We claim:

1. A double-stranded ribonucleic acid (dsRNA) for inhibiting expression of ALAS1, wherein ~~said~~ the dsRNA comprises a sense strand and an antisense strand, wherein the antisense strand comprise~~s~~ing a region of complementarity to an ALAS1 RNA transcript ~~(e.g., SEQ ID NO:1)~~, ~~which~~ wherein the antisense strand comprises at least ~~20~~15 contiguous nucleotides from the antisense sequence GAAGAUGAGACACUCUUUCUGGU (SEQ ID NO: 3579)~~of UAAGAUGAGACACUCUUUCUGGU (SEQ ID NO: 4153) or UAAGAUGAGACACUCTUUCUGGU (SEQ ID NO: 4154)~~.

~~2. A double-stranded ribonucleic acid (dsRNA) for inhibiting expression of ALAS1, wherein said dsRNA comprises a sense strand and an antisense strand, the antisense strand comprising a region of complementarity to an ALAS1 RNA transcript (e.g., SEQ ID NO:1), which antisense strand comprises at least 20 contiguous nucleotides from (i) an antisense sequence listed in any one of Tables 21 to 40, or (ii) an unmodified version of an antisense sequence listed in any one of Tables 21 to 40 (SEQ ID NOs: 4172 to 5237).~~

~~3~~2. The dsRNA of claim ~~2~~1, wherein said dsRNA comprises at least one modified nucleotide.

3. The dsRNA of claim 2, wherein the modified nucleotide is selected from the group consisting of: a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, a terminal nucleotide linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group, a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide.

4. The dsRNA of any one of the preceding claims, wherein the dsRNA has one or more of the following:

(a) comprises a duplex region of 15 to 30 base pairs in length;

(b) at least one strand comprises a 3' overhang of at least 2 nucleotides; and/or

(c) each strand is no more than 26 nucleotides in length.

5. The dsRNA of claim 4, wherein the duplex region is 21-23 nucleotide pairs or 21 nucleotide pairs in length.

6. The dsRNA of any one the preceding claims, wherein the antisense strand comprises the sequence of GAAGAUGAGACACUCUUUCUGGU (SEQ ID NO: 3579).

7. The dsRNA of any one the preceding claims, wherein the antisense strand consists of the sequence of GAAGAUGAGACACUCUUUCUGGU (SEQ ID NO: 3579).

8. The dsRNA of any one the preceding claims, wherein the sense strand comprises the sequence of CAGAAAGAGUGUCUCAUCUUC (SEQ ID NO: 3578).

9. The dsRNA of any one the preceding claims, wherein the sense strand consists of the sequence of CAGAAAGAGUGUCUCAUCUUC (SEQ ID NO: 3578).

~~4. The dsRNA of any one of the preceding claims, wherein the duplex region is 17-23 nucleotide pairs in length.~~

~~5. The dsRNA of any one of the preceding claims, wherein at least one strand comprises a 3' overhang of at least 2 nucleotides.~~

~~6. The dsRNA of any one of the preceding claims, wherein each strand is no more than 26 nucleotides in length.~~

~~7. The dsRNA of claim 3, wherein at least one modified nucleotide is chosen from a 2′-O-methyl, a 2′ fluoro modified nucleotide, and optionally one or more 5' phosphorothioate groups, or any combination thereof.~~

810. The dsRNA of any one of the preceding claims, further comprising a ligand.

11. The dsRNA of claim 10, wherein the ligand has one or more of the following:, optionally wherein the ligand

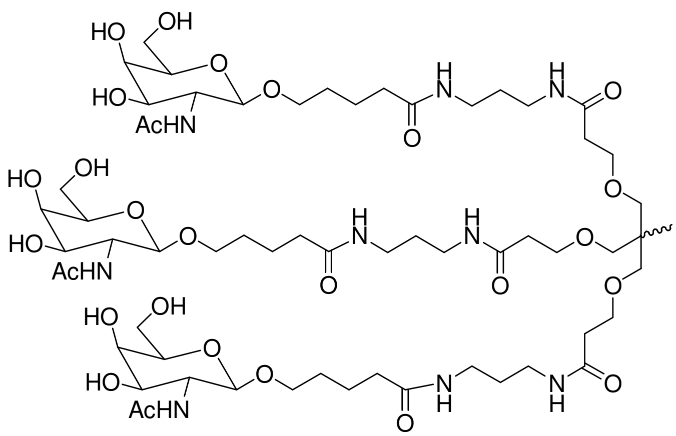
(a) is conjugated to the 3’ end of the sense strand of the dsRNA;.

9. The dsRNA of claim 8, wherein the ligand(b) comprises a carbohydrate,; and/or

(c) optionally targets the dsRNA to hepatocytes.

12. The dsRNA of claim 10, wherein the ligand is a GalNAc ligand.

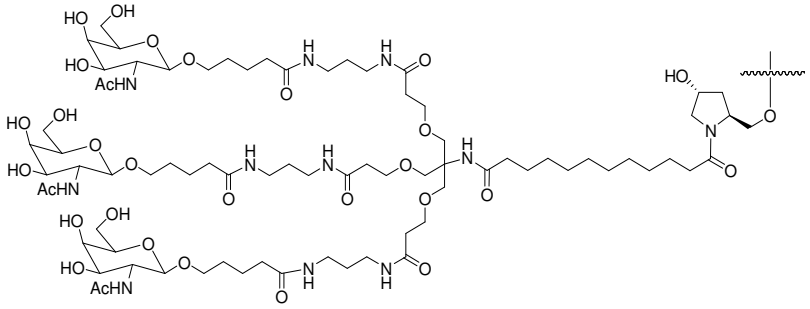
1013. The dsRNA of claim 912, wherein the ligand is



.

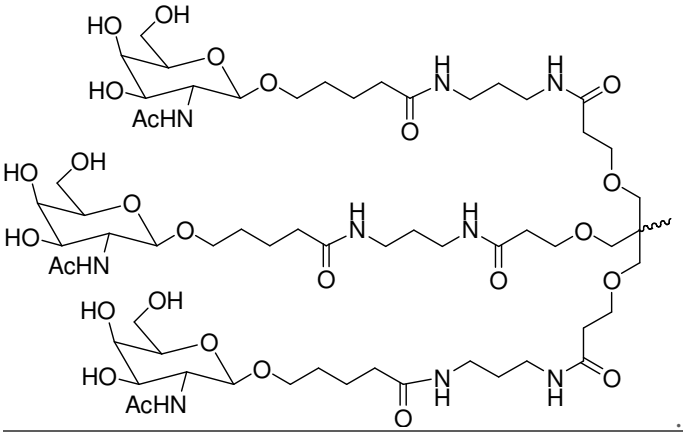
1114. The dsRNA of any one of claims 8-10, wherein the ligand is attached via a bivalent or trivalent branched linker.

~~12~~15. The dsRNA of claim ~~11~~14, wherein the ligand and the linker are as shown in Formula XXIV:



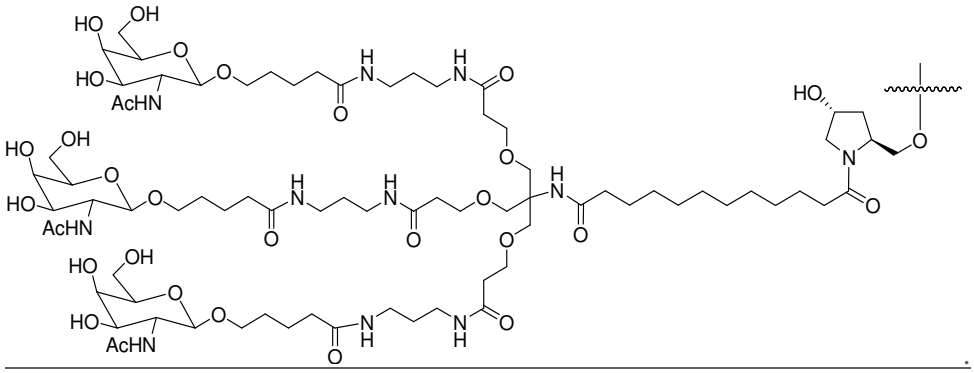
16. A double-stranded ribonucleic acid (dsRNA) for inhibiting expression of ALAS1, wherein the dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises a region of complementarity to an ALAS1 RNA transcript, wherein the antisense strand comprises the nucleotide sequence of GAAGAUGAGACACUCUUUCUGGU (SEQ ID NO: 3579) and the sense strand comprises the nucleotide sequence of CAGAAAGAGUGUCUCAUCUUC (SEQ ID NO: 3578), wherein all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand are modified nucloetides selected from a 2'-O-methyl modified nucleotide and a 2'-O-fluoro modified nucleotide and wherein the 3'-end of the sense strand is conjugated to a GalNAc ligand.

17. The dsRNA of claim 16, wherein the ligand is



18. The dsRNA of claim 17, wherein the ligand is attached via a bivalent or trivalent branched linker.

19. The dsRNA of claim 18, wherein the ligand and the linker are as shown in Formula XXIV:



.

20. The dsRNA of claim 1, wherein:

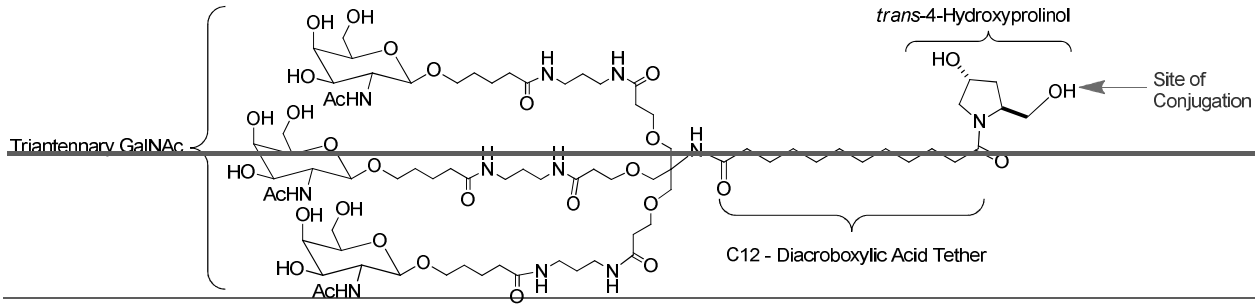
(i) the antisense strand comprises the sequence of usAfsaGfaUfgAfgAfcacUfcUfuUfcUfgsgsu (SEQ ID NO: 4157), and/or the sense strand comprises the sequence of CfsasGfaAfaGfaGfUfGfuCfuCfaUfcUfuAf (SEQ ID NO: 4156);

(ii) the antisense strand consists of the sequence of usAfsaGfaUfgAfgAfcacUfcUfuUfcUfgsgsu (SEQ ID NO: 4157), and/or the sense strand consists of the sequence of CfsasGfaAfaGfaGfUfGfuCfuCfaUfcUfuAf (SEQ ID NO: 4156);

(iii) the antisense strand comprises the sequence of usAfsaGfaUfgAfgAfcacUfcdTuUfcUfgsgsu (SEQ ID NO: 4165), and/or the sense strand comprises the sequence of csasgaaaGfaGfuGfuCfuCfaucuuaL96 (SEQ ID NO: 4164); or

(ii) the antisense strand consists of the sequence of usAfsaGfaUfgAfgAfcacUfcdTuUfcUfgsgsu (SEQ ID NO: 4165), and/or the sense strand consists of the sequence of csasgaaaGfaGfuGfuCfuCfaucuuaL96 (SEQ ID NO: 4164).

~~13. The dsRNA of claim 8, wherein the dsRNA is conjugated to ligand L96 via a linker as shown below~~



~~14. The dsRNA of any one of claims 8 to 13, wherein the ligand targets the dsRNA to hepatocytes.~~

~~15. The dsRNA of any one of the preceding claims, wherein the dsRNA comprises a sense strand consisting of a sense sequence selected from the sense sequences listed in Tables 21 to 40, and an antisense strand consisting of an antisense sequence selected from the antisense sequences listed in Tables 21 to 40.~~

~~16. The dsRNA of any one of the preceding claims, wherein the dsRNA has an IC_{50} of less than 1 nM, less than 0.05 nM, less than 0.02 nM, or less than 0.01 nM.~~

~~17. The dsRNA of any one of the preceding claims, wherein the dsRNA has a single dose ED50 of less than about 10 mg/kg or less than about 5 mg/kg.~~

18. The dsRNA of any one of the preceding claims, wherein the dsRNA shows improved activity compared with AD-58632 or AD-60489, optionally wherein the dsRNA is selected from the dsRNAs listed in Tables 21 to 40.

19. The dsRNA of any one of the preceding claims, wherein the sense strand comprises or consists of the sequence of CAGAAAGAGUGUCUCAUCUUA (SEQ ID NO: 4155).

20. The dsRNA of claim 1 or 2, wherein:

(i) the antisense strand comprises the antisense sequence of AD-60519, wherein the antisense sequence comprises all of the modified nucleotides of AD-60519;

(ii) the antisense strand consists of the antisense sequence of AD-60519, wherein the antisense sequence comprises all of the modified nucleotides of AD-60519;

(iii) the sense strand comprises the sense sequence of AD-60519, wherein the sense sequence comprises all of the modified nucleotides of AD-60519;

(iv) the sense strand consists of the sense sequence of AD-60519, wherein the sense sequence comprises all of the modified nucleotides of AD-60519;

(v) the sense strand comprises the sense sequence of AD-60519, and the antisense strand comprises the antisense sequence of AD-60519, wherein the sense and antisense sequences comprise all of the modified nucleotides of AD-60519, or

(vi) the sense strand consists of the sense sequence of AD-60519, and the antisense strand consists of the antisense sequence of AD-60519, wherein the sense and antisense sequences comprise all of the modified nucleotides of AD-60519.

~~21. The dsRNA of claim 1 or 2, wherein:~~

~~(i) the antisense strand comprises the antisense sequence of AD-60489, and/or the sense strand comprises the sense sequence of AD-60489, wherein the antisense and/or sense sequence comprises all of the modified nucleotides of AD-60489, or~~

~~(ii) the antisense strand consists of the antisense sequence of AD-60489, and/or the sense strand consists of the sense sequence of AD-60489, wherein the antisense and/or sense sequence comprises all of the modified nucleotides of AD-60489.~~

~~22. The dsRNA of claim 1 or 2, wherein:~~

~~(i) the antisense strand comprises the antisense sequence of AD-61193, and/or the sense strand comprises the sense sequence of AD-61193, wherein the antisense and/or sense sequence comprises all of the modified nucleotides of AD-61193, or~~

~~(ii) the antisense strand consists of the antisense sequence of AD-61193, and/or the sense strand consists of the sense sequence of AD-61193, wherein the antisense and/or sense sequence comprises all of the modified nucleotides of AD-61193.~~

~~23. The dsRNA of claim 2, wherein:~~

~~(i) the antisense strand comprises the antisense sequence of AD-60819, and/or the sense strand comprises the sense sequence of AD-60819, wherein the antisense and/or sense sequence comprises all of the modified nucleotides of AD-60819, or~~

~~(ii) the antisense strand consists of the antisense sequence of AD-60819, and/or the sense strand consists of the sense sequence of AD-60819, wherein the antisense and/or sense sequence comprises all of the modified nucleotides of AD-60819.~~

~~24. A vector encoding at least one strand of a dsRNA of any one of claims 1 to 23.~~

~~25~~21. A cell comprising the dsRNA of any one of claims 1 to ~~23~~20 ~~or the vector of claim 24~~.

~~26~~22. A pharmaceutical composition ~~for inhibiting expression of an ALAS1 gene, the composition~~ comprising the dsRNA of any one of claims 1 to ~~23~~20.

~~27~~23. The pharmaceutical composition of claim ~~26~~22, wherein the composition comprises ~~dsRNA is administered in an unbuffered saline or~~ water ~~solution~~ for injection.

~~28~~24. The pharmaceutical composition of claim ~~26~~22 or ~~27~~23, wherein ~~said~~ the composition is suitable for subcutaneous administration.

~~29. A method of inhibiting ALAS1 expression in a cell, the method comprising:~~

~~(a) introducing into the cell the dsRNA of any one of claims 1 to 23, and~~

~~(b) maintaining the cell of step (a) for a time sufficient to obtain degradation of the mRNA transcript of an ALAS1 gene, thereby inhibiting expression of the ALAS1 gene in the cell, optionally wherein the expression of ALAS1 is inhibited by at least 20% or at least 30%.~~

~~30. A method for decreasing a level of a porphyrin or a porphyrin precursor (e.g., ALA or PBG) in a cell (e.g., a hepatocyte), comprising contacting the cell with the dsRNA of any one of claims 1 to 23, in an amount effective to decrease the level of the porphyrin or the porphyrin precursor in the cell.~~

~~31~~25. Use of the dsRNA of any one of claims 1 to 20, or the composition of any one of claims 22 to 24, in the manufacture of a medicament ~~A method of~~ treating a porphyria in~~, the method comprising administering to~~ a subject~~. in need of such treatment a therapeutically effective amount of~~

~~(i) the dsRNA of any one of claims 1 to 23 or~~

~~(ii) the composition of any one of claims 26 to 28,~~
~~thereby treating the porphyria.~~

~~32. The method of claim 31, wherein the subject is at risk for developing, or is diagnosed with, a porphyria.~~

~~33~~26. The ~~method~~ use of claim ~~31 or 32~~25, wherein the porphyria is hepatic porphyria or an acute intermittent porphyria (AIP) ~~or ALA-dehydratase deficiency porphyria~~.

27. The use of claim 26, wherein the porphyria is acute hepatic porphyria.

28. The use of any one of claims 25 to 27, wherein the use has one or more of the following:
(a) the subject is at risk for developing, or is diagnosed with, a porphyria;
(b) the porphyria is acute intermittent porphyria or ALA-dehydratase deficiency porphyria;
(c) (i) the dsRNA or composition comprising dsRNA is to be administered after an acute attack of porphyria; (ii) the dsRNA or composition is to be administered during an acute attack of porphyria, or (iii) the dsRNA or composition is to be administered prophylactically to prevent an acute attack of porphyria;
(d) the dsRNA is to be administered at a dose of 0.05 to 50 mg/kg bodyweight of the subject, or at a dose of 0.01 mg/kg to 5 mg/kg bodyweight of the subject;
(e) the use (i) decreases a level of a porphyrin or a porphyrin precursor in the subject, or wherein the level is decreased by at least 30%; or wherein the level is decreased by at least 40%, and/or (ii) inhibits ALAS1 expression in the subject;
(I) said use (i) ameliorates a symptom associated with an ALAS1 related disorder, (ii) decreases frequency of acute attacks of symptoms associated with a porphyria in the subject, and/or (v) decreases incidence of acute attacks of symptoms associated with a porphyria in the subject when the subject is exposed to a precipitating factor;
(g) the dsRNA or composition comprising the dsRNA is to be administered according to a dosing regimen; or wherein the dsRNA is to be administered monthly at a dose of 2.5 mg/kg bodyweight of the subject;

(h) the dsRNA is to be administered before an acute attack of porphyria;

(i) the subject has an elevated level of ALA and/or PBG or wherein the subject suffers from chronic pain;

(j) the use decreases the elevated level of ALA and/or PBG;

(k) the use decreases or prevents pain, neuropathy, and/or nerve damage;

(l) the method prevents acute attacks of porphyria; or

(m) the dsRNA or composition comprising the dsRNA is to be administered repeatedly.

29. A dsRNA of any one of claims 1 to 20, or a pharmaceutical composition of any one of claims 22 to 24 for use in treating a disease or disorder.

~~34. The method of any one of claims 31 to 33, wherein (i) the dsRNA or composition comprising dsRNA is administered after an acute attack of porphyria, (ii) the dsRNA or composition comprising dsRNA is administered during an acute attack of porphyria, or (iii) the dsRNA or composition comprising dsRNA is administered prophylactically to prevent an acute attack of porphyria.~~

~~35. The method of any one of claims 31 to 34, wherein the dsRNA is administered at a dose of 0.05 to 50 mg/kg bodyweight of the subject, e.g, at a dose of 0.01 mg/kg to 5 mg/kg bodyweight of the subject.~~

~~36. The method of any one of claims 31 to 35, wherein the method~~

~~(i) decreases a level of a porphyrin or a porphyrin precursor (e.g., δ-aminolevulinic acid (ALA) or porphopilinogen (PBG)) in the subject, optionally wherein the level is decreased by at least 30% and/or~~

~~(ii) inhibits ALAS1 expression in the subject.~~

~~37. The method of any one of claims 31 to 36, wherein said method (i) ameliorates a symptom associated with an ALAS1 related disorder (e.g., a porphyria), (ii) decreases frequency of acute attacks of symptoms associated with a porphyria in the subject, and/or (iii) decreases incidence~~

of acute attacks of symptoms associated with a porphyria in the subject when the subject is exposed to a precipitating factor, e.g., the premenstrual phase.

38. The method of claim any one of claims 31 to 37, wherein the dsRNA or composition comprising the dsRNA is administered according to a dosing regimen, e.g., weekly, biweekly, or monthly.

39. The method of any one of claims 31 to 38, wherein the dsRNA is administered before an acute attack of porphyria, e.g., during a prodrome.

40. The method of claim any one of claims 31 to 39, wherein the subject has an elevated level (e.g., plasma or urine level) of ALA and/or PBG and optionally wherein the subject suffers from chronic pain.

41. The method of any one of claims 31 to 40, wherein the method decreases the elevated level of ALA and/or PBG.

42. The method of any one of claims 31 to 41, wherein the method decreases or prevents pain, neuropathy, and/or nerve damage.

43. The method of any one of claims 31 to 42, wherein the method prevents acute attacks of porphyria.

44. The method of any one of claims 31 to 43, wherein the dsRNA or composition comprising the dsRNA is administered repeatedly.

45. A method of treating a subject with an elevated level of ALA and/or PBG, the method comprising administering to a subject in need of such treatment a therapeutically effective amount of

(i) the dsRNA of any one of claims 1 to 23 or

(ii) the composition of any one of claims 26 to 28.

optionally, wherein the method is effective to decrease the level of ALA and/or PBG.

46. A method of treating a subject having an increased level of ALA and/or PBG, the method comprising administering the dsRNA of any one of claims 1 to 23 at 1 mg/kg, 2.5 mg/kg, or 5 mg/kg once per week for at least ten weeks, thereby reducing the level of ALA and/or PBG in said subject.

47. A method of treating a human patient with AIP who has suffered from multiple recurrent attacks, the method comprising administering the dsRNA of any one of claims 1 to 23 at a dose of 2.5 mg/kg for at least 6 months, thereby treating said patient,

optionally wherein said method

(i) reduces the frequency of attacks,

(ii) reduces hematin use,

(iii) reduces hospitalization, and/or

(iv) improves quality of life.

48. A method for assaying the level of circulating extracellular ALAS1 mRNA in a subject, said method comprising:

detecting (*e.g.*, measuring) the level of ALAS1 mRNA in a biological fluid sample (e.g., a blood sample, a plasma sample, a serum sample, or a urine sample) from the subject, said biological fluid sample comprising the ALAS1 mRNA,

thereby assaying the level of circulating extracellular ALAS1 mRNA in the subject.

49. A method for assaying the level of circulating extracellular ALAS1 mRNA in a subject, said method comprising

(i) providing RNA (*e.g.*, extracellular RNA) from a biological fluid sample (e.g., a blood sample, a plasma sample, a serum sample, or urine sample) from the subject, said biological fluid sample comprising the ALAS1 mRNA;

(ii) obtaining an ALAS1 cDNA from the ALAS1 mRNA;

(iii) contacting the ALAS1 cDNA with a nucleic acid complementary (e.g., probe and/or primer) to the ALAS1 cDNA or a portion thereof, thereby producing a reaction mix; and

(iv) detecting (e.g., measuring) the level of ALAS1 cDNA in the reaction mix, wherein the ALAS1 cDNA level is indicative of the ALAS1 mRNA level,

thereby assaying the level of circulating extracellular ALAS1 mRNA in the subject, optionally wherein

(a) the method comprises PCR, qPCR or 5'-RACE,

(b) the nucleic acid is a probe or primer, and/or

(c) the nucleic acid comprises a detectable moiety and the level of ALAS1 mRNA is determined by detection of the amount of the detectable moiety.

50. The method of claim 49, wherein the efficacy of a porphyria treatment is assessed based on a comparison of the level of circulating extracellular ALAS1 mRNA in the subject with a reference value.

51. The method of claim 49, wherein a decrease in the level of of circulating extracellular ALAS1 mRNA in the subject in response to the porphyria treatment, relative to the reference value, indicates that the porphyria treatment is efficacious.

52. The method of any one of claims 49 to 51, further comprising acquiring the biological fluid sample from a subject, optionally wherein the biological fluid sample is separate from the tissue, and wherein the biological fluid sample contains exosomes.

53. A composition comprising the dsRNA of claim 20 and water for injection.

54. The composition of claim 53, comprising about 200 mg/mL of the dsRNA of claim 20.

55. The composition of claim 53 or 54, wherein the composition has a pH of 6.0-7.5, e.g., about 7.0.

~~56. The composition of any one of claims 53 to 55, wherein the composition is formulated for subcutaneous injection.~~

~~57. A method of treating a subject having a porphyria (e.g., AIP) or an elevated level of ALA and/or PBG, the method comprising subcutaneously administering the composition of any one of claims 53 to 56 to the subject.~~

~~58. The method of claim 57, wherein the composition is administered at a dose of 0 to 5 mg/kg, e.g., at a dose of 2.5 mg/kg or less or at a dose of 1 to 2.5 mg/kg.~~

~~59. The method of claim 58, wherein the composition is administered weekly.~~