74; Amara et al. *J Virol.* 2002, 76(15):7625-31; Egan et al. *AIDS Research and Human Retroviruses*. 2005, 21(7):629-43; Hanke et al. *J Virol.* 1999, 73(9):7524-32; Ramsburg et al. *Journal of Virology*. 2004, 78(8):3930-40; Santra et al. *J Virol.* 2007; Xu et al. *Journal of Virology*. 2009, 83(19):9813-23). Serotype switching of rVSV vectors, achieved by swapping the surface G protein with that of a different vesiculovirus serotype, also enhances immunogenicity in prime-boost regimens in mice (Rose et al. *Journal of Virology*. 2000, 74(23):10903-10). However, cross-reactivity of cellular immune responses directed towards rVSV core proteins may limit this approach.

[005] In view of these observations and potential limitations, there is a need for additional heterologous vectors for use either alone or in conjunction with rVSV vectors.

## SUMMARY

[006] Embodiments of the invention include immunogenic compositions and methods related to vesiculoviruses, such as Isfahan virus (ISFV) alone or in combination with vesicular stomatitis virus (VSV) and their use as therapeutics and/or prophylactics. Certain aspects include methods and immunogenic compositions comprising a recombinant vesiculovirus encoding one or more heterologous polypeptides. "Recombinant virus" refers to any viral genome or virion that is the same as or different than a wild-type virus due to a rearrangement, deletion, insertion, or substitution of one or more nucleotides in the wild-type viral genome. In particular, the term includes recombinant viruses generated by the intervention of a human. In certain aspects the vesiculovirus is a recombinant Isfahan virus (rISFV). In certain aspects the rISFV is a replication competent virus. As applied to a recombinant virus, "replication competent" means that the virus is capable of cell infection; replication of the viral genome; and production and release of new virus particles; although one or more of these characteristics need not occur at the same rate as they occur in the same cell type infected by a wild-type virus, and may occur at a faster or slower rate.

[007] In a further aspect the rISFV comprises one or more of (i) an N protein having an amino acid sequence that is 90, 95, 98, or 100% identical to the amino acid sequence of SEQ ID

74; Amara et al. *J Virol.* 2002, 76(15):7625-31; Egan et al. *AIDS Research and Human Retroviruses.* 2005, 21(7):629-43; Hanke et al. *J Virol.* 1999, 73(9):7524-32; Ramsburg et al. *Journal of Virology.* 2004, 78(8):3930-40; Santra et al. *J Virol.* 2007; Xu et al. *Journal of Virology.* 2009, 83(19):9813-23). Serotype switching of rVSV vectors, achieved by swapping the surface G protein with that of a different vesiculovirus serotype, also enhances immunogenicity in prime-boost regimens in mice (Rose et al. *Journal of Virology.* 2000, 74(23):10903-10). However, cross-reactivity of cellular immune responses directed towards rVSV core proteins may limit this approach.

[005] In view of these observations and potential limitations, there is a need for additional heterologous vectors for use either alone or in conjunction with rVSV vectors.

## SUMMARY

[006] Embodiments of the invention include immunogenic compositions and methods related to vesiculoviruses, such as Isfahan virus (ISFV) alone or in combination with vesicular stomatitis virus (VSV) and their use as therapeutics and/or prophylactics. Certain aspects include methods and immunogenic compositions comprising a recombinant vesiculovirus encoding one or more heterologous polypeptides. "Recombinant virus" refers to any viral genome or virion that is the same as or different than a wild-type virus due to a rearrangement, deletion, insertion, or substitution of one or more nucleotides in the wild-type viral genome. In particular, the term includes recombinant viruses generated by the intervention of a human. In certain aspects the vesiculovirus is a recombinant Isfahan virus (rISFV). In certain aspects the rISFV is a replication competent virus. As applied to a recombinant virus, "replication competent" means that the virus is capable of cell infection; replication of the viral genome; and production and release of new virus particles; although one or more of these characteristics need not occur at the same rate as they occur in the same cell type infected by a wild-type virus, and may occur at a faster or slower rate.

[006a] In one aspect, there is provided a recombinant replication competent Isfahan virus comprising an N protein gene, a P protein gene, an M protein gene, a G protein gene, and an L protein gene; and

further comprising a heterologous polynucleotide sequence encoding a heterologous polypeptide.

[006b] In one aspect, there is provided a host cell comprising the Isfahan virus as described herein.

[006c] In one aspect, there is provided an immunogenic composition comprising a recombinant replication competent Isfahan virus comprising an N protein gene, a P protein gene, an M protein gene, a G protein gene, and an L protein gene; and

further comprising a heterologous polynucleotide sequence, wherein said heterologous polynucleotide sequence encodes a heterologous polypeptide;

and a pharmaceutically acceptable diluent, excipient or carrier.

[006d] In one aspect, there is provided the use of the immunogenic composition as described herein, in the manufacture of a medicament for inducing an antigen-specific immune response to an antigen in a mammalian subject.

[006e] In one aspect, there is provided an immunization kit for inducing an antigen-specific immune response in a mammalian subject, said kit comprising:

(a) a priming composition comprising a recombinant replication competent Isfahan virus encoding an N protein gene, a P protein gene, an M protein gene, a G protein gene, an L protein gene, and a heterologous polynucleotide sequence, wherein said heterologous polynucleotide sequence encodes a heterologous polypeptide, wherein said heterologous polynucleotide sequence (i) is flanked by a transcription start signal and a transcription stop signal, and (ii) encodes a heterologous polypeptide; and a pharmaceutically acceptable diluent, excipient or carrier; and

(b) a boosting composition comprising a recombinant replication competent vesicular stomatitis virus encoding an N protein gene, a P protein gene, an M protein gene, a G protein gene, an L protein gene, and a heterologous polynucleotide sequence, wherein said heterologous polynucleotide sequence (i) is flanked by a transcription start signal and a transcription stop signal, and (ii) encodes a heterologous polypeptide; and a pharmaceutically acceptable diluent, excipient or carrier.

[006f] In one aspect, there is provided an immunization kit for inducing an antigen-specific immune response in a mammalian subject, said kit comprising:

(a) a priming composition comprising a recombinant replication competent vesicular stomatitis virus (VSV) encoding an N protein gene, a P protein gene, an M protein gene, a G protein gene, an L protein gene, and a heterologous polynucleotide sequence, wherein said heterologous polynucleotide sequence encodes a heterologous polypeptide, wherein said heterologous polynucleotide sequence (i) is flanked by a transcription start signal and a transcription stop signal, and (ii) encodes a heterologous polypeptide; and a pharmaceutically acceptable diluent, excipient or carrier; and

(b) a boosting composition comprising a recombinant replication competent Isfahan virus (ISFV) encoding an N protein gene, a P protein gene, an M protein gene, a G protein gene, an L protein gene, and a heterologous polynucleotide sequence, wherein said heterologous polynucleotide sequence encodes a heterologous polypeptide, wherein said heterologous polynucleotide sequence (i) is flanked by a transcription start signal and a transcription stop signal, and (ii) encodes a heterologous polypeptide; and a pharmaceutically acceptable diluent, excipient or carrier.

[006g] In one aspect, there is provided a recombinant replication competent Isfahan virus comprising an N protein gene, a P protein gene, an M protein gene, a heterologous viral surface protein gene(s), and an L protein gene.

[006h] In one aspect, there is provided an oncolytic viral composition comprising a recombinant replication competent Isfahan virus comprising an N protein gene, a P protein gene, an M protein gene, a G protein gene, and an L protein gene, wherein said Isfahan virus is used as an anti-cancer therapeutic.

[007] In a further aspect the rISFV comprises one or more of (i) an N protein having an amino acid sequence that is 90, 95, 98, or 100% identical to the amino acid sequence of SEQ ID

CLAIMS

1. A recombinant replication competent Isfahan virus comprising an N protein gene, a P protein gene, an M protein gene, a G protein gene, and an L protein gene; and

further comprising a heterologous polynucleotide sequence encoding a heterologous polypeptide.

2. The Isfahan virus of claim 1, wherein the heterologous polynucleotide sequence is flanked by a transcription start signal and a transcription stop signal.

3. The Isfahan virus of claim 1, wherein the heterologous polynucleotide encodes an immunogenic polypeptide.

4. The Isfahan virus of claim 1, wherein the heterologous polynucleotide encodes one or more antigens.

5. The Isfahan virus of claim 4, wherein the antigen is a viral antigen, a bacterial antigen, a tumor-specific or cancer antigen, a parasitic antigen or an allergen.

6. The Isfahan virus of claim 5, wherein the antigen is a viral antigen.

7. The Isfahan virus of claim 1, wherein the heterologous polynucleotide sequence is located at position 1, 2, 3, 4, 5 or 6 of the Isfahan virus genome.

8. The Isfahan virus of claim 1, wherein the N protein gene is located at position 1, 2, 3, 4 or 5 of the Isfahan virus genome.

9. The Isfahan virus of claim 1, wherein the G protein gene encodes a G protein having a carboxy-terminal truncation.

10. The Isfahan virus of claim 9, wherein the G protein has a carboxy-terminal truncation of 20 to 25 amino acids.

11. The Isfahan virus of claim 1, wherein the heterologous polynucleotide sequence is located at position 5 and the N protein gene is located at position 4 of the Isfahan virus genome.

12. A host cell comprising the Isfahan virus of claim 1.

13. An immunogenic composition comprising a recombinant replication competent Isfahan virus comprising an N protein gene, a P protein gene, an M protein gene, a G protein gene, and an L protein gene; and

further comprising a heterologous polynucleotide sequence, wherein said heterologous polynucleotide sequence encodes a heterologous polypeptide;

and a pharmaceutically acceptable diluent, excipient or carrier.

14. The immunogenic composition of claim 13, wherein the heterologous polynucleotide sequence is flanked by a transcription start signal and a transcription stop signal.

15. A method of inducing an antigen-specific immune response to an antigen in a mammalian subject comprising administering the immunogenic composition of claim 14.

16. An immunization kit for inducing an antigen-specific immune response in a mammalian subject, said kit comprising:

(a) a priming composition comprising a recombinant replication competent Isfahan virus encoding an N protein gene, a P protein gene, an M protein gene, a G protein gene, an L protein gene, and a heterologous polynucleotide sequence, wherein said heterologous polynucleotide sequence encodes a heterologous polypeptide, wherein said heterologous polynucleotide sequence (i) is flanked by a transcription start signal and a

transcription stop signal, and (ii) encodes a heterologous polypeptide; and a pharmaceutically acceptable diluent, excipient or carrier; and

(b) a boosting composition comprising a recombinant replication competent vesicular stomatitis virus encoding an N protein gene, a P protein gene, an M protein gene, a G protein gene, an L protein gene, and a heterologous polynucleotide sequence, wherein said heterologous polynucleotide sequence (i) is flanked by a transcription start signal and a transcription stop signal, and (ii) encodes a heterologous polypeptide; and a pharmaceutically acceptable diluent, excipient or carrier.

17. A method of inducing an antigen-specific immune response in a mammalian subject comprising administering the immunogenic compositions of claim 16.

18. An immunization kit for inducing an antigen-specific immune response in a mammalian subject, said kit comprising:

(a) a priming composition comprising a recombinant replication competent vesicular stomatitis virus (VSV) encoding an N protein gene, a P protein gene, an M protein gene, a G protein gene, an L protein gene, and a heterologous polynucleotide sequence, wherein said heterologous polynucleotide sequence encodes a heterologous polypeptide, wherein said heterologous polynucleotide sequence (i) is flanked by a transcription start signal and a transcription stop signal, and (ii) encodes a heterologous polypeptide; and a pharmaceutically acceptable diluent, excipient or carrier; and

(b) a boosting composition comprising a recombinant replication competent Isfahan virus (ISFV) encoding an N protein gene, a P protein gene, an M protein gene, a G protein gene, an L protein gene, and a heterologous polynucleotide sequence, wherein said heterologous polynucleotide sequence encodes a heterologous polypeptide, wherein said heterologous polynucleotide sequence (i) is flanked by a transcription start signal and a transcription stop signal, and (ii) encodes a heterologous polypeptide; and a pharmaceutically acceptable diluent, excipient or carrier.

19. A method of inducing an antigen-specific immune response in a mammalian subject comprising administering the immunogenic compositions of claim 18.

20. A recombinant replication competent Isfahan virus comprising an N protein gene, a P protein gene, an M protein gene, a heterologous viral surface protein gene(s), and an L protein gene.

21. The recombinant replication competent Isfahan virus of claim 20, further comprising a second heterologous polynucleotide sequence, wherein said heterologous polynucleotide sequence encodes a second heterologous polypeptide.

22. The recombinant replication competent Isfahan virus of claim 20, wherein the heterologous viral surface protein gene replaces the Isfahan virus G protein gene.

23. The recombinant replication competent Isfahan virus of claim 20, wherein the heterologous viral surface protein gene is a G protein gene of vesicular stomatitis virus.

24. An oncolytic viral composition comprising a recombinant replication competent Isfahan virus comprising an N protein gene, a P protein gene, an M protein gene, a G protein gene, and an L protein gene, wherein said Isfahan virus is used as an anti-cancer therapeutic.

25. The Isfahan virus of claim 6, wherein the viral antigen is from Chikungunya virus.

CLAIMS

1. A recombinant replication competent Isfahan virus comprising an N protein gene, a P protein gene, an M protein gene, a G protein gene, and an L protein gene; and

further comprising a heterologous polynucleotide sequence encoding a heterologous polypeptide.

2. The Isfahan virus of claim 1, wherein the heterologous polynucleotide sequence is flanked by a transcription start signal and a transcription stop signal.

3. The Isfahan virus of claim 1, wherein the heterologous polynucleotide encodes an immunogenic polypeptide.

4. The Isfahan virus of claim 1, wherein the heterologous polynucleotide encodes one or more antigens.

5. The Isfahan virus of claim 4, wherein the antigen is a viral antigen, a bacterial antigen, a tumor-specific or cancer antigen, a parasitic antigen or an allergen.

6. The Isfahan virus of claim 5, wherein the antigen is a viral antigen.

7. The Isfahan virus of claim 1, wherein the heterologous polynucleotide sequence is located at position 1, 2, 3, 4, 5 or 6 of the Isfahan virus genome.

8. The Isfahan virus of claim 1, wherein the N protein gene is located at position 1, 2, 3, 4 or 5 of the Isfahan virus genome.

9. The Isfahan virus of claim 1, wherein the G protein gene encodes a G protein having a carboxy-terminal truncation.

10. The Isfahan virus of claim 9, wherein the G protein has a carboxy-terminal truncation of 20 to 25 amino acids.

11. The Isfahan virus of claim 1, wherein the heterologous polynucleotide sequence is located at position 5 and the N protein gene is located at position 4 of the Isfahan virus genome.

12. A host cell comprising the Isfahan virus of claim 1.

13. An immunogenic composition comprising a recombinant replication competent Isfahan virus comprising an N protein gene, a P protein gene, an M protein gene, a G protein gene, and an L protein gene; and

further comprising a heterologous polynucleotide sequence, wherein said heterologous polynucleotide sequence encodes a heterologous polypeptide;

and a pharmaceutically acceptable diluent, excipient or carrier.

14. The immunogenic composition of claim 13, wherein the heterologous polynucleotide sequence is flanked by a transcription start signal and a transcription stop signal.

15. Use of the immunogenic composition of claim 14, in the manufacture of a medicament for inducing an antigen-specific immune response to an antigen in a mammalian subject.

16. An immunization kit for inducing an antigen-specific immune response in a mammalian subject, said kit comprising:

(a) a priming composition comprising a recombinant replication competent Isfahan virus encoding an N protein gene, a P protein gene, an M protein gene, a G protein gene, an L protein gene, and a heterologous polynucleotide sequence, wherein said heterologous polynucleotide sequence encodes a heterologous polypeptide, wherein said heterologous polynucleotide sequence (i) is flanked by a transcription start signal and a transcription stop signal, and (ii) encodes a heterologous polypeptide; and a pharmaceutically acceptable diluent, excipient or carrier; and

(b) a boosting composition comprising a recombinant replication competent vesicular stomatitis virus encoding an N protein gene, a P protein gene, an M protein gene, a G protein gene, an L protein gene, and a heterologous polynucleotide sequence, wherein said heterologous polynucleotide sequence (i) is flanked by a transcription start signal and a transcription stop signal, and (ii) encodes a

heterologous polypeptide; and a pharmaceutically acceptable diluent, excipient or carrier.

17. Use of the immunogenic compositions of claim 16, in the manufacture of a medicament for inducing an antigen-specific immune response in a mammalian subject.

18. An immunization kit for inducing an antigen-specific immune response in a mammalian subject, said kit comprising:

(a) a priming composition comprising a recombinant replication competent vesicular stomatitis virus (VSV) encoding an N protein gene, a P protein gene, an M protein gene, a G protein gene, an L protein gene, and a heterologous polynucleotide sequence, wherein said heterologous polynucleotide sequence encodes a heterologous polypeptide, wherein said heterologous polynucleotide sequence (i) is flanked by a transcription start signal and a transcription stop signal, and (ii) encodes a heterologous polypeptide; and a pharmaceutically acceptable diluent, excipient or carrier; and

(b) a boosting composition comprising a recombinant replication competent Isfahan virus (ISFV) encoding an N protein gene, a P protein gene, an M protein gene, a G protein gene, an L protein gene, and a heterologous polynucleotide sequence, wherein said heterologous polynucleotide sequence encodes a heterologous polypeptide, wherein said heterologous polynucleotide sequence (i) is flanked by a transcription start signal and a transcription stop signal, and (ii) encodes a heterologous polypeptide; and a pharmaceutically acceptable diluent, excipient or carrier.

19. Use of the immunogenic compositions of claim 18, in the manufacture of a medicament for inducing an antigen-specific immune response in a mammalian subject.

20. A recombinant replication competent Isfahan virus comprising an N protein gene, a P protein gene, an M protein gene, a heterologous viral surface protein gene(s), and an L protein gene.

21. The recombinant replication competent Isfahan virus of claim 20, further comprising a second heterologous polynucleotide sequence, wherein said heterologous polynucleotide sequence encodes a second heterologous polypeptide.

22. The recombinant replication competent Isfahan virus of claim 20, wherein the heterologous viral surface protein gene replaces the Isfahan virus G protein gene.

23. The recombinant replication competent Isfahan virus of claim 20, wherein the heterologous viral surface protein gene is a G protein gene of vesicular stomatitis virus.

24. An oncolytic viral composition comprising a recombinant replication competent Isfahan virus comprising an N protein gene, a P protein gene, an M protein gene, a G protein gene, and an L protein gene, wherein said Isfahan virus is used as an anti-cancer therapeutic.

25. The Isfahan virus of claim 6, wherein the viral antigen is from Chikungunya virus.