Abstract 744

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

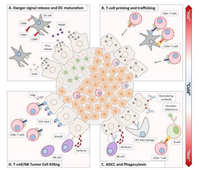
Lina S. Franco, Lino E. Torres-Dominguez, Heather Hrach, Christopher Fraser, Ana L. de Matos, Mario Abrantes, Nicole Grigaitis, Jack St. Peter, Zachary Tacner, Dillon McCrite, Rachel Dutcher, Leslie L. Sharp
OncoMyx Therapeutics, 850 N. 5th Street, Suite 402, Phoenix, AZ 85004

OncoMyx Therapeutics

BACKGROUND

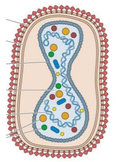
Despite improvements with new therapeutics, multiple myeloma (MM) patients still relapse and become refractory. Myxoma viruses (MYXV) selectively replicate in human tumor cells and stimulate the immune system. MYXV can infect and kill MM cells. This represents a promising therapeutic option for MM patients 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 model of mouse MM in vivo.

Oncolytic Virus



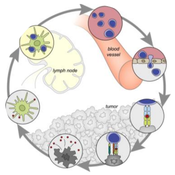
Oncolytic Viruses:
- Kill cancer cells directly
- Release tumor antigens
- Promote inflammation to turn cold tumors hot

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 REPLICATES IN U266 HUMAN MULTIPLE MYELOMA CELL LINE.

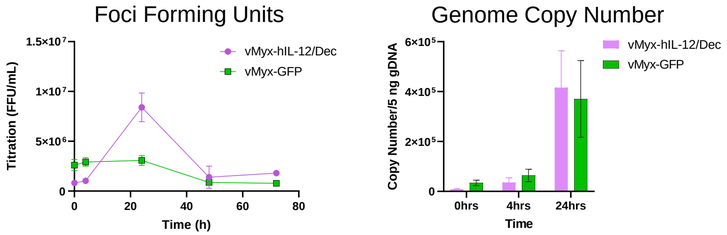


Figure 1. Multi-armed myxoma virus replicates in U266 human multiple myeloma cell line similarly as the unarmed virus.
Replication of each virus was evaluated by infecting U266 at MOI 10 and samples were taken at the designated time points. Each sample was divided and used for both assays. Infectious foci forming units were counted in Vero cells using serial dilution after homogenizing the infected U266 cells (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.

MULTI-ARMED MYXV EXPRESS TRANSGENES AND INDUCE GROWTH INHIBITION IN U266 MULTIPLE MYELOMA CELLS

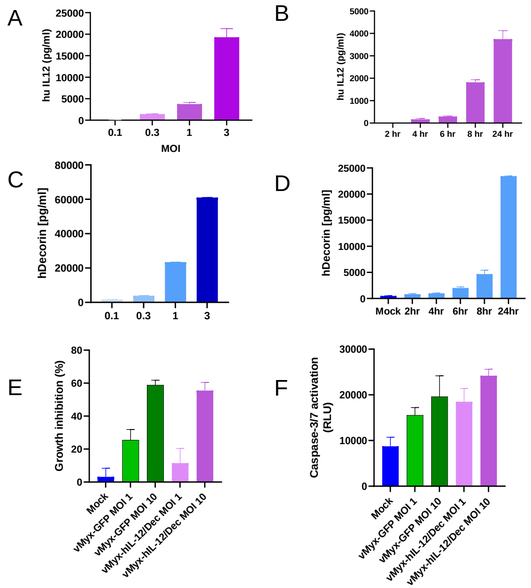


Figure 2. Multi-armed MYXV infects, expresses transgenes, and inhibits growth of the U266 MM cell line

U266 cells were infected with vMyx-hIL-12/Dec at the indicated MOI for 24 hours and supernatants collected for cytokine analysis A) for human IL-12 or C) for human decorin; Or infected at MOI 1 and supernatant was collected at the indicated times and subjected to ELISA for B) human IL-12 or D) human decorin. E) U266 cells were exposed to vMyx-hIL-12/Dec or vMyx-GFP at MOIs 1 and 10, tested by MTS at 24- and 48- hours. F) Apoptosis induction by vMyx-GFP and vMyx-hIL-12/Dec was tested by Caspase-Glo® 3/7 Assay in U266 cells infected at MOIs 1 and 10 at 48h.

MULTI-ARMED MYXV EXPRESS TRANSGENES AND INHIBIT GROWTH OF HUMAN MULTIPLE MYELOMA CELL LINES

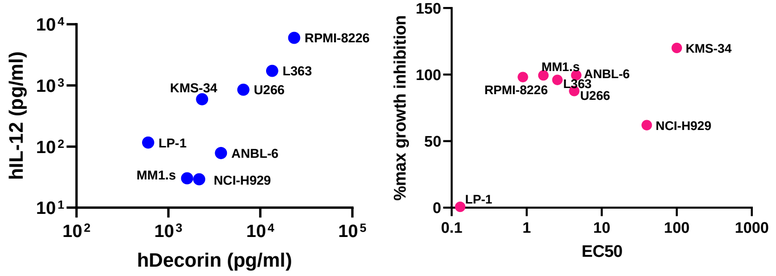


Figure 3. Multi-armed myxoma virus expresses human IL-12 and decorin, and inhibits growth of human multiple myeloma cell lines
Human multiple myeloma cell lines were infected with vMyx-hIL-12/dec and transgene expression in supernatants determined via ELISA after infection at a MOI 1 for 24hrs. EC50 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 72 hours.

DO NOT POST

MULTI-ARMED MYXV INHIBITS MOUSE MULTIPLE MYELOMA CELL LINE 5TGM1 GROWTH IN VITRO AND IN VIVO

A

Virus	EC50 (MOI)
vMyx-mIL-12/Dec	24.6

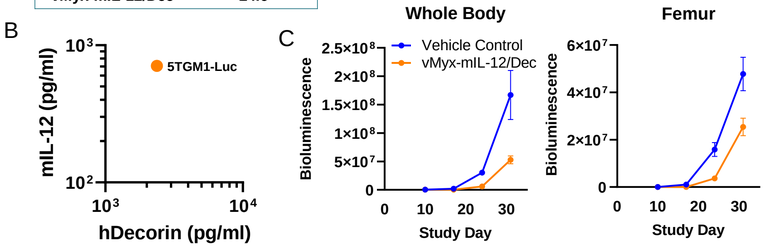


Figure 4. MYXV expressing mIL-12 and hDecorin inhibits 5TGM1-luc murine multiple myeloma cell line growth in vitro and in vivo

A) 5TGM1-luc growth inhibition EC50 was determined via Cell Titer Glow assay after infection with vMyx-mIL-12/Dec in a 9-point multiplicity of infection (MOI) response curve. B) In vitro transgene expression of virus infected 5TGM1-luc cell supernatant by ELISA. Infection was performed at a MOI 1 for 24hrs. C) C57BL/KaLwRijHsd mice injected IV with $5x10^6$ 5TGM-1-Luc via tail vein. Animals were treated with vehicle control or $1x10^8$ FFU/dose of vMyx-mIL-12/Dec IV Q4Dx4 starting on day 3. Bioluminescence imaging was recorded on days 10, 17, 24 and 31. This study was approved by LabCorp IACUC.

MULTI-ARMED MYXV INFECTS MYELOMA PLASMA CELLS IN A 3D CULTURE OF HUMAN PRIMARY MULTIPLE MYELOMA BONE MARROW

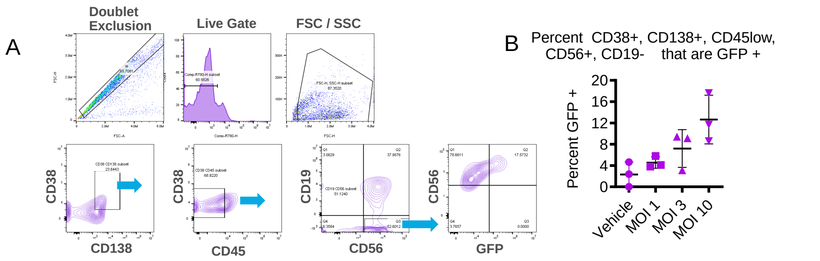


Figure 5. Multi-armed myxoma virus infects primary bone marrow multiple myeloma cells in vitro. Bone marrow from a multiple myeloma patient was incubated in a 3D culture (zPredicta) for 3 days in the presence of vMyx-hIL-12/Dec/GFP or vehicle and analyzed by flow cytometry. A) gating strategy and GFP expression. B) Phenotype of GFP+ cells are CD38+, CD138+, CD45low, CD56+, CD19neg. [This study was approved by Sterling IRB Ethics Board, approval number 3764]

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 virus expressing IL-12 and decorin can infect and kill human and mouse multiple myeloma cell lines in vitro, demonstrates efficacy in a mouse model of multiple myeloma, and can infect primary human multiple myeloma cells in vitro.

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.