

CLAIMS

We claim:

1. A method of monitoring gene therapy, the method comprising a step of:
detecting, in a biological sample from a subject who has received integrating gene therapy treatment, a level or activity of a biomarker generated by integration of the integrating gene therapy treatment, as a surrogate for one or more characteristics of the status of the gene therapy treatment, wherein the one or more characteristics of the status of the gene therapy treatment is selected from the group consisting of level of a payload, activity of a payload, level of integration of the gene therapy treatment in a population of cells, and combinations thereof.
2. A method of monitoring delivery, level and/or activity of a payload in a subject who has received a gene-integrating composition that delivers the payload, the method comprising a step of:
detecting, in a biological sample from the subject, level or activity of a biomarker generated by integration of the gene-integrating composition, as a surrogate for delivery, level and/or activity of the payload.
3. The method of claim 1 or claim 2, wherein the payload is or comprises a peptide expressed intracellularly.
4. The method of claim 1 or claim 2, wherein the payload is or comprises a peptide that is secreted extracellularly.
5. The method of any one of claims 1-4, wherein the payload is or comprises a peptide that has cell-intrinsic or cell-extrinsic activity that promotes a biological process to treat a medical condition.
6. The method of any one of claims 1-5, wherein the payload is or comprises a peptide that is normally expressed in liver cells.

7. The method of any one of claims 1-5, wherein the payload is or comprises a peptide that is ectopically expressed in liver cells.
8. The method of any one of claims 1-5, wherein the payload is or comprises methylmalonyl-CoA mutase or human Factor IX.
9. The method of any one of the above claims, wherein the integrating gene therapy treatment or gene-integrating composition achieves integration of a nucleic acid element comprising a sequence that encodes the payload into a target site in the genome of the subject.
10. The method of claim 9, wherein the target site encodes a polypeptide.
11. The method of claim 10, wherein integration of the nucleic acid element occurs at the 5' or 3' end of a gene that encodes the polypeptide.
12. The method of claim 9, wherein the target site encodes albumin.
13. The method of any one of claims 9-11, wherein integration of the nucleic acid element does not significantly disrupt expression of the polypeptide encoded at the target site.
14. The method of any one of the above claims wherein the biological sample is or comprises hair, skin, feces, blood, plasma, serum, cerebrospinal fluid, urine, saliva, tears, vitreous humor, or mucus.
15. The method of any one of the above claims wherein the step of detecting comprises an immunological assay or a nucleic acid amplification assay.
16. The method of any one of the above claims wherein the biomarker comprises a detectable moiety that, after translation of the polypeptide encoded by the target site, becomes fused to the polypeptide encoded by the target site.

17. The method of any one of the above claims wherein the biomarker comprises a detectable moiety that, after translation of the polypeptide encoded by the target site, becomes fused to the polypeptide encoded by the payload.
18. The method of any one of the above claims wherein the biomarker comprises a detectable moiety that is a 2A peptide or a Furin cleavage motif.
19. The method of claim 18, wherein the 2A peptide is selected from the group consisting of P2A, T2A, E2A and F2A.
20. The method of any one of the above claims wherein the subject receives a single dose of the gene therapy treatment or gene-integrating composition.
21. The method of any one of the above claims wherein the detecting step is performed 1, 2, 3, 4, 5, 6, 7, 8 or more weeks after the subject has received the gene therapy treatment or gene-integrating composition.
22. The method of any one of the above claims wherein the detecting step is performed at multiple time points after the subject has received the gene therapy treatment or gene-integrating composition.
23. The method of any one of the above claims wherein the detecting step is performed over a period of at least 3 months after the subject has received the gene therapy treatment or gene-integrating composition.
24. The method of any one of the above claims wherein the subject receives the gene therapy treatment or gene-integrating composition as an infant.
25. The method of any one of claims 1-23, wherein the subject receives the gene therapy treatment or gene-integrating composition before reaching adulthood.

26. The method of any one of claims 1-23 wherein the subject receives the gene therapy treatment or gene-integrating composition as an adult.

27. The method of any one of the above claims wherein the method further comprises monitoring the subject for autoimmune response to the gene therapy.

28. A method of determining one or more characteristics of the status of gene therapy treatment in a subject who has received an integrating gene therapy treatment, said method comprising:

- a) providing a biological sample from the subject;
- b) determining a level of a biomarker, wherein the biomarker is generated by integration of the gene therapy in the genome of the subject; and
- c) based on the determined level of the biomarker, establishing one or more characteristics of the status of gene therapy treatment in the subject, wherein the determined level of the biomarker corresponds one or more characteristics of the status of gene therapy treatment.

29. A method of delivering a gene therapy treatment to a subject in need thereof, comprising the steps of:

- a. administering an integrating gene therapy treatment to the subject; and
- b. determining in a biological sample from the subject a level of a biomarker that is generated by integration of the gene therapy treatment in the genome of the subject.

30. The method of claim 29, further comprising

administering an additional treatment to the subject if the level of the biomarker is lower than would indicate a therapeutically effective amount of the integrating gene therapy has been achieved.

31. The method of any one of claims 28-30, wherein the integrating gene therapy treatment achieves integration of a nucleic acid element comprising a sequence that encodes a payload into a target site in the genome of the subject.

32. The method of claim 31, wherein the target site encodes a polypeptide.
33. The method of claim 32, wherein integration of the nucleic acid element occurs at the 5' or 3' end of a gene that encodes the polypeptide.
34. The method of claim 31, wherein the target site encodes albumin.
35. The method of any one of claims 31-33, wherein integration of the nucleic acid element does not significantly disrupt expression of the polypeptide encoded at the target site.
36. The method of any one of claims 28-35 wherein the biological sample is or comprises hair, skin, feces, blood, plasma, serum, cerebrospinal fluid, urine, saliva, tears, vitreous humor, or mucus.
37. The method of any one of claims 28-36, wherein the step of determining comprises an immunological assay or a nucleic acid amplification assay.
38. The method of any one of claims 28-37, wherein the biomarker comprises a detectable moiety that, after translation of the polypeptide encoded by the target site, becomes fused to the polypeptide encoded by the target site.
39. The method of any one of claims 28-38, wherein the biomarker comprises a detectable moiety that, after translation of the polypeptide encoded by the target site, becomes fused to the polypeptide encoded by the payload.
40. The method of any one of claims 28-39, wherein the biomarker comprises a detectable moiety that is a 2A peptide.
41. The method of claim 40, wherein the 2A peptide is selected from the group consisting of P2A, T2A, E2A and F2A.

42. The method of any one of claims 28-41, wherein the subject receives a single dose of the gene therapy treatment.
43. The method of any one of claims 28-42, wherein the determining step is performed 1, 2, 3, 4, 5, 6, 7, 8 or more weeks after the subject has received the gene therapy treatment.
44. The method of any one of claims 28-43, wherein the determining step is performed at multiple time points after the subject has received the gene therapy treatment.
45. The method of any one of claims 28-44, wherein the determining step is performed over a period of at least 3 months after the subject has received the gene therapy treatment.
46. The method of any one of claims 28-45, wherein the subject receives the gene therapy treatment as an infant.
47. The method of any one of claims 28-45, wherein the subject receives the gene therapy treatment before reaching adulthood.
48. The method of any one of claims 28-45, wherein the subject receives the gene therapy treatment as an adult.
49. The method of any one of claims 28-48, wherein the method further comprises monitoring the subject for autoimmune response to the gene therapy.
50. The method of any one of claims 1-49, wherein the method further comprises delivering an additional treatment to the subject that reduces or inhibits expression of a payload delivered by the gene therapy treatment if the level of the biomarker exceeds a level that is indicative of an optimal or safe level of the payload.