PRECISE EXCISION OF HTLV-1 PROVIRUS WITH DESIGNER-RECOMBINASES

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Background:
The oncogenic Human T-cell leukemia virus type 1 (HTLV-1) is the etiologic agent of the incurable disease adult T-cell leukemia/ lymphoma (ATL/ L), after decades of viral latency. In recent years, genome editing tools have emerged as promising antiviral agents. In this study, we engineered a Cre-derived site-specific recombinase to target HTLV-1 infected cells.

Methods:
Substrate linked directed evolution, flow cytometry, Western blot, quantitative PCR (qPCR)

Results:
To excise the HTLV-1 provirus, we identified a conserved *loxP*-like sequence (*hoxLTR*) present in the long terminal repeats (LTR) of the virus. Using substrate linked directed evolution, we isolated two designer-recombinases (E5 and G9), which efficiently recombine the *hoxLTR* sequence in bacteria. Furthermore, E5 and G9 show recombination activity in human cells ($CD4^+$ Jurkat T-cells, 293T), not only in the context of a fluorescent reporter or an HTLV-1 oncoprotein Tax expression vector, but also when challenged with the full proviral sequence. Moreover, in HTLV-1 chronically infected SP cells, expression of G9 led to the excision of proviral DNA from the host genome.

Conclusion:
In summary, our data suggest that recombinase mediated excision of the HTLV-1 provirus represents a promising strategy to reverse HTLV-1 infections and bears potential for future clinical applications.

Disclosure of Interest Statement:
Frank Buchholz and Joachim Hauber declare competing financial interests.

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