

## CELL & GENE THERAPY INSIGHTS

THE LATEST DEVELOPMENTS  
IN VIRAL & NON-VIRAL VECTOR  
MANUFACTURING

SPOTLIGHT

### INNOVATOR INSIGHT

# Standardizing viral vector manufacture: maximizing production with the TRiP System™

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The use of viral vectors to create novel gene- and cell-based medicines is now a reality. As gene therapy matures into a new era, the industry will need to adopt improvements to viral vector manufacturing to meet the demand for GMP grade material. The activities of process optimisation/characterisation with any given viral vector entering into clinical development are considerable. The cell culture (Upstream) and purification/concentration (Downstream) aspects of this process are multi-faceted. The amount of vector produced during Upstream can vary depending on the transgene encoded, especially if the active protein is expressed in the production cell when constitutive or leaky tissue specific promoters are employed. Oxford BioMedica has developed the Transgene Repression In vector Production (TRiP) System™ to recover vector titres compromised by transgene expression. The system utilises the bacterial protein TRAP and its short RNA binding sequence – inserted within the transgene leader sequence – to repress transgene mRNA translation during vector production only, leaving expression unaffected in target cells. The TRiP System™ has been used to fully recover titres of Lenti, Adeno and AAV based vectors, and is expected to be universally applicable to any viral vector/vaccine platform. We anticipate that the TRiP System™ will enable new gene therapies to be considered, and assist those already in development to become commercially viable. Given that only TRAP and the viral proteins of the vector platform being employed will be expressed during Upstream, the TRiP System™ opens the door to ‘plug-and-play’ manufacturing, greatly minimising the burden of process development within a given pipeline.

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“The TRiP System™ is on its way to become a new standard in viral vector manufacturing, implemented across diverse viral vector systems.”

The development of new gene therapies for genetic disorders and cancer is increasing at a rapid pace. Several promising therapies have recently received regulatory approval and are now commercialized, including Kymriah™ and Yescarta® for the treatment of certain B-cell malignancies, and Luxturna™ for the treatment of RPE65-mediated inherited retinal disease [1-3].

The contemporary vectors of choice are derived from engineered viruses including those based on gamma-retroviruses (RVs), lentiviruses (LVs), adeno-associated viruses (AAVs) and adenoviruses (AdVs), although a variety of non-viral vector approaches are now gaining greater momentum [4]. Given the diverse complexities associated with the broad range of indications being targeted by vectored gene therapies, it is likely that different viral vectors will ‘find their niche’ and that no single viral vector will become the de facto ‘industry standard’. As the successes of these approaches in clinical trials begin to build towards regulatory approval and commercialization, attention has focused on the emerging bottleneck in mass production of GMP grade vector material [5]. A way to overcome this challenge is to find new ways to maximize titer during viral vector production. Oxford BioMedica has developed the TRiP System™, a new technology that allows suppression of the

transgene expression in the production cell, essentially mitigating any side effects of the therapeutic protein on the manufacturing process. The TRiP System™ is on its way to become a new standard in viral vector manufacturing, implemented across diverse viral vector systems.

### VIRUS TO VECTOR

The current break-through gene therapy products owe their success partly to the previous three decades of viral vector platform development, wherein complex virus genomes have been stripped down to their minimal functional sequences, and components separated onto multiple plasmids, to enable safe, efficient and stable delivery of transgenic cassettes to primary cells. In many cases, this has involved removal of various auxiliary and accessory genes specific to the virus/vector platform that are considered redundant in ‘single-round’ transduction-competent viral vectors [6-8]. Additionally, removal of certain functions, such as the multiple gene-regulator tat from HIV-1 based LVs, were important safety steps in which Oxford BioMedica played an important role [9]. Adenoviral vector development from first to third generations started with progressive deletion of regulatory and immune-regulatory genes (E1, E2, E3, E4) from the vector genome, and has essentially finished at completely ‘guttled’ AdVs, although these remain dependent on helper AdVs that are difficult to remove entirely from final vector material [10]. Similarly, AAV vectors have been engineered such that only the cis-acting viral

sequences that allow production and packaging of the vector genome are present with transgenic sequences. A key advancement in retroviral vector development was the ‘self-inactivating’ (SIN) modification to the vector genome to remove viral enhancer-promoter sequences, greatly limiting the chances activation of proto-oncogenes within the neighbouring chromosome [11]. Together with sequence optimization processes, such as codon-optimization of packaging cassettes (minimizes recombination between components) [12], these modifications have led to the current state-of-art viral vector platforms in which no replicating competent entities have been detected to date [13–15]. Whilst there remains a theoretical risk of insertional mutagenesis by SIN-LVs, a realization of such an event has not been empirically observed in patients. In cases where only short term transgene expression is required, integration-defective LVs (ID-LVs) harboring mutation(s) in the integrase protein and/or in cis-acting elements on the vector genome are being used [16], as well as AAV vectors, which integrate into the host genome at low levels (~0.1%) [17]. Such rational design of viral vectors continues alongside the burgeoning field of ‘library-enhanced’ viral vectors, for example DNA shuffling of AAV capsid sequences for improved targeting and escape of pre-existing immunity [18]. Examples of rational design have led to the principle of targeting of enveloped viral vectors by pseudotyping with different viral glycoproteins [19], as well as microRNA-regulated vectors [20], and the development of novel vector architecture [21].

## THE CHALLENGES OF VIRAL VECTOR PRODUCTION

Historically, the main choice of viral vector cell line has been those based on the HEK293 cell line developed by Frank Graham and colleagues several decades ago [22], and later the HEK293T cell line that expresses the SV40 large T antigen [23]. Effectively, researchers have selected these cells by trial-and-error to find that they typically yield higher titers than other cell lines. This is perhaps in part due to their high transfection efficiency but in hindsight, also due to their relatively low (or absent) expression levels of viral restriction factors that have subsequently been identified by the research community over the last 15 years [24]. Only relatively recently have other cell lines been developed to provide alternative base cell lines for vector production [25,26]. Whatever the choice of production cell, the output of viral vector titers during the ‘Upstream’ process phase can be affected by several different factors, for example [27]:

- ▶ Viral serotype/pseudotype employed;
- ▶ Transgenic sequence composition and size;
- ▶ Media composition/gassing/pH;
- ▶ Transfection reagent/process;
- ▶ Chemical induction and vector harvest timings;
- ▶ Cell fragility/viability;
- ▶ Bioreactor shear-forces; and,
- ▶ Impurities

“...it is probable that biologically active transgene proteins will impact on some aspect of vector product; from cell viability/vitality, to vector assembly/activity, to drug product purity”

The potential variability in the composition of ‘crude’ harvest material typically impacts on the performance of the different steps taken during the ‘Downstream’ process, for example ion-exchange chromatography, size-exclusion and (sterile) filtration steps [28]. Both Upstream and Downstream processes ultimately determine the purity of the drug product being administered. Certain gene therapies for direct *in vivo* administration will likely require high drug product titers in order to achieve target doses. This requires large concentration factors over the process, and if crude harvest titer is limiting this will necessarily result in ‘over’-concentration of impurities such as residual DNA.

However, one single factor that affects many of these considerations is the potential expression of the transgene product during the Upstream process. This often means that the entire viral vector process will be bespoken for a given transgene-expressing vector, which places a considerable burden of resource and time for process characterization and validation.

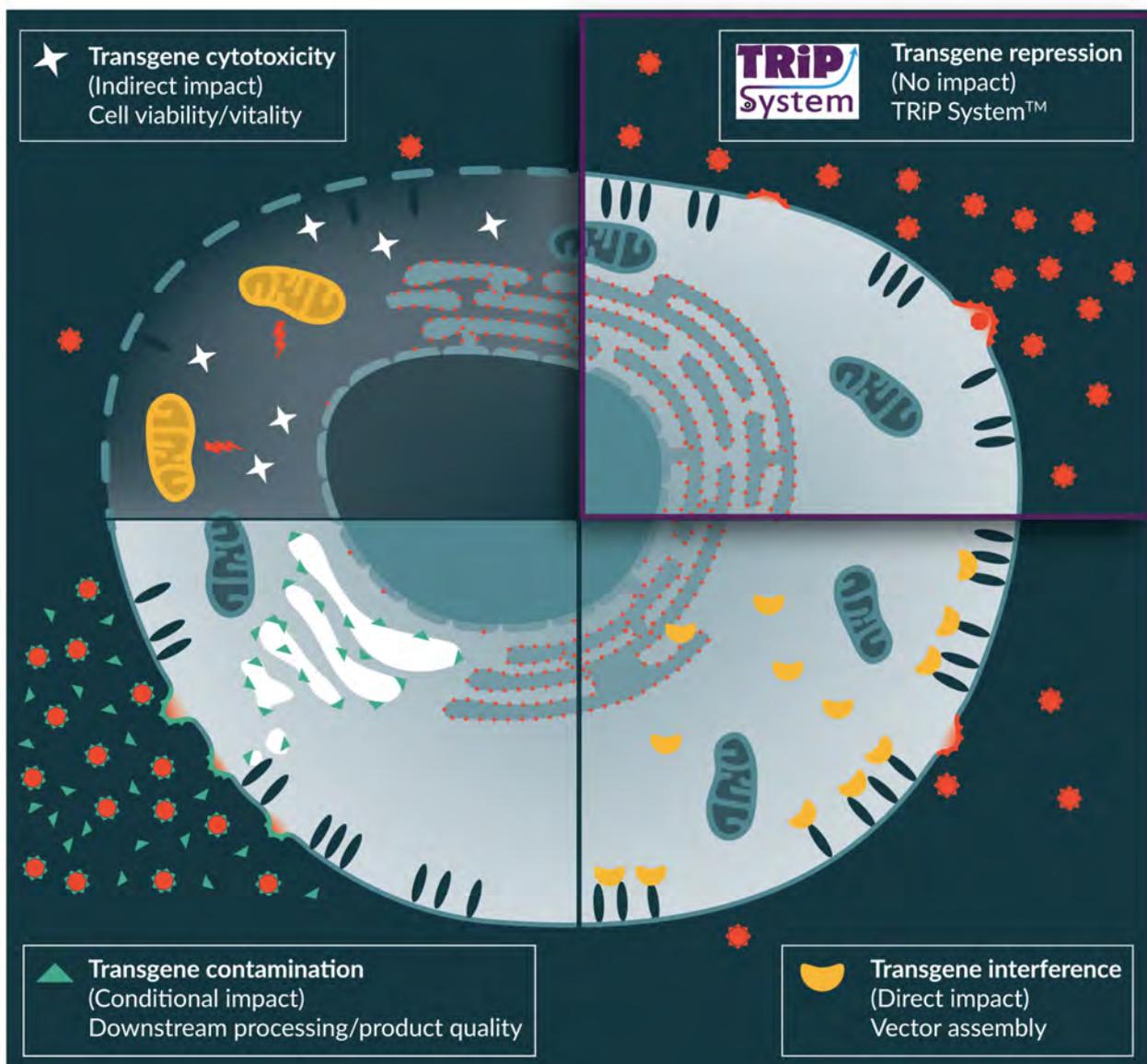
#### HIGH TRANSGENE EXPRESSION: BE CAREFUL WHAT YOU WISH FOR

The level of transgene expression within vector production cells is

dictated by several cis-acting nucleotide sequences designed into the expression cassette: promoter/enhancer elements (transcription activity) [29]; introns/polynucleotide length-composition/polyadenylation (mRNA export/stability) [30,31]; and Kozak sequence/UTR length-composition/codon usage (translation efficiency) [32–34]. Clearly, there are other factors in the make-up of the protein itself that contribute to its half-life and activity; indeed, it is protein activity that is ultimately the most important factor both from the perspective of *in vivo* efficacy and the unwanted activity during vector production. Typically, the researcher will aim to optimize all of these sequences in order to maximize activity in the target cell to increase chance of therapeutic benefit and to potentially allow lower dosing levels. However, unwanted expression during vector production often remains as a ‘brut fact’ after this optimization process. The impact of transgene activity during viral vector production can sometimes be predicted from the outset (such as a known cytotoxic protein) but others cannot (Figure 1). ‘Benchmark’ vector titers are often reported at high levels for GFP or other inert reporter-encoding vectors but it is probable that biologically active transgene proteins will impact on some aspect of vector product; from cell viability/vitality, to vector assembly/activity, to drug product purity. It is our experience, and of others within the field, that LVs expressing transgenes such as certain CARs (encoding signaling domains) and CRISPR-cas9 cassettes can be well below benchmark levels [35]. We previously demonstrated that constitutive Factor VIII expression limited functional virion

► **FIGURE 1**

The impact of problematic transgene expression during viral vector production.



Viral vector production is a complex process involving many cellular processes. Unwanted expression of the transgene during vector production can have unpredictable impact on the manufacturing process.

Upper left panel: Transgene cytotoxicity may have an indirect impact on vector production by affecting cellular viability or vitality.

Lower left panel: Transgene contamination may have a conditional impact on the manufacturing process. For example if the transgene protein is a membrane or secreted protein it will likely become imbedded or associated with the vector virions, possibly impacting some aspect of Downstream processing, even if no observable reduction in crude vector titers are observed.

Lower right panel: Transgene interference may have a direct impact on vector assembly, leading to reduced virion production or reduce activity.

Upper right panel: Transgene repression (by the TRiP system™) minimizes unwanted impacts imposed by the transgene protein.

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assembly during LV production [36]. Alternatively, the transgene may have no significant impact on measurable output vector titers but may result in reduced cell integrity leading to increased contaminants

inputting to Downstream processes and possibly into the drug product. Conversely, there are many published examples of biologically active transgenes successfully produced and delivered by viral vectors,

indicating no significant impact on vector production or purification; but this perhaps tells only half of the story – what about all the failed attempts to produce viral vectors encoding genes x, y and z, that did not make it to the editors inbox?

It is usual, when conducting early proof-of-efficacy experiments in pre-clinical models to consider 3-to-5 fold lower vector titers (compared to benchmark values) as practicable levels of vector production at research scales, without contemplating the knock-on consequences for scale-up and cost-of-goods at GMP production scales. This ultimately may determine whether a promising therapy can be realized commercially. Still other conceptually promising approaches may be terminated during these early development stages because the transgene activity is so potently detrimental to viral vector titers. Therefore, the ability to repress transgene protein expression/activity during vector production becomes extremely desirable. To do so may allow previous viral vector therapies – once hampered by poor titers – to be fully evaluated for clinical development. It would also represent a significant step to standardized viral vector manufacturing processes whereby only the viral vector components are expressed during the Upstream stage.

### TRANSGENE: QUIET PLEASE!

There are a number of approaches to minimize transgene expression during viral vector production. The development of next generation tissue-specific promoters is increasing within the gene therapy field. Whilst minimizing transgene

expression within vector production cells is achievable with use of certain tissue specific promoters, leaky expression is possible and will always need to be determined empirically. For example, the Adenovirus E1a and SV40 Large T gene products expressed in HEK293T cells have been shown to be promiscuous transactivators of housekeeping and tissue-specific genes [37–40]. Current manufacturing methods that require large quantities of plasmid DNA per cell at transfection may result in a substantial amount of transgene expression from even very ‘quiet’ tissue specific promoters. The initial cost and time investment in order to develop a bespoke tissue specific promoter (and for it to be validated in all the specific models of choice in pre-clinical studies) may be too great to justify, especially when there may be proven alternatives immediately available. Moreover, the validation of specificity/activity of a bespoke promoter within animal models may not necessarily translate in the clinic. Additionally, there are settings in which the use of completely silent tissue specific promoters will not quench transgene expression. For example, we have shown that the genomic RNA of RVs/LVs is a proficient mRNA for translation of ORFs directed by IRES elements, leading to substantial transgene expression even when the transgene cassette has no promoter [41]. Finally, it actually may be desirable to use strong constitutive promoters to maximise expression in a wide number of target tissues if such an approach is required to realize clinical efficacy.

Other molecular tools available that can achieve various degrees of transgene repression include: tet/cumate ON/OFF and tetR/lacR

repressor systems (transcription control) [42–45], ribozyme switches/microRNA (mRNA stability) [46] and protein-degron systems (protein stability) [47,48]. The caveats to such approaches typically fall into at least one of four categories: difficult to engineer (e.g., inserting tet-operator sequences into promoter of choice without compromising promoter functionality); modest levels of repression achieved; impact on vector utility (titers, capacity); and risk of non-human protein sequences being expressed or appended to the transgene protein *in vivo*.

### TRANSGENE REPRESSION IN VECTOR PRODUCTION (TRIP) SYSTEM™

Oxford BioMedica recently reported the development of the TRiP System™, a universal transgene repression system for limiting translation of transgene ORFs during viral vector production [49]. The TRiP System™ is based on previous characterisation of the bacterial protein ‘tryptophan RNA-binding attenuation protein’ (TRAP) [50]. In bacteria such as *Bacillus subtilis*, TRAP is a component of the L-tryptophan (L-trp) synthase operon feed-back loop [51]. TRAP is a small protein of ~8kDa in size, which self-assembles into a toroidal structure composing typically 11 monomers. The resulting tyre-like scaffold contains 11 L-trp-binding pockets, which when bound by L-trp causes a conformational change such that TRAP binds to its target RNA sequence around the ‘tyre tread’. The trp operon is regulated by TRAP both at the level of pre-mature RNA polymerase termination and at the level of

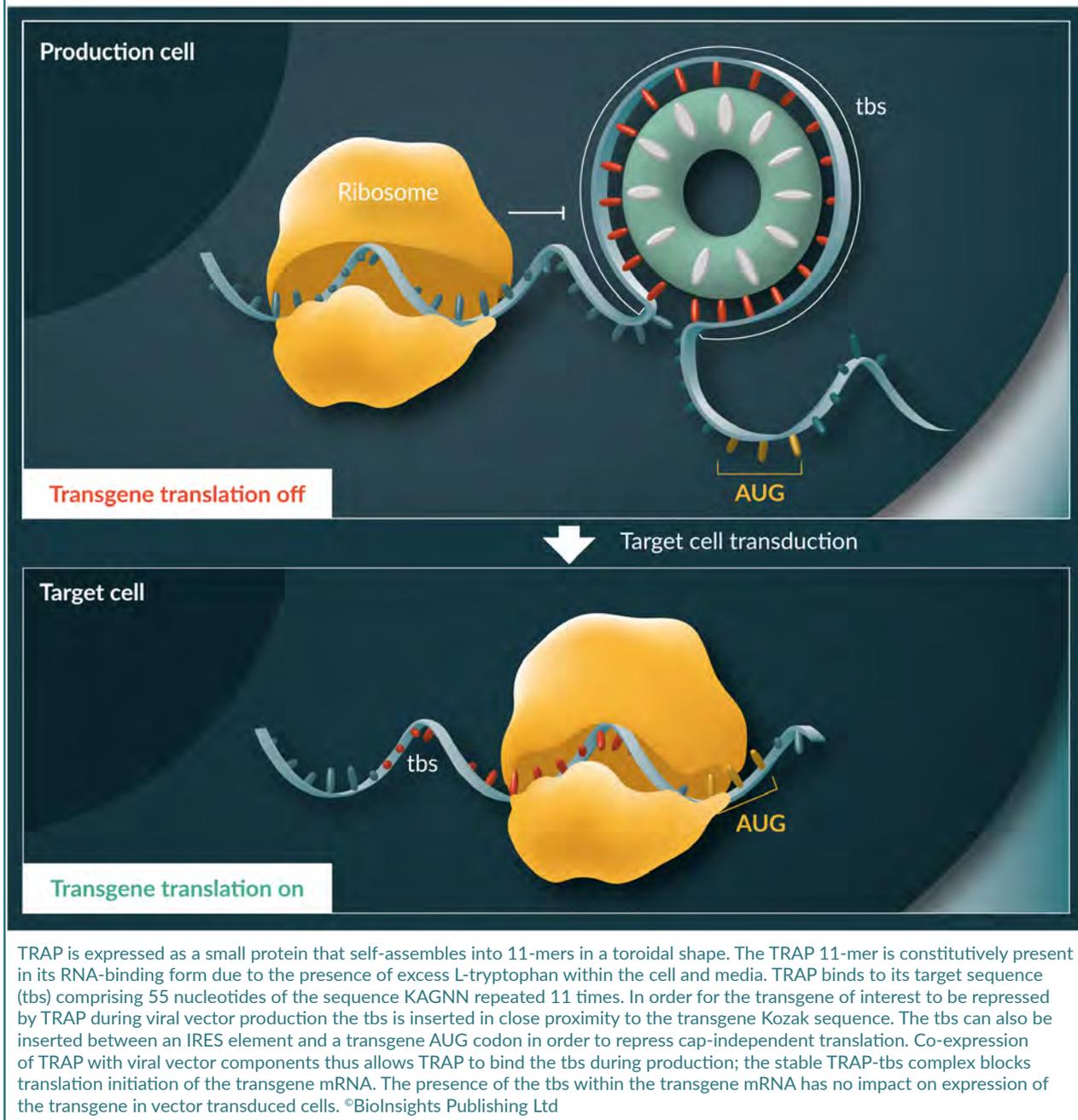
‘The TRiP System™ is consistently capable of achieving transgene repression levels up to two orders of magnitude...’

translation initiation via binding to the TRAP binding sequence (tbs) within the RNA leader of a number of genes in the operon.

In the TRiP System™, the tbs is inserted close to the initiation codon of the transgene of interest, and the resulting stable TRAP-tbs complex that forms upstream results in inhibition of translation of the protein (Figure 2). *In vitro* studies have determined maximal TRAP affinity (Kd) for its optimal target sequence in the nanomolar range [52]. The optimal consensus sequence for the tbs in the TRiP System™ is [KAGNN]<sub>11</sub>, and when it is inserted in close proximity to the Kozak sequence. The TRiP System™ is consistently capable of achieving transgene repression levels up to two orders of magnitude [49], and we are now developing second generation cassettes capable of over 1000-fold repression levels [Unpublished Data]. Being such a short sequence, the tbs does not significantly impact on vector genome length, and this also ensures a relatively short leader to maintain robust expression in target cells. The fact that repression acts at the level of translation means that TRiP has been used to repress expression from a variety of constitutive promoters, and could also address any leaky expression from

► **FIGURE 2**

The TRiP system™ components.



tissue specific promoters. We have also demonstrated that the TRiP System™ can be used to repress translation from IRES elements, paving the way for application of TRiP to multicistronic viral vectors and enabling researchers to deliver ever more complex transgene payloads.

Importantly, the simple components of the TRiP System™ do not appear to impact on the fundamental biology of the classic vector platforms, namely LVs, AAV and AdV vectors. In principle it could be applied to other viral vectors and ‘armed’ vaccines. Of further importance is the ability

to produce stable cell lines constitutively expressing the TRAP protein, indicating that there is no obvious cytotoxicity associated with this RNA-binding protein. At Oxford BioMedica we have recently leveraged a bespoke, automated high through-put cell line isolation/screening platform to isolate serum-free, suspension TRAP-expressing cell lines that maintain high levels of transgene repression over several weeks.

The TRiP System™ enhances output titres of LVs encoding CARs, as well as fully rescuing AAV and AdVs expressing pro-apoptotic factors to benchmark levels [49]. Table 1 presents examples of the levels of recoveries in vector titers capable in leveraging the TRiP System™ across different platforms. Further benefits of the TRiP System™ were exemplified by evaluation of an LV vector constitutively expressing the Cyclo-oxygenase-2 (COX-2) gene under control of the CMV promoter (for treatment of Glaucoma). This vector typically produces up to 1000 times lower vector titers compared to the GFP control. The TRiP

‘The TRiP System™ enhances output titres of LVs encoding CARs, as well as fully rescuing AAV and AdVs expressing pro-apoptotic factors to benchmark levels.’

System™ rescued vector output of the LV-CMV-COX-2 vector to the same levels as the GFP vector, and remarkably also improved the protein profile of the concentrated LV material [49]. The high expression of COX-2 during LV production had a broad impact on the abundance and type of host cell proteins associated with concentrated LV particles, including the amount of VSVG envelope protein incorporated. Repressing COX-2 expression resulted in a similar protein profile to that of a GFP-expressing LV, as well as rescuing VSVG-incorporation into virions.

The protein profiling analysis also revealed that the TRAP protein itself is of high abundance within LV material, presumably

▶ **TABLE 1** — Examples of typical viral vector titer recoveries when utilizing the TRiP System™.

Platform	Transgene/vector	Titer recovery
TRiPLenti	Cox-2/EIAV	600-fold
TRiPLenti	FVIII/EIAV	10-fold
TRiPLenti	FPR/EIAV	10-fold
TRiPLenti	VEGF-B/EIAV	10-fold
TRiPLenti	CAR/HIV-1	30-fold
TRiPAAV	Bax/scAAV2	>10-fold*
TRiPAdeno	Bax-IRES-GFP/Ad5	100,000-fold

The TRiP System™ is applicable to many viral vector platforms, including Lenti-, Adeno- and AAV-based vectors. The table presents observed increases in vector titers across these three vector platforms using different transgenes, all expressed by the potent CMV promoter [49].

Transgenes: Cyclooxygenase-2, Factor VIII, Prostaglandin receptor (FPR), Vascular endothelial growth factor B, a Chimeric antigen receptor, Bcl-2-associated X.

\*Likely underestimate due to assay LOQ.

due to passive incorporation of this abundant cytoplasmic protein into virions during the budding process. Clearly this initial finding represented an open question as to whether the presence of TRAP protein with LV material might compromise a direct *in vivo* administration gene therapy approach. Given that re-dosing of patients with LVs is not likely to be required (and may not be possible given the likely immune responses raised to vector envelopes such as VSVG [53]), the most likely theoretical hindrance to gene delivery by LVs produced using the TRiP System™ would be pre-existing antibody responses to TRAP protein. *Bacillus* is a genus of Gram-positive, rod-shaped bacteria found in soil and water [54], and so it's highly likely that most humans will have been exposed to this organism from a young age. However, it is also a gut-commensal organism suggesting that humans may have been generally tolerised [55]; such pre-existing immune responses to TRAP would therefore be unlikely. In our initial work, no immune response to TRAP was detected when evaluating the LV-COX-2 vector in a small rat study [49].

To empirically determine if such responses exist in humans, we have subsequently initiated screening of patient sera for pre-existing antibody responses to the TRAP protein. Within the limits of sensitivity of the assay employed, we have not detected antibody responses from 25 patient sera to native or non-native TRAP, either in its 11-mer or monomeric form [Unpublished Data]. It is of note that similar techniques employed to determine

pre-existing antibody responses to Cas9 protein, detected responses in 79% of donors probing against SaCas9 (*S. aureus*) and 65% of donors probing against SpCas9 (*S. pyogenes*) [56]. Whilst later reports using assays based on ELISA have suggested that more accurate response rates to Cas9 may be lower at  $\leq 10\%$  [57], the lack of any demonstrable antibody responses to TRAP in our ongoing study already suggests that the presence of TRAP protein within viral vector material will not be an issue for the general population.

## TAKING A TRIP TO THE LAB

It is exciting to see other researchers now start to evaluate the The TRiP System™ for use in generation of 'difficult' vectors. For example, the Jenner Institute in Oxford is developing novel vaccination approaches against a wide range of pathogens, such as Malaria, Influenza, Ebola and HIV-1, using the simian (chimpanzee) adenovirus (ChAdV) vector platform in combination with modified vaccinia virus Ankara (MVA). Professor Tomáš Hanke, one of the collaborators in the TRiPAdeno evaluation, is taking an approach to develop HIV-1 vaccines by designing novel immunogens composed of multiple, M-group conserved regions of the virus, and has already reported some promising results [58]. However, production of the recombinant ChAdV vectors expressing these highly artificial proteins has proven to be difficult in some cases, and the TRiP System™ is giving new hope:



“The HIV-1 based immunogens that we are developing are bioinformatics-informed, computed chimeric proteins derived from the most conserved regions of the viral proteome and are of over 700 amino acids in length. These can be expressed with or without the tPA-leader secretory sequence. We have found that ChAdVs expressing these types of protein mosaics, which do not have any natural folding, can destabilize the recombinant vector during amplification. In other contexts where transgene ORFs are stable, we find that downstream processing steps can also be differentially affected depending on the transgene encoded. As a result, the recombinant vectors may be hard to rescue and sequencing of the transgene ORF within the vector genomes may reveal small nucleotide insertions/deletions causing a shift in the reading frame, expression of irrelevant amino acids and premature termination. This has previously led us to employ the tetR repression system, which in some cases has proven successful. However, we have found that even using the tetR system, some recombinant vectors expressing certain difficult products can be hard to rescue due to the leakiness of the tetR repression. This goes to show how unpredictable the impact of expression of some of these proteins can be on Adenoviral vector biology. We’re therefore enthusiastic about the ongoing collaboration with Oxford BioMedica to evaluate the TRiP System™ in production of recombinant ChAdVs, and our initial results look promising.”

– **Professor Tomáš Hanke**, Jenner Institute, University of Oxford, UK

Still others have leveraged the TRiP System™ as a helpful research tool:



“Our goal was to understand whether a particular transgene was responsible for low lentiviral vector production efficiencies; was the transgene causing cytotoxicity, and reducing vector output from the producer cells? The TRiP System™ allowed us to control transgene expression in the transfected HEK293T cells. We used a tbs-containing transgene cassette, and tested a GFP LV genome in parallel as a control. Co-expression of TRAP effectively switched off GFP and our transgene expression, allowing us to clearly show that in this case transgene expression itself was not responsible for poor vector production. We eventually worked out that another feature of the transgene RNA was responsible, allowing us to fix this through further construct design. Overall, the TRiP System™ was a tractable and straightforward system for repression transgene expression in producer cells, and we were very pleased with how it worked.”

– **Professor Greg Towers**, University College London, UK

We anticipate that the TRiP System™ will be employable in other areas of research, such as expanding the complexity in vectored cDNA libraries, especially those encoding novel modified or synthetic proteins. Typically, vectored libraries are produced by transient transfection of vector production cells with a complex mix of DNA comprising the entire cloned/synthesized library; each vector genome expressing an individual clone. Given that

each production cell will likely be transfected with multiple plasmids, then the expression of just one of these clones expressing a gene that impacts on cell viability/vitality or vector assembly will compromise the production of all the other clones transfected into that cell. Currently, the only way to mitigate this problem is to massively up-scale production of the vector library to ensure that ‘somewhere’ within the transfected culture every vectored

clone is produced from cells that are not expressing a problematic gene. Therefore repression of apoptotic, toxic or dominant-negative genes using the TRiP System™ during the initial production of the viral vector library will expedite the production of maximal library complexity that would otherwise be limited by the activities of those proteins.

In summary, the TRiP System™ is fundamentally an approach to rescue or restore viral vectors at or close to the ‘benchmark’ titers achievable with any given viral vector platform, and could be of immediate benefit to researchers programs already in clinical development. However, its application will also help to prevent early stage gene therapy product failures related to transgene protein expression,

and also represents the first steps towards ‘plug-and-play’ viral vector manufacture within commercial pipelines, avoiding continual process development for each new product. The TRiP System™ will pave the way to faster and more consistent translation of gene therapy strategies from theory to clinical success.

#### FINANCIAL & COMPETING INTERESTS DISCLOSURE

*Dan Farley is an employee of Oxford BioMedica, which has funded the subject matter and materials discussed in this manuscript. He also has stock ownership and options in the Company and also holds patents and patents pending. No writing assistance was utilized in the production of this manuscript.*



#### REFERENCES

- Leahy AB, Elgarten CW, Grupp SA, Maude SL, Teachey DT. Tisagenlecleucel for the treatment of B-cell acute lymphoblastic leukemia. *Expert Rev. Anticancer Ther.* 2018; 18(10): 959–71.
- Ghobadi A. Chimeric antigen receptor T cell therapy for non-Hodgkin lymphoma. *Curr. Res. Transl. Med.* 2018; 66(2): 43–9.
- Ameri H. Prospect of retinal gene therapy following commercialization of voretigene neparvovec-rzyl for retinal dystrophy mediated by RPE65 mutation. *J. Curr. Ophthalmol.* 2018; 30(1): 1–2.
- Chira S, Jackson CS, Oprea I *et al.* Progresses towards safe and efficient gene therapy vectors. *Oncotarget* 2015; 6(31): 30675–703.
- Van der Loo JCM, Wright JF. Progress and Challenges in Viral Vector Manufacturing.” *Hum. Mol. Genetics* 25 2016; R1: R42–R52.
- Dormond E, Perrier M, Kamen A *et al.* From the first to the third generation adenoviral vector: what parameters are governing the production yield? *Biotechnol. Adv.* 2009; 27(2): 133–44.
- Durand S, Cimarelli A. The Inside Out of Lentiviral Vectors. *Viruses* 2011; 3(2): 132–59.
- Naso MF, Tomkowicz B, Perry WL 3rd, Strohl WR. Adeno-Associated Virus (AAV) as a Vector for Gene Therapy. *Biodrugs* 2017; 31(4): 317–34.
- Kim VN, Mitrophanous K, Kingsman SM, Kingsman AJ. Minimal Requirement for a Lentivirus Vector Based on Human Immunodeficiency Virus Type 1. *J. Virol.* 1998; 72(1): 811–6.
- Dormond E, Perrier M, Kamen A. From the first to the third generation adenoviral vector: what parameters are governing the production yield? *Biotechnol. Adv.* 2009; 27(2): 133–44.
- Yu SF, von Rüden T, Kantoff PW *et al.* Self-inactivating retroviral vectors designed for transfer of whole genes into mammalian cells. *Proc. Natl Acad. Sci. USA* 1986; 83(10): 3194–8.

12. Kotsopoulou, E, Kim VN, Kingsman AJ, Kingsman SM, Mitrophanous KA. A Rev-Independent Human Immunodeficiency Virus Type 1 (HIV-1)-Based Vector That Exploits a Codon-Optimized HIV-1 gag-Pol Gene. *J. Virol.* 2000; 74(10): 4839–52.
13. Farley DC, Bannister R, Leroux-Carlucci MA, Evans NE, Miskin JE, Mitrophanous KA. Development of an equine-tropic replication-competent lentivirus assay for equine infectious anemia virus-based lentiviral vectors. *Hum. Gene Ther. Methods* 2012; 23(5): 309–23.
14. Tareen SU, Nicolai CJ, Campbell DJ *et al.* A Rev-Independent gag/pol Eliminates Detectable Psi-Gag Recombination in Lentiviral Vectors. *BioResearch Open Access* 2013; 2.6: 421–30.
15. Farley DC, McCloskey L, Thorne BA *et al.* Development of a replication-competent lentivirus assay for dendritic cell-targeting lentiviral vectors. *Mol. Ther. Methods Clin. Dev.* 2015; 2: 15017.
16. Albershardt TC, Campbell DJ, Parsons AJ, Slough MM, Ter Meulen J, Berglund P. LV305, a dendritic cell-targeting integration-deficient ZVex(TM)-based lentiviral vector encoding NY-ESO-1, induces potent anti-tumor immune response. *Mol. Ther. Oncolytics* 2016; 3: 16010.
17. Deyle, DR, Russell DW. Adeno-Associated Virus Vector Integration. *Curr. Opin. Mol. Ther.* 2009; 11.4: 442–7.
18. Michelfelder S, Trepel M. Adeno-associated viral vectors and their redirection to cell-type specific receptors. *Adv. Genet.* 2009; 67: 29–60.
19. Joglekar AV, Sandoval S. Pseudotyped Lentiviral Vectors: One Vector, Many Guises. *Hum. Gene Ther. Methods* 2017; 28(6): 291–301.
20. Geisler A, Fechner H. MicroRNA-regulated viral vectors for gene therapy. *World J. Exp. Med.* 2016; 6(2): 37–54.
21. Vink CA, Counsell JR, Perocheau DP *et al.* Eliminating HIV-1 Packaging Sequences from Lentiviral Vector Proviruses Enhances Safety and Expedites Gene Transfer for Gene Therapy. *Mol. Ther.* 2017; 25(8): 1790–1804.
22. Graham FL, Smiley J, Russell WC, Nairn R. Characteristics of a human cell line transformed by DNA from human adenovirus type 5. *J. Gen. Virol.* 1977; 36(1): 59–74. (1977).
23. DuBridge RB, Tang P, Hsia HC, Leong PM, Miller JH, Calos MP. Analysis of mutation in human cells by using an Epstein-Barr virus shuttle system. *Mol. Cell Biol.* 1987; 7(1): 379–87.
24. Chemudupati M, Kenney AD, Bonifati S *et al.* From APOBEC to ZAP: Diverse mechanisms used by cellular restriction factors to inhibit virus infections. *Biochim. Biophys. Acta Mol. Cell Res.* 2018; pii: S0167-4889(18)30416-6.
25. Fallaux FJ, Bout A, van der Velde I *et al.* New helper cells and matched early region 1-deleted adenovirus vectors prevent generation of replication-competent adenoviruses. *Hum. Gene Ther.* 1998; 9: 1909–17.
26. Wölfel J, Ruth Essers, Corinna Bialek, Sabine Hertel, Nadine Scholz-Neumann, Gudrun Schiedner. CAP-T cell expression system: a novel rapid and versatile human cell expression system for fast and high yield transient protein expression. *BMC Proc.* 2011; 5(Suppl. 8): P133.
27. Merten O-W, Schweizer M, Chahal P, Kamen AA. A. Manufacturing of viral vectors for gene therapy: part I. Upstream processing. *Pharmaceut. Bioprocess* 2014; 2: 183–203.
28. Merten O-W, Merten O-W, Schweizer M, Chahal P, Kamen AA. A. Manufacturing of viral vectors for gene therapy: part II. Downstream processing and safety aspects. *Pharmaceut. Bioprocess* 2014; 2: 237–51.
29. Papadakis ED, Nicklin SA, Baker AH, White SJ. Promoters and control elements: designing expression cassettes for gene therapy. *Curr. Gene Ther.* 2004; 4(1): 89–113.
30. Valencia P, Dias AP, Reed R. Splicing promotes rapid and efficient mRNA export in mammalian cells. *Proc. Natl Acad. Sci. USA* 2008; 105: 3386–91. .
31. Proudfoot NJ. Ending the message: poly(A) signals then and now. *Genes Dev.* 2011; 25(17): 1770–82.
32. Polychronakos C. Gene expression as a quantitative trait: what about translation? *J. Med. Genet.* 2012; 49(9): 554–7.
33. Leppke K, Das R, Barna M. Functional 5' UTR mRNA structures in eukaryotic translation regulation and how to find them. *Nat. Rev. Mol. Cell Biol.* 2018; 19(3): 158–74.
34. Hanson G, Collier J. Codon optimality, bias and usage in translation and mRNA decay. *Nat. Rev. Mol. Cell Biol.* 2018; 19(1): 20–30.
35. Sanjana NE, Shalem O, Zhang F. Improved Vectors and Genome-Wide Libraries for CRISPR Screening. *Nat. Methods* 2014; 11(8): 783–4.
36. Radcliffe PA, Sion CJ, Wilkes FJ *et al.* Analysis of factor VIII mediated

- suppression of lentiviral vector titres. *Gene Ther.* 2008; 15(4): 289–97.
37. Zhao H, Granberg F, Elfineh L, Pettersson U, Svensson C. Strategic Attack on Host Cell Gene Expression during Adenovirus Infection. *J. Virol.* 77(20): 11006–15.
  38. Fukamizu A, Sagara M, Sugiyama F, Yagami K, Murakami K. Tissue-specific trans-activation of renin gene by targeted expression of adenovirus E1A in transgenic mice. *Biochem. Biophys. Res. Commun.* 1994; 199(1): 183–90.
  39. Gruda MC, Zabolotny JM, Xiao JH, Davidson I, Alwine JC. Transcriptional activation by simian virus 40 large T antigen: interactions with multiple components of the transcription complex. *Mol. Cell. Biol.* 1993; 13(2): 961–9.
  40. Cantalupo PG, *et al.* Cell-type Specific Regulation of Gene Expression by Simian Virus 40 T antigens. *Virology* 386(1):183-191. (2009)
  41. Supplementary information of ref 49
  42. Gossen M, Bujard H. Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proc. Natl Acad. Sci. USA* 1992; 89(12): 5547–51.
  43. Cottingham MG, Carroll F, Morris SJ *et al.* Preventing Spontaneous Genetic Rearrangements in the Transgene Cassettes of Adenovirus Vectors. *Biotechnol. Bioeng.* 2012; 109(3): 719–28.
  44. Mullick A, Xu Y, Warren R *et al.* The cumate gene-switch: a system for regulated expression in mammalian cells. *BMC Biotechnol.* 2006; 6: 43.
  45. Hu MC, Davidson N. The inducible lac operator-repressor system is functional in mammalian cells. *Cell* 1987; 48(4): 555–66.
  46. Strobel B, Klauser B, Hartig JS, Lamla T, Gantner F, Kreuz S. Riboswitch-mediated Attenuation of Transgene Cytotoxicity Increases Adeno-associated Virus Vector Yields in HEK-293 Cells. *Mol. Ther.* 2015; 23(10): 1582–91.
  47. Neklesa TK, Winkler JD, Crews CM. Targeted protein degradation by PROTACs. *Pharmacol. Ther.* 2017; 174: 138–44.
  48. Chung HK, Jacobs CL, Huo Y *et al.* Tunable and reversible drug control of protein production via a self-excising degron. *Nat. Chem. Biol.* 2015; 11(9): 713–20.
  49. Maunder HE, Wright J, Kolli BR *et al.* Enhancing titres of therapeutic viral vectors using the transgene repression in vector production (TRiP) system. *Nat. Commun.* 2017; 8: 14834.
  50. Nie M, Htun H. Different modes and potencies of translational repression by sequence-specific RNA-protein interaction at the 5' -UTR. *Nucleic Acids Res.* 2006; 34: 5528–40.
  51. Gollnick P, Babitzke P, Antson A, Yanofsky C. Complexity in regulation of tryptophan biosynthesis in *Bacillus subtilis*. *Annu. Rev. Genet.* 2005; 39: 47–68.
  52. Elliott MB, Gottlieb PA, Gollnick P. Probing the TRAP-RNA interaction with nucleoside analogs. *RNA* 1999; 5(10): 1277–89.
  53. Palfi S, Gurruchaga JM, Ralph GS *et al.* Long-term safety and tolerability of ProSavin, a lentiviral vector-based gene therapy for Parkinson's disease: a dose escalation, open-label, phase 1/2 trial. *Lancet* 2014; 383: 1138–46.
  54. Traxler ME, Kolter R. Natural products in soil microbe interactions and evolution. *Nat. Prod. Rep.* 2015; 32(7): 956–70.
  55. Chistiakov DA, Bobryshev YV, Kozarov E, Sobenin IA, Orekhov AN. Intestinal Mucosal Tolerance and Impact of Gut Microbiota to Mucosal Tolerance. *Front. Microbiol.* 2014; 5: 781.
  56. Charlesworth CT, Deshpande PS, Dever DP *et al.* Identification of pre-existing adaptive immunity to Cas9 proteins in humans. *bioRxiv* 2018; doi:10.1101/243345.
  57. Simhadri VL, McGill J, McMahon S *et al.* Prevalence of Pre-existing Antibodies to CRISPR-Associated Nuclease Cas9 in the USA Population. *Mol. Ther. Methods Clin. Dev.* 2018; 10: 105–12.
  58. Ondondo B, Murakoshi H, Clutton G *et al.* Novel Conserved-region T-cell Mosaic Vaccine With High Global HIV-1 Coverage Is Recognized by Protective Responses in Untreated Infection. *Mol. Ther.* 2016; 24(4): 832–42.

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