SUMMARY

Thus, in accordance with the present disclosure, there is described a method of inhibiting immune cell activation comprising administering to a mammalian subject in need thereof a viral RNA segment comprising a T cell immune-inhibitory domain. The viral RNA segment may comprise about 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 75, 100, 150, 175, 200, 225, 250, 300, 400 or 500 consecutive bases of the T cell immune-inhibitory domain. The viral RNA segment may encode HCV E2 sequences, GBV-C E2 sequences, YFV envelope protein or HIV gp41 or gp120/160 sequences. The viral RNA segment may further encode non-HCV E2 sequences, non-GBV-C E2 sequences, non-YFV env sequences or non-HIV gp41 sequences. The T cell may be a helper T cell suppressor T cell, or a killer T cell. The subject may be a human or a non-human mammal. The immune cell activation may in particular be T cell activation or T cell receptor signaling.

Administering may comprise intravenous, intra-arterial, oral, subcutaneous, topical or intraperitoneal administration. The method may further comprise administering a second anti-inflammatory agent, such as a steroid or a COX-2 inhibitor. The second anti-inflammatory agent may be contacted prior to or after said viral RNA segment, or at the same time as said viral RNA segment. The viral RNA segment may be provided in combination with gene therapy. The viral RNA segment may comprise at least one non-natural base. The viral RNA segment may comprise a Dicer substrate.

The viral RNA segment may be administered at 0.1-500 mg/kg/d. The viral RNA segment may be administered daily or weekly, such as daily for 7 days, 2 weeks, 3 weeks, 4 weeks, one month, 6 weeks, 8 weeks, two months, 12 weeks, or 3 months, or weekly for 2 weeks, 3 weeks, 4 weeks, 6 weeks, 8 weeks, 10 weeks, or 12 weeks. The viral RNA segment may be derived from Human Immunodeficiency Virus envelope gp41 or gp120/160, Yellow Fever Virus envelope protein, Bovine Viral Diarrhea Virus envelope protein, Classical Swine Fever Virus envelope protein, influenza envelope protein, Dengue Virus envelope protein, West Nile Virus envelope protein, and Japanese Encephalitis Virus envelope protein.

In another example, there is disclosed a composition comprising a viral RNA segment comprising a T cell immune-inhibitory domain, formulated with a pharmaceutically acceptable carrier buffer or diluent. The viral RNA segment may comprise about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 30, 35, 40, 45, 50, 51, 75, 100 consecutive bases of the native viral genome from which it is derived. The viral RNA segment may be about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 30, 35,

40, 45, 50, 51, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 400 or 500 bases in length. The viral RNA segment may be fused to a non-viral sequence. The composition may be formulated for pharmaceutical administration, such as topical, cutaneous, subcutaneous, alimentrary or parenteral administration.

In yet another example, there is disclosed a method of inducing an immune response in a mammalian subject comprising administering to said subject a viral RNA segment wherein said viral RNA segment comprises one or more modified T cell immune-inhibitory domains. The modified site may comprise Dicer substrate. The RNA virus may be from the *Reoviridae*, *Atroviridae*, *Caliciviridae*, *Hepeviridae*, *Picornaviridae*, *Togaviridae*, *Flaviviridae*, *Coronaviridae*, *Orthomyxoviridae*, *Arenaviridae*, *Bunyaviridae*, *Paramyxoviridae*, *Filoviridae*, *Rabdoviridae*, or *Retroviridae* family. The RNA virus may be GBV-C, Hepatitis C Virus, Human Immunodeficiency Virus, influenza virus, Dengue Virus, West Nile Virus, Japanese Encephalitis Virus, Bovine Viral Diarrhea Virus, Classical Swine Fever Virus or Yellow Fever Virus. The viral RNA segment may be free from other viral sequences.

The viral RNA segment may be delivered via expression from an expression vector, such as a viral expression vector. The viral RNA segment may be comprised in lipid vehicle or nanoparticle. The viral RNA segment may be administered with a second viral RNA segment from a distinct serotype or strain of said virus. The viral RNA segment may be administered more than once. The viral RNA segment may be formulated with an adjuvant. The viral RNA segment may comprise a modification to an immunomodulatory domain in a viral glycoprotein. The viral RNA segment may be from a HCV E2-coding region, and HIV gp41- or gp120/160-coding region or a GBV-C E2-coding region. The site comprises a dicer substrate. The subject may be a human subject or a non-human animal subject.

Also disclosed is a vaccine comprising a viral RNA segment having a modification in a T cell immune-inhibitory domain. The modification may comprise a deleted segment or a mutated segment. The viral RNA segment may comprise about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 30, 35, 40, 45, 50, 51, 75, or 100 consecutive residues of the native viral genome from which it is derived. The viral RNA segment may be about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 30, 35, 40, 45, 50, 51, 75, 100, 125, 150, 175, 200, 219 or 250 bases in length. The viral RNA segment may be fused to a non-viral sequence. The vaccine may be formulated with an adjuvant. The viral RNA segment may be from an HCV E2-, YFV Env, HIV gp41-, HIV pg120/160 or GBV-C E2-coding region.

In one aspect, the present invention refers to use of a viral RNA segment comprising a T cell immune-inhibitory domain in the manufacture of a medicament for inhibiting immune cell activation. In another aspect, the present invention refers to a composition comprising a viral RNA segment comprising a T cell immune-inhibitory domain, formulated with a pharmaceutically acceptable carrier buffer or diluent. In yet another aspect, the present invention refers to use of a viral RNA segment in the manufacture of a medicament for inducing an immune response in a mammalian subject, wherein said viral RNA segment comprises one or more modified T cell immune-inhibitory domains. In a further example, the present invention refers to a vaccine comprising a viral RNA segment having a modification in a T cell immune-inhibitory domain. In one aspect, the present invention refers to use of a) an expression cassette comprising a heterologous gene segment under the control of a promoter operable in cells of said subject and b) a viral RNA segment comprising a T cell immune-inhibitory domain in the manufacture of a medicament for performing gene transfer in a subject. In another aspect, the present invention refers to a pharmaceutical composition comprising a nucleic acid having the sequence CTCACGCCAAGGTGCCTGATCGACTACCCCTACAGGCTCTGGCATTATCC (SEQ ID NO: 3) or CTCACGCCAAGGTGCCTGATCGACTACCCCTACAGGCTCTGGCATTACCCC (SEQ ID NO: 4) or GACAACAACCTTTACAAACTACATGGT (SEQ ID NO: 5). In a further aspect, the present invention refers to a pharmaceutical composition comprising a nucleic acid sequence selected from GCATTATCC (SEQ ID NO: 38), GCAUUAUCC (SEQ ID NO: 39), GCATTACCCC (SEQ ID NO: 40), GCAUUACCCC (SEQ ID NO: 41), CUUUACAAAA (SEQ ID NO: 42), or CTTTACAAA (SEQ ID NO: 43), wherein said nucleic acid consists essentially of at least about 20 base pairs and no more than about 55 base pairs.

Another example comprises a method of performing gene transfer into a subject comprising administering to said subject an expression cassette comprising a heterologous gene segment under the control of a promoter operable in cells of said subject, wherein said expression cassette further comprises a viral RNA segment comprising a T cell immune-inhibitory domain. The viral RNA segment may comprise about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 30, 35, 40, 45, 50, 51, 75, 100 consecutive bases of the native viral genome from which it is derived. The viral RNA segment may be about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 30, 35, 40, 45, 50, 51, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 400 or 500 bases in length. The viral RNA segment may be fused to a non-viral sequence. The composition may be formulated for pharmaceutical administration, such as topical, cutaneous, subcutaneous, alimentrary or parenteral administration.

In yet another example, there is disclosed a method of inducing an immune response in a mammalian subject comprising administering to said subject a viral RNA segment wherein said viral RNA segment comprises one or more modified T cell immune-inhibitory domains. The modified site may comprise Dicer substrate. The RNA virus may be from the *Reoviridae*, *Atroviridae, Caliciviridae, Hepeviridae, Picornaviridae, Togaviridae, Flaviviridae, Coronaviridae, Orthomyxoviridae, Arenaviridae, Bunyaviridae, Paramyxoviridae, Filoviridae, Rabdoviridae,* or *Retroviridae* family. The RNA virus may be GBV-C, Hepatitis C Virus, Human Immunodeficiency Virus, influenza virus, Dengue Virus, West Nile Virus, Japanese Encephalitis Virus, Bovine Viral Diarrhea Virus, Classical Swine Fever Virus or Yellow Fever Virus. The viral RNA segment may be free from other viral sequences. The viral RNA segment may comprise a Dicer substrate.

In yet another example, the target of the HCV RNA has been identified as a novel T cell immune modulator. This target is a phosphatase (protein tyrosine phosphatase receptor type E or epsilon, abbreviated PTPRE). PTPRE is reduced by both HCV RNA envelope E2 and YFV envelope RNA, and complete knockdown of this gene renders the cells non-viable. However, HCV and YFV RNA does not have complete complementarity, and thus merely reduces PTPRE protein expression, which in turn reduces T cell activation and functional responses. Since this partial T cell inhibition does not lead to profound immune deficiency, but does impair T cell responses, PTPRE is identified as a novel target for immune suppressant therapy. Threus, partial inhibition using nucleic acid-based methodologies (miRNA, siRNA, shRNA, antisense, *etc.*) can be achieved using only routine experimentation, *i.e.*, starting with molecules having complete or high complementarity, and

making sequential changes to reduce complementarity, thereby adjusting the level of inhibition such that immune function is blunted, but not completely eliminated (*i.e.*, rendering the cells non-viable). It is expected that in contexts involving inhibitory RNAs that rely on a "seed sequence" for recognition, the seed sequence will remain completely or highly complementary, with other sequences being reduced in complementarity. For example, seed sequence complementarity might be greater than about 80%, while other regions would be 20-60% complementary.

Also disclosed are pharmaceutical compositions comprising a nucleic acid having the sequence:

CTCACGCCAAGGTGCCTGATCGACTACCCCTACAGGCTCTGGCATTATCC (SEQ ID NO: 3),

CTCACGCCAAGGTGCCTGATCGACTACCCCTACAGGCTCTGGCATTACCCC (SEQ ID NO: 4)

or

GACAACAACCTTTACAAACTACATGGT (SEQ ID NO: 5).

The pharmaceutical composition may also comprise a nucleic acid sequence selected from GCATTATCC (SEQ ID NO: 38), GCAUUAUCC (SEQ ID NO: 39), GCATTACCCC (SEQ ID NO: 40), GCAUUACCCC (SEQ ID NO: 41), CUUUACAAAA (SEQ ID NO: 42), or CTTTACAAA (SEQ ID NO: 43), wherein said nucleic acid consists essentially of at least about 20 base pairs and no more than about 55 base pairs. Specific lengths include 22, 27 and 51 nucleotides.

The use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one." Furthermore, where multiple steps of a method of process are recited, it is understood that the steps are not required to be performed in the particular order recited unless one of skill in the art is not be able to practice the method in a different order.

Other objects, features, and advantages of the present disclosure will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating some embodiments of the disclosure, are given by way of illustration only, since various changes and modifications within the spirit and scope of the disclosure will become apparent to those skilled in the art from this detailed description.

WHAT IS CLAIMED IS:

1. Use of a viral RNA segment comprising a T cell immune-inhibitory domain in the manufacture of a medicament for inhibiting immune cell activation .

2. The use of claim 1, wherein said viral RNA segment comprises about 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 30, 35, 40, 45, 50, 51, 75, 100, 150, 175, 200, 225, 250, 300, 400 or 500 consecutive bases of the T cell immune-inhibitory domain.

3. The use of claim 1, wherein the viral RNA segment encodes HCV E2 sequences, GBV-C E2, YFV envelope or HIV gp41 sequences.

4. The use of claim 3, wherein the viral RNA segment further encodes non-HCV E2 sequences, non-GBV-C E2 sequences, non-YFV env sequences or non-HIV gp41 sequences.

5. The use of claim 1, wherein the T cell is a helper T cell suppressor T cell, or a killer T cell.

6. The use of claim 1, wherein said subject is a non-human mammal.

7. The use of claim 1, wherein said subject is a human.

8. The use of claim 1, wherein the medicament is to be administered intravenously, intra-arterially, orally, subcutaneously, topically or intraperitoneally.

9. The use of claim 1, further comprising a second anti-inflammatory agent to be administered.

10. The use of claim 9, wherein said second anti-inflammatory agent is a steroid or a COX-2 inhibitor.

11. The use of claim 9, wherein said second anti-inflammatory agent is to be contacted prior to or after said viral RNA segment.

12. The use of claim 9, wherein said second anti-inflammatory agent is to be contacted at the same time as said viral RNA segment.

13. The use of claim 1, wherein said viral RNA segment is provided in combination with a gene therapy vector.

14. The use of claim 1, further comprising a gene therapy vector to be administered.

15. The use of claim 13 or 14, wherein said gene therapy vector comprises a viral gene therapy vector.

16. The use of claim 15, wherein said viral gene therapy vector comprises an adeno-associated virus (AAV) vector.

17. The use of claim 1, wherein said viral RNA segment comprises at least one non-natural base.

18. The use of claim 1, wherein said viral RNA segment comprises a Dicer substrate.

19. The use of claim 1, wherein said viral RNA segment is to be administered at 0.1-500 mg/kg/d.

20. The use of claim 1, wherein said viral RNA segment is to be administered daily or weekly.

21. The use of claim 18, wherein said viral RNA segment is to be administered daily for 7 days, 2 weeks, 3 weeks, 4 weeks, one month, 6 weeks, 8 weeks, two months, 12 weeks, or 3 months, or weekly for 2 weeks, 3 weeks, 4 weeks, 6 weeks, 8 weeks, 10 weeks, or 12 weeks.

22. The use of claim 1, wherein said viral RNA segment is derived from Human Immunodeficiency Virus envelope gp120/160, Yellow Fever Virus envelope protein, Bovine Viral Diarrhea Virus envelope protein, Classical Swine Fever Virus envelope

protein, influenza envelope protein, Dengue Virus envelope protein, West Nile Virus envelope protein, and Japanese Encephalitis Virus envelope protein.

23. A composition comprising a viral RNA segment comprising a T cell immune-inhibitory domain, formulated with a pharmaceutically acceptable carrier buffer or diluent.

24. The composition of claim 21, wherein said viral RNA segment comprises about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 30, 35, 40, 45, 50, 51, 75, 100 consecutive bases of the native viral genome from which it is derived.

25. The composition of claim 21, wherein said viral RNA segment is about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 30, 35, 40, 45, 50, 51, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 400 or 500 bases in length.

26. The composition of claim 21, wherein said viral RNA segment is fused to a non-viral sequence.

27. The composition of claim 21, formulated for pharmaceutical administration, such as topically, cutaneously, subcutaneously, alimentrarily or parenterally.

28. Use of a viral RNA segment in the manufacture of a medicament for inducing an immune response in a mammalian subject, wherein said viral RNA segment comprises one or more modified T cell immune-inhibitory domains.

29. The use of claim 28, wherein said modified site comprises Dicer substrate.

30. The use of claim 28, wherein said RNA virus is from the *Reoviridae*, *Atroviridae, Caliciviridae, Hepeviridae, Picornaviridae, Togaviridae, Flaviviridae, Coronaviridae, Orthomyxoviridae, Arenaviridae, Bunyaviridae, Paramyxoviridae, Filoviridae, Rabdoviridae,* or *Retroviridae* family.

31. The use of claim 28, wherein said RNA virus is GBV-C, Hepatitis C Virus, Human Immunodeficiency Virus, influenza virus, Dengue Virus, West Nile Virus, Japanese

Encephalitis Virus, Bovine Viral Diarrhea Virus, Classical Swine Fever Virus or Yellow Fever Virus.

32. The use of claim 28, wherein said viral RNA segment is free from other viral sequences.

33. The use of claim 28, wherein said viral RNA segment is delivered via expression from an expression vector.

34. The use of claim 33, wherein said expression vector is a viral expression vector.

35. The use of claim 28, wherein said viral RNA segment is comprised in lipid vehicle or nanoparticle.

36. The use of claim 28, wherein said viral RNA segment is to be administered with a second viral RNA segment from a distinct serotype or strain of said virus.

37. The use of claim 28, wherein said viral RNA segment is to be administered more than once.

38. The use of claims 32, 33, 34 or 35, wherein said viral RNA segment is formulated with an adjuvant.

39 The use of claim 28, wherein said viral RNA segment is from HCV E2.

40. The use of claim 28, wherein said viral RNA segment is from YFV env.

41. The use of claim 28, wherein said viral RNA segment is from GBV-C E2.

42. The use of claim 28, wherein a modified domain comprises a deletion or a substitution as compared to the natural sequence.

43. The use of claim 42, wherein said subject is a human subject.

44. The use of claim 42, wherein said subject is a non-human animal subject.

45. A vaccine comprising a viral RNA segment having a modification in a T cell immune-inhibitory domain.

46. The vaccine of claim 45, wherein said modification comprises a deleted segment or a mutated segment.

47. The vaccine of claim 45, wherein said viral RNA segment comprises about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 30, 35, 40, 45, 50, 51, 75, or 100 consecutive residues of the native viral genome from which it is derived.

48. The vaccine of claim 45, wherein said viral RNA segment is about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 30, 35, 40, 45, 50, 51, 75, 100, 125, 150, 175, 200, 219 or 250 bases in length.

49. The vaccine of claim 45, wherein said viral RNA segment is fused to a non-viral sequence.

50. The vaccine of claim 45, formulated with an adjuvant.

51. The vaccine of claim 45, wherein said viral RNA segment is from an HCV coding region.

52. Use of

a) an expression cassette comprising a heterologous gene segment under the control of a promoter operable in cells of said subject and

b) a viral RNA segment comprising a T cell immune-inhibitory domain

in the manufacture of a medicament for performing gene transfer in a subject.

53. The use of claim 52, wherein said expression cassette further comprises the viral RNA segment comprising the T cell immune-inhibitory domain.

54. The use of claim 52, wherein said expression cassette comprises a viral vector.

55. The use of claim 52, wherein said viral vector comprises an adeno-associated virus (AAV) vector.

56. A pharmaceutical composition comprising a nucleic acid having the sequence:

CTCACGCCAAGGTGCCTGATCGACTACCCCTACAGGCTCTGGCATTATCC (SEQ ID NO: 3)

or

CTCACGCCAAGGTGCCTGATCGACTACCCCTACAGGCTCTGGCATTACCCC (SEQ ID NO: 4).

or

GACAACAACCTTTACAAACTACATGGT (SEQ ID NO: 5).

57. A pharmaceutical composition comprising a nucleic acid sequence selected from GCATTATCC (SEQ ID NO: 38), GCAUUAUCC (SEQ ID NO: 39), GCATTACCCC (SEQ ID NO: 40), GCAUUACCCC (SEQ ID NO: 41), CUUUACAAAA (SEQ ID NO: 42), or CTTTACAAA (SEQ ID NO: 43), wherein said nucleic acid consists essentially of at least about 20 base pairs and no more than about 55 base pairs.