Claims

1. Use of a conjugate of a protein having substantial L-asparagine aminohydrolase activity and polyethylene glycol (PEG) in the manufacture of a medicament for use in the treatment of a disease treatable by L-asparagine depletion in a patient, wherein an effective amount of the medicament is to be administered to the patient, wherein the polyethylene glycol has a molecular weight less than or equal to about 5000 Da, wherein the protein is a L-asparaginase from *Erwinia*.

2. The use of claim 1 wherein the L-asparaginase has at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid of SEQ ID NO: 1.

3. The use of claim 1 wherein the conjugate comprises an L-asparaginase from *Erwinia* having at 100% sequence identity to the amino acid of SEQ ID NO: 1.

4. The use of any preceding claim wherein the PEG has a molecular weight of about 5000 Da, 4000, Da, 3000 Da, 2500 Da, or 2000 Da.

5. The use of any preceding claim wherein the conjugate has an in vitro activity of at least 60%, 65%, 70%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% as compared to the L-asparaginase when not conjugated to PEG.

6. The use of any preceding claim wherein the conjugate has an L-asparagine depletion activity at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 times more potent than the L-asparaginase when not conjugated to PEG.

7. The use of any preceding claim wherein the conjugate depletes plasma L-asparagine levels to an undetectable level for at least about 12, 24, 48, 96, 108, or 120 hours.

8. The use of any preceding claim wherein the conjugate has a longer in vivo circulating half-life compared to the L-asparaginase when not conjugated to PEG.

9. The use of any preceding claim wherein the conjugate has a longer t½ than pegaspargase administered at an equivalent protein dose.

10. The use of any preceding claim wherein the conjugate has a t½ of at least about 58 to about 65 hours at a dose of about 50 µg/kg on a protein content basis, and a t½ of at least about 34 to about 40 hours at a dose of about 10 µg/kg on a protein content basis, following iv administration in mice.

11. The use of any preceding claim wherein the conjugate has a t½ of at least about 100 to about 200 hours at a dose ranging from about 10,000 to about 15,000 IU/m^2 (about 20-30 mg protein/m^2).

12. The use of any preceding claim wherein the conjugate has a greater area under the curve (AUC) compared to the L-asparaginase when not conjugated to PEG.

13. The use of any preceding claim wherein the conjugate has a mean AUC that is at least about 3 times greater than pegaspargase at an equivalent protein dose.

14. The use of any preceding claim wherein the PEG is covalently linked to one or more amino groups of the L-asparaginase.

15. The use of claim 14 wherein the PEG is covalently linked to the one or more amino groups by an amide bond.

16. The use of any preceding claim wherein the PEG is covalently linked to at least from about 40% to about 100% of the accessible amino groups or at least from about 40% to about 90% of total amino groups.

17. The use of any preceding claim wherein the conjugate has the formula:

$$Asp\text{-}[NH\text{-}CO\text{-}(CH_2)_x\text{-}CO\text{-}NH\text{-}PEG]_n$$

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

18. The use of any preceding claims wherein the PEG is monomethoxy-polyethylene glycol (mPEG).

19. The use of any preceding claim wherein disease is a cancer.

20. The use of claim 19, wherein the cancer is selected from the group consisting of lymphoma, large cell immunoblastic lymphoma, non-Hodgkin's lymphoma, diffuse large B-cell lymphoma, NK lymphoma, Hodgkin's disease, acute myelocytic Leukemia, acute promyelocytic Leukemia, acute myelomonocytic Leukemia, acute monocytic Leukemia, acute T-cell Leukemia, acute myeloid Leukemia (AML), biphenotypic B-cell myelomonocytic Leukemia and chronic lymphocytic Leukemia.

21. The use of any of claims 1-18 wherein disease is selected from the group consisting of renal cell carcinoma, renal cell adenocarcinoma, glioblastoma including glioblastoma multiforma and glioblastoma astrocytoma, medulloblastoma, rhabdomyosarcoma, malignant melanoma, epidermoid carcinoma, squamous cell carcinoma, lung carcinoma including large cell lung carcinoma and small cell lung carcinoma, endometrial carcinoma, ovarian adenocarcinoma, ovarian tetratocarcinoma, cervical adenocarcinoma, breast carcinoma, breast adenocarcinoma, breast ductal carcinoma, pancreatic adenocarcinoma, pancreatic ductal carcinoma, colon carcinoma, colon adenocarcinoma, colorectal

adenocarcinoma, bladder transitional cell carcinoma, bladder papilloma, prostate carcinoma, osteosarcoma, epitheloid carcinoma of the bone, prostate carcinoma, and thyroid cancer.

22. The use of any preceding claim wherein the conjugate is to be administered at an amount of about 5 U/kg body weight to about 50 U/kg body weight.

23. The use of any preceding claim wherein the conjugate is to be administered at a dose ranging from about 10,000 to about 15,000 IU/m^2.

24. The use of any preceding claim wherein the medicament is to be administered intravenously or intramuscularly once per week, twice per week, or three times per week.

25. The use of any preceding claim wherein the conjugate is to be administered as monotherapy.

26. The use of claims 1-23 wherein the conjugate is to be administered as part of a combination therapy.

27. The use of claim 25 wherein the conjugate is to be administered as part of a combination therapy with Oncaspar®, daunorubicin, cytarabine, Vyxeos®, ABT-737, Venetoclax, dactolisib, bortezomib, carfilzomib, vincristine, prednisolone, everolimus, and/or CB-839.

28. The use of any preceding claim wherein the patient receiving treatment has had a previous hypersensitivity to an *E. coli* asparaginase or PEGylated form thereof or to an *Erwinia* asparaginase.

29. The use of any preceding claim wherein the patient receiving treatment has had a disease relapse, in particular a relapse that occurs after treatment with an *E. coli* asparaginase or PEGylated form thereof.