Abstract 409

Armed Myxoma Virus Demonstrates Therapeutic Efficacy in Xenograft Models

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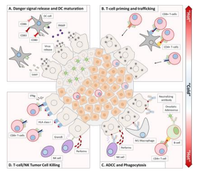
BACKGROUND

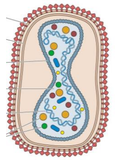
Oncolytic viruses (OV) selectively replicate in and lyse tumor cells and provide stimulation to the immune system. This represents a promising therapeutic option for cancer patients that do not respond well to treatment with immune checkpoint inhibitors. Myxoma virus (MYXV) is a member of the Poxviridae family of double stranded DNA 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



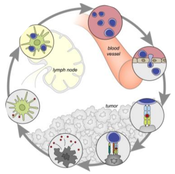
Oncolytic viruses:
- Kill cancer cells directly
- Release tumor antigens
- Promote inflammation to turn cold tumors hot

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
- Complementary to approved immune checkpoint inhibitors

MULTI-ARMED MYXOMA VIRUS REPLICATES AND KILLS TUMOR CELLS

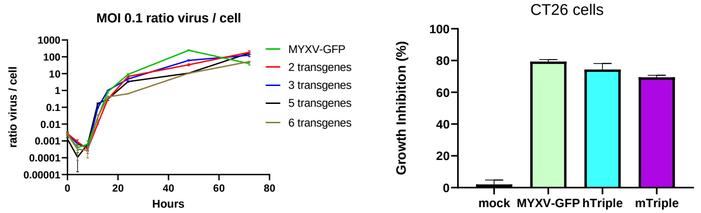


Figure 1. Multi-armed myxoma virus has similar replicative and oncolytic capacity as parental virus

Left panel: Vero cells were infected with myxoma viruses containing different numbers of transgenes at a multiplicity of infection (MOI) MOI=0.1 and incubated for up to 72 hours. Cells and culture supernatant was harvested at the indicated time. The titration was calculated by seeding serial dilutions in Vero cells and counting the number of foci forming units at 48 hours post infection.

Right panel: CT26 mouse cancer cells were incubated with viruses containing GFP only (MYXV-GFP), or GFP+ three additional human and/or mouse transgenes at MOI=10. Cell growth inhibition was measured using MTS assay at 48 hours post infection

MULTI-ARMED MYXOMA EFFICIENTLY PRODUCES MULTIPLE TRANSGENES

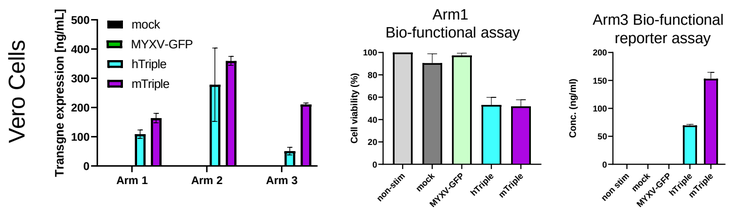


Figure 2. Multi-armed myxoma virus produces functional transgenes in Vero cells

Vero cells were incubated with the indicated virus at MOI=1 for 24 hours. Cell culture supernatant was harvested at 24 hours and subjected to ELISA for each of the transgenes (Left panel), a bio-functional assay related to the mechanism of action of arm 1 (middle panel) or a bio-functional reporter assay specific for Arm 2 (right panel).

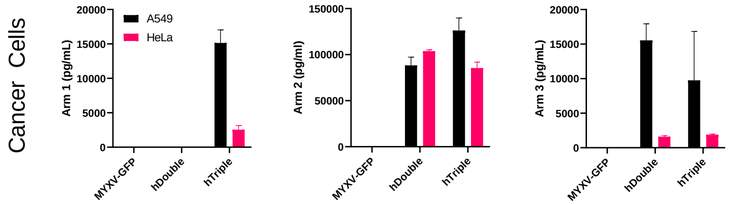


Figure 3. Multi-armed myxoma virus produces transgenes in cancer cells

A549 human lung carcinoma (black) or HeLa human cervical adenocarcinoma (pink) were incubated with the Myxoma virus containing GFP (MYXV-GFP), a single transgene encoding 2 (hDouble), or 3 (hTriple) additional transgenes, at MOI=1 for 24 hours. Cell culture supernatant was harvested at 24 hours and subjected to ELISA for each of the arm 1 transgenes (Left panel), Arm 2 transgene (middle panel), or Arm 3 transgene (right panel).

MULTI-ARMED MYXOMA IS CYTOTOXIC VS. MULTIPLE HUMAN TUMOR TYPES IN VITRO

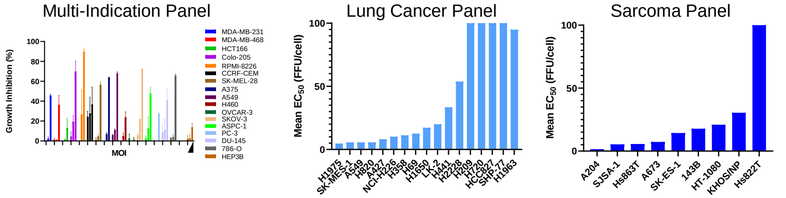


Figure 4. Multi-armed myxoma virus is oncolytic across a wide variety of human cancer cell lines in vitro

Left Panel: The indicated cell line was incubated with MOI=0.1, 1, or 10 FFU/cell for 72 hours. Cell viability was determined using Cell Titer Glow (CTG). Surviving fraction was determined by dividing the mean luminescence values so the Test agents (T) by the mean luminescence of Controls (C). Percent growth inhibition was calculated as (1-T/C) x100%

Middle Panel (lung cancer) and Right Panel (sarcoma): The indicated cell line assessed for cell proliferation vs. a nine-point MOI dose response curve following 72 hours of co-incubation. Cell viability was determine using CTG. Data is expressed as the 50% of the maximum response inhibition to control determined from the CTG luminescence signal. The surviving fraction of cells was determined by dividing the mean luminescence values of the test agents by the mean luminescence values of untreated controls.

DO NOT POST

MULTI-ARMED MYXOMA DEMONSTRATES ANTI-TUMOR EFFICACY IN HUMAN XENOGRAFT MODELS

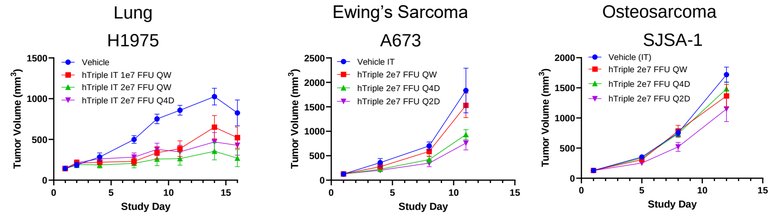


Figure 5. Multi-armed myxoma virus demonstrates anti-tumor efficacy in multiple human xenograft models

The indicated cell line, NCI-H1975 (left panel), A673 (middle panel), or SJSA-1 (right panel) were inoculated into the flank of immunodeficient mice. When tumors reached ~100-150mm^3 tumor bearing animals were randomized into treatment groups and injected intratumorally with vehicle (PBS) or the indicated dose of multi-armed myxoma virus containing three human transgenes (hTriple) on the indicated schedule until the end of the study. Tumor volume was measured via calipers three times per week.

INCREASED NECROSIS FOLLOWING TREATMENT WITH MULTI-ARMED MYXOMA VIRUS

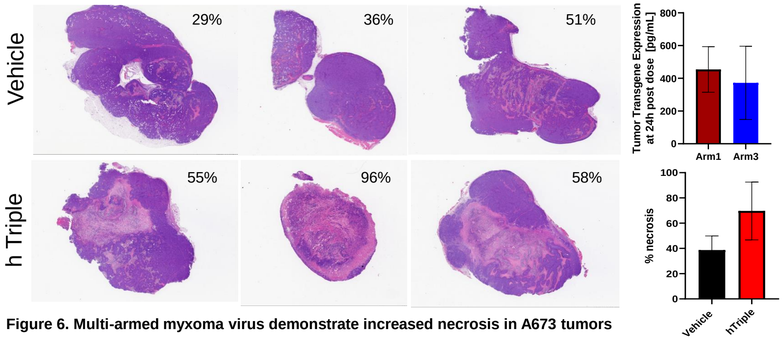


Figure 6. Multi-armed myxoma virus demonstrate increased necrosis in A673 tumors

FFPE samples from A673 tumors treated with Vehicle or hTriple multi-armed myxoma virus were generated and stained with hematoxylin and eosin. Necrosis was measured using pattern recognition with computer trained image analysis (Qpath software). Area based measurements after pattern recognition of tumor and necrotic regions are shown.

Transgene expression of transgenes 1 and 3 at 24-hour post dose was determined via MSD multiplex ELISA.

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 is able to produce multiple, bio-functional transgenes *in vitro* and *in vivo*

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

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