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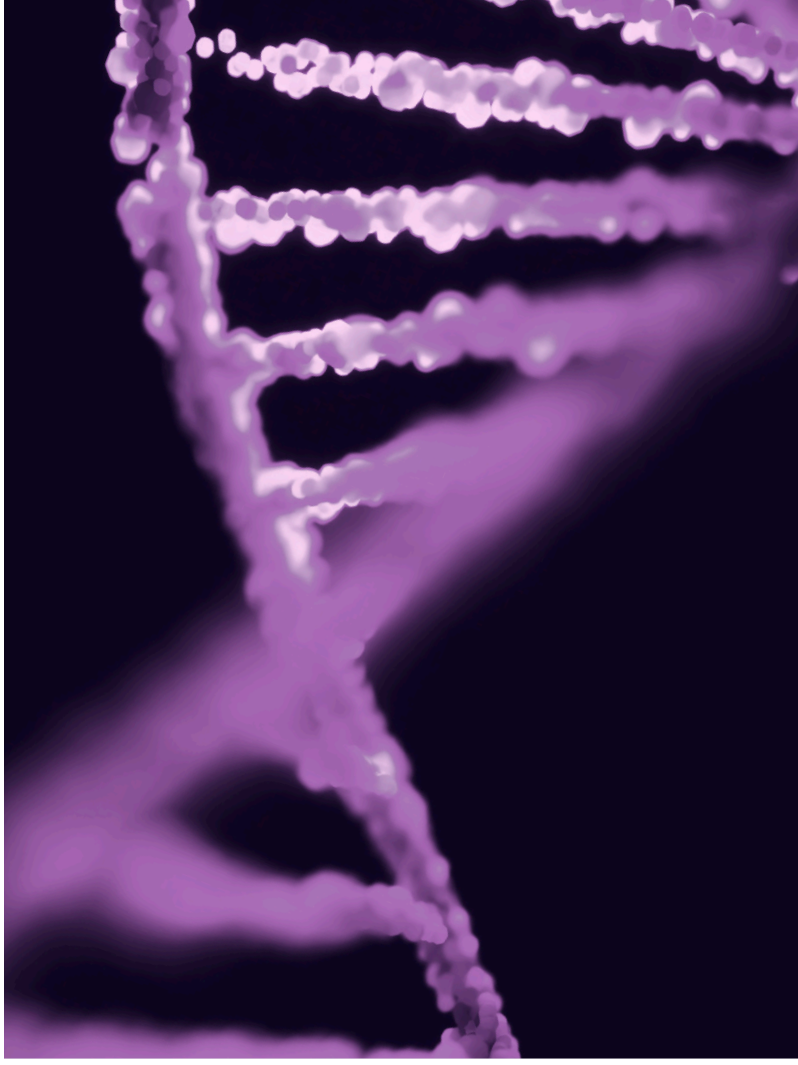
Non-viral Gene Modification for *Ex-vivo* Cell Therapy

Ludger Altrogge, PhD,
Head of Transfection R&D, Lonza

In this interview Ludger Altrogge, Director Transfection R&D at Lonza, is discussing questions around advantages of non-viral transfection technologies for CRISPR- or transposon-based genetic modification cell and gene therapy and how Lonza's Nucleofector® Technology can support this approach.



Q: *Why would one use a non-viral technology for ex-vivo cell therapy?*

A: Viral transduction has been used for *ex-vivo* and *in-vivo* gene therapy approaches for several decades. In-vivo gene therapies rely on viral vectors to transport the therapeutic gene to the target cells in selective tissues. In contrast, tissue or target cell specificity in *ex-vivo* cell therapy is achieved by isolating the required cells from a donor, modifying them in culture and transferring them back into a patient to treat a disease. Historically, most *ex-vivo* processes also used viral transduction for genetic modification of cells. More recently non-viral alternatives are being increasingly deployed, since those may have some advantages over viral transduction due to fewer safety concerns, lower costs, fewer limitations in genetic payload and co-delivery capabilities. Non-viral methods provide flexibility with regards to the type of cargo/substrate used. For instance, it can not only deliver nucleic acids (DNA, mRNA) but also proteins such as Cas9 ribonucleoproteins (RNPs) for CRISPR-based gene editing. Non-viral methods are suitable for both, transient expression of a therapeutic gene or stable genetic engineering of cells. The first can be achieved by delivering plasmid DNA or mRNA, while transposase systems (e.g. Sleeping Beauty™ or piggyBac®), as well as engineered nucleases for more targeted integrations (e.g. zinc-finger nucleases, TALEN or CRISPR) lead to stable genetic engineering of cells. With the latter, a safer and more controlled modification can be achieved. Viral- and transposase-based modifications are generally more efficient, but are less controllable as integration occurs randomly in the genome. Delivering the engineered nucleases as mRNA or protein may allow for a better dosage control of the modification.

Q: *Can you briefly describe the non-viral gene transfer technology that Lonza offers?*

A: The key for a successful implementation of a non-viral technology is to combine high transfection efficiencies that can be achieved by viruses with the flexibility of a non-viral technology. Our solution is an improved electroporation technology, the Nucleofector® Technology, originally introduced into the market by Amaxa in 2001. Optimized electrical parameters combined with cell-type specific solutions enable transfer of a molecule directly into the cells nucleus. It has been optimized for highly efficient transfection of primary cells,including those relevant for *ex-vivo* cell therapy. Since it does not rely on proliferation, it can even transfect non-dividing cells, like resting T cells.

It is based on three key components: firstly, a Nucleofector® Instrument that generates unique electrical pulses. Secondly, specified Nucleofection® Vessels used in combination with cell-type specific Nucleofector® Solutions acting as a supportive environment for high transfection efficiency, cell viability and it is particularly beneficial for hard-to-transfect cells. Primary or stem cells typically used for cell therapies, like primary T cells, hematopoietic stem cells, or natural killer cells, are notoriously hard to transfect by non-viral methods. The Nucleofector® Technology has proven to efficiently transfect these cell types, as well as, being well suited to generate or transfect induced pluripotent stem cells. The predominant cell therapy applications generated using the Nucleofector® Technology are chimeric antigen receptor expressing T cells (CAR-T cells), engineered T cell receptor (TCR) and hematopoietic stem cells (HSCs). Natural killer cells might be the next big thing.

Successful generation of CAR-T cells has been shown for Nucleofection® in combination with Sleeping Beauty™ or piggyBac® transposon- transposase systems. As large amounts of DNA can be toxic for T cells, the use of minimalistic DNA vectors encoding transposon and transposase, so called mini-circles, might be a promising alternative. For the Sleeping Beauty™ system, researchers demonstrated that transfection of mini-circle CAR transposons with mini-circle- or mRNA-based transposase provided significantly higher transfection efficiency and less toxicity compared to a plasmid-based approach, while keeping functional effects comparable to viral vectors. DNA toxicity as well as non- specific, random integration into the genome can also be overcome by transfecting CAR mRNA. Furthermore, using transiently expressed CAR can temporally limit the CAR-T activity and thus reduce off-tissue toxicity affecting normal tissue.

More recently, CRISPR/Cas9 has been used for targeted insertion of the CAR sequence into the TCR locus. This allowed for endogenous control of CAR expression with parallel knockout of the TCR. CRISPR/Cas9 has also been used to knock out the inhibitory checkpoint PD-1 receptor in T cells to potentially improve the efficiency of CAR-T cell-based therapeutics.

Q: *Is the Nucleofector® Technology scalable to manufacturing needs?*

A: Ability to scale up is an important consideration for GMP manufacturing and clinical translation of cell therapies. Lonza's large-scale 4D-Nucleofector® LV Unit is designed with this need in mind. Transfection protocols established on the smaller-scale Nucleofector® Units can be efficiently transferred to the large-scale LV unit without the need for extensive re-optimization.

A highly skilled Scientific Support Team is available for any optimization or fine-tuning. The 4D-Nucleofector® LV Unit can handle up to 1 billion cells and supports most autologous cell therapy applications. The system can be closed via weldable connections to upstream and downstream equipment. This was recently demonstrated in a **Lonza case study** of the Cocoon® Platform, where the 4D-Nucleofector® LV Unit and X-VIVO® 15 Medium were integrated in a closed and automated workflow for non-viral gene delivery into primary T cells and subsequent cell expansion.

Q: *Can the technology be used in a GMP manufacturing process?*

A: The use of the 4D-Nucleofector® LV Unit as manufacturing equipment in a GMP cell therapy process is supported by various means. The unit itself can be equipped with a 21CFR part 11 compliant software to fulfill documentation needs in a GMP environment and Lonza offers IQ/OQ services for equipment qualification. In addition, TheraPEAK® Nucleofector® Solutions and Vessels are available for GMP manufacturing. Early clinical trials are already ongoing involving the use of this technology.

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Contact Us

North America
Customer Service: + 1 800 638 8174 (toll free)
order.us@lonza.com
Scientific Support: + 1 800 521 0390 (toll free)
scientific.support@lonza.com

Europe
Customer Service: + 32 87 321 611
order.europe@lonza.com
Scientific Support: + 32 87 321 611
scientific.support.eu@lonza.com

International
Contact your local Lonza Distributor
Customer Service: + 1 301 898 7025
Fax: + 1 301 845 8291
scientific.support@lonza.com

Lonza Walkersville, Inc. – Walkersville, MD 21793

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