A NEW ONCOLYTIC VIRUS PLATFORM TO TREAT CANCERS WITH MYXOMA VIRUS

CROSS REFERENCE

[0001] This application claims the benefit of U.S. provisional application No. 62/894,925, filed September 2, 2019, and U.S. provisional application No. 62/944,233, filed December 5, 2019, each of which is incorporated herein by reference in its entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on September 1, 2020, is named 42206-721_601_SL.txt and is 72,925 bytes in size.

STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

[0003] This invention was made with government support under P50 CA186781 awarded by the National Institutes of Health. The government has certain rights in the invention.

FIELD

[0004] Disclosed herein are recombinant myxoma viruses (MYXVs), nucleic acid constructs encoding recombinant MYXVs, and methods of use thereof.

BACKGROUND

[0005] Current treatments used to treat various types of cancer tend to work by poisoning or killing the cancerous cell, but treatments that are toxic to cancer cells typically tend to be toxic to healthy cells as well. Moreover, the heterogenous nature of tumors is one of the primary reasons that effective treatments for cancer remain elusive. Current mainstream therapies such as chemotherapy and radiotherapy tend to be used within a narrow therapeutic window of toxicity. These types of therapies have limited applicability due to the varying types of tumor cells and the limited window in which these treatments can be administered.

SUMMARY

[0006] In one aspect, provided herein~~Disclosed herein in some aspects~~ is a myxoma virus (MYXV) having enhanced anti-cancer activity, wherein the myxoma virus is genetically engineered to attenuate an activity or expression level of its M153 protein.

[0007] In certain ~~embodiment~~examples, the activity or the expression level of the M153 protein is attenuated at least 80%. Alternatively and/or additionally, MYXV is engineered to introduce a mutation in a nucleic acid encoding the M153 protein, wherein the mutation comprises an

insertion, deletion, or substitution mutation. In some ~~embodiment~~examples, the mutation enhances cell immune response activity in relation to a wild-type M153 protein. In certain ~~embodiment~~examples, at least a portion of a nucleic acid encoding the M153 protein in MYXV genome is knocked out. In some ~~embodiment~~examples, the MYXV comprises an inhibitory molecule targeting M153 transcript that thereby attenuates the M153 protein expression. In some ~~embodiment~~examples, the inhibitory molecule is an inhibitory RNA. In some ~~embodiment~~examples, the inhibitory RNA comprises dsRNA, siRNA, antisense RNA, or miRNA. In some ~~embodiment~~examples, the MYXV further comprises a nucleic acid encoding a non-viral molecule. In some ~~embodiment~~examples, the non-viral molecule is tumor necrosis factor alpha (TNFα). In some ~~embodiment~~examples, the TNFα is human TNFα. In some ~~embodiment~~examples, the TNFα is a soluble peptide. In some ~~embodiment~~examples, the TNFα is a membrane- or surface-bound peptide. In some ~~embodiment~~examples, the TNFα further enhances the anti-cancer activity of the MYXV by activating anti-tumor immune cells or inducing cancer cell death. In some ~~embodiment~~examples, the non-viral molecule is interleukin-12 (IL-12). In some ~~embodiment~~examples, the IL-12 is human IL 12. In some ~~embodiment~~examples, the IL-12 is a soluble peptide. In some ~~embodiment~~examples, the IL-12 is a membrane- or surface-bound peptide. In some ~~embodiment~~examples, the IL-12 further enhances the anti-cancer activity of the MYXV by promoting immune cell differentiation or eliciting immune cell cytotoxicity. In some ~~embodiment~~examples, the IL-12 comprises an IL-12α subunit and an IL-12β subunit. In some ~~embodiment~~examples, the IL-12α subunit and the IL-12β subunit are joined by a polypeptide linker. In some ~~embodiment~~examples, the polypeptide linker is an elastin linker. In some ~~embodiment~~examples, the non-viral molecule is decorin. In some ~~embodiment~~examples, the decorin is human decorin. In some ~~embodiment~~examples, the decorin is a soluble peptide. In some ~~embodiment~~examples, the decorin is a membrane- or surface-bound peptide. In some ~~embodiment~~examples, the decorin further enhances the anti-cancer activity of the MYXV by blocking or decreasing TGF-β signaling. In some ~~embodiment~~examples, the nucleic acid encodes at least two molecules selected from a group consisting of TNFα, IL-12, and decorin. In some ~~embodiment~~examples, the nucleic acid encodes TNFα, IL-12, and decorin. In some ~~embodiment~~examples, the MYXV is derived from a Lausanne strain.

[0008] Disclosed herein, in some ~~aspect~~examples, is a composition comprising a MYXV of any of the above ~~embodiment~~examples, and a pharmaceutically acceptable carrier or excipient.

[0009] In some ~~embodiment~~examples, the composition is formulated for systemic administration. In some ~~embodiment~~examples, the composition is formulated for local administration. In some ~~embodiment~~examples, the composition is formulated for parenteral administration.

[0010] Disclosed herein, in some ~~aspect~~examples is a composition comprising a plurality of cells treated *ex vivo* by the MYXV of any of the above ~~embodiment~~examples, wherein the plurality of cells comprises peripheral blood mononuclear cells (PBMCs), bone marrow (BM) cells, or a combination thereof.

[0011] Disclosed herein, in some ~~aspect~~examples is a method of inhibiting, alleviating, or preventing a cancer in a subject in need thereof, comprising administering to the subject the MYXV or the composition of any one of the above ~~embodiment~~examples.

[0012] In some ~~embodiment~~examples, the subject has, is suspected of having, or is at risk of having the cancer, and wherein the method further comprises selecting the subject. In some ~~embodiment~~examples, the subject is a human. In some ~~embodiment~~examples, the administration is systemic administration. In some ~~embodiment~~examples, the administration reduces cancer cell viability, or activates immunogenic cell death in the cancer. In some ~~embodiment~~examples, the administration improves the subject's survival. In some ~~embodiment~~examples, the cancer comprises a solid tumor. In some ~~embodiment~~examples, the cancer is an osteosarcoma, triple negative breast cancer, or melanoma. In some ~~embodiment~~examples, the cancer has metastasized to a location in the subject. In some ~~embodiment~~examples, the location comprises a lung, a brain, a liver and/or a lymph node of the subject. In some ~~embodiment~~examples, the method further comprises administering to the subject an additional therapeutic agent. In some ~~embodiment~~examples, the additional therapeutic agent is an immune checkpoint modulator. In some ~~embodiment~~examples, the additional therapeutic agent is administered to the subject before administering the composition. In some ~~embodiment~~examples, the additional therapeutic agent is administered to the subject after administering the composition. In some ~~embodiment~~examples, the additional therapeutic agent is administered to the subject as a combination with the composition. In some ~~embodiment~~examples, the composition comprising the plurality of cells is administered to the subject, wherein the plurality of cells comprises cells that are autologous to the subject. In some ~~embodiment~~examples, the composition comprising the plurality of cells is administered to the subject, wherein the plurality of cells comprises cells that are allogenic to the subject.

[0013] Disclosed herein, in some ~~aspect~~examples is a recombinant nucleic acid comprising at least a portion of MYXV genome, wherein the MYXV genome is modified to reduce expression of M153 gene. In some ~~embodiment~~examples, the recombinant nucleic acid comprises DNA. In some ~~embodiment~~examples, the portion of MYXV genome is modified to knock out at least a portion of the M153 gene in the portion of MYXV genome. In some ~~embodiment~~examples, the recombinant MYXV genome comprises a first nucleic acid encoding TNFα. In some ~~embodiment~~examples, the TNFα is human TNFα. In some ~~embodiment~~examples, the first nucleic acid replaces or is adjacent to an M135R gene of the MYXV genome. In some ~~embodiment~~examples, the first nucleic acid is inserted between an M135R gene and an M136R gene of the MYXV genome. In some ~~embodiment~~examples, expression of the first nucleic acid is driven by a poxvirus synthetic early/late (sE/L) promoter. In some ~~embodiment~~examples, the recombinant MYXV genome comprises a second nucleic acid encoding interleukin-12 subunit alpha (IL-12α). In some ~~embodiment~~examples, the IL-12α is human IL-12α. In some ~~embodiment~~examples, expression of IL-12α is driven by an internal ribosome entry site (IRES). In some ~~embodiment~~examples, the second nucleic acid

IL-12α disrupts the expression of the M153 gene of the MYXV genome. In some ~~embodiment~~examples, the recombinant MYXV genome comprises a third nucleic acid encoding an interleukin-12 subunit beta (IL-12β). In some ~~embodiment~~examples, the IL-12β is human IL-12β. In some ~~embodiment~~examples, expression of the third nucleic acid is driven by an sE/L promoter. In some ~~embodiment~~examples, the third nucleic acid disrupts the expression of the M153 gene of the MYXV genome. In some ~~embodiment~~examples, the recombinant MYXV genome comprises a fourth nucleic acid encoding decorin. In some ~~embodiment~~examples, the decorin is human decorin. In some ~~embodiment~~examples, expression of the fourth nucleic acid is driven by an sE/L promoter. In some ~~embodiment~~examples, the fourth nucleic acid disrupts expression of the M153 gene of the MYXV genome. In some ~~embodiment~~examples, the recombinant MYXV genome further comprises a fifth nucleic acid encoding a reporter tag. In some ~~embodiment~~examples, the reporter tag comprises a green fluorescent protein (GFP). In some ~~embodiment~~examples, expression of the fifth nucleic acid is driven by an sE/L promoter. In some ~~embodiment~~examples, the recombinant nucleic acid further comprises a sixth nucleic acid encoding a second reporter tag. In some ~~embodiment~~examples, the second reporter tag comprises a red fluorescent protein (RFP). In some ~~embodiment~~examples, expression of the sixth nucleic acid is driven by a poxvirus P11 late promoter. In some ~~embodiment~~examples, the recombinant nucleic acid comprises a vMyx-hTNFa cassette, optionally comprising GFP. In some ~~embodiment~~examples, the recombinant nucleic acid comprises an hDecorin-hIL-12 cassette, optionally comprising dsRed. In some ~~embodiment~~examples, the recombinant nucleic acid comprises or consists of a vMyx-hTNFa-hDecorin-hIL-12-M153KO (vMyx-Triple) cassette, optionally comprising dsRed and/or GFP.

[0014] Disclosed herein, in some ~~aspect~~examples, is a recombinant MYXV comprising the recombinant nucleic acid of any of the above ~~embodiment~~examples.

[0015] Disclosed herein, in some ~~aspect~~examples, is a method of inhibiting, alleviating, or preventing a cancer in a subject in need thereof, comprising administering to the subject the recombinant nucleic acid or the recombinant MYXV of any one of the preceding ~~embodiment~~examples,

[0016] In some ~~embodiment~~examples, the subject has, is suspected of having, or is at risk of having the cancer, and wherein the method further comprises selecting the subject. In some ~~embodiment~~examples, the subject is a human. In some ~~embodiment~~examples, the administration is systemic administration. In some ~~embodiment~~examples, the administration reduces cell viability, or activates immunogenic cell death in the cancer. In some ~~embodiment~~examples, the administration is performed in a dose and a schedule effective to increase expression of at least two cytokines in PBMCs of the subject. In some ~~embodiment~~examples, the administration is performed in a dose and a schedule effective to increase expression of at least two cytokines in cancer cells in the subject. In some ~~embodiment~~examples, the at least two cytokines comprise IFN-γ, IL-2, IL-6, IL-10, IL-12, or TNF-α. In some ~~embodiment~~examples, the administration is performed in a dose and a schedule effective to reduce volume of the cancer

by at least 10%. In some ~~embodiment~~examples, wherein the administration is performed in a dose and a schedule effective to reduce growth of the cancer by at least 10%.

[0017] Disclosed herein, in some ~~aspect~~examples, is a myxoma virus (MYXV) having enhanced anti-cancer activity, wherein the MYXV is genetically engineered to attenuate an activity or expression level of its M153 protein.

[0018] In some ~~embodiment~~examples, the activity or the expression level of the M153 protein is attenuated at least 80%. In some ~~embodiment~~examples, the MYXV is engineered to introduce a mutation in a nucleic acid encoding the M153 protein, wherein the mutation comprises an insertion, deletion, or substitution mutation. In some ~~embodiment~~examples, at least a portion of a nucleic acid encoding the M153 protein in MYXV genome is knocked out. In some ~~embodiment~~examples, the MYXV comprises an inhibitory molecule targeting M153 transcript that thereby attenuates the M153 protein expression, wherein the inhibitory molecule comprises dsRNA, siRNA, antisense RNA, or miRNA. In some ~~embodiment~~examples, the MYXV is further genetically engineered to express a non-viral molecule. In some ~~embodiment~~examples, the non-viral molecule is a cytokine or a cell matrix protein. In some ~~embodiment~~examples, the non-viral molecule is tumor necrosis factor alpha (TNFα), interleukin-12 subunit alpha (IL-12α), interleukin-12 subunit beta (IL-12β), or decorin. In some ~~embodiment~~examples, the non-viral molecule is a human protein. In some ~~embodiment~~examples, the MYXV expresses at least two non-viral molecules selected from a group consisting of TNFα, IL 12α, IL-12β, and decorin.

[0019] Disclosed herein, in some ~~aspect~~examples is a composition comprising a plurality of cells treated ex vivo by a MYXV, wherein the MYXV is genetically engineered to attenuate an activity or expression level of its M153 protein, and to express a non-viral molecule.

[0020] In some ~~embodiment~~examples, the plurality of cells comprises peripheral blood mononuclear cells (PBMCs), bone marrow (BM) cells, or a combination thereof.

[0021] Disclosed herein, in some ~~aspect~~examples, is recombinant nucleic acid comprising at least a portion of MYXV genome, wherein the portion of the MYXV genome is modified to reduce expression of M153 gene.

[0022] In some ~~embodiment~~examples, the portion of MYXV genome is modified to knock out at least a portion of the M153 gene in the portion of MYXV genome. In some ~~embodiment~~examples, the recombinant nucleic acid comprises a nucleic acid encoding a non-viral molecule. In some ~~embodiment~~examples, the non-viral molecule is human TNFα, human IL-12α (hTNFα), human IL-12β, or human decorin. In some ~~embodiment~~examples, the nucleic acid encoding the non-viral molecule comprises a vMyx-hTNFα cassette, and the nucleic acid encoding the non-viral molecule replaces or is adjacent to an M135R gene of the MYXV genome. In some ~~embodiment~~examples, the nucleic acid encoding the non-viral molecule comprises a vMyx-hTNFα cassette, and the nucleic acid encoding the non-viral molecule is inserted between an M135R gene and an M136R gene of the

MYXV genome. In some ~~embodiment~~examples, the nucleic acid encoding the non-viral molecule comprises an hDecorin-hIL-12 cassette, and the nucleic acid encoding the non-viral molecule replaces at least a portion of the M153 gene of the MYXV genome. In some ~~embodiment~~examples, the nucleic acid encoding the non-viral molecule comprises a vMyx-hTNFα-hDecorin-hIL-12-M153KO (vMyx-Triple) cassette.

[0023] Disclosed herein, in some ~~aspect~~examples is a method of inhibiting, alleviating, or preventing a cancer in a subject in need thereof, comprising administering to the subject a MYXV or a plurality of cells treated with the MYXV, wherein the MYXV is genetically engineered to attenuate an activity or expression level of its M153 protein.

[0024] In some ~~embodiment~~examples, the MYXV is further genetically engineered to express a non-viral molecule. In some ~~embodiment~~examples, the non-viral molecule is tumor necrosis factor alpha (TNFα), interleukin-12 subunit alpha (IL-12α), interleukin-12 subunit beta (IL-12β), or decorin. In some ~~embodiment~~examples, the MYXV or the plurality of cells is administered by systemic administration. In some ~~embodiment~~examples, the administration reduces tumor cell viability, or activates immunogenic cell death in the cancer. In some ~~embodiment~~examples, the cancer is a solid tumor, an osteosarcoma, triple negative breast cancer, or melanoma. In some ~~embodiment~~examples, the cancer has metastasized to a lung, a brain, a liver or a lymph node in the subject. In some ~~embodiment~~examples, the method further comprises administering to the subject an immune checkpoint modulator. In some ~~embodiment~~examples, the administration is performed in a dose and a schedule effective to increase expression of at least two cytokines in PBMC or cancer cells of the subject, wherein the at least two cytokines comprise IFN-γ, IL-2, IL-6, IL-10, IL-12, or TNF-α. In some ~~embodiment~~examples, the administration is performed in a dose and a schedule effective to reduce volume of the cancer at least 10%.

[0025] Disclosed herein, in some ~~aspect~~examples, is a myxoma virus (MYXV) having enhanced anti-cancer activity, wherein the myxoma virus is genetically engineered to attenuate an activity or expression level of its M153 protein.

[0026] In some ~~embodiment~~examples, the activity or the expression level of the M153 protein is attenuated at least 80%. In some ~~embodiment~~examples, the MYXV is engineered to introduce a mutation to in a nucleic acid encoding the M153 protein, wherein the mutation comprises an insertion, deletion, or substitution mutation. In some ~~embodiment~~examples, at least a portion of a nucleic acid encoding the M153 protein in MYXV genome is knocked out. In some ~~embodiment~~examples, the MYXV comprises an inhibitory molecule targeting M153 transcript that thereby attenuates the M153 protein expression, wherein the inhibitory molecule comprises dsRNA, siRNA, antisense RNA, or miRNA. In some ~~embodiment~~examples, the MYXV further comprises a nucleic acid encoding a non-viral molecule. In some ~~embodiment~~examples, the non-viral molecule is tumor necrosis factor alpha (TNFα), interleukin-12 subunit alpha (IL-12α), interleukin-12 subunit beta (IL-12β), or decorin. In some

~~embodiment~~examples, the non-viral molecule is a human protein. In some ~~embodiment~~examples, the nucleic acid encodes at least two non-viral molecules selected from a group consisting of TNFα, IL 12α, IL-12β, and decorin.

[0027] Disclosed herein, in some ~~aspect~~examples, is a composition comprising a plurality of cells treated ex vivo by a MYXV, wherein the MYXV is genetically engineered to attenuate an activity or expression level of its M153 protein, and wherein the plurality of cells comprises peripheral blood mononuclear cells (PBMCs), bone marrow (BM) cells, or a combination thereof.

[0028] In some ~~embodiment~~examples, the composition is for use in inhibiting, alleviating, or preventing a cancer in a subject in need thereof, wherein the plurality of cells comprises cells that are autologous to the subject. In some ~~embodiment~~examples, the composition is for use in inhibiting, alleviating, or preventing a cancer in a subject in need thereof, wherein the plurality of cells comprises cells that are allogeneic to the subject.

[0029] Disclosed herein, in some ~~aspect~~examples, is a MYXV for use in inhibiting, alleviating, or preventing a cancer in a subject in need thereof, wherein the MYXV is genetically engineered to attenuate an activity or expression level of its M153 protein.

[0030] In some ~~embodiment~~examples, the MYXV is administered to the subject by systemic administration. In some ~~embodiment~~examples, the MYXV reduces cancer cell viability, or activates immunogenic cell death in the cancer. In some ~~embodiment~~examples, the cancer is a solid tumor, an osteosarcoma, triple negative breast cancer, or melanoma. In some ~~embodiment~~examples, the cancer has metastasized to a lung, a brain, a liver or a lymph node in the subject. In some ~~embodiment~~examples, the MYXV is administered to the subject with an immune checkpoint modulator. In some ~~embodiment~~examples, the MYXV is administered in a dose and a schedule effective to increase expression of at least two cytokines in PBMC or cancer cells of the subject, wherein the at least two cytokines comprise IFN-γ, IL-2, IL-6, IL-10, IL-12, or TNF-α. In some ~~embodiment~~examples, the MYXV is administered in a dose and a schedule effective to reduce volume of the cancer at least 10%.

[0031] Disclosed herein, in some ~~aspect~~examples is a recombinant nucleic acid comprising at least a portion of MYXV genome, wherein the portion of the MYXV genome is modified to reduce expression of M153.

[0032] In some ~~embodiment~~examples, the portion of MYXV genome is modified to knock out at least a portion of the M153 gene in the portion of MYXV genome. In some ~~embodiment~~examples, the recombinant nucleic acid comprises a nucleic acid encoding a non-viral molecule. In some ~~embodiment~~examples, the non-viral molecule is tumor necrosis factor alpha (TNFα), interleukin-12 subunit alpha (IL-12α), interleukin-12 subunit beta (IL-12β), or decorin. In some ~~embodiment~~examples, the non-viral molecule is a human protein. In some ~~embodiment~~examples, the nucleic acid encoding the non-viral molecule is inserted between an M135R gene and an M136R gene of the MYXV

genome. In some ~~embodiment~~examples, the nucleic acid encoding the non-viral molecule is inserted to replace at least a portion of the M153 gene of the MYXV genome. In some ~~embodiment~~examples, the nucleic acid encoding the non-viral molecule comprises a vMyx-hTNFα cassette, optionally comprising GFP, and the nucleic acid encoding the non-viral molecule replaces or is adjacent to an M135R gene of the MYXV genome. In some ~~embodiment~~examples, the nucleic acid encoding the non-viral molecule comprises a vMyx-hTNFα cassette, and the nucleic acid encoding the non-viral molecule is inserted between an M135R gene and an M136R gene of the MYXV genome. In some ~~embodiment~~examples, the nucleic acid encoding the non-viral molecule comprises an hDecorin-hIL-12 cassette, optionally comprising dsRed, and the nucleic acid encoding the non-viral molecule replaces at least a portion of the M153 gene of the MYXV genome. In some ~~embodiment~~examples, the nucleic acid encoding the non-viral molecule comprises a vMyx-hTNFα-hDecorin-hIL-12-M153KO (vMyx-Triple) cassette, optionally comprising dsRed.

BRIEF DESCRIPTION OF THE DRAWINGS

[0033] The novel features of certain embodiments of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0034] Fig. 1A is a schematic diagram showing construction of recombinant nucleic acids that can be used to generate myxoma viruses (MYXV) disclosed herein. The top image depicts a recombinant nucleic acid that comprises a vMyx-hTNFa-GFP cassette, and an M153 locus. The recombinant nucleic acid can be present in the genome of a MYXV of the disclosure, for example, a vMyx-hTNFa-GFP virus. The middle image shows a recombinant nucleic acid comprising the hDecorin-hIL-12-dsRed cassette with flanking sequences that correspond to sequences that flank the M153 gene in the MYXV genome (M152 and M154). This recombinant nucleic acid can be present in a recombination plasmid that can be introduced into MYXV to generate a modified recombinant virus. The bottom image shows a modified vMyx-Triple recombinant nucleic acid. The recombinant nucleic acid can be present in the genome of a MYXV of the disclosure. For example, the recombination plasmid depicted in the middle image can be introduced into a MYXV (e.g., by infecting RK13 cells with vMyx-hTNFa-GFP virus, and transfecting the cells with the recombination plasmid). Recombination can occur to generate the vMyx-Triple recombinant nucleic acid. Recombinant viruses that comprise the vMyx-Triple recombinant nucleic acid can be identified and purified based on a selection marker, for example, dsRed.

CLAIMS

WHAT IS CLAIMED IS:

1. A myxoma virus (MYXV) having enhanced anti-cancer activity, wherein the myxoma virus is genetically engineered to attenuate an activity or expression level of its M153 protein.

2. The MYXV of claim 1, wherein the activity or the expression level of the M153 protein is attenuated at least 80%.

3. The MYXV of claim 1 or claim 2, wherein the MYXV is engineered to introduce a mutation in a nucleic acid encoding the M153 protein, wherein the mutation comprises an insertion, deletion, or substitution mutation.

4. The MYXV of claim 3, wherein the mutation enhances cell immune response activity in relation to a wild-type M153 protein.

5. The MYXV of claim 1, wherein at least a portion of a nucleic acid encoding the M153 protein in MYXV genome is knocked out.

6. The MYXV of claim 1, wherein the MYXV comprises an inhibitory molecule targeting M153 transcript that thereby attenuates the M153 protein expression.

7. The MYXV of claim 6, wherein the inhibitory molecule is an inhibitory RNA.

8. The MYXV of claim 7, wherein the inhibitory RNA comprises dsRNA, siRNA, antisense RNA, or miRNA.

9. The MYXV of any one of claims 1-8, further comprising a nucleic acid encoding a non-viral molecule.

10. The MYXV of claim 9, wherein the non-viral molecule is tumor necrosis factor alpha (TNFα).

11. The MYXV of claim 10, wherein the TNFα is human TNFα.

12. The MYXV of claim 10 or 11, wherein the TNFα is a soluble peptide.

13. The MYXV of any of claims 10-12, wherein the TNFα is a membrane- or surface-bound peptide.

14. The MYXV of any of claims 10-13, wherein the TNFα further enhances the anti-cancer activity of the MYXV by activating anti-tumor immune cells or inducing cancer cell death.

15. The MYXV of claim 9, wherein the non-viral molecule is interleukin-12 (IL-12).

16. The MYXV of claim 15, wherein the IL-12 is human IL-12.

17. The MYXV of claim 15 or claim 16, wherein the IL-12 is a soluble peptide.

18. The MYXV of any one of claims 15-17, wherein the IL-12 is a membrane- or surface-bound peptide.

19. The MYXV of any of claims 15-18, wherein the IL-12 further enhances the anti-cancer activity of the MYXV by promoting immune cell differentiation or eliciting immune cell cytotoxicity.

20. The MYXV of claim 9, wherein the non-viral molecule is decorin.

~~21. The MYXV of claim 20, wherein the decorin is human decorin.~~

~~22. The MYXV of claim 20 or 21, wherein the decorin is a soluble peptide.~~

~~23. The MYXV of any of claims 20-22, wherein the decorin is a membrane- or surface-bound peptide.~~

~~24. The MYXV of any of claims 20-23, wherein the decorin further enhances the anti-cancer activity of the MYXV by blocking or decreasing TGF-β signaling.~~

~~25. The MYXV of claim 9, wherein the nucleic acid encodes at least two molecules selected from a group consisting of TNFα, IL-12, and decorin.~~

~~26. The MYXV of claim 9, wherein the nucleic acid encodes TNFα, IL-12, and decorin.~~

~~27. The MYXV of any of claims 1-24, wherein the MYXV is derived from a *Lausanne* strain.~~

~~28. A composition comprising a MYXV of any of claims 1-27, and a pharmaceutically acceptable carrier or excipient.~~

~~29. The composition of claim 28, formulated for systemic administration.~~

~~30. The composition of claim 28, formulated for local administration.~~

~~31. The composition of any one of claims 28-30, formulated for parenteral administration.~~

~~32. A composition comprising a plurality of cells treated *ex vivo* by the MYXV of any one of claims 1-27, wherein the plurality of cells comprises peripheral blood mononuclear cells (PBMCs), bone marrow (BM) cells, or a combination thereof.~~

~~33. A method of inhibiting, alleviating, or preventing a cancer in a subject in need thereof, comprising administering to the subject the MYXV of any one of claim 1-27 or the composition of any one of claims 28-32.~~

~~34. The method of claim 33, wherein the subject has, is suspected of having, or is at risk of having the cancer, and wherein the method further comprises selecting the subject.~~

~~35. The method of claim 33 or claim 34, wherein the subject is a human.~~

~~36. The method of any one of the claims 33-35, wherein the administration is systemic administration.~~

~~37. The method of any one of the claims 33-36, wherein the administration reduces cancer cell viability, or activates immunogenic cell death in the cancer.~~

~~38. The method of any one of the claims 33-37, wherein the administration improves the subject's survival.~~

~~39. The method of any one of the claims 33-38, wherein the cancer comprises a solid tumor.~~

~~40. The method of any one of the claims 33-38, wherein the cancer is an osteosarcoma, triple negative breast cancer, or melanoma.~~

~~41. The method of any one of the claims 33-40, wherein the cancer has metastasized to a location in the subject.~~

~~42. The method of claim 41, wherein the location comprises a lung, a brain, a liver and/or a lymph node of the subject.~~

~~43. The method of any one of the claims 33-42, further comprising administering to the subject an additional therapeutic agent.~~

~~44. The method of claim 43, wherein the additional therapeutic agent is an immune checkpoint modulator.~~

~~45. The method of claim 43 or claim 44, wherein the additional therapeutic agent is administered to the subject before administering the composition.~~

~~46. The method of claim 43 or claim 44, wherein the additional therapeutic agent is administered to the subject after administering the composition.~~

~~47. The method of claim 43 or claim 44, wherein the additional therapeutic agent is administered to the subject as a combination with the composition.~~

~~48. The method of any one of claims 33-47, wherein the composition comprising the plurality of cells is administered to the subject, wherein the plurality of cells comprises cells that are autologous to the subject.~~

~~49. The method of any one of claims 33-47, wherein the composition comprising the plurality of cells is administered to the subject, wherein the plurality of cells comprises cells that are allogenic to the subject.~~

~~50. A recombinant nucleic acid comprising at least a portion of MYXV genome, wherein the MYXV genome is modified to reduce expression of M153 gene.~~

~~51. The recombinant nucleic acid of claim 50, wherein the recombinant nucleic acid comprises DNA.~~

~~52. The recombinant nucleic acid of claim 50 or claim 51, wherein the portion of MYXV genome is modified to knock out at least a portion of the M153 gene in the portion of MYXV genome.~~

~~53. The recombinant nucleic acid of any of claims 50-52, wherein the recombinant MYXV genome comprises a first nucleic acid encoding TNFα.~~

54. The recombinant nucleic acid of claim 53, wherein the TNFα is human TNFα.

55. The recombinant nucleic acid of claim 53 or claim 54, wherein the first nucleic acid replaces or is adjacent to an M135R gene of the MYXV genome.

56. The recombinant nucleic acid of any of claims 53-55, wherein first nucleic acid is inserted between an M135R gene and an M136R gene of the MYXV genome.

57. The recombinant nucleic acid of any of claims 53-56, wherein expression of the first nucleic acid is driven by a poxvirus synthetic early/late (sE/L) promoter.

58. The recombinant nucleic acid of any of claims 50-57, wherein the recombinant MYXV genome comprises a second nucleic acid encoding interleukin-12 subunit alpha (IL-12α).

59. The recombinant nucleic acid of claim 58, wherein the IL-12α is human IL-12α.

60. The recombinant nucleic acid of claim 58 or claim 59, wherein expression of IL-12α is driven by an internal ribosome entry site (IRES).

61. The recombinant nucleic acid of any of claims 58-60, wherein the second nucleic acid IL-12α disrupts the expression of the M153 gene of the MYXV genome.

62. The recombinant nucleic acid of any of claims 50-61, wherein the recombinant MYXV genome comprises a third nucleic acid encoding an interleukin-12 subunit beta (IL-12β).

63. The recombinant nucleic acid of claim 62, wherein the IL-12β is human IL-12β.

64. The recombinant nucleic acid of claim 62 or claim 63, wherein expression of the third nucleic acid is driven by an sE/L promoter.

65. The recombinant nucleic acid of any of claims 62-64, wherein the third nucleic acid disrupts the expression of the M153 gene of the MYXV genome.

66. The recombinant nucleic acid of any of claims 50-65, wherein the recombinant MYXV genome comprises a fourth nucleic acid encoding decorin.

67. The recombinant nucleic acid of claim 66, wherein the decorin is human decorin.

68. The recombinant nucleic acid of claim 66 or claim 67, wherein expression of fourth nucleic acid is driven by an sE/L promoter.

69. The recombinant nucleic acid of any of claims 66-68, wherein the fourth nucleic acid disrupts expression of the M153 gene of the MYXV genome.

70. The recombinant nucleic acid of any of claims 50-69, further comprising a fifth nucleic acid encoding a reporter tag.

71. The recombinant nucleic acid of claim 70, wherein the reporter tag comprises a green fluorescent protein (GFP).

72. The recombinant nucleic acid of claim 70 or claim 71, wherein expression of the fifth nucleic acid is driven by an sE/L promoter.

73. The recombinant nucleic acid of claim 70, further comprising a sixth nucleic acid encoding a second reporter tag.

74. The recombinant nucleic acid of claim 73, wherein the second reporter tag comprises a red fluorescent protein (RFP).

75. The recombinant nucleic acid of claim 73 or claim 74, wherein expression of the sixth nucleic acid is driven by a poxvirus P11 late promoter.

76. The recombinant nucleic acid of any of claims 50-75, wherein the recombinant nucleic acid comprises a vMyx-hTNFa cassette, optionally comprising GFP.

77. The recombinant nucleic acid of any of claims 50-76, wherein the recombinant nucleic acid comprises an hDecorin-hIL-12 cassette, optionally comprising dsRed.

78. The recombinant nucleic acid of any of claims 50-77, wherein the recombinant nucleic acid comprises or consists of a vMyx-hTNFa-hDecorin-hIL-12-M153KO (vMyx-Triple) cassette, optionally comprising dsRed and/or GFP.

79. A recombinant MYXV comprising the recombinant nucleic acid of any of claims 50-78.

80. A method of inhibiting, alleviating, or preventing a cancer in a subject in need thereof, comprising administering to the subject the recombinant nucleic acid of any one of claims 50-79 or the recombinant MYXV of claim 79.

81. The method of claim 80, wherein the subject has, is suspected of having, or is at risk of having the cancer, and wherein the method further comprises selecting the subject.

82. The method of claims 80 or 81, wherein the subject is a human.

83. The method of any one of claims 80-82, wherein the administration is systemic administration.

84. The method of any one of the claims 80-83, wherein the administration reduces cell viability, or activates immunogenic cell death in the cancer.

85. The method of any one of the claims 35-49 or any one of claims 80-84, wherein the administration is performed in a dose and a schedule effective to increase expression of at least two cytokines in PBMCs of the subject.

86. The method of any one of the claims 35-49 or any one of claims 80-84, wherein the administration is performed in a dose and a schedule effective to increase expression of at least two cytokines in cancer cells in the subject.

87. The method of claim 85 or claim 86, wherein the at least two cytokines comprise IFN-γ, IL-2, IL-6, IL-10, IL-12, or TNF-α.

~~88. The method of any one of the claims 35-49 or any one of claims 80-87, wherein the administration is performed in a dose and a schedule effective to reduce volume of the cancer by at least 10%.~~

~~89. The method of any one of the claims 35-49 or any one of claims 80-87, wherein the administration is performed in a dose and a schedule effective to reduce growth of the cancer by at least 10%.~~

~~90. The MYXV of claim 15, wherein the IL-12 comprises an IL-12α subunit and an IL-12β subunit.~~

~~91. The MYXV of claim 90, wherein the IL-12α subunit and the IL-12β subunit are joined by a polypeptide linker.~~

~~92. The MYXV of claim 91, wherein the polypeptide linker is an elastin linker.~~

~~93. The MYXV of claim 15, wherein the MYXV expresses a low level of the IL-12.~~

~~94. The MYXV of claim 15, wherein the MYXV expresses a high level of the IL-12.~~