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BRIEF SUMMARY

In one aspect of the present disclosure, there is provided a therapeutic composition comprising an isolated arginine deiminase (ADI) ~~, 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~~ADI comprises the amino acid sequence set forth in any one of SEQ ID Nis: 2, 6, 8, or 14, or a sequence at least 95% sequence identity to any one of SEQ ID NOs: 2, 6, 8, or 14 having arginine deiminase activity.

~~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 therapeutic 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~~another 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 a further ~~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.~~

The present 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

CLAIMS

What is claimed is:

1. A therapeutic composition comprising an isolated arginine deiminase (ADI) ~~, 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~~ADI comprises the amino acid sequence set forth in any one of SEQ ID NOs: 2, 6, 8, or 14, or a sequence at least 95% sequence identity to any one of SEQ ID NOs: 2, 6, 8, or 14 having arginine deiminase activity.

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

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

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

~~5. The therapeutic composition of claim 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 claim 1 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 1 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 1 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 1 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 1 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 1 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 claim 1 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.~~

~~13. The therapeutic composition of claim 1 wherein the isolated arginine deiminase comprises the amino acid sequence set forth in any one of SEQ ID NOs:2-32.~~

~~14~~2. The therapeutic composition of ~~any one of the preceding claims~~claim 1 wherein the isolated ~~arginine deiminase~~ADI has been modified to remove at least one pegylation site.

~~15~~3. The therapeutic composition of ~~any one of the preceding claims~~claim 1 or 2 wherein at least one lysine residue has been modified by an amino acid substitution.

~~16~~4. The therapeutic composition of claim ~~15~~ 3 wherein at least 5 lysine residues have been modified by an amino acid substitution.

~~17~~5. The therapeutic composition of claim ~~15~~ 3 wherein at least 10 lysine residues have been modified by an amino acid substitution.

~~18~~6. The therapeutic composition of claim ~~15~~ 3 wherein at least 15 lysine residues have been modified by an amino acid substitution.

~~19~~7. The therapeutic composition of claim ~~15~~ 3 wherein at least 20 lysine residues have been modified by an amino acid substitution.

~~20~~8. The therapeutic composition of any one of ~~the preceding~~ claims 1-4 wherein the ~~arginine deiminase~~ADI is covalently bonded via a linker to ~~a~~ at least one PEG molecule.

~~21~~9. The therapeutic composition of claim ~~20~~ 5 wherein the ~~arginine deiminase~~ADI is covalently bonded to more than one PEG molecule.

~~22~~10. The therapeutic composition of claim ~~20~~ 5 wherein the ~~arginine deiminase~~ADI is covalently bonded to ~~about~~ 1 to ~~about~~ 10 PEG molecules.

~~23~~11. The therapeutic composition of claim ~~20~~ 5 wherein the ~~arginine deiminase~~ADI is covalently bonded to ~~about~~ 2 to ~~about~~ 8 PEG molecules.

~~24~~12. The therapeutic composition of claim ~~20~~ 5 wherein the PEG molecules are straight chain or branch chain PEG molecules.

~~25~~13. The therapeutic composition of claim ~~20~~ 5 wherein the PEG has a total weight average molecular weight of from ~~about~~ 1,000 to ~~about~~ 40,000; or

~~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~~14. The therapeutic composition of claim ~~20~~ 5 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~~15. The therapeutic composition of claim ~~27~~ 9 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 30 wherein the isolated arginine deiminase is from an organism listed in Table 1.

32. The isolated arginine deiminase of claim 29 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 claim 29 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 29 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 29 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 29 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 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 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 the preceding claims 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 wherein the arginine deiminase is covalently bonded to about 1 to about 10 PEG molecules.

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

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

53. The isolated arginine deiminase of claim 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 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 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 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~~16. The therapeutic composition of any one of claims 1 to ~~28~~ 10 further comprising a chemotherapeutic agent.

~~61~~17. The therapeutic composition of claim ~~60~~ 11 wherein the chemotherapeutic agent is selected from the group consisting of docetaxel, carboplatin, cyclophosphamide, gemcitabine, cisplatin, sorafenib, sunitinib and everolimus.

~~62~~18. Use of a therapeutic composition of any one of claims 1 to ~~28~~ 17 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~~.

~~63~~19. The use of claim ~~62~~ 18 wherein the patient in need thereof has been determined to have anti-ADI-PEG 20 antibodies.

~~64~~20. The use of claim ~~62~~ 18 or ~~63~~ 19 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~~21. Use of a therapeutic composition comprising ADI-PEG 20 and a therapeutic composition of any one of claims 1 to ~~28~~ 17 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.

~~66~~22. The use of claim ~~65~~ 21 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~~23. The use of claim ~~65~~ 21 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~~24. A therapeutic composition of any one of claims 1 to ~~28~~ 17 for use in treating, ameliorating the symptoms of, or inhibiting the progression of a cancer.

~~70~~25. A composition comprising ADI-PEG 20 and a therapeutic composition of any one of claims 1 to ~~28~~ 17 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.~~