Do mutations in the *tat* exonic splice enhancer contribute to HIV-1 latency?

Nicholas Norton, Hoi Ping Mok, Jack Hirst and Andrew Lever

Latent infection of long-lived memory CD4+ T cells is thought to be a major barrier to the eradication of HIV-1. Globally there is a substantial research effort aimed at disrupting latency to enable the purging of the latent reservoir. Despite maximal activation a significant proportion of viruses are not reactivated from latency (Ho et al. 2013).

We examined sequences deposited by the Siliciano lab from non-induced proviruses found in patient samples. All sequences contained mutations in a recently described exonic splice enhancer involved in the regulation of *tat* mRNA splicing (Erkelenz et al. 2015). By comparison with over 2000 subtype B sequences deposited in the Los Alamos database we identified mutations which are highly enriched in the latent sequences. We hypothesise that mutations in this region could result in inefficient splicing of *tat* mRNA; preventing the establishment of the Tat-TAR feedback loop and leading to silencing. One of the mutations corresponding to a G to A mutation at position 5817 in HXB2 was found in 11/18 (60%) of the latent sequences but only 10% of subtype B sequences

To investigate this further, we have cloned this mutation into NL4-3 and NL4-3 expressing GFP in *env*. We compared the replicative capacity of the mutant virus compared to wild type. We also studied the effect of this single mutation on viral RNA splicing pattern and the dynamics of viral gene expression in a primary cell model of latency. Results of our studies will be presented in the meeting.