

Poster Sessions – Abstract P174

Universal Tre (uTre) recombinase specifically targets the majority of HIV-1 isolates

Karpinski, Janet¹; Chemnitz, Jan²; Hauber, Ilona²; Abi-Ghanem, Josephine³; Paszkowski-Rogacz, Maciej¹; Surendranath, Vineeth⁴; Chakrabort, Debojyoti¹; Hackmann, Karl⁵; Schröck, Evelin⁵; Teresa Pisabarro, María³; Hauber, Joachim² and Buchholz, Frank¹

¹Medical Systems Biology, Medical Faculty, TU Dresden, Dresden, Germany. ²Antiviral Strategies, Heinrich Pette Institute – Leibniz Institute for Experimental Virology, Hamburg, Germany. ³Structural Bioinformatics, TU Dresden, Dresden, Germany. ⁴Molecular Cell Biology and Genetics, Max Planck Institute, Dresden, Germany. ⁵Clinical Genetics, Medical Faculty, TU Dresden, Dresden, Germany.

Current drugs against HIV can suppress the progression to AIDS but cannot clear the patient from the virus. Because of potential side effects of these drugs and the possible development of drug resistance, finding a cure for HIV infection remains a high priority of HIV/AIDS research. We recently generated a recombinase (termed Tre) tailored to efficiently eradicate the provirus from the host genome of HIV-1 infected cells by specifically targeting a sequence that is present in the long terminal repeats (LTRs) of the viral DNA [1]. In vivo analyses in HIV-infected humanized mice demonstrated highly significant antiviral effects of Tre recombinase [2]. However, the fact that Tre recognizes a particular HIV-1 subtype A strain may limit its broad therapeutic application. To advance our Tre-based strategy towards a universally efficient cure, we have engineered a new, universal recombinase (uTre) applicable to the majority of HIV-1 infections by the various virus strains and subtypes. We employed the search tool SeLOX [3] in order to find a well-conserved HIV-1 proviral sequence that could serve as target site for a universal Tre from sequences compiled in the Los Alamos HIV Sequence Database. We selected a candidate (termed loxLTRu) with a mean conservation rate of 94% throughout the major HIV-1 subtype groups A, B and C. We applied loxLTRu as substrate in our established substrate-linked protein evolution (SLiPE) process [4] and evolved the uTre recombinase in 142 evolution cycles. Highly specific enzymatic activity on loxLTRu is demonstrated for uTre in both *Escherichia coli* and human cells. Naturally occurring viral variants with single mutations within the loxLTRu sequence are also shown to be efficiently targeted by uTre, further increasing the range of applicability of the recombinase. Potential off-target sites in the human genome are not recombined by uTre. Furthermore, uTre expression in primary human T cells shows no obvious Tre-related cytopathic or genotoxic effects. Finally, uTre expressing mice show no undesired phenotypes during their normal lifespan. We have developed a broad-range HIV-1 LTR specific recombinase that has the potential to be effective against the vast majority of HIV-1 strains and to cure HIV-1 infected cells from the infection. These results strongly encouraged us in our confidence that a Tre recombinase-mediated HIV eradication strategy may become a valuable component of a future therapy for HIV-infected patients.

References

1. Sarkar I, Hauber I, Hauber J, Buchholz F. HIV-1 proviral DNA excision using an evolved recombinase. *Science*. 2007;316(5833):1912–5.
2. Hauber I, Hofmann-Sieber H, Chemnitz J, et al. Highly Significant Antiviral Activity of HIV-1 LTR-Specific Tre-Recombinase in Humanized Mice. *PLoS pathogens*. 2013;9(9):e1003587.
3. Surendranath V, Chusainow J, Hauber J et al. SeLOX—a locus of recombination site search tool for the detection and directed evolution of site-specific recombination systems. *Nucleic acids research*. 2010;38(Web Server issue):W293–8.
4. Buchholz F, Stewart AF. Alteration of Cre recombinase site specificity by substrate-linked protein evolution. *Nature biotechnology*. 2001;19(11):1047–52.

Published 2 November 2014

Copyright: © 2014 Karpinski J et al; licensee International AIDS Society. This is an Open Access article distributed under the terms of the Creative Commons Attribution 3.0 Unported (CC BY 3.0) License (<http://creativecommons.org/licenses/by/3.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.