CLAIMS:

1. A conjugate comprising an L-asparaginase from*Erwinia* having at least 80% identity to the amino acid of SEQ ID NO: 1 and polyethylene glycol (PEG), wherein the PEG has a molecular weight less than or equal to about 5000 Da.

2. The conjugate of claim 1, wherein said L-asparaginase has at least from 90% to 99% identity to the amino acid of SEQ ID NO: 1.

3. The conjugate of claim 1, wherein said L-asparaginase comprises the amino acid sequence of SEQ ID NO: 1.

4. The conjugate of claim 1, wherein said PEG has a molecular weight of less than or equal to about 5000 Da.

5. The conjugate of claim 1, wherein said conjugate has an *in vitro* activity of at least from 60% to 90% as compared to the L-asparaginase when not conjugated to PEG.

6. The conjugate of claim 1, wherein said conjugate has an L-asparagine depletion activity at least about 50 times more potent than the L-asparaginase when not conjugated to PEG.

7. The conjugate of claim 1, wherein said conjugate depletes plasma L-asparagine levels to an undetectable level for at least between 48 and 96 hours.

8. The conjugate of claim 1, wherein said conjugate has a longer *in vivo* circulating half life compared to the L-asparaginase when not conjugated to PEG.

9. The conjugate of claim 1, wherein said conjugate has a longer $t_{1/2}$ than pegaspargase at an equivalent protein dose.

10. The conjugate of claim 9, wherein said conjugate has a $t_{1/2}$ of at least from about 58 hours to about 65 hours at a dose of about 50 μg protein/kg.

11. The conjugate of claim 9, wherein said conjugate has a $t_{1/2}$ of at least from about 34 hours to about 40 hours at a dose of about 10 μg protein/kg.

12. The conjugate of claim 1, wherein the PEG is covalently linked to one or more amino of said L-asparaginase.

13. The conjugate of claim 12, wherein the PEG is covalently linked to said one or more amino groups by an amide bond.

14. The conjugate of claim 12, wherein the PEG is covalently linked to at least from about 40% to about 100% of the accessible amino groups.

15. The conjugate of claim 1 having the formula:

Asp-[NH-CO-(CH2)x-CO-NH-PEG]n

wherein Asp is the L-asparaginase, NH is one or more of the NH groups of the lysine residues and/or the N-terminus in the Asp, PEG is a polyethylene glycol moiety, n is a number that represents at least 40% to about 100% of the accessible amino groups in the Asp, and x is an integer ranging from 1 to 8.

16. The conjugate of claim 1, wherein said PEG is monomethoxy-polyethylene glycol.

17. A method of making the conjugate of claim 1, said method comprising combining an amount of said PEG with an amount of said L-asparaginase in a buffered solution for a time period sufficient to covalently link said PEG to said L-asparaginase.

18. The method of claim 17, wherein said buffered solution has a pH value of between about 7.0 and about 9.0.

19. The method of claim 17, wherein the amount of said L-asparaginase is a protein concentration of between about 0.5 mg/mL and about 25 mg/mL.

20. The method of claim 17, wherein the amount of said PEG is a molar excess of polymer over amino groups in said L-asparaginase of less than about 20:1.

21. The method of claim 17, wherein said PEG is monomethoxy-polyethylene glycol.

22. A method of treating a disease treatable by L-asparagine depletion in a patient, said method comprising administering to said patient an effective amount of the conjugate of claim 1.

23. The method of claim 22, wherein said disease treatable by L-asparagine depletion is a cancer.

24. The method of claim 23, wherein said cancer is selected from the group consisting of Acute Lymphoblastic Leukemia (ALL), non-Hodgkin's lymphoma, NK lymphoma, and pancreatic cancer.

25. The method of claim 24, wherein said cancer is ALL.

26. The method of claim 25, wherein said conjugate is administered at an amount of about 5 U/kg to about 25 U/kg.

27. The method of claim 25, wherein said conjugate is administered in a dose that depletes L-asparagine to undetectable levels for a period of about 3 days to about 10 days.

28. The method of claim 25, wherein said conjugate elicits a lower immunogenic response in said patient compared to the unconjugated L-asparaginase.

29. The method of claim 25, wherein said conjugate has a longer *in vivo* circulating half-life after a single dose compared to the unconjugated L-asparaginase.

30. The method of claim 22, wherein said conjugate is administered intravenously or intramuscularly.

31. The method of claim 22, wherein said conjugate is administered once or twice per week.

32. The method of claim 22, wherein said conjugate is administered less than once per week.

33. The method of claim 22, wherein said conjugate is administered as monotherapy.

34. The method of claim 33, wherein said conjugate is not administered with an asparagine synthetase inhibitor.

35. The method of claim 22, wherein said patient has had a previous hypersensitivity to an L-asparaginase selected from the group consisting of an *E. coli* L-asparaginase, *Erwinia* L-asparaginase and PEGylated form thereof.

36. The method of claim 35, wherein said hypersensitivity is selected from the group consisting of allergic reaction, anaphylactic shock, and silent hypersensitivity.

37. The method of claim 22, wherein said patient has had a disease relapse.

38. The method of claim 37, wherein said disease relapse occurs after treatment with an *E. coli* L-asparaginase or PEGylated form thereof.

39. A pharmaceutical composition comprising the conjugate of claim 1.

CLAIMS

1. A conjugate comprising an L-asparaginase from *Erwinia* having at least 80% identity to the amino acid of SEQ ID NO:1 and polyethylene glycol (PEG), wherein the PEG has a molecular weight less than or equal to about 5000 Da.

2. The conjugate of claim 1, wherein said L-asparaginase has at least 90% identity to the amino acid of SEQ ID NO:1, or at least 99% identity to the amino acid of SEQ ID NO:1.

3. The conjugate of claim 1, wherein said L-asparaginase is identical to SEQ ID NO:1.

4. The conjugate of any of claims 1 to 3, wherein said PEG has a molecular weight within the range of at least 500 Da.

5. The conjugate of claim 3, wherein said PEG has a molecular weight of about 5000 Da, less than about 5000 Da, less than about 4000 Da, less than about 3000 Da, or less than about 2500 Da.

6. The conjugate of claim 1, wherein said L-asparaginase is identical to SEQ ID NO:1 and said PEG has a molecular weight of about 5000 Da.

7. The conjugate of any one of claims 1 to 6 wherein the PEG is covalently linked to one or more amino groups of said L-asparaginase.

8. The conjugate of claim 7, wherein the PEG is covalently linked to said one or more amino groups by an amide bond.

9. The conjugate of claim 7 or 8, wherein the PEG is covalently linked to from 40% to 100% of the accessible amino groups, such from 40% to about 90% of total amino groups.

10. The conjugate of one of claims 1 to 9 having the formula:

Asp-[NH-CO-(CH2)x-CO-NH-PEG]n

wherein Asp is the L-asparaginase, NH is one or more of the NH groups of the lysine residues and/or the N-terminus in the Asp, PEG is a polyethylene glycol moiety, n is a

number that represents at least 40% to about 100% of the accessible amino groups (*e.g.*, lysine residues and/or the N-terminus) in the Asp, and x is an integer ranging from 1 to 8, such as wherein x is an integer ranging from 2 to 5.

11. A method of making the conjugate of any one of claims 1 to 10, said method comprising combining an amount of a PEG reagent with an amount of said L-asparaginase in a buffered solution for a time period sufficient to covalently link said PEG reagent to said L-asparaginase.

12. The method of claim 11, wherein said buffered solution has a pH value of between 7.0 and 9.0, such as between 7.5 and 8.5.

13. The method of claim 11, wherein the amount of said L-asparaginase is a protein concentration of between 0.5 mg/mL and 25 mg/mL, such as between 2 mg/mL and 20 mg/mL, such as between 3 mg/mL and 15 mg/mL.

14. The method of claim 11, wherein the amount of said PEG is a molar excess of polymer over amino groups in said L-asparaginase of less than 20:1, such as less than 10:1, such as less than 8:1.

15. The conjugate of any of claims 1 to 10, or the method of any of claims 11 to 14, wherein said PEG is monomethoxy-polyethylene glycol.

16. The conjugate of any of claims 1 to 10 for use in medicine.