

INTERVIEW

Non-viral methods for *ex vivo* cell & gene therapy: is the future non-viral?

In this episode, **Abi Pinchbeck**, Assistant Editor, *BioInsights*, speaks two industry experts from Lonza, **Valeria Annibaldi**, Group Leader of R&D Transfection, and **Andrea Toell**, Director, Senior Product Manager. They discuss the types and benefits of non-viral methods for *ex vivo* cell and gene therapy in addition to the readiness of electroporation-based technologies for use in GMP manufacturing.



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Q What non-viral methods are currently used in the cell and gene therapy environment, and do you see their use evolving?

VA: Non-viral methods can be grouped into two main categories: carrier-mediated and methods without a carrier. An example of the first group is lipid nanoparticles

(LNPs), which form a complex with the cargo molecule and then are taken up by the cells. Other types of vehicles explored in the field include polymeric nanoparticles and exosomes.

Alternatively, cell permeabilization can be achieved by physical or chemical perturbation of the cell membrane. For example, electroporation is based on the electrical stimulation of the cells and sonoporation utilizes acoustic waves to deliver material into the cells. Other methods rely on mechanical and hydrodynamic forces, for example, in microfluidic squeezing, cells are forced to pass through a constriction and the squeezing makes the cell permeable enabling the cargo to enter. Another microfluidic method is based on vortex shedding, whereby miniaturized posts cause a vortex in the cell solution in a microfluidic channel. This vortex shedding alters the membrane permeability, enabling the cargo to enter cells. Chemical approaches can also be effective, such as Solupore® technology, whereby a solution containing a low concentration of ethanol is delivered to the cells in a specific embodiment enabling the cargo to enter the cells. Electroporation and LNPs are already well established, but some of these other technologies have made big steps forward in the last couple of years, and they are ready or almost ready for clinical use.

Q What are the advantages and disadvantages of utilizing non-viral technology for *ex vivo* cell therapy?

VA: Viral transduction represents the current standard for cell engineering, and it has been investigated and used for both *ex vivo* and *in vivo* gene therapy approaches for decades. There is extensive literature and products on the market in which cell modification was achieved with viral transduction. However, the use of viruses also poses safety concerns related to the random nature of viral integration.

Although significant work has been done to improve the safety and efficacy of viral vectors, in recent years non-viral alternatives are being increasingly adopted because they may provide some advantages over viral transduction due to, for example, lower safety concerns. With non-viral technologies, the risk of insertional mutagenesis is lower or non-existent. Non-viral methods can also offer the option of transient expression, which is considered safer. They can also be used to achieve precise genome editing, which is also a safer option, and they cause less immunogenicity and less toxicity to the cells.

Another crucial aspect is the lower cost of good manufacturing practice (GMP) manufacturing of the therapeutic product. The production of clinical-grade viral vectors can be expensive, time-consuming, and challenging to scale, and there might be long lead times for viral manufacturing. In addition, non-viral methods also offer flexibility with regard to the type of cargo used. In comparison to viral transduction, non-viral delivery has fewer limitations regarding the type and size of the payloads that can be delivered. Moreover, with some technologies, co-delivery of multiple payloads is possible for complex cell modifications.

Regarding potential drawbacks of non-viral methods, in the past, lower efficiency and thus lower expression of the transgene has been seen, and the nature of the expression was normally only transient. However, non-viral technologies have moved forwards tremendously in

recent years and low efficiency is no longer an issue. Transient expression is now considered an advantage in some applications, and if it is not desired, can be overcome by exploring genome editing tools like transposon/transposase systems or engineered nucleases like ZFN-, TALEN- or CRISPR.

Q Cell types used in cell therapy applications can be difficult to transfect using non-viral means. How can these challenges be overcome?

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– Valeria Annibaldi

VA: Non-viral methods are often used for blood-related disorders such as leukemia and lymphoma. Primary cells, which are the focus of those therapies, like primary T cells, hematopoietic stem cells (HSCs), or natural killer (NK) cells are historically known to be hard to transfect by non-viral methods. However, this mainly refers to traditional chemical methods. Electroporation can significantly improve efficiency but also requires higher doses of payload, which may be toxic to cells, especially in the case of DNA. Improved electroporation-based techniques like our Nucleofector® Technology can overcome this drawback to some extent by requiring less payload. In addition, by combining non-viral methods with genome editing tools, stable integration can be achieved, with efficiency which is similar or in some cases higher than viruses. Alternatively, in case only transient expression is preferred, mRNA can also be transfected into cells.

Q Can you tell me about the non-viral gene transfer technology that Lonza offers?

AT: The key to the successful implementation of a non-viral technology is to combine the high transfection efficiencies that can typically be achieved by viruses, with the flexibility of non-viral technology. Our solution is an improved electroporation technology, the Nucleofector Technology, which was originally introduced into the market by Amaxa™ in 2001. With this technology, optimized electrical parameters combined with cell type-specific solutions enable the transfer of a molecule directly into the cell's nucleus. Since it does not rely on proliferation due to this nuclear transfer, it can even transfect non-dividing cells like resting T cells. This nuclear transfer makes it particularly beneficial for hard-to-transfect cells and allows for highly efficient transfection of primary cells, including those relevant for *ex vivo* cell therapy.

This technology is based on three key components: firstly, a Nucleofector Instrument that generates unique electrical pulses. Secondly, specified Nucleofection® Vessels are used in

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combination with cell type specific Nucleofector Solutions acting as a supportive environment for high transfection efficiency and cell viability.

The predominant cell therapy applications generated using the Nucleofector Technology are T cells expressing a chimeric antigen receptor (CAR-T cells) or expressing an engineered T cell receptor. It can also be used for genetically modified HSCs or genetically modified induced pluripotent stem cells. The latter can also be generated with the help of Nucleofection by doing the reprogramming step in a non-viral fashion. Natural killer cells might be the next big thing.

Q What unique advantages does an electroporation-based method offer?

AT: Electroporation is relatively easy to establish and can be very efficient, but it needs to be balanced out with toxicity. Here, Nucleofection can be beneficial because less payload is required, for example in case of DNA which can be quite toxic to cells. Differently from other non-viral methods, especially lipid or chemical-based methods, when using electroporation, the naked cargo is directly delivered into the cell through transient pores in the cell membrane. With chemical methods, you may rely on the endosomal pathway for cargo release, which can trigger toll-like receptor pathways and affect the cells in a negative way. Furthermore, electroporation is flexible, as it can deliver nucleic acids like DNA or mRNA, in addition to proteins such as Cas9 ribonucleoproteins for CRISPR-based gene editing, or even combinations of these as required. For example, when performing CRISPR-based knock-ins, you have to co-transfect Cas9 ribonucleoprotein together with a DNA or PCR donor template. As a non-viral method, Nucleofection is suited for both. You can either do transient expression of a therapeutic gene by delivering plasmid DNA or mRNA or aim for stable genetic engineering of cells by combining it with transposon-based systems like Sleeping Beauty™ or piggyBac®, as well as engineered nucleases for more targeted integrations like zinc finger nucleases, TALEN, or CRISPR Cas9.

When using such engineering tools, a few things may require consideration. Similar to viruses, transposon-based modifications are generally more efficient but are less controllable as integration occurs randomly in the genome. In addition, the large amounts of DNA that are

typically part of these transposon-based modifications can be toxic for cells, especially T cells. Researchers have demonstrated that the use of minimalistic DNA vectors encoding transposon and transposase (so-called minicircles) or transfecting the transposase as an mRNA might be a promising alternative that provides significantly higher transfection efficiency and less toxicity compared to plasmid-based approaches while keeping functional effects comparable to viral vectors. With engineered nucleases, like Cas9, a safer and more controlled modification can be achieved because it can be targeted to a specific locus. Delivering the engineered nuclease as an mRNA or protein would allow for better dosage control of the modification. Another alternative to reduce DNA toxicity can be to transfect CAR mRNA. Such transiently expressed CAR can temporarily limit the CAR-T activity and thus reduce off-tissue toxicity affecting normal tissue.

Q The ability to scale up is an important consideration for GMP manufacturing and clinical translation of cell therapies. Is this technology scalable to meet the needs of the industry?

AT: Our large-scale platform, the 4D-Nucleofactor LV Unit, is designed with this need in mind. The LV Unit can handle up to 1–2 billion cells depending on the size of the cell type and thus supports most autologous cell therapy applications. Transfection protocols can be established on the smaller scale Nucleofactor units, and then transferred to the large-scale LV Unit without the need for extensive re-optimization. In some cases, re-optimization might be required, and in those cases, a highly skilled scientific support team is available at Lonza, that can help with any optimization or fine-tuning. Furthermore, the use of the LV Unit as manufacturing equipment in a GMP process is supported by various means. For example, the unit itself can be equipped with 21 CFR part 11 compliant software to fulfill documentation needs in a GMP environment. In addition, Lonza offers IQ/OQ services for equipment qualification and also Nucleofactor Solutions and Vessels manufactured according to GMP or ISO 13485 rules are available. The system can be closed via weldable connections to upstream and downstream equipment, for example our Cocoon Platform. Early clinical trials are already ongoing involving the use of this technology.

Q What non-viral technologies are currently being used in clinical or commercial applications based on *ex vivo* modifications?

VA: Except for electroporation and LNPs, most of the non-viral methods that I mentioned initially are rapidly evolving but have not yet reached the clinical stage. However, the number of immunotherapy products based on non-viral methods in the pipelines of cell and gene therapy companies has more than doubled over the last 7–8 years. In particular, there are several clinical trials ongoing combining electroporation with Sleeping Beauty or piggyBac transposon/transpose systems, or even CRISPR-Cas9.

Q What are your predictions for the future of this space as non-viral technologies continue to develop?

VA: Up to now, the vast majority of genetic modification has been done with viruses, especially for *in vivo* therapies, but also for *ex vivo* therapies. Nonetheless, there is an increasing interest in non-viral technologies due to the advantages that I mentioned earlier. I expect that in the future, both approaches will coexist, with multiple offerings in the non-viral space, because there may not be a one-size-fits-all method. The type of application will probably dictate the technology of choice, for example, the size of the therapeutic dose, the target indication, or whether the therapeutic approach is autologous or allogeneic. Whether the modification needs to be permanent or if a transient product is desirable could affect the choice. The specifications of the drug product could also play a significant role. For complex gene editing requiring multiple modifications, there are already companies exploring combinations of different technologies, for example, viral delivery and electroporation. Market research shows that growth is expected for both viral and non-viral technologies in the future as the industry evolves and cell and gene therapy approaches become increasingly established.

BIOGRAPHIES

DR VALERIA ANNIBALDI joined Lonza Cologne GmbH, Germany, in 2021 as a Group Leader in the R&D Transfection Team. The team develops innovative solutions for transfection of cells, with emphasis on primary cells. In her current role, she works on the development of next generation Nucleofector Platforms and Consumables, with the focus on cellular therapy applications and large-scale electroporation. Prior to joining Lonza, she worked in the CGT field developing and evaluating transfection technologies suitable for immunotherapy. In her previous positions she gained experience in product and process development formulation and primary T cells. Valeria is a qualified pharmacist, she holds a master's degree in Pharmaceutical Chemistry and Technology from Sapienza University of Rome, Italy and completed a PhD in Electrochemistry, at Maynooth University, Ireland. Results of her studies were published in peer-reviewed journals and presented at scientific conferences.

ANDREA TOELL is a Senior Product Manager at Lonza Cologne GmbH and is responsible for Lonza's Nucleofector Transfection Portfolio. In her current role she is focusing on large volume transfection and cell and gene therapy applications (CAR-T cells, genome editing, GMP manufacturing). In her 20 years' experience with the technology she gained a broad knowledge about transfection technologies and factors that are important for successful transfection experiments. Andrea joined the Lonza team (legacy amaxa) as Scientific Support Specialist in 2001 before moving into Product Management in 2004. She holds a Diploma in Biology from the University of Münster, Germany, and a PhD in Biology from the University of Düsseldorf, Germany.

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AUTHORSHIP & CONFLICT OF INTEREST

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