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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 ADI activity, and a pharmaceutically-acceptable carrier, wherein the isolated arginine deiminase has reduced cross-reactivity with patient anti-ADI-PEG 20 antibodies.

In another aspect of the present disclosure, there is provided 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.

In a further aspect of the present disclosure, there is provided a polynucleotide encoding an isolated arginine deiminase as disclosed herein.

In another aspect of the present disclosure, there is provided a vector comprising a polynucleotide as disclosed herein.

In a further aspect of the present disclosure, there is provided an isolated host cell comprising a vector as disclosed herein.

In another aspect of the present disclosure, there is provided a use of a therapeutic composition as 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 need thereof.

In a further aspect of the present disclosure, there is provided a use of a composition comprising ADI-PEG 20 and a therapeutic composition as 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 need thereof, wherein said therapeutic composition is to be administered after said composition comprising ADI-PEG 20 after a period of time.

In another aspect of the present disclosure, there is provided a use of an isolated arginine deiminase as disclosed herein in the manufacture of a medicament for treating, ameliorating the symptoms of, or inhibiting the progression of a cancer.

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

In another aspect of the present disclosure, there is provided a composition comprising ADI-PEG 20 and a therapeutic composition as disclosed herein for use in treating, ameliorating the symptoms of, or inhibiting the progression of a cancer.

In a further aspect of the present disclosure, there is provided an isolated arginine deiminase as disclosed herein for use treating, ameliorating the symptoms of, or inhibiting the progression of a cancer.

~~One aspect of t~~The present ~~invention~~ disclosure provides 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 embodiments, the composition is sterile and/or substantially free of pyrogens such as endotoxins. In one embodiment, the isolated arginine deiminase having reduced cross-reactivity with patient anti-ADI-PEG 20 antibodies is not from *M. hominis*. In another embodiment, 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 embodiments 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 one embodiment, the isolated arginine deiminase having reduced cross-reactivity with patient anti-ADI-PEG 20 antibodies, has at least 20 surface residue changes as compared to *M. hominis* arginine deiminase. In another embodiment, the isolated arginine deiminase having reduced cross-reactivity with patient anti-ADI-PEG 20 antibodies has between 20 and 135 surface residue

changes, between 40 and 100 surface residue changes, between 30 and 60 surface residue changes, between 80 and 100 surface residues changes, or between 100 and 120 surface residues changes, as compared to *M. hominis* arginine deiminase.

In another embodiment, the isolated arginine deiminase having reduced cross-reactivity with patient anti-ADI-PEG 20 antibodies is from *M. arginini, M. arthritidis*, *M. phocicerebrale*, *M. gateae, M. phocidae, M. columbinum, M. iowae, M. crocodyli, M. alligatoris, H. orenii,* or *M. bovis*. Illustrative arginine deiminase having reduced cross-reactivity with patient anti-ADI-PEG 20 antibodies comprise the amino acid sequence set forth in any one of SEQ ID NOs:2-32.

In another embodiment, 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 another embodiment 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 embodiments, at least 5 lysine residues, at least 10 lysine residues, or at least 20 lysine residues have been modified by an amino acid substitution.

In another embodiment, 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 those embodiments where the PEG is covalently bonded to the ADIr of the present ~~invention~~disclosure, 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 one embodiment, the source of the succinyl group is succinimidyl succinate.

~~Another aspect of t~~The present ~~invention~~disclosure further provides a polynucleotide encoding an isolated arginine deiminase described herein, vectors comprising the polynucleotide, and isolated host cells comprising the vectors.

~~An additional aspect of t~~The present ~~invention~~disclosure further provides 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. In certain embodiments, the compositions further comprise a chemotherapeutic agent. Exemplary themotherapeutic agents include, but are not limited to, docetaxel, carboplatin, cyclophosphamide, gemcitabine, cisplatin, sorafenib, sunitinib, and everolimus.

~~Another aspect of t~~The present ~~invention~~disclosure further provides a method 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 embodiments, the patient in need thereof has been determined to have anti-ADI-PEG 20 antibodies. In another embodiment, 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.

~~Another aspect of t~~The ~~invention~~present disclosure further provides a method 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. In this regard, 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

18. The therapeutic composition of claim 15 wherein at least 15 lysine residues have been modified by an amino acid substitution.

19. The therapeutic composition of claim 15 wherein at least 20 lysine residues have been modified by an amino acid substitution.

20. The therapeutic composition of any one of the preceding claims wherein the arginine deiminase is covalently bonded via a linker to a PEG molecule.

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

22. The therapeutic composition of claim 20 wherein the arginine deiminase is covalently bonded to about 1 to about 10 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. The therapeutic composition of claim 20 wherein the PEG molecules are straight chain or branch chain PEG molecules.

25. The therapeutic composition of claim 20 wherein the PEG has a total weight average molecular weight of from about 1,000 to about 40,000.

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. The therapeutic composition ~~isolated arginine deiminase~~ of claim 20 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. The therapeutic composition of claim 27 wherein the source of the succinyl group is succinimidyl succinate.

38. The isolated arginine deiminase of claim 29 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 29 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 claim 29 wherein the isolated arginine deiminase is from *M. arginini, M. arthritidis*, *M. phocicerebrale*, *M. gateae, M. phocidae, M. columbinum, M. iowae, M. crocodyli, M. alligatoris, H. orenii*, or *M. bovis*.

41. The isolated arginine deiminase of claim 29 wherein the isolated arginine deiminase comprises the amino acid sequence set forth in any one of SEQ ID NOs:2-32.

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

43. The isolated arginine deiminase of any one of the preceding claims 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 ~~15~~ 43 wherein at least 10 lysine residues have been modified by an amino acid substitution.

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

47. The isolated arginine deiminase of claim ~~15~~ 43 wherein at least 20 lysine residues have been modified by an amino acid substitution.

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

49. The isolated arginine deiminase of claim ~~20~~ 48 wherein the arginine deiminase is covalently bonded to more than one PEG molecule.

50. The isolated arginine deiminase of claim ~~20~~ 48 wherein the arginine deiminase is covalently bonded to about 1 to about 10 PEG molecules.

51. The isolated arginine deiminase of claim ~~20~~ 48 wherein the arginine deiminase is covalently bonded to about 2 to about 8 PEG molecules.

52. The isolated arginine deiminase of claim ~~20~~ 48 wherein the PEG molecules are straight chain or branch chain PEG molecules.

53. The isolated arginine deiminase of claim ~~20~~ 48 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 ~~20~~ 48 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 claim ~~20~~ 48 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 ~~27~~ 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. The therapeutic composition of any one of ~~the preceding~~ claims 1 to 28 further comprising a chemotherapeutic agent.

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

62. Use of a therapeutic composition of any one of claims 1 to 28 in the manufacture of a medicament for ~~A method of~~ treating, ameliorating the symptoms of, or inhibiting the progression of a cancer ~~comprising administering to~~ in a patient in need thereof ~~a therapeutically effective amount of a therapeutic composition of any of the preceding claims, thereby treating, ameliorating the symptoms of, or inhibiting the progression of the cancer~~.

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

64. The ~~method~~ use of claim 62 or 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. Use of a composition comprising ADI-PEG 20 and a therapeutic composition of any one of claims 1 to 28 in the manufacture of a medicament for ~~A method of~~ treating, ameliorating the symptoms of, or inhibiting the progression of a cancer ~~comprising administering to~~ in a patient in need thereof, wherein said therapeutic composition is to be administered after said ~~a therapeutically effective amount of a~~ composition comprising ADI-PEG 20, ~~and~~ after a period of time, ~~administering to the patient a therapeutic composition of any of the preceding claims, thereby treating, ameliorating the symptoms of, or inhibiting the progression of the cancer~~.

66. The ~~method~~ use of claim 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. The ~~method~~ use of claim 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. Use of an isolated arginine deiminase of any of the preceding claims in the manufacture of a medicament for treating, ameliorating the symptoms of, or inhibiting the progression of a cancer.

69. A therapeutic composition of any one of claims 1 to 28 for use in treating, ameliorating the symptoms of, or inhibiting the progression of a cancer.

70. A composition comprising ADI-PEG 20 and a therapeutic composition of any one of claims 1 to 28 for use in treating, ameliorating the symptoms of, or inhibiting the progression of a cancer.

71. The isolated arginine deiminase of any of claims 29 to 56 for use treating, ameliorating the symptoms of, or inhibiting the progression of a cancer.