cancer cells. The present invention provides this and other advantages for the treatment of cancers.

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BRIEF SUMMARY

In a first aspect of the present disclosure, there is provided a use of a liquid composition for the manufacture of a medicament for the treatment of a cancer that exhibits reduced expression of argininosuccinate synthetase, wherein the liquid composition comprises pegylated arginine deiminase ADI-PEG 20, where the liquid composition is sterile and at a pH of 6.6 to 7.0, the composition comprising

(a) histidine-HCl which is from 0.0035M Histidine-HCl to 0.35M Histidine-HCl, or

(b) 10 to 12 mg of ADI-PEG 20, 20 to 40 mg of polyethylene glycol, 1.27 mg ±5% monobasic sodium phosphate, 3 mg ±5% dibasic sodium phosphate, 7.6 mg ±5% sodium chloride, and 1-2 ml of water for injection.

In a second aspect of the present disclosure, there is provided a use of a compound comprising arginine deiminase covalently bonded via a linking group to polyethylene glycol for the manufacture of a medicament for the treatment of:

(a) a pancreatic cancer, small cell lung cancer (SCLC), or mesothelioma;

(b) a cancer in a patient, wherein the cancer is pancreatic cancer, a sarcoma, or small cell lung cancer, wherein the medicament is to be administered in combination with an autophagy inhibitor, wherein the autophagy inhibitor is optionally selected from the group consisting of chloroquine, 3-methyladenine, hydroxychloroquine, bafilomycin A1, 5-amino-4-imidazole carboxamide riboside (AICAR), okadaic acid, N6-mercaptopurine riboside, wortmannin, and vinblastine, and optionally wherein the arginine deiminase and the autophagy inhibitor act synergistically;

(c) melanoma, wherein the medicament is to be administered to a patient in combination with cisplatin;

(d) non-small cell lung cancer, head and neck cancer or prostate cancer, wherein the medicament is to be administered to a patient in combination with docetaxel; or

(e) renal cell carcinoma, wherein the medicament is to be administered to a patient in combination with rapamycin,

wherein the cancer exhibits reduced expression of argininosuccinate synthetase.

In a third aspect of the present disclosure, there is provided a liquid composition for use in treating a cancer that exhibits reduced expression of argininosuccinate synthetase, comprising pegylated arginine deiminase ADI-PEG 20, where the liquid composition is sterile and at a pH of 6.6 to 7.0, the composition comprising

(a) histidine-HCl which is from 0.0035M Histidine-HCl to 0.35M Histidine-HCl, or

(b) 10 to 12 mg of ADI-PEG 20, 20 to 40 mg of polyethylene glycol, 1.27 mg ±5% monobasic sodium phosphate, 3 mg ±5% dibasic sodium phosphate, 7.6 mg ±5% sodium chloride, and 1-2 ml of water for injection.

In a fourth aspect of the present disclosure, there is provided a compound comprising arginine deiminase covalently bonded via a linking group to polyethylene glycol:

(a) for use in treating a pancreatic cancer, small cell lung cancer (SCLC), or mesothelioma;

(b) for use in treating a cancer in a patient in combination with an autophagy inhibitor, wherein the cancer is pancreatic cancer, a sarcoma, or small cell lung cancer, wherein the autophagy inhibitor is optionally selected from the group consisting of chloroquine, 3-methyladenine, hydroxychloroquine, bafilomycin A1, 5-amino-4-imidazole carboxamide riboside (AICAR), okadaic acid, N6-mercaptopurine riboside, wortmannin, and vinblastine, and optionally wherein the arginine deiminase and the autophagy inhibitor act synergistically;

(c) for use in treating melanoma in a patient in combination with cisplatin;

(d) for use in treating non-small cell lung cancer, head and neck cancer or prostate cancer in a patient in combination with docetaxel; or

(e) for use in treating renal cell carcinoma in a patient in combination with rapamycin,

wherein the cancer exhibits reduced expression of argininosuccinate synthetase.

One ~~aspect~~ embodiment of the present ~~invention~~ disclosure provides a method of treating leukemia in a patient comprising administering to the patient a compound comprising ADI covalently bonded via a linking group to polyethylene glycol. In one embodiment, the leukemia is acute myeloid leukemia or relapsed acute myeloid leukemia. In a further embodiment, the leukemia is not lymphocytic leukemia or chronic myelogenous leukemia. However in further embodiments the leukemia may include lymphocytic leukemia or chronic myelogenous leukemia. In another embodiment, the arginine deiminase is covalently bonded to 5±1.5 straight chain PEG molecules and the compound is formulated in a composition comprising less than about 0.5% native ADI, less than about 5% free PEG, or both; wherein the arginine deiminase is administered weekly at a dose of about 160 IU/m^2 to about 640 IU/m^2; and wherein the leukemia exhibits reduced expression of ASS.

Another embodiment ~~aspect~~ of the present disclosure ~~invention~~ provides a method of treating a cancer in a patient comprising administering to the patient an autophagy inhibitor and a compound comprising ADI covalently bonded via a linking group to

polyethylene glycol, wherein the cancer is pancreatic cancer or small cell lung cancer. In this regard an autophagy inhibitor is selected from the group consisting of chloroquine, 3-methyladenine, hydroxychloroquine, bafilomycin A1, 5-amino-4-*imidazole carboxamide riboside (AICAR), okadaic acid*, N6-*mercaptopurine riboside*, wortmannin, and vinblastine. Other autophagy inhibitors known in the art are contemplated for use in the methods herein. In certain embodiments, the ADI and the autophagy inhibitor act additively or synergistically. In another embodiment, the arginine deiminase is covalently bonded to 5±1.5 straight chain PEG molecules and the compound is formulated in a composition comprising less than about 0.5% native ADI, less than about 5% free PEG, or both; wherein the arginine deiminase is administered weekly at a dose of about 160 IU/m2 to about 640 IU/m2; and wherein the cancer exhibits reduced expression of ASS.

One embodiment ~~aspect~~ of the present disclosure ~~invention~~ provides a method of treating a cancer in a patient comprising administering to the patient a compound comprising ADI covalently bonded via a linking group to polyethylene glycol, wherein the cancer is selected from the group consisting of 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. In one embodiment, the arginine deiminase is covalently bonded to 5±1.5 straight chain PEG molecules and the compound is formulated in a composition comprising less than about 0.5% native ADI, less than about 5% free PEG, or both; wherein the arginine deiminase is administered weekly at a dose of about 160 IU/m2 to about 640 IU/m2; and wherein the cancer exhibits reduced expression of ASS.

One embodiment ~~aspect~~ of the present disclosure ~~invention~~ provides a method of treating melanoma in a patient comprising administering to the patient a compound comprising ADI covalently bonded via a linking group to polyethylene glycol, *in combination with cisplatin. In one embodiment, the arginine deiminase is covalently* bonded to 5±1.5 straight chain PEG molecules and the compound is formulated in a composition comprising less than about 0.5% native ADI, less than about 5% free PEG, or both; wherein the arginine deiminase is administered weekly at a dose of about 160 IU/m2 to about 640 IU/m2; and wherein the melanoma exhibits reduced expression of ASS.

Another embodiment ~~aspect~~ of the present disclosure ~~invention~~ provides a method of treating a cancer in a patient comprising administering to the patient a compound comprising ADI covalently bonded via a linking group to polyethylene glycol, wherein the cancer is not melanoma, pancreatic cancer, prostate cancer, small cell lung cancer, mesothelioma, lymphocytic leukemia, chronic myelogenous leukemia, lymphoma, hepatoma, and sarcoma. In one embodiment, the arginine deiminase is covalently bonded to 5±1.5 straight chain PEG molecules and the compound is formulated in a composition comprising less than about 0.5% native ADI, less than about 5% free PEG, or both; wherein the arginine deiminase is administered weekly at a dose of about 160 IU/m2 to about 640 IU/m2; and wherein the cancer exhibits reduced expression of ASS.

Another embodiment ~~aspect~~ of the present disclosure provides a method of treating non-small cell lung cancer, head and neck cancer or prostate cancer in a patient comprising administering a therapeutically effective amount of a compound comprising arginine deiminase covalently bonded via a linking group to polyethylene glycol, in combination with docetaxel. In one embodiment, the arginine deiminase is covalently bonded to 5±1.5 straight chain PEG molecules and the compound is formulated in a composition comprising less than about 0.5% native ADI, less than about 5% free PEG, or both; wherein the arginine deiminase is administered weekly at a dose of about 160 IU/m2 to about 640 IU/m2; and wherein the non-small cell lung cancer, head and neck cancer or prostate cancer exhibits reduced expression of ASS.

Another embodiment ~~aspect~~ of the present disclosure provides a method of treating renal cell carcinoma in a patient comprising administering a therapeutically effective amount of a compound comprising arginine deiminase covalently bonded via a linking group to polyethylene glycol, in combination with rapamycin. In one embodiment, the arginine deiminase is covalently bonded to 5±1.5 straight chain PEG molecules and the compound is formulated in a composition comprising less than about 0.5% native ADI, less than about 5% free PEG, or both; wherein the arginine deiminase is administered weekly at a dose of about 160 IU/m2 to about 640 IU/m2; and wherein the cancer exhibits reduced expression of ASS.

In certain embodiments of the present disclosure ~~invention~~ the ADI is covalently bonded to more than one polyethylene glycol molecule, or to about 9 to about 12

polyethylene glycol molecules. In another embodiment of the present disclosure ~~invention~~ the polyethylene glycol has a total weight average molecular weight of from about 1,000 to about 40,000, has a total weight average molecular weight of from about 10,000 to about 30,000, and in certain embodiments, the polyethylene glycol has a molecular weight of 20,000.

In certain embodiments of the present disclosure the linking group is a succinimide group. In certain embodiments, the succinimide group may be succinimidyl succinate, succinimidyl propionate, succinimidyl carboxymethylate, succinimidyl succinamide, N-hydroxy succinimide or combinations thereof. In one embodiment, the succinimide group is succinimidyl succinate, succinimidyl propionate or combinations thereof.

In certain embodiments of the present disclosure the ADI is not isolated from *Mycoplasma arginini*. In other embodiments, the ADI is isolated from *Mycoplasma hominis*. In one particular embodiment, the ADI has been modified to be free of at least one lysine at position 112, 374, 405 or 408 of SEQ ID NO:1, and in another embodiment, the ADI comprises the amino acid sequence set forth in SEQ ID NO: 2. In one embodiment, the arginine deiminase is covalently bonded to 5±1.5 PEG molecules, and in certain embodiments the PEG is straight chain PEG.

In some embodiments of the present methods, the ADI is administered from about twice a week to about once every 2 weeks, and in a particular embodiment the ADI is administered weekly.

In one embodiment, the ADI is administered at a dose of between about 80 IU/m^2 and about 640 IU/m^2, and in one particular embodiment is administered at a dose of about 160 IU/m^2. In another embodiment, the ADI is administered weekly at a dose of about 160 IU/m^2.

In one embodiment, the compound used in the methods described herein comprising arginine deiminase covalently bonded via a linking group to polyethylene glycol, is formulated in a composition comprising less than about 0.5% native ADI, less than about 5% free PEG, or both.

Another embodiment ~~aspect~~ of the present disclosure ~~invention~~ provides a method of treating GVHD in a patient comprising administering to the patient a compound comprising ADI covalently bonded via a linking group to polyethylene glycol.

A further embodiment ~~aspect~~ of the present disclosure ~~invention~~ provides a method of treating a cancer in a patient comprising administering to the patient a compound comprising ADI covalently bonded via a linking group to polyethylene glycol, wherein the cancer exhibits of ASS. In one embodiment, the cancer is selected from the group consisting of 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. In a further embodiment, the reduced expression of ASS results from methylation of the argininosuccinate synthetase promoter. In another embodiment, the reduced expression of ASS results from a DNA mutation or deletion. In one particular embodiment, reduced expression of ASS results from deletion or transposition of the 9q34 locus as part of the "Philadelphia chromosome". In certain embodiments, the cancer exhibits reduced expression of ASS.

Yet a further embodiment ~~aspect~~ of the present disclosure ~~invention~~ provides a method of treating a cancer in a patient comprising administering to the patient a compound comprising ADI covalently bonded via a linking group to polyethylene glycol, wherein the cancer exhibits reduced expression of argininosuccinate lyase. In one embodiment, the cancer is selected from the group consisting of 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. In one embodiment, reduced expression of ASL results from methylation of the argininosuccinate lyase promoter. In certain embodiments, the reduced expression of ASL results from a DNA mutation or deletion. In one particular embodiment, the cancer is ASL negative.

In certain embodiments of the present disclosure~~invention~~, the treatment with ADI-PEG inhibits NO synthesis, inhibits angiogenesis, induces apoptosis in tumor cells, or a combination thereof, *in vivo*. In certain embodiments the treatment results in stable disease. In other embodiments, the treatment increases progression free

CLAIMS

What is claimed is:

1. A compound comprising arginine deiminase covalently bonded via a linking group to polyethylene glycol, for use in treating leukemia.

2. The compound of claim 1 wherein the leukemia is acute myeloid leukemia.

3. The compound of claim 1 wherein the leukemia is relapsed acute myeloid leukemia.

4. The compound of claim 1 wherein the leukemia is not lymphocytic leukemia or chronic myelogenous leukemia.

5. A compound comprising arginine deiminase covalently bonded via a linking group to polyethylene glycol, in combination with an autophagy inhibitor, for use in treating a cancer in a patient, wherein the cancer is pancreatic cancer, a sarcoma, or small cell lung cancer.

6. The compound of claim 5 wherein the autophagy inhibitor is selected from the group consisting of chloroquine, 3-methyladenine, hydroxychloroquine, bafilomycin A1, 5-amino-4-imidazole carboxamide riboside (AICAR), okadaic acid, N6-mercaptopurine riboside, wortmannin, and vinblastine.

7. A compound comprising arginine deiminase covalently bonded via a linking group to polyethylene glycol, for treating a cancer in a patient, wherein the cancer is selected from the group consisting of 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.

8. A compound comprising arginine deiminase covalently bonded via a linking group to polyethylene glycol, for treating a cancer in a patient, wherein the cancer is not melanoma, pancreatic cancer, prostate cancer, small cell lung cancer, mesothelioma, lymphocytic leukemia, chronic myelogenous leukemia, lymphoma, hepatoma, or sarcoma.

9. A compound comprising arginine deiminase covalently bonded via a linking group to polyethylene glycol, in combination with cisplatin, for treating melanoma in a patient.

10. A compound comprising arginine deiminase covalently bonded via a linking group to polyethylene glycol, in combination with docetaxel, for treating non-small cell lung cancer, head and neck cancer or prostate cancer in a patient.

11. A compound comprising arginine deiminase covalently bonded via a linking group to polyethylene glycol, in combination with rapamycin, for treating renal cell carcinoma in a patient.

12. The compound of any one of claims 1, 5, and 7 - 11 wherein the arginine deiminase is covalently bonded to more than one polyethylene glycol molecule.

13. The compound of any one of claims 1, 5, and 7 - 11 wherein the arginine deiminase is covalently bonded to about 9 to about 12 polyethylene glycol molecules.

14. The compound of any one of claims 1, 5, and 7 - 11 wherein the arginine deiminase is covalently bonded to 5±1.5 PEG molecules.

15. The compound of claim 14, wherein the PEG molecules are straight chain PEG molecules.

16. The compound of any one of claims 1, 5, and 7 - 11 wherein the polyethylene glycol has a total weight average molecular weight of from about 1,000 to about 40,000.

17. The compound of any one of claims 1, 5, and 7 - 11 wherein the polyethylene glycol has a total weight average molecular weight of from about 10,000 to about 30,000.

18. The compound of any one of claims 1, 5, and 7 - 11 wherein the polyethylene glycol has a molecular weight of 20,000.

19. The compound of claim 17 wherein the polyethylene glycol is straight chain polyethylene glycol.

20. The compound of any one of claims 1, 5, and 7 - 11 wherein the linking group is a succinimide group.

21. The compound of claim 20 wherein the succinimide group is succinimidyl succinate, succinimidyl propionate, succinimidyl carboxymethylate, succinimidyl succinamide, N-hydroxy succinimide or combinations thereof.

22. The compound of claim 20 wherein the succinimide group is succinimidyl succinate, succinimidyl propionate or combinations thereof.

23. The compound of any one of claims 1, 5, and 7 - 11 wherein the arginine deiminase is not isolated from *Mycoplasma arginini*.

24. The compound of any one of claims 1, 5, and 7 - 11 wherein the arginine deiminase is isolated from *Mycoplasma hominis*.

25. The compound of claim 24 wherein the arginine deiminase has been modified to be free of at least one lysine at position 112, 374, 405 or 408 of SEQ ID NO:1.

26. The compound of any one of claims 1, 5, and 7 - 11 wherein the compound is formulated in a composition comprising less than about 0.5% native ADI, less than about 5% free PEG, or both.

27. The compound of claim 24 wherein the arginine deiminase comprises the amino acid sequence set forth in SEQ ID NO: 2.

28. The compound of any one of claims 1, 5, and 7 - 11 wherein the arginine deiminase is administered from about twice a week to about once every 2 weeks.

29. The compound of any one of claims 1, 5, and 7 - 11 wherein the arginine deiminase is administered weekly.

30. The compound of any one of claims 1, 5, and 7 - 11 wherein the arginine deiminase is administered at a dose of between about 80 IU/m^2 and about 650 IU/m^2.

31. The compound of claim 30 wherein the arginine deiminase is administered at a dose of about 160 IU/m^2.

32. The compound of any one of claims 1, 5, and 7 - 11 wherein the arginine deiminase is administered weekly at a dose of about 160 IU/m^2.

33. A compound comprising arginine deiminase covalently bonded via a linking group to polyethylene glycol, for treating GVHD in a patient.

34. A compound comprising arginine deiminase covalently bonded via a linking group to polyethylene glycol, for treating a cancer in a patient, wherein the cancer exhibits reduced expression of argininosuccinate synthetase.

35. The compound of claim 34, wherein the cancer is selected from the group consisting of 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.

36. The compound of claim 34 wherein the reduced expression of argininosuccinate synthetase results from methylation of the argininosuccinate synthetase promoter.

37. The compound of claim 34 wherein the reduced expression of argininosuccinate synthetase results from a DNA mutation or deletion.

38. The compound of claim 37 wherein the cancer is argininosuccinate synthetase negative.

39. A compound comprising arginine deiminase covalently bonded via a linking group to polyethylene glycol, for treating a cancer in a patient, wherein the cancer exhibits reduced expression of argininosuccinate lyase.

40. The compound of claim 39, wherein the cancer is selected from the group consisting of 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.

41. The compound of claim 39 wherein the reduced expression of argininosuccinate lyase results from methylation of the argininosuccinate lyase promoter.

42. The compound of claim 39 wherein the reduced expression of argininosuccinate lyase results from a DNA mutation or deletion.

43. The compound of claim 42 wherein the cancer is argininosuccinate lyase negative.

44. The compound of any one of claims 1, 5, 7 - 11, 34 and 39 wherein the treatment inhibits NO synthesis, inhibits angiogenesis, induces apoptosis in tumor cells, or a combination thereof, *in vivo*.

45. The compound of any one of claims 1, 5, 7 - 11, 34 and 39 wherein the treatment results in stable disease.

46. The compound of any one of claims 1, 5, 7 - 11, 34 and 39 wherein the treatment increases progression free survival time in the patient.

47. The compound of any one of claims 1, 5, 7 - 11, 34 and 39 wherein plasma arginine is depleted for at least one month.

48. The compound of any one of claims 1, 5, 7 - 11, 34 and 39 wherein plasma arginine is depleted for more than 2 months.

49. The compound of claim 5 wherein the arginine deiminase and the autophagy inhibitor act synergistically.

50. The compound any one of claims 1, 5, 7 - 8, 10 - 11, 34 and 39 further comprising administration of a chemotherapeutic agent.

51. The compound of claim 50 wherein the chemotherapeutic agent is selected from the group consisting of cyclophosphamide, gemcitabine, cisplatin, sorafenib, sunitinib and everolimus.

52. The compound of claim 1, 5, 7 - 11, and 39 wherein:

(a) wherein the arginine deiminase is covalently bonded to 5±1.5 straight chain PEG molecules and the compound is formulated in a composition comprising less than about 0.5% native ADI, less than about 5% free PEG, or both;

(b) wherein the arginine deiminase is administered weekly at a dose of about 160 IU/m^2 to about 640 IU/m^2; and

(c) wherein the cancer exhibits reduced expression of ASS.

CLAIMS

What is claimed is:

1. Use of a liquid composition for the manufacture of a medicament for the treatment of a cancer that exhibits reduced expression of argininosuccinate synthetase, wherein the liquid composition comprises pegylated arginine deiminase ADI-PEG 20, where the liquid composition is sterile and at a pH of 6.6 to 7.0, the composition comprising

(a) histidine-HCl which is from 0.0035M Histidine-HCl to 0.35M Histidine-HCl, or

(b) 10 to 12 mg of ADI-PEG 20, 20 to 40 mg of polyethylene glycol, 1.27 mg ±5% monobasic sodium phosphate, 3 mg ±5% dibasic sodium phosphate, 7.6 mg ±5% sodium chloride, and 1-2 ml of water for injection.

2. The use according to claim 1(a), wherein the liquid composition comprises 0.035 M Histidine-HCl at pH 6.8 with 0.13 M sodium chloride.

3. The use according to claim 1 or 2, wherein the cancer is:

(a) pancreatic cancer, small cell lung cancer (SCLC), mesothelioma, or hepatocellular carcinoma;

(b) pancreatic cancer, a sarcoma, or small cell lung cancer, wherein the autophagy inhibitor is optionally selected from the group consisting of chloroquine, 3-methyladenine, hydroxychloroquine, bafilomycin A1, 5-amino-4-imidazole carboxamide riboside (AICAR), okadaic acid, N6-mercaptopurine riboside, wortmannin, and vinblastine, and optionally wherein the arginine deiminase and the autophagy inhibitor act synergistically, wherein the medicament is to be administered to a patient in combination with an autophagy inhibitor;

(c) melanoma, wherein the medicament is to be administered to a patient in combination with cisplatin;

(d) non-small cell lung cancer, head and neck cancer or prostate cancer, wherein the medicament is to be administered to a patient in combination with docetaxel;

(e) renal cell carcinoma, wherein the medicament is to be administered to a patient in combination with rapamycin; or

(f) selected from the group consisting of 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.

4. The use according to any one of claims 1-3, wherein the medicament is to be administered from twice a week to once every 2 weeks.

5. The use according to any one of claims 1-3, wherein the medicament is to be administered weekly.

6. The use according to any one of claims 1-3, wherein the medicament is to be administered at a dose of between 80 IU/m^2 and 650 IU/m^2.

7. The use according to any one of claims 1-3, wherein the medicament is to be administered at a dose of 160 IU/m^2.

8. The use according to any one of claims 1-3, wherein the medicament is to be administered weekly at a dose of 160 IU/m^2.

9. The use according to any one of claims 1-3, wherein the medicament is to be administered weekly at a dose of 160 IU/m^2 to 640 IU/m^2.

10. The use according to any one of claims 1-9, wherein the reduced expression of argininosuccinate synthetase results from methylation of the argininosuccinate synthetase promoter, or from a DNA mutation or deletion.

11. The use according to claim 10, wherein the cancer is argininosuccinate synthetase negative.

12. The use according to any one of claims 1-11, wherein the treatment inhibits NO synthesis, inhibits angiogenesis, induces apoptosis in tumor cells, or a combination thereof, *in vivo*.

13. The use according to any one of claims 1-11, wherein the treatment results in stable disease or increases progression free survival time in the patient.

14. The use according to any one of claims 1-11, wherein the treatment increases progression free survival time in the patient.

15. The use according to any one of claims 1-14, wherein plasma arginine is depleted for at least one month.

16. The use according to any one of claims 1-14, wherein plasma arginine is depleted for more than 2 months.

17. The use according to any one of claims 1-16, wherein the medicament is to be administered in combination with an additional chemotherapeutic agent.

18. The use according to claim 17, wherein the chemotherapeutic agent is selected from the group consisting of cyclophosphamide, gemcitabine, cisplatin, sorafenib, sunitinib and everolimus.

19. Use of a compound comprising arginine deiminase covalently bonded via a linking group to polyethylene glycol for the manufacture of a medicament for the treatment of:

(a) a pancreatic cancer, small cell lung cancer (SCLC), or mesothelioma;

(b) a cancer in a patient, wherein the cancer is pancreatic cancer, a sarcoma, or small cell lung cancer, wherein the medicament is to be administered in combination with an autophagy inhibitor, wherein the autophagy inhibitor is optionally selected from the group consisting of chloroquine, 3-methyladenine, hydroxychloroquine, bafilomycin A1, 5-amino-4-imidazole carboxamide riboside (AICAR), okadaic acid, N6-mercaptopurine riboside, wortmannin, and vinblastine, and optionally wherein the arginine deiminase and the autophagy inhibitor act synergistically;

(c) melanoma, wherein the medicament is to be administered to a patient in combination with cisplatin;

(d) non-small cell lung cancer, head and neck cancer or prostate cancer, wherein the medicament is to be administered to a patient in combination with docetaxel; or

(e) renal cell carcinoma, wherein the medicament is to be administered to a patient in combination with rapamycin,

wherein the cancer exhibits reduced expression of argininosuccinate synthetase.

20. The use of claim 19 wherein the arginine deiminase is covalently bonded to:

(a) more than one polyethylene glycol molecule;

(b) about 9 to about 12 polyethylene glycol molecules; or

(d) 5±1.5 PEG molecules.

21. The use of claim 19 or 20 wherein:

(a) the polyethylene glycol has a total weight average molecular weight of from about 1,000 to about 40,000;

(b) the polyethylene glycol has a total weight average molecular weight of from about 10,000 to about 30,000; or

(d) the polyethylene glycol has a molecular weight of 20,000.

22. The use of any of claims 19 to 21 wherein the polyethylene glycol(s) are straight chain polyethylene glycol(s).

23. The use of any one of claims 19 to 22 wherein the linking group is a succinimide group, optionally selected from the group consisting of succinimidyl succinate, succinimidyl propionate, succinimidyl carboxymethylate, succinimidyl succinamide, N-hydroxy succinimide, and combinations thereof.

24. The use of any one of claims 19 to 23 wherein the arginine deiminase is not isolated from *Mycoplasma arginini.*

25. The use of any one of claims 19 to 24 wherein the arginine deiminase is isolated from *Mycoplasma hominis*.

26. The use of claim 25 wherein the arginine deiminase has been modified to be free of at least one lysine at position 112, 374, 405 or 408 of SEQ ID NO:1, or wherein the arginine deiminase comprises the amino acid sequence set forth in SEQ ID NO:2.

27. The use of any one of claims 19 to 26 wherein the compound is formulated in a composition comprising less than about 0.5% native ADI, less than about 5% free PEG, or both.

28. The use of any one of claims 19 to 27 wherein the arginine deiminase is to be administered:

(a) from about twice a week to about once every 2 weeks;

(b) weekly;

(c) at a dose of between about 80 IU/m^2 and about 650 IU/m^2;

(d) at a dose of about 160 IU/m^2; or

(e) weekly at a dose of about 160 IU/m^2.

29. The use of any one of claims 19 to 28 wherein the treatment:

(a) inhibits NO synthesis, inhibits angiogenesis, induces apoptosis in tumor cells, or a combination thereof, *in vivo*;

(b) results in stable disease or increases progression free survival time in the patient; or

(c) increases progression free survival time in the patient.

30. The use of any one of claims 19 to 28 wherein plasma arginine is:

(a) depleted for at least one month; or

(b) depleted for more than 2 months.

31. The use any one of claims 19 to 28, wherein a chemotherapeutic agent is to be further administered, wherein the chemotherapeutic agent is optionally selected

from the group consisting of cyclophosphamide, gemcitabine, cisplatin, sorafenib, sunitinib and everolimus.

32. The use of any one of claims 19 to 31 wherein:

(a) the arginine deiminase is covalently bonded to 5±1.5 straight chain PEG molecules and the compound is formulated in a composition comprising less than about 0.5% native ADI, less than about 5% free PEG, or both; and

(b) the arginine deiminase is administered weekly at a dose of about 160 IU/m^2 to about 640 IU/m^2.

33. A liquid composition for use in treating a cancer that exhibits reduced expression of argininosuccinate synthetase, comprising pegylated arginine deiminase ADI-PEG 20, where the liquid composition is sterile and at a pH of 6.6 to 7.0, the composition comprising

(a) histidine-HCl which is from 0.0035M Histidine-HCl to 0.35M Histidine-HCl, or

(b) 10 to 12 mg of ADI-PEG 20, 20 to 40 mg of polyethylene glycol, 1.27 mg ±5% monobasic sodium phosphate, 3 mg ±5% dibasic sodium phosphate, 7.6 mg ±5% sodium chloride, and 1-2 ml of water for injection.

34. The liquid composition for use according to claim 33(a), comprising 0.035 M Histidine-HCl at pH 6.8 with 0.13 M sodium chloride.

35. The liquid composition for use according to claim 33 or 34, for use in treating:

(a) pancreatic cancer, small cell lung cancer (SCLC), mesothelioma, or hepatocellular carcinoma;

(b) a patient in combination with an autophagy inhibitor, wherein the cancer is pancreatic cancer, a sarcoma, or small cell lung cancer, wherein the autophagy inhibitor is optionally selected from the group consisting of chloroquine, 3-methyladenine,

hydroxychloroquine, bafilomycin A1, 5-amino-4-imidazole carboxamide riboside (AICAR), okadaic acid, N6-mercaptopurine riboside, wortmannin, and vinblastine, and optionally wherein the arginine deiminase and the autophagy inhibitor act synergistically;

(c) melanoma in a patient in combination with cisplatin;

(d) non-small cell lung cancer, head and neck cancer or prostate cancer in a patient in combination with docetaxel;

(e) renal cell carcinoma in a patient in combination with rapamycin; or

(f) a cancer selected from the group consisting of 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.

36. The liquid composition for use according to any one of claims 33-35, which is administered from twice a week to once every 2 weeks.

37. The liquid composition for use according to any one of claims 33-35, which is administered weekly.

38. The liquid composition for use according to any one of claims 33-35, which is administered at a dose of between 80 IU/m^2 and 650 IU/m^2.

39. *The liquid composition for use according to any one of claims* 33-35, which is administered at a dose of 160 IU/m^2.

40. The liquid composition for use according to any one of claims 33-35, which is administered weekly at a dose of 160 IU/m^2.

41. The liquid composition for use according to any one of claims 33-35, which is administered weekly at a dose of 160 IU/m^2 to 640 IU/m^2.

42. The liquid composition for use according to any one of claims 33-41 wherein the reduced expression of argininosuccinate synthetase results from methylation of the argininosuccinate synthetase promoter, or from a DNA mutation or deletion.

43. The liquid composition for use according to claim 42, wherein the cancer is argininosuccinate synthetase negative.

44. The liquid composition for use according to any one of claims 33-43, wherein the treatment inhibits NO synthesis, inhibits angiogenesis, induces apoptosis in tumor cells, or a combination thereof, *in vivo*.

45. The liquid composition for use according to any one of claims 33-43, wherein the treatment results in stable disease or increases progression free survival time in the patient.

46. The liquid composition for use according to any one of claims 33-43, wherein the treatment increases progression free survival time in the patient.

47. The liquid composition for use according to any one of claims 33-46, wherein plasma arginine is depleted for at least one month.

48. The liquid composition for use according to any one of claims 33-46, wherein plasma arginine is depleted for more than 2 months.

49. The liquid composition for use according to any one of claims 33-48, for use in combination with an additional chemotherapeutic agent

50. The liquid composition for use according to claim 49, wherein the chemotherapeutic agent is selected from the group consisting of cyclophosphamide, gemcitabine, cisplatin, sorafenib, sunitinib and everolimus.

51. A compound comprising arginine deiminase covalently bonded via a linking group to polyethylene glycol:

(a) for use in treating a pancreatic cancer, small cell lung cancer (SCLC), or mesothelioma;

(b) for use in treating a cancer in a patient in combination with an autophagy inhibitor, wherein the cancer is pancreatic cancer, a sarcoma, or small cell lung cancer, wherein the autophagy inhibitor is optionally selected from the group consisting of chloroquine, 3-methyladenine, hydroxychloroquine, bafilomycin A1, 5-amino-4-imidazole carboxamide riboside (AICAR), okadaic acid, N6-mercaptopurine riboside, wortmannin, and vinblastine, and optionally wherein the arginine deiminase and the autophagy inhibitor act synergistically;

(c) for use in treating melanoma in a patient in combination with cisplatin;

(d) for use in treating non-small cell lung cancer, head and neck cancer or prostate cancer in a patient in combination with docetaxel; or

(e) for use in treating renal cell carcinoma in a patient in combination with rapamycin,

wherein the cancer exhibits reduced expression of argininosuccinate synthetase.

52. The compound of claim 51 wherein the arginine deiminase is covalently bonded to:

(a) more than one polyethylene glycol molecule;

(b) about 9 to about 12 polyethylene glycol molecules; or

(d) 5±1.5 PEG molecules.

53. The compound of claim 51 or 52 wherein:

(a) the polyethylene glycol has a total weight average molecular weight of from about 1,000 to about 40,000;

(b) the polyethylene glycol has a total weight average molecular weight of from *about 10,000 to about 30,000; or*

(d) the polyethylene glycol has a molecular weight of 20,000.

54. The compound of any of claims 51 to 53 wherein the polyethylene glycol(s) are straight chain polyethylene glycol(s).

55. The compound of any one of claims 51 to 54 wherein the linking group is a succinimide group, optionally selected from the group consisting of succinimidyl succinate, succinimidyl propionate, succinimidyl carboxymethylate, succinimidyl succinamide, N-hydroxy succinimide, and combinations thereof.

56. The compound of any one of claims 51 to 55 wherein the arginine deiminase is not isolated from *Mycoplasma arginini*.

57. The compound of any one of claims 51 to 56 wherein the arginine deiminase is isolated from *Mycoplasma hominis*.

58. The compound of claim 57 wherein the arginine deiminase has been modified to be free of at least one lysine at position 112, 374, 405 or 408 of SEQ ID NO:1, or wherein the arginine deiminase comprises the amino acid sequence set forth in SEQ ID NO:2.

59. The compound of any one of claims 51 to 58 wherein the compound is formulated in a composition comprising less than about 0.5% native ADI, less than about 5% free PEG, or both.

60. The compound of any one of claims 51 to 59 wherein the arginine deiminase is administered:

(a) from about twice a week to about once every 2 weeks;

(b) weekly;

(c) at a dose of between about 80 IU/m^2 and about 650 IU/m^2;

(d) at a dose of about 160 IU/m^2; or

(e) weekly at a dose of about 160 IU/m^2.

61. The compound of any one of claims 51 to 60 wherein the treatment:

(a) inhibits NO synthesis, inhibits angiogenesis, induces apoptosis in tumor cells, or a combination thereof, *in vivo*;

(b) results in stable disease or increases progression free survival time in the patient; or

(c) increases progression free survival time in the patient.

62. The compound of any one of claims 51 to 60 wherein plasma arginine is:

(a) depleted for at least one month; or

(b) depleted for more than 2 months.

63. The compound any one of claims 51 to 60 further comprising administration of a chemotherapeutic agent, wherein the chemotherapeutic agent is optionally selected from the group consisting of cyclophosphamide, gemcitabine, cisplatin, sorafenib, sunitinib and everolimus.

64. The compound of any one of claims 51 to 63 wherein:

(a) the arginine deiminase is covalently bonded to 5±1.5 straight chain PEG molecules and the compound is formulated in a composition comprising less than about 0.5% native ADI, less than about 5% free PEG, or both; and

(b) the arginine deiminase is administered weekly at a dose of about 160 IU/m^2 to about 640 IU/m^2.