RNA-directed epigenetic silencing protects humanised mice during HIV challenge

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Background:
The HIV-1 latent reservoir is maintained by specific epigenetic modifications, such as increased histone methylation and decreased histone acetylation. Using RNA-directed epigenetic silencing to induce and enforce super-latency, we aim to mimic natural virus latency in an HIV-1 functional cure "block and lock" approach. We have previously shown novel siRNAs induce potent HIV-1 silencing in various cell lines *in vitro* and provide protection from virus challenge in a humanized mouse model of **acute** HIV-1 infection. We now investigate their potential for gene therapy using shRNA-transduced CD34+ haematopoietic stem cells in a humanized mouse model of **chronic** HIV-1 infection.

Methods:
Human CD34+ cells were transduced using GFP-labelled lentivirus expressing the promoter-targeted shRNA, shPromA or dual construct shPromA/shCCR5 or mock-transduced or empty shRNA-transduced and transplanted into irradiated NSG mice. Transduction efficiencies ranged between 40-70%. At 17 wks post-engraftment mice expressing GFP in ~4000 CD4+ T cells/mL were challenged with CCR5-tropic HIV-$1_{JR\text{-}FL}$. Mice were bled at wks 3, 5, 7 and 10 post-infection (p.i.), and then received ART for 8 wks, following which ART was interrupted to measure virus rebound for 4 wks prior to sacrifice and assessment of CD4+ T cells/GFP expression by flow cytometry and viral load using RT-PCR.

Results:
Transduced mice expressing shPromA or dual shPromA/shCCR5 showed up to 90% CD4+ GFP expression, with means of ~30-40%, respectively, over wks 3, 5 and 7 p.i. This correlated with a decrease in viraemia in transduced mice vs mock at wks 3, 5 and 7, between 1 and 3 logs, depending on the individual mouse CD4+ GFP expression. Transduced mice also showed a >1 log increase in CD4+ T cell numbers compared to mock at 10 wk p.i. in spleen, bone marrow and blood. RNAscope and immunostaining of lymph nodes is currently underway.

Conclusion:
Preliminary data from this study demonstrates RNA-directed epigenetic silencing by shPromA/shCCR5 delivered by *ex vivo* gene therapy can protect against HIV-1 in a humanized mouse model.

Disclosure of Interest Statement:
CA, AK and GS hold siRNA patents. GS is an employee of CSL. All other authors report no conflict of interest.