Armed Myxoma Virus Demonstrates Transgene Expression, Efficacy, and Immune System Modulation in Syngeneic Tumor Models

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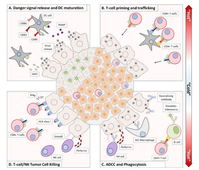
BACKGROUND

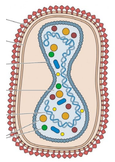
Oncolytic viruses (OV) selectively replicate in and lyse tumor cells and provide stimulation to the immune system. This represents a promising therapeutic option for cancer patients that do not respond well to treatment with immune checkpoint inhibitors. Myxoma virus (MYXV) is a member of the Poxviridae family of double stranded DNA viruses. The natural host of MYXV is a subset of lagomorphs, but MYXV can infect cancer cell lines of humans and other species. The genome of MYXV is relatively large and is amenable to engineering for expression of transgenes making it an excellent oncolytic virus for introduction of immunomodulatory proteins. The current work describes the oncolytic activity, transgene production capability, in vivo activity, and immunomodulatory mechanism of actions following intratumoral (IT) and intravenous (IV) administration of armed myxoma viruses in murine cancer models.

Oncolytic Virus



Oncolytic viruses:
- Kill cancer cells directly
- Release tumor antigens
- Promote inflammation to turn cold tumors hot

Myxoma



Myxoma is a rabbit pox virus:
- Large, dsDNA allows engineering of multiple payload genes
- Not pathogenic to humans
- Suitable for IV delivery

Arming

Arming selected that:
- Target additional points around cancer immunity cycle
- IL-12: T- & NK-cell modulator
- Decorin: multifunctional microenvironment modulator

MULTI-ARMED MYXOMA PRODUCES MULTIPLE TRANSGENES IN DOSE AND TIME RESPONSIVE MANNER

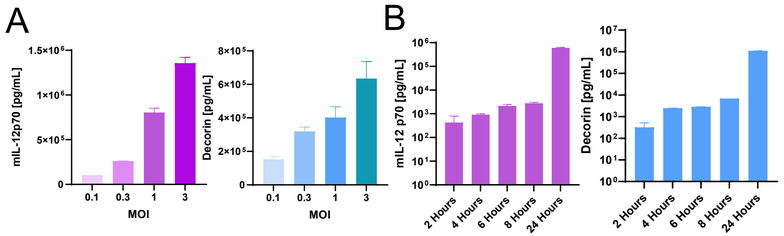


Figure 1. Multi-armed myxoma virus produces functional transgenes in a dose and time responsive manner in Vero cells

A) Vero cells were incubated with vMYX-hIL-12/Dec at the indicated MOI for 24 hours. Cell culture supernatant was harvested at 24 hours and subjected to ELISA for the indicated transgenes. B) Vero cells were incubated with vMYX-hIL-12/Dec at the MOI=1 for the indicated time. Cell culture supernatant was harvested and subjected to ELISA for the indicated transgenes.

MULTI-ARMED MYXOMA IS EFFICACIOUS IN SYNGENEIC MODELS FOLLOWING IT OR IV DELIVERY

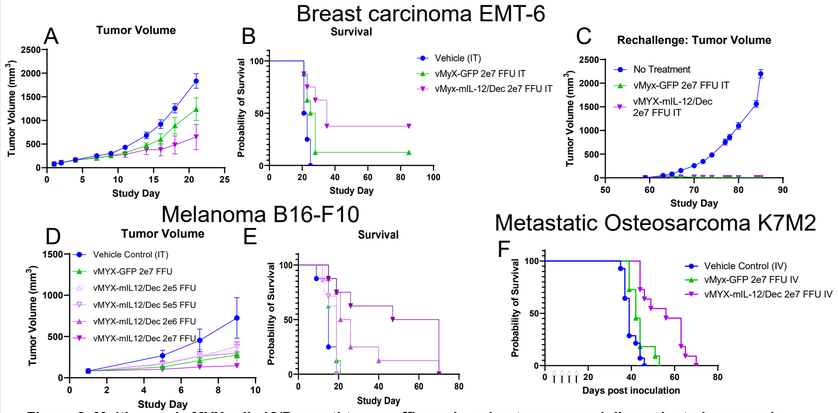


Figure 2. Multi-armed vMYX-mIL-12/Dec anti-tumor efficacy in subcutaneous and disseminated syngeneic murine tumor models

Anti-tumor efficacy and resistance to rechallenge following treatment with armed myxoma virus. Balb/c mice were implanted subcutaneously with of $1x10^6$ EMT-6 cells in the right flank. Tumor bearing animals were randomized into treatment groups of 8 animals per group with an average tumor volume of 60-90 mm^3. A) Animals were treated via intratumoral (IT) injection of $2x10^7$ FFU/dose once every four days for four doses post-randomization with the indicated myxoma virus. B) Animals treated in A were assessed for survival. Survival endpoints were met when tumor volume ≥ $1500mm^3$ for individual animals or when animals met IACUC guidelines for terminal sacrifice. C) Animals that were still surviving at 59 Days post initial treatment were implanted subcutaneously with of $1x10^6$ EMT-6 cells on the left flank as a tumor rechallenge. Tumor volume measurements were recorded three times per week. Animals treated with MYXV and armed MYXV were resistant to tumor rechallenge.
Dose responsive anti-tumor efficacy of vMYX mouse double in subcutaneous B16-F10 mouse melanoma. C57BL/6 mice were implanted subcutaneously with $1x10^6$ B16-F10 melanoma cells. Tumor bearing animals were randomized into treatment groups of 8 animals per group with an average tumor volume of 75-100 mm^3. D) Animals were treated via intratumoral (IT) injection of the indicated FFU/dose on Day 1 and Day 8 post-randomization with the indicated dose of the indicated virus. E) Animals treated in A were assessed for survival. Survival endpoints were met when tumor volume ≥ $1500mm^3$ for individual animals or when animals met IACUC guidelines for terminal sacrifice.
Anti-tumor efficacy of vMYX mouse double armed virus following intravenous delivery in K7M2-Luc mouse disseminated osteosarcoma model. F) Balb/c mice were implanted with $2x10^6$ K7M2-Luc osteosarcoma cells via intravenous injection in the tail vein. Animals were treated via intravenous injection of the indicated virus at $2x10^7$ FFU/dose once every 4 days for 4 doses (Q4Dx4) with the indicated myxoma virus beginning on Day 3 post injection of tumor cells. Animals on both studies were assessed for survival. Survival endpoints were met when clinical symptoms of animals met clinical score symptoms or IACUC guidelines for terminal sacrifice.

In vivo studies were performed by Translational Drug Development (TD2) or Arizona State University and were governed by the corresponding IACUC protocols. Special thanks to Jessica Dalsing-Hernandez at TD2.

MULTI-ARMED MYXOMA MINIMALLY INDUCES ANTI-MYXV nAb RESPONSE AS COMPARED TO ANTI-MYXV TOTAL IgG

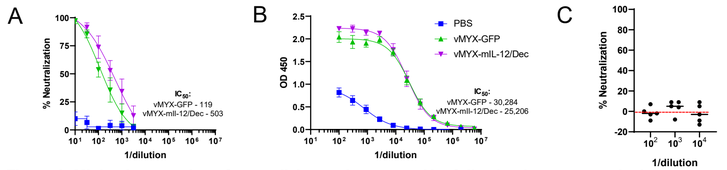


Figure 3. Minimal generation of neutralizing anti-myxoma Abs following intravenous dosing

Non-tumor bearing C57BL/6 mice were dosed IV with $2x10^7$ FFU of the indicated myxoma virus on d1 and d8. Serum samples were collected on day 0, 15, 22 and 29 and analyzed for anti-MYXV neutralizing antibodies via PRNT and anti-MYXV total IgG via ELISA.
A) d29 serum samples from each group were diluted from 1:10 (10^{-1}) to 1:3,162 ($10^{-3.5}$) and mixed with equal amount of myxoma virus. Neutralization was allowed to proceed for 1 h and this serum/virus mixture was then used to inoculate vero cells. Following the addition of an overlay, the infected cells were incubated for 48 h at which time the number of foci per treatment was counted and % neutralization calculated. Data are represented as mean ± STD of triplicate wells for each animal (N = 5 per group). B) $1x10^8$ FFU/ml of vMYX-GFP virus was coated overnight and challenged with d29 serum samples from each treatment group. Sera were diluted from 1:100 to 1: 5,904,900 and total anti-MYXV IgG was detected using HRP-conjugated anti-mouse IgG. Data are represented as mean ± STD (N = 5 per group). C) Human sera evaluated for anti-MYXV nAb via PRNT as described in (A). Data are represented as mean of duplicate wells representing 5 individual donors.

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MULTI-ARMED MYXOMA ACTIVATES IMMUNE SYSTEM

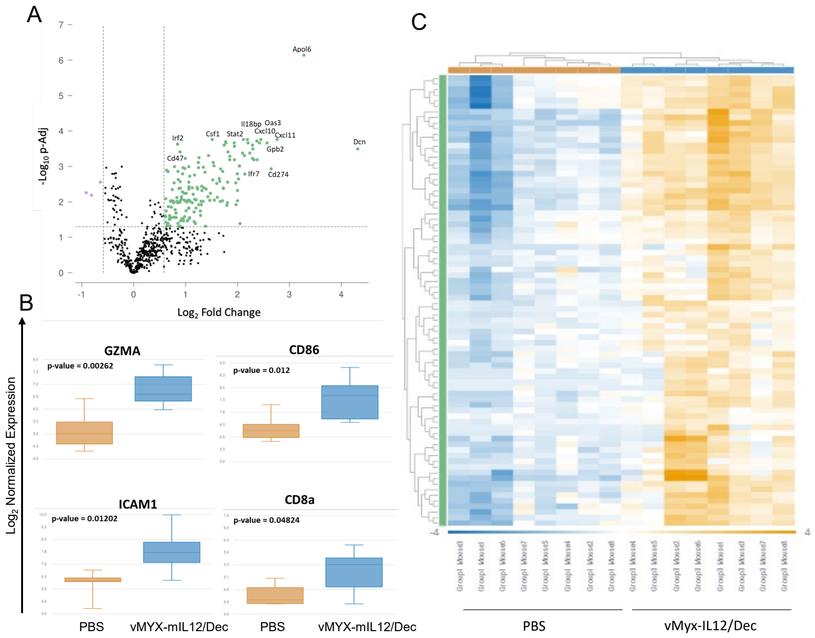

Figure 4. Multi-armed vMYX-mIL-12/Dec upregulates genes associated with immune response

B16-F10 tumor bearing C57BL/6 mice were treated via IT injection with $2x10^7$ FFU/dose of the indicated myxoma virus on d1 and d7. Tumor samples were harvested on d8 and gene expression profile was assessed using the Nanostring PanCancer IO 360 panel and analyzed using ROSALIND (https://rosalind.onramp.bio/).
A) Gene expression profile showing distribution of upregulated genes (green upper right section) and down regulated genes (purple, upper left section) in multi-armed MYXV as compared to vehicle. B) Examples of upregulated genes associated with T cell activation, following multi-armed MYXV treatment. Expression in samples from PBS treated animals are show in yellow and from vMYX-mIL-12/Dec are shown in blue. C) Summary of gene expression profile from BioPlanet Immune System pathway, comparing multi-armed MYXV to vehicle treated mice (N = 8 per group). Fold-change of ±1.5 = p-Adj ≤ 0.05.

CONCLUSIONS

Myxoma is a large dsDNA pox virus suitable for oncolytic virotherapy, is engineerable to carry multiple transgenic payloads, and is not pathogenic to humans

Multi-armed myxoma demonstrates efficacy in multiple subcutaneous and metastatic syngeneic tumor models following intratumoral or intravenous delivery

Minimal generation of neutralizing anti-myxoma Abs following intravenous administration of multi-armed myxoma virus in mice. Additionally, humans do not exhibit pre-existing nAb against MYXV.

Evidence of modulation of the immune response to favor anti-tumor immunity including upregulation of IFNα, IFNγ, and IL-12 response pathways following administration of multi-armed myxoma virus