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Editorial

The Pharmacology of Gene and Cell Therapy

The marketing authorization of Glybera and Strimvelis by the European Medicines Agency (EMA) marked the end of the long and often troubled road of gene therapy from biological concept to medical practice. Glybera is a recombinant adeno-associated virus (AAV) vector designed for gene therapy of lipoprotein lipase deficiency,[1] while Strimvelis is a genetically modified hematopoietic stem cell preparation for the treatment of severe combined immunodeficiency.[2] The US Food and Drug Administration (FDA) recently filled the gap for the US market by approving in rapid succession Ymlygic, a genetically modified oncolytic herpes virus, Kymriah and Yeskarta, two forms of genetically manipulated autologous T cells for immunotherapy of cancer, and Luxturna, a recombinant AAV vector for gene therapy of a rare form of retinal dystrophy. Many more products are about to follow because pivotal clinical trials are showing efficacy and safety in addressing rare genetic disorders as well as refractory neoplastic diseases. Nevertheless, gene therapy technology is still in its infancy: manufacturing processes are far from being industrialized and quality and potency attributes are continuously improving thanks to innovation and a rapidly evolving analytical toolbox. The pharmacology of gene therapy is a new science, applying concepts developed for chemical drugs to extremely complex biological products for which terms such as active principle, dose, purity, strength, toxicity, biodistribution, shedding, environmental risk, pharmacokinetics, and pharmacodynamics must be redefined in a creative, though equally rigorous, fashion. As an example, the very definition of "drug" is ill-adapted to heterogeneous preparations of genetically modified autologous cells, which are individualized therapies by definition. Regulatory agencies around the world are trying to cope by providing guidelines and regulations intended to enable development and marketing of gene therapy in a safe, sensible fashion to avoid unnecessary delays in making innovative products available to patients.[3]

This issue of *Molecular Therapy – Methods & Clinical Development* provides an update on some outstanding issues in the pharmacology of gene therapy products. Six articles authored by experts in the field address manufacturing of vectors and genetically modified cells, vector-host interactions, and the use of animal models to analyze activity, potency, biodistribution, pharmacokinetics, toxicity, and immunogenicity of gene therapy products. Rather than providing exhaustive reviews, the articles assess the state-of-the-art and identify controversy and future needs in this continuously evolving field, with reference to the most relevant regulations and guidelines in Europe and the US. This special issue is meant to raise awareness on the complexity associated with defining and characterizing the biological and pharmacological properties of gene and cell therapy products. AAV vectors, lentiviral vectors, and chimeric antigen receptor (CAR)-T cells serve as examples of different classes of therapeutics that already proved their market fitness.

AAV vectors are a relatively simple and flexible tool to deliver genes or gene-modifying nucleic acids to an array of target tissues for a variety of indications and are widely used in clinical applications by academia and industry. Pennaud-Budloo et al.[4] provide an overview of the current technology used to produce clinical-grade AAV vectors batches in compliance with the good manufacturing practices (GMPs) required by both the FDA and EMA. They describe the two most commonly used production systems (transient transfection in human HEK293 cells and infection of insect cells by baculovirus expression vectors), the challenges associated with large-scale production, and the potential advantages offered by mammalian stable cell lines. Much emphasis is given to product characterization, the type of impurities associated with the different production and purification systems, and the analytical tools required to detect them in a quantitative fashion. The quality of the AAV particles and the amount of defective or "empty" capsids varies with the different production systems and is discussed in terms of both safety and potency of the vector preparations. Empty capsids and their impact on the immune response to AAV vectors are discussed by Colella et al.[5] in the context of the overall immunological consequences of AAV vector administration to humans. The authors focus on liver-directed gene therapy as a paradigm of a therapeutic platform that associates proven clinical efficacy to specific vector-host interactions and the double-edged sword of immunization/tolerization. Particular emphasis is given to the design of transgene expression cassettes and new AAV capsids and to the pre-clinical studies required to assess efficacy, potency, toxicity, and humoral and cytotoxic immune responses against vector and transgene products. The impact of pre-existing anti-capsid immunity on the safety and efficacy of AAV-mediated gene therapy and the correlation between organ growth and long-term vector persistence are discussed in the context of the existing clinical data and future clinical trial design. The challenges of building a pre-clinical package for gene therapy medicinal products and the use of animal models for biodistribution and toxicity studies are discussed by Silva Lima and Videira,[6] focusing, in particular, on EMA requirements for clinical trial authorization in Europe. The relevance of the animal model and the factors determining its suitability for any intended pre-clinical assessment are given particular emphasis and described in the context of pharmacology, pharmacokinetics, and toxicology studies. The use of a risk-based approach is described by taking two specific cases as examples (Glybera and Imlygic), and analyzing how choices made in pre-clinical studies impacted the registration process for both products.

Genetically modified cells are a different class of products with respect to injectable viral vectors because they are made of a patient-derived component, most commonly hematopoietic stem cells or T cells, and a viral vector that mediates integration of a gene expression cassette in the cell's DNA. Integration, most frequently obtained by an HIV-derived lentiviral vector, is a potentially genotoxic event by definition, as it interrupts the continuity of the genome and may potentially disrupt (or interfere with the regulation of) endogenous genes. Insertional oncogenesis has been seen in the past as a severe side effect of

gene therapies based on older-generation retroviral vectors but remains a safety concern for any integrating vector. Poletti and Mavilio[7] describe the molecular basis of target site selection by retroviruses and retroviral vectors, a knowledge derived from over 15 years of research on the biology of HIV and the severe adverse events observed in gene therapy clinical trials. The development of high-throughput DNA sequencing and genome-wide analysis of chromatin structure and configuration enables detailed understanding of the complexity of retrovirus-genome interactions, including how integration preferences of each virus impact the potential genotoxicity of the vectors derived from them. Biasco et al.[8] describe the tools and analytics available to analyze the integration characteristics and the potential genotoxicity of vectors developed for clinical applications in both cell culture and *in vivo* in the context of pre-clinical studies. They also describe how to monitor genetically modified cells and their clonal dynamics in treated patients. These analyses are still relatively complex and depend on bioinformatic tools that are neither standardized nor validated. Nevertheless, they provide an important biosafety readout and, in the case of genetic modification of long-lived stem cells, crucial information on their fate and, ultimately, on the efficacy of the therapy. In the case of CAR-T cells, which are revolutionizing cancer therapy despite their relatively short clinical history, integration-driven genotoxicity is much less of a concern. CAR-T cells are relatively short-lived and there is little evidence that a mature T cell or even a T cell progenitor can be "transformed" by a vector-driven event. On the other hand, their efficacy depends on their pharmacological properties, which are very different from those of viral vectors or more conventional biologics. Milone and Bhoj[9] review the different types of CAR-T cells currently under clinical investigation, their cell kinetics after infusion, and the relationship between kinetic properties and anti-cancer activity. They also discuss the toxicity associated with T cell therapies and the relationship between toxicity and therapeutic efficacy, an intriguing though poorly understood aspect of this form of gene therapy.

The age of molecular therapies and medicines has arrived. Recent initial approvals highlight a future wherein we will be able to treat and cure disease with molecular technologies. We can now look back at Carl Sagan's "pale blue dot" that gene therapy once was and look forward to the expanding possibilities ahead. To accomplish these goals, we will need to develop a cohesiveness in subspecialties of the field (CAR-T cells, DNA, or RNA viruses, etc.), including the methods used for determining attributes, such as active principle, dose, purity, strength, toxicity, biodistribution, shedding, environmental risk, pharmacokinetics and pharmacodynamics, both in animal and human studies. Standardizing these methods will provide for more data that can be directly compared throughout the field and used to predict outcomes, thereby reducing preclinical studies and the risk-benefit ratio, while increasing the efficacy of early clinical studies.

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