The TetraVecta™ System: Optimisation and Exemplification of OXB's Next Generation Lentiviral Vector (LV)

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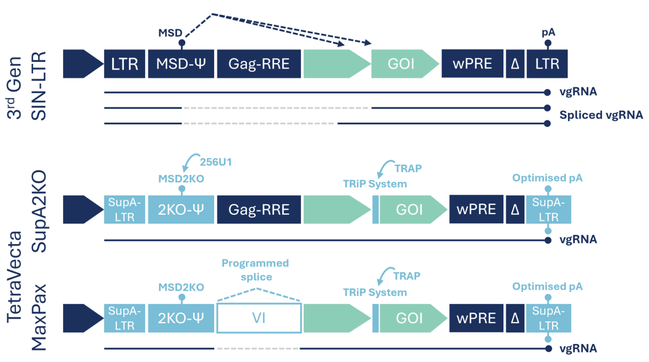
Introduction

HIV-1 based lentiviral vectors (LVs) are the mainstay for stable gene delivery to non-/dividing cells. However, LV architecture has largely unchanged over the past 20 years, with 3rd generation LVs remaining gold-standard. As therapeutics become more complex, and the move to *in vivo* applications increase, advancement of LV design will be necessary. Enhancements to eliminate transgene expression during LV production could improve purity, batch consistency, and *in vivo* efficacy. Advancements in viral composition, viral genome RNA (vgRNA) integrity, and integrated LV insulation are also vital for further LV development. Here we describe OXB's TetraVecta™ System, the new generation of LVs, with focus on enhancements to the TRiP System™[A].

TetraVecta™ System: A New Generation of LVs

OXB have extensively redesigned the 3rd Generation LV, culminating in the TetraVecta™ system; a 4th Generation LV comprising improvements in LV quality, safety, capacity and performance (Fig.1).

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



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

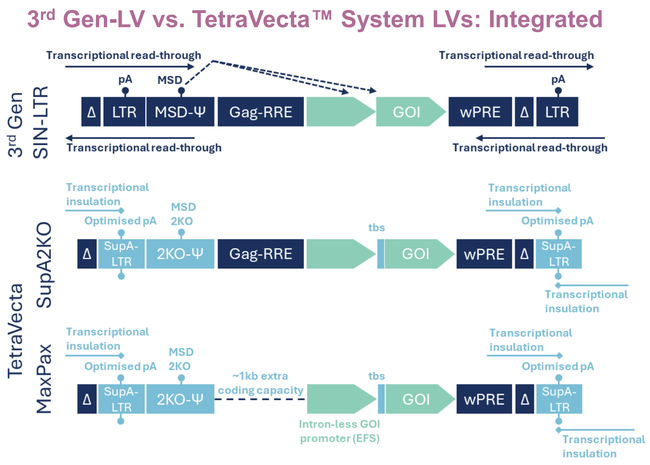


Fig.1. OXBs TetraVecta™ System

2KO-Genome™: Simplified vgRNA biogenesis

- Inactivation of the HIV-1 Major Splice Donor (MSD) in 2KO-LVs reduces promiscuous splicing, thus increasing the availability of full-length vgRNA species during production[B].
- 2KO-LVs are produced in the presence of modified U1 snRNA (256U1) to enhance vgRNA stability (not needed for MaxPax™).
- Induction with OXBs proprietary small molecular enhancer, Ingenol-3-Angelate (I3A)[C] a PKC agonist, works synergistically with U1 snRNA to achieve 9-fold increases in titre.

SupA-LTR™: Enhanced safety in target cells

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

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 a further 1 kb in cargo capacity.
- Simplified, Rev-independent LV production reduces plasmid needs during manufacture.

TRiP System™: Transgene Repression in Production

- Expression of therapeutic transgenes during LV production can impact producer/packaging cell health, interfere with vector assembly, and contaminate the vector.
- This can cause reduced vector yield and quality, as well downstream processing inconsistencies. **Additionally, expression of therapeutic targets on LV surfaces could cause issues with off-target cell transduction and immunogenicity *in vivo*[E].**
- The TRiP system™ (Fig.2) offers a translational block during LV production, allowing vgRNA to be produced in the absence of transgene expression.

The TRiP System™ in action

OXBs TRiP system™ co-expresses *Bacillus* bacterial tryptophan RNA binding attenuating protein (TRAP) in production cells (transiently or stably) (Fig.2). TRAP binds to an optimised 55nt TRAP binding sequence (tbs), upstream of the transgene ORF in the vector genome. In production cells, in excess of L-tryptophan, TRAP binds to the tbs sequence, forming a stable RNP complex, and blocks translation initiation of the transgene mRNA. Transduction of the vector in target cells has no impact on transgene expression.

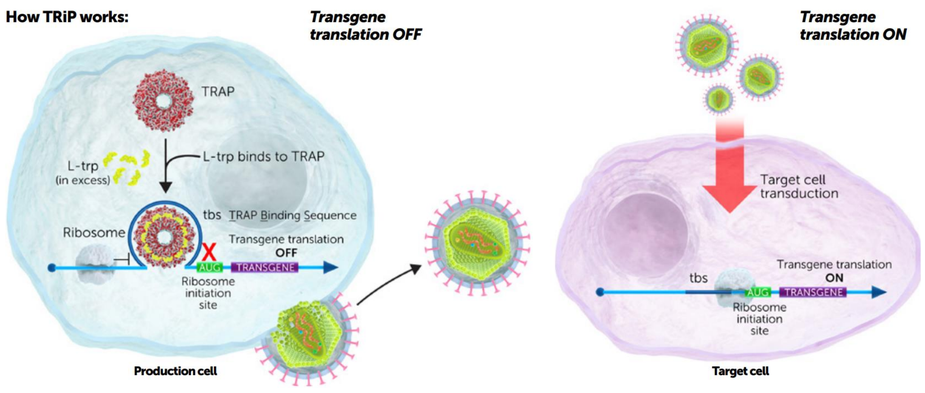


Fig.2. OXBs TRiP system™

TRiP 5' UTR design

- OXB have engineered a tbs sequence, containing [KAGNN]X11, to overlap with the transgene Kozak sequence, occluding the primary AUG from the scanning ribosome in the TRAP-tbs complex (Fig.3).
- The TRiP 5'UTR of intron-less promoters (i.e EFS) contain the L12 leader sequence, the first 12 nucleotides (nt) of exon 1 of EF1α.
- Promoters with introns, (i.e EF1α) have TRiP 5'UTR with Exon 1 of EF1α and the first 12 nt of exon 2.

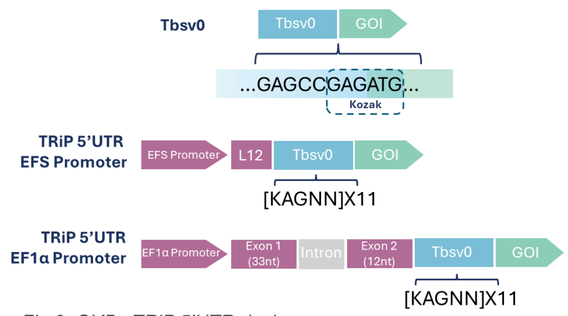


Fig.3. OXBs TRiP 5'UTR design.

SupA2KO is the optimal genome for the tbsV0 TRiP System™

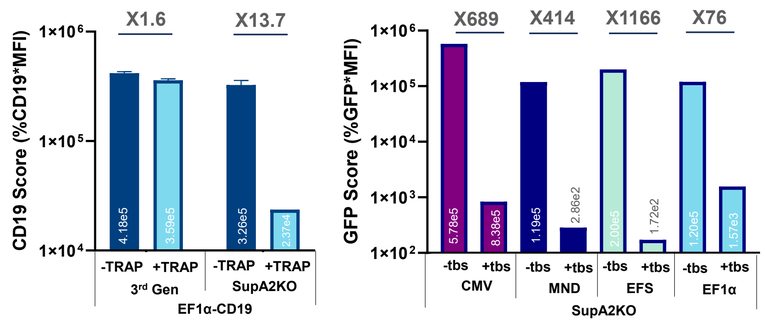
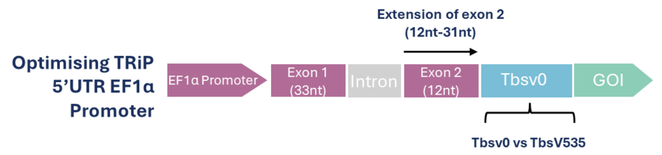


Fig.4. SupA2KO genomes repress CAR and reporter transgenes with the OXB TRiP System™

- SupA2KO with U1 is the optimal genome for the tbsV0 TRiP System™ (**8-fold improvement**) (Fig.4).
- The TRiP system™ has been verified with a range of constitutive promoters, including CMV, MND and EFS.
- Can repression with EF1alpha genomes, particularly for CARs, be further refined?

Optimising 5'UTR to improve TRiP repression in EF1α genomes



1) **Explore increased EF1α exon 2 length**
2) **Explore optimisation of the tbs sequences, maintaining the [KAGNN]X11 consensus**

Top two candidates improve TRiP CAR repression in EF1α genomes

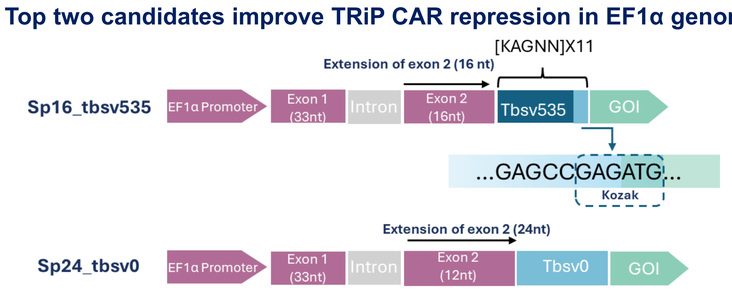
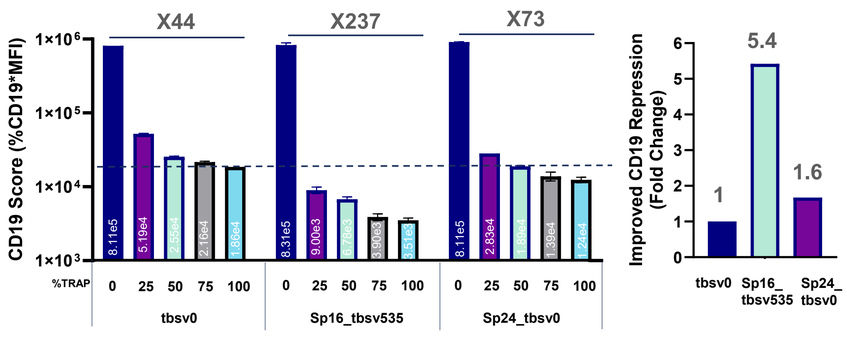


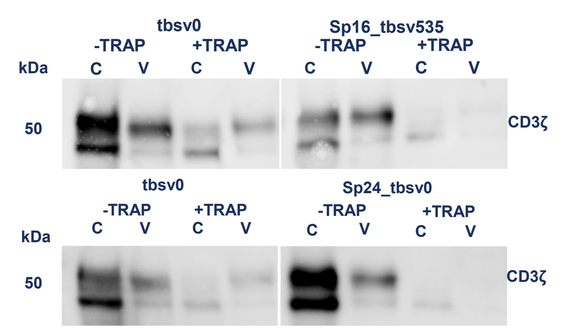
Fig.5. Re-engineering the Ef1α TRiP 5'UTR.

OXB preliminary studies produced **two top candidates** to take forward for vector production with EF1α CAR and GFP transgenes (Fig.5):

1) sp16_tbsv535 2) sp24_tbsv0



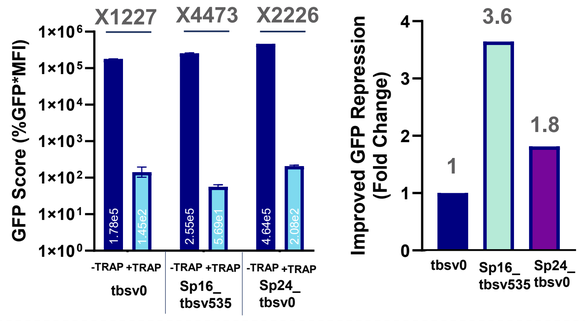
- CD19 repression (CD19 flow) improved by both candidates, but optimal with sp16_tbsv535 (**x5.4 improvement**) (Fig.6).
- Improvement in repression is clear in CD3ζ western blot showing end of production cell (C) and crude vector (V).



- A lower input of pTRAP produces CD19 repression levels better than tbsv0 at 100% pTRAP (25% pTRAP for sp16_tbsv535), with potential to titrate pTRAP further.
- This offers a greater ability to optimise pDNA ratios to maximise titre.
- Although, implementation of the stable TRAP cell line is preferable.

Fig.6.Extension of EF1α exon 2 and alternate tbs variant improves CD19 repression in producer cells.

Top two candidates improve TRiP GFP reporter repression in EF1α genomes



- GFP repression (GFP flow) improved by both candidates, but optimal with sp16_tbsv535 (**x3.6 improvement**).
- Sp16_tbsv535 to be taken forward for larger scale studies.

Fig.7.Extension of EF1α exon 2 and alternate tbs variant improves GFP repression in producer cells.

Summary

- OXB have optimised the TRiP 5'UTR, **improving EF1α repression of CD19 by approx. 5-fold compared to tbsV0 TRiP 5' UTR, totalling in an approx.60-fold increase in repression compared to 3rd Gen tbsV0**.
- With improved vector quality, safety and efficacy these data emphasise the potential of TetraVecta™ to be the LV platform of choice for the next generation of gene therapies, particularly when moving forward to *in vivo* therapies.

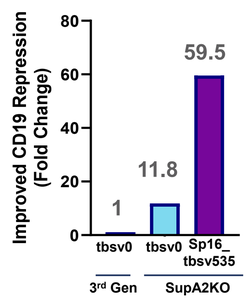


Fig.8. Approximately 60-fold improvement in CD19 repression with 4th Gen SupA2KO sp16_tbsv535 compared to 3rd Gen tsbv0 control.

[A] Maunder, H. E., et al., (2017). Enhancing titres of therapeutic viral vectors using the transgene repression in vector production (TRiP) system. Nature Communications, 8

[B] Wright, J., et al., (2025). Improved Production and Quality of Lentiviral Vectors By Major-Splice-Donor Mutation and Co-Expression of a Novel U1 Snrna-Based Enhancer. Heliyon 11, e43732.

[C] Moore-Kelly, et al., (2025). Enhancing titres of therapeutic lentiviral vectors using PKC agonists. Mol Ther Methods Clin Dev. 33.

[D] Wright, J., [In revision] Enhanced transcriptional insulation of lentiviral vectors using 'sequence-upgraded polyA long terminal repeats' (supA-LTRs) for publication in Molecular Therapy Methods & Clinical Development.

[E] Coradin,T., et al., (2025). Efficient in vivo generation of CAR T cells using a retargeted fourth-generation lentiviral vector. Mol Ther. [Preprint].