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 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 intramuscularly or intravenously, wherein the polyethylene glycol has a molecular weight less than or equal to 5000 Da, wherein the protein is a L-asparaginase from Erwinia, and wherein the disease treatable by L-asparagine depletion is selected from the group consisting of: colon carcinoma, colon adenocarcinoma, and colorectal carcinoma.

2. The use of the conjugate of claim 1, wherein the L-asparaginase has an amino acid sequence identical to SEQ ID NO: 1.

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

4. The use of the conjugate of claim 1, wherein the PEG has a molecular weight of 5000 Da, 4000 Da, 3000 Da, 2500 Da, or 2000 Da.

5. The use of the conjugate of claim 1, 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 the conjugate of claim 1, wherein the conjugate has an L-asparagine depletion activity at least 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 the conjugate of claim 1, wherein the conjugate depletes plasma L-asparagine levels to an undetectable level for at least 12, 24, 48, 96, 108, or 120 hours.

8. The use of the conjugate of claim 1, 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 the conjugate of claim 1, wherein the conjugate has a longer t½ than pegaspargase administered at an equivalent protein dose.

10. The use of the conjugate of claim 1, wherein the conjugate has a t½ of at least 58 to 65 hours at a dose of 50 µg/kg on a protein content basis, and a t½ of at least 34 to 40 hours at a dose of 10 µg/kg on a protein content basis, following IV administration in mice.

11. The use of the conjugate of claim 1, wherein the conjugate has a t½ of at least 100 to 200 hours at a dose ranging from 10,000 to 15,000 IU/m^2 (20-30 mg protein/m^2).

12. The use of the conjugate of claim 1, 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 the conjugate of claim 1, wherein the conjugate has a mean AUC that is at least 3 times greater than pegaspargase at an equivalent protein dose.

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

15. The use of the conjugate of claim 1, wherein the PEG is covalently linked to the one or more amino groups by an amide bond.

16. The use of the conjugate of claim 1, wherein the PEG is covalently linked to at least from 40% to 100% of the accessible amino groups or at least from 40% to 90% of total amino groups.

17. The use of the conjugate of claim 1, 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 40% to 100% of the accessible amino groups in the Asp, and x is an integer ranging from 1 to 8.

18. The use of the conjugate of claim 1, wherein the PEG is monomethoxy-polyethylene glycol (mPEG).

19. The use of the conjugate of claim 1, wherein the medicament is to be administered at an amount of 5 U/kg body weight to 50 U/kg body weight.

20. The use of the conjugate of claim 1, wherein the medicament is to be administered at a dose ranging from 10,000 to 15,000 IU/m^2.

21. The use of the conjugate of claim 1, wherein the administration is to be performed intravenously or intramuscularly and is once per week, twice per week, or three times per week.

22. The use of the conjugate of claim 1, wherein the medicament is to be administered as monotherapy.

23. The use of the conjugate of claim 1, wherein the medicament is to be administered as part of a combination therapy.

24. The use of the conjugate of claim 23, wherein the medicament 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.

25. The use of the conjugate of claim 1, wherein the patient who is to receive treatment has had a previous hypersensitivity to an *E. coli* asparaginase or PEGylated form thereof or to an *Erwinia* asparaginase.

26. The use of the conjugate of claim 1, wherein the patient who is to receive treatment has had a disease relapse.

27. The use of the conjugate of claim 26, wherein the relapse occurs after treatment with an *E. coli* asparaginase or PEGylated form thereof.

28. The use of claim 1, wherein the colon carcinoma, colon adenocarcinoma, or colorectal carcinoma is associated with a mutation in a gene selected from NRAS, PTEN, ARHGAP35, MLH1, CHD4, CDKN2A,

COND1, ASXL1, ERBB2, KEAP1, ACVR1B, SMARCA4, FBXW7, MSH6, PCU2F2, PPMID, NSD1, TNFAIP3, ARID1A, CTCF, JAK1, MLL4, EP300, STIK11, SPEN, CTNNB1, SMC3, KRAS, LRP1B, or a combination thereof.

29. The use of claim 1, wherein the colon carcinoma, colon adenocarcinoma, or colorectal carcinoma is associated with a mutation in an NRAS gene.

30. The use of claim 1, wherein the colon carcinoma, colon adenocarcinoma, or colorectal carcinoma is associated with a mutation in a PTEN gene.