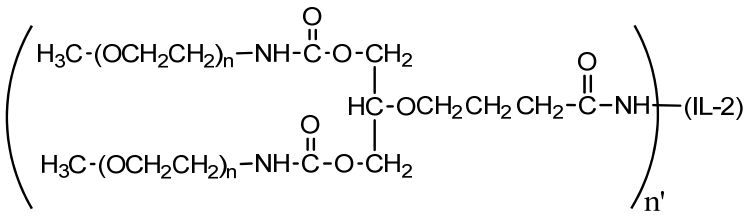
compositions for the treatment of autoimmune diseases and inflammatory disorders. RUR_{20kD}-IL-2 compositions induce durable responses in immune inflammatory disorders by activating and expanding antigen specific T regulatory cells. Treatment of autoimmune disorders with low dose subcutaneous administration of an RUR_{20kD}-IL-2 composition may provide a means to selectively restore Treg homeostasis, with minimal impact on conventional T cell function, thereby providing an alternative and/or improved approach to alleviate these disorders.

In one aspect, the present disclosure refers to a composition comprising PEGylated IL-2 conjugates having a structure:



wherein;

IL-2 is an interleukin-2;

n is independently at each occurrence an integer from about 3 to about 4000; and

n’ is 1 and 2 and 3.

In a further aspect, the present disclosure refers to a use of a composition disclosed herein in the manufacture of a medicament for treating autoimmune or inflammatory diseases in a subject, wherein the treatment comprises increasing the ratio of regulatory T cells to effector T cells in the subject.

In another aspect, the present disclosure refers to a use of a composition disclosed herein in the manufacture of a medicament for treating a subject having an autoimmune disease.

In yet another aspect, the present disclosure refers to a composition disclosed herein for use in therapy.

In a further aspect, the present disclosure refers to a composition disclosed herein for use in the treatment of an autoimmune disease.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGs. 1A and 1B are representative reverse phase HPLC plots illustrating the general composition of an RUR_{20kD}-IL-2 composition, the preparation of which is described in Examples 1 and 1A. Moving from left to right along the x-axis (elution times, minutes), the purified conjugate composition comprises primarily di-PEGylated and tri-PEGylated rIL-2.

FIG. 2 is the amino acid sequence of aldesleukin (125-L-serine-2-133 interleukin-2, a recombinant non-glycosylated interleukin-2 expressed in *E. coli*).

FIGs. 3A and 3B are plots demonstrating the results of a pharmacodynamic analysis of mouse Tregs in blood (**FIG. 3A**) and spleen (**FIG. 3B**) following administration of a single-dose of an RUR_{20kD}-IL-2 composition in mice as described in Example 2.

FIGs. 4A, 4B and 4C are plots showing levels of NK cells, CD4 T cells, and CD8 T cells, respectively, in blood, following administration of a single-dose of an RUR_{20kD}-IL-2 composition in mice as described in Example 2.

FIGs. 5A and 5B are plots of Treg function and activity as measured by the mean fluorescence intensity (MFI) of CD25 and Foxp3 following administration of a single-dose of an RUR_{20kD}-IL-2 composition in mice as described in Example 2.

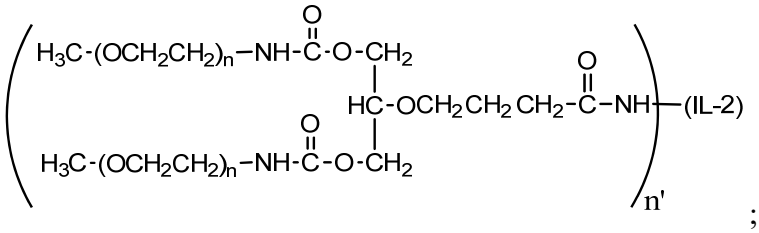
FIGs. 6A-D are plots of splenic Treg isolated from vehicle treated mice at 1 and 4 days in an *in vitro* Treg suppression assay as described in Example 3.

FIG. 7 is a plot demonstrating the relative suppressive capacity of isolated Treg cultured with Tcon (conventional T cells) at a ratio of 1:2 assessed over time as described in Example 3.

FIGs. 8A and 8B demonstrate the extent of ear swelling in mice treated with an RUR_{20kD}-IL-2 composition; the study was conducted to assess the ability of Treg induction by RUR_{20kD}-IL-2

WHAT IS CLAIMED:

1. A composition comprising PEGylated IL-2 conjugates of a formula:



;

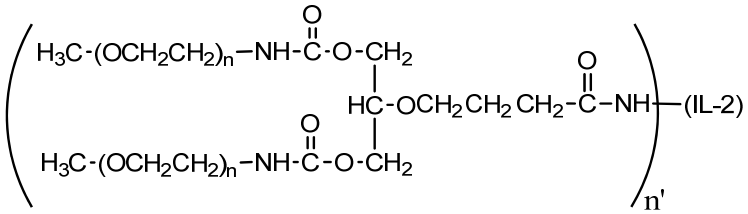
wherein:

IL-2 is an interleukin-2;

n is independently at each occurrence an integer from about 3 to about 4000; and

n' is 1 and 2 and 3.

2. The composition of claim 1 comprising PEGylated IL-2 conjugates of the formula:



;

wherein:

IL-2 is an interleukin-2;

n is independently at each occurrence an integer from about 3 to about 4000; and

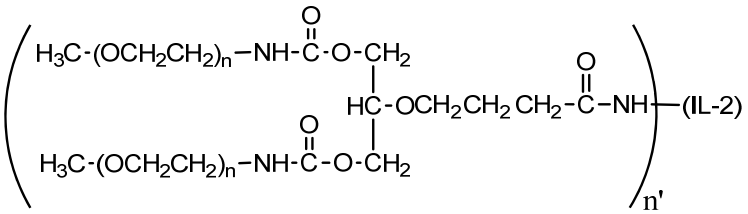
n' is 1 and 2 and 3;

wherein the PEGylated IL-2 conjugates comprise conjugates having n' equal to 1 in an amount of no more than 20 mole percent (within plus or minus 10%) and a combined n' equal to 2 and n' equal to 3 in an amount of at least 80 mole percent (within plus or minus 10%).

3. The composition of claim 2 comprising PEGylated IL-2 conjugates having n' equal to 2 and n' equal to 3 at a molar ratio ranging from 1.4:1 to 1:1.4 (within plus or minus 10%).

4. The composition of claim 2 comprising PEGylated IL-2 conjugates having n' equal to 2 and n' equal to 3 at a molar ratio chosen from 1.4: 1, 1.3:1, 1.2:1, 1.1:1, 1:1, 1:1.1, 1: 1.2, 1:1.3, and 1:1.4 (within plus or minus 10%).

5. The composition of claim 1 comprising PEGylated IL-2 conjugates of the formula:



;

wherein:

IL-2 is an interleukin-2;

n is independently at each occurrence an integer from about 3 to about 4000; and

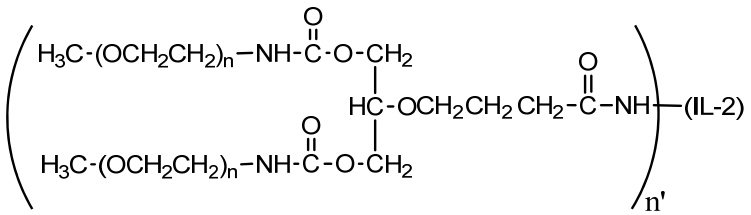
n' is 1 and 2 and 3;

wherein the PEGylated IL-2 conjugates having n' equal to 1 in an amount of no more than 10 mole percent (within plus or minus 10%) at a molar ratio ranging from 1.4:1 to 1:1.4 (within plus or minus 10%).

6. The composition of claim 5 comprising PEGylated IL-2 conjugates having n' = 2 and n' = 3 at a molar ratio chosen from 1.4:1, 1.3:1, 1.2:1, 1.1:1, 1:1, 1:1.1, 1: 1.2, 1:1.3, and 1:1.4 (within plus or minus 10%).

7. The composition of claim 1, wherein IL-2 is aldesleukin.

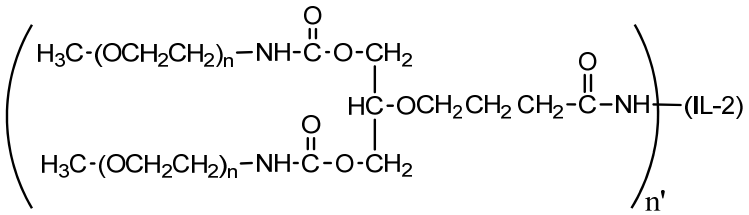
8. The composition of claim 2, wherein the composition comprises no more than about 20 mole percent of PEGylated IL-2 conjugates, when considered collectively, encompassed by the formula



,

wherein n' is selected from 1, 4, 5.

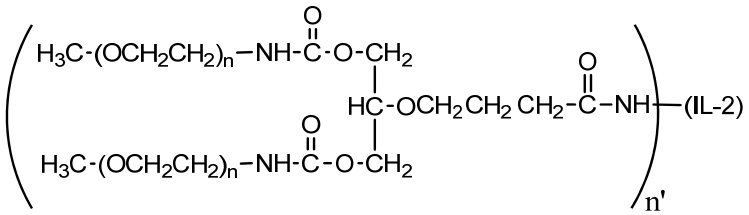
9. The composition of claim 3, wherein the composition comprises no more than about 15 mole percent of PEGylated IL-2 conjugates, when considered collectively, encompassed by the formula



,

wherein n' is selected from 1, 4, 5.

10. The composition of claim 3, wherein the composition comprises no more than about 10 mole percent of PEGylated IL-2 conjugates, when considered collectively, encompassed by the formula



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wherein n' is selected from 1, 4, 5.

11. The composition of any one of claims 1 to 10, comprising no more than about 10 mole percent of PEGylated IL-2 conjugates having n' equal to 1.

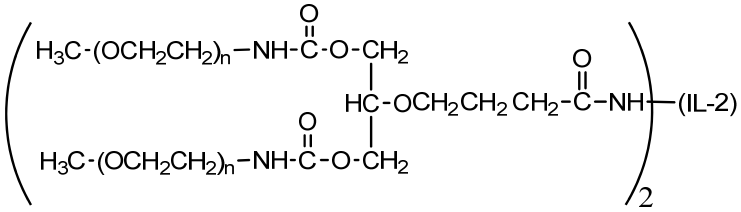
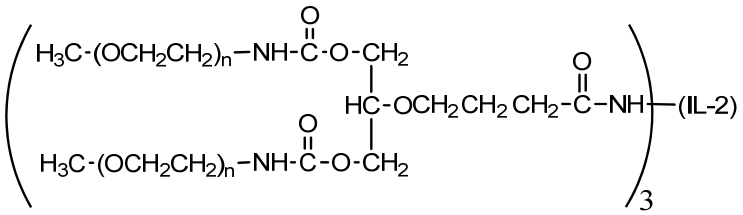
12. The composition of any one of claims 1 to 10, comprising no more than about 7 mole percent of PEGylated IL-2 conjugates having n' equal to 1.

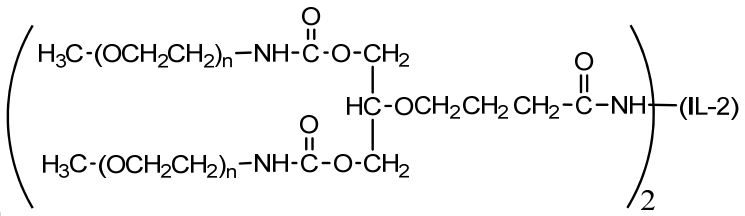
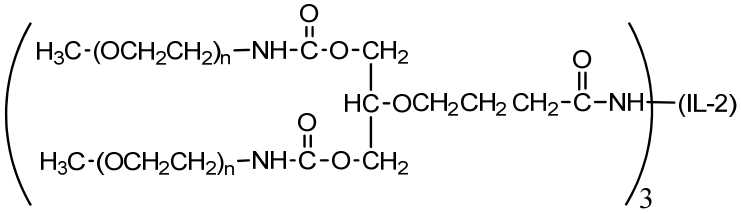
13. The composition of any one of claims 3-5, comprising no more than about 5 mole percent of PEGylated IL-2 conjugates having n' equal to 1.

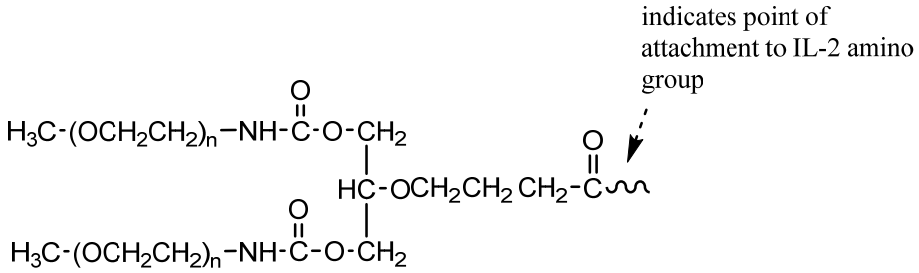
14. The composition of any one of claims 8 to 10, comprising no more than about 10 mole percent of PEGylated IL-2 conjugates having n' equal to 4.

15. The composition of any one of claims 8 to 10, comprising no more than about 7 mole percent of PEGylated IL-2 conjugates having n' equal to 4.

16. The composition of any one of claims 8 to 10, comprising no more than about 5 mole percent of PEGylated IL-2 conjugates having n' equal to 4.

17. The composition of claim 1, wherein the composition comprises approximately equimolar amounts of  (II), and  (III).

18. The composition of claim 7, wherein the composition comprises PEGylated IL-2 conjugates having a formula  (II); and  (III); wherein the molar ratio of (II)/(III) is selected from 1.4:1; 1.3:1; 1.2:1; 1.1:1; 1:1; 1:1.1; 1:1.2; 1:1.3; and 1:1.4.

19. The composition of claim 18, having an average number of branched polyethylene glycol moieties, 

, per aldesleukin is selected from the group consisting of 2; 2.1; 2.2; 2.3; 2.4; 2.5; 2.6; 2.7; 2.8; 2.9; or 3.

20. The composition of claim 19, wherein the average number of branched polyethylene glycol moieties per aldesleukin is about 2.5.

21. The composition of any one of claims 1 to 20, wherein the value of n in each occurrence ranges from 5-2000.

22. The composition of any one of claims 1 to 20, wherein the value of n in each occurrence ranges from 10-1000.

23. The composition of any one of claims 1 to 20, wherein the value of n in each occurrence ranges from 10-750.

24. The composition of any one of claims 1 to 20, wherein the value of n in each occurrence ranges from 10-500.

25. The composition of any one of claims 1 to 20, wherein the value of n in each occurrence ranges from 20-250.

26. The composition of any one of claims 1 to 20, wherein the average value of n in each occurrence is about 226.

27. The composition of any one of claims 1 to 20, wherein the nominal average molecular weight of each branched polyethylene glycol moiety is in a range of from about 250 daltons to about 90,000 daltons.

28. The composition of any one of claims 1 to 20, wherein the nominal average molecular weight of each branched polyethylene glycol moiety is in a range of from about 1000 daltons to about 60,000 daltons.

29. The composition of any one of claims 1 to 20, wherein the nominal average molecular weight of each branched polyethylene glycol moiety is in a range of from about 5,000 daltons to about 60,000 daltons.

30. The composition of any one of claims 1 to 20, wherein the nominal average molecular weight of each branched polyethylene glycol moiety is in a range of from about 10,000 daltons to about 55,000 daltons.

31. The composition of claim 1 or 7 comprising, on a molar basis, about 5 mole percent or less mono-PEGylated IL-2 conjugates, and from about 28 mole percent to about 60 mole percent di-PEGylated IL-2 conjugates, and from about 24 mole percent to about 65 mole percent tri-PEGylated IL-2 conjugates, and about 12 mole percent or less of higher PEGylated IL-2 conjugates, and wherein the nominal average molecular weight of each branched polyethylene glycol moiety is about 20,000 daltons.

32. The composition of claim 31 which further comprises, with respect to PEGylated IL-2 conjugates comprised in the composition, 80 mole percent or greater combined di- and tri-PEGylated IL-2 conjugates.

33. The composition of claim 1 or 7 comprising, on a molar basis, from about 2.5 to about 4.5 mole percent mono-PEGylated IL-2 conjugates, and from about 35 to about 50 mole percent di-PEGylated IL-2 conjugates, and from about 38 to about 46 mole percent tri-PEGylated IL-2 conjugates, and from about 3 to about 10 mole percent higher

PEGylated IL-2 conjugates, and wherein the nominal average molecular weight of each branched polyethylene glycol moiety is about 20,000 daltons.

34. The composition of claim 33 which further comprises a combined total of di-PEGylated and tri-PEGylated IL-2 conjugates from about 80 to about 95 mole percent.

35. The composition of claim 1 or 7 comprising, on a molar basis, from about 2.8 to about 3.8 mole percent mono-PEGylated IL-2 conjugates, and from about 44 to about 48 mole percent di-PEGylated IL-2 conjugates, and from about 41 to about 44 mole percent tri-PEGylated IL-2 conjugates, and from about 7 to about 9 mole percent higher PEGylated IL-2 conjugates, and wherein the nominal average molecular weight of each branched polyethylene glycol moiety is about 20,000 daltons.

36. The composition of claim 35 which further comprises a combined total of di-PEGylated and tri-PEGylated IL-2 conjugates from about 87 to about 90 mole percent.

37. The composition of claim 7 comprising, on a molar basis, from about 2.8 to about 3.8 mole percent mono-PEGylated IL-2 conjugates, and from about 44 to about 48 mole percent di-PEGylated IL-2 conjugates, and from about 41 to about 44 mole percent tri-PEGylated IL-2 conjugates, and from about 7 to about 9 mole percent higher PEGylated IL-2 conjugates, and wherein said composition comprises a mixture of mono-PEGylated IL-2 conjugates which have a PEG moiety attached at one of lysine K7 or K8 or K31 or K75, and wherein the nominal average molecular weight of each branched polyethylene glycol moiety is about 20,000 daltons.

38. The composition of claim 37 which further comprises a combined total of di-PEGylated and tri-PEGylated IL-2 conjugates from about 87 to about 90 mole percent.

39. The composition of claim 7 comprising, on a molar basis, from about 2.8 to about 3.8 mole percent mono-PEGylated IL-2 conjugates, and from about 44 to about 48 mole percent di-PEGylated IL-2 conjugates, and from about 41 to about 44 mole percent tri-

PEGylated IL-2 conjugates, and from about 7 to about 9 mole percent higher PEGylated IL-2 conjugates, and wherein said composition comprises mono-PEGylated IL-2 conjugates which have a PEG moiety attached at lysine K7, wherein the nominal average molecular weight of each branched polyethylene glycol moiety is about 20,000 daltons.

40. The composition of claim 39 which further comprises a combined total of di-PEGylated and tri-PEGylated IL-2 conjugates from about 87 to about 90 mole percent.

41. The composition of any one of claims 31 to 40, further comprising a pharmaceutically acceptable excipient.

42. The composition of any one of claims 31 to 40, in a form suitable for parenteral administration.

43. The composition of any one of claims 31 to 40, in a form suitable for subcutaneous administration.

44. The composition of claim 43, comprising an aqueous diluent.

45. The composition of claim 44, having a pH of about 5.

46. The composition of claim 45, further comprising sodium acetate, sodium chloride and sucrose.

47. The composition of claim 46, comprising 1.5 mg/ml IL-2 protein equivalent, 10 mM sodium acetate, 110 mM sodium chloride, 2% sucrose (w/v), pH 5.0.

48. Use of a composition according to any one of claims 1 to 47 in the manufacture of a medicament for treating autoimmune or inflammatory diseases in a subject, wherein

the treatment comprises increasing the ratio of regulatory T cells to effector T cells in the subject.

49. The use of claim 48, wherein the regulatory T cells are selected from Foxp3+ and CD25+ cells.

50. The use of claim 49, wherein the effector T cells are selected from CD4+ and CD8+ cells.

51. The use of any one of claims 49, wherein the fold-increase in regulatory T cells, when compared to baseline, reaches a value of at least about 2 when evaluated in an in-vivo mouse model.

52. The use of any one of claims 49, wherein the fold increase in regulatory T cells, when compared to baseline, reaches a value of at least about 4 when evaluated in an in-vivo mouse model.

53. The use of any one of claims 52, wherein the increase in regulatory T cell numbers is sustained above baseline levels for at least 3 days post-administration.

54. The use of claim 49, wherein the increase in regulatory T cell numbers is sustained above baseline levels for at least 5 days post-administration.

55. Use of a composition according to any one of the claims 1 to 47 in the manufacture of a medicament for treating a subject having an autoimmune disease.

56. The use of claim 55, wherein the medicament is to be administered by subcutaneous injection.

57. The use of any one of claims 48, 56 or 57, wherein the medicament is to be administered once every 2 weeks or once every 4 weeks.

58. The use of any one of claims 48, 56, 57 or 58, wherein the medicament is to be administered at a dose of between 3-24 μg/kg, on a protein basis which only counts the mass contributed by the protein component and not that contributed by the PEG moieties, once every two weeks.

59. A composition according to any one of claims 1 to 47 for use in therapy.

60. A composition according to any one of claims 1 to 47 for use in the treatment of an autoimmune disease.