

Lentiviral Delivered VRX496 Long Antisense Sequence Exerts Molecular Pressures on HIV-1 in Human Subjects

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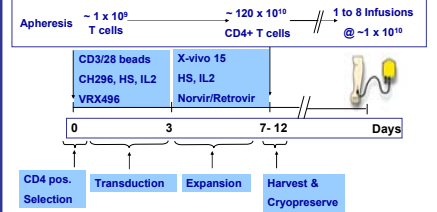
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Background:

In a phase II open label clinical trial, forty HIV infected subjects were infused with their own CD4 T cells previously transduced ex-vivo with VRX496, a lentiviral vector encoding for a 937 nt antisense against the *env* gene. VRX496 molecular pressures on the diversity and the replicative fitness of the HIV-1 quaspecies pre- and post-infusions were investigated.

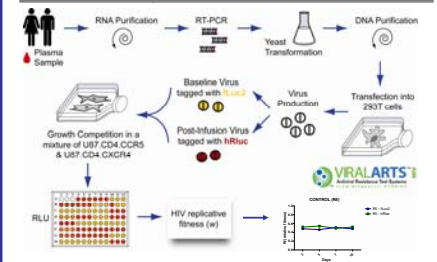
VRX496 Cellular Processing



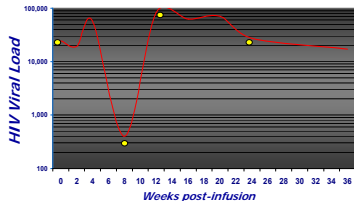
Methods:

Plasma samples were collected at different time points during the course of the study. Samples from nine subjects were randomly included in this study. HIV-1 *env* sequences were amplified by RT-PCR, cloned into expression vectors, sequenced for phylogenetic studies and viral replicative fitness analyzed.

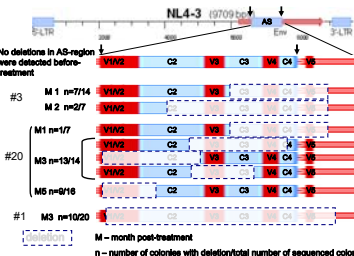
DHI's Fitness Assay Protocol



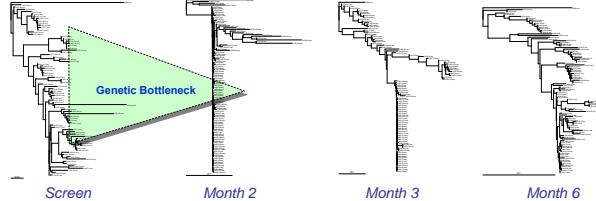
Time points studied for quaspecies analysis



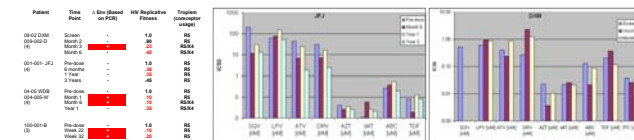
Mapping of the Envelope Deletions after VRX496 Treatment



Broad Variations in Quaspecies Diversity in Few Months



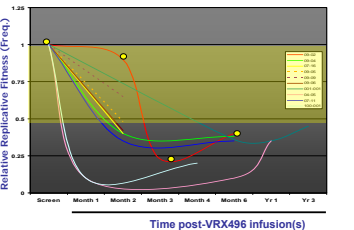
Co-Receptor Usages and Drug Susceptibilities are not affected



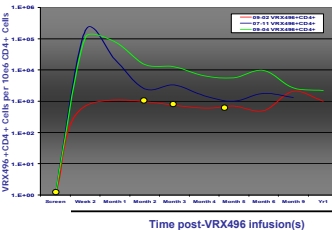
Other Results (not shown)

Found wt-HIV heterogeneity decreased after VRX496 therapy for all analyzed patients
Found different and unusual nucleotide changes in area targeted by antisense (G to A) versus untargeted areas (A to G) in 4/5 patients

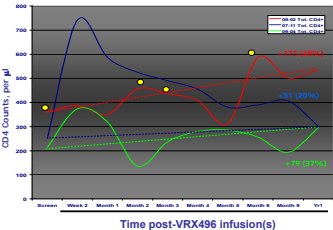
Reduced Replicative Fitness After VRX496 Treatment



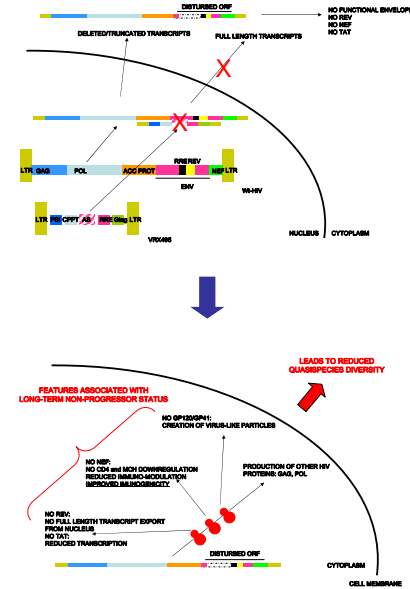
Long Term Persistence of VRX496 Modified Cells



Overall CD4 counts are increasing



Mechanism of Action: Working Hypothesis



Conclusions:

This is the first *in vivo* demonstration of anti-HIV antisense activity in cells delivered by a lentiviral vector. Antisense pressures resulted in *env* deleted mutants, massive quaspecies reductions and impaired viral replicative fitness. The parameters studied indicate that VRX496 exerts high pressures on wt-HIV, which were not reflected by the conventional read out methods to monitor HIV infection.