Combination Therapy with Pseudotyped MG1 and SMAC Mimetics to Selectively Kill HIV Infected Cells

Bengisu Molyer[1,2], Yasmeen Ameeriar[1,3], Jonathan B. Angel[,1,2,4*]

[1]Chronic Disease Program, Ottawa Hospital Research Institute, Ottawa, Ontario, Canada

[2]Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, Ontario, Canada,

[3]Translational and Molecular Medicine, University of Ottawa, Ottawa, Ontario, Canada

[4]Division of Infectious Diseases, Ottawa Hospital, Ottawa, Ontario, Canada

Contact:bmoly053@uottawa.ca

Abstract

Background: Targeting the latently infected $CD4^+$ T-cells is one of the main challenges in finding a cure for HIV. Although these cells cannot be phenotypically distinguished from their uninfected counterparts, we have demonstrated that the cells latently/persistently infected with HIV have impaired interferon signalling. This defect makes them susceptible to be selectively infected and killed by the oncolytic virus (OV) MG1. Both increasing its specificity to infected cells and sensitizing these cells to its killing can be expected to make MG1 a more effective therapeutic. This project aims to pseudotype MG1 with HIV envelope protein gp160, to enhance targeting of infected cells, and determine if combining MG1 with the SMAC mimetics (SM) LCL-161 and AEG-40730 can increase killing of cells that are latently infected with HIV.

Methodology: For pseudotyping experiments, restriction enzyme cloning, and Gibson Assembly were used to create full length and modified gp160 containing MG1 clones. For SM and MG1 combination experiments, Jurkat cells and their latently HIV infected counterpart J1.1 cells were infected with MG1 and treated with the SM LCL-161 and AEG-40730, and their viability and infection percentage were assessed 24h and 48h post treatment via flow cytometry.

Results: MG1 clones have been verified by sequence analysis and are in the process of being rescued by transfection. The divalent SM AEG-40730 has greater killing effect compared to the monovalent LCL-161 in both cell lines, but neither SM show a preferential killing of J1.1 cells compared to Jurkat cells. Combination of a low dose of LCL-161 and MG1 infection shows significantly increased killing of J1.1 cells.

Conclusion: Both OV and SM therapy are promising therapeutic agents in the field of HIV. A combination of OV and SM therapy can potentially be a new strategy to eliminate cells latently infected with HIV.

Conflict of Interest Disclosure: I have no conflicts of interest

Introduction

- CD4+ T-cells latently infected with HIV produce low or no amount of transcripts and cannot be distinguished from their healthy counterparts, making them a major target in HIV cure research.(Siliciano et al, 2011)
- Type 1 interferon (IFN) responses are impaired in latently infected CD4+ T-cells, making them susceptible to killing by the IFN sensitive oncolytic virus, MG1. (Ranganath et al., 2018)

- We can improve MG1 therapy by:

1. Enhancing MG1 selectivity

- MG1 entry into cells is by using LDL receptor, which is expressed in many different cell types.
- MG1's tropism can be restricted to HIV's target cells by pseudotyping MG1 with the HIV envelope protein gp160.

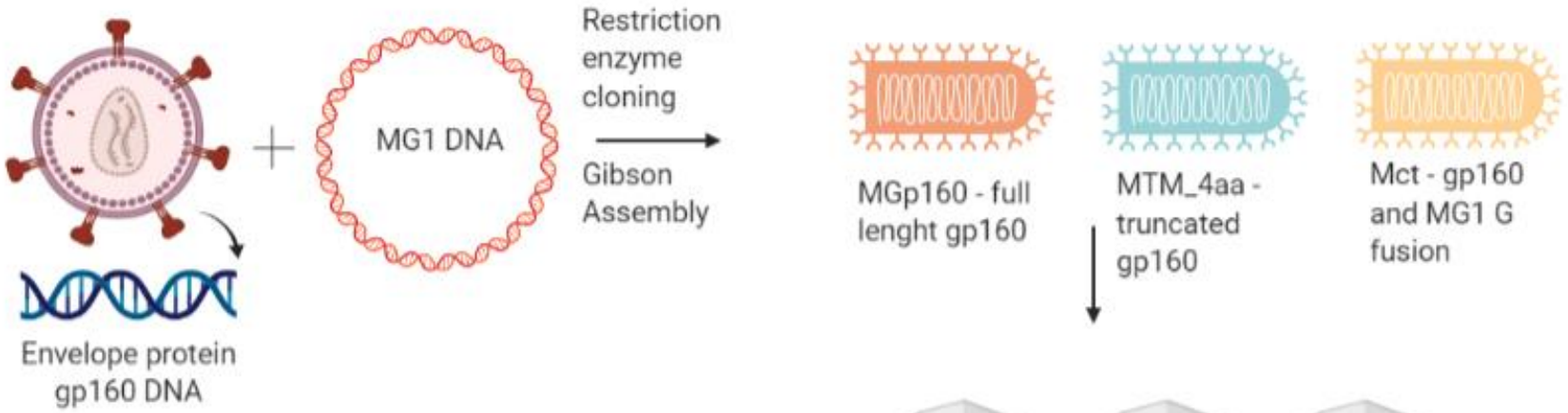
2. Enhancing MG1 killing

- Studies in cancer have shown that oncolytic virus killing can be enhanced by using SMAC mimetics.
- SMAC mimetics (SM) are pro-apoptotic proteins that inhibit indigenous SMAC proteins. They inhibit inhibitors of apoptosis proteins cIAP1/2 and XIAP.
- SMAC mimetics have also been shown to selectively kill HIV infected cells. (Reviewed by Molyer et al., 2021)

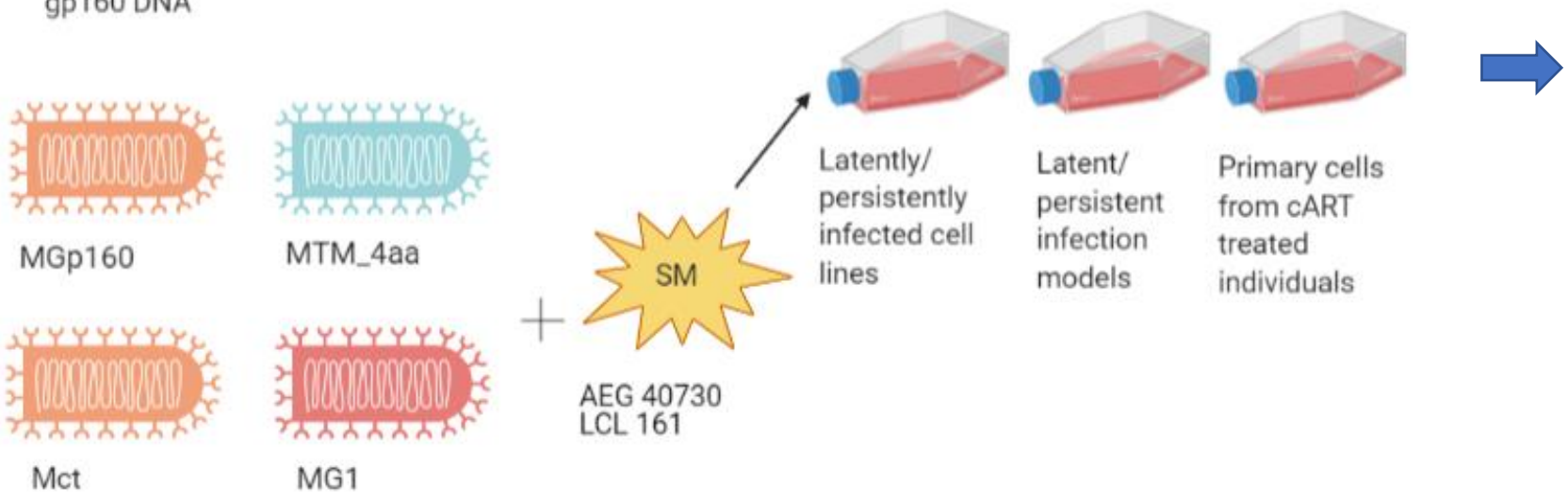
Hypothesis and objectives

- Combination of pseudotyped MG1 and SMAC mimetics can be used to enhance MG1 infection and killing of HIV-infected cells

Aim 1



Aim 2



Assess infection and killing

Made with BioRender.com

Results

MG1 and MG1 clone plasmids

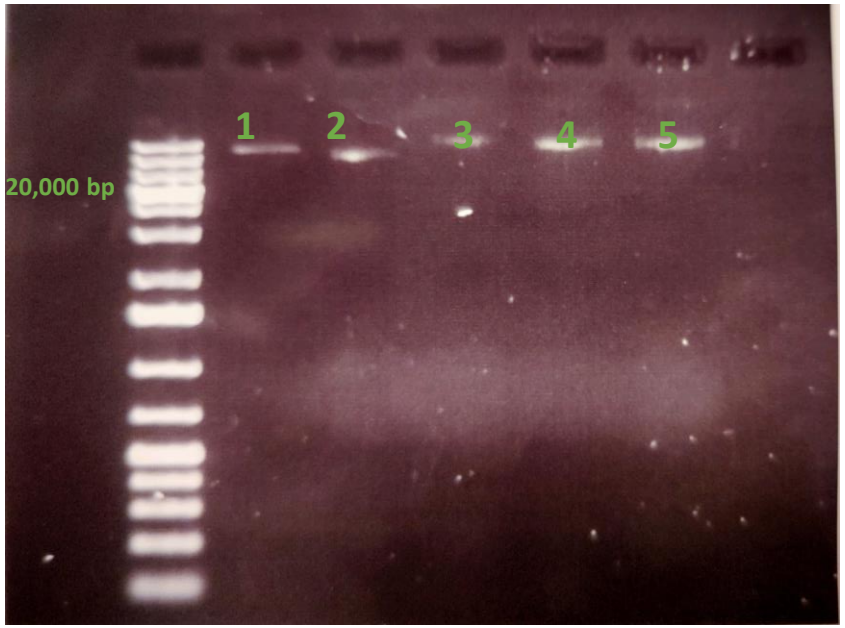


Figure 1 showing MG1 and MG1 clone plasmids on agarose gel

1. MG1: 14,023 bp
2. MG1 G-less: 12,440 bp
3. Mgp160: 15,536 bp
   Pseudotyped with full length gp160
4. Mct: 14,686 bp
   Pseudotyped with gp160 truncated to four amino acids after the transmembrane region.
5. MTM_4aa: 14,607 bp
   Pseudotyped with gp120 and CT tail of MG1 G

Concurrent SMAC mimetic treatment with MG1 infection

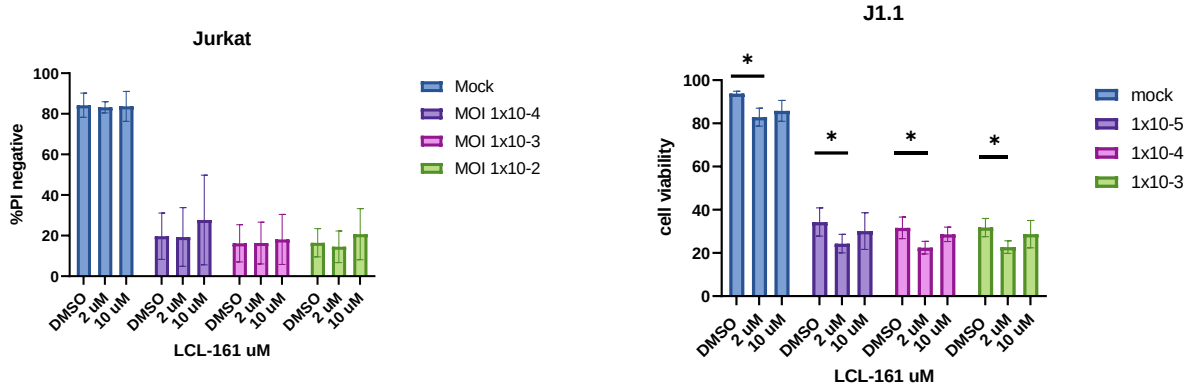


Figure 2. Cell viability (%PI negative) of Jurkat (n=3) and J1.1 (n=4) cells following MG1 infection and concurrent monovalent SM LCL-161 treatment at 48h post infection. A slight increase in cell death in HIV infected J1.1 cells when treated with the SM LCL-161 at 2 µM can be seen while no such affect can be observed in their uninfected Jurkat cell counterpart. (*$p<0.01$ *by* 2-way ANOVA with Dunnet's multiple comparisons test).

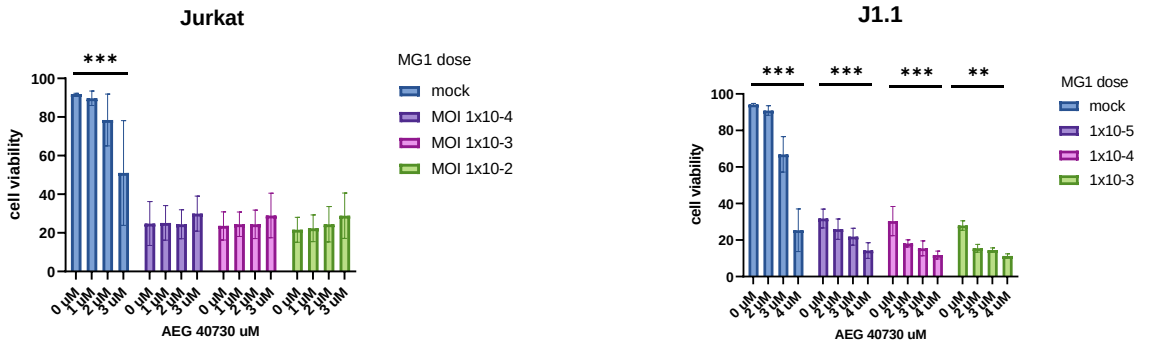


Figure 3. Cell viability (%PI negative) of Jurkat (n=4) and J1.1 (n=4) cells following MG1 infection and concurrent divalent SM AEG-40730 treatment at 48h post infection. A significant increase in cell death in the HIV infected J1.1 cells at all concentrations of SM AEG-40730 can be while this is not observed in their uninfected Jurkat cells counterpart (**$p<0.001$, ***$p< 0.0001$ *by* 2-way ANOVA with Dunnet's multiple comparisons test)

Conclusion

- MG1 clones containing full length HIV envelope (Mgp160), truncated HIV envelope (MTM_4aa), and HIV envelope fused to MG1 G (Mct) have been generated by restriction enzyme cloning and Gibson assembly.
- The clones are in the process of being rescued by a modified VSV reverse genetics rescue protocol. The pseudotyped viruses will be tested on cell lines latently infected with HIV, CD4 T-cell latency models and memory CD4+ T-cells from cART treated patients and the cytopathic ability of the MG1 clones will be compared to that of MG1.
- There is increase in MG1 mediated cell death when HIV infected J1.1 cells are concurrently treated with SM AEG-40730 at all doses and only at the lower dose of SM LCL-161. However, no such increase is observed in the healthy Jurkat cells. Other strategies where MG1 infection is followed by SM treatment or SM treatment is followed by MG1 infection will be investigated to see the importance of the timing of MG1 infection and SM treatment.
- A combination therapy with SM and OV is a novel cure strategy that has the potential to eliminate latently infected cells with HIV.

References

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