Gene targeted editing to disable the oncogenic retrovirus HTLV-1

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Background:
HTLV-1 encodes two genes that are essential for transformation and proliferation. *Tax* is essential for *de novo* infection and cellular immortalization. *Hbz* supports proliferation and survival of infected cells in both its protein and mRNA forms. Together, these two viral genes are essential to the pathophysiology of both ATL and HAM/TSP. Additionally, HTLV-1 persists in infected hosts through mitotic host cell division, and the viral genome is highly conserved. Given this conservation, genomic editing has strong potential as a treatment option for HTLV-1-mediated diseases. We hypothesize that abrogating the function or expression of *tax* and/or *hbz* by genome editing may disable HTLV-1-infected cell growth/survival and prevent immune modulatory effects and ultimately HTLV-1-associated disease.

Methods:
We constructed a library of 163 gRNAs covering Tax, Hbz, and the LTRs. These gRNAs were sub-cloned into a lentiviral vector expressing Cas9 and puromycin resistance. VSV-G pseudotyped lentivirus was produced and transduced into an HTLV-1-infected T-cell line (Hut-102) and an ATL-derived T-cell line (ATL-ED). Following puromycin selection to eliminate cells not expressing Cas9, cellular proliferation rates were analyzed by MTS assay. *Tax*, *hbz*, and *gag* gene expression was measured in each CRISPR-edited cell line.

Results:
Our results suggest numerous gRNAs targeting *Tax* (27), LTR (24), and/or *Hbz* (21) significantly decreased cellular proliferation in Hut-102 cells. A total of 16 gRNAs targeting the LTR or *Hbz* affected cellular proliferation in ATL-ED cells. Of these gRNAs, a total of 36 and 17 affected *tax*, *hbz,* or *gag* gene expression in Hut-102 or ATL-ED, respectively.

Conclusion:
After off-target analysis, the top five gRNA candidates per viral gene/LTR will be selected for NSG sequencing and applied in our *in vivo* transplantation NOG mouse model. Our experiments will determine the effectiveness of CRISPR genome editing for disabling HTLV-1 and further inform genome editing strategies for HTLV-1 treatment.

Disclosure of Interest Statement:
None.

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