



uOttawa

# Development and Characterization of a newly constructed cytokine producing oncolytic virus

Marina Fukano<sup>1,2</sup>, Sarwat T. Khan<sup>2,3</sup>, Michael A. Kennedy<sup>2</sup>, Rebecca C. Auer<sup>2-4</sup>

<sup>1</sup>Department of Chemistry and Biomolecular Sciences, University of Ottawa,

<sup>2</sup>Centre for Innovative Cancer Research, Ottawa Hospital Research Institute, Ottawa, ON

<sup>3</sup>Department of Biochemistry, Microbiology and Immunology, University of Ottawa

<sup>4</sup>Department of Surgery, Division of General Surgery, The Ottawa Hospital

## Abstract

Maraba is an oncolytic rhabdovirus that is currently being developed for the treatment of a variety of cancers due to the ability to selectively infect and replicate in cancer cells. Previous work from the Auer lab has shown that engineering MG1 to express Interleukin-12 (IL-12) triggers drastic anti-tumoural effects including the recruitment and activation of NK and T cells when delivered as part of an infected cell vaccine. Since the effect of MG1-IL12 in anti-tumour immunity is not applicable to all tumour types, a new MG1 containing additional immunostimulatory genes is being constructed, and the characterization of this virus (Virus X due to proprietary information) is being carried out such as: viral replication, cytotoxicity, and transgene expression, have performed.

## Introduction

### Immunotherapy

- ❖ Harnesses the patients' own immune system to fight cancers and other malignancies.
- ❖ Achieves this by stimulating immune responses, resulting in an increased level of natural killer (NK) cells and T cells activities.

### Maraba Virus MG1

- ❖ An engineered variant of the Maraba oncolytic viruses with mutations potentiating antitumor effects and safety profile.
- ❖ Developed for use in an infected cell vaccine (ICV) platform for treatment of peritoneal carcinomatosis by the Auer Lab
- ❖ Has an ability to selectively replicate in and kill cancerous cells without affecting normal cells.

### Natural Killer (NK) and T cells

- ❖ Potent lymphocytes of the innate immune system
- ❖ Play an important role in a long-term cancer surveillance,
- ❖ Reduced NK and T-cell activity can induce the formation of metastases, and recurrence after surgical debulking of solid tumors.

### Interleukin-12 (IL-12)

- ❖ A type of the cytokine expressed by leukocytes,
- ❖ Has a significant role in boosting the cytotoxic function of NK cells and T-cells
- ❖ The efficacy of this interleukin can be stimulated by other interleukins.

## Hypothesis

Newly developed MG-1 variant expressing immunostimulatory genes will increase efficacy of the ICV platform.

3'- N P M G mIL-12 Linker Gene X L -5'

A schematic of a newly engineered MG1 genome.

## Methodology

### Creating the virus

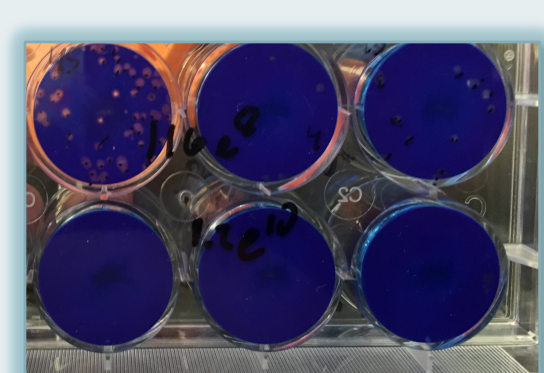
- ❖ Designing a plasmid
- ❖ Cloning the plasmid into MG1 backbone (Digestion, Ligation, Transformation, Transfection)



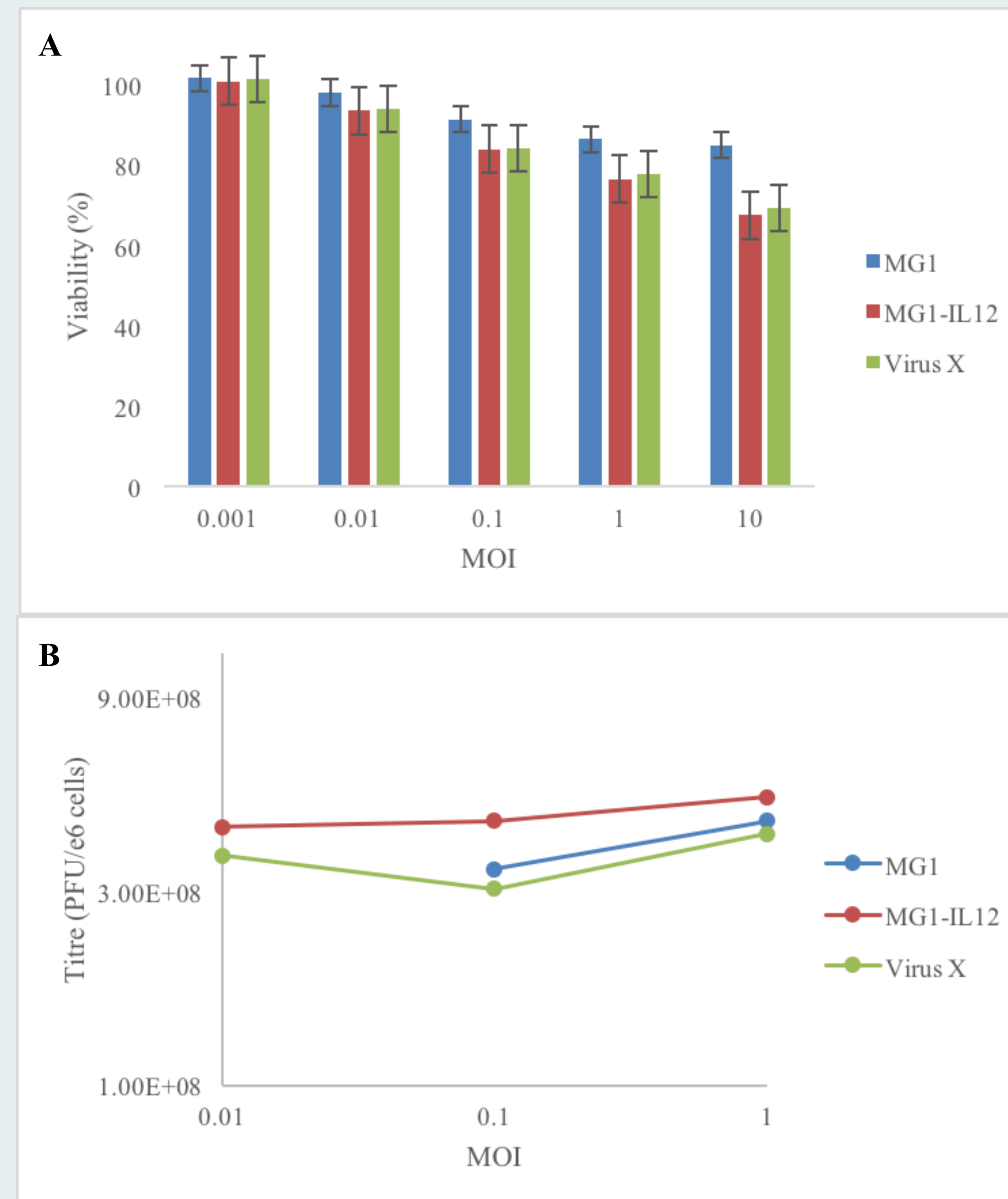
Rescuing the virus

### Characterizing the rescued virus

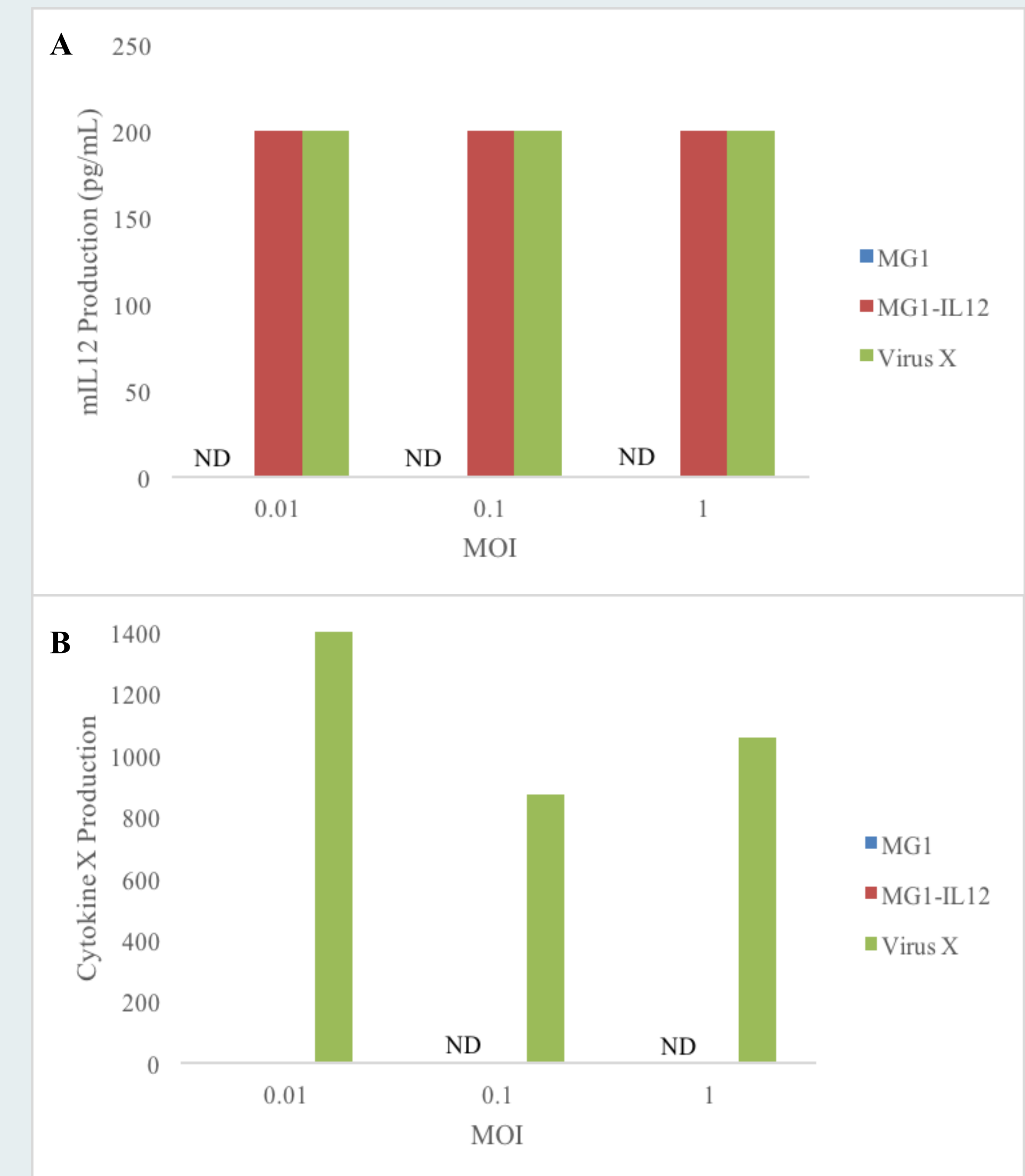
- ❖ Viral growth
- ❖ Cytotoxicity
- ❖ Transgene expression



## Results



**Figure 1. Cytotoxicity and vital titre of Virus X, compared to MG1 and MG1-IL12 viruses.** A) Cytotoxicity of MG1, MG1-IL12 and Virus X were determined in MC38 cells by measuring fluorescence intensities of metabolized Alamar Blue at the range of MOIs 24 hours post-infection. B) Viral titres of MC38 cells infected with MG1, MG1-IL12 and Virus X at the MOI of 0.01, 0.1 and 1, were measured via plaque assays in Veros. Data Courtesy of Mike A. Kennedy



**Figure 2. Quantifying the amount of IL-12 and cytokine X, secreted from MG1, MG1-IL12 and Virus X.** A) The level of mIL-12 production was examined via mIL-12 p70 subunit ELISA by collecting supernatants 24 hours post-infection of irradiated MC38. B) The level of cytokine X production was measured via ELISA from supernatants collected 24 hours post-infection of irradiated MC38. Data Courtesy of Mike A. Kennedy

## Conclusions

1. Virus X has a tendency to decrease in the viability of tumors as MOI increases, which is the similar trend as that of MG1 and MG1-IL12,
2. All three viruses have relatively consistent levels of viral titres at the indicated MOIs,
3. The amount of mIL-12 secreted from MG1-IL12 virus and X virus were both greater than the highest concentration on the standard curve (200pg/mL); the levels of mIL12 production in both of the viruses were very high,
4. An average of 1110 pg/mL of cytokine X was secreted from virus X.

The data above confirm that the insertion of an additional immunostimulatory gene in the MG1 virus does not alter its viral characteristics.

Furthermore, the production of the cytokine of interest was verified.

### Further Approaches:

- ❖ Performing more assays for the virus characterization,
- ❖ Testing effectiveness of this virus in stimulating NK and T cell activations in vivo.

These positive results from this study may give an insight into future researches in the exciting field of oncolytic virotherapy.

## References

- ❖ Alkayyal, A., Tai, L., Kennedy, M., de Souza, C., Zhang, J., Lefebvre, C., Sahi, S., Ananth, A., Mahmoud, A., Makrigiannis, A., Cron, G., Macdonald, B., Marginean, E., Stojdl, D., Bell, J. and Auer, R. (2017). NK-Cell Recruitment Is Necessary for Eradication of Peritoneal Carcinomatosis with an IL12-Expressing Maraba Virus Cellular Vaccine. *Cancer Immunology Research*, 5(3), pp.211-221.
- ❖ Tai, L.-H., Zhang, J., & Auer, R. C. (2013). Preventing surgery-induced NK cell dysfunction and cancer metastases with influenza vaccination. *Oncotarget*, 2(11), e26618. doi:10.4161/onc.26618
- ❖ Lemay, C. G., Rintoul, J. L., Kus, A., Paterson, J. M., Garcia, V., Falls, T. J., ... Auer, R. C. (2012). Harnessing Oncolytic virus-mediated Antitumor immunity in an infected cell vaccine. *Molecular Therapy*, 20(9), 1791-1799. doi:10.1038/mt.2012.128

## Acknowledgements

### I would like to thank:

- Dr. Mike Kennedy and Dr. Rebecca Auer for making this project happen
- I would also like to thank Sarwat Khan and Meaghan Chapados for help in running experiments and analyzing data.
- All of the lab members in Auer lab for great support.

### Contact Information:

Email: mfuka092@uottawa.ca  
Phone Number: (819) 661-4959

