RNA-directed epigenetic silencing protects humanised mice during HIV challenge

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Background: The block and lock HIV functional cure approach aims to block virus transcription and lock the latent reservoir in a super-latent state, resistant to reactivation. We have previously shown short interfering (si)RNAs therapeutics induce potent HIV silencing using this approach in various cell lines *in vitro* and in primary CD4+ T cells *in vivo,* when delivered as a gene therapy using shRNA-transduced CD34+ haematopoietic stem cells, in a humanized mouse model of HIV-1 infection. We have extended this study to include RNAscope and immunostaining analysis to determine the potential protective effect in HIV tissue reservoirs, including lymph nodes and spleen.

Methods: Human CD34+ cells were transduced using GFP-labelled lentivirus expressing the promoter-targeted shRNAs, shPromA or dual construct shPromA/shCCR5, 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-CD4+ T cells were challenged with CCR5-tropic HIV-$1_{JR\text{-}FL.}$ and bled at wks 3, 5, 7 and 10 post-infection (p.i.). Mice were then treated with ART for 8 wks, followed by an ART interruption to measure virus rebound for 4 wks prior to sacrifice and assessment of CD4+ T cells/GFP expression by flow cytometry, viral load using RT-qPCR and RNAscope/immunostaining analysis of virus RNA in CD4+ T cells located in lymph node and spleen tissue.

Results: Transduced mice expressing shPromA or dual shPromA/shCCR5 showed up to 90% CD4+ GFP expression, which correlated with a >1 log increase in CD4+ T cell numbers compared to mock in blood, spleen and bone marrow at sacrifice. Following ART interruption, we also observed a delay in virus bound in the gene modified shPromA and dual shPromA/shCCR5 mouse groups compared to mock. RNAscope/immunostaining also indicated a reduction in virus located in CD4+ T cells in the lymph nodes in dual shPromA/CCR5 gene modified mouse groups and in spleen tissue in the shPromA gene modified mouse groups.

Conclusion: This is the first study to demonstrate a delayed virus rebound induced by anti-HIV RNA therapeutics and the reduction of virus-infected CD4+ T cells in the HIV latent reservoir lymph and spleen tissue sites. These exciting data further support the block and lock approach for achieving a permanent HIV cure.

Disclosure of Interest Statement:
CA, AK hold siRNA patents. All other authors report no conflict of interest.
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