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

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

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

[0010] Disclosed herein, in some examples is a composition comprising a plurality of cells treated *ex vivo* by the MYXV of any of the above 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 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 examples.

[0012] In some 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 examples, the subject is a human. In some examples, the administration is systemic administration. In some examples, the administration reduces cancer cell viability, or activates immunogenic cell death in the cancer. In some examples, the administration improves the subject's survival. In some examples, the cancer comprises a solid tumor. In some examples, the cancer is an osteosarcoma, triple negative breast cancer, or melanoma. In some examples, the cancer has metastasized to a location in the subject. In some examples, the location comprises a lung, a brain, a liver and/or a lymph node of the subject. In some examples, the method further comprises administering to the subject an additional therapeutic agent. In some examples, the additional therapeutic agent is an immune checkpoint modulator. In some examples, the additional therapeutic agent is administered to the subject before administering the composition. In some examples, the additional therapeutic agent is administered to the subject after administering the composition. In some examples, the additional therapeutic agent is administered to the subject as a combination with the composition. In some 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 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 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 examples, the recombinant nucleic acid comprises DNA. In some 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 examples, the recombinant MYXV genome comprises a first nucleic acid encoding TNFα. In some examples, the TNFα is human TNFα. In some examples, the first nucleic acid replaces or is adjacent to an M135R gene of the MYXV genome. In some examples, the first nucleic acid is inserted between an M135R gene and an M136R gene of the MYXV genome. In some examples, expression of the first nucleic acid is driven by a poxvirus synthetic early/late (sE/L) promoter. In some examples, the recombinant MYXV genome comprises a second nucleic acid encoding interleukin-12 subunit alpha (IL-12α). In some examples, the IL-12α is human IL-12α. In some examples, expression of IL-12α is driven by an internal ribosome entry site (IRES). In some examples, the second nucleic acid

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

[0015] Disclosed herein, in some 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 examples,

[0016] In some 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 examples, the subject is a human. In some examples, the administration is systemic administration. In some examples, the administration reduces cell viability, or activates immunogenic cell death in the cancer. In some 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 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 examples, the at least two cytokines comprise IFN-γ, IL-2, IL-6, IL-10, IL-12, or TNF-α. In some examples, the administration is performed in a dose and a schedule effective to reduce volume of the cancer

by at least 10%. In some 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 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 examples, the activity or the expression level of the M153 protein is attenuated at least 80%. In some 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 examples, at least a portion of a nucleic acid encoding the M153 protein in MYXV genome is knocked out. In some 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 examples, the MYXV is further genetically engineered to express a non-viral molecule. In some examples, the non-viral molecule is a cytokine or a cell matrix protein. In some 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 examples, the non-viral molecule is a human protein. In some 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 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 examples, the plurality of cells comprises peripheral blood mononuclear cells (PBMCs), bone marrow (BM) cells, or a combination thereof.

[0021] Disclosed herein, in some 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 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 examples, the recombinant nucleic acid comprises a nucleic acid encoding a non-viral molecule. In some examples, the non-viral molecule is human TNFα, human IL-12α (hTNFα), human IL-12β, or human decorin. In some 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 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 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 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 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 examples, the MYXV is further genetically engineered to express a non-viral molecule. In some 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 examples, the MYXV or the plurality of cells is administered by systemic administration. In some examples, the administration reduces tumor cell viability, or activates immunogenic cell death in the cancer. In some examples, the cancer is a solid tumor, an osteosarcoma, triple negative breast cancer, or melanoma. In some examples, the cancer has metastasized to a lung, a brain, a liver or a lymph node in the subject. In some examples, the method further comprises administering to the subject an immune checkpoint modulator. In some 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 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 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 examples, the activity or the expression level of the M153 protein is attenuated at least 80%. In some 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 examples, at least a portion of a nucleic acid encoding the M153 protein in MYXV genome is knocked out. In some 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 examples, the MYXV further comprises a nucleic acid encoding a non-viral molecule. In some 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

examples, the non-viral molecule is a human protein. In some 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 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 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 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 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 examples, the MYXV is administered to the subject by systemic administration. In some examples, the MYXV reduces cancer cell viability, or activates immunogenic cell death in the cancer. In some examples, the cancer is a solid tumor, an osteosarcoma, triple negative breast cancer, or melanoma. In some examples, the cancer has metastasized to a lung, a brain, a liver or a lymph node in the subject. In some examples, the MYXV is administered to the subject with an immune checkpoint modulator. In some 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 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 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 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 examples, the recombinant nucleic acid comprises a nucleic acid encoding a non-viral molecule. In some 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 examples, the non-viral molecule is a human protein. In some 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 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 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 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 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 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.