %, 130 %, 135 %, 140 %, 145 %, 150 %, 155 %, 160 %, 165 %, 170 %, 175 %, 180 %, 185 %, 190 %, 195 %, or 200 % of that of a PEGylated or non-PEGylated hSDH enzyme comprising SEQ ID NO: 1. In some instances, the serine and/or

threonine degrading activity of a PEGylated or non-PEGylated engineered hSDH enzyme can have a K_M from 30 to 50 %, from 40 to 60 %, from 50 to 70 %, from 60 to 80 %, from 70 to 90 %, from 80 to 100 %, from 90 to 110 %, from 100 to 150 %, from 120 to 180 %, from 150 to 200 %, from 170 to 220 %,from 200 to 250 %, from 220 to 270 %, from 250 to 350 %, from 300 to 400 %, from 350 to 450 %, or from 400 to 500 % of that of a PEGylated or non-PEGylated hSDH enzyme comprising SEQ ID NO: 1. In some instances, the serine and/or threonine degrading activity of a PEGylated or non-PEGylated engineered hSDH enzyme can have a K_M no more than twice, or 200 %, of that of a PEGylated or non-PEGylated hSDH enzyme comprising SEQ ID NO: 1.

[00115] In some instances, the serine and/or threonine degrading activity of a PEGylated or non-PEGylated engineered hSDH enzyme can have a k_{cat} 30 %, 40 %, 50 %, 60 %, 70 %, 80 %, 90 %, 100 %, 110 %, 120 %, 130 %, 140 %, 150 %, 160 %, 170 %, 180 %, 190 %, 200 %, 210 %, 220 %, 230 %, 240 %, 250 %, 260 %, 270 %, 280 %, 290 %, 300 %, 310 %, 320 %, 330 %, 340 %, 350 %, 360 %, 370 %, 380 %, 390 %, 400 %, 410 %, 420 %, 430 %, 440 %, 450 %, 460 %, 470 %, 480 %, 490 %, 500 %, 510 %, 520 %, 530 %, 540 %, 550 %, 560 %, 570 %, 580 %, 590 %, 600 %, 610 %, 620 %, 630 %, 640 %, 650 %, 660 %, 670 %, 680 %, 690 %, 700 %, 710 %, 720 %, 730 %, 740 %, 750 %, 760 %, 770 %, 780 %, 790 %, 800 %, 810 %, 820 %, 830 %, 840 %, 850 %, 860 %, 870 %, 880 %, 890 %, 900 %, 910 %, 920 %, 930 %, 940 %, 950 %, 960 %, 970 %, 980 %, 990 %, or 1000 % of that of a PEGylated or non-PEGylated hSDH enzyme comprising SEQ ID NO: 1. In some instances, the serine and/or threonine degrading activity of a PEGylated or non-PEGylated engineered hSDH enzyme can have a k_{cat} from 30 to 50 %, from 40 to 60 %, from 50 to 70 %, from 60 to 80 %, from 70 to 90 %, from 80 to 100 %, from 90 to 110 %, from 100 to 150 %, from 120 to 180 %, from 150 to 200 %, from 170 to 220 %,from 200 to 250 %, from 220 to 270 %, from 250 to 350 %, from 300 to 400 %, from 350 to 450 %, from 400 to 500 %, from 450 to 550 %, from 500 to 600 %, from 550 to 650 %, from 600 to 700 %, from 650 to 750 %, from 700 to 800 %, from 750 to 850 %, from 800 to 900 %, from 850 to 950 %, or from 900 to 1,000 % of that of a PEGylated or non-PEGylated hSDH enzyme comprising SEQ ID NO: 1. In some instances, the serine and/or threonine degrading activity of a PEGylated or non-PEGylated engineered hSDH enzyme can have a k_{cat} at least 50 % of that of a PEGylated or non-PEGylated hSDH enzyme comprising SEQ ID NO: 1.

[00116] In some instances, the serine and/or threonine degrading activity of a PEGylated or non-PEGylated engineered hSDH enzyme can have a k_{cat}/K_M 30 %, 40 %, 50 %, 60 %, 70 %, 80 %, 90 %, 100 %, 110 %, 120 %, 130 %, 140 %, 150 %, 160 %, 170 %, 180 %, 190 %, 200 %, 210 %, 220 %, 230 %, 240 %, 250 %, 260 %, 270 %, 280 %, 290 %, 300 %, 310 %, 320 %, 330 %, 340 %, 350 %, 360 %, 370 %, 380 %, 390 %, 400 %, 410 %, 420 %, 430 %, 440 %, 450 %, 460 %, 470 %, 480 %, 490 %, 500 %, 510 %, 520 %, 530 %, 540 %, 550 %, 560 %, 570 %, 580 %, 590 %, 600 %, 610 %, 620 %, 630 %, 640 %, 650 %, 660 %, 670 %, 680 %, 690 %, 700 %, 710 %, 720 %, 730 %, 740 %, 750 %, 760 %, 770 %, 780 %, 790 %, 800 %, 810 %, 820 %, 830 %, 840 %, 850 %, 860 %, 870 %, 880 %, 890 %, 900 %, 910 %, 920 %, 930 %, 940 %, 950 %, 960 %, 970 %, 980 %, 990 %, or 1000 % of that of a PEGylated or non-PEGylated hSDH enzyme comprising SEQ ID NO: 1. In some instances, the serine and/or threonine degrading activity of a PEGylated or non-PEGylated engineered hSDH enzyme can have a k_{cat}/K_M from 30 to 50 %, from 40 to 60 %, from 50 to 70 %, from 60 to 80 %, from 70 to 90 %, from 80 to 100 %, from 90 to 110 %, from 100 to 150 %, from 120 to 180 %, from 150 to 200 %, from 170 to 220 %,from 200 to 250 %, from 220 to 270 %, from 250 to 350 %, from 300 to 400 %, from 350 to 450 %, from 400 to 500 %, from 450 to 550 %, from 500 to 600 %, from 550 to 650 %, from 600 to 700 %, from 650 to 750 %, from 700 to 800 %, from 750 to 850 %, from 800 to 900 %, from 850 to 950 %, or from 900 to 1,000 % of that of a PEGylated or non-PEGylated hSDH enzyme comprising SEQ ID NO: 1. In some instances, the serine and/or threonine degrading activity of a PEGylated or non-PEGylated engineered hSDH enzyme can have a k_{cat}/K_M at least 50 % of that of a PEGylated or non-PEGylated hSDH enzyme comprising SEQ ID NO: 1.

[00117] In some cases, PEGylation of an engineered hSDH enzyme does not alter the secondary or tertiary structure of the engineered hSDH enzyme. In other cases, PEGylation of an engineered hSDH enzyme can alter the secondary or tertiary structure of the engineered hSDH enzyme, without affecting serine and/or threonine degrading activity. In some instances, PEGylation of an engineered hSDH enzyme may alter the molecular size, charge, or receptor-binding capabilities of the engineered hSDH enzyme. In some cases, PEGylation of an engineered hSDH enzyme can reduce clearance of the engineered hSDH enzyme by the reticuloendothelial system (RES), kidney, spleen, or liver. In some cases, PEGylation of an engineered hSDH enzyme can increase the circulatory time of the engineered hSDH enzyme. In some cases, PEGylation of an engineered hSDH enzyme can create a steric hindrance, mask, or shield of the engineered hSDH enzyme that prevent its

access by an enzyme or protein. In some cases, a steric hindrance, mask, or shield can also decrease the immunogenicity or antigenicity of the engineered hSDH enzyme. In other cases, a PEG molecule for PEGylation of an engineered hSDH enzyme can comprise a hydrophilic, flexible, and biocompatible spacer. In other instances, a PEG molecule for PEGylation of an engineered hSDH enzyme can also comprise maleimide, vinyl sulfones, pyridyl disulfide, amine, carboxylic acids, or NHS esters.

[00118] In some cases, PEGylation of an engineered hSDH enzyme can modulate the serum half-life of the engineered hSDH enzyme. In other cases, PEGylation of an engineered hSDH enzyme can increase the serum half-life of the engineered hSDH enzyme. PEGylation of an engineered hSDH enzyme, in some cases, can increase the hydrodynamic radius of the enzyme and hence increase the serum persistence.

[00119] In some embodiments, PEGylation of an engineered hSDH enzyme can increase the hydrodynamic radius of the engineered hSDH enzyme by at least about 30 %, 40 %, 50 %, 60 %, 70 %, 80 %, 90 %, 100 %, 120 %, 140 %, 160 %, 180 %, 200 %, 250 %, 300 %, 350 %, 400 %, 500 %, 600 %, 700 %, 800 %, 900 %, or 1000 %. In some instances, PEGylation of an engineered hSDH enzyme can increase the hydrodynamic radius of the engineered hSDH enzyme by an amount from 30 to 50 %, from 40 to 60 %, from 50 to 70 %, from 60 to 80 %, from 70 to 90 %, from 80 to 100 %, from 90 to 110 %, from 100 to 150 %, from 120 to 180 %, from 150 to 200 %, from 170 to 220 %,from 200 to 250 %, from 220 to 270 %, from 250 to 350 %, from 300 to 400 %, from 350 to 450 %, from 400 to 500 %, from 450 to 550 %, from 500 to 600 %, from 550 to 650 %, from 600 to 700 %, from 650 to 750 %, from 700 to 800 %, from 750 to 850 %, from 800 to 900 %, from 850 to 950 %, or from 900 to 1,000 %.

[00120] In some embodiments, a PEGylated engineered hSDH enzyme can have a serum half-life of at least about 1 hours, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 18 hours, 24 hours, 36 hours, 48 hours, 49 hours, 50 hours, 51 hours, 52 hours, 53 hours, 54 hours, 55 hours, 56 hours, 57 hours, 58 hours, 59 hours, 60 hours, 61 hours, 62 hours, 63 hours, 64 hours, 65 hours, 66 hours, 67 hours, 68 hours, 69 hours, 70 hours, 71 hours,72 hours, 84 hours, 96 hours, 108 hours, 120 hours, 132 hours, 144 hours, 156 hours, 168 hours, 180 hours, 190 hours, or 200 hours. In other cases, In some embodiments, a PEGylated engineered hSDH enzyme can have a serum half-life of from 1 to 5 hours, from 2 to 6 hours, from 3 to 7 hours, from 4 to 8 hours, from 5

to 9 hours, from 6 to 10 hours, from 7 to 11 hours, from 8 to 12 hours, from 9 to 13 hours, from 10 to 14 hours, from 11 to 15 hours, from 12 to 16 hours, from 13 to 17 hours, from 14 to 18 hours, from 12 to 24 hours, from 18 to 30 hours, from 24 to 36 hours, from 30 to 42 hours, from 36 to 48 hours, from 42 to 46 hours, from 43 to 47 hours, from 44 to 48 hours, from 45 to 49 hours, from 46 to 50 hours, from 47 to 51 hours, from 48 to 52 hours, from 49 to 53 hours, from 50 to 54 hours, from 51 to 55 hours, from 52 to 56 hours, from 53 to 57 hours, from 54 to 58 hours, from 55 to 59 hours, from 56 to 60 hours, from 57 to 61 hours, from 58 to 62 hours, from 59 to 63 hours, from 60 to 64 hours, from 61 to 65 hours, from 62 to 66 hours, from 63 to 67 hours, from 64 to 68 hours, from 65 to 69 hours, from 66 to 70 hours, from 67 to 71 hours, from 68 to 72 hours, from 66 to 78 hours, from 72 to 84 hours, from 78 to 90 hours, from 84 to 96 hours, from 90 to 102 hours, from 96 to 108 hours, from 102 to 114 hours, from 108 to 120 hours, from 114 to 126 hours, from 120 to 132 hours, from 126 to 138 hours, from 132 to 144 hours, from 138 to 150 hours, from 144 to 156 hours, from 150 to 162 hours, from 156 to 168 hours, from 162 to 174 hours, from 168 to 180 hours, from 174 to 186 hours, from 180 to 192 hours, from 186 to 198 hours, or from 192 to 204 hours.

[00121] In some instances, a PEGylated engineered hSDH enzyme can have a serum half-life of at least about 10 %, 20 %, 30 %, 40 %, 50 %, 60 %, 70 %, 80 %, 90 %, 100 %, 150 %, 200 %, 250 %, 300 %, 350 %, 400 %, 450 %, 500 %, 550 %, 600 %, 650 %, 700 %, 750 %, 800 %, 850 %, 900 %, 950 %, or 1000 % longer than that of an engineered hSDH enzyme not PEGylated. In some instances, a PEGylated engineered hSDH enzyme can have a serum half-life from 10 to 100 %, from 50 to 150 %, from 100 to 200 %, from 150 to 250 %, from 200 to 300 %, from 250 to 350 %, from 300 to 400 %, from 350 to 450 %, from 400 to 500 %, from 450 to 550 %, from 500 to 600 %, from 550 to 650 %, from 600 to 700 %, from 650 to 750 %, from 700 to 800 %, from 750 to 850 %, from 800 to 900 %, from 850 to 950 %, or from 900 to 1000 %, longer than that of an engineered hSDH enzyme not PEGylated.

[00122] In some instances, a PEGylated engineered hSDH enzyme can have an extracellular half-life of at least about 10 %, 20 %, 30 %, 40 %, 50 %, 60 %, 70 %, 80 %, 90 %, 100 %, 150 %, 200 %, 250 %, 300 %, 350 %, 400 %, 450 %, 500 %, 550 %, 600 %, 650 %, 700 %, 750 %, 800 %, 850 %, 900 %, 950 %, 1000 % longer than that of an engineered hSDH enzyme not PEGylated. In some instances, a PEGylated engineered hSDH enzyme can have an extracellular half-life from 10 to 100 %, from 50 to 150 %, from 100 to 200 %, from 150 to 250 %, from 200 to 300 %, from 250 to 350 %, from 300 to 400 %, from 350 to 450

%, from 400 to 500 %, from 450 to 550 %, from 500 to 600 %, from 550 to 650 %, from 600 to 700 %, from 650 to 750 %, from 700 to 800 %, from 750 to 850 %, from 800 to 900 %, from 850 to 950 %, or from 900 to 1000 %, longer than that of an engineered hSDH enzyme not PEGylated.

[00123] A PEGylated engineered hSDH enzyme, in some cases, can comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or more PEG molecules. In some instances, a PEGylated engineered hSDH enzyme can comprise from 1 to 10, from 2 to 11, from 3 to 12, from 4 to 13, from 5 to 14, from 6 to 15, from 7 to 16, from 8 to 17, from 9 to 18, from 10 to 19, from 11 to 20, from 12 to 21, from 13 to 22, from 14 to 23, from 15 to 24, from 16 to 25, from 17 to 26, from 18 to 27, from 19 to 28, from 20 to 29, from 21 to 30, from 22 to 31, from 23 to 32, from 24 to 33, from 25 to 34, from 26 to 35 PEG molecules.

[00124] In some instances, said polyethylene glycol has an average molecular weight of about 500 kDa to about 1000 kDa, about 800 kDa to about 1600 kDa, about 1500 kDa to about 3000 kDa, about 2000 kDa to about 4000 kDa, about 2500 kDa to about 5000 kDa, about 3000 kDa to about 6000 kDa, about 4,000 kDa to about 8,000 kDa, about 6,000 kDa to about 12,000 kDa, about 10,000 kDa to about 20,000 kDa, or about 15,000 kDa to about 30,000 kDa.

B. Fusion Proteins

[00125] In some instances, an engineered hSDH enzyme can comprise a heterologous object. In some cases, a heterologous object can comprise a heterologous peptide. In some cases, an engineered hSDH enzyme and a heterologous peptide can be linked as a fusion protein. In some cases, a fusion protein comprising an engineered hSDH enzyme and a heterologous peptide can be constituted in the same translation unit, wherein the engineered hSDH enzyme and the heterologous peptide can share the same ATG start codon. In other cases, an engineered hSDH enzyme and a heterologous peptide can be linked by a covalent bond. Sun a covalent bond can comprise a peptide bond. In other cases, an engineered hSDH enzyme and a heterologous peptide can have a non-covalent bond. In some cases, an engineered hSDH enzyme and a heterologous peptide may not be constituted in the same translation unit.

[00126] Fusion proteins are provided in which the engineered hSDH enzyme may be linked at the N- or C-terminus to a heterologous domain. For example, fusions may also employ leader sequences from other species to permit the recombinant expression of a protein in a heterologous host. Another useful fusion includes the addition of a protein affinity tag, such as a serum albumin affinity tag or six histidine residues, or an immunologically active domain, such as an antibody epitope, preferably cleavable, to facilitate purification of the fusion protein. Non-limiting affinity tags include polyhistidine, chitin binding protein (CBP), maltose binding protein (MBP), and glutathione-S-transferase (GST).

[00127] In some cases, the engineered hSDH enzyme may be linked to a peptide that increases the *in vivo* half-life, such as an XTEN polypeptide (Schellenberger *et al.*, 2009), IgG Fc domain, albumin, or an albumin binding peptide.

[00128] In some cases, an engineered hSDH enzyme can comprise cell-targeting moieties comprising an antibody, a growth factor, a hormone, a peptide, an aptamer, a chemical, a drug, a nucleic acid, a cytokine, any derivatives herein and thereof, any biological equivalents herein and thereof, or any combination herein and thereof. For instance, a cell targeting moiety according the embodiments may bind to a cancer cell.

[00129] For example, an engineered hSDH enzyme comprising a cell targeting moiety can be directed to breast cancer cells. In some cases, a cell targeting moiety can bind to Her-2/neu. In other cases, an engineered hSDH enzyme may comprise an anti-Her-2/neu antibody. In some instances, a fusion protein comprising an engineered hSDH enzyme and a targeting moiety comprising a single chain anti-Her-2/neu antibody scFv23. In other instances, a fusion protein comprising an engineered hSDH enzyme and a targeting moiety comprising a scFv(FRP5) that bind to Her-2/neu may also be used in the compositions and methods of the current embodiments.

[00130] In some cases, a cell targeting moiety can bind to multiple types of cancer cells. In some instances, an 8H9 monoclonal antibody and the single chain antibodies derived therefrom that bind to a glycoprotein expressed on breast cancers, sarcomas and neuroblastomas can be used as a targeting moiety. In other cases, a cell targeting moiety can comprise the cell targeting agents described in U.S. patent application no. 2004/005647 that bind to MUC-1, an antigen that is expressed on a variety cancer types. In some cases, it is

understood that in certain embodiments, cell targeting constructs according the embodiments may be targeted against a plurality of cancer or tumor types.

[00131] Certain cell surface molecules are highly expressed in tumor cells, comprising hormone receptors such as human chorionic gonadotropin receptor and gonadotropin releasing hormone receptor. In some cases, a hormone peptide binding to a hormone receptor expressed by a cancer cell can be used as a cell targeting moiety that specifically targets cancer in cancer therapy.

C. Conjugation via Linkers

[00132] In some cases, an engineered hSDH enzyme can be chemically conjugated to a heterologous object by a bifunctional cross-linking reagent. In some cases, an engineered hSDH enzyme can be chemically conjugated to a heterologous object by a peptide linker. In some cases, a suitable peptide linker comprises a Gly-Ser linker.

[00133] Bifunctional cross-linking reagent have been extensively used for a variety of purposes and well known in the art, comprising preparation of affinity matrices, modification and stabilization of diverse structures, identification of ligand and receptor binding sites, and structural studies.

[00134] In some cases, a bifunctional cross-linking reagent can comprise a homobifunctional reagent. In some instances, a homobifunctional reagent carrying two identical functional groups can be highly efficient in inducing cross-linking between identical and different macromolecules or subunits of a macromolecule and linking of polypeptide ligands to their specific binding sites. In some cases, a bifunctional cross-linking reagent can comprise a heterobifunctional reagent that contains two different functional groups. In some cases, a heterobifunctional reagent can control a cross-linking selectively and sequentially with the differential reactivities of the two different functional groups. In some instances, a bifunctional cross-linking reagent can be divided according to the specificity of their functional groups, e.g., amino-, sulfhydryl-, guanidine-, indole-, carboxyl-specific groups. In some instances, cross-linking reagents directed to free amino can be used based on their commercial availability, ease of synthesis, and the mild reaction conditions under which they can be applied.

[00135] In some cases, a heterobifunctional cross-linking reagent can comprise a primary amine-reactive group and a thiol-reactive group. In another example, heterobifunctional cross-linking reagents and methods of using the cross-linking reagents are described (U.S. Pat. No. 5,889,155, specifically incorporated herein by reference in its entirety). The cross-linking reagents combine a nucleophilic hydrazide residue with an electrophilic maleimide residue, allowing coupling, in one example, of aldehydes to free thiols. The cross-linking reagent can be modified to cross-link various functional groups.

[00136] Additionally, any other linking/coupling agents and/or mechanisms known to those of skill in the art may be used to combine an engineered hSDH enzyme, comprising antibody-antigen interaction, avidin biotin linkages, amide linkages, ester linkages, thioester linkages, ether linkages, thioether linkages, phosphoester linkages, phosphoramide linkages, anhydride linkages, disulfide linkages, ionic and hydrophobic interactions, bispecific antibodies and antibody fragments, or combinations thereof.

[00137] In some instances, a cross-linker having reasonable stability in blood can be employed. Numerous types of disulfide-bond containing linkers are known that can be successfully employed to conjugate targeting and therapeutic/preventative agents. In some cases, a linker comprising a disulfide bond that is sterically hindered can give greater stability of a molecule being conjugated to the linker in vivo. These linkers are thus one group of linking agents.

[00138] In some cases, a non-hindered linker can also be employed in accordance herewith. Other useful cross-linkers, not considered to contain or generate a protected disulfide, comprise SATA, SPDP, and 2-iminothiolane. The use of such cross-linkers is well understood in the art. In some cases, a flexible linker can be used.

[00139] In some cases, once chemically conjugated, a peptide can be purified to separate the conjugate from unconjugated agents and from other contaminants. In some instances, a large number of purification techniques are available for use in providing conjugates of a sufficient degree of purity to render them clinically useful. In some cases, purification methods can comprise methods based upon size separation, such as gel filtration, gel permeation, or high-performance liquid chromatography, will generally be of most use. Other chromatographic techniques, such as Blue-Sepharose separation, may also be used.

Conventional methods to purify the fusion proteins from inclusion bodies may be useful, such as using weak detergents, such as sodium N-lauroyl-sarcosine (SLS).

D. Protein Purification

[00140] Protein purification techniques, in some instances, can comprise techniques involved, at one level, the homogenization and crude fractionation of the cells, tissue, or organ to polypeptide and non-polypeptide fractions. In some instances, the protein or polypeptide of interest may be further purified using chromatographic and electrophoretic techniques to achieve partial or complete purification (or purification to homogeneity) unless otherwise specified. In some cases, analytical methods particularly suited to the preparation of a pure peptide are ion-exchange chromatography, gel exclusion chromatography, polyacrylamide gel electrophoresis, affinity chromatography, immunoaffinity chromatography, and isoelectric focusing can also be used. In some case, a method of purifying peptides can be fast-performance liquid chromatography (FPLC) or even high-performance liquid chromatography (HPLC).

[00141] In some cases, a purified protein or peptide can comprise a composition, isolatable from other components, wherein the protein or peptide is purified to any degree relative to its naturally obtainable state. In other cases, an isolated or purified protein or peptide can also comprise a protein or peptide free from the environment in which it may naturally occur. In some cases, a purified protein can comprise a protein or peptide composition that has been subjected to fractionation to remove various other components, and which composition substantially retains its expressed biological activity. In other cases, a protein being purified can comprise a protein or peptide forming the major component of a composition. In some cases, a major component of a composition can comprise about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, or more of the proteins in the composition.

[00142] Protein purification, in some cases, can comprise precipitation with ammonium sulphate, PEG, antibodies and the like, or by heat denaturation, followed by centrifugation; chromatography steps comprising ion exchange, gel filtration, reverse phase, hydroxyapatite, and affinity chromatography; isoelectric focusing; gel electrophoresis; and combinations of these and other techniques. As is generally known in the art, it is believed that the order of conducting the various purification steps may be changed, or that certain

steps may be omitted, and still result in a suitable method for the preparation of a substantially purified protein or peptide.

[00143] Various methods for quantifying the degree of purification of the protein or peptide are known to those of skill in the art in light of the present disclosure. These methods can, in some cases, comprise determining the specific activity of an active fraction, or assessing the amount of polypeptides within a fraction by SDS/PAGE analysis. In some cases, a method can comprise assessing the purity of a fraction is to calculate the specific activity of the fraction, to compare it to the specific activity of the initial extract, and to thus calculate the degree of purity therein, assessed by a "-fold purification number". The actual units used to represent the amount of activity, in some cases, can be dependent upon the particular assay technique chosen to follow the purification, and whether or not the expressed protein or peptide exhibits a detectable activity.

[00144] In some cases, a protein or peptide may not always be provided in its most purified state. In some cases, a less-than-most purified products may have utility in certain embodiments. In some cases, partial purification may be accomplished by using fewer purification steps in combination, or by utilizing different forms of the same general purification scheme. For example, it is appreciated that a cation-exchange column chromatography performed utilizing an HPLC apparatus will generally result in a greater "-fold" purification than the same technique utilizing a low pressure chromatography system. Methods exhibiting a lower degree of relative purification may have advantages in total recovery of protein product, or in maintaining the activity of an expressed protein.

[00145] In certain embodiments, a protein or peptide may be isolated or purified, for example, an engineered hSDH enzyme or PEGylated engineered hSDH enzyme. For example, a His tag or an affinity epitope may be comprised in such an engineered hSDH enzyme or PEGylated engineered hSDH enzyme to facilitate purification. Affinity chromatography is a chromatographic procedure that relies on the specific affinity between a substance to be isolated and a molecule to which it can specifically bind. This is a receptor-ligand type of interaction. The column material is synthesized by covalently coupling one of the binding partners to an insoluble matrix. The column material is then able to specifically adsorb the substance from the solution. Elution occurs by changing the conditions to those in which binding will not occur (*e.g.*, altered pH, ionic strength, temperature, *etc.*). The matrix should be a substance that does not adsorb molecules to any significant extent and that has a

broad range of chemical, physical, and thermal stability. The ligand should be coupled in such a way as to not affect its binding properties. The ligand should also provide relatively tight binding. It should be possible to elute the substance without destroying the sample or the ligand.

[00146] Size exclusion chromatography (SEC) is a chromatographic method in which molecules in solution are separated based on their size, or in more technical terms, their hydrodynamic volume. It is usually applied to large molecules or macromolecular complexes, such as proteins and industrial polymers. Typically, when an aqueous solution is used to transport the sample through the column, the technique is known as gel filtration chromatography, versus the name gel permeation chromatography, which is used when an organic solvent is used as a mobile phase.

[00147] The underlying principle of SEC is that particles of different sizes will elute (filter) through a stationary phase at different rates. This results in the separation of a solution of particles based on size. Provided that all the particles are loaded simultaneously or near simultaneously, particles of the same size should elute together. Each size exclusion column has a range of molecular weights that can be separated. The exclusion limit defines the molecular weight at the upper end of this range and is where molecules are too large to be trapped in the stationary phase. The permeation limit defines the molecular weight at the lower end of the range of separation and is where molecules of a small enough size can penetrate into the pores of the stationary phase completely and all molecules below this molecular mass are so small that they elute as a single band.

[00148] High-performance liquid chromatography (or high-pressure liquid chromatography, HPLC) is a form of column chromatography used frequently in biochemistry and analytical chemistry to separate, identify, and quantify compounds. HPLC utilizes a column that holds chromatographic packing material (stationary phase), a pump that moves the mobile phase(s) through the column, and a detector that shows the retention times of the molecules. Retention time varies depending on the interactions between the stationary phase, the molecules being analyzed, and the solvent(s) used.

III. Nucleic Acids

[00149] In some instances, a nucleic acid encoding an engineered hSDH enzyme can comprise a DNA, RNA, LNA, PNA, or any derivatives herein and thereof that

can encode the engineered hSDH enzyme. In some cases, a nucleic acid comprising SEQ ID NO: 8 encodes an engineered hSDH enzyme comprising SEQ ID NO: 2. In some cases, a nucleic acid comprising SEQ ID NO: 9 encodes an engineered hSDH enzyme comprising SEQ ID NO: 3. In some cases, a nucleic acid comprising SEQ ID NO: 10 encodes an engineered hSDH enzyme comprising SEQ ID NO: 4. In some cases, a nucleic acid comprising SEQ ID NO: 11 encodes an engineered hSDH enzyme comprising SEQ ID NO: 5. In some cases, a nucleic acid comprising SEQ ID NO: 12 encodes an engineered hSDH enzyme comprising SEQ ID NO: 6.

[00150] In some cases, nucleic acids encoding an engineered hSDH enzyme can also be codon-optimized based on the organism used to express the polypeptide. For example, if the engineered hSDH enzyme is derived from human serine dehydratase and contains multiple codons that are rarely utilized in *E. coli*, then that may interfere with expression. Therefore, the respective genes or variants thereof may be codon optimized for *E. coli* expression using freely available software to design coding sequences free of rare codons.

IV. Vectors

[00151] The nucleic acids provided herein can be delivered by any suitable means. In some cases, a suitable means comprises a vector. Any vector system can be used utilized, including but not limited to: plasmid vectors, minicircle vectors, linear DNA vectors, doggy bone vectors, retroviral vectors, lentiviral vectors, adenovirus vectors, poxvirus vectors; herpesvirus vectors and adeno-associated virus vectors, a liposome, a nanoparticle, an exosome, an extracellular vesicle, a nanomesh, modified versions thereof, good manufacturing practices versions thereof, chimeras thereof, and any combination thereof. In some cases, a vector can be used to introduce a polynucleotide provided herein. In some cases, the polynucleotide comprises a targeting sequence that hybridizes to a region of an RNA provided herein. In some embodiments, a nanoparticle vector can comprise a polymeric-based nanoparticle, an amino lipid-based nanoparticle, a metallic nanoparticle (such as gold-based nanoparticle), a portion of any of these, or any combination thereof.

[00152] In some cases, a vector may not be a viral vector. Non-viral methods can comprise naked delivery of compositions comprising polynucleotides and the like. In some cases, modifications provided herein can be incorporated into polynucleotides to

increase stability and combat degradation when being delivered as naked polynucleotides. In other cases, a non-viral approach can harness use of nanoparticles, liposomes, and the like.

V. Host Cells

[00153] Host cells may be any that may be transformed to allow the expression and secretion of engineered hSDH enzyme and conjugates thereof. The host cells may be bacteria, mammalian cells, yeast, or filamentous fungi. Bacteria may comprise *Escherichia* and *Bacillus*. In some instances, bacteria can comprise *Escherichia coli* (*E. coli*) or *Salmonella enterica*. Yeasts belonging to the genera *Saccharomyces*, *Kiuyveromyces*, *Hansenula*, or *Pichia* may be used as host cells. Various species of filamentous fungi may be used as expression hosts, including the following genera: *Aspergillus*, *Trichoderma*, *Neurospora*, *Penicillium*, *Cephalosporium*, *Achlya*, *Podospora*, *Endothia*, *Mucor*, *Cochliobolus*, and *Pyricularia*.

[00154] Examples of usable host organisms include bacteria, *e.g.*, *Escherichia coli* MC1061, derivatives of *Bacillus subtilis* BRB1, *Staphylococcus aureus* SAI123 or *Streptococcus lividans*; yeasts, *e.g.*, *Saccharomyces cerevisiae* AH 22 or *Schizosaccharomyces pombe*; and filamentous fungi, *e.g.*, *Aspergillus nidulans*, *Aspergillus awamori*, or *Trichoderma reesei*.

[00155] In some cases, an engineered hSDH enzyme can be expressed in a mammalian host cell. Examples of mammalian host cells include Chinese hamster ovary cells (CHO-K1; American Type Culture Collection (ATCC) No. CCL61), rat pituitary cells (GH1; ATCC No. CCL82), HeLa S3 cells (ATCC No. CCL2.2), rat hepatoma cells (H-4-II-E; ATCC No. CRL-1548), SV40-transformed monkey kidney cells (COS-1; ATCC No. CRL-1650), and murine embryonic cells (NIH-3T3; ATCC No. CRL-1658).

[00156] Mammalian host cells expressing an engineered hSDH enzymes may be cultured under conditions typically employed to culture the parental cell line. Generally, mammalian host cells expressing an engineered hSDH enzyme are cultured in a standard medium containing physiological salts and nutrients, such as standard RPMI, MEM, IMEM, or DMEM, typically supplemented with 5%-10% serum, such as fetal bovine serum. Culture conditions may comprise incubation at 37 °C in stationary or roller cultures until desired levels of the proteins are achieved.

[00157] In some cases, insect host cell can be used to express an engineered hSDH enzyme. In some cases, insect host cells can comprise Sf9, Sf21, High Five, or S2 cells. In other cases, insect expression host cells can comprise baculovirus expression systems.

VI. Pharmaceutical Compositions

[00158] It is contemplated that an engineered hSDH enzyme can be administered systemically or locally. They can be administered using any routes described herein and thereof.

[00159] It is not intended that the present invention be limited by the particular nature of the therapeutic preparation. For example, such compositions can be provided in formulations together with physiologically tolerable liquid, gel, or solid carriers, diluents, and excipients. These therapeutic preparations can be administered to mammals for veterinary use, such as with domestic animals, and clinical use in humans in a manner similar to other therapeutic agents. In general, the dosage required for therapeutic efficacy will vary according to the type of use and mode of administration, as well as the particularized requirements of individual subjects.

[00160] Such compositions are typically prepared as liquid solutions or suspensions, as injectables. Suitable diluents and excipients are, for example, water, saline, dextrose, glycerol, or the like, and combinations thereof. In addition, if desired, the compositions may contain minor amounts of auxiliary substances, such as wetting or emulsifying agents, stabilizing agents, or pH buffering agents.

[00161] Generally, pharmaceutical compositions may comprise an effective amount of one or more engineered hSDH enzyme or additional agents dissolved or dispersed in a pharmaceutically acceptable carrier. The phrases "pharmaceutical or pharmacologically acceptable" refers to molecular entities and compositions that do not produce an adverse, allergic, or other untoward reaction when administered to an animal, such as, for example, a human, as appropriate. The preparation of a pharmaceutical composition that contains at least one engineered hSDH enzyme isolated by the method disclosed herein, or additional active ingredient will be known to those of skill in the art in light of the present disclosure, as exemplified by Remington's Pharmaceutical Sciences, 18th Ed., 1990, incorporated herein by reference. Moreover, for animal (*e.g.*, human) administration, it will be understood that

preparations should meet sterility, pyrogenicity, general safety, and purity standards as required by the FDA Office of Biological Standards.

[00162] As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, surfactants, antioxidants, preservatives (*e.g.*, antibacterial agents, antifungal agents), isotonic agents, absorption delaying agents, salts, preservatives, drugs, drug stabilizers, gels, binders, excipients, disintegration agents, lubricants, sweetening agents, flavoring agents, dyes, such like materials and combinations thereof, as would be known to one of ordinary skill in the art (see, for example, Remington's Pharmaceutical Sciences, 18th Ed., 1990, incorporated herein by reference). Except insofar as any conventional carrier is incompatible with the active ingredient, its use in the pharmaceutical compositions is contemplated.

[00163] Certain embodiments of the present invention may comprise different types of carriers depending on whether it is to be administered in solid, liquid, or aerosol form, and whether it needs to be sterile for the route of administration, such as injection. The compositions can be administered intravenously, intradermally, transdermally, intrathecally, intraarterially, intraperitoneally, intranasally, intravaginally, intrarectally, intramuscularly, subcutaneously, mucosally, orally, topically, locally, by inhalation (*e.g.*, aerosol inhalation), by injection, by infusion, by continuous infusion, by localized perfusion bathing target cells directly, *via* a catheter, *via* a lavage, in lipid compositions (*e.g.*, liposomes), or by other methods or any combination of the forgoing as would be known to one of ordinary skill in the art (see, for example, Remington's Pharmaceutical Sciences, 18th Ed., 1990, incorporated herein by reference).

[00164] The modified polypeptides may be formulated into a composition in a free base, neutral, or salt form. Pharmaceutically acceptable salts include the acid addition salts, *e.g.*, those formed with the free amino groups of a proteinaceous composition, or which are formed with inorganic acids, such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, or mandelic acid. Salts formed with the free carboxyl groups can also be derived from inorganic bases, such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides; or such organic bases as isopropylamine, trimethylamine, histidine, or procaine. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage

forms, such as formulated for parenteral administrations, such as injectable solutions, or aerosols for delivery to the lungs, or formulated for alimentary administrations, such as drug release capsules and the like.

[00165] Further in accordance with certain aspects of the present invention, the composition suitable for administration may be provided in a pharmaceutically acceptable carrier with or without an inert diluent. The carrier should be assimilable and includes liquid, semi-solid, *i.e.*, pastes, or solid carriers. Except insofar as any conventional media, agent, diluent, or carrier is detrimental to the recipient or to the therapeutic effectiveness of a composition contained therein, its use in administrable composition for use in practicing the methods is appropriate. Examples of carriers or diluents include fats, oils, water, saline solutions, lipids, liposomes, resins, binders, fillers, and the like, or combinations thereof. The composition may also comprise various antioxidants to retard oxidation of one or more component. Additionally, the prevention of the action of microorganisms can be brought about by preservatives, such as various antibacterial and antifungal agents, including but not limited to parabens (*e.g.*, methylparabens, propylparabens), chlorobutanol, phenol, sorbic acid, thimerosal or combinations thereof.

[00166] In accordance with certain aspects of the present invention, the composition is combined with the carrier in any convenient and practical manner, *i.e.*, by solution, suspension, emulsification, admixture, encapsulation, absorption, and the like. Such procedures are routine for those skilled in the art.

[00167] In a specific embodiment of the present invention, the composition is combined or mixed thoroughly with a semi-solid or solid carrier. The mixing can be carried out in any convenient manner, such as grinding. Stabilizing agents can be also added in the mixing process in order to protect the composition from loss of therapeutic activity, *i.e.*, denaturation in the stomach. Examples of stabilizers for use in a composition include buffers, amino acids, such as glycine and lysine, carbohydrates, such as dextrose, mannose, galactose, fructose, lactose, sucrose, maltose, sorbitol, mannitol, *etc.*

[00168] In further embodiments, the present invention may concern the use of a pharmaceutical lipid vehicle composition that includes engineered hSDH enzyme, one or more lipids, and an aqueous solvent. As used herein, the term "lipid" will be defined to include any of a broad range of substances that is characteristically insoluble in water and

extractable with an organic solvent. This broad class of compounds is well known to those of skill in the art, and as the term "lipid" is used herein, it is not limited to any particular structure. Examples include compounds that contain long-chain aliphatic hydrocarbons and their derivatives. A lipid may be naturally occurring or synthetic (*i.e.*, designed or produced by man). However, a lipid is usually a biological substance. Biological lipids are well known in the art, and include for example, neutral fats, phospholipids, phosphoglycerides, steroids, terpenes, lysolipids, glycosphingolipids, glycolipids, sulphatides, lipids with ether- and ester-linked fatty acids, polymerizable lipids, and combinations thereof. Of course, compounds other than those specifically described herein that are understood by one of skill in the art as lipids are also encompassed by the compositions and methods.

[00169] One of ordinary skill in the art would be familiar with the range of techniques that can be employed for dispersing a composition in a lipid vehicle. For example, the engineered hSDH enzyme or a fusion protein thereof may be dispersed in a solution containing a lipid, dissolved with a lipid, emulsified with a lipid, mixed with a lipid, combined with a lipid, covalently bonded to a lipid, contained as a suspension in a lipid, contained or complexed with a micelle or liposome, or otherwise associated with a lipid or lipid structure by any means known to those of ordinary skill in the art. The dispersion may or may not result in the formation of liposomes.

VII. Methods of Treating Cancer

[00170] In some cases, an engineered hSDH enzyme can be used to treat cancer. For example, patients with luminal breast cancer exhibiting deficient PSAT1 levels or patients with any cancer type identified to have low levels of PHGDH, PSAT1, or PSPH or mutations/deletions in PHGDH, PSAT1, or PSPH genes could be specifically targeted with engineered hSDH variants that systemically deplete serine and threonine in serum. As such, a patient may be selected for treatment with an engineered hSDH enzyme based on hypermethylation of the *PSAT1*, *PHGDH*, or *PSPH* genes, based on mutation of the *PSAT1*, *PHGDH*, or *PSPH* genes, or based on decreased expression levels of PSAT1 (phosphoserine aminotransferase 1; NCBI Entrez Gene: 29968; GenBank references: NG_012165.1, NM_021154.5, NP_066977.1, NM_058179.4, NP_478059.1, each of which is incorporated herein by reference), PHGDH (Phosphoglycerate Dehydrogenase; NCBI Entrez Gene: 26227; GenBank references: NG_009188.1; NM_006623.4; NP_006614.2, each of which is incorporated herein by reference), or PSPH (Phosphoserine Phosphatase; NCBI Entrez Gene:

5723; GenBank references: NG_011473.1, NM_004577.4, NP_004568.2, each of which is incorporated herein by reference). In some cases, the expression level of *PSAT1*, *PHGDH*, and/or *PSPH* may be reduced by at least 10 %, 20 %, 30 %, 40 %, 50 %, 60 %, 70 %, 80 %, or 90 % relative to a reference level. The reference level may be a level in a non-cancerous cell obtained from the patient. The reference level may be a level in a sample obtained from a healthy subject or a population of healthy subjects.

[00171] In some cases, treatment with an engineered hSDH enzyme or PEGylated engineered hSDH enzyme reduces the extracellular concentration of serine and/or threonine in the tumor microenvironment by at least 10 %, 20 %, 30 %, 40 %, 50 %, 60 %, 70 %, 80 %, or 90 %. In some embodiments, a PEGylated engineered hSDH enzyme can have a low immunogenicity risk. In other cases, an engineered hSDH enzyme can have no low immunogenicity risk.

[00172] A cancer, in some instances, can comprise malignant cell type, such as those found in a solid tumor or a hematological tumor. In some case, a cancer can comprise a tumor of an organ selected from the group consisting of pancreas, colon, cecum, stomach, gallbladder, skin, brain, head, neck, ovary, kidney, larynx, sarcoma, lung, bladder, melanoma, prostate, and breast. In some case, a cancer can comprise hematological tumors include tumors of the bone marrow, T or B cell malignancies, leukemias, lymphomas, blastomas, myelomas, and the like. In some case, a cancer can also comprise carcinoma, lymphoma, blastoma, sarcoma, leukemia, squamous cell cancer, lung cancer (including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung), cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer (including gastrointestinal cancer and gastrointestinal stromal cancer), pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, gallbladder cancer, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, renal cell carcinoma, prostate cancer, vulval cancer, thyroid cancer, various types of head and neck cancer, head and neck squamous cell carcinoma, melanoma, superficial spreading melanoma, lentigo malignant melanoma, acral lentiginous melanomas, nodular melanomas, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell

NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's macroglobulinemia), chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), Hairy cell leukemia, multiple myeloma, acute myeloid leukemia (AML) and chronic myeloblastic leukemia.

[00173] In some case, a cancer can comprise neoplasm, malignant; carcinoma; carcinoma, undifferentiated; giant and spindle cell carcinoma; small cell carcinoma; papillary carcinoma; squamous cell carcinoma; lymphoepithelial carcinoma; basal cell carcinoma; pilomatrix carcinoma; transitional cell carcinoma; papillary transitional cell carcinoma; adenocarcinoma; gastrinoma, malignant; cholangiocarcinoma; hepatocellular carcinoma; combined hepatocellular carcinoma and cholangiocarcinoma; trabecular adenocarcinoma; adenoid cystic carcinoma; adenocarcinoma in adenomatous polyp; adenocarcinoma, familial polyposis coli; solid carcinoma; carcinoid tumor, malignant; branchiolo-alveolar adenocarcinoma; papillary adenocarcinoma; chromophobe carcinoma; acidophil carcinoma; oxyphilic adenocarcinoma; basophil carcinoma; clear cell adenocarcinoma; granular cell carcinoma; follicular adenocarcinoma; papillary and follicular adenocarcinoma; nonencapsulating sclerosing carcinoma; adrenal cortical carcinoma; endometroid carcinoma; skin appendage carcinoma; apocrine adenocarcinoma; sebaceous adenocarcinoma; ceruminous adenocarcinoma; mucoepidermoid carcinoma; cystadenocarcinoma; papillary cystadenocarcinoma; papillary serous cystadenocarcinoma; mucinous cystadenocarcinoma; mucinous adenocarcinoma; signet ring cell carcinoma; infiltrating duct carcinoma; medullary carcinoma; lobular carcinoma; inflammatory carcinoma; paget's disease, mammary; acinar cell carcinoma; adenosquamous carcinoma; adenocarcinoma w/squamous metaplasia; thymoma, malignant; ovarian stromal tumor, malignant; thecoma, malignant; granulosa cell tumor, malignant; androblastoma, malignant; sertoli cell carcinoma; leydig cell tumor, malignant; lipid cell tumor, malignant; paraganglioma, malignant; extra-mammary paraganglioma, malignant; pheochromocytoma; glomangiosarcoma; malignant melanoma; amelanotic melanoma; superficial spreading melanoma; malignant melanoma in giant pigmented nevus; epithelioid cell melanoma; blue nevus, malignant; sarcoma; fibrosarcoma; fibrous histiocytoma, malignant; myxosarcoma; liposarcoma; leiomyosarcoma; rhabdomyosarcoma; embryonal rhabdomyosarcoma; alveolar rhabdomyosarcoma; stromal sarcoma; mixed tumor, malignant; mullerian mixed tumor; nephroblastoma; hepatoblastoma; carcinosarcoma; mesenchymoma, malignant; brenner tumor, malignant; phyllodes tumor, malignant; synovial sarcoma; mesothelioma, malignant; dysgerminoma; embryonal

carcinoma; teratoma, malignant; struma ovarii, malignant; choriocarcinoma; mesonephroma, malignant; hemangiosarcoma; hemangioendothelioma, malignant; kaposi's sarcoma; hemangiopericytoma, malignant; lymphangiosarcoma; osteosarcoma; juxtacortical osteosarcoma; chondrosarcoma; chondroblastoma, malignant; mesenchymal chondrosarcoma; giant cell tumor of bone; ewing's sarcoma; odontogenic tumor, malignant; ameloblastic odontosarcoma; ameloblastoma, malignant; ameloblastic fibrosarcoma; pinealoma, malignant; chordoma; glioma, malignant; ependymoma; astrocytoma; protoplasmic astrocytoma; fibrillary astrocytoma; astroblastoma; glioblastoma; oligodendroglioma; oligodendroblastoma; primitive neuroectodermal; cerebellar sarcoma; ganglioneuroblastoma; neuroblastoma; retinoblastoma; olfactory neurogenic tumor; meningioma, malignant; neurofibrosarcoma; neurilemmoma, malignant; granular cell tumor, malignant; malignant lymphoma; hodgkin's disease; hodgkin's; paragranuloma; malignant lymphoma, small lymphocytic; malignant lymphoma, large cell, diffuse; malignant lymphoma, follicular; mycosis fungoides; other specified non-hodgkin's lymphomas; malignant histiocytosis; multiple myeloma; mast cell sarcoma; immunoproliferative small intestinal disease; leukemia; lymphoid leukemia; plasma cell leukemia; erythroleukemia; lymphosarcoma cell leukemia; myeloid leukemia; basophilic leukemia; eosinophilic leukemia; monocytic leukemia; mast cell leukemia; megakaryoblastic leukemia; myeloid sarcoma; and hairy cell leukemia.

A. Detection

[00174] In some aspects, the present disclosure concerns immunodetection methods for detecting expression of *PSAT1*, *PHGDH*, or *PSPH*. A wide variety of assay formats are contemplated for detecting protein products, including immunohistochemistry, enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA), immunoradiometric assay, fluoroimmunoassay, chemiluminescent assay, bioluminescent assay, dot blotting, FACS analyses, and Western blot to mention a few. The steps of various useful immunodetection methods have been described in the scientific literature. In general, the immunobinding methods include obtaining a sample of the cancer, and contacting the sample with an antibody specific for the protein to be detected, as the case may be, under conditions effective to allow the formation of immunocomplexes. In general, the detection of immunocomplex formation is well known in the art and may be achieved through the application of numerous approaches. These methods are generally based upon the detection of a label or marker, such as any of those radioactive, fluorescent, biological and enzymatic

tags. Of course, one may find additional advantages through the use of a secondary binding ligand such as a second antibody and/or a biotin/avidin ligand binding arrangement, as is known in the art.

[00175] The antibody employed in the detection may itself be linked to a detectable label, wherein one would then simply detect this label, thereby allowing the amount of the primary immune complexes in the composition to be determined. Alternatively, the first antibody that becomes bound within the primary immune complexes may be detected by means of a second binding ligand that has binding affinity for the antibody. In these cases, the second binding ligand may be linked to a detectable label. The second binding ligand is itself often an antibody, which may thus be termed a "secondary" antibody. The primary immune complexes are contacted with the labeled, secondary binding ligand, or antibody, under effective conditions and for a period of time sufficient to allow the formation of secondary immune complexes. The secondary immune complexes are then generally washed to remove any non-specifically bound labeled secondary antibodies or ligands, and the remaining label in the secondary immune complexes is then detected.

[00176] With regard to detecting expression of mRNA products, or a corresponding cDNA, mRNA is first extracted from the cancer cells. The extracted mRNA may be amplified prior to detection. Alternatively, the extracted mRNA may be reverse transcribed to cDNA prior to amplification of the cDNA. Any nucleic acid amplification assay which can be utilized, including but not limited to the polymerase chain reaction (RT-PCR), branched DNA signal amplification, ligase chain reaction, isothermal nucleic acid sequence based amplification (NASBA), Q-beta replication, transcription-based amplification, amplifiable RNA reporters, boomerang DNA amplification, strand displacement activation, cycling probe technology, and other self-sustained sequence replication assays, as well as variations on these including methods for nucleic acid enrichment such as by using restriction digestion with polymerase chain reaction and the use of nested primers. Similarly, any method capable of detecting an amplified nucleic acid product, including but not limited to agarose gel electrophoresis, fluorescence-based detection methods, real-time PCR, ELISA detection methods, electrochemiluminescence, high performance liquid chromatography, reverse dot blot methods, and nucleic acid sequencing methods, may be used.

[00177] With regard to detecting methylation of a gene, there are mainly techniques known which are used for the detection of the methylation of gene. The methylation status of specific CpG dinucleotides can be determined using isoschizomers of bacterial restriction endonucleases which are characterized by different sensitivities vis-à-vis 5-methylcytosine. Examples thereof are the enzymes HpaII and MspI—both cut CCGG sequences, HpaII however only if the internal cytosine is not methylated. The treatment of double-stranded genomic DNA with sodium bisulfate leads to the deamination of unmethylated cytosine residues into uracil residues and to the formation of two single strands that are no longer complementary. During this treatment, 5-methyl cytosine is maintained. The differences in sequence produced in this way form the basis of the differentiation between methylated and unmethylated DNA. DNA treated with bisulfite can be used directly in PCR in which uracil residues (previously unmethylated cytosine) and thymidine residues are amplified as thymidine and only 5-methylcytosine residues are amplified as cytosine residues. Depending on the application, the primers used for the PCR differentiate between methylated and unmethylated sequences or amplify fragments independently of the methylation status. PCR fragments which have been amplified using non-discriminating primers can, for instance, be sequenced directly to determine the share in methylated and unmethylated CpGs. Antibodies against 5-methyl cytosine recognize CpG methylation in denatured, single-stranded DNA are used mainly for the immunohistochemical staining of the CpG methylation on the chromosomes of individual, fixed cells.

[00178] As used herein, the term "sample" refers to any sample suitable for the detection methods provided by the present invention. The sample may be any sample that includes material suitable for detection or isolation. Sources of samples include blood, pleural fluid, peritoneal fluid, urine, saliva, malignant ascites, broncho-alveolar lavage fluid, synovial fluid, and bronchial washes. In one aspect, the sample is a blood sample, including, for example, whole blood or any fraction or component thereof. A blood sample suitable for use with the present invention may be extracted from any source known that includes blood cells or components thereof, such as venous, arterial, peripheral, tissue, cord, and the like. For example, a sample may be obtained and processed using well-known and routine clinical methods (*e.g.*, procedures for drawing and processing whole blood). In one aspect, an exemplary sample may be peripheral blood drawn from a subject with cancer. In some aspects, the biological sample comprises a plurality of cells. In certain aspects, the biological sample comprises fresh or frozen tissue. In specific aspects, the biological sample comprises

formalin fixed, paraffin embedded tissue. In some aspects, the biological sample is a tissue biopsy, fine needle aspirate, blood, serum, plasma, cerebral spinal fluid, urine, stool, saliva, circulating tumor cells, exosomes, or aspirates and bodily secretions, such as sweat. In some aspects, the biological sample contains cell-free DNA.

B. Administration

[00179] In some cases, administration of an engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition herein and thereof can comprise packing the engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition herein and thereof into a composition or formulation for delivery or administration in a subject. In some cases, an administration of an engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition herein and thereof can refer to methods that can be used to enable delivery of the engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition herein and thereof to the desired site of biological action. Delivery can comprise direct application to the affect tissue or region of the body. An engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition herein and thereof herein can be administered by any method. A method of administration can be by inhalation, intraarterial injection, intracerebroventricular injection, intracisternal injection, intramuscular injection, infraorbital injection, intraparenchymal injection, intraperitoneal injection, intraspinal injection, intrathecal injection, intravenous injection, intraventricular injection, stereotactic injection, subcutaneous injection, or any combination thereof. Delivery can include parenteral administration (including intravenous, subcutaneous, intrathecal, intraperitoneal, intramuscular, intravascular or gradual infusion), oral administration, inhalation administration, intraduodenal administration, rectal administration. Delivery can include topical administration (such as a lotion, a cream, an ointment) to an external surface of a surface, such as a skin. In some cases, administration is by parenchymal injection, intra-thecal injection, intra-ventricular injection, intra-cisternal injection, intravenous injection, or intranasal administration or any combination thereof. In some instances, a subject can administer the composition in the absence of supervision. In some instances, a subject can administer the composition under the supervision of a medical professional (e.g., a physician, nurse, physician's assistant, orderly, hospice worker, etc.). A medical professional can administer the composition. In some cases, an engineered hSDH enzyme, nucleic acid

encoding the engineered hSDH enzyme, or pharmaceutical composition herein and thereof can also be delivered by peristaltic means, injected directly into the urinary tract, or administered by a pump connected to a catheter that may contain a potential biosensor for serine and/or threonine. In some cases, an engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition herein and thereof can also be administered intratumorally, intravenously, intradermally, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostaticaly, intrapleurally, intratracheally, intraocularly, intranasally, intravitreally, intravaginally, intrarectally, intramuscularly, subcutaneously, subconjunctival, intravesicularlly, mucosally, intrapericardially, intraumbilically, orally, by inhalation, by injection, by infusion, by continuous infusion, by localized perfusion bathing target cells directly, via a catheter, or via a lavage.

[00180] The methods of treating an individual with cancer described herein can comprise administration of an engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition herein and thereof in an individual with a cancer or suspected of a cancer. The methods of treating an individual with cancer described herein can also comprise administration of an engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition herein and thereof in an individual without a cancer or suspected of a cancer.

[00181] Administration or application of an engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition herein and thereof can be performed for a treatment duration of at least about at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 days consecutive or nonconsecutive days. A treatment duration can be from about 1 to about 30 days, from about 2 to about 30 days, from about 3 to about 30 days, from about 4 to about 30 days, from about 5 to about 30 days, from about 6 to about 30 days, from about 7 to about 30 days, from about 8 to about 30 days, from about 9 to about 30 days, from about 10 to about 30 days, from about 11 to about 30 days, from about 12 to about 30 days, from about 13 to about 30 days, from about 14 to about 30 days, from about 15 to about 30 days, from about 16 to about 30 days, from about 17 to about 30 days, from about 18 to about 30 days, from about 19 to

about 30 days, from about 20 to about 30 days, from about 21 to about 30 days, from about 22 to about 30 days, from about 23 to about 30 days, from about 24 to about 30 days, from about 25 to about 30 days, from about 26 to about 30 days, from about 27 to about 30 days, from about 28 to about 30 days, or from about 29 to about 30 days.

[00182] In some instances, administration or application of an engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition herein and thereof can be performed for a treatment duration of at least about 1 week, at least about 1 month, at least about 1 year, at least about 2 years, at least about 3 years, at least about 4 years, at least about 5 years, at least about 6 years, at least about 7 years, at least about 8 years, at least about 9 years, at least about 10 years, at least about 15 years, at least about 20 years, or more. Administration can be performed repeatedly over a lifetime of a subject, such as once a month or once a year for the lifetime of a subject. Administration can be performed repeatedly over a substantial portion of a subject's life, such as once a month or once a year for at least about 1 year, 5 years, 10 years, 15 years, 20 years, 25 years, 30 years, or more.

[00183] In some cases, an administration of any engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition herein and thereof to reduce a symptom of a disease or condition and/or to reduce a disease or condition. In some instances, an effective amount can be sufficient to achieve a desired effect. In the context of therapeutic or prophylactic applications, the effective amount will depend on the type and severity of the condition at issue and the characteristics of the individual subject, such as general health, age, sex, body weight, and tolerance to the engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition herein and thereof.

C. Dosing

[00184] Administration or application of an engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition herein and thereof can be performed at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 times a day. In some cases, administration or application of pharmaceutical compositions disclosed herein can be performed at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 times a week. In some cases, administration or application of an engineered hSDH enzyme, nucleic acid encoding the engineered hSDH

enzyme, or pharmaceutical composition herein and thereof can be performed at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, or 90 times a month.

[00185] An engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition herein and thereof can be administered/applied as a single dose or as divided doses. In some cases, the engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition herein and thereof can be administered at a first time point and a second time point. In some cases, an engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition herein and thereof can be administered such that a first administration is administered before the other with a difference in administration time of 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 1 day, 2 days, 4 days, 7 days, 2 weeks, 4 weeks, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year or more.

[00186] In some cases, an engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition comprising the engineered hSDH enzyme described herein and thereof can reduce the size of a tumor. In other cases, an engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition can decrease the size of a tumor by 0 %, 5 %, 10 %, 15 %, 20 %, 25 %, 30 %, 35 %, 40 %, 45 %, 50 %, 55 %, 60 %, 65 %, 70 %, 75 %, 80 %, 85 %, 90 %, 95 %, or 100 % of the size of the tumor before the administration of the engineered hSDH enzyme. In some instances, an engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition can decrease the size of a tumor by 1-10 %, 5-15 %, 10-20 %, 15-25 %, 20-30 %, 25-35 %, 30-40 %, 35-45 %, 40-50 %, 45-55 %, 50-60 %, 55-65 %, 60-70 %, 65-75 %, 70-80 %, 75-85 %, 80-90 %, 85-95 %, or 90-100 % of the size of the tumor before the administration of the engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition.

[00187] In some instances, an effective amount of an engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition can reduce the number of cancer cells. In some cases, an engineered hSDH enzyme, nucleic

acid encoding the engineered hSDH enzyme, or pharmaceutical composition described herein and thereof can reduce the number of cancer cells by 0 %, 5 %, 10 %, 15 %, 20 %, 25 %, 30 %, 35 %, 40 %, 45 %, 50 %, 55 %, 60 %, 65 %, 70 %, 75 %, 80 %, 85 %, 90 %, 95 %, or 100 % of number of the cancer cells before the administration of the engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition. In some instances, an effective amount of an engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition herein and thereof can decrease the number of cancer cells by 1-10 %, 5-15 %, 10-20 %, 15-25 %, 20-30 %, 25-35 %, 30-40 %, 35-45 %, 40-50 %, 45-55 %, 50-60 %, 55-65 %, 60-70 %, 65-75 %, 70-80 %, 75-85 %, 80-90 %, 85-95 %, or 90-100 % of the number of the cancer cells before the administration of the engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition.

[00188] In some cases, an engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition comprising the engineered hSDH enzyme described herein and thereof can decrease or prevent metastasis of a tumor. In other cases, an engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition can decrease or prevent metastasis of a tumor by 0 %, 5 %, 10 %, 15 %, 20 %, 25 %, 30 %, 35 %, 40 %, 45 %, 50 %, 55 %, 60 %, 65 %, 70 %, 75 %, 80 %, 85 %, 90 %, 95 %, or 100 % of that of a tumor without the administration of the engineered hSDH enzyme. In some instances, an engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition can decrease or prevent metastasis of a tumor by 1-10 %, 5-15 %, 10-20 %, 15-25 %, 20-30 %, 25-35 %, 30-40 %, 35-45 %, 40-50 %, 45-55 %, 50-60 %, 55-65 %, 60-70 %, 65-75 %, 70-80 %, 75-85 %, 80-90 %, 85-95 %, or 90-100 % of that the tumor without the administration of the engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition. In some instances, an effective amount of an engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition herein and thereof can delay the metastasis of a tumor by 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, 30 days, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 1 year, 2 years, 3 years, 4 years, 5 years, 6 years, 7 years, 8 years, 9 years, 10 years, 11 years, 12 years, 13 years, 14 years, 15 years, 16 years,

17 years, 18 years, 19 years, 20 years, 21 years, 22 years, 23 years, 24 years, 25 years, 26 years, 27 years, 28 years, 29 years, 30 years, 31 years, 32 years, 33 years, 34 years, 35 years, 36 years, 37 years, 38 years, 39 years, 40 years, 41 years, 42 years, 43 years, 44 years, 45 years, 46 years, 47 years, 48 years, 49 years, 50 years, or more than 50 years. In some instances, an effective amount of an engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition herein and thereof can delay the metastasis of a tumor by an amount of time from 1 day to 1 month, from 25 days to 6 months, from 5 months to 12 months, from 10 months to 2 years, from 1 year to 5 years, from 4 years to 10 years, from 9 years to 15 years, from 14 years to 20 years, from 19 years to 25 years, from 24 years to 30 years, from 29 years to 35 years, from 34 years to 40 years, from 39 years to 45 years, or from 44 years to 50 years.

[00189] In some cases, an effective amount of an engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition herein and thereof can extend the life-span of a subject administered with the engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition. In some instances, an effective amount of an engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition herein and thereof can extend the life-span of a subject administered with engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition by 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, 30 days, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 1 year, 2 years, 3 years, 4 years, 5 years, 6 years, 7 years, 8 years, 9 years, 10 years, 11 years, 12 years, 13 years, 14 years, 15 years, 16 years, 17 years, 18 years, 19 years, 20 years, 21 years, 22 years, 23 years, 24 years, 25 years, 26 years, 27 years, 28 years, 29 years, 30 years, 31 years, 32 years, 33 years, 34 years, 35 years, 36 years, 37 years, 38 years, 39 years, 40 years, 41 years, 42 years, 43 years, 44 years, 45 years, 46 years, 47 years, 48 years, 49 years, 50 years, or more than 50 years. In some instances, an effective amount of an engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition herein and thereof can extend the life-span of a subject administered with the engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition by an amount of time from 1 day

to 1 month, from 25 days to 6 months, from 5 months to 12 months, from 10 months to 2 years, from 1 year to 5 years, from 4 years to 10 years, from 9 years to 15 years, from 14 years to 20 years, from 19 years to 25 years, from 24 years to 30 years, from 29 years to 35 years, from 34 years to 40 years, from 39 years to 45 years, or from 44 years to 50 years.

[00190] In some cases, an effective amount of an engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition herein and thereof can delay the onset of a cancer of a subject administered with the engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition. In some instances, an effective amount of an engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition herein and thereof can delay the onset of a cancer of a subject administered with the engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition by 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, 30 days, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 1 year, 2 years, 3 years, 4 years, 5 years, 6 years, 7 years, 8 years, 9 years, 10 years, 11 years, 12 years, 13 years, 14 years, 15 years, 16 years, 17 years, 18 years, 19 years, 20 years, 21 years, 22 years, 23 years, 24 years, 25 years, 26 years, 27 years, 28 years, 29 years, 30 years, 31 years, 32 years, 33 years, 34 years, 35 years, 36 years, 37 years, 38 years, 39 years, 40 years, 41 years, 42 years, 43 years, 44 years, 45 years, 46 years, 47 years, 48 years, 49 years, 50 years, or more than 50 years. In some instances, an effective amount of an engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition herein and thereof can delay the onset of a cancer of a subject administered with the engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition by an amount of time from 1 day to 1 month, from 25 days to 6 months, from 5 months to 12 months, from 10 months to 2 years, from 1 year to 5 years, from 4 years to 10 years, from 9 years to 15 years, from 14 years to 20 years, from 19 years to 25 years, from 24 years to 30 years, from 29 years to 35 years, from 34 years to 40 years, from 39 years to 45 years, or from 44 years to 50 years.

[00191] In some cases, an effective amount of an engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition herein

and thereof can decrease the serum serine level by 10 %, 20 %, 30 %, 40 %, 50 %, 60 %, 70 %, 80 %, 90 %, 100 %, 150 %, 200 %, 250 %, 300 %, 350 %, 400 %, 450 %, 500 %, 550 %, 600 %, 650 %, 700 %, 750 %, 800 %, 850 %, 900 %, 950 %, 1000 %, 2000 %, 3000 %, 4000 %, 5000 %, 6000 %, 7000 %, 8000 %, 9000 %, 10000 %, 20000 %, 30000 %, 40000 %, 50000 %, 60000 %, 70000 %, 80000 %, 90000 %, or 100000 %. In some cases, an effective amount of an engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition herein and thereof can decrease the serum serine level from 10 to 30 %, from 20 to 40 %, from 30 to 50 %, from 40 to 60 %, from 50 to 70 %, from 60 to 80 %, from 70 to 90 %, from 80 to 100 %, from 90 to 150 %, from 100 to 200 %, from 150 to 250 %, from 200 to 300 %, from 250 to 350 %, from 300 to 400 %, from 350 to 450 %, from 400 to 500 %, from 450 to 550 %, from 500 to 600 %, from 550 to 650 %, from 600 to 700 %, from 650 to 750 %, from 700 to 800 %, from 750 to 850 %, from 800 to 900 %, from 850 to 950 %, from 900 to 1000 %, from 950 to 2000 %, from 1500 to 2500 %, from 2000 to 3000 %, from 2500 to 3500 %, from 3000 to 4000 %, from 3500 to 4500 %, from 4000 to 5000 %, from 4500 to 5500 %, from 5000 to 6000 %, from 5500 to 6500 %, from 6000 to 7000 %, from 6500 to 7500 %, from 7000 to 8000 %, from 7500 to 8500 %, from 8000 to 9000 %, from 8500 to 9500 %, from 9000 to 10000 %, from 9500 to 20000 %, from 15000 to 25000 %, from 20000 to 30000 %, from 25000 to 35000 %, from 30000 to 40000 %, from 35000 to 45000 %, from 40000 to 50000 %, from 45000 to 55000 %, from 50000 to 60000 %, from 55000 to 65000 %, from 60000 to 70000 %, from 65000 to 75000 %, from 70000 to 80000 %, from 75000 to 85000 %, from 80000 to 90000 %, from 85000 to 95000 %, or from 90000 to 100000 %.

[00192] In some cases, an effective amount of an engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition herein and thereof can decrease the serum threonine level by 10 %, 20 %, 30 %, 40 %, 50 %, 60 %, 70 %, 80 %, 90 %, 100 %, 150 %, 200 %, 250 %, 300 %, 350 %, 400 %, 450 %, 500 %, 550 %, 600 %, 650 %, 700 %, 750 %, 800 %, 850 %, 900 %, 950 %, 1000 %, 2000 %, 3000 %, 4000 %, 5000 %, 6000 %, 7000 %, 8000 %, 9000 %, 10000 %, 20000 %, 30000 %, 40000 %, 50000 %, 60000 %, 70000 %, 80000 %, 90000 %, or 100000 %. In some cases, an effective amount of an engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition herein and thereof can decrease the serum threonine level from 10 to 30 %, from 20 to 40 %, from 30 to 50 %, from 40 to 60 %, from 50 to 70 %, from 60 to 80 %, from 70 to 90 %, from 80 to 100 %, from 90 to 150 %, from 100 to 200 %,

from 150 to 250 %, from 200 to 300 %, from 250 to 350 %, from 300 to 400 %, from 350 to 450 %, from 400 to 500 %, from 450 to 550 %, from 500 to 600 %, from 550 to 650 %, from 600 to 700 %, from 650 to 750 %, from 700 to 800 %, from 750 to 850 %, from 800 to 900 %, from 850 to 950 %, from 900 to 1000 %, from 950 to 2000 %, from 1500 to 2500 %, from 2000 to 3000 %, from 2500 to 3500 %, from 3000 to 4000 %, from 3500 to 4500 %, from 4000 to 5000 %, from 4500 to 5500 %, from 5000 to 6000 %, from 5500 to 6500 %, from 6000 to 7000 %, from 6500 to 7500 %, from 7000 to 8000 %, from 7500 to 8500 %, from 8000 to 9000 %, from 8500 to 9500 %, from 9000 to 10000 %, from 9500 to 20000 %, from 15000 to 25000 %, from 20000 to 30000 %, from 25000 to 35000 %, from 30000 to 40000 %, from 35000 to 45000 %, from 40000 to 50000 %, from 45000 to 55000 %, from 50000 to 60000 %, from 55000 to 65000 %, from 60000 to 70000 %, from 65000 to 75000 %, from 70000 to 80000 %, from 75000 to 85000 %, from 80000 to 90000 %, from 85000 to 95000 %, or from 90000 to 100000 %.

[00193] In some cases, a reduction of serine and/or threonine level can be conducted in vivo in the circulation of a mammal, in vitro in cases where serine and/or threonine reduction in tissue culture or other biological mediums is desired, and in ex vivo procedures where biological fluids, cells, or tissues are manipulated outside the body and subsequently returned to the body of the patient mammal. In some case, a reduction of serine and/or threonine from circulation, culture media, biological fluids, or cells can be conducted to reduce the amount of serine and/or threonine accessible to the material being treated, and therefore comprises contacting the material to be depleted with a serine and/or threonine-degrading amount of an engineered hSDH enzyme under serine and/or threonine-degrading conditions as to degrade the ambient serine and/or threonine in the material being contacted.

[00194] In some instances, the serine and/or threonine-degrading efficiency of an engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition herein and thereof can vary widely depending upon the application; and can depend upon the amount of serine and/or threonine present in the material, the desired rate of depletion, and the tolerance of the material for exposure to the administered materials. In some cases, serine and/or threonine levels in a material, and therefore rates of serine and/or threonine depletion from the material, can readily be monitored by a variety of chemical and biochemical methods well known in the art. In some instances, serine and/or threonine-degrading amounts can be described further herein, and can

range from 0.001 to 100 units (U) of engineered hSDH enzyme, preferably about 0.01 to 10 U, and more preferably about 0.1 to 5 U engineered hSDH enzyme per milliliter (mL) of material to be treated.

[00195] In some cases, the conditions for serine and/or threonine-degrading can comprise buffer and temperature conditions compatible with the biological activity of an engineered hSDH enzyme, comprise moderate temperature, salt, and pH conditions compatible with the enzyme, for example, physiological conditions. Exemplary conditions can comprise about 4-40°C, ionic strength equivalent to about 0.05 to 0.2 M NaCl, and a pH of about 5 to 9, while physiological conditions are included.

[00196] In some case, an engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition herein and thereof can be conventionally administered intravenously, as by injection of a unit dose.

[00197] In some cases, the quantity to be administered depends on the subject to be treated, capacity of the subject's system to utilize the enzyme, and degree of therapeutic effect desired. In some instances, precise amounts of enzyme required to be administered can depend on the judgment of the practitioner and are peculiar to each individual. In some cases, suitable dosage ranges for systemic application are disclosed herein and can depend on the route of administration. In some instances, suitable regimes for initial administration and booster shots can also be contemplated and be typified by an initial administration followed by repeated doses at one or more hour intervals by a subsequent injection or other administration. In some instances, administrations are described herein and are particularly preferred to maintain continuously high serum and tissue levels of engineered hSDH enzyme and conversely low serum and tissue levels of serine and/or threonine. In some cases, continuous intravenous infusion sufficient to maintain concentrations in the blood in the ranges specified for in vivo therapies can be contemplated.

[00198] In some cases, an effective amount of an engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition can be adjusted based on the material being administered. In some instances, the effective amount of an engineered hSDH enzyme, nucleic acid encoding the engineered hSDH enzyme, or pharmaceutical composition can vary based on the therapeutic desirable outcome described herein and thereof being sought.

[00199] In some cases, a therapeutically effective amount of an engineered hSDH enzyme can comprise a predetermined amount calculated to achieve the desired effect comprising a reduction of serine and/or threonine in a subject's circulation. Thus, the dosage ranges for the administration of an engineered hSDH enzyme are those large enough to produce the desired effect. The dosage should not be so large as to cause adverse side effects, such as hyperviscosity syndromes, pulmonary edema, congestive heart failure, and the like. Generally, the dosage will vary with age of, condition of, sex of, and extent of the disease in the patient and can be determined by one of skill in the art. The dosage can be adjusted by the individual physician in the event of any complication.

[00200] In other cases, a therapeutically effective amount of an engineered hSDH enzyme may be an amount such that when administered in a physiologically tolerable composition is sufficient to achieve a intravascular (plasma) or local concentration of from about 0.001 to about 100 units (U) per mL, preferably above about 0.1 U, and more preferably above 1 U engineered hSDH enzyme per mL. Typical dosages can be administered based on body weight and are in the range of about 5-1000 U/kilogram (kg)/day, preferably about 5-100 U/kg/day, more preferably about 10-50 U/kg/day, and more preferably about 20-40 U/kg/day.

[00201] In some embodiments, a dose may also comprise about 1x10^1 microgram/kg/body weight, about 2x10^1 microgram/kg/body weight, about 3x10^1 microgram/kg/body weight, about 4x10^1 microgram/kg/body weight, about 5x10^1 microgram/kg/body weight, about 6x10^1 microgram/kg/body weight, about 7x10^1 microgram/kg/body weight, about 8x10^1 microgram/kg/body weight, about 9x10^1 microgram/kg/body weight, about 1x10^2 microgram/kg/body weight, about 2x10^2 microgram/kg/body weight, about 3x10^2 microgram/kg/body weight, about 4x10^2 microgram/kg/body weight, about 5x10^2 microgram/kg/body weight, about 6x10^2 microgram/kg/body weight, about 7x10^2 microgram/kg/body weight, about 8x10^2 microgram/kg/body weight, about 9x10^2 microgram/kg/body weight, about 1x10^3 microgram/kg/body weight, about 2x10^3 microgram/kg/body weight, about 3x10^3 microgram/kg/body weight, about 4x10^3 microgram/kg/body weight, about 5x10^3 microgram/kg/body weight, about 6x10^3 microgram/kg/body weight, about 7x10^3 microgram/kg/body weight, about 8x10^3 microgram/kg/body weight, about 9x10^3 microgram/kg/body weight, about 1x10^4 microgram/kg/body weight, about 2x10^4

microgram/kg/body weight, about 3x10^4 microgram/kg/body weight, about 4x10^4 microgram/kg/body weight, about 5x10^4 microgram/kg/body weight, about 6x10^4 microgram/kg/body weight, about 7x10^4 microgram/kg/body weight, about 8x10^4 microgram/kg/body weight, about 9x10^4 microgram/kg/body weight, about 1x10^5 microgram/kg/body weight, about 2x10^5 microgram/kg/body weight, about 3x10^5 microgram/kg/body weight, about 4x10^5 microgram/kg/body weight, about 5x10^5 microgram/kg/body weight, about 6x10^5 microgram/kg/body weight, about 7x10^5 microgram/kg/body weight, about 8x10^5 microgram/kg/body weight, about 9x10^5 microgram/kg/body weight, about 1x10^6 microgram/kg/body weight, about 2x10^6 microgram/kg/body weight, about 3x10^6 microgram/kg/body weight, about 4x10^6 microgram/kg/body weight, about 5x10^6 microgram/kg/body weight, about 6x10^6 microgram/kg/body weight, about 7x10^6 microgram/kg/body weight, about 8x10^6 microgram/kg/body weight, about 9x10^6 microgram/kg/body weight, about 1x10^7 microgram/kg/body weight, about 2x10^7 microgram/kg/body weight, about 3x10^7 microgram/kg/body weight, about 4x10^7 microgram/kg/body weight, about 5x10^7 microgram/kg/body weight, about 6x10^7 microgram/kg/body weight, about 7x10^7 microgram/kg/body weight, about 8x10^7 microgram/kg/body weight, about 9x10^7 microgram/kg/body weight, about 1x10^8 microgram/kg/body weight, about 2x10^8 microgram/kg/body weight, about 3x10^8 microgram/kg/body weight, about 4x10^8 microgram/kg/body weight, about 5x10^8 microgram/kg/body weight, about 6x10^8 microgram/kg/body weight, about 7x10^8 microgram/kg/body weight, about 8x10^8 microgram/kg/body weight, about 9x10^8 microgram/kg/body weight, about 1x10^9 microgram/kg/body weight, about 2x10^9 microgram/kg/body weight, about 3x10^9 microgram/kg/body weight, about 4x10^9 microgram/kg/body weight, about 5x10^9 microgram/kg/body weight, about 6x10^9 microgram/kg/body weight, about 7x10^9 microgram/kg/body weight, about 8x10^9 microgram/kg/body weight, about 9x10^9 microgram/kg/body weight, about 1x10^10 microgram/kg/body weight, about 2x10^10 microgram/kg/body weight, about 3x10^10 microgram/kg/body weight, about 4x10^10 microgram/kg/body weight, about 5x10^10 microgram/kg/body weight, about 6x10^10 microgram/kg/body weight, about 7x10^10 microgram/kg/body weight, about 8x10^10 microgram/kg/body weight, or about 9x10^10 microgram/kg/body weight. In some embodiments, a dose may also comprise from 0.5x10^1 to 2x10^1 microgram/kg/body weight, from 1.5x10^1 to 3x10^1 microgram/kg/body weight, from 2.5x10^1 to 4x10^1 microgram/kg/body weight, from 3.5x10^1 to 5x10^1 microgram/kg/body weight, from

4.5x10^1 to 6x10^1 microgram/kg/body weight, from 5.5x10^1 to 7x10^1 microgram/kg/body weight, from 6.5x10^1 to 8x10^1 microgram/kg/body weight, from 7.5x10^1 to 9x10^1 microgram/kg/body weight, from 8.5x10^1 to 1x10^1 microgram/kg/body weight, from 0.5x10^2 to 2x10^2 microgram/kg/body weight, from 1.5x10^2 to 3x10^2 microgram/kg/body weight, from 2.5x10^2 to 4x10^2 microgram/kg/body weight, from 3.5x10^2 to 5x10^2 microgram/kg/body weight, from 4.5x10^2 to 6x10^2 microgram/kg/body weight, from 5.5x10^2 to 7x10^2 microgram/kg/body weight, from 6.5x10^2 to 8x10^2 microgram/kg/body weight, from 7.5x10^2 to 9x10^2 microgram/kg/body weight, from 8.5x10^2 to 1x10^3 microgram/kg/body weight, from 0.5x10^3 to 2x10^3 microgram/kg/body weight, from 1.5x10^3 to 3x10^3 microgram/kg/body weight, from 2.5x10^3 to 4x10^3 microgram/kg/body weight, from 3.5x10^3 to 5x10^3 microgram/kg/body weight, from 4.5x10^3 to 6x10^3 microgram/kg/body weight, from 5.5x10^3 to 7x10^3 microgram/kg/body weight, from 6.5x10^3 to 8x10^3 microgram/kg/body weight, from 7.5x10^3 to 9x10^3 microgram/kg/body weight, from 8.5x10^3 to 1x10^4 microgram/kg/body weight, from 0.5x10^4 to 2x10^4 microgram/kg/body weight, from 1.5x10^4 to 3x10^4 microgram/kg/body weight, from 2.5x10^4 to 4x10^4 microgram/kg/body weight, from 3.5x10^4 to 5x10^4 microgram/kg/body weight, from 4.5x10^4 to 6x10^4 microgram/kg/body weight, from 5.5x10^4 to 7x10^4 microgram/kg/body weight, from 6.5x10^4 to 8x10^4 microgram/kg/body weight, from 7.5x10^4 to 9x10^4 microgram/kg/body weight, from 8.5x10^4 to 1x10^5 microgram/kg/body weight, from 0.5x10^5 to 2x10^5 microgram/kg/body weight, from 1.5x10^5 to 3x10^5 microgram/kg/body weight, from 2.5x10^5 to 4x10^5 microgram/kg/body weight, from 3.5x10^5 to 5x10^5 microgram/kg/body weight, from 4.5x10^5 to 6x10^5 microgram/kg/body weight, from 5.5x10^5 to 7x10^5 microgram/kg/body weight, from 6.5x10^5 to 8x10^5 microgram/kg/body weight, from 7.5x10^5 to 9x10^5 microgram/kg/body weight, from 8.5x10^5 to 1x10^6 microgram/kg/body weight, from 0.5x10^6 to 2x10^6 microgram/kg/body weight, from 1.5x10^6 to 3x10^6 microgram/kg/body weight, from 2.5x10^6 to 4x10^6 microgram/kg/body weight, from 3.5x10^6 to 5x10^6 microgram/kg/body weight, from 4.5x10^6 to 6x10^6 microgram/kg/body weight, from 5.5x10^6 to 7x10^6 microgram/kg/body weight, from 6.5x10^6 to 8x10^6 microgram/kg/body weight, from 7.5x10^6 to 9x10^6 microgram/kg/body weight, from 8.5x10^6 to 1x10^7 microgram/kg/body weight, from 0.5x10^7 to 2x10^7 microgram/kg/body weight, from

1.5x10^7 to 3x10^7 microgram/kg/body weight, from 2.5x10^7 to 4x10^7 microgram/kg/body weight, from 3.5x10^7 to 5x10^7 microgram/kg/body weight, from 4.5x10^7 to 6x10^7 microgram/kg/body weight, from 5.5x10^7 to 7x10^7 microgram/kg/body weight, from 6.5x10^7 to 8x10^7 microgram/kg/body weight, from 7.5x10^7 to 9x10^7 microgram/kg/body weight, from 8.5x10^7 to 1x10^8 microgram/kg/body weight, from 0.5x10^8 to 2x10^8 microgram/kg/body weight, from 1.5x10^8 to 3x10^8 microgram/kg/body weight, from 2.5x10^8 to 4x10^8 microgram/kg/body weight, from 3.5x10^8 to 5x10^8 microgram/kg/body weight, from 4.5x10^8 to 6x10^8 microgram/kg/body weight, from 5.5x10^8 to 7x10^8 microgram/kg/body weight, from 6.5x10^8 to 8x10^8 microgram/kg/body weight, from 7.5x10^8 to 9x10^8 microgram/kg/body weight, from 8.5x10^8 to 1x10^9 microgram/kg/body weight, from 0.5x10^9 to 2x10^9 microgram/kg/body weight, from 1.5x10^9 to 3x10^9 microgram/kg/body weight, from 2.5x10^9 to 4x10^9 microgram/kg/body weight, from 3.5x10^9 to 5x10^9 microgram/kg/body weight, from 4.5x10^9 to 6x10^9 microgram/kg/body weight, from 5.5x10^9 to 7x10^9 microgram/kg/body weight, from 6.5x10^9 to 8x10^9 microgram/kg/body weight, from 7.5x10^9 to 9x10^9 microgram/kg/body weight, from 8.5x10^9 to 1x10^10 microgram/kg/body weight, from 0.5x10^10 to 2x10^10 microgram/kg/body weight, from 1.5x10^10 to 3x10^10 microgram/kg/body weight, from 2.5x10^10 to 4x10^10 microgram/kg/body weight, from 3.5x10^10 to 5x10^10 microgram/kg/body weight, from 4.5x10^10 to 6x10^10 microgram/kg/body weight, from 5.5x10^10 to 7x10^10 microgram/kg/body weight, from 6.5x10^10 to 8x10^10 microgram/kg/body weight, from 7.5x10^10 to 9x10^10 microgram/kg/body weight, or from 8.5x10^10 to 1x10^10 microgram/kg/body weight.

[00202] The actual dosage amount of a composition administered to an animal patient can be determined by physical and physiological factors, such as body weight, severity of condition, the type of disease being treated, previous or concurrent therapeutic interventions, idiopathy of the patient, and on the route of administration. Depending upon the dosage and the route of administration, the number of administrations of a preferred dosage and/or an effective amount may vary according to the response of the subject. The practitioner responsible for administration will, in any event, determine the concentration of active ingredient(s) in a composition and appropriate dose(s) for the individual subject.

[00203] In certain embodiments, pharmaceutical compositions may comprise, for example, at least about 0.1% of an active compound. In other embodiments, an active compound may comprise between about 2% to about 75% of the weight of the unit, or between about 25% to about 60%, for example, and any range derivable therein. Naturally, the amount of active compound(s) in each therapeutically useful composition may be prepared in such a way that a suitable dosage will be obtained in any given unit dose of the compound. Factors, such as solubility, bioavailability, biological half-life, route of administration, product shelf life, as well as other pharmacological considerations, will be contemplated by one skilled in the art of preparing such pharmaceutical formulations, and as such, a variety of dosages and treatment regimens may be desirable.

[00204] In other non-limiting examples, a dose may also comprise from about 1 microgram/kg/body weight, about 5 microgram/kg/body weight, about 10 microgram/kg/body weight, about 50 microgram/kg/body weight, about 100 microgram/kg/body weight, about 200 microgram/kg/body weight, about 350 microgram/kg/body weight, about 500 microgram/kg/body weight, about 1 milligram/kg/body weight, about 5 milligram/kg/body weight, about 10 milligram/kg/body weight, about 50 milligram/kg/body weight, about 100 milligram/kg/body weight, about 200 milligram/kg/body weight, about 350 milligram/kg/body weight, about 500 milligram/kg/body weight, to about 1000 milligram/kg/body weight or more per administration, and any range derivable therein. In non-limiting examples of a derivable range from the numbers listed herein, a range of about 5 milligram/kg/body weight to about 100 milligram/kg/body weight, about 5 microgram/kg/body weight to about 500 milligram/kg/body weight, etc., can be administered, based on the numbers described above.

VIII. Combination Treatments

[00205] In certain embodiments, the compositions and methods of the present embodiments involve administration of an engineered hSDH enzyme in combination with a second or additional therapy. The methods and compositions, including combination therapies, enhance the therapeutic or protective effect, and/or increase the therapeutic effect of another therapy. Therapeutic and prophylactic methods and compositions can be provided in a combined amount effective to achieve the desired effect. This process may involve administering both an engineered hSDH enzyme and a second therapy. A tissue, organ, or cell can be exposed to one or more compositions or pharmacological formulation(s)

comprising one or more of the agents (*i.e.*, an engineered hSDH enzyme or a second agent), or by contacting the tissue, organ, and/or cell with two or more distinct compositions or formulations, wherein one composition provides 1) an engineered hSDH enzyme, 2) a second agent, or 3) both an engineered hSDH enzyme and a second agent. Also, it is contemplated that such a combination therapy can be used in conjunction with surgical therapy.

[00206] An engineered hSDH enzyme may be administered before, during, after, or in various combinations relative to a second treatment. The administrations may be in intervals ranging from concurrently to minutes to days to weeks. In embodiments where the engineered hSDH enzyme is provided to a patient separately from a second agent, one would generally ensure that a significant period of time did not expire between the time of each delivery, such that the two treatments would still be able to exert an advantageously combined effect on the patient. In such instances, it is contemplated that one may provide a patient with the engineered hSDH enzyme and the second therapy within about 12 to 24 or 72 h of each other and, more particularly, within about 6-12 h of each other. In some situations it may be desirable to extend the time period for treatment significantly where several days (2, 3, 4, 5, 6, or 7) to several weeks (1, 2, 3, 4, 5, 6, 7, or 8) lapse between respective administrations.

[00207] In certain embodiments, a course of treatment will last 1-90 days or more (this such range includes intervening days). It is contemplated that the engineered hSDH enzyme may be given on any day of day 1 to day 90 (this such range includes intervening days) or any combination thereof, and another treatment is given on any day of day 1 to day 90 (this such range includes intervening days) or any combination thereof. Within a single day (24-hour period), the patient may be given one or multiple administrations of the treatment(s). Moreover, after a course of treatment, it is contemplated that there is a period of time at which no treatment is administered. This time period may last 1-7 days, and/or 1-5 weeks, and/or 1-12 months or more (this such range includes intervening days), depending on the condition of the patient, such as their prognosis, strength, health, *etc.* *I*t is expected that the treatment cycles would be repeated as necessary.

[00208] Various combinations may be employed. For the example below an engineered hSDH enzyme is "A" and a second therapy is "B":

A/B/A B/A/B B/B/A A/A/B A/B/B B/A/A A/B/B/B B/A/B/B

B/B/B/A B/B/A/B A/A/B/B A/B/A/B A/B/B/A B/B/A/A
B/A/B/A B/A/A/B A/A/A/B B/A/A/A A/B/A/A A/A/B/A

[00209] Administration of any compound or therapy of the present embodiments to a patient will follow general protocols for the administration of such compounds, taking into account the toxicity, if any, of the agents. Therefore, in some embodiments there is a step of monitoring toxicity that is attributable to combination therapy.

A. Chemotherapy

[00210] A wide variety of chemotherapeutic agents may be used in accordance with the present embodiments. The term "chemotherapy" refers to the use of drugs to treat cancer. A "chemotherapeutic agent" is used to connote a compound or composition that is administered in the treatment of cancer. These agents or drugs are categorized by their mode of activity within a cell, for example, whether and at what stage they affect the cell cycle. Alternatively, an agent may be characterized based on its ability to directly cross-link DNA, to intercalate into DNA, or to induce chromosomal and mitotic aberrations by affecting nucleic acid synthesis.

[00211] Examples of chemotherapeutic agents include alkylating agents, such as thiotepa and cyclosphosphamide; alkyl sulfonates, such as busulfan, improsulfan, and piposulfan; aziridines, such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines, including altretamine, triethylenemelamine, trietylenephosphoramide, triethiylenethiophosphoramide, and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards, such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, and uracil mustard; nitrosureas, such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics, such as the enediyne antibiotics (*e.g.,* calicheamicin, especially calicheamicin gammaII and calicheamicin omegaI1); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related

chromoprotein enediyne antiobiotic chromophores, aclacinomysins, actinomycin, authrarnycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, such as mitomycin C, mycophenolic acid, nogalarnycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, and zorubicin; anti-metabolites, such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues, such as denopterin, pteropterin, and trimetrexate; purine analogs, such as fludarabine, 6-mercaptopurine, thiamiprine, and thioguanine; pyrimidine analogs, such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, and floxuridine; androgens, such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, and testolactone; anti-adrenals, such as mitotane and trilostane; folic acid replenisher, such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids, such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSKpolysaccharide complex; razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; taxoids, *e.g.*, paclitaxel and docetaxel gemcitabine; 6-thioguanine; mercaptopurine; platinum coordination complexes, such as cisplatin, oxaliplatin, and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; irinotecan (*e.g.*, CPT-11); topoisomerase inhibitor RFS 2000; difluorometlhylornithine (DMFO); retinoids, such as retinoic acid; capecitabine; carboplatin, procarbazine,plicomycin, gemcitabien, navelbine, farnesyl-protein tansferase inhibitors, transplatinum, and pharmaceutically acceptable salts, acids, or derivatives of any of the above.

B. Radiotherapy

[00212] Other factors that cause DNA damage and have been used extensively include what are commonly known as γ-rays, X-rays, and/or the directed delivery of radioisotopes to tumor cells. Other forms of DNA damaging factors are also contemplated, such as microwaves, proton beam irradiation (U.S. Patents 5,760,395 and 4,870,287), and UV-irradiation. It is most likely that all of these factors affect a broad range of damage on DNA, on the precursors of DNA, on the replication and repair of DNA, and on the assembly and maintenance of chromosomes. Dosage ranges for X-rays range from daily doses of 50 to 200 roentgens for prolonged periods of time (3 to 4 wk), to single doses of 2000 to 6000 roentgens. Dosage ranges for radioisotopes vary widely, and depend on the half-life of the isotope, the strength and type of radiation emitted, and the uptake by the neoplastic cells.

C. Immunotherapy

[00213] The skilled artisan will understand that immunotherapies may be used in combination or in conjunction with methods of the embodiments. In the context of cancer treatment, immunotherapeutics, generally, rely on the use of immune effector cells and molecules to target and destroy cancer cells. Rituximab (RITUXAN®) is such an example. The immune effector may be, for example, an antibody specific for some marker on the surface of a tumor cell. The antibody alone may serve as an effector of therapy or it may recruit other cells to actually affect cell killing. The antibody also may be conjugated to a drug or toxin (chemotherapeutic, radionuclide, ricin A chain, cholera toxin, pertussis toxin, *etc.*) and serve merely as a targeting agent. Alternatively, the effector may be a lymphocyte carrying a surface molecule that interacts, either directly or indirectly, with a tumor cell target. Various effector cells include cytotoxic T-cells and NK cells.

[00214] In one aspect of immunotherapy, the tumor cell must bear some marker that is amenable to targeting, *i.e.*, is not present on the majority of other cells. Many tumor markers exist and any of these may be suitable for targeting in the context of the present embodiments. Common tumor markers include CD20, carcinoembryonic antigen, tyrosinase (p97), gp68, TAG-72, HMFG, Sialyl Lewis Antigen, MucA, MucB, PLAP, laminin receptor, erb B, and p155. An alternative aspect of immunotherapy is to combine anticancer effects with immune stimulatory effects. Immune stimulating molecules also exist including: cytokines, such as IL-2, IL-4, IL-12, GM-CSF, gamma-IFN, chemokines, such as MIP-1, MCP-1, IL-8, and growth factors, such as FLT3 ligand.

[00215] Examples of immunotherapies currently under investigation or in use are immune adjuvants, *e.g.*, *Mycobacterium bovis*, *Plasmodium falciparum*, dinitrochlorobenzene, and aromatic compounds (U.S. Patents 5,801,005 and 5,739,169; Hui and Hashimoto, 1998; Christodoulides *et al.*, 1998); cytokine therapy, *e.g.*, interferons α, β, and γ, IL-1, GM-CSF, and TNF (Bukowski *et al.*, 1998; Davidson *et al.*, 1998; Hellstrand *et al.*, 1998); gene therapy, *e.g.*, TNF, IL-1, IL-2, and p53 (Qin *et al.*, 1998; Austin-Ward and Villaseca, 1998; U.S. Patents 5,830,880 and 5,846,945); and monoclonal antibodies, *e.g.*, anti-CD20, anti-ganglioside GM2, and anti-p185 (Hollander, 2012; Hanibuchi *et al.*, 1998; U.S. Patent 5,824,311). It is contemplated that one or more anti-cancer therapies may be employed with the antibody therapies described herein.

[00216] In some embodiments, the immunotherapy may be an immune checkpoint inhibitor. Immune checkpoints either turn up a signal (*e.g.*, co-stimulatory molecules) or turn down a signal. Immune checkpoints either turn up a signal (*e.g.*, co-stimulatory molecules) or turn down a signal. Immune checkpoint proteins that may be targeted by immune checkpoint blockade include adenosine A2A receptor (A2AR), B7-H3 (also known as CD276), B and T lymphocyte attenuator (BTLA), CCL5, CD27, CD38, CD8A, CMKLR1, cytotoxic T-lymphocyte-associated protein 4 (CTLA-4, also known as CD152), CXCL9, CXCR5, glucocorticoid-induced tumour necrosis factor receptor-related protein (GITR), HLA-DRB1, ICOS (also known as CD278), HLA-DQA1, HLA-E, indoleamine 2,3-dioxygenase 1 (IDO1), killer-cell immunoglobulin (KIR), lymphocyte activation gene-3 (LAG-3, also known as CD223), Mer tyrosine kinase (MerTK), NKG7, OX40 (also known as CD134), programmed death 1 (PD-1), programmed death-ligand 1 (PD-L1, also known as CD274), PDCD1LG2, PSMB10, STAT1, T-cell immunoreceptor with Ig and ITIM domains (TIGIT), T-cell immunoglobulin domain and mucin domain 3 (TIM-3), V-domain Ig suppressor of T-cell activation (VISTA, also known as C10orf54), and 4-1BB (CD137). In particular, the immune checkpoint inhibitors target the PD-1 axis and/or CTLA-4.

[00217] The immune checkpoint inhibitors may be drugs, such as small molecules, recombinant forms of ligand or receptors, or antibodies, such as human antibodies (*e.g.*, International Patent Publication WO2015/016718; Pardoll, *Nat Rev Cancer*, 12(4): 252-264, 2012; both incorporated herein by reference). Known inhibitors of the immune checkpoint proteins or analogs thereof may be used, in particular chimerized, humanized, or

human forms of antibodies may be used. As the skilled person will know, alternative and/or equivalent names may be in use for certain antibodies mentioned in the present disclosure. Such alternative and/or equivalent names are interchangeable in the context of the present disclosure. For example, it is known that lambrolizumab is also known under the alternative and equivalent names MK-3475 and pembrolizumab.

[00218] In some embodiments, a PD-1 binding antagonist is a molecule that inhibits the binding of PD-1 to its ligand binding partners. In a specific aspect, the PD-1 ligand binding partners are PD-L1 and/or PD-L2. In another embodiment, a PD-L1 binding antagonist is a molecule that inhibits the binding of PD-L1 to its binding partners. In a specific aspect, PD-L1 binding partners are PD-1 and/or B7-1. In another embodiment, a PD-L2 binding antagonist is a molecule that inhibits the binding of PD-L2 to its binding partners. In a specific aspect, a PD-L2 binding partner is PD-1. The antagonist may be an antibody, an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or an oligopeptide. Exemplary antibodies are described in U.S. Patent Nos. 8,735,553, 8,354,509, and 8,008,449, all of which are incorporated herein by reference. Other PD-1 axis antagonists for use in the methods provided herein are known in the art, such as described in U.S. Patent Application Publication Nos. 2014/0294898, 2014/022021, and 2011/0008369, all of which are incorporated herein by reference.

[00219] In some embodiments, a PD-1 binding antagonist is an anti-PD-1 antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody). In some embodiments, the anti-PD-1 antibody is selected from the group consisting of nivolumab, pembrolizumab, and CT-011. In some embodiments, the PD-1 binding antagonist is an immunoadhesin (*e.g.*, an immunoadhesin comprising an extracellular or PD-1 binding portion of PD-L1 or PD-L2 fused to a constant region (*e.g.*, an Fc region of an immunoglobulin sequence)). In some embodiments, the PD-1 binding antagonist is AMP- 224. Nivolumab, also known as MDX-1106-04, MDX-1106, ONO-4538, BMS-936558, and OPDIVO®, is an anti-PD-1 antibody described in WO2006/121168. Pembrolizumab, also known as MK-3475, Merck 3475, lambrolizumab, KEYTRUDA®, and SCH-900475, is an anti-PD-1 antibody described in WO2009/114335. CT-011, also known as hBAT or hBAT-1, is an anti-PD-1 antibody described in WO2009/101611. AMP-224, also known as B7-DCIg, is a PD-L2-Fc fusion soluble receptor described in WO2010/027827 and WO2011/066342.

[00220] Another immune checkpoint protein that can be targeted in the methods provided herein is the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), also known as CD152. The complete cDNA sequence of human CTLA-4 has the Genbank accession number L15006. CTLA-4 is found on the surface of T-cells and acts as an "off" switch when bound to CD80 or CD86 on the surface of antigen-presenting cells. CTLA-4 is similar to the T-cell co-stimulatory protein, CD28, and both molecules bind to CD80 and CD86, also called B7-1 and B7-2 respectively, on antigen-presenting cells. CTLA-4 transmits an inhibitory signal to T-cells, whereas CD28 transmits a stimulatory signal. Intracellular CTLA-4 is also found in regulatory T-cells and may be important to their function. T-cell activation through the T-cell receptor and CD28 leads to increased expression of CTLA-4, an inhibitory receptor for B7 molecules.

[00221] In some embodiments, the immune checkpoint inhibitor is an anti-CTLA-4 antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody), an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide. Anti-human-CTLA-4 antibodies (or VH and/or VL domains derived therefrom) suitable for use in the present methods can be generated using methods well known in the art. Alternatively, art recognized anti-CTLA-4 antibodies can be used. For example, the anti-CTLA-4 antibodies disclosed in US Patent No. 8,119,129; PCT Publn. Nos. WO 01/14424, WO 98/42752, WO 00/37504 (CP675,206, also known as tremelimumab; formerly ticilimumab); U.S. Patent No. 6,207,156; Hurwitz *et al.* (1998) *Proc Natl Acad Sci USA*, 95(17): 10067-10071; Camacho *et al.* (2004) *J Clin Oncology*, 22(145): Abstract No. 2505 (antibody CP-675206); and Mokyr *et al.* (1998) *Cancer Res*, 58:5301-5304 can be used in the methods disclosed herein. The teachings of each of the aforementioned publications are hereby incorporated by reference. Antibodies that compete with any of these art-recognized antibodies for binding to CTLA-4 can also be used. For example, a humanized CTLA-4 antibody is described in International Patent Application No. WO2001/014424, WO2000/037504, and U.S. Patent No. 8,017,114; all incorporated herein by reference.

[00222] An exemplary anti-CTLA-4 antibody is ipilimumab (also known as 10D1, MDX- 010, MDX- 101, and Yervoy®) or antigen binding fragments and variants thereof (see, *e.g.*, WO 01/14424). In other embodiments, the antibody comprises the heavy and light chain CDRs or VRs of ipilimumab. Accordingly, in one embodiment, the antibody comprises the CDR1, CDR2, and CDR3 domains of the VH region of ipilimumab, and the

CDR1, CDR2, and CDR3 domains of the VL region of ipilimumab. In another embodiment, the antibody competes for binding with and/or binds to the same epitope on CTLA-4 as the above-mentioned antibodies. In another embodiment, the antibody has an at least about 90% variable region amino acid sequence identity with the above-mentioned antibodies (*e.g.*, at least about 90%, 95%, or 99% variable region identity with ipilimumab). Other molecules for modulating CTLA-4 include CTLA-4 ligands and receptors such as described in U.S. Patent Nos. 5844905, 5885796 and International Patent Application Nos. WO1995001994 and WO1998042752; all incorporated herein by reference, and immunoadhesins such as described in U.S. Patent No. 8329867, incorporated herein by reference.

[00223] Another immune checkpoint protein that can be targeted in the methods provided herein is lymphocyte-activation gene 3 (LAG-3), also known as CD223. The complete protein sequence of human LAG-3 has the Genbank accession number NP-002277. LAG-3 is found on the surface of activated T-cells, natural killer cells, B cells, and plasmacytoid dendritic cells. LAG-3 acts as an "off" switch when bound to MHC class II on the surface of antigen-presenting cells. Inhibition of LAG-3 both activates effector T-cells and inhibitor regulatory T-cells. In some embodiments, the immune checkpoint inhibitor is an anti-LAG-3 antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody), an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide. Anti-human-LAG-3 antibodies (or VH and/or VL domains derived therefrom) suitable for use in the present methods can be generated using methods well known in the art. Alternatively, art recognized anti-LAG-3 antibodies can be used. An exemplary anti-LAG-3 antibody is relatlimab (also known as BMS-986016) or antigen binding fragments and variants thereof (see, *e.g.*, WO 2015/116539). Other exemplary anti-LAG-3 antibodies include TSR-033 (see, *e.g.*, WO 2018/201096), MK-4280, and REGN3767. MGD013 is an anti-LAG-3/PD-1 bispecific antibody described in WO 2017/019846. FS118 is an anti-LAG-3/PD-L1 bispecific antibody described in WO 2017/220569.

[00224] Another immune checkpoint protein that can be targeted in the methods provided herein is V-domain Ig suppressor of T-cell activation (VISTA), also known as C10orf54. The complete protein sequence of human VISTA has the Genbank accession number NP_071436. VISTA is found on white blood cells and inhibits T-cell effector function. In some embodiments, the immune checkpoint inhibitor is an anti-VISTA3 antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody), an antigen

binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide. Anti-human-VISTA antibodies (or VH and/or VL domains derived therefrom) suitable for use in the present methods can be generated using methods well known in the art. Alternatively, art recognized anti-VISTA antibodies can be used. An exemplary anti-VISTA antibody is JNJ-61610588 (also known as onvatilimab) (see, *e.g.*, WO 2015/097536, WO 2016/207717, WO 2017/137830, WO 2017/175058). VISTA can also be inhibited with the small molecule CA-170, which selectively targets both PD-L1 and VISTA (see, *e.g.*, WO 2015/033299, WO 2015/033301).

[00225] Another immune checkpoint protein that can be targeted in the methods provided herein is indoleamine 2,3-dioxygenase (IDO). The complete protein sequence of human IDO has Genbank accession number NP_002155. In some embodiments, the immune checkpoint inhibitor is a small molecule IDO inhibitor. Exemplary small molecules include BMS-986205, epacadostat (INCB24360), and navoximod (GDC-0919).

[00226] Another immune checkpoint protein that can be targeted in the methods provided herein is CD38. The complete protein sequence of human CD38 has Genbank accession number NP_001766. In some embodiments, the immune checkpoint inhibitor is an anti-CD38 antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody), an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide. Anti-human-CD38 antibodies (or VH and/or VL domains derived therefrom) suitable for use in the present methods can be generated using methods well known in the art. Alternatively, art recognized anti-CD38 antibodies can be used. An exemplary anti-CD38 antibody is daratumumab (see, *e.g.*, U.S. Pat. No. 7,829,673).

[00227] Another immune checkpoint protein that can be targeted in the methods provided herein is ICOS, also known as CD278. The complete protein sequence of human ICOS has Genbank accession number NP_036224. In some embodiments, the immune checkpoint inhibitor is an anti-ICOS antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody), an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide. Anti-human-ICOS antibodies (or VH and/or VL domains derived therefrom) suitable for use in the present methods can be generated using methods well known in the art. Alternatively, art recognized anti-ICOS antibodies can be used. Exemplary anti-ICOS antibodies include JTX-2011 (see, *e.g.*, WO 2016/154177, WO 2018/187191) and GSK3359609 (see, *e.g.*, WO 2016/059602).

[00228] Another immune checkpoint protein that can be targeted in the methods provided herein is T-cell immunoreceptor with Ig and ITIM domains (TIGIT). The complete protein sequence of human TIGIT has Genbank accession number NP_776160. In some embodiments, the immune checkpoint inhibitor is an anti-TIGIT antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody), an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide. Anti-human-TIGIT antibodies (or VH and/or VL domains derived therefrom) suitable for use in the present methods can be generated using methods well known in the art. Alternatively, art recognized anti-TIGIT antibodies can be used. An exemplary anti-TIGIT antibody is MK-7684 (see, *e.g.*, WO 2017/030823, WO 2016/028656).

[00229] Another immune checkpoint protein that can be targeted in the methods provided herein is OX40, also known as CD134. The complete protein sequence of human OX40 has Genbank accession number NP_003318. In some embodiments, the immune checkpoint inhibitor is an anti-OX40 antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody), an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide. Anti-human-OX40 antibodies (or VH and/or VL domains derived therefrom) suitable for use in the present methods can be generated using methods well known in the art. Alternatively, art recognized anti-OX40 antibodies can be used. An exemplary anti-OX40 antibody is PF-04518600 (see, *e.g.*, WO 2017/130076). ATOR-1015 is a bispecific antibody targeting CTLA4 and OX40 (see, *e.g.*, WO 2017/182672, WO 2018/091740, WO 2018/202649, WO 2018/002339).

[00230] Another immune checkpoint protein that can be targeted in the methods provided herein is glucocorticoid-induced tumour necrosis factor receptor-related protein (GITR), also known as TNFRSF18 and AITR. The complete protein sequence of human GITR has Genbank accession number NP_004186. In some embodiments, the immune checkpoint inhibitor is an anti-GITR antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody), an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide. Anti-human-GITR antibodies (or VH and/or VL domains derived therefrom) suitable for use in the present methods can be generated using methods well known in the art. Alternatively, art recognized anti-GITR antibodies can be used. An exemplary anti-GITR antibody is TRX518 (see, *e.g.*, WO 2006/105021).

[00231] Another immune checkpoint protein that can be targeted in the methods provided herein is T-cell immunoglobulin and mucin-domain containing-3 (TIM3), also known as HAVCR2. The complete protein sequence of human TIM3 has Genbank accession number NP_116171. In some embodiments, the immune checkpoint inhibitor is an anti-TIM3 antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody), an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide. Anti-human-TIM3 antibodies (or VH and/or VL domains derived therefrom) suitable for use in the present methods can be generated using methods well known in the art. Alternatively, art recognized anti-TIM3 antibodies can be used. Exemplary anti-TIM3 antibodies include LY3321367 (see, *e.g.*, WO 2018/039020), MBG453 (see, *e.g.*, WO 2015/117002) and TSR-022 (see, *e.g.*, WO 2018/085469).

[00232] Another immune checkpoint protein that can be targeted in the methods provided herein is 4-1BB, also known as CD137, TNFRSF9, and ILA. The complete protein sequence of human 4-1BB has Genbank accession number NP_001552. In some embodiments, the immune checkpoint inhibitor is an anti-4-1BB antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody), an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide. Anti-human-4-1BB antibodies (or VH and/or VL domains derived therefrom) suitable for use in the present methods can be generated using methods well known in the art. Alternatively, art recognized anti-4-1BB antibodies can be used. An exemplary anti-4-1BB antibody is PF-05082566 (utomilumab; see, *e.g.*, WO 2012/032433).

[00233] In some embodiment, the immune therapy could be adoptive immunotherapy, which involves the transfer of autologous antigen-specific T-cells generated *ex vivo*. The T-cells used for adoptive immunotherapy can be generated either by expansion of antigen-specific T-cells or redirection of T-cells through genetic engineering (Park, Rosenberg et al. 2011). Isolation and transfer of tumor specific T-cells has been shown to be successful in treating melanoma. Novel specificities in T-cells have been successfully generated through the genetic transfer of transgenic T-cell receptors or chimeric antigen receptors (CARs) (Jena, Dotti et al. 2010). CARs are synthetic receptors consisting of a targeting moiety that is associated with one or more signaling domains in a single fusion molecule. In general, the binding moiety of a CAR consists of an antigen-binding domain of a single-chain antibody (scFv), comprising the light and variable fragments of a monoclonal

antibody joined by a flexible linker. Binding moieties based on receptor or ligand domains have also been used successfully. The signaling domains for first generation CARs are derived from the cytoplasmic region of the CD3zeta or the Fc receptor gamma chains. CARs have successfully allowed T-cells to be redirected against antigens expressed at the surface of tumor cells from various malignancies including lymphomas and solid tumors (Jena, Dotti et al. 2010).

[00234] In one embodiment, the present application provides for a combination therapy for the treatment of cancer wherein the combination therapy comprises adoptive T-cell therapy and a checkpoint inhibitor. In one aspect, the adoptive T-cell therapy comprises autologous and/or allogeneic T-cells. In another aspect, the autologous and/or allogeneic T-cells are targeted against tumor antigens. The engineered hSDH enzyme may be administered to the patient prior to and/or simultaneously with the administration of the adoptive T-cell therapy. In another aspect, the autologous and/or allogeneic T-cells may be engineered to express the engineered hSDH enzyme.

D. Surgery

[00235] Approximately 60% of persons with cancer will undergo surgery of some type, which includes preventative, diagnostic or staging, curative, and palliative surgery. Curative surgery includes resection in which all or part of cancerous tissue is physically removed, excised, and/or destroyed and may be used in conjunction with other therapies, such as the treatment of the present embodiments, chemotherapy, radiotherapy, hormonal therapy, gene therapy, immunotherapy, and/or alternative therapies. Tumor resection refers to physical removal of at least part of a tumor. In addition to tumor resection, treatment by surgery includes laser surgery, cryosurgery, electrosurgery, and microscopically-controlled surgery (Mohs' surgery).

[00236] Upon excision of part or all of cancerous cells, tissue, or tumor, a cavity may be formed in the body. Treatment may be accomplished by perfusion, direct injection, or local application of the area with an additional anti-cancer therapy. Such treatment may be repeated, for example, every 1, 2, 3, 4, 5, 6, or 7 days, or every 1, 2, 3, 4, and 5 weeks or every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months. These treatments may be of varying dosages as well.

E. Other Agents

[00237] It is contemplated that other agents may be used in combination with certain aspects of the present embodiments to improve the therapeutic efficacy of treatment. These additional agents include agents that affect the upregulation of cell surface receptors and GAP junctions, cytostatic and differentiation agents, inhibitors of cell adhesion, agents that increase the sensitivity of the hyperproliferative cells to apoptotic inducers, or other biological agents. Increases in intercellular signaling by elevating the number of GAP junctions would increase the anti-hyperproliferative effects on the neighboring hyperproliferative cell population. In other embodiments, cytostatic or differentiation agents can be used in combination with certain aspects of the present embodiments to improve the anti-hyperproliferative efficacy of the treatments. Inhibitors of cell adhesion are contemplated to improve the efficacy of the present embodiments. Examples of cell adhesion inhibitors are focal adhesion kinase (FAKs) inhibitors and Lovastatin. It is further contemplated that other agents that increase the sensitivity of a hyperproliferative cell to apoptosis, such as the antibody c225, could be used in combination with certain aspects of the present embodiments to improve the treatment efficacy.

IX. Kits

[00238] Provided are kits, such as therapeutic kits. For example, a kit may comprise one or more therapeutic composition as described herein and optionally instructions for their use. Kits may also comprise one or more devices for accomplishing administration of such compositions. For example, a subject kit may comprise a therapeutic composition and catheter for accomplishing direct intravenous injection of the composition into a target tissue. A kit may comprise pre-filled ampoules of an engineered hSDH enzyme, optionally formulated as a therapeutic composition, or lyophilized, for use with a delivery device.

[00239] Kits may comprise a container with a label. Suitable containers include, for example, bottles, vials, and test tubes. The containers may be formed from a variety of materials, such as glass or plastic. The container may hold a composition that includes an engineered hSDH enzyme that is effective for therapeutic or non-therapeutic applications, such as described above. The label on the container may indicate that the composition is used for a specific therapy or non-therapeutic application, and may also indicate directions for either *in vivo* or *in vitro* use, such as those described above. Kits will typically comprise the container described above and one or more other containers

comprising materials desirable from a commercial and user standpoint, including buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

X. Examples

[00240] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1 – Human Serine Dehydratase as a Scaffold for Engineering Studies

[00241] Human serine dehydratase (hSDH, EC 4.3.1.17) (SEQ ID NO: 1) catalyzes the irreversible deamination reactions of L-serine to pyruvate and L-threonine to α-ketobutyrate with a shared second product: ammonia[25]. SDH activity has been shown to be attenuated concomitant with increased serine biosynthesis activity in rat sarcoma and human colon carcinoma, hypothesized to channel serine utilization towards nucleotide biosynthesis[26]. As mentioned before, despite its poor kinetic properties, hSDH was chosen as a promising enzyme scaffold for these genetically guided mutagenesis and engineering studies for the following reasons: 1) It catalyzes an irreversible reaction for serine degradation[25]. 2) It does not require a co-substrate to degrade serine as seen with other serine-degrading enzymes: Serine hydroxymethyltransferase[27] and Serine-pyruvate aminotransferase[28]. 3) It also degrades threonine, which is a minor contributor to glycine production and therefore one-carbon metabolism[29]. 4) Further, a therapeutic enzyme derived from a human sequence is critical in order to avoid immunogenic hazards that arises from the use of non-human proteins.

Example 2 – Gene Synthesis of Human Serine Dehydratase and Its Variants

[00242] The wild type hSDH gene with *E. coli* codon optimization was purchased as a gblock from Integrated DNA Technologies with an N-terminal hexahistidine tag and was amplified by PCR. Specific hSDH mutants were synthesized by overlap

extension PCR using wildtype or generated variant genes as the template. Briefly, an initial round of PCRs were conducted, utilizing gene-specific primers to encode the desired mutations coupled with plasmid-specific primers. Subsequently, a second PCR was initiated, using the products from the mutagenic first PCRs and the plasmid-specific primers. After the second PCR, the product was purified via gel extraction. The gel extracted DNA was digested using NcoI and EcoRI, after which it was ligated into the pET28a plasmid using the T7 ligase protocol. Ligated plasmid was then transformed into DH10β *E. coli* cells and plated on an antibiotic selective agar plate. Individual colonies were picked and their identity confirmed via Sanger sequencing. Confirmed plasmids were re-transformed into BL21(DE3) *E. coli* cells and again plated on antibiotic selective agar plates. Colonies were picked and used to inoculate overnight cultures, which were then frozen for subsequent usage.

[00243] Mutant enzyme libraries were constructed using a pET28a plasmid containing the hSDH gene. Libraries were constructed according to structural analyses, convolutional neural network analysis (MutCompute: https://mutcompute.com) and randomly. Libraries based on structural analyses were constructed via overlap extension PCR utilizing NNS codons to mutate specific residues using mutagenic primers listed in Table 1. Random library of mutants generated by error-prone PCR utilizing Taq DNA polymerase and resulting mutations listed in Table 1.

Table 1. Mutagenesis primers and introduced mutations into hSDH

Mutagenesis Method	**Enzyme ID and their mutations**	**Primers**
NNS codon saturation using structural analysis	Variant 1 (K221F, A222S) (SEQ ID NOs: 2 and 8)	Fwd: 5' GAGCGTAGCGNNSNNSCTGGGCGTGAA 3' (SEQ ID NO: 13) Rev: 5' TTCACGCCCAGSNNSNNCGCTACGCTC 3' (SEQ ID NO: 14)
Error-prone PCR	Variant 2 (S26N, R51H, S63P, M70I, L94P, G129D, A204S, K221R, A222S) (SEQ ID NOs: 3 and 9)	Fwd: 5' TGTGAGCGGATAACAATTCC 3' (SEQ ID NO: 15) Rev: 5' TCGACGGAGCTCGAATTC 3' (SEQ ID NO: 16)
NNS codon saturation using MutCompute analysis	Variant 3 (S26N, R51H, S63P, M70I, L94P, G129D, A204S, K221R,	Fwd: 5' AGTGGGCGAGNNSCTGGAAGAAGCG 3' (SEQ ID NO: 17) Rev: 5' CGCTTCTTCCAGSNNCTCGCCCACT 3' (SEQ ID NO: 18)

	A222S + L112S) (SEQ ID NOs: 4 and 10)	
Site-directed Mutagenesis using MutCompute analysis	Variant 4 (I13T, S26N, R51H, I96T, D114E, G129D, K221R, A222S, W157R, D261L, L112S) (SEQ ID NOs: 5 and 11)	1. W157R: Fwd: 5' GGAAACCCTGaggGAAAAACCTG 3' (SEQ ID NO: 19) Rev: 5' CAGGTTTTTCcctCAGGGTTTCC 3' (SEQ ID NO: 20) 2. D261L: Fwd: 5' GTTCGTGGACcttGAAAAGATACTGGTGGAACC 3' (SEQ ID NO: 21) Rev: 5' GGTTCCACCAGTATCTTTTCaagGTCCACGAAC 3' (SEQ ID NO: 22) 3. L112S: Fwd: 5' AGTGGGCGAGtcgCTGGAAGAAGCG 3' (SEQ ID NO: 23) Rev: 5' CGCTTCTTCCAGcgaTCGCCCACT 3' (SEQ ID NO: 24)

Example 3 – 96-well Plate Screen for Human Serine Dehydratase Activity

[00244] A colorimetric assay for the detection of pyruvate using 3-methyl-2-benzothiazolinone hydrazone (MBTH)[30] was scaled to a 96-well plate format for screening libraries to isolate clones with the higher serine/threonine degrading activity. Clones displaying higher activity than parental enzyme controls are selected for further characterization.

[00245] Single colonies containing hSDH variants are picked into 96-well culture plates containing 75 µL of TB media/well containing 50 µg/ml kanamycin. These cultures are then grown at 37 °C on a plate shaker until reaching an OD_{600} of 0.8–1. After cooling to 25 °C, an additional 75 µL of media/well containing 50 µg/ml kanamycin and 2 mM IPTG is added. Expression is performed at 25 °C with shaking for at least 2 hours, following which 100 µL of culture/well is transferred to a 96 well assay plate. The assay plates are then centrifuged to pellet the cells, the media is removed, and the cells are lysed by addition of 50 µL/well of B-PER protein extraction reagent (Pierce). After clearing by centrifugation, the lysate is incubated with 2-20 mM serine and 100 µM PLP at 37 °C for 2-4 hours. The reaction is then derivatized by addition of 3 parts of 0.03 % MBTH solution in 1

M sodium acetate pH 5. The plates are heated at 50 °C for 40 min and after cooling are read at λ = 320 nm in a microtiter plate reader.

Example 4 – Expression and Purification of Human Serine Dehydratase Variants

[00246] Human SDH variants were expressed and purified for subsequent biophysical characterization. In brief, hSDH variants in the pET28a plasmid was transformed into BL21 (DE3) for expression. The cells were grown overnight at 37°C in 5 mL of TB media with 50 µg/mL of kanamycin. The subsequent morning, cultures were inoculated, at a 1:1000 dilution, into 0.5 L of fresh TB media containing 50 µg/mL of kanamycin. Upon reaching an A600 of 0.6 to 0.8, the culture was moved to 25°C and isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to a final concentration of 1 mM to induce T7 RNA polymerase expression, and subsequently hSDH variant protein expression. After overnight incubation, cells were harvested via centrifugation at 5,500 rpm for 20 minutes. The cell pellet, after removing the supernatant, was resuspended using 25 mL of pET lysis buffer (10 mM NaH_2PO_4, 30 mM imidazole, 300 mM NaCl, pH 8.0). The cell suspension was then lysed by sonication and cleared by centrifugation at 14,000 rpm for 40 minutes. The supernatant from this centrifugation step was then filtered using a 0.22 µm membrane. The clarified supernatant was then applied to 1 mL of nickel-nitrotriacetic acid (Ni-NTA) resin that had been pre-equilibrated with 20 mL of pET lysis buffer. The resin was washed with 20 mL (20 column volumes) of pET lysis buffer. Afterward, the resin was incubated with 3 mL of pET elution buffer (10 mM NaH_2PO_4, 250 mM imidazole, 300 mM NaCl, pH 8.0), equilibrated for 5 minutes, and then eluted. The elution step was then repeated with 2 ml of extra elution buffer to increase recovery. The total elution was then buffer exchanged against storage buffer (10 mM Tris-HCl, 100 mM KCl, and 1 mM DTT) utilizing dialysis membranes overnight. After completing the buffer exchange, glycerol was added to a final concentration of 10% v/v, aliquoted, flash frozen with liquid nitrogen, and stored at -80°C. Purity of the proteins were analyzed via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 4% to 20% pre-cast Tris-Glycine gel run under reducing conditions (2.5% β-Mercaptoethanol (BME). Protein concentration was determined via A280 under denaturing conditions using a calculated extinction coefficient (ε_{280} =33960 M^{-1} cm^{-1}) from Geneious software (v. 8.0.5).

Example 5 – Characterization of wild-type Human Serine Dehydratase and Its Variants

[00247] Purified wild type and mutant enzymes were characterized using MBTH assay in 200 µL scale in a 96 well plate. Briefly, 44 µL serine or threonine (1-100 mM range) serial dilutions were incubated at 37 °C for 10-15 minutes. Then, 1-2 µM enzymes were added to the substrate and mixed briefly. After 20 seconds of reaction time, reactions were quenched by addition of 50% trichloroacetic acid solution. Pre-quenched controls were also included at each substrate concentration to subtract the background signals. The reaction was then derivatized by addition of 3 parts of 0.03 % MBTH solution in 1 M sodium acetate, pH 5. The reactions were heated at 50 °C for 40 min and after cooling were read at λ=320 nm in a microtiter plate reader. k_{cat}/K_m values were calculated for both substrates as can be seen FIG. 1 and Table 2.

Table 2. Calculated kcat/Km values of wt hSDH and its engineered variants.

Enzyme	Serine K_{cat}/K_m ($mM^{-1}*s^{-1}$)	Threonine K_{cat}/K_m ($mM^{-1}*s^{-1}$)
wt hSDH	0.6 ± 0.04	1.1 ± 0.05
Variant 1	3.0 ± 0.3	3.8 ± 0.3
Variant 2	2.7 ± 0.2	2.0 ± 0.05
Variant 3	3.0 ± 0.04	2.8 ± 0.2
Variant 4	4.7 ± 0.2	6.8 ± 0.7
Variant 5	3.2 ± 0.1	1.7 ± 0.1

Example 6 – *In Vitro* Cytotoxicity of wild-type hSDH and Variant 3 Against MCF7 Breast Cancer Cell Line

[00248] Cell viability of cancer cells was confirmed by Alamar Blue assay (Thermo-Fisher Scientific, Cat. No: A50100) according to manufacturer's instructions. Briefly, MCF7 breast cancer cells were seeded 1000-5000 cell per well in fluorescence compatible 96 well plates. Edges of the 96 well plate were not used to prevent evaporation issues. Cells were incubated for 24 h for cell attachment and then different concentrations of hSDH enzymes were added to cell culture. Cells were observed for viability for 1-3 days by adding 10% v/v Alamar blue dye. Fluorescence intensities of each sample were read using a plate reader at excitation 550 nm and emission 585 nm. The cytotoxic effect of hSDH in the viability of cancer cell lines was reported as percentage changes of fluorescence intensities in wells incubated with the enzyme compared to control wells (FIG. 2). As an inactive enzyme

control, variant 3 was deactivated by incubation at 100 °C for 20 minutes followed by centrifugation (16000 x g for 5 minutes) to remove protein precipitates. Analysis of the resulting data yielded an apparent IC_{50} value of ~ 0.4 μM for MCF7 human breast cancer cells with the addition of engineered enzymes. WT hSDH addition into media including MCF7 breast cancer cells did not affect the viability of cancer cells except for the highest concentration (2.5 μM) suggesting improved activity for the engineered variant. In contrast there was a significant impact on viability by the addition of only 0.6 μM of variant 3 (FIG. 2).

Example 7 – Pharmacological Preparation of Engineered Human Serine Dehydratase

[00249] *E.coli*-BL21(DE3) cells harboring plasmids of corresponding variant 3 were grown in TB media containing 50 μg/mL kanamycin at 37°C to an OD600 of ~0.5 at which time IPTG was added to a final concentration of 1 mM, followed by overnight expression at 25°C. Cells were collected by centrifugation and re-suspended in immobilized metal affinity chromatography (IMAC) buffer (10 mM $NaPO_4$/ 30 mM imidazole/ 300 mM NaCl, pH: 8) additionally containing PLP (1 mM) and 25 U/mL universal nuclease. The re-suspended cells were then lysed by a homogenizer, and the lysates were centrifuged at 14,000 x g for 1 hour at 4°C. The resulting supernatant was applied to a nickel IMAC column. Importantly, glass columns were cleaned with 1 M NaOH prior to use for all purifications since low endotoxin material is needed for *in vivo* experiments. Then supernatant washed with 10-20 column volumes of IMAC buffer (containing 0.5 mM PLP) followed by a second wash using 100 column volumes of Dulbecco's PBS (dPBS) additionally containing 0.1 mM PLP and 0.1% Triton X114 (Sigma, MO) in order to remove endotoxin using a peristaltic pump. Protein was eluted with dPBS containing 250 mM imidazole. All chromatography was performed at 4°C.

[00250] Eluted enzyme was then incubated with 10 mM PLP for a minimum of 1 hour at 4°C, followed by buffer exchange into 100 mM potassium phosphate buffer, pH 8.4 using a Pall Corporation Minimate TFF diafiltration system (10,000 MWCO). Enzyme was subsequently conjugated to lysyl residues using methoxy PEG succinimidyl carboxymethyl ester of MW 5000 (PEG-5K; NOF America Corporation) by adding PEG-5K at a 20:1 molar ratio to enzyme and allowed to react for half an hour at 25°C in a glass vial with gentle stirring. After the reaction had reached completion, diafiltration was again used (100,000 MWCO) to remove excess PEG-5K and buffer exchange into endotoxin free dPBS, 10%

glycerol (pH 7.4). After sufficient buffer exchange, aliquots of variant 3 were flash frozen in liquid nitrogen and stored at -80 °C. All PEGylated enzymes were analyzed for lipopolysaccharide (LPS) content using the commercially available Limulus Amebocyte Lysate (LAL) kit (Pierce). Purity and PEGylation efficiency of the proteins were assessed by SDS-PAGE.

Example 8 – Pharmacodynamic Effect of Engineered Human Serine Dehydratase Enzymes

[00251] Administration of PEGylated engineered enzyme, variant 3 at 50 mg/kg dosing in mice resulted in a marked decrease in serine and threonine levels (FIG. 3). Serine and threonine levels in mice serum were analyzed via OPA derivatization on an Agilent 1200 series HPLC with a ZORBAX Eclipse Plus C18 column. Derivatization and chromatography were adapted from the referenced literature[31]. Data were analyzed using GraphPad Prism software (GraphPad, San Diego, CA) and presented as mean values ± s.e.m. Statistical analyses were performed using unpaired two-tailed Student's t-test.

Example 9 – Antitumoral Effect of Engineered Human Serine Dehydratase Enzymes

[00252] Engineered PEGylated enzyme, variant 3 was administrated at 50 mg/kg to mice bearing MC38 human colon cancers. Variant 3 was injected every 48 hrs for a total of four doses. Administration of PEG-hSDH variant 3 resulted in marked suppression of tumor growth compared to controls and a significant survival advantage (FIG. 4). All animal work was conducted with preapproval from the University of Texas at Austin Institutional Animal Care and Use Committee. C57BL/6 mice were bred-in house and maintained in house in pathogen-free, ventilated cages with irradiated food and autoclaved water ad libitum. All mice were allowed to acclimate for at least 1 week before use in experiments. Experimental mice were euthanized by CO_2 asphyxiation and cervical dislocation when tumor size surpassed 1500 mm^3, or if the animals showed any signs of hypersensitivity reactions. For all studies, after the tumors were palpable, mice were randomly divided into treatments groups such that each group had approximately the same starting average tumor size before treatment was initiated. Tumor size was measured every three days using a digital caliper, and tumor volume was calculated by the formula: $0.5236*D1(D2)^2$, where D1 and D2 are the long and short diameter, respectively.

* * *

[00253] All of the methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

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The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

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WHAT IS CLAIMED IS:

1. An engineered human serine dehydratase enzyme comprising at least one substitution relative to a native human serine dehydratase enzyme according to SEQ ID NO: 1, wherein the modified enzyme has greater serine and/or threonine degrading activity than the native human serine dehydratase enzyme.

2. The enzyme of claim 1, comprising an amino acid sequence at least 90% identical to SEQ ID NO: 1.

3. The enzyme of claim 1 or 2, comprising a kcat/Km for degrading serine that is greater than 0.6 mM-1*s-1.

4. The enzyme of any one of claims 1-3, comprising a kcat/Km for degrading threonine that is greater than 1.1 mM-1*s-1.

5. The enzyme of any one of claims 1-4, comprising at least one substitution at a position corresponding to a position of SEQ ID NO: 1 selected from the group consisting of: I13, S26, R51, S63, M70, L94, I96, L112, D114, G129, A204, K221, A222, W157, D261, and a combination thereof.

6. The enzyme of any one of claims 1-5, comprising at least one substitution selected from the group consisting of: I13T, S26N, R51H, S63P, M70I, L94P, I96T, L112S, D114E, G129D, A204S, K221F, K221R, A222G, A222S, W157R, D261L, and a combination thereof.

7. The enzyme of any one of claims 1-6, comprising a set of substitutions at positions corresponding to positions of SEQ ID NO: 1, wherein the set of substitutions is selected from the group consisting of:

(a) K221 and A222;

(b) S26, R51, S63, M70, L94, G129, A204, K221, and A222;

(c) S26, R51, S63, M70, L94, G129, A204, K221, A222, and L112; and

(d) I13, S26, R51, I96, D114, G129, K221, A222, W157, D261, and L112.

8. The enzyme of any one of claims 1-7, comprising a set of substitutions selected from the group consisting of:

(a) K221F and A222S;

(b) S26N, R51H, S63P, M70I, L94P, G129D, A204S, K221R, and A222S;

(c) S26N, R51H, S63P, M70I, L94P, G129D, A204S, K221R, A222S, and L112S;

(d) I13T, S26N, R51H, I96T, D114E, G129D, K221R, A222S, W157R, D261L, and L112S; and

(e) I13T, S26N, R51H, I96T, D114E, G129D, K221R, A222G, W157R, D261L, and L112S.

9. The enzyme of any one of claims 1-8, comprising the sequence of any one of SEQ ID NOs: 2-6, optionally excluding the initial methionine.

10. The enzyme of any one of claims 1-9, wherein the enzyme comprises an initial methionine.

11. The enzyme of any one of claims 1-9, wherein the enzyme does not comprise an initial methionine.

12. The enzyme of any one of claims 1-11, wherein the enzyme is coupled to at least one polyethylene glycol (PEG).

13. The enzyme of claim 12, wherein the enzyme is coupled to the at least one PEG via one or more lysine residues.

14. The enzyme of claim 12 or 13, wherein the at least one PEG is PEG-5K.

15. A nucleic acid comprising a nucleotide sequence encoding the enzyme of any one of claims 1-11.

16. The nucleic acid of claim 15, wherein the nucleic acid is codon optimized for expression in bacteria, fungus, insects, or mammals.

17. The nucleic acid of claim 16, wherein the bacteria are *E. coli*.

18. An expression vector comprising the nucleic acid of any one of claims 15-17.

19. A host cell comprising the nucleic acid of any one of claims 15-17.

20. The host cell of claim 19, wherein the host cell is a bacterial cell, a fungal cell, an insect cell, or a mammalian cell.

21. A pharmaceutical formulation comprising an enzyme of any one of claims 1-14, or the nucleic acid of any one of claims 15-17, in a pharmaceutically acceptable carrier.

22. The pharmaceutical formulation of claim 21, for use in the treatment of a subject having a cancer.

23. A method of lowering the blood/plasma serine levels in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a formulation of claim 21.

24. The method of claim 23, wherein the method reduces the concentration of serine/threonine in the subject's serum by at least 50% within 24 hours of administration.

25. A method of treating a subject having cancer, the method comprising administering to the subject a therapeutically effective amount of a formulation of claim 21.

26. The formulation for use of claim 22 or method of any one of claims 23-25, wherein the subject is maintained on a serine restricted diet.

27. The formulation for use of claim 22 or method of any one of claims 23-25, wherein the subject is maintained on a normal diet.

28. The formulation for use of claim 22 or method of any one of claims 23-27, wherein the subject is a human subject.

29. The formulation for use of claim 22 or method of any one of claims 23-28, wherein the formulation is administered intratumorally, intravenously, intradermally, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostaticaly, intrapleurally, intratracheally, intraocularly, intranasally, intravitreally, intravaginally, intrarectally, intramuscularly, subcutaneously, subconjunctival, intravesicularlly, mucosally, intrapericardially, intraumbilically, orally, by inhalation, by injection, by infusion, by continuous infusion, by localized perfusion bathing target cells directly, via a catheter, or via a lavage.

30. The formulation for use of claim 22 or method of any one of claims 25-28, wherein the formulation is administered to a nutrient medium of the cancer.

31. The formulation for use of claim 22 or method of claim 30, wherein the nutrient medium is blood, lymphatic fluid, or spinal fluid.

32. The formulation for use of claim 22 or method of any one of claims 25-31, further comprising administering at least a second anti-cancer therapy to the subject.

33. The formulation for use of claim 22 or method of claim 32, wherein the second anti-cancer therapy is a surgical therapy, chemotherapy, radiation therapy, cryotherapy, hormone therapy, immunotherapy or cytokine therapy.

34. The formulation for use of claim 22 or method of claim 32, wherein the second anti-cancer therapy comprises a serine synthesis inhibitor.

35. The formulation for use of claim 22 or method of any one of claims 25-34, wherein the method reduces the level of serine and/or threonine in the subject's plasma and/or serum.

36. The formulation for use of claim 22 or method of any one of claims 25-35, wherein the method suppresses and/or delays tumor growth in the subject.

37. The formulation for use of claim 22 or method of any one of claims 25-36, wherein the subject's cancer is characterized as addicted to extracellular serine.

38. The formulation for use of claim 22 or method of any one of claims 25-37, wherein the subject's cancer is characterized as dependent upon external sources of serine.

39. The formulation for use of claim 22 or method of any one of claims 25-38, wherein the subject's cancer has been determined to express a decreased level of *PGDGH*, *PSAT1*, and/or *PSPH* compared to a reference level.

40. The formulation for use of claim 22 or method of claim 39, wherein the method comprises:

(a) determining or having determined an expression level of *PGDGH*, *PSAT1*, and/or *PSPH* in the subject's cancer; and

(b) selecting or having selected the subject for treatment when the cancer expresses a decreased level of *PGDGH*, *PSAT1*, and/or *PSPH* compared to a reference level.

41. The formulation for use of claim 22 or method of claim 40, wherein step (a) comprises (i) obtaining or having obtained a biological sample from the cancer; and (ii)

performing or having performed an assay on the biological sample to determine an expression level of *PGDGH*, *PSAT1*, and/or *PSPH*.

42. The formulation for use of claim 22 or method of claim 40, wherein determining the expression level of *PGDGH*, *PSAT1*, and/or *PSPH* in the cancer comprises detecting a PGDGH, PSAT1, and/or PSPH protein in the cancer.

43. The formulation for use of claim 22 or method of claim 42, wherein the protein is detected by mass spectrometry, western blot, immunohistochemistry, ELISA, or RIA.

44. The formulation for use of claim 22 or method of claim 40, wherein determining the expression level of *PGDGH*, *PSAT1*, and/or *PSPH* in the cancer comprises detecting a PGDGH, PSAT1, and/or PSPH mRNA in the cancer.

45. The formulation for use of claim 22 or method of claim 44, wherein the mRNA is detected by microarray, RT-PCR, qRT-PCR, nanostring assay, or in situ hybridization.

46. The formulation for use of claim 22 or method of claim 41, wherein the sample is a formalin-fixed, paraffin-embedded sample.

47. The formulation for use of claim 22 or method of claim 41, wherein the sample is a fresh frozen sample.

48. The formulation for use of claim 22 or method of any one of claims 25-38, wherein the subject's cancer has been determined to have a mutation in the *PGDGH* gene, *PSAT1* gene, and/or *PSPH* gene.

49. The formulation for use of claim 22 or method of any one of claims 25-38, wherein a promoter, untranslated region, and/or gene body of the *PGDGH* gene, *PSAT1* gene, and/or *PSPH* gene is methylated in the subject's cancer.

50. The formulation for use of claim 22 or method of claim 49, wherein the method comprises:

(a) determining or having determined whether the promoter, untranslated region, and/or gene body of *PGDGH*, *PSAT1*, and/or *PSPH* is methylated in the subject's cancer; and

(b) selecting or having selected the subject for treatment when the promoter, untranslated region, and/or gene body of *PGDGH*, *PSAT1*, and/or *PSPH* is methylated in the cancer.

51. The formulation for use of claim 22 or method of claim 50, wherein step (a) comprises (i) obtaining or having obtained a biological sample from the cancer; and (ii) performing or having performed an assay on the biological sample to determine whether the promoter, untranslated region, and/or gene body of *PGDGH*, *PSAT1*, and/or *PSPH* is methylated.

52. The formulation for use of claim 22 or method of claim 51, wherein the sample is a formalin-fixed, paraffin-embedded sample.

53. The formulation for use of claim 22 or method of claim 51, wherein the sample is a fresh frozen sample.

54. The formulation for use of claim 22 or method of any one of claims 25-53, wherein the cancer is a non-small cell lung cancer, a pancreatic adenocarcinoma, a breast cancer, a prostate cancer, a melanoma, a colon cancer, a leukemia, a lymphoma, a glioblastoma, an osteosarcoma, an oral cavity cancer, an ovarian cancer, a uterine cancer, a bone cancer, a brain cancer, a prostate cancer, a kidney cancer, a stomach cancer, an esophageal cancer, a rectal cancer, a bladder cancer, a testicular cancer, or a liver cancer.

55. The formulation for use of claim 22 or method of claim 54, wherein the cancer is a breast cancer or a colon cancer.

56. Use of an enzyme of any one of claims 1 to 14 or a composition comprising said enzyme for the manufacture of a medicament for therapeutic application to a subject having cancer.

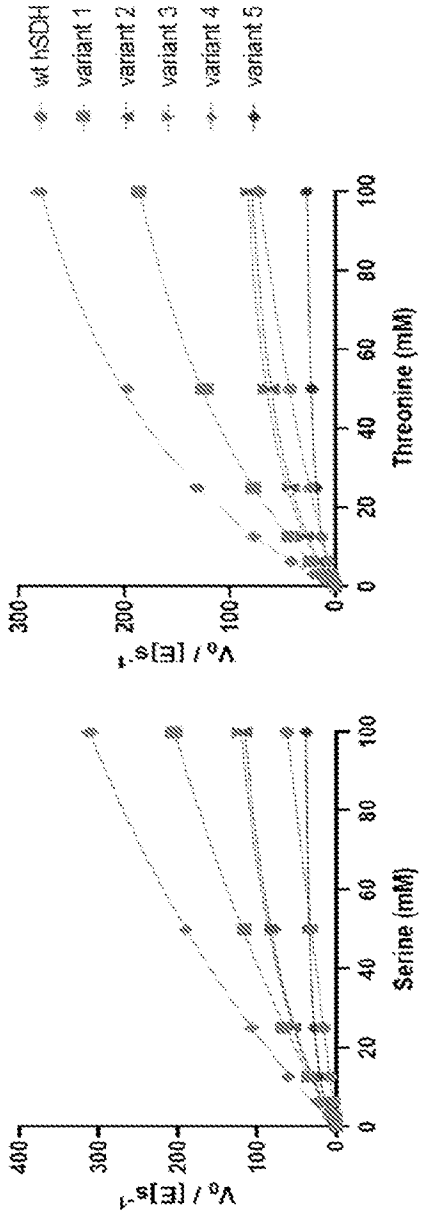

wt hSDH
variant 1
variant 2
variant 3
variant 4
variant 5
Threonine (mM)
Serine (mM)
V_0 / [E]s^{-1}
V_0 / [E]s^{-1}

FIG. 1

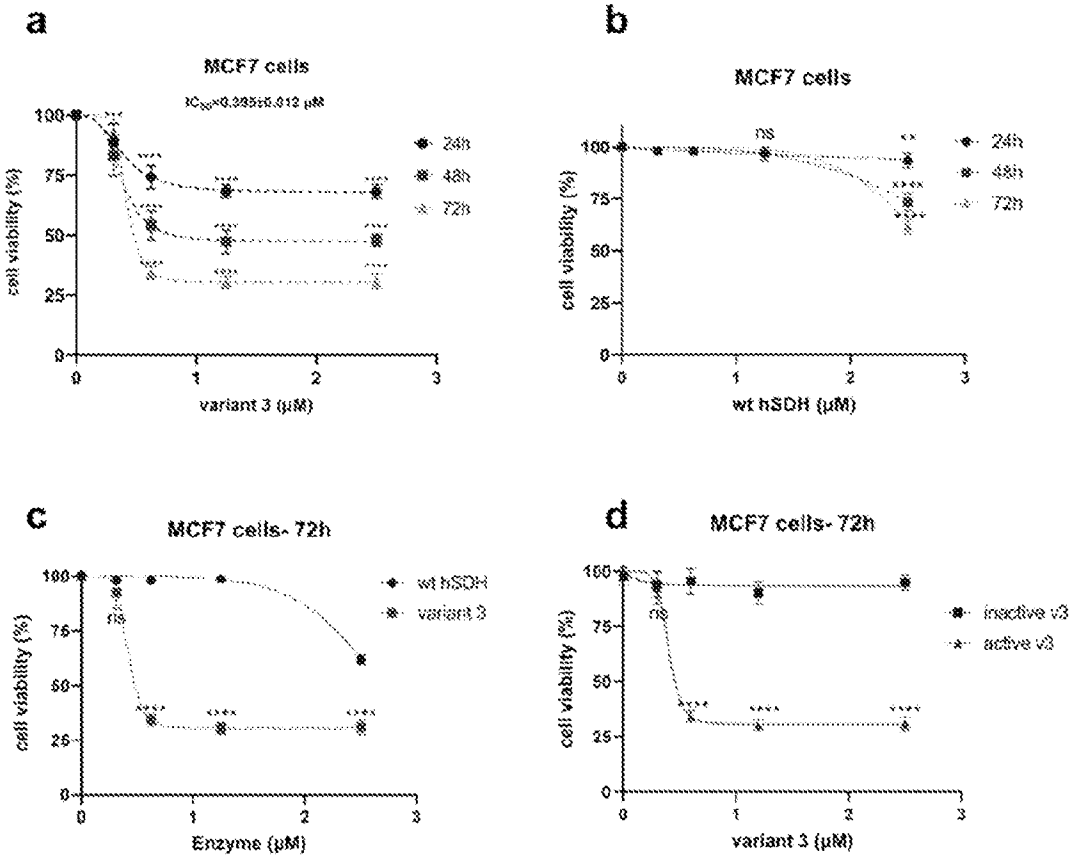

a
MCF7 cells
cell viability (%)
variant 3 (μM)
24h
48h
72h
b
MCF7 cells
cell viability (%)
wt hSDH (μM)
ns
24h
48h
72h
c
MCF7 cells- 72h
cell viability (%)
Enzyme (μM)
wt hSDH
variant 3
d
MCF7 cells- 72h
cell viability (%)
variant 3 (μM)
inactive v3
active v3

FIG. 2

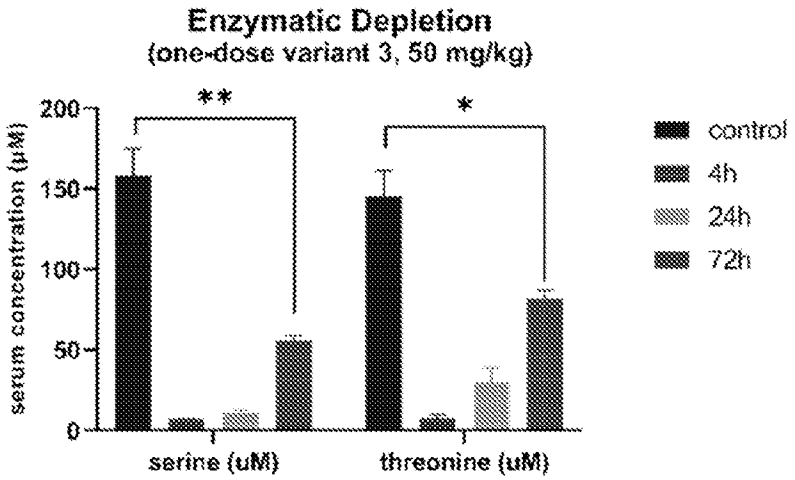

Enzymatic Depletion
(one-dose variant 3, 50 mg/kg)
serum concentration (μM)
**
*
control
4h
24h
72h
serine (uM)
threonine (uM)

FIG. 3

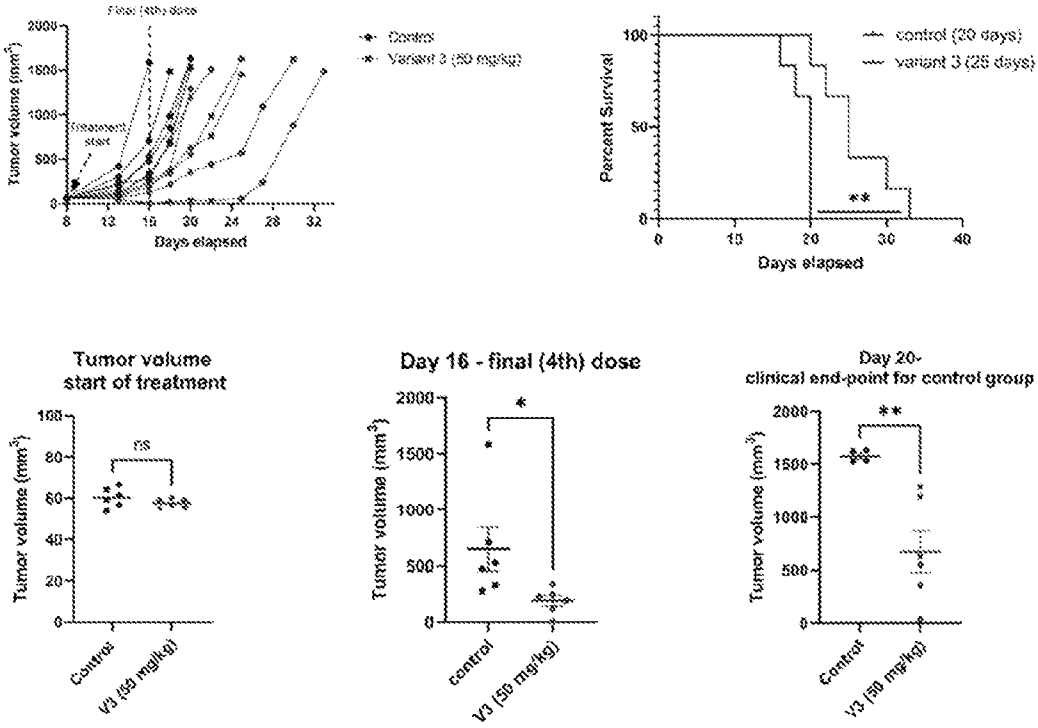

Tumor volume (mm³)
Days elapsed
Control
Variant 3 (50 mg/kg)
Percent Survival
control (20 days)
variant 3 (25 days)
**
Tumor volume start of treatment
ns
Control
V3 (50 mg/kg)
Day 16 - final (4th) dose
*
control
V3 (50 mg/kg)
Day 20- clinical end-point for control group
**
Control
V3 (50 mg/kg)

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 23/69477

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. C12Q 1/527 (2023.01)
ADD. C12N 9/88, A61K 38/00 (2023.01)

CPC - INV. C12Q 1/527

ADD. G01N 2500/02, G01N 2500/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	YAMADA et al. A catalytic mechanism that explains a low catalytic activity of serine dehydratase like-1 from human cancer cells: Crystal structure and site-directed mutagenesis studies. Biochimica et Biophysica Acta 19 February 2008, Vol 1780, pg 809–818. pg 815, col 1, para 2; pg 815, Table 3	1-3
Y	WO 2003/040325 A2 (CURAGEN CORP) 15 May 2003 (15.05.2003) Table A	1-3

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 October 2023	NOV 06 2023
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Kari Rodriquez Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 23/69477

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as fi led.

   b. [ ] furnished subsequent to the international fi ling date for the purposes of international search (Rule 13ter.1(a)),

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 23/69477

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-56
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.