Comparing the Efficacy of Tat-Dependent and Tat-Independent Lentiviral Vectors

Chantelle McIntyre[1-3], Fiona Craig[1,2], Patricia Cmielewski[1-4], Martin Donnelley[1-4], Nigel Farrow[1-4], David Parsons[1-4]

[1]Department of Respiratory and Sleep Medicine, Women's and Children's Hospital, North Adelaide, SA, Australia; [2]Robinson Research Institute, University of Adelaide, Adelaide, SA, Australia; [3]School of Paediatrics and Reproductive Health, Adelaide, SA, Australia; [4]Center for Stem Cell Research, and Children's Research Center, University of Adelaide, Adelaide, SA, Australia.

Correspondence to: chantelle.mcintyre@adelaide.edu.au

Introduction:

The tat HIV-1 accessory protein plays a role in both transcriptional initiation and elongation of RNA transcripts and provirus. For historical “safety” reasons, clinically focused HIV-1 vectors avoid the use of tat. In development of lentiviral-mediated gene therapy for the treatment of cystic fibrosis (CF) airway disease, we have utilised a self-inactivating, tat-dependent, HIV-1 lentiviral vector for transfer of reporter and therapeutic genes into the airway epithelium of animal models. Since later generation, tat-independent, lentiviral vector systems have become commercially available and increasingly popular in recent years, the aim of this experiment was to compare the efficacy of our tat-dependent vector with a tat-independent vector system, to ensure that the most effective vector is used for the further development of gene therapy for CF.

Methods:

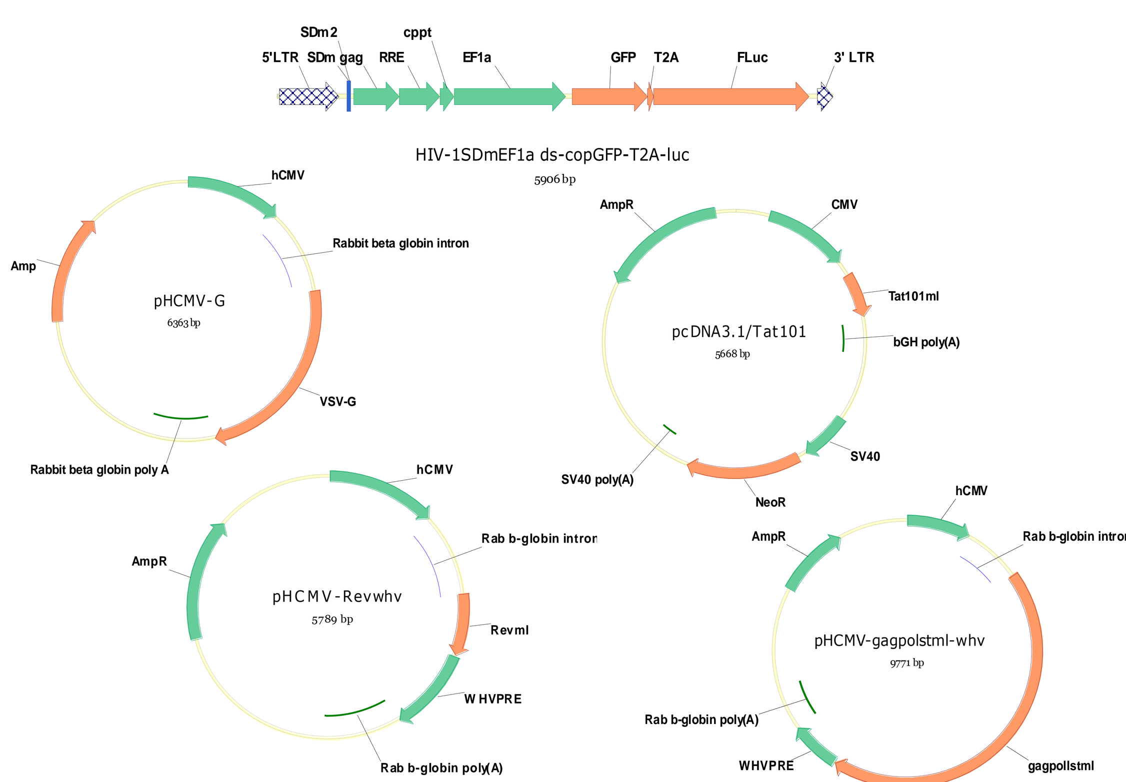

Figure 1. Tat-dependent system

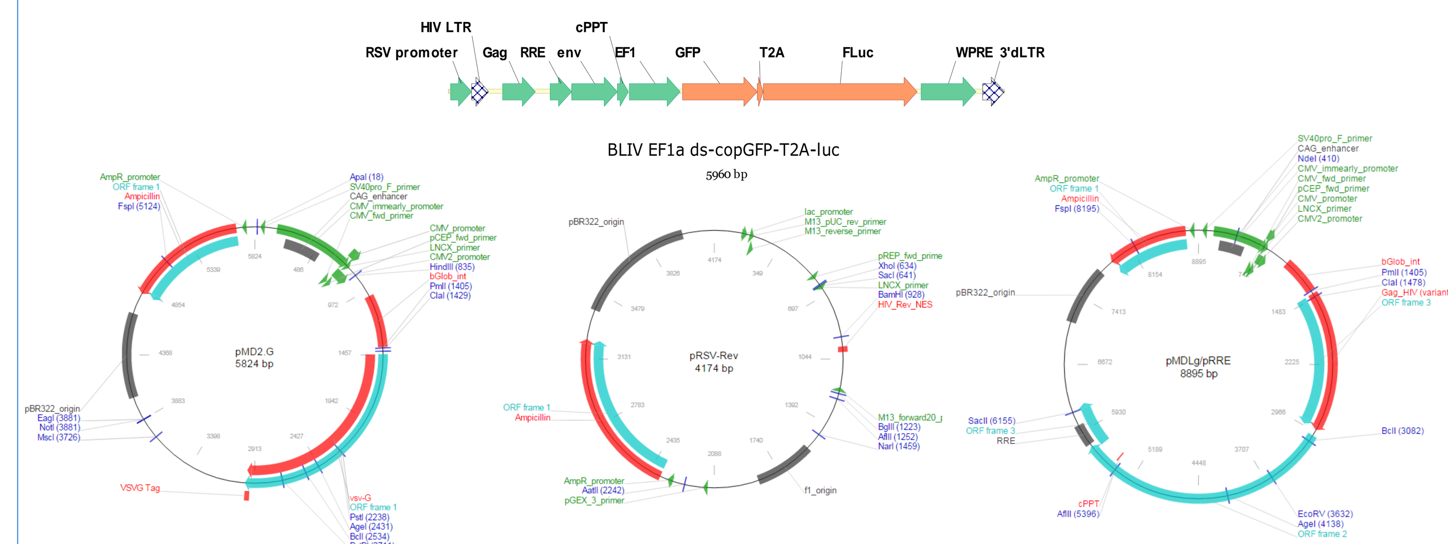

Figure 2. Tat-independent system (helper plasmid maps from addgene; https://www.addgene.org)

Virus production: Virus, expressing both GFP and luciferase, was made in 6-well plates of 293T cells by calcium phosphate mediated coprecipitation and endocytosis of DNA. The quantities of DNA used to make virus are outlined in tables 1 and 2. Cell culture medium was replaced with Opti-Pro SFM medium supplemented with 1% (v/v) glutamine and 1% (v/v) penicillin/streptomycin sulphate 8 hours after transfection. Plates were incubated for a further 48 hours before the media was collected and 0.45 µm syringe filtered.

Virus assays: Virus made in 6-well plates was put onto new 293T cells in 6-well plates. Transduced cells were assessed by FACS to quantify GFP positive cells, and by IVIS imaging to measure luciferase bioluminescence. RNA titre was determined using a commercial qPCR lentivirus titration kit (abm) according to the manufacturer's instructions. p24 concentrations in virus supernatants were determined using a commercial p24 HIV antigen ELISA (PerkinElmer) according to the manufacturer's instructions.

N= 6 virus preps per vector, and n = 3 for controls. Data is expressed as mean ±1 standard deviation of the mean

Results:

Tat-dependent system	DNA quantity per well (µg)	Tat-independent system	DNA quantity per well (µg)
pHIV-1SDmEF1a ds-copGFP-T2A-luc	3	pBLIV EF1a ds-copGFP-T2A-luc	3
pcDNA3.1/Tat101	0.06	pRSVrev	1.5
pHCMV-Revwhv	0.06	pMDLg/pRRE	1.5
pHCMV-gagpolstml-whv	0.3	pMD2.G	1.5
pHCMV-G	0.15		

Table 1. DNA quantities used for transfection

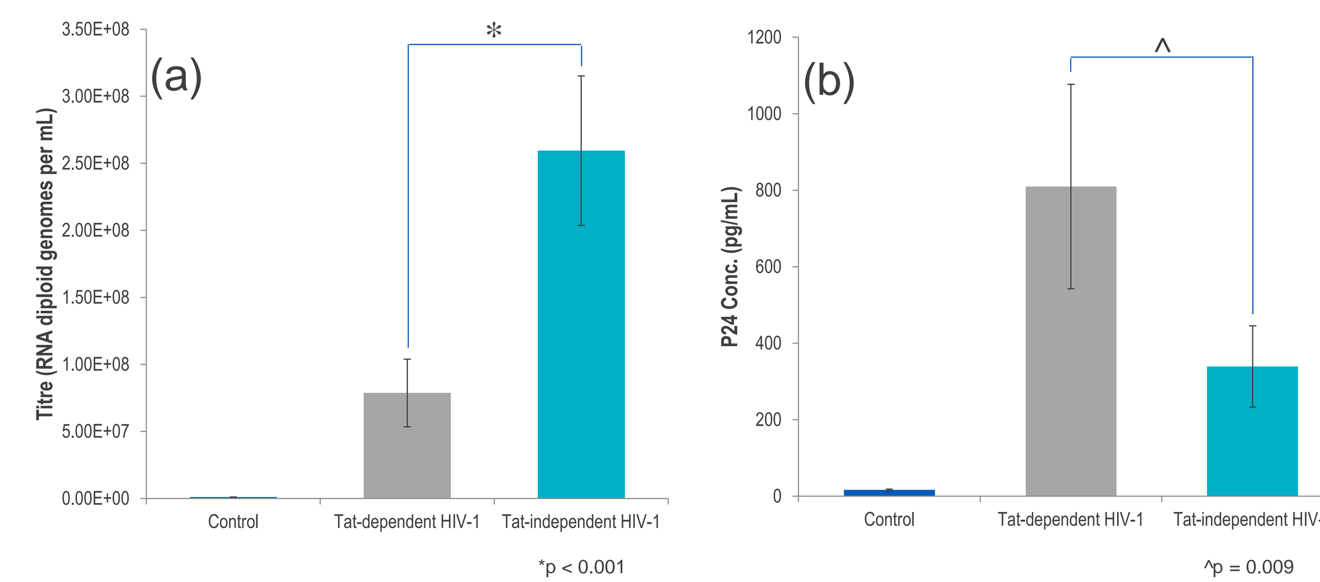

Figure 3. Titre determination by (a) qPCR against viral genomic RNA revealed higher titres for the tat-independent system, while (b) p24 ELISA suggested higher levels of viral protein production by the tat-dependent system

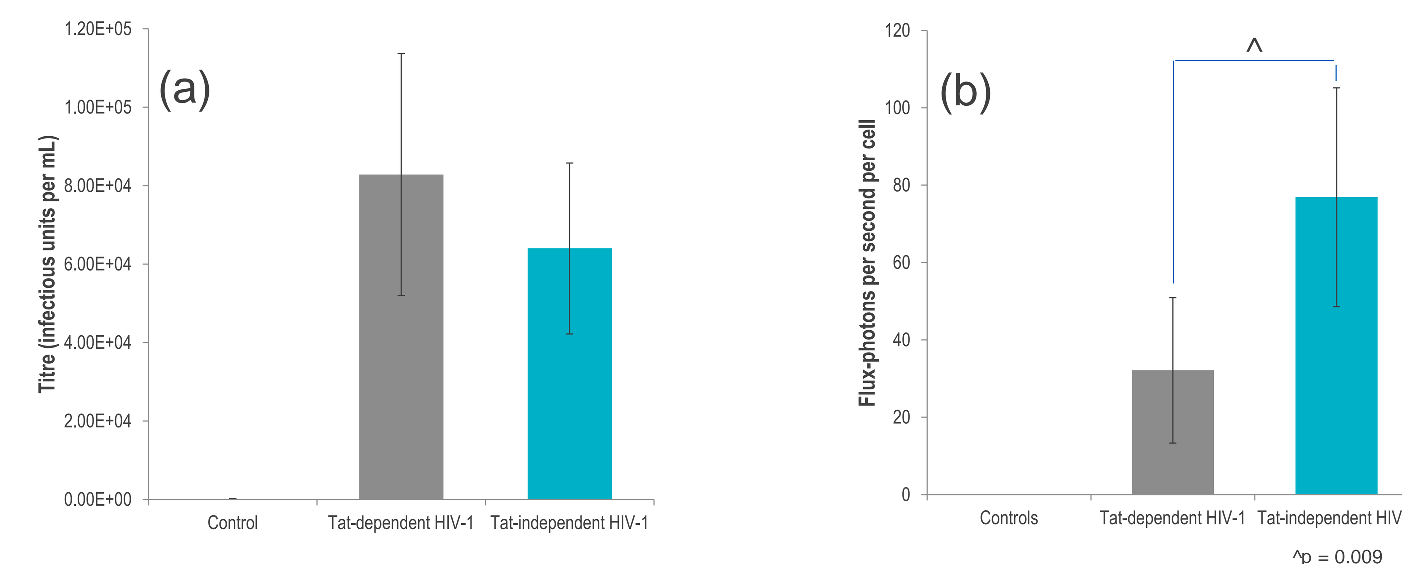

Figure 4. Titre determination by (a) FACS of GFP positive cells suggested similar titres between the two systems, while (b) bioluminescence of luciferase was highest for the tat-independent vector

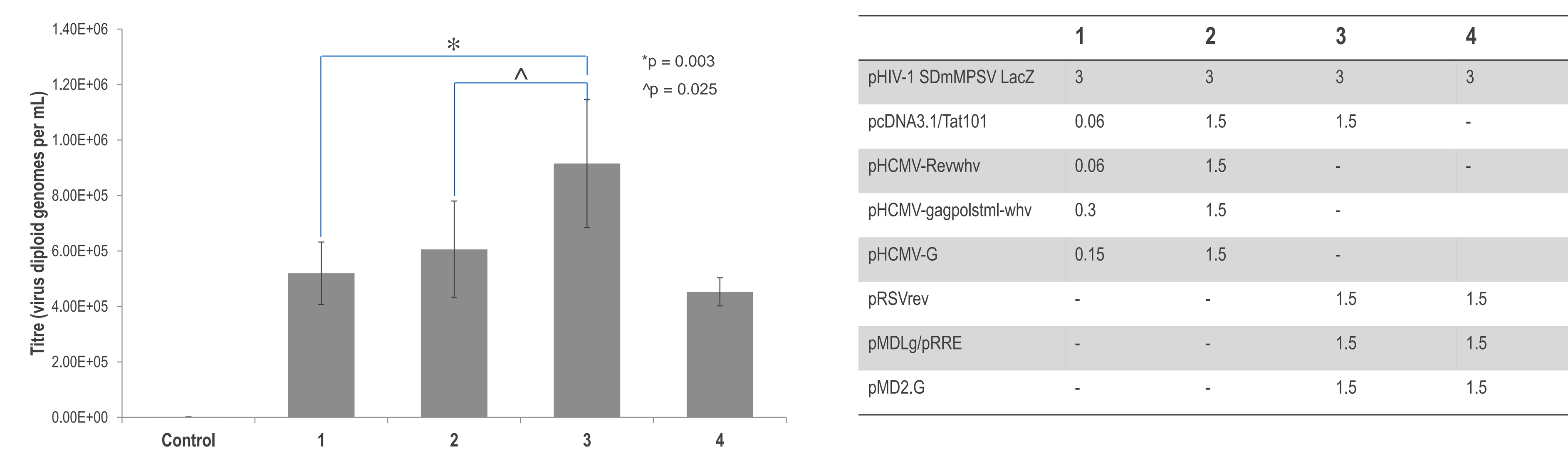

Figure 5. Tat-dependent vector production was most effective when using helper plasmids from the tat-independent vector system

	1	2	3	4
pHIV-1 SDmMPSV LacZ	3	3	3	3
pcDNA3.1/Tat101	0.06	1.5	1.5	-
pHCMV-Revwhv	0.06	1.5	-	-
pHCMV-gagpolstml-whv	0.3	1.5	-	
pHCMV-G	0.15	1.5	-	
pRSVrev	-	-	1.5	1.5
pMDLg/pRRE	-	-	1.5	1.5
pMD2.G	-	-	1.5	1.5

Table 2. DNA quantities used for transfection (per well) to produce virus with titres shown in Figure 5

Discussion :

Although titres measured by p24 ELISA were highest for the tat-dependent vector, the tat-independent vector system was approximately 3 times more effective at producing genomic RNA, compared with the tat-dependent system. Although neither qPCR against genomic RNA, nor p24 ELISA, discriminated between infective and non-infective virus particles here, it is logical to assume that the quantification of RNA genomes provides the more accurate reflection of titre in terms of infectious units.

Since each of the two vectors shared the same expression cassette (internal promoter and transgenes), it is unclear why their FACS titres appeared similar, in contrast to the titres achieved by qPCR, while luciferase expression was clearly higher for the tat-independent vector, as might be predicted by the qPCR titres. Notably though, FACS analysis does not account for multiple vector copies per cell, which might explain this discrepancy.

For packaging the tat-dependent vector, the helper plasmids from the tat-independent vector system were superior to the helper plasmids from the tat-dependent system, however, as expected, the presence of the tat-plasmid remained necessary for the efficient production of tat-dependent virus.

Conclusions:

Future development of gene therapy for CF will likely utilize a tat-independent lentiviral vector, rather than a tat-dependent lentiviral vector, although studies comparing the efficacy of each system *in vivo*, after large-scale virus production and purification, are necessary before final conclusions (as to which system is most effective) can be made.

Acknowledgements :

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