Developing Stable Lentiviral Cell Lines

OXGENE
A WuXi Advanced Therapies Company

Introduction

Lentiviral vectors (LVV) are commonly used as gene delivery tools for cell and gene therapies, notably chimeric antigen receptor (CAR) T cell therapies. Like other retroviruses, lentiviruses can convert their single-stranded RNA genome into double-stranded DNA when integrating into the genome of a cell. Unlike other retroviruses, lentiviruses can transduce non-dividing and quiescent cells, which makes them highly suitable for use in cell therapy.

The positive effects of novel cell therapies on malignancies, followed by the approvals granted by regulatory bodies such as the FDA and EMA, led to an increased interest in cell therapies. This in turn has led to an increased number of clinical trials and a growing demand for lentiviral manufacture. Process scalability and robustness are therefore essential to ensure consistent and reproducible production, maximise yields and lower costs.

Lentiviral vectors are often produced in HEK293 cells transiently transfected with four (or more) plasmids that contain all the genetic material required for vector production (GagPol, VSV-G, Rev and Lentiviral genome gene of interest (LV_Genome_GOI)). However, while transient systems are useful in some contexts, transient systems can often be subject to more variability, a higher cost and larger amounts of complexity than stable systems.

The development of stable packaging and producer cell lines, which integrate some or all of the lentivirus elements into the host cell genome, could provide a simpler, more scalable alternative to transient systems, reducing the variability associated with transient transfection and generating more consistent titres across manufacturing runs.

OXGENE, a WuXi Advanced Therapies Company, is a biotechnology company based in Oxford, UK. OXGENE provides end-to-end contract services to cell and gene therapy companies seeking to discover, develop, manufacture and test innovative drug candidates at scale for global commercialization. Building on years of experience with the transient system, OXGENE's LentiVEX™ packaging and producer cell lines contain the same proven genetic sequence as their LentiVEX™ transient system and start from the same HEK293 clonal suspension cell lines. This facilitates a smooth transition to this more scalable technology while retaining the benefits optimised within the transient system.

LentiVEX™ packaging cell lines contain all the packaging elements integrated in the genome, with VSV-G and GagPol under the control of a tetracycline-regulated promoter (TetR) and Rev under a constitutive or inducible promoter. These cell lines require transfection of only one plasmid carrying the lentivirus genome with the therapeutic gene of interest (LV_Genome_GOI) and doxycycline induction to produce lentiviral vectors. This platform also forms a basis for the development of lentiviral producer cell lines. LentiVEX™ producer cell lines stably integrate the LV_Genome_GOI and only require induction to produce lentiviral vectors, allowing a transfection-free manufacturing process.

Integration of lentiviral elements into the genome of a host cell line has historically been very challenging, with lower yields often reported compared to the transient system. Now more than ever, we need stable cell lines which can match the viral yield of a transient production method, but with the scalability and robustness of a stable system. This is what we have been working toward at OXGENE and WuXi Advanced Therapies.

Development of a stable lentiviral packaging cell line

Engineering and development of OXGENE's lentiviral packaging HEK293 cell line required two consecutive rounds of integration. The first involved integration of a TetRepressor and VSV-G and GagPol genes with a doxycycline-inducible promoter by random integration of linear DNA. The second involved integration of a Rev gene, either with a constitutive or doxycycline-inducible promoter, by random integration of linear DNA. The resulting stable pools were then submitted to a single cell cloning and screening campaign to allow identification of the best performing clonal cell lines.

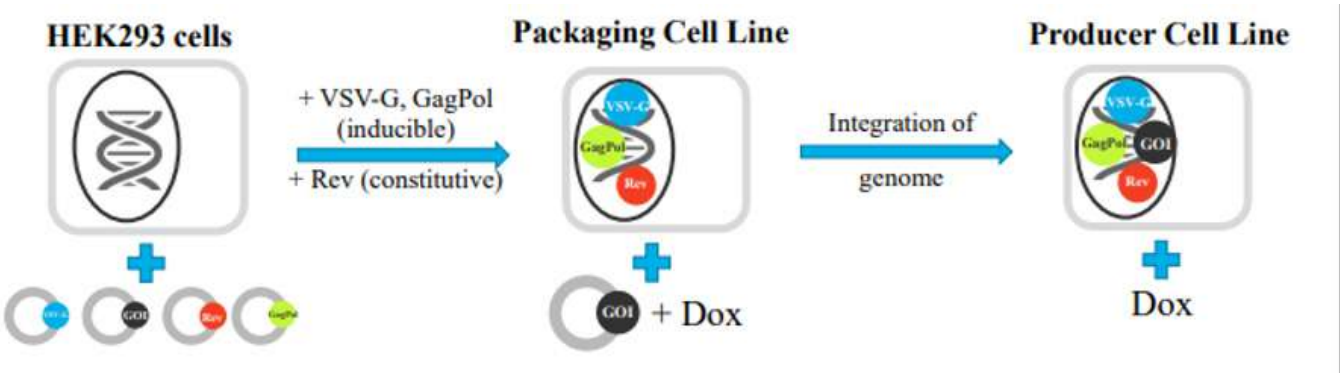

Figure 1: OXGENE's approach to generation of stable lentiviral packaging and producer cell lines.

Using transient transfection of a single LV_Genome plasmid carrying green fluorescent protein (GFP) as a gene of interest, LentiVEX™ packaging cell line version 1.0 was able to achieve lentiviral titres of up to 6.0E+07 TU/mL, with the highest productivity attained by maintaining cells in antibiotic throughout the subculture period. The high titres were also consistent across later generations (up to passage 21).

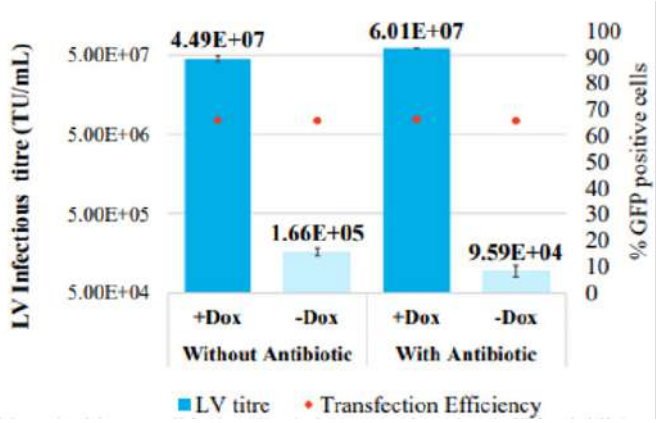

Figure 2: LV production with packaging cell line platform V1.0 cultured with and without antibiotics at passage 21 according to standard protocol and titrated by transduction of HEK293T and analysis by flow cytometry. LV genome transfected is GFP.

Despite the high titre achieved with such a platform, batch-to-batch variability was still observed. OXGENE's acquisition by WuXi AppTec to become part of WuXi Advanced Therapies has given the team access to a new HEK293 suspension cell line platform which shows increased robustness and high viral production titres. The engineering work is enabling development of packaging cell line platform version 2.0. Upon successful retention of packaging elements and long-term functionality, the new platform will be eligible to support manufacturing processes whereby only a single plasmid transfection is required.

Development of a Stable Lentiviral Producer Cell Line with GFP as Gene of Interest

Considering the high yields reached by packaging cell line platform version 1.0, OXGENE employed it for development of LentiVEX™ producer cell lines. This required integration of a lentiviral genome into HEK293 cells: this approach has been prototyped using GFP as a gene of interest.

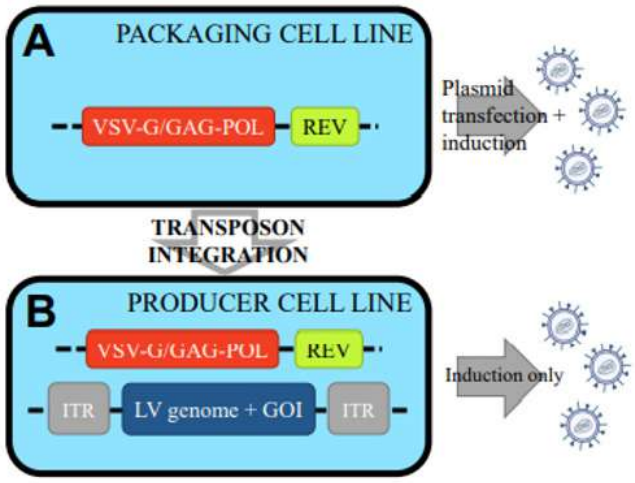

Figure 3: OXGENE's use of transposons for producer cell line development. (A) Packaging cell lines, which encode VSV-G, gag-pol and rev, require plasmid transfection and induction for lentiviral vector production. Transposon integration of the lentiviral genome yields producer cell lines, (B), which only require induction for lentiviral vector production. ITR = transposon inverted terminal repeats.

The scientists used a transposon-based integration method, (Wild-type PiggyBac), to develop producer cell lines. Transposons, known as 'jumping genes', encode a transposase enzyme, which copies and pastes the transposon gene from one genetic location to another. They can be modified to allow any gene to be integrated into a genome, and OXGENE used transposons to integrate the lentiviral genome and gene of interest into a packaging cell line, resulting in a stable LentiVEX™ producer cell line.

Transposon-based integration offers a number of benefits over random integration. Copy number is usually higher and integration is preferentially targeted to transcriptionally active sites: this approach is useful to ensure more copies of the LV_Genome_GOI are integrated in the host genome, which should therefore result in higher lentiviral vector production.

The resulting lentiviral producer cell line, with GFP encoded as the gene of interest, achieves lentiviral titres of up to 1–2E+08 TU/mL. This titre range is comparable with the titres achieved using four-plasmid transient transfection approaches, indicating that OXGENE's LentiVEX™ technology can provide a solution for a stable system with high viral titres. Long-term stability tests are ongoing to monitor the titre and the GFP copy number.

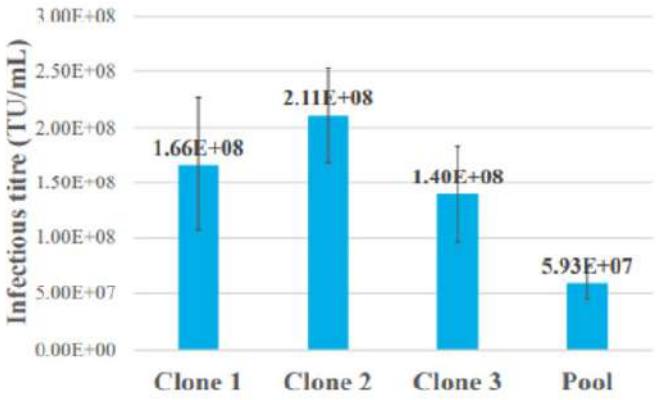

Figure 4: LV production before and after clonal isolation: LV infectious titre generated by transduction of HEK293T and flow cytometry analysis. Gene of interest = GFP. N = two production replicates at passage numbers 10 and 15; two flask replicates at each production. Error bars indicate 4 SD.

Development of a Stable Lentiviral Producer Cell Line with a Therapeutically Relevant Gene of Interest

Next, the OXGENE scientists aimed to demonstrate that their lentiviral producer cell lines could produce high titres with a therapeutically relevant gene of interest, and would thereby offer a robust solution for manufacture of LVV-based cell and gene therapies.

Using the same transposon-based method, the team is developing a lentiviral producer cell line with CAR-CD19 as the gene of interest.

The resulting stable pools yielded titres of approximately 3–5E+07 TU/mL (comparable to titres achieved with the GFP pool in development, explained in section 3, Figure 4). The development of a clonal cell line is ongoing.

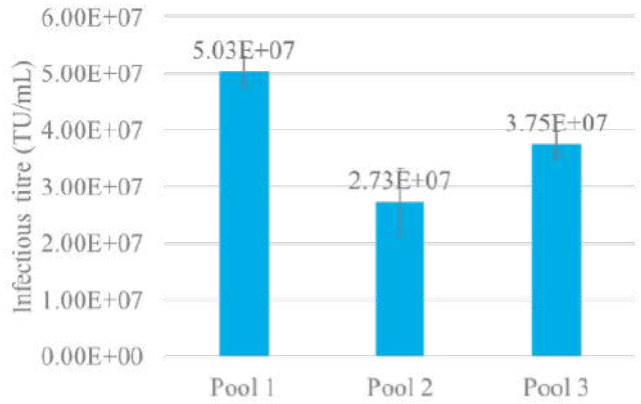

Figure 5: LV production. LV infectious titration by transduction of HEK293T and integrated vector copy number analysis (IVCN by qPCR). Gene of interest = CAR-CD19.

Conclusion

The speed of production of lentiviral vectors needs to keep pace with the accelerating demands for cell therapies. A substantial challenge is to develop manufacturing technologies that can produce safe lentiviral vectors at a consistently high titre and with reduced manufacturing costs.

OXGENE has developed a stable lentiviral packaging cell line, useful for screening multiple targets at early clinical stages, but which can also be adopted for later stages of cell therapy manufacture. This LentiVEX™ packaging cell line forms the basis of OXGENE's LentiVEX™ producer cell lines. These producer cell lines have been created using GFP and a therapeutically-relevant transgene as genes of interest, and can produce lentiviral yields comparable with transient systems.

OXGENE works with customers to understand their gene of interest and the expression system of their novel cell therapy, so they can optimise integration of this custom transgene into a high-yielding, stable lentiviral producer cell line, with the option for seamless internal technology transfer to WuXi Advanced Therapies for further process development, scale up and GMP manufacture and testing. Together, WuXi Advanced Therapies and OXGENE offer end-to-end support, from pre-clinical discovery to commercialisation, for innovators developing novel cell therapies.

In conclusion, OXGENE has developed a LentiVEX™ platform system to address the challenge of lentiviral manufacture from transient to fully-stable lentiviral production systems. Their technology can maintain consistently high yields across different platforms and offers a cost-effective solution for large-scale manufacture of lentivirus-based therapies. This, in turn, will help meet the increasing demands for lentiviral vectors for novel cell therapies.

Corinne Branciaroli

Corinne joined OXGENE in 2017, and, after a brief period working within the Molecular Biology team, moved onto Cell Line Engineering, developing cell line platforms with enhanced characteristics for viral vector manufacture (rAAV, LVV, AdV). As a Group Leader of Viral Cell Line Development, Corinne continues to optimise solutions for both commercial and internal R&D projects, the main focus being development of lentivirus packaging and producer cell lines. Prior to OXGENE, Corinne gained extensive experience in molecular biology, protein purification and immunoassays working in both academia and industry settings. Corinne holds a Bachelor Degree from Universita' degli studi dell'Aquila and a Master Degree from Universita' Politecnica delle Marche (Italy).

Dr. Katie Roberts

Dr. Katie Roberts is Content Manager at OXGENE, a WuXi Advanced Therapies company. She has been writing about science and communicating science, first as a Medical Writer and then within biotech marketing, for most of the last five years. Before this, she completed her PhD in cellular signalling at the University of Manchester, helping to decode oscillatory cell signalling patterns using molecular biology, live-cell confocal microscopy, and mathematical modelling.