Abstract 5618

Multi-Armed Myxoma Virus has Therapeutic Potential for Treatment of Multiple Myeloma

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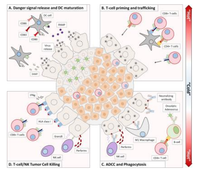
BACKGROUND

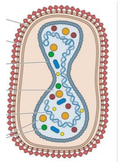
Despite improvements with new therapeutics, multiple myeloma (MM) patients still relapse and become refractory. Myxoma viruses (MYXV) selectively replicate in human tumor cells and stimulate the immune system. MYXV can infect and kill MM cells. This represents a promising therapeutic option for MM patients that do not respond well to immunotherapy. Immune dysfunction in MM is caused by multiple factors that potentially may be overcome by therapeutic approaches. MYXV can be multi-armed without impacting viral function or replication. We generated MYXV carrying IL-12 and decorin. IL-12 is an immune modulator. Responses to decorin include tumor cell intrinsic signaling effects and microenvironment modulation. We hypothesized that MYXV armed with IL-12 and decorin could be an effective anti-MM therapy. We show that armed MYXV infects and kills human MM cells *in vitro* and reduces growth of a disseminated syngeneic and xenograft models MM *in vivo*.

Oncolytic Virus



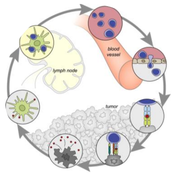
Oncolytic Viruses:
- Kill cancer cells directly
- Release tumor antigens
- Promote inflammation to turn cold tumors hot

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 INFECTS AND EXPRESSES TRANSGENES IN MOUSE MULTIPLE MYELOMA CELL LINE MOPC 315

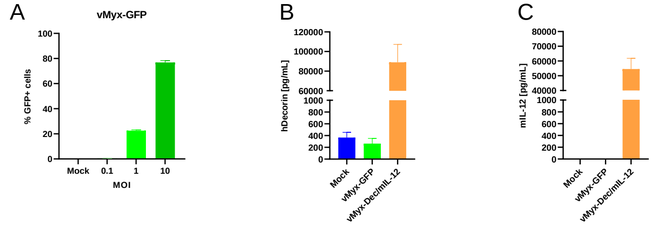
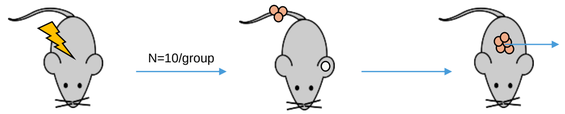


Figure 1. Multi-armed MYXV infects and expresses transgenes in mouse MM cell line MOPC 315

A) MOPC 315 cells were infected with vMyx-GFP for 24 hours at the indicated multiplicity of infection (MOI) and GFP expression was measured by flow cytometry. Supernatants from infections with vMyx-GFP and vMyx-Dec/mIL-12 for 24 hours at MOI 1 were collected and analyzed by ELISA for B) human Decorin and C) mouse IL-12. Biological and technical replicates were performed in duplicate.

MULTI-ARMED MYXV INDUCES AN IMMUNOMODULATORY RESPONSE IN A MULTIPLE MYELOMA SYNGENEIC MOUSE MODEL



sublethally irradiated
Day -14

5x10^6 MOPC 315 IV
Day - 13

1x10^8 FFU/dose IV
Days 1, 5, 9 and 12

Day 13
Bone marrow harvest for flow cytometry

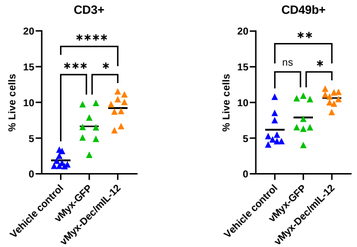


Figure 2. Multi-armed MYXV induces an immunomodulatory response in MOPC 315 mouse MM model

MOPC 315 cells were inoculated intravenously (IV) one day after Balb/c mice were sublethally irradiated (450 cGy). Fourteen days later (Day 1), animals were IV injected with vehicle control or 1x10^8 FFU/dose of the indicated virus. Dosing was repeated on Days 5, 9 and 12. Bone marrow was harvested 24 hours after the last dose treatment, stained for T (CD3) and NK (CD49b) cells markers and analyzed by flow cytometry.

In vivo studies were performed by Translational Drug Development (TD2) and governed by TD2 IACUC protocol. Special thanks to Jessica Dalsing-Hernandez at TD2.

MULTI-ARMED MYXV INFECTS AND EXPRESSES TRANSGENES IN A 3D CULTURE OF HUMAN PRIMARY MULTIPLE MYELOMA BONE MARROW

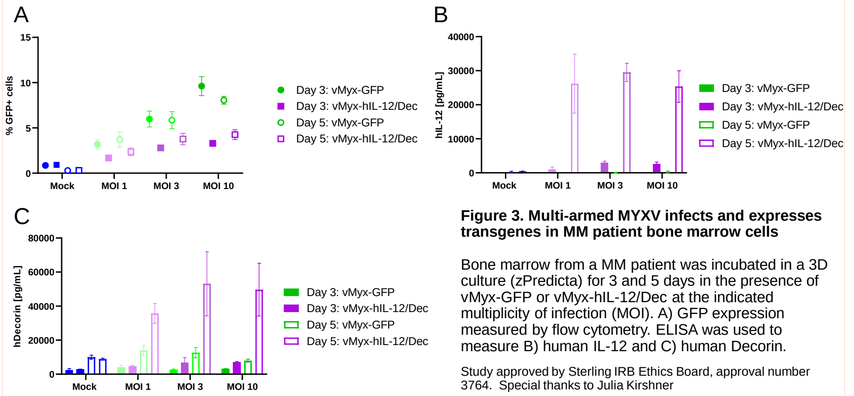


Figure 3. Multi-armed MYXV infects and expresses transgenes in MM patient bone marrow cells

Bone marrow from a MM patient was incubated in a 3D culture (zPredicta) for 3 and 5 days in the presence of vMyx-GFP or vMyx-hIL-12/Dec at the indicated multiplicity of infection (MOI). A) GFP expression measured by flow cytometry. ELISA was used to measure B) human IL-12 and C) human Decorin.

Study approved by Sterling IRB Ethics Board, approval number 3764. Special thanks to Julia Kirshner

DO NOT POST

MULTI-ARMED MYXV EXPRESSES TRANSGENES AND INHIBITS HUMAN MULTIPLE MYELOMA CELL LINE MM1.S GROWTH IN VITRO

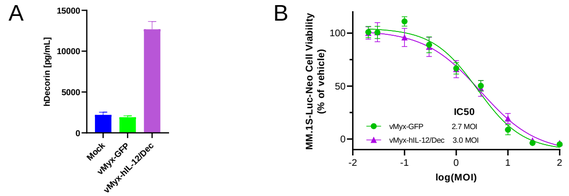
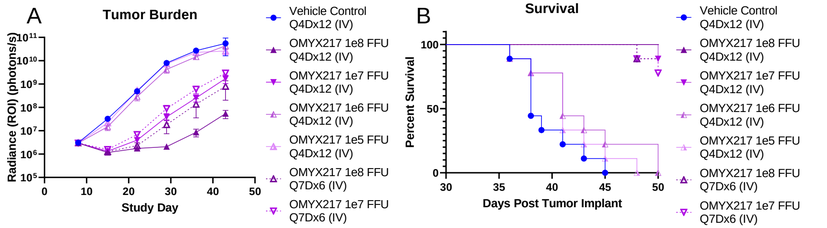


Figure 4. Multi-armed MYXV expresses transgenes and inhibits human MM cell line MM1.S growth *in vitro*

A) Human Decorin was measured by ELISA in the supernatants of MM1.S-Luc-Neo cells infected with vMyx-GFP and vMyx-hIL-12/Dec for 24 hours at multiplicity of infection (MOI) 1. B) MM1.S-Luc-Neo cells growth inhibition EC50 was determined via CellTiter-Glo® assay after a 72-hour infection with vMyx-GFP and vMyx-hIL-12/Dec in a 9-point MOI response curve.

MULTI-ARMED MYXV IS EFFICACIOUS IN A XENOGRAFT MULTIPLE MYELOMA MODEL IN A DOSE RESPONSIVE MANNER



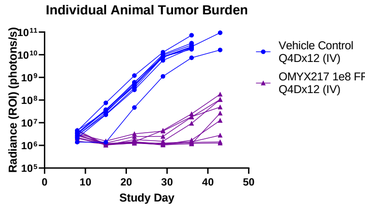


Figure 5. Multi-armed MYXV is efficacious in MM1.S-Luc-Neo human multiple myeloma in SCID Beige mice in a dose responsive manner

SCID Beige mice were intravenously (IV) implanted with 5x10^6 MM1.S-Luc-Neo cells. Animals were randomized by BLI on Day 8 and treated with vehicle control or vMyx-hIL-12/Dec IV at the indicated concentrations and dosage schedules (N=9/group). A) Disease was assessed via whole body bioluminescence imaging once per week. Group mean (top) and individual animal for control and high dose groups (bottom) are shown B) Animals were assessed for survival. Survival endpoints were met when animals met IACUC approved study protocol guidelines for terminal sacrifice.

Study approved by LabCorp IACUC.
Special thanks to Mariana Gonzalez Rodriguez.

CONCLUSIONS

MYXV 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 expressing IL-12 and decorin can infect a mouse MM cell line *in vitro* and efficiently induce an immunomodulatory response in the same MM mouse model. It infects human primary MM bone marrow cells and a human MM cell line *in vitro*. Finally, it demonstrates efficacy in a xenograft MM model.

Our data suggest there is significant value in pursuing vMYX-hIL-12/Dec and other armed MYXV as a new approach towards multiple myeloma therapy.