Pseudotyping the Oncolytic Virus MG1 to Enhance Its Ability to Selectively Kill HIV Infected Cells

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Abstract

Background: The CD4+ T-cells latently infected with HIV cannot be phenotypically distinguished from their uninfected counterparts, making them difficult to target for therapy. However, we have recently demonstrated that the CD4+ T-cells latently infected with HIV have impaired interferon signalling and this defect allows them to be selectively infected and killed by the interferon sensitive oncolytic virus MG1. As MG1 has broad tropism, it is important to make it more specific to its target, if it is to be used therapeutically. This project aims to pseudotype MG1 with the HIV envelope protein gp160 to restrict its tropism to CD4 expressing cells.

Methodology: Full length gp160 insert was generated from p96zm651 expression vector by adding the cut-sites of Acc651 restriction enzyme and artificial start and stop codons by PCR. The insert was ligated into MG1 G-less backbone by T4 ligase. The truncated insert was cut up to 4 amino acids after the transmembrane region of gp160. MG1 G-less backbone was linearized by inverse PCR and overlapping regions to both the insert and the backbone were added by PCR. The truncated clone was generated by Gibson Assembly.

Results: As verified by sequence analysis, the first MG1 clone contains the full length gp160 insert and is named Mgp160, and the second clone contains the truncated gp160 insert and is named MG1_4aa. Both of the MG1 clones have the inserts between the M and eGFP genes. The clones are in the process of being rescued by transfection.

Conclusion: The pseudotyped MG1 clones have been designed to specifically target CD4 positive cells. These will now be tested for their ability to selectively kill HIV infected cells in different models of HIV latency. This work will to potentially identify a novel strategy to target the viral HIV reservoir.

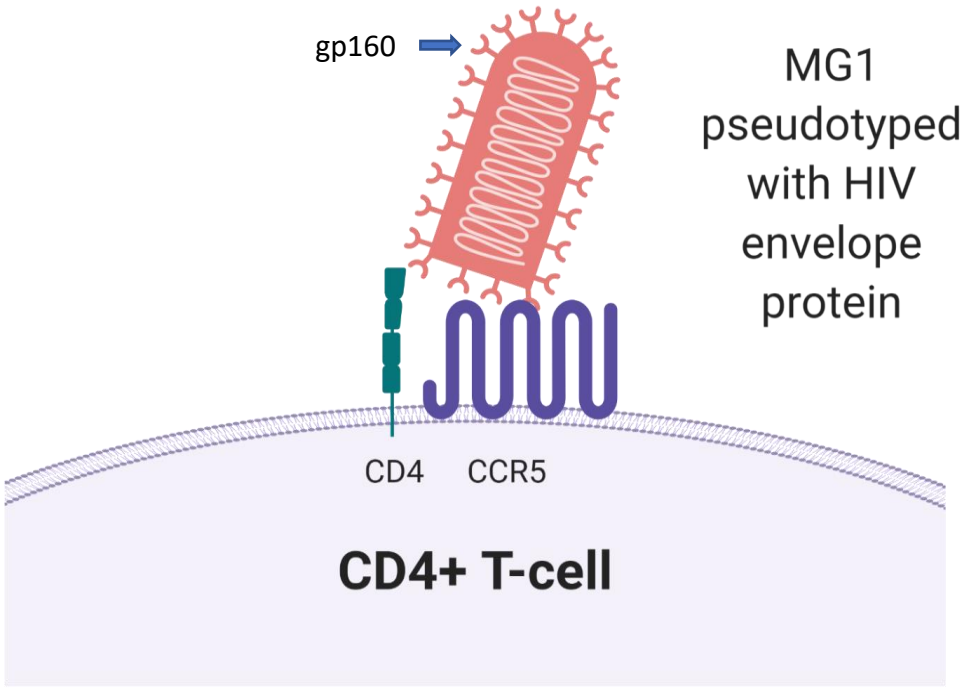
Conflict of Interest Disclosure: I have no conflicts of interest

Introduction

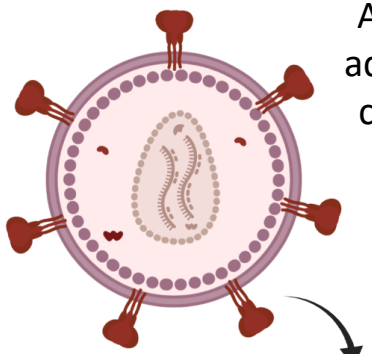
- Latency is established early on in CD4+ T-cells and cells latently infected with HIV produce low or no amount of transcripts. (Siliciano et al., 2011)
- The cells latently infected with HIV cannot be distinguished from uninfected CD4+ T-cells but our lab has shown that type 1 interferon responses are impaired in cells latently infected with HIV (Ranganath et al., 2016).
- The interferon sensitive oncolytic virus MG1 has a more robust killing activity of latently infected CD4+ T-cells compared to their healthy counterparts. (Ranganath et al., 2018)
- MG1 has the ability to infect many different cell types. Therefore, its tropism needs to be restricted to HIV's target cells if it is to be used as a potential therapy.

Hypothesis

- Pseudotyping MG1 with HIV envelope gp160 protein will restrict its tropism to CD4+ expressing cells.



Methodology - Overview



Envelope protein gp160 DNA

(CCR5 tropic)

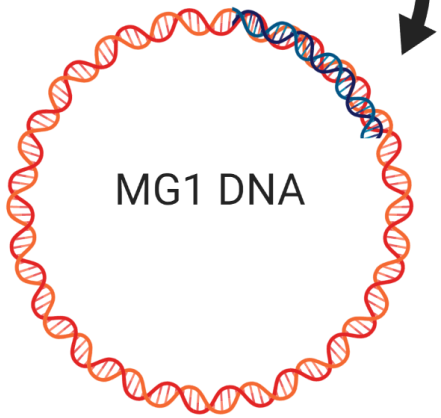
Amplify and add necessary cut-sites for enzymes

Full length gp160 and a truncated version (TM+4aa)

Proceed to Gibson Assembly for truncated clone (MTM_4aa)

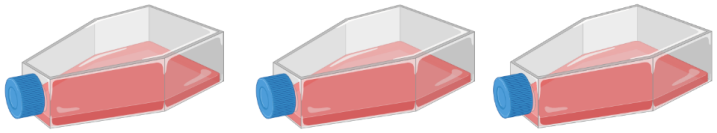
Restrict and dephosphorylate vector backbone for restriction enzyme cloning (full length clone, Mgp160)

Ligate



Rescue

Test and compare with MG1

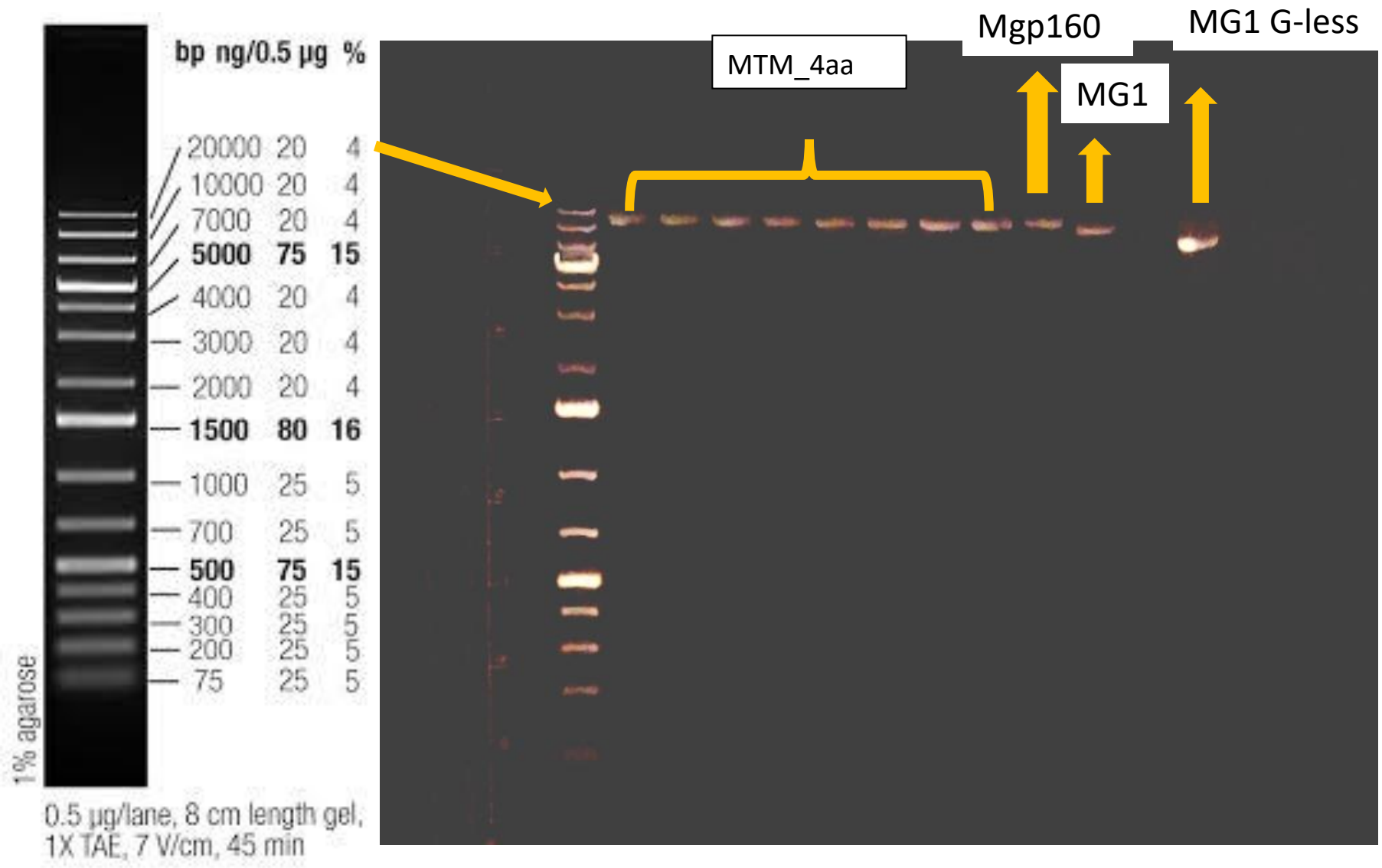


Jurkat, J-lat and OM10.1 Cell lines

CD4+ T-cell models of latency

Memory CD4+ T-cells from cART treated patients

Results



- MGp160: 15,070 bp (full length gp160)
- MTM_4aa: 14,578 bp (truncated gp160)
- MG1_eGFP: 14,023 bp
- MG1 G-less: 12,440 bp

Agarose Gel Electrophoresis image of MG1 clone plasmids

Conclusion

- MG1 clones containing full length HIV envelope (MGp160) and truncated HIV envelope (MTM_4aa) 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 rescued clones 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.
- Targeted MG1 oncolytic virus therapy is a novel cure strategy to eliminate cells latently infected with HIV.

References

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2. Ranganath, N., Sandstrom, T. S., Fadel, S., Côté, S. C. & Angel, J. B. Type I interferon responses are impaired in latently HIV infected cells. *Retrovirology* **13**, 66 (2016).
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