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A Challenge: Controlling the Quality of Cell and Gene Therapies

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Cell and gene therapies are revolutionizing the treatment of patients with cancers or genetic diseases, but they are complex: The respective starting materials, *e.g.* blood cells, have an impact on the quality and variability of the final product. Tailored analytical methods need to properly control and understand the starting materials, the process, and final product. In order to adhere to commercial current Good Manufacturing Practice, CQAs (Critical Quality Attributes) must be identified and their dependence from the CMAs and CPPs (Clinical Process Parameters) must be clear. Safety and efficacy requirements must be met and quality controlled.

Viral vectors (VVs) are the key vehicles to introduce therapeutic benefit into human cells. Various types can be employed such as Adeno-Associated Viruses (most common vectors used), Lentiviruses, or oncolytic viruses. Functional and potency assays need to be tailored for the respective virus types. Testing the strength includes cell count and viability, physical titer ddPCR and qPCR, HPLC titer, optical density titer, and infectious titer. Identification is done with cell line identification, Sanger sequencing, next generation sequencing, and restriction enzyme mapping. Purity testing includes residual Benzonase, Triton testing. For potency, ddPCR and ELISA are employed as well as *in vivo* testing. Safety testing includes methods for detection of retrovirus, adventitious agents, sterility, mycoplasma, endotoxin, and bioburden.

Autologous therapies use the patient's own T-cells which are reprogrammed making use of VVs. The patient is at the beginning and end of the supply chain. The quality of cells differs, so even though it is not possible to produce a standard, the product must meet certain specifications. Process comparability is more frequently required with a more complex design, which requires adequate analytical methodologies capturing the CQAs. Rapid disease progression may be an issue, and product shelf-life can be short: quality control release testing is time-critical. A step-wise release process, *e.g.* interim sterility, may be needed. Automated plate-reading technologies allow faster turn-around times (TAT). Generally, methods applied include: Identity: PCR testing for transgene presence. Safety: Endotoxin, sterility, mycoplasma, and virus DNA qPCR. Purity: Cellular phenotyping by flow cytometry for viable T-cell percentage. Transduction efficiency is tested by CAR, qPCR, and cell viability test. Impurities: Microscopy to check for residual beads. Potency: Release of Interferon Gamma in response to CD19-expressing target cells (Cluster of Differentiation 19, B-lymphocyte antigen).

Fig. 1. The Lonza Cocoon® system for the manufacturing of autologous cell therapies.

Allogenic therapies allow for off-the-shelf centralized manufacturing. They stand out for their scalability with challenges similar to those of traditional biologics. Induced pluripotent stem cells can differentiate into any of the 220 plus cell types found in the human body. This allows for tissue material standardization and analytical method standardization. Allogenic CAR-T has the potential to solve the major CAR-T challenge of donor variability with a pool of well-characterized reference donors. CQAs to be tested are less variable. There are multiplex gene editing options, and a combination of viral and non-viral methods can be employed.

Exosomes function as intercellular messengers and are key mediators of cell-to-cell communication by transfer of nucleic acids, proteins, and lipids and accordingly influence functional aspects of recipient cells. All cells produce and absorb these round nanovesicles. They play fundamental roles in altering the activity of recipient cells as a response to physiological stress and disease. Tailored functional assays are required for Exosome quality control testing.

Generally, analytical quality control methods must be capable of testing for safety, identity, strength, purity, and overall quality (SISPQ). The methods must be validated for specificity, accuracy, precision, linearity, range, limit of detection, and limit of quantitation in accordance to the regulatory guidances. Robustness criteria need to be met.

All main areas in cell and gene therapy are unique and with specific needs for both manufacturing and the analytical sciences. The requirements on product quality and the analytical methodologies remain the same as with traditional treatments.

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References

EudraLex Vol. 4, 'Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products', **2017**.
ICH Guidances: www.ich.org (International Conference on Harmonization).

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