The Human phosphoglycerate kinase promoter in a Lentiviral Vector for Correction of Adenosine Deaminase Deficiency

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Background

- Adenosine deaminase (ADA) deficiency is a defect in purine metabolism, leading to Severe Combined Immune Deficiency (SCID) with paucity of T, B and Natural Killer (NK) cells.
- Current standard of care is to initiate pegylated ADA enzyme replacement while undergoing workup for definitive treatment with allogeneic hematopoietic stem cell transplant or autologous stem cell transplant with gene modified cells (ASCT-GT).
- ASCT-GT has been successfully used in the treatment of ADA-SCID utilizing a self-inactivating lentiviral vector containing the human ADA cDNA and an elongation factor 1alpha constitutive promoter (EFS-ADA) (Fig.1).
    - While this construct was therapeutically effective, there are many constitutive promoters which may prove to be similarly efficacious.
- We hypothesize that a shortened version of the human phosphoglycerate kinase (μPGK) promoter in a lentiviral vector construct will produce similar titer and expression results to a construct containing the EFS promoter.

Design & Methods

- The μPGK promoter was developed by removing about 120bp and 80bp from the 5' and 3' ends respectively (Figure 2).
- The lentiviral vector was constructed with Gibson Assembly, inserting the μPGK promoter and ADA cDNA into a backbone previously used by our lab.
- HEK293T cells were transfected with the target, packaging and envelope plasmids using a five-day packaging protocol. GFP is a packaging control.
- The resulting viral vectors were used to transduce HT-29 cells in a five-day titering protocol for titer and vector copy number (VCN) via ddPCR.
- Lastly, transduced HT-29 cells were maintained in culture for a total of 14 days prior to harvesting for additional VCN analysis and ADA activity determination using an ADA assay.

Lentiviral Vector Design

Fig. 1

EFS-ADA (3900 bp)

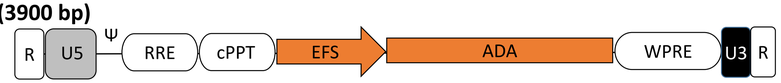


Fig. 2

μPGK-ADA (3924 bp)

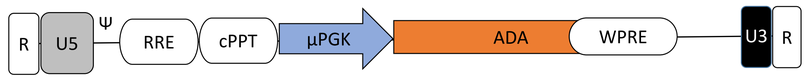
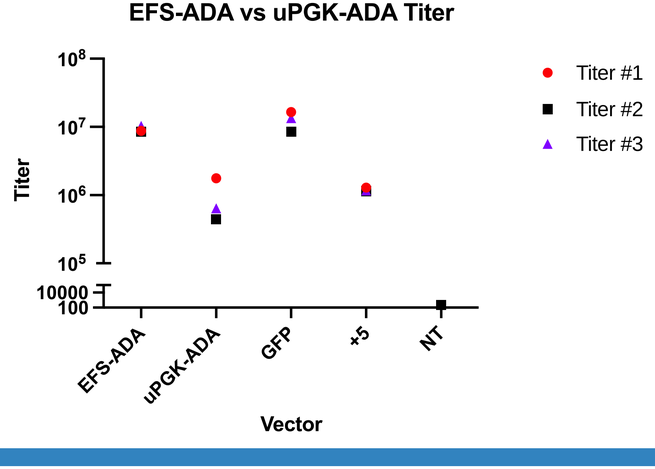
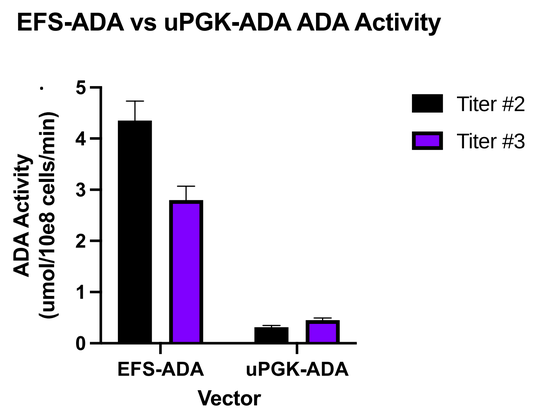
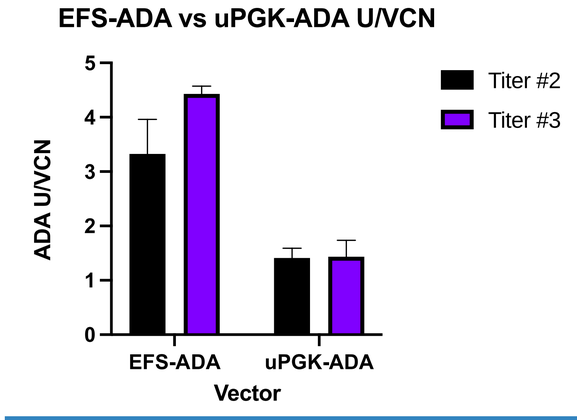


Fig. 1 & Fig 2: R – repeat region. U5 – unique 5' region. RRE – Rev response element. cPPT – central polypurine tract. WPRE – woodchuck hepatitis virus post-ranscriptional regulatory element. U3 – unique 3' region.

Results







Conclusion

The μPGK-ADA vector had titers 2.5 times lower than that of EFS-ADA (Fig. 3). ADA assay results demonstrated much lower ADA activity (Fig.4), 2.5 – 3 times lower, and overall lower activity units per VCN (U/VCN) for the μPGK-ADA vector (Fig. 5).

The μPGK-ADA vector is not likely a good alternative candidate for use in ASCT-GT for patients with ADA-SCID. Future research will focus on comparing to both EFS-ADA and μPGK-ADA vectors to a series of new vectors with various constitutive promoters including full-length human PGK, intronless ubiquitin C (UbC) and a shortened UbC promoter.