Model of Gene Therapy Against Retroviruses Exemplified by CRISPR-Cas9 Targeting of HTLV-1 Tax

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BACKGROUND

Human T-lymphotropic virus type 1 (HTLV-1), a deltaretrovirus, is etiologically linked to severe clinical pathologies, such as Adult T-cell Leukemia/Lymphoma (ATLL), and a neurological diseases, named HTLV-1-associated myelopathy (HAM), an aggressive malignancy and chronic myelopathy with limited therapeutic options. Unlike Human Immunodeficiency Virus (HIV), which maintains its reservoir through continuous replication cycles, HTLV-1 persists primarily through immortalized clonal expansion of infected lymphocytes and periods of transcriptional latency. These mechanisms create a remarkably stable viral reservoir and pose a significant barrier to treatment.

At the core of HTLV-1-mediated pathogenesis is the viral oncoprotein Tax. Tax is a potent transactivator of viral and cellular gene expression. It constitutively activates the NF-κB signaling pathway, giving infected cells increased survival, proliferative capacity, and resistance to apoptosis. However, these same properties increase the immunogenicity of *tax*-expressing cells, leading to their elimination by host cytotoxic T lymphocytes (CTLs). This creates a biological paradox in which the virus must balance immune evasion with malignant transformation, a dynamic that influences disease progression over decades. While *tax* expression is often suppressed in established ATLL tumors to evade immune surveillance, its transient or sporadic expression is considered crucial for initiating and maintaining the oncogenic process.

In this context, gene editing emerges as a promising therapeutic alternative. The CRISPR-Cas9 system, in particular, allows precise inactivation of critical viral genes. Targeting the *tax* gene could not only abrogate constitutive NF-κB activation but also curtail clonal proliferation and mitigate oncogenic potential. The reliance of HTLV-1 on an integrated provirus, with its highly conserved genetic sequence, makes this strategy particularly attractive compared to its application against hypervariable retroviruses like HIV.

METHODS

MT-2 cells, an HTLV-1–transformed T-cell line, were used as an in vitro model to evaluate CRISPR-Cas9–mediated editing of the *tax* gene. Gene delivery was performed by nucleofection using two approaches: (i) plasmid-based Cas9-GFP constructs to assess transfection efficiency and optimize pulse conditions, and (ii) Cas9-GFP/sgRNA RNP complexes targeting four conserved regions of *tax*, homologous between HTLV-1 and STLV-1. Forty-eight hours post-transfection, GFP expression was quantified by flow cytometry, and edited cells were assessed by sequencing. GFP⁺ clones were expanded in 96-well plates, and indels were confirmed by PCR and Sanger sequencing. A single GFP⁺ clone with verified tax indels was subjected to three additional nucleofection pulses to enhance editing efficiency, followed by reanalysis of indels

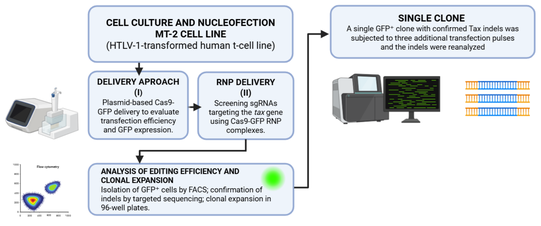


Figure 1. Workflow of CRISPR-Cas9–mediated editing of the HTLV-1 tax gene in MT-2 cells

RESULTS

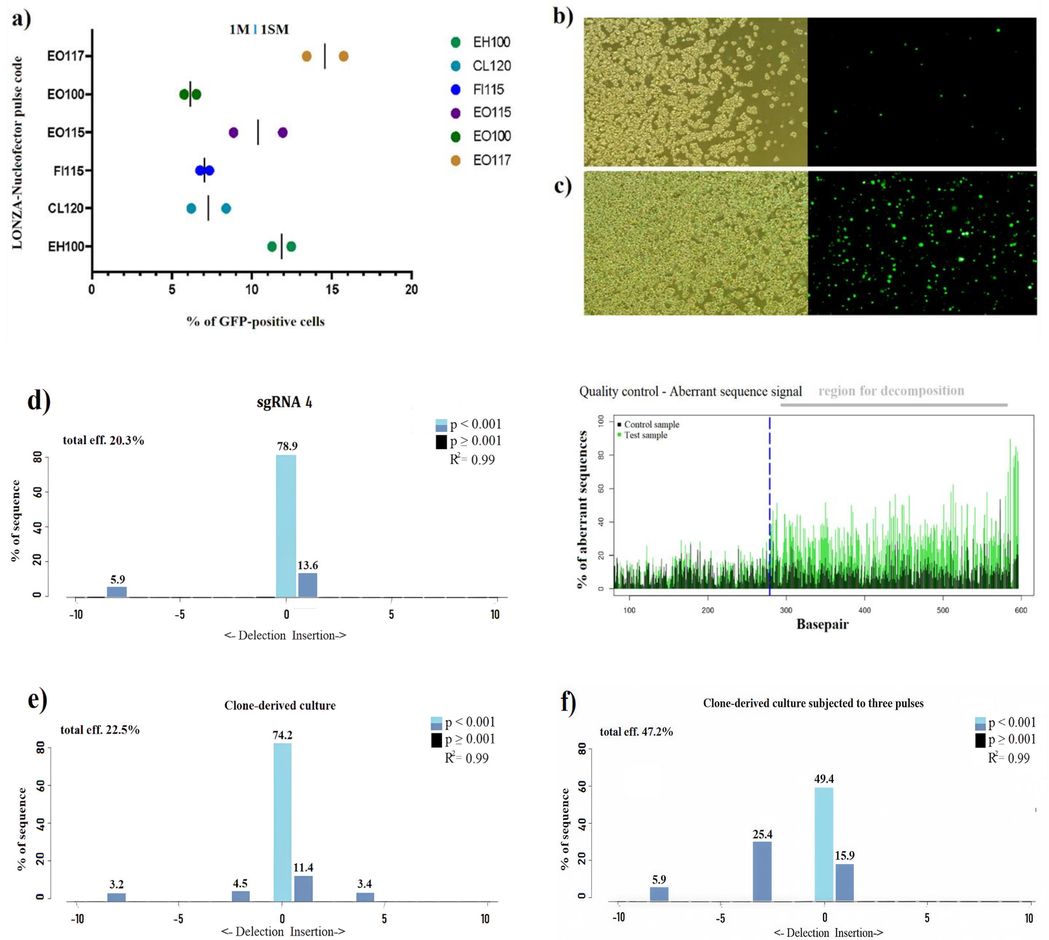


Figure 2. Validation of CRISPR-Cas9 targeting of the *tax* gene in HTLV-1-infected MT-2 cells. a) Nucleofection efficiency using Cas9/GFP plasmid (control, without sgRNA) under different pulse protocols (LONZA Nucleofector program codes); b) MT-2 cells nucleofected with the 9,223 bp plasmid; c) MT-2 cells nucleofected with the 3,486 bp plasmid. Left: brightfield; Right: GFP fluorescence. d) Gene-editing efficiency using Cas9/sgRNA 4 ribonucleoprotein (RNP) complex, showing a total indel efficiency of 20.3%; e) Example of indel spectrum in cultures derived from a single clone nucleofected with sgRNA 4, with total efficiencies of 22.5%. f) The indel spectrum after three pulses, showing a total of 47.2% indels

CONCLUSIONS

This study demonstrates that CRISPR-Cas9-mediated gene editing effectively targets the integrated HTLV-1 provirus, disrupting the viral oncoprotein Tax. Despite a maximum nucleofection efficiency of approximately 20% in MT-2 cells, the generation of diverse indels across multiple proviral copies underscores the precision and robustness of this approach. The moderate efficiency likely reflects limitations of nucleofection, including transient membrane permeabilization, cellular stress, and DNA damage responses that affect viability and stable editing. Interestingly, after the third nucleofection pulse, editing efficiency exceeded approximately 47% at the most suitable target site, further supporting the feasibility of achieving substantial proviral disruption under optimized conditions

CRISPR-Cas9–mediated editing of integrated HTLV-1 proviral sequences generated disruptive indels in the *tax* locus, demonstrating that viral oncogenic elements embedded in the host genome can be selectively inactivated. Repeated delivery enhanced editing across multiple proviral copies, indicating that progressive reduction of functional provirus is achievable. This strategy provides a framework to impair NF-κB activation, reduce the survival advantage of infected clones, and modulate molecular pathways associated with HTLV-1–driven leukemogenesis.

PERSPECTIVES

- Extend the strategy from *tax* to *hbz*, targeting conserved regions shared with its simian homolog *sbz*;
- Perform combined disruption of *tax* and *hbz/sbz* for in vivo therapeutic intervention in Chlorocebus aethiops primates living with the ancestral HTLV-1 isolate; https://doi.org/10.3389/fmed.2025.1616406
- Assess the effects of CRISPR-Cas9 editing on viral persistence, immune evasion, and the leukemic phenotype;
- Compare the functional outcomes of disrupting *tax* and *hbz/sbz* to strengthen the translational relevance of the model.

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