BRIEF SUMMARY

Certain embodiments 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 embodiments, the composition is sterile and/or substantially free of pyrogens such as endotoxins. In some embodiments, the isolated arginine deiminase having reduced cross-reactivity with patient anti-ADI-PEG 20 antibodies is not from *M. hominis*. In some embodiments, the isolated arginine deiminase having reduced cross-reactivity with patient anti-ADI-PEG 20 antibodies is from an organism listed in Table 1.

In a first 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 and is from an organism listed in the following table:

Selected ADI Sequences with Varying Degrees of Similarity to *M. hominis* ADI			
ORGANISM	PERCENT IDENTITY	SEQUENCE ACCESSION NUMBER	SEQ ID NO:
Mycoplasma gallinarum	58.06	gi|653082428	10
Mycoplasma iners	60.20	gi|657715567	9
Mycoplasma salivarium	81.95	gi|639205998	2
Mycoplasma spumans	81.51	gi|652844751	3
Mycoplasma canadense	81.22	gi|780004832	20
Mycoplasma auris	78.54	gi|490558584	4
Mycoplasma hyosynoviae	78.78	gi|738491959	5
Mycoplasma cloacale	78.97	gi|657216914	6
Mycoplasma anseris	78.73	gi|697347301	21
Mycoplasma alkalescens	77.67	gi|488970417	7
Mycoplasma orale	79.27	gi|551316378	8
Mycoplasma meleagridis	59.1	gi|803632330	22
Mycoplasma alvi	58.46	gi|697091625	23
Mycoplasma penetrans	58.1	gi|768722109	24
Mycoplasma pirum	56.05	gi|652846075	11
Mycoplasma primatum	56.86	gi|657716867	12
Mycoplasma fermentans	56.11	gi|753838319	25
Mycoplasma lipofaciens	55.64	gi|652840093	13

Mycoplasma felifaucium	55.85	gi|653084388	14
Mycoplasma imitans	53.85	gi|652843200	15
Mycoplasma opalescens	54.25	gi|659858924	16
Mycoplasma moatsii	50.24	gi|551314758	17
Mycoplasma elephantis	52.63	gi|653084064	18
Mycoplasma pneumoniae	51.97	gi|12643471	26
Mycoplasma testudinis	49.50	gi|652838532	19
Mycoplasma sp. CAG:877	39.28	gi|524101565	27
Mycoplasma sp. CAG:472	38.07	gi|524735679	28

In a second 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 and is from an organism listed in the following table:

Selected ADI Sequences with Varying Degrees of Similarity to *M. hominis* ADI			
ORGANISM	PERCENT IDENTITY	SEQUENCE ACCESSION NUMBER	SEQ ID NO:
Mycoplasma gallinarum	58.06	gi|653082428	10
Mycoplasma iners	60.20	gi|657715567	9
Mycoplasma salivarium	81.95	gi|639205998	2
Mycoplasma spumans	81.51	gi|652844751	3
Mycoplasma canadense	81.22	gi|780004832	20
Mycoplasma auris	78.54	gi|490558584	4
Mycoplasma hyosynoviae	78.78	gi|738491959	5
Mycoplasma cloacale	78.97	gi|657216914	6
Mycoplasma anseris	78.73	gi|697347301	21
Mycoplasma alkalescens	77.67	gi|488970417	7
Mycoplasma orale	79.27	gi|551316378	8
Mycoplasma meleagridis	59.1	gi|803632330	22
Mycoplasma alvi	58.46	gi|697091625	23
Mycoplasma penetrans	58.1	gi|768722109	24
Mycoplasma pirum	56.05	gi|652846075	11
Mycoplasma primatum	56.86	gi|657716867	12
Mycoplasma fermentans	56.11	gi|753838319	25
Mycoplasma lipofaciens	55.64	gi|652840093	13
Mycoplasma felifaucium	55.85	gi|653084388	14
Mycoplasma imitans	53.85	gi|652843200	15
Mycoplasma opalescens	54.25	gi|659858924	16
Mycoplasma moatsii	50.24	gi|551314758	17
Mycoplasma elephantis	52.63	gi|653084064	18
Mycoplasma pneumoniae	51.97	gi|12643471	26
Mycoplasma testudinis	49.50	gi|652838532	19
Mycoplasma sp. CAG:877	39.28	gi|524101565	27
Mycoplasma sp. CAG:472	38.07	gi|524735679	28

In a third aspect of the present disclosure, there is provided a polynucleotide encoding the isolated arginine deiminase of the second aspect.

In a fourth aspect of the present disclosure, there is provided a vector comprising the polynucleotide of the third aspect.

In a fifth aspect of the present disclosure, there is provided an isolated host cell comprising the vector of the fourth aspect.

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

In a seventh aspect of the present disclosure, there is provided a use of a ADI-PEG 20 and a therapeutic composition of the first aspect 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 an eighth aspect of the present disclosure, there is provided a therapeutic composition of the first aspect for use in treating, ameliorating the symptoms of, or inhibiting the progression of a cancer.

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 some embodiments, 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 embodiments, 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 embodiments, 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.

CLAIMS

1. 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 and is from an organism listed in the following table:

Selected ADI Sequences with Varying Degrees of Similarity to *M. hominis* ADI			
ORGANISM	PERCENT IDENTITY	SEQUENCE ACCESSION NUMBER	SEQ ID NO:
Mycoplasma gallinarum	58.06	gi|653082428	10
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Mycoplasma spumans	81.51	gi|652844751	3
Mycoplasma canadense	81.22	gi|780004832	20
Mycoplasma auris	78.54	gi|490558584	4
Mycoplasma hyosynoviae	78.78	gi|738491959	5
Mycoplasma cloacale	78.97	gi|657216914	6
Mycoplasma anseris	78.73	gi|697347301	21
Mycoplasma alkalescens	77.67	gi|488970417	7
Mycoplasma orale	79.27	gi|551316378	8
Mycoplasma meleagridis	59.1	gi|803632330	22
Mycoplasma alvi	58.46	gi|697091625	23
Mycoplasma penetrans	58.1	gi|768722109	24
Mycoplasma pirum	56.05	gi|652846075	11
Mycoplasma primatum	56.86	gi|657716867	12
Mycoplasma fermentans	56.11	gi|753838319	25
Mycoplasma lipofaciens	55.64	gi|652840093	13
Mycoplasma felifaucium	55.85	gi|653084388	14
Mycoplasma imitans	53.85	gi|652843200	15
Mycoplasma opalescens	54.25	gi|659858924	16
Mycoplasma moatsii	50.24	gi|551314758	17
Mycoplasma elephantis	52.63	gi|653084064	18
Mycoplasma pneumoniae	51.97	gi|12643471	26
Mycoplasma testudinis	49.50	gi|652838532	19
Mycoplasma sp. CAG:877	39.28	gi|524101565	27
Mycoplasma sp. CAG:472	38.07	gi|524735679	28

2. 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.

3. The therapeutic composition of claim 2 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.

4. The therapeutic composition of any one of claims 1-3 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.*

5. The therapeutic composition of any one of claims 1-4 wherein the isolated arginine deiminase comprises the amino acid sequence set forth in any one of SEQ ID NOs:2-28, or a variant thereof that displays at least 90% sequence identity to any one of SEQ ID NOs:2-28.

6. The therapeutic composition of any one of claims 1-5 wherein the isolated arginine deiminase has been modified to remove at least one pegylation site.

7. The therapeutic composition of any one of claims 1-6 wherein at least one lysine residue has been modified by an amino acid substitution.

8. The therapeutic composition of any one of claims 1 to 7 wherein at least 5 lysine residues have been modified by an amino acid substitution.

9. The therapeutic composition of any one of claims 1 to 8 wherein at least 10 lysine residues have been modified by an amino acid substitution.

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

11. The therapeutic composition of any one of claims 1 to 10 wherein at least 20 lysine residues have been modified by an amino acid substitution.

12. The therapeutic composition of any one of claims 1-11 wherein the arginine deiminase is covalently bonded via a linker to a PEG molecule.

13. The therapeutic composition of claim 12 wherein the arginine deiminase is covalently bonded to more than one PEG molecule.

14. The therapeutic composition of claim 12 or 13 wherein the arginine deiminase is covalently bonded to about 1 to about 10 PEG molecules, or about 2 to about 8 PEG molecules.

15. The therapeutic composition of any one of claims 12 to 14 wherein the PEG molecules are straight chain or branch chain PEG molecules.

16. The therapeutic composition of any one of claims 12 to 15 wherein the PEG has a total weight average molecular weight of from about 1,000 to about 40,000, or from about 10,000 to about 30,000.

17. The therapeutic composition of any one of claims 12 to 16 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.

18. The therapeutic composition of claim 17 wherein the source of the succinyl group is succinimidyl succinate.

19. 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

and is from an organism listed in the following table:

Selected ADI Sequences with Varying Degrees of Similarity to *M. hominis* ADI			
ORGANISM	PERCENT IDENTITY	SEQUENCE ACCESSION NUMBER	SEQ ID NO:
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Mycoplasma cloacale	78.97	gi|657216914	6
Mycoplasma anseris	78.73	gi|697347301	21
Mycoplasma alkalescens	77.67	gi|488970417	7
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Mycoplasma alvi	58.46	gi|697091625	23
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Mycoplasma pirum	56.05	gi|652846075	11
Mycoplasma primatum	56.86	gi|657716867	12
Mycoplasma fermentans	56.11	gi|753838319	25
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Mycoplasma moatsii	50.24	gi|551314758	17
Mycoplasma elephantis	52.63	gi|653084064	18
Mycoplasma pneumoniae	51.97	gi|12643471	26
Mycoplasma testudinis	49.50	gi|652838532	19
Mycoplasma sp. CAG:877	39.28	gi|524101565	27
Mycoplasma sp. CAG:472	38.07	gi|524735679	28

20. A polynucleotide encoding the isolated arginine deiminase of claim 19.

21. A vector comprising the polynucleotide of claim 20.

22. An isolated host cell comprising the vector of claim 21.

23. The therapeutic composition of one any of claims 1 to 18 further comprising a chemotherapeutic agent.

24. The therapeutic composition of claim 23 wherein the chemotherapeutic agent is selected from the group consisting of docetaxel, carboplatin, cyclophosphamide, gemcitabine, cisplatin, sorafenib, sunitinib and everolimus.

25. Use of a therapeutic composition of any one of claims 1 to 18, and 23-24 in the manufacture of a medicament for treating, ameliorating the symptoms of, or inhibiting the progression of a cancer in a patient.

26. The use of claim 25 wherein the patient in need thereof has been determined to have anti-ADI-PEG 20 antibodies.

27. The use of claim 25 or 26 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.

28. Use of a ADI-PEG 20 and a therapeutic composition of any one of claims 1 to 18, and 23-24 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.

29. The use of claim 28 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.

30. The use of claim 28 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.

31. The therapeutic composition of any one of claims 1 to 18, and 23-24 for use in treating, ameliorating the symptoms of, or inhibiting the progression of a cancer.