Optimising the production of the 4th generation lentiviral vector (LV) TetraVecta™ System: towards plug-and-play LV manufacturing.

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Introduction

HIV-1 based lentiviral vectors (LVs) have remained largely unchanged over the past 20 years. As therapeutic applications become more complex, there will be a need to advance LV design. For example, enhancements to virion composition, vgRNA integrity and integrated LV insulation will become ever more important. Repression of transgene expression during production may also result in increased titre and improved batch to batch consistency. Here we describe the development of, and enhancements to, OXB's next generation of LVs.

TetraVecta™ System: A New Feneration of LVs

OXB have extensively redesigned the 3rd Generation LV, culminating in TetraVecta™ system; a 4th Generation LV comprising improvements in LV quality, safety, capacity and performance.

2KO-Genome™: Simplified vRNA biogenesis

- Inactivation of the HIV-1 Major Splice Donor (MSD) in 2KO-LVs reduces unwanted splicing and increases availability of a single, full-length vRNA species during production.
- 2KO-LVs[A] are produced in presence of modified U1 snRNA (256U1) to enhance vRNA stability.

SupA-LTR™: Enhanced safety in target cells

- SupA-LTR™ LVs contain reengineered LTRs with improved polyadenylation activity when integrated in target cells.
- Over 10-fold reduced transcriptional read-in to the integrated LV and enhanced transgene expression due to enhanced mRNA stability.

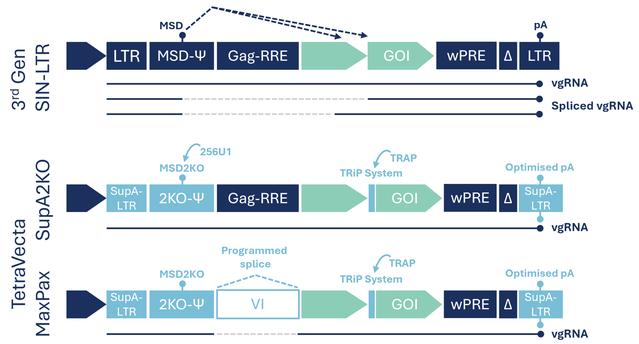
TRiP System™: Transgene Repression in Production

- Expression of therapeutic transgenes during LV production can impact producer cell health and lead to downstream processing inconsistencies. The TRiP system™[B] offers a translational block during LV production, allowing vgRNA to be produced in the absence of transgene expression.
- Reduced expression of Chimeric Antigen Receptors (CARs) on the surface of LVs may reduce risk of off-target cell transduction when LVs are used in vivo.

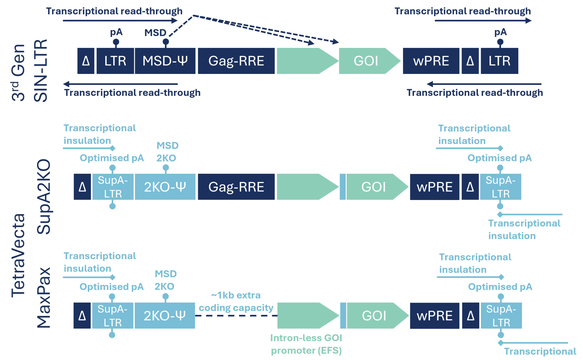
MaxPax™: Reduction of HIV *cis*-acting elements in LVs

- MaxPax™ LVs utilise a unique nuclear export system, with the Rev Response Element (RRE) being replaced by a synthetic 'vector intron' (VI). This replacement, in addition to minimization of other cis-acting elements, liberates an additional 1 kb in cargo capacity
- Simplified, Rev- and U1256-independent LV production reduces plasmid needs during manufacture

3rd Gen-LV vs. TetraVecta™ System LVs: In Production

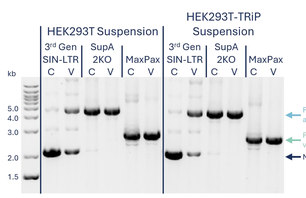


3rd Gen-LV vs. TetraVecta™ System LVs: Integrated



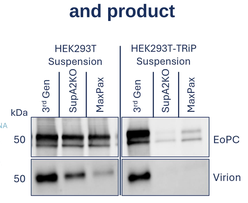
Case Study: 5L Production of 3rd Gen-LVs and TetraVecta™ System LVs Encoding a CAR Transgene

Simplified vgRNA biosynthesis



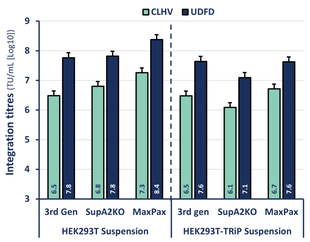
TetraVecta™ LVs comprise exclusively full length vgRNA in Production cells (C) and virions (V). Assessed by RT-PCR. 3rd Gen and SupA2KO = EF1a, MaxPax = EFS

CAR repression in production and product



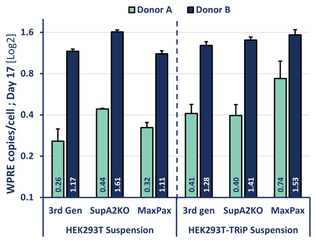
Western blot analysis confirmed CAR expression in production cells is reduced in HEK293T-TRiP cells when encoded using a TetraVecta™ LV.

High titres for all OXB LV variants



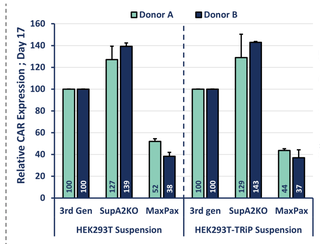
TetraVecta™ LVs achieved comparable titres to its 3rd Generation counterpart when produced in 5L bioreactors. The TRiP system had negligible impact on output titres. Averaged data from 2 bioreactor runs per condition (±SD)

Comparable T-cell transduction



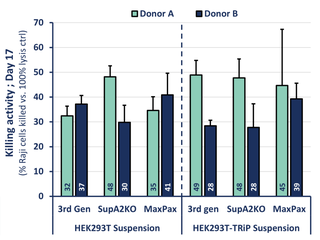
293T & 293T-TRiP produced TetraVecta™ LVs transduced primary T cells with similar efficiency across two donors. Average integration events achieved using purified LV from 2 bioreactor runs (±SD). T-cells transduced at MOI = 3

Enhanced T-cell CAR expression



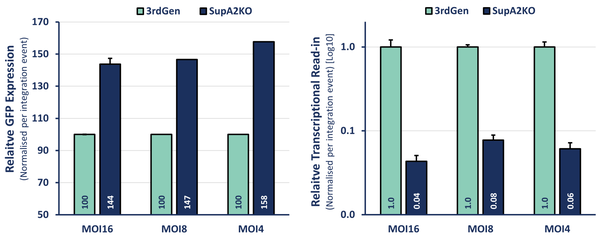
SupA2KO LVs exhibit 20-40% higher CAR expression in transduced T-cells. Averaged data from 2 bioreactor runs per condition (±SD). Note: MaxPax encodes a weaker intron-less promoter.

T cell killing assay



CAR-T cells generated by OXB's 3rd Gen and TetraVecta™ LVs exhibit similar potency. TetraVecta™ LVs perform comparably to 3rd Gen LVs. Averaged killing data from 2 bioreactors per condition (±SD).

SupA2KO-LVs: Superior Transcriptional Insulation

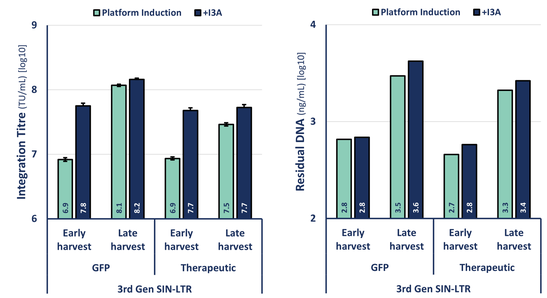


SupA2KO LVs boasted a 40-60% increase in GFP expression in Primary Human Dermal Fibroblasts (HDFs) across a range of MOIs. MFI normalised to integration events (% vs. 3rd Gen control ±SD).

SupA2KO LVs exhibit >10-fold reduced transcriptional read in events in HDFs vs. 3rd Gen SIN-LTR LVs. Psi copies assessed by qPCR, normalised to integration events (fold change vs. 3rd Gen control ±SD)

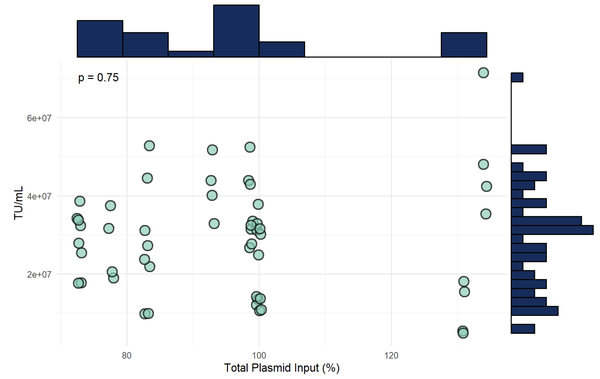
Recent Developments & Platform Robustness

Inducer I3A enables early crude harvest with lower residuals



Addition of OXB's proprietary small molecule enhancer, Ingenol-3-Angelate (I3A)[C] (see P1364), enables earlier vector harvest with minimal impact on output titres. LV harvested earlier displays significantly reduced levels of residual DNA compared to late harvest samples. Performed at small scale in the absence of a nuclease step.

SupA2KO-LV + I3A: broadening optimal pDNA input 'space'



As shown above when SupA2KO TU/mL data is filtered for +I3A, standard harvest time and standard plasmid conditions (±35%), there is no change or trend apparent in TU/mL (p=0.75) indicating robust titre over a range of conditions.

Summary

OXB's TetraVecta™ system encompasses multiple modifications, which improve LV quality, safety, capacity and performance. Here, we validated TetraVecta™ LV performance at 5L bioreactor scale, compared against OXB's current 3rd Generation platform, obtaining an improved vRNA splicing profile, comparable LV infectivity, and comparable activity of transduced T-cells. SupA2KO-LVs also exhibited increased transgene expression and transcriptional insulation in target cells. Finally, we show some recent advancements in vector production, suggesting that earlier harvests, in the presence of I3A, may be beneficial by obtaining comparable titres to late harvest with significantly reduced residual DNA.

[A] Wright, J., et al., (preprint). Improved Production and Quality of Lentiviral Vectors By Major-Splice-Donor Mutation and Co-Expression of a Novel U1 Snrna-Based Enhancer. *Heliyon*
[B] Maunder, H. E., et al., (2017). Enhancing titres of therapeutic viral vectors using the transgene repression in vector production (TRiP) system. *Nature Communications, 8*
[C] Moore-Kelly, et al., (2025). Enhancing titres of therapeutic lentiviral vectors using PKC agonists. *Mol Ther Methods Clin Dev.* [Manuscript accepted]

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