What is claimed is:

1. A method for producing purified recombinant cobalt-substituted human Arginase, wherein the recombinant human Arginase (rhARG) comprises an amino acid sequence that is at least 98% identical to SEQ ID NO: 1, the method comprising:
    a. in a bioreactor, culturing *E. coli* cells that produce rhARG;
    b. lysing the *E. coli* cells;
    c. removing cell debris from the lysate;
    d. loading the cell lysate on a cation exchange column;
    e. eluting the rhARG with a high salt solution;
    f. incubating the eluted rhARG with a cobalt salt to form cobalt-substituted rhARG (Co-rhARG);
    g. applying said Co-rhARG to an anion exchange column and collecting the flow-through;
    h. applying the flow-through onto a third chromatography column; and
    i. eluting the Co-rhARG from the third chromatography column with a high salt solution.
2. The method of claim 1, wherein up to 60 grams of rhARG is loaded onto the cation exchange column per liter of cation exchange resin.
3. The method of claim 1 or 2, wherein eluting the rhARG from the cation exchange column uses a high salt solution of up to about 0.5 M salt concentration.
4. The method of claim 1 or 2, wherein eluting the rhARG from the cation exchange column uses a high salt solution of about 0.1 M salt concentration.
5. The method of claim 1 or 2, wherein eluting the rhARG from the cation exchange column uses a gradient of about 0.0 to about 0.5 M salt concentration.
6. The method of claim 1 or 2, wherein eluting the rhARG from the cation exchange column uses a gradient of about 0.0 to about 0.2M salt concentration.
7. The method of any one of claims 1-6, wherein the cobalt salt comprises a Co^{2+} salt.
8. The method of any one of claims 1-7, wherein the cobalt salt comprises $CoCl_2$.
9. The method of any one of claims 1-8, wherein the third chromatography column comprises a multimodal chromatography (MMC) column.

10. The method of any one of claims 1-9, further comprising reacting the rhARG or Co-rhARG with a PEGylation reactant to provide a PEGylated protein.

11. The method of claim 10, wherein the PEGylated protein comprises one or more of PEGylated amino acid residues at K16, K32, K38, K40, K47, K67, K74, K82, L87, K88, K152, K154, K171, K222, K223, K312 and K321.

12. The method of claim 11, wherein the PEGylated protein comprises one of more of about 15% to about 60% of K16, about 35% to about 80% of K32, about 20% to about 85% of K38, about 10% to about 60% of K40, about 10% to about 60% of K47, about 40% to about 90% of K67, about 30% to about 95% of K74, about 30% to about 98% of K82, about 15% to about 65% of K87, about 25% to about 70% of K88, about 25% to about 85% of K152, about 15% to about 65% of K154, about 20% to about 75% of K171, 0% to about 30% of K222, 0% to about 35% of K223, 0% to about 45% of K312, and 0% to about 45% of K321 is PEGylated.

13. The method of any one of claims 10-12, wherein the PEGylated protein comprises PEGylated amino acid residues at least at K16, K32, K38, K40, K47, K67, K74, K82, L87, K88, K152, K154, K171, K312 and K321.

14. The method of any one of claims 10-13, wherein the PEGylated protein does not have PEGylated amino acid residues at K3, K149, K190, K195, K29, K265 and K283.

15. A method for producing purified PEGylated recombinant cobalt-substituted human Arginase, wherein the recombinant human Arginase (rhARG) comprises an amino acid sequence that is at least 98% identical to SEQ ID NO: 1, the method comprising:

a. in a bioreactor, culturing *E. coli* that produce rhARG;
b. lysing the *E. coli* cells;
c. removing cell debris from the lysate;
d. loading the cell lysate on a cation exchange column;
e. eluting the rhARG with a high salt solution;
f. incubating the eluted rhARG with 10 mM $CoCl_2$ to form cobalt-substituted rhARG (Co-rhARG);
g. applying the Co-rhARG to an anion column and collecting the flow-through;
h. applying the flow-through onto a multimodal chromatography (MMC) column;
i. eluting the Co-rhARG from the MMC column with a high salt solution;

j. adding a molar excess of methoxy PEG succinimidyl carboxymethyl ester;

k. removing excess PEG.

16. A method for producing purified recombinant cobalt-substituted human Arginase, wherein the recombinant human Arginase (rhARG) comprises an amino acid sequence that is at least 98% identical to SEQ ID NO: 1, the method comprising:

a. in a bioreactor, culturing *E. coli* cells that produce rhARG at about 36°C to about 38°C , and pH of between about 7.0 and about 7.4, with agitation and aeration;

i. adjusting the temperature of the bioreactor to about 29°C;

ii. inducing *E. coli* cells to produce for rhARG;

iii. culturing the *E. coli* cells for about 18 hours;

iv. harvesting the *E. coli* cells by centrifugation;

b. lysing the *E. coli* cells by high pressure homogenization in 25 mM HEPES at a pH of between about 7.2 to about 7.6, and at or below about 15°C ;

c. removing cell debris from the lysate by centrifugation at or below 15°C , then filtering the lysate with a 0.8 micron filter then filtering with a 0.5 micron filter;

d. loading the cell lysate on a cation exchange column then washing the column with 25 mM HEPES, pH 7.2-7.6;

e. eluting the rhARG at room temperature with a high salt solution comprising 25mM HEPES, 0.1M NaCl, pH 7.2-7.6;

f. incubating the eluted rhARG with 10 mM $CoCl_2$ at room temperature for about 2 to about 8 hours to form cobalt-substituted rhARG (Co-rhARG);

i. exchanging Co-rhARG into 50mM Tris, pH 8.1-8.5;

g. applying the Co-rhARG to an anion exchange column and collecting the flow-through;

h. applying the flow-through onto a Capto multimodal chromatography (MMC) column;

i. eluting the Co-rhARG from the MMC column with a high salt solution comprising 50mM Tris, 250mM NaCl, pH 8.1-8.5.

17. A method for producing purified recombinant PEGylated recombinant cobalt-substituted human Arginase, wherein the recombinant human Arginase (rhARG)

comprises an amino acid sequence that is at least 98% identical to SEQ ID NO: 1, the method comprising:

a. in a bioreactor, culturing *E. coli* that produce rhARG at about 36°C and about 38°C, and pH of between about 7.0 and about 7.4, with agitation and aeration;
    i. adjusting the temperature of the bioreactor to about 29°C;
    ii. inducing *E. coli* to produce for rhARG;
    iii. culturing the *E. coli* for about 18 hours;
    iv. harvesting the *E. coli* by centrifugation;
b. lysing the *E. coli* cells by high pressure homogenization in 25 mM HEPES at a pH of about 7.2 to about 7.6, and at or below about 15°C;
c. removing cell debris from the lysate by centrifugation at or below 15°C, then filtering the lysate with an about 0.8 micron filter then filtering with an about 0.5 micron filter;
d. loading the cell lysate on a cation exchange column then washing the column with 25 mM HEPES, pH 7.2-7.6;
e. eluting the rhARG at room temperature with a high salt solution comprising 25mM HEPES, 0.1M NaCl, pH 7.2-7.6;
f. incubating the eluted rhARG with 10 mM CoCl2 at room temperature for about 2 to about 8 hours to form cobalt-substituted rhARG (Co-rhARG);
    i. exchanging Co-rhARG into 50mM Tris, pH 8.1-8.5;
g. applying the Co-rhARG to an anion exchange column and collecting the flow-through;
h. applying the flow-through onto a Capto multimodal chromatography (MMC) column;
i. eluting the Co-rhARG from the MMC column with a high salt solution comprising 50mM Tris, 250mM NaCl, pH 8.1-8.5 (MMC Buffer);
    i. exchanging the MMC Buffer for 20mM Sodium Phosphate, 50mM NaCl, 1.5% Glycerol, pH 7.4 (Buffer 1), and adjusting the concentration of protein to about 5.0 mg/mL;
    ii. exchanging Buffer 1 for 0.1M sodium phosphate, pH 8.1-8.5 (Buffer 2), and concentrating the protein concentration to about 10.0 mg/mL;

j. adding a molar excess of methoxy PEG succinimidyl carboxymethyl ester 5,000 Da approximate molecular weight, wherein about 19 moles of methoxy PEG succinimidyl carboxymethyl ester is added for each mole of protein and incubating this mixture for about 30 minutes to about 4 hours at approximately pH 8.4;

k. removing excess PEG by exchanging Buffer 2 for 20mM Sodium Phosphate, 50mM NaCl, 1.5% Glycerol, pH 7.4.

~~18. A composition comprising Co-rhARG or Co-rhARG-PEG produced by the methods of any one of claims 1-17.~~

~~19. The composition of claim 18, wherein the protein is covalently linked to a polyethylene glycol at one or more of K16, K32, K38, K40, K47, K67, K74, K82, L87, K88, K152, K154, K171, K222, K223, K312 and K321.~~

~~20. A composition comprising a recombinant human Arginase (rhARG) protein, wherein the protein comprises an amino acid sequence that is at least 98% identical to SEQ ID NO: 1, wherein the protein is in a complex with a non-native metal cofactor, wherein the non-native metal cofactor is cobalt, and wherein the protein is covalently linked to a polyethylene glycol at one or more of K16, K32, K38, K40, K47, K67, K74, K82, L87, K88, K152, K154, K171, K222, K223, K312 and K321.~~

~~21. The composition of any one of claims 18-20, wherein the recombinant human Arginase (rhARG) comprises an amino acid substitution at a position selected from the group consisting of: H100, D123, H125, D127, D231, D233, W121, D180, S229, C302, and E255.~~

~~22. The composition of any one of claims 18-21, wherein the recombinant human Arginase (rhARG) comprises at least one amino acid substitution selected from the group consisting of: D180S, S229C, S229G, C302F, C302I, E255Q, D180E, and S229A.~~

~~23. The composition of any one of claims 18-22, wherein the recombinant human Arginase (rhARG) comprises the at least one amino acid substitution is C302.~~

~~24. The composition of any one of claims 18-23, wherein the recombinant human Arginase (rhARG) comprises at least two amino acid substitutions.~~

~~25. The composition of any one of claims 18-24, wherein the recombinant human Arginase (rhARG) is a truncated arginase I protein.~~

26. The composition of any one of claims 18-25, wherein the recombinant human Arginase (rhARG) further comprises an exogenous protein fragment.

27. The composition of claim 26, wherein the exogenous protein fragment comprising the Fc region of an immunoglobulin or a portion of the Fc region of an immunoglobulin.

28. The composition of any one of claims 18-27, wherein the specific activity of Co-rhARG-PEG is in the range of about 400 U/mg to about 700 U/mg.

29. The composition of any one of claims 18-28, wherein the protein displays a k_{cat}/K_m for the hydrolysis of arginine in the range of about 200 $mM^{-1}\cdot s^{-1}$ to about 4,000 $mM^{-1}\cdot s^{-1}$ at pH 7.4 when assayed *in vitro*.

30. The composition of claim 29, wherein the protein displays a k_{cat}/K_M for the hydrolysis of arginine in the range of about 400 $mM^{-1}\cdot s^{-1}$ to about 2,500 $mM^{-1}\cdot s^{-1}$ at pH 7.4 when assayed *in vitro*.

31. The composition of any one of claims 18-30, wherein the molar ratio of PEG:Co-rhARG is in the range of about 7 moles/mole to about 15 moles/mole.

32. The composition of any one of claims 18-31, wherein the free PEG concentration is less than or equal to 100 µg/mL.

33. The composition of any one of claims 18-32, wherein total cobalt content of the composition is in the range of about 9 µg/mL to about 15 µg/mL.

34. The composition of any one of claims 18-33, wherein the composition produces at least 9 peaks when loaded on imaging capillary isoelectric focusing (iCIEF), wherein peak 1 is less than 20%, peak 2 is less than 30%, Peak 3+4 is in the range of 10-30%, peak 5 is in the range of 15-30%, peak 6 is in the range of 10-25%, peak 7 is less than 25%, peak 8 is less than 15%, and peak 9 is less than 8%.

35. The composition of any one of claims 18-34, wherein the composition produces at least 9 peaks when loaded on icIEF, wherein peak 1 is in the range of 5-7%, peak 2 is in the range of 8-11%, Peak 3+4 in the range of 16-20%, peak 5 in the range of 21-24%, peak 6 in the range of 21-22%, peak 7 in the range of 14-15%, peak 8 in the range of 5-8%, and peak 9 in the range of 2-3%.

36. A pharmaceutical composition comprising the Co-rhARG or Co-rhARG-PEG of any one of claims 18-35 and a pharmaceutically acceptable carrier.

37. The pharmaceutical composition of claim 36, wherein the composition is formulated for intravenous or subcutaneous administration.

38. The pharmaceutical composition of claim 36 or 37, wherein the composition comprises potassium phosphate, sodium chloride and glycerol.

39. The pharmaceutical composition of any one of claims 36-38, wherein the composition comprises about 50 mM NaCl, about 1 mM K_2HPO_4, about 4 mM KH_2PO_4, and about 1.5% w/v glycerol.

40. A method of treating Arginase 1 deficiency, the method comprising administering the pharmaceutical composition of any one of claims 36-39 to a patient.

41. The method of claim 40, wherein the pharmaceutical composition is administered intravenously.

42. The method of claim 40, wherein the pharmaceutical composition is administered subcutaneously.

43. The method of any one of claims 40-42, wherein administration the pharmaceutical composition is initiated at a dose of 0.1 mg/kg based on the weight of unPEGylated enzyme.

44. The method of any one of claims 40-43, further comprising monitoring the patient's plasma arginine levels.

45. The method of any one of claims 40-44, wherein the dose is adjusted according to the following algorithm:

a. If the plasma arginine level is >150 μM, a single 168-hour sample will be used to increase the dose by 2 dose levels in the table below (not to exceed 0.20 mg/kg) if the 2 doses prior to this sample were a) the same dose level in mg/kg, and b) consecutive (with no missed doses).

b. If the plasma arginine levels from 2 sequential 168-hour samples (regardless of missed doses) are both <50 μM, the dose is decreased by 1 dose level in the table below, not to decrease below 0.05 mg/kg.

Dose Level [a]	Dose
1 (Minimum Possible Dose)	0.05 mg/kg
2 (Starting Dose)	0.10 mg/kg
3	0.15 mg/kg
4 (Maximum Possible Dose)	0.20 mg/kg

(a) Pegzilarginase dosing starts at level 2, 0.10 mg/kg. Dose increases, when required, are by 2 dose levels. Dose decreases are by 1 dose level.