BRIEF SUMMARY

In one aspect of the present disclosure, there is provided a therapeutic composition comprising an isolated arginine deiminase, or a fragment thereof having arginine deiminase (ADI) activity, and a pharmaceutically-acceptable carrier, wherein the isolated arginine deiminase has reduced cross-reactivity with patient anti-ADI-PEG 20 antibodies and comprises the amino acid sequence set forth in SEQ ID NO: 9 or SEQ ID NO: 22, or a variant thereof that displays at least 90% sequence identity to SEQ ID NO: 9 or SEQ ID NO: 22.

In another aspect of the present disclosure, there is provided a use of a therapeutic composition disclosed herein in the manufacture of a medicament for treating, ameliorating the symptoms of, or inhibiting the progression of a cancer in a patient.

In a further aspect of the present disclosure, there is provided a use of a ADI-PEG 20 and a therapeutic composition disclosed herein in the manufacture of a medicament for treating, ameliorating the symptoms of, or inhibiting the progression of a cancer in a patient, wherein said ADI-PEG 20 is to be administered to the patient, and after a period of time, said therapeutic composition is to be administered to the patient.

In another aspect of the present disclosure, there is provided a therapeutic composition disclosed herein for use in treating, ameliorating the symptoms of, or inhibiting the progression of a cancer.

Certain ~~embodiment~~examples relate to an isolated arginine deiminase, wherein the isolated arginine deiminase has reduced cross-reactivity with patient anti-ADI-PEG 20 antibodies. Also included are therapeutic or pharmaceutical compositions comprising an isolated arginine deiminase or a fragment thereof having ADI activity, and a pharmaceutically-acceptable carrier. In certain ~~embodiment~~examples, the composition is sterile and/or substantially free of pyrogens such as endotoxins. In some ~~embodiment~~examples, the isolated arginine deiminase having reduced cross-reactivity with patient anti-ADI-PEG 20 antibodies is not from *M. hominis*. In some ~~embodiment~~examples, the isolated arginine deiminase having reduced cross-reactivity with patient anti-ADI-PEG 20 antibodies is from an organism listed in Table 1.

In certain ~~embodiment~~examples the isolated arginine deiminase having reduced cross-reactivity with patient anti-ADI-PEG 20 antibodies has one or more properties comparable to or better than those of ADI-PEG 20. In this regard, the one or more properties includes, but is not limited to, Kcat, Km, pH optimum, stability, *in vivo* proteolytic stability, or no requirement for ions or cofactors that are not already present in blood, or any combination thereof. In some ~~embodiment~~examples, the isolated arginine deiminase having reduced cross-reactivity with patient anti-ADI-PEG 20 antibodies, has at least 5, 10, 15, or 20 surface residue changes as compared to *M. hominis* arginine deiminase. In certain ~~embodiment~~examples,

the isolated arginine deiminase having reduced cross-reactivity with patient anti-ADI-PEG 20 antibodies has between about 20 and 135 surface residue changes, between about 40 and 100 surface residue changes, between about 30 and 60 surface residue changes, between about 80 and 100 surface residues changes, or between about 100 and 120 surface residues changes, as compared to *M. hominis* arginine deiminase.

In certain ~~embodiment~~example~~s~~, the isolated arginine deiminase having reduced cross-reactivity with patient anti-ADI-PEG 20 antibodies is from *Mycoplasma salivarium*, *Mycoplasma spumans*, *Mycoplasma canadense, Mycoplasma auris, Mycoplasma hyosynoviae, Mycoplasma cloacale, Mycoplasma anseris, Mycoplasma alkalescens, Mycoplasma orale, Mycoplasma iners, Mycoplasma meleagridis, Mycoplasma alvi, Mycoplasma penetrans, Mycoplasma gallinarum, Mycoplasma pirum, Mycoplasma primatum, Mycoplasma fermentans, Mycoplasma lipofaciens, Mycoplasma felifaucium, Mycoplasma imitans, Mycoplasma opalescens, Mycoplasma moatsii, Mycoplasma elephantis, Mycoplasma pneumoniae*, *Mycoplasma testudinis, Mycoplasma sp. CAG:877, or Mycoplasma sp. CAG:472*. Illustrative arginine deiminase having reduced cross-reactivity with patient anti-ADI-PEG 20 antibodies comprise any one or more of the amino acid sequences set forth in SEQ ID NOs:2-28.

In some ~~embodiment~~example~~s~~, the isolated arginine deiminase having reduced cross-reactivity with patient anti-ADI-PEG 20 antibodies has been modified to remove at least one pegylation site. In certain ~~embodiment~~example~~s~~ of the arginine deiminase having reduced cross-reactivity with patient anti-ADI-PEG 20 antibodies, at least one lysine residue has been modified by an amino acid substitution. In this regard, in certain ~~embodiment~~example~~s~~, at least about 5 lysine residues, at least about 10 lysine residues, or at least about 20 lysine residues have been modified by an amino acid substitution.

In some ~~embodiment~~example~~s~~, the arginine deiminase having reduced cross-reactivity with patient anti-ADI-PEG 20 antibodies is covalently bonded via a linker to a PEG molecule. In this regard, the arginine deiminase having reduced cross-reactivity with patient anti-ADI-PEG 20 antibodies may be covalently bonded to one or more PEG molecule, such as to about 1 to about 10 or about 2 to about 8 PEG molecules. The PEG molecules may be straight chain or branch chain PEG molecules and may have a total weight average molecular weight of from about 1,000 to about 40,000, or a total weight average molecular weight of from about 10,000 to about 30,000. In some ~~embodiment~~example~~s~~, where the PEG is covalently bonded to an ADIr described herein via a linker, the linker may comprise a succinyl group, an amide group, an imide group, a carbamate group, an ester group, an epoxy group, a carboxyl group, a hydroxyl group, a carbohydrate, a tyrosine group, a cysteine group, a histidine group, a methylene group, or any combinations thereof. In specific ~~embodiment~~example~~s~~, the source of the succinyl group is succinimidyl succinate.

Also included are polynucleotides encoding an isolated arginine deiminase described herein, vectors comprising the polynucleotide, and isolated host cells comprising the vectors.

Certain ~~embodiment~~example~~s~~ relate to compositions comprising an isolated arginine deiminase having reduced cross-reactivity with patient anti-ADI-PEG 20 antibodies as described herein, and a physiologically acceptable carrier. In certain ~~embodiment~~example~~s~~, the compositions further comprise a chemotherapeutic agent. Exemplary chemotherapeutic agents include, but are not limited to, docetaxel, carboplatin, cyclophosphamide, gemcitabine, cisplatin, sorafenib, sunitinib, and everolimus.

Also included are methods of treating, ameliorating the symptoms of, or inhibiting the progression of a cancer comprising administering to a patient in need thereof a therapeutically effective amount of a composition comprising the isolated arginine deiminase having reduced cross-reactivity with patient anti-ADI-PEG 20 antibodies as described herein and a physiologically acceptable carrier, thereby treating, ameliorating the symptoms of, or inhibiting the progression of the cancer. In certain ~~embodiment~~example~~s~~, the patient in need thereof has been determined to have anti-ADI-PEG 20 antibodies. In some ~~embodiment~~example~~s~~, the cancer is selected from the group consisting of hepatocellular carcinoma, melanoma including metastatic melanoma, pancreatic cancer, prostate cancer, small cell lung cancer,

mesothelioma, lymphocytic leukemia, chronic myelogenous leukemia, lymphoma, hepatoma, sarcoma, leukemia, acute myeloid leukemia, relapsed acute myeloid leukemia, breast cancer, ovarian cancer, colorectal cancer, gastric cancer, glioma, glioblastoma multiforme, non-small cell lung cancer (NSCLC), kidney cancer, bladder cancer, uterine cancer, esophageal cancer, brain cancer, head and neck cancers, cervical cancer, testicular cancer, and stomach cancer.

Some ~~embodiment~~example**s** include methods of treating, ameliorating the symptoms of, or inhibiting the progression of a cancer comprising administering to a patient in need thereof a therapeutically effective amount of a composition comprising ADI-PEG 20, and after a period of time, administering to the patient a composition comprising the isolated arginine deiminase having reduced cross-reactivity with patient anti-ADI-PEG 20 antibodies as described herein and a physiologically acceptable carrier, thereby treating, ameliorating the symptoms of, or inhibiting the progression of the cancer. The period of time may be determined, for example, by detecting a predetermined level of anti-ADI-PEG 20 antibodies in the patient and/or measuring or otherwise observing ADI activity in the patient, wherein the composition comprising the isolated arginine deiminase having reduced cross-reactivity with patient anti-ADI-PEG 20 antibodies is administered following detection of the predetermined level of said anti-ADI-PEG 20 antibodies and/or measurement or observation of a predetermined level of ADI activity in the patient.

Also included are isolated arginine deiminase proteins described herein for use in the preparation or manufacture of a medicament for treating, ameliorating the symptoms of, or inhibiting the progression of a cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1D illustrate a variety of cysteine-reactive PEG molecules that can be conjugated to the ADIr enzymes described herein.

DETAILED DESCRIPTION

Embodiments of the present invention relate to selected ADI enzymes, which in some embodiments are conjugated with PEG through a linker, e.g., a stable linker. In some embodiments, the ADI enzymes are engineered or selected to have a small or otherwise reduced number of surface lysine residues, for example, relative to a wild-type sequence or a reference sequence (see, e.g., Table 1). The selected ADI enzymes are chosen from a large number of ADI enzymes, from different organisms, based on their beneficial properties. These properties include the ability of the enzyme to establish and maintain low arginine concentrations in human blood through ADI conversion of arginine to citrulline and ammonia. In some embodiments, the selected ADI molecules have reduced cross-reactivity toward

Claims

1. A therapeutic composition comprising an isolated arginine deiminase, or a fragment thereof having arginine deiminase (ADI) activity, and a pharmaceutically-acceptable carrier, wherein the isolated arginine deiminase has reduced cross-reactivity with patient anti-ADI-PEG 20 antibodies and comprises the amino acid sequence set forth in SEQ ID NO: 9 or SEQ ID NO: 22, or a variant thereof that displays at least 90% sequence identity to SEQ ID NO: 9 or SEQ ID NO: 22.

~~2. The therapeutic composition of claim 1 wherein the isolated arginine deiminase is not from *M. hominis*.~~

~~3. The therapeutic composition of claim 1 or 2 wherein the isolated arginine deiminase is from an organism listed in Table 1.~~

~~4.~~2. The therapeutic composition of ~~any one of~~ claim~~s~~ 1~~-3~~ wherein the isolated arginine deiminase has one or more properties comparable to or better than those of ADI-PEG 20.

~~5.~~3. The therapeutic composition of claim 2~~4~~ wherein the one or more properties is Kcat, Km, pH optimum, stability, *in vivo* proteolytic stability, or no requirement for ions or cofactors that are not already present in blood, or any combination thereof.

~~6. The therapeutic composition of any one of claims 1-5 wherein the isolated arginine deiminase has at least 20 surface residue changes as compared to *M. hominis* arginine deiminase.~~

~~7. The therapeutic composition of claim 6 wherein the isolated arginine deiminase has between 20 and 135 surface residue changes as compared to *M. hominis* arginine deiminase.~~

~~8. The therapeutic composition of claim 6 wherein the isolated arginine deiminase has between 40 and 100 surface residue changes as compared to *M. hominis* arginine deiminase.~~

~~9. The therapeutic composition of claim 6 wherein the isolated arginine deiminase has between 30 and 60 surface residue changes as compared to *M. hominis* arginine deiminase.~~

~~10. The therapeutic composition of claim 6 wherein the isolated arginine deiminase has between 80 and 100 surface residues changes as compared to *M. hominis* arginine deiminase.~~

~~11. The therapeutic composition of claim 6 wherein the isolated arginine deiminase has between 100 and 120 surface residues changes as compared to *M. hominis* arginine deiminase.~~

~~12. The therapeutic composition of any one of claims 1-11 wherein the isolated arginine deiminase is from Mycoplasma salivarium, Mycoplasma spumans, Mycoplasma canadense, Mycoplasma auris, Mycoplasma hyosynoviae, Mycoplasma cloacale, Mycoplasma anseris, Mycoplasma alkalescens, Mycoplasma orale, Mycoplasma iners, Mycoplasma meleagridis, Mycoplasma alvi, Mycoplasma penetrans, Mycoplasma gallinarum, Mycoplasma pirum,~~

~~Mycoplasma primatum, Mycoplasma fermentans, Mycoplasma lipofaciens, Mycoplasma felifaucium, Mycoplasma imitans, Mycoplasma opalescens, Mycoplasma moatsii, Mycoplasma elephantis, Mycoplasma pneumoniae, Mycoplasma testudinis, Mycoplasma sp. CAG:877, or Mycoplasma sp. CAG:472.~~

~~13. The therapeutic composition of any one of claims 1-12 wherein the isolated arginine deiminase comprises the amino acid sequence set forth in any one of SEQ ID NOs:2-28.~~

~~14.~~4. The therapeutic composition of any one of claims 1-~~1~~3 wherein the isolated arginine deiminase has been modified to remove at least one pegylation site.

~~15.~~5. The therapeutic composition of any one of claims 1-~~4~~14 wherein at least one lysine residue has been modified by an amino acid substitution.

~~16.~~6. The therapeutic composition of any one of claims 1 to 5~~claim 15~~ wherein at least 5 lysine residues have been modified by an amino acid substitution.

~~17.~~7. The therapeutic composition of any one of claims 1 to 6~~claim 15~~ wherein at least 10 lysine residues have been modified by an amino acid substitution.

~~18.~~8. The therapeutic composition of any one of claims 1 to 7~~claim 15~~ wherein at least 15 lysine residues have been modified by an amino acid substitution.

~~19.~~9. The therapeutic composition of any one of claims 1 to 8~~claim 15~~ wherein at least 20 lysine residues have been modified by an amino acid substitution.

~~20.~~10. The therapeutic composition of any one of claims 1-~~9~~19 wherein the arginine deiminase is covalently bonded via a linker to a PEG molecule.

~~21.~~11. The therapeutic composition of claim 10~~20~~ wherein the arginine deiminase is covalently bonded to more than one PEG molecule.

~~22.~~12. The therapeutic composition of claim 10 or 11~~20~~ wherein the arginine deiminase is covalently bonded to ~~about~~ 1 to ~~about~~ 10 PEG molecules, or 2 to 8 PEG molecules.

~~23. The therapeutic composition of claim 20 wherein the arginine deiminase is covalently bonded to about 2 to about 8 PEG molecules.~~

~~24.~~13. The therapeutic composition of any one of claims 10 to 12~~claim 20~~ wherein the PEG molecules are straight chain or branch chain PEG molecules.

~~25.~~14. The therapeutic composition of any one of claims 10 to 13~~claim 20~~ wherein the PEG has a total weight average molecular weight of from ~~about~~ 1,000 to ~~about~~ 40,000, or from 10,000 to 30,000.

~~26. The therapeutic composition of claim 20 wherein the PEG has a total weight average molecular weight of from about 10,000 to about 30,000.~~

~~27.~~15. The therapeutic composition~~isolated arginine deiminase~~ of any one of claims 10 to 14~~20-26~~ wherein the linker is a succinyl group, an amide group, an imide group, a carbamate group, an ester group, an epoxy group, a carboxyl group, a hydroxyl group, a carbohydrate, a tyrosine group, a cysteine group, a histidine group, a methylene group, or any combinations thereof.

~~28.~~16. The therapeutic composition of claim 15~~27~~ wherein the source of the succinyl group is succinimidyl succinate.

~~29. An isolated arginine deiminase, or a fragment thereof having ADI activity, wherein the isolated arginine deiminase has reduced cross-reactivity with patient anti-ADI-PEG 20 antibodies.~~

~~30. The isolated arginine deiminase of claim 29 wherein the isolated arginine deiminase is not from *M. hominis*.~~

~~31. The isolated arginine deiminase of claim 29 or 30 wherein the isolated arginine deiminase is from an organism listed in Table 1.~~

~~32. The isolated arginine deiminase of one any of claims 29-31 wherein the isolated arginine deiminase has one or more properties comparable to or better than those of ADI-PEG 20.~~

~~33. The isolated arginine deiminase of claim 32 wherein the one or more properties is Kcat, Km, pH optimum, stability, *in vivo* proteolytic stability, or no requirement for ions or cofactors that are not already present in blood, or any combination thereof.~~

~~34. The isolated arginine deiminase of one of claims 29-33 wherein the isolated arginine deiminase has at least 20 surface residue changes as compared to *M. hominis* arginine deiminase.~~

~~35. The isolated arginine deiminase of claim 34 wherein the isolated arginine deiminase has between 20 and 135 surface residue changes as compared to *M. hominis* arginine deiminase.~~

~~36. The isolated arginine deiminase of claim 34 wherein the isolated arginine deiminase has between 40 and 100 surface residue changes as compared to *M. hominis* arginine deiminase.~~

~~37. The isolated arginine deiminase of claim 34 wherein the isolated arginine deiminase has between 30 and 60 surface residue changes as compared to *M. hominis* arginine deiminase.~~

~~38. The isolated arginine deiminase of claim 34 wherein the isolated arginine deiminase has between 80 and 100 surface residues changes as compared to *M. hominis* arginine deiminase.~~

~~39. The isolated arginine deiminase of claim 34 wherein the isolated arginine deiminase has between 100 and 120 surface residues changes as compared to *M. hominis* arginine deiminase.~~

~~40. The isolated arginine deiminase of any one of claims 29-39 wherein the isolated arginine deiminase is from Mycoplasma salivarium, Mycoplasma spumans, Mycoplasma canadense,~~

~~Mycoplasma auris, Mycoplasma hyosynoviae, Mycoplasma cloacale, Mycoplasma anseris, Mycoplasma alkalescens, Mycoplasma orale, Mycoplasma iners, Mycoplasma meleagridis, Mycoplasma alvi, Mycoplasma penetrans, Mycoplasma gallinarum, Mycoplasma pirum, Mycoplasma primatum, Mycoplasma fermentans, Mycoplasma lipofaciens, Mycoplasma felifaucium, Mycoplasma imitans, Mycoplasma opalescens, Mycoplasma moatsii, Mycoplasma elephantis, Mycoplasma pneumoniae, Mycoplasma testudinis, Mycoplasma sp. CAG:877, or Mycoplasma sp. CAG:472.~~

~~41. The isolated arginine deiminase of any one of claims 29-40 wherein the isolated arginine deiminase comprises the amino acid sequence set forth in any one of SEQ ID NOs:2-28.~~

~~42. The isolated arginine deiminase of any one of claims 29-41 wherein the isolated arginine deiminase has been modified to remove at least one pegylation site.~~

~~43. The isolated arginine deiminase of any one of claims 29-42 wherein at least one lysine residue has been modified by an amino acid substitution.~~

~~44. The isolated arginine deiminase of claim 43 wherein at least 5 lysine residues have been modified by an amino acid substitution.~~

~~45. The isolated arginine deiminase of claim 43 wherein at least 10 lysine residues have been modified by an amino acid substitution.~~

~~46. The isolated arginine deiminase of claim 43 wherein at least 15 lysine residues have been modified by an amino acid substitution.~~

~~47. The isolated arginine deiminase of claim 43 wherein at least 20 lysine residues have been modified by an amino acid substitution.~~

~~48. The isolated arginine deiminase of any one of claims 29-47 wherein the arginine deiminase is covalently bonded via a linker to a PEG molecule.~~

~~49. The isolated arginine deiminase of claim 48 wherein the arginine deiminase is covalently bonded to more than one PEG molecule.~~

~~50. The isolated arginine deiminase of claim 48 or 49 wherein the arginine deiminase is covalently bonded to about 1 to about 10 PEG molecules.~~

~~51. The isolated arginine deiminase of claim 50 wherein the arginine deiminase is covalently bonded to about 2 to about 8 PEG molecules.~~

~~52. The isolated arginine deiminase of any one of claims 48-51 wherein the PEG molecules are straight chain or branch chain PEG molecules.~~

~~53. The isolated arginine deiminase of any one of claims 48-52 wherein the PEG has a total weight average molecular weight of from about 1,000 to about 40,000.~~

~~54. The isolated arginine deiminase of claim 53 wherein the PEG has a total weight average molecular weight of from about 10,000 to about 30,000.~~

~~55. The isolated arginine deiminase of any one of claims 48-54 wherein the linker is a succinyl group, an amide group, an imide group, a carbamate group, an ester group, an epoxy group, a carboxyl group, a hydroxyl group, a carbohydrate, a tyrosine group, a cysteine group, a histidine group, a methylene group, or any combinations thereof.~~

~~56. The isolated arginine deiminase of claim 55 wherein the source of the succinyl group is succinimidyl succinate.~~

~~57. A polynucleotide encoding the isolated arginine deiminase of any one of the preceding claims.~~

~~58. A vector comprising the polynucleotide of claim 57.~~

~~59. An isolated host cell comprising the vector of claim 58.~~

~~60.~~17. The therapeutic composition of one any of ~~the preceding~~ claims 1 to 16 further comprising a chemotherapeutic agent.

~~61.~~18. The therapeutic composition of claim 17~~60~~ wherein the chemotherapeutic agent is selected from the group consisting of docetaxel, carboplatin, cyclophosphamide, gemcitabine, cisplatin, sorafenib, sunitinib and everolimus.

~~62.~~19. Use of a therapeutic composition of any one of claims 1 to 18 in the manufacture of a medicament for~~A method of~~ treating, ameliorating the symptoms of, or inhibiting the progression of a cancer in ~~comprising administering to~~ a patient ~~in need thereof a therapeutically effective amount of a therapeutic composition or isolated arginine deiminase of any one of the preceding claims, thereby treating, ameliorating the symptoms of, or inhibiting the progression of the cancer~~.

~~63.~~20. The use~~method~~ of claim 19~~62~~ wherein the patient in need thereof has been determined to have anti-ADI-PEG 20 antibodies.

~~64.~~21. The use~~method~~ of claim 19~~62~~ or 20~~63~~ wherein the cancer is selected from the group consisting of hepatocellular carcinoma, melanoma, metastatic melanoma, pancreatic cancer, prostate cancer, small cell lung cancer, mesothelioma, lymphocytic leukemia, chronic myelogenous leukemia, lymphoma, hepatoma, sarcoma, leukemia, acute myeloid leukemia, relapsed acute myeloid leukemia, breast cancer, ovarian cancer, colorectal cancer, gastric cancer, glioma, glioblastoma multiforme, non-small cell lung cancer (NSCLC), kidney cancer, bladder cancer, uterine cancer, esophageal cancer, brain cancer, head and neck cancers, cervical cancer, testicular cancer, and stomach cancer.

~~65.~~22. Use of a ADI-PEG 20 and a therapeutic composition of any one of claims 1 to 18 in the manufacture of a medicament for~~A method of~~ treating, ameliorating the symptoms of, or inhibiting the progression of a cancer in ~~comprising administering to~~ a patient, wherein said ADI-PEG 20 is to be administered to the patient ~~in need thereof a therapeutically effective amount of~~

~~a composition comprising ADI-PEG 20~~, and after a period of time, saidadministering to the patient ~~a~~ therapeutic composition is to be administered to~~or arginine deiminase of any one of the preceding claims, thereby treating, ameliorating~~ the patient~~symptoms of, or inhibiting the progression of the cancer~~.

~~66.~~23. The use~~method~~ of claim 22~~65~~ wherein the period of time is determined by detecting a predetermined level of anti-ADI-PEG 20 antibodies in the patient, wherein the therapeutic composition is to be administered following detection of the predetermined level of said anti-ADI-PEG 20 antibodies.

~~67.~~24. The use~~method~~ of claim 22~~65~~ wherein the period of time is determined by detecting ADI activity in the patient, wherein the therapeutic composition is to be administered following detection of a predetermined or reduced level of ADI activity.

~~68.~~25. The~~Use of a~~ therapeutic composition ~~or an isolated arginine deiminase~~ of any one of ~~the preceding~~ claims 1 to 18 for use in~~in the manufacture of a medicament for~~ treating, ameliorating the symptoms of, or inhibiting the progression of a cancer.