Abstract 742

Multi-Armed Myxoma Virus Induces a Potent Anti-Tumor Response in vitro and in vivo

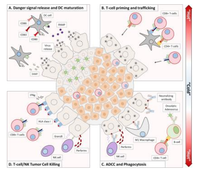
Lina S. Franco, Wazir Abdullahi, Heather Hrach, Mario Abrantes, Nicole Grigaitis, Zachary Tacner, Dillon McCrite, Rachel Dutcher Lino E. Torres-Dominguez, Christopher Fraser, Leslie L. Sharp
OncoMyx Therapeutics, 850 N. 5th Street, Suite 402, Phoenix, AZ 85004

OncoMyx Therapeutics

BACKGROUND

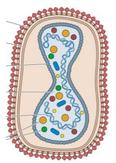
Myxoma virus (MYXV) has been shown to selectively infect cancer cells in humans in vitro and inhibit tumor growth in mice. The genome of MYXV is amenable to engineering for expression of transgenes. We armed MYXV with mouse or human IL-12 and human decorin. IL-12 is an immune modulator. Cellular responses to decorin include tumor cell intrinsic signaling effects, tumor matrix remodeling, and inhibition of the TGF-β pathway. We hypothesized that MYXV armed with decorin and IL-12 would be an effective anti-tumor therapy. The current work describes oncolytic activity and transgene expression following exposure to multi-armed MYXV to human cancer cell lines in vitro, and in vivo efficacy in murine models, as single agents and in combination with immune checkpoint inhibition.

Oncolytic Virus



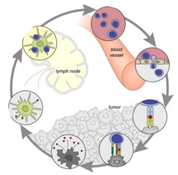
Oncolytic Viruses:
- Kill cancer cells directly
- Release tumor antigens
- Promote inflammation to turn cold tumors hot

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
- Complementary to approved immune checkpoint inhibitors

MULTI-ARMED MYXV REPLICATES IN MULTIPLE HUMAN CANCER CELL LINES

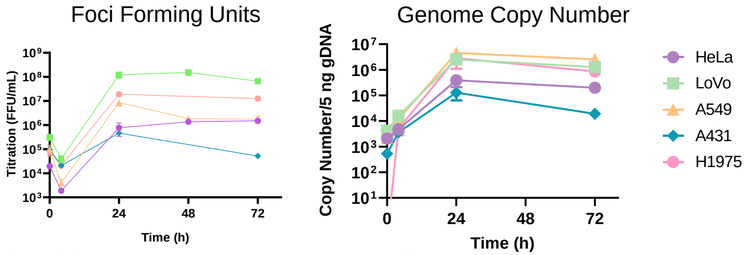


Figure 1. Replication of multi-armed myxoma virus in different human cancer cell lines

Replication of vMyx-hIL-12/Dec was evaluated by determining the infectious foci forming units (FFU) in Vero cells using serial dilution after homogenizing the infected cells from each cell line (left). Genome copy number (right) was determined via qPCR using 5ng of total genomic DNA isolated from infected cells. Absolute quantification of vial genome copy number was calculated using a standard curve. Biological and technical replicate were evaluated in triplicate. Each cell line was infected with MOI 10 and samples were taken at the designated time points. Each sample was divided to be used for the two assays.

MULTI-ARMED MYXV EXPRESS TRANSGENES AND INDUCE GROWTH INHIBITION IN MULTIPLE HUMAN CANCER CELL LINES

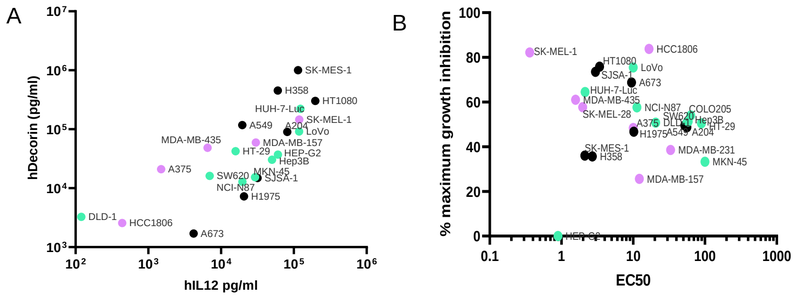


Figure 2. Multi-armed myxoma virus expresses hIL-12 and decorin, and inhibits growth of multiple human cancer cell lines

A) Human cancer cell lines were infected with vMyx-hIL-12/dec and transgene expression in supernatants determined via ELISA after infection at a MOI 1 for 24hrs. B) EC50 and maximum growth inhibition were determined via Cell Titer Glow assay after infection with vMyx-hIL-12/Dec in a 9-point multiplicity of infection (MOI) response curve after 72hrs. Lung and sarcoma cell lines are represented in black; breast and melanoma cancer cell lines are in purple; and colon, gastric and liver cancer cell lines are in green.

MULTI-ARMED MYXV TRANSGENES INHIBIT TGF–β SIGNALING AND INDUCE CASPASE-3 ACTIVATION

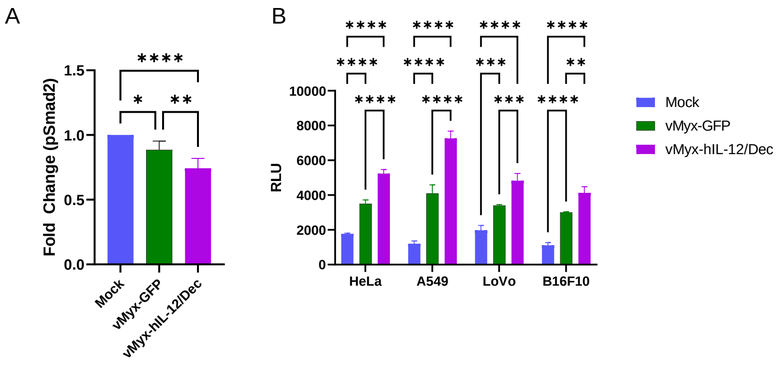


Figure 3 Transgene produced decorin suppresses TGF-β signaling and induces caspase-3 activity.

A.) Inhibition of TGFβ signaling: Vero cells were infected with vMyx-GFP or vMyx-hIL-12/Dec at MOI of 1 for 48 h in serum-free media. Cell culture supernatant was then harvested and passed through 0.1uM filter to generate virus-free supernatant which was used to treat HeLa cells. 1 h post treatment, cell lysate was harvested using RIPA buffer and pSmad2 level was measured via ELISA. B.) Induction of caspase-3 activity: Vero cells were infected with vMyx-GFP or vMyx-hIL-12/Dec at MOI of 3 for 72 h in complete media. Cell culture supernatant was then harvested and passed through 0.1uM filter to generate virus-free supernatant which was used to treat the indicated human and mouse tumor cells lines. Treatment was allowed to proceed for 24 h at 37°C and the level of caspase-3 activity was measured via luminescence

DO NOT POST

MULTI-ARMED MYXV IS EFFICACIOUS IN SYNGENEIC AND XENOGRAFT MOUSE CANCER MODELS

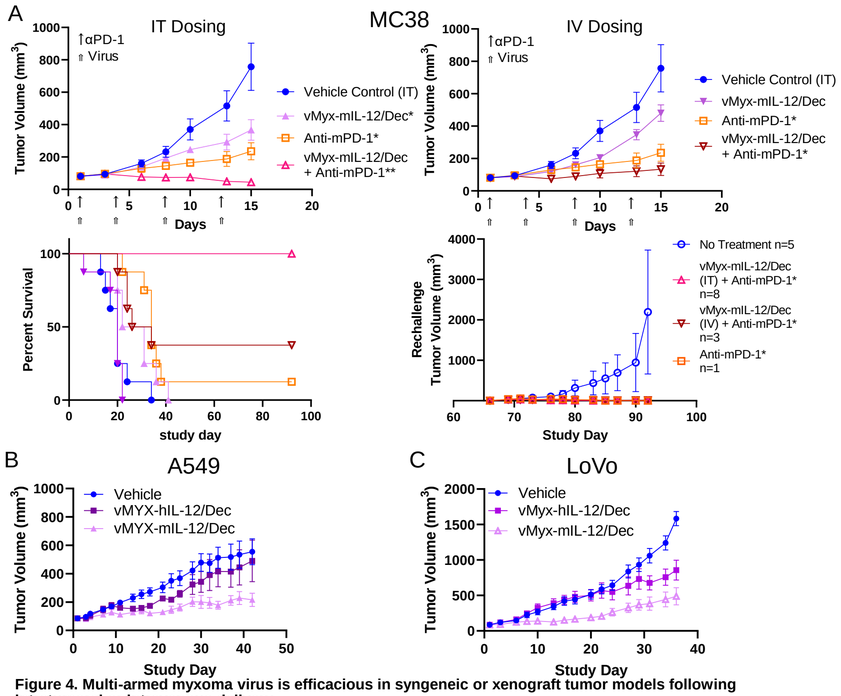


Figure 4. Multi-armed myxoma virus is efficacious in syngeneic or xenograft tumor models following intratumoral or intravenous delivery.

A) C57BL/6 mice were implanted subcutaneously with $1x10^6$ MC38 adenocarcinoma cells. Tumor bearing animals were randomized into treatment groups of n=8 with an average tumor volume of 75-100 mm^3. Animals were treated with virus at $2x10^7$ FFU/dose IT Q4Dx7, $1x10^8$ FFU/dose IV Q4Dx7, and/or Anti-PD-1 dosed 10mg/kg IP Q4Dx4 as indicated. 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. Complete responders were rechallenged with MC38 tumor cells at Day 66. B) Athymic nude mice were implanted subcutaneously with $5x10^6$ A549 cells. Tumor bearing animals were randomized into treatment groups of n=8 with an average tumor volume of 85 mm^3 and dosed intravenously with the indicated virus at $1x10^8$ FFU/dose IV Q4D. C) Athymic nude mice were implanted subcutaneously with $1x10^7$ LoVo cells. Tumor bearing animals were randomized into treatment groups of n=8 with an average tumor volume of 85 mm^3 and dosed with the indicated virus at $2x10^7$ FFU/dose IT Q4D. These studies were approved by TD2 IACUC.

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 MYXV effectively infects multiple human tumor cell lines, expresses transgenes, suppresses TGFβ signaling, and induces caspase-3 activity

Multi-armed MYXV demonstrates efficacy in syngeneic and xenograft tumor models following intratumoral or intravenous delivery and combinatorial efficacy with immune checkpoint inhibitors

Immune modulation is a key component of the anti-tumor activity of multi-armed MYXV *in vivo*