Armed Myxoma Virus Demonstrates Transgene Production, Function, and Therapeutic Activity in Xenograft Models

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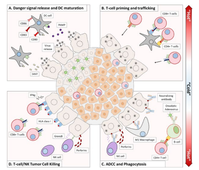
BACKGROUND

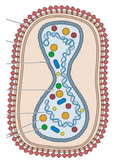
Oncolytic viruses (OV) selectively replicate in and lyse tumor cells and provide stimulation to the immune system. This represents a promising therapeutic option for cancer patients that do not respond well to treatment with immune checkpoint inhibitors. Myxoma virus (MYXV) is a member of the Poxviridae family of double stranded DNA (dsDNA) 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, and in vivo activity following administration of armed vMYX-hIL-12/Decorin (Dec) in human 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 poxvirus:
- 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 vMYX-hIL-12/Dec PRODUCES MULTIPLE TRANSGENES IN DOSE AND TIME RESPONSIVE MANNER

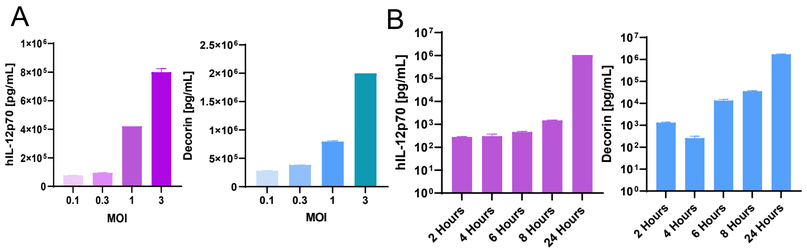


Figure 1. Multi-armed myxoma virus produces functional transgenes in a dose and time responsive manner in Vero cells

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 vMYX-hIL-12/Dec PRODUCES MULTIPLE TRANSGENES IN MULTIPLE CANCER CELLS

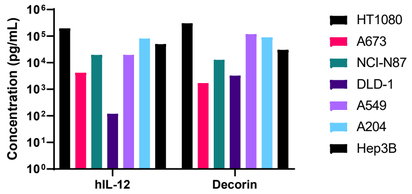


Figure 2. Transgene expression of the virus in human cancer cell lines.

The indicated cell lines were infected with vMYX-hIL-12/Dec at MOI=1 and cell culture supernatants were collected after 24hrs of infection. Transgene expression was evaluated via ELISA and numbers indicate pg/mL.

MULTI-ARMED MYXOMA vMYX-hIL-12/Dec PRODUCES FUNCTIONAL TRANSGENES

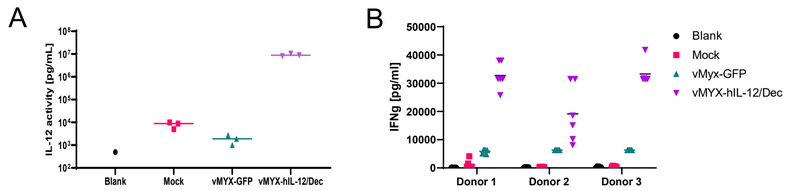


Figure 3. Multi-armed myxoma virus produces biofunctional hIL-12

Biofunctional expression of hIL-12 was determined by the activation of downstream proteins. A) Vero cells were infected for 24 hours at MOI=1 with vMYX-hIL-12/Dec and cell culture supernatant was collected at the end of the incubation period. Cell culture supernatant was measured for functional IL-12 activity using an IL-12 responsive reporter cell line. B) Infection of Vero cells with vMYX-hIL-12/Dec was performed at a MOI 1. Supernatants of Vero cells were collected 24hrs later and filtered to eliminate virus in suspension. Human PBMCs isolated from whole blood were posteriorly exposed to the mentioned supernatants for 7 days. Supernatants of human PBMCs were collected and analyzed for IFNγ production using ELISA.

MULTI-ARMED MYXOMA vMYX-hIL-12/Dec IS CYTOTOXIC IN HUMAN CANCER CELL LINES IN VITRO

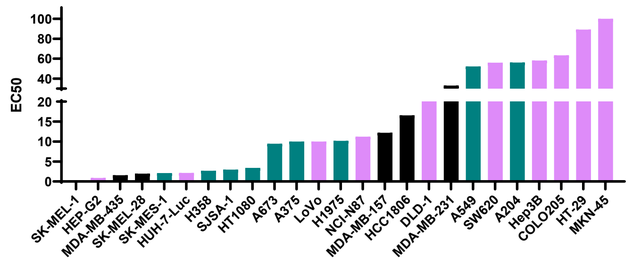


Figure 4. Multi-armed myxoma virus is cytotoxic in a wide variety of human cancer cell lines in vitro

EC50 of different human cancer cell lines was determined by measuring growth inhibition via Cell Tier Glow assay after infection with vMYX-hIL-12/Dec in a 9-point multiplicity of infection dose response curve. Lung and sarcoma cancer cell lines are represented in green; breast and melanoma cancer cell lines are in black; and, colon, gastric, and liver cancer cell lines are in purple.

MULTI-ARMED MYXOMA DEMONSTRATES ANTI-TUMOR EFFICACY AND TRANSGENE PRODUCTION IN A HUMAN XENOGRAFT MODEL

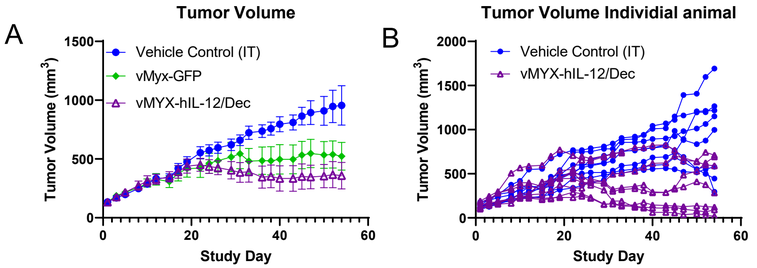


Figure 5. Anti-tumor efficacy of multi-armed vMYX-hIL-12/Dec in a xenograft A549 human lung cancer.

Immunodeficient animals were implanted with $5x10^6$ A549 human non-small cell lung cancer cells subcutaneously on the flank. Tumor bearing animals were randomized when the mean tumor volume was 100-150 mm^3, n=8/group. Tumor bearing animals were treated via intratumoral (IT) injection of $2x10^7$ FFU/dose of the indicated virus once every 4 days to end. Survival endpoints were met when tumor volume ≥ 1500mm^3 for individual animals or when animals met IACUC guidelines for terminal sacrifice. A) Average tumor volume, error bars represent SEM B) Individual animal tumor volumes following treatment with vehicle or vMYX-hIL-12/Dec.

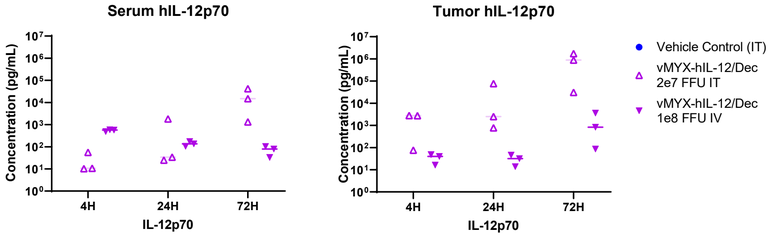


Figure 6. Multi-armed myxoma virus expresses transgenic proteins in vivo.

Immunodeficient animals were implanted with $5x10^6$ A549 human non-small cell lung cancer cells subcutaneously on the flank. Tumor bearing animals were randomized when the mean tumor volume was 100-150 mm^3. Tumor bearing animals were treated via intratumoral (IT) injection of $2x10^7$ FFU/dose of the virus or intravenous (IV) injection of $1x10^8$ FFU/dose on Day 1 (n=3 animals per group). Serum (left panel) and tumor (right panel) samples were collected at 4-, 24-, or 72- hours post dose and processed for cytokine sampling. Cytokine analysis was performed using MesoScale Discovery (MSD) U-Plex 6-assay 96-Well SECTOR plates. Symbols represent individual animals, line represents mean.

CONCLUSIONS

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

Multi-armed myxoma produces multiple, biofunctional transgenes in dose and time responsive manner in Vero cells

Multi-armed myxoma demonstrates efficacy and transgene production in multiple *in vitro* and *in vivo* human tumor models

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