

## Topic: Accelerating research & development for advanced therapy medicinal products

**All information regarding future IMI Call topics is indicative and subject to change. Final information about future IMI Calls will be communicated after approval by the IMI Governing Board.**

### Topic details

Action type	Research and Innovation Action (RIA)
Submission and evaluation process	2 stages

### Specific challenges to be addressed

Curative or near curative therapies for rare and orphan diseases have been a long-held desire for many in the biomedical research and development arena including patients. Rare diseases are often very severe, genetically driven illnesses with high morbidity and mortality that place a large burden on families of patients and healthcare systems. Though these diseases are relatively rare, the costs of medicines are high, even for many that provide only marginal benefit. Gene therapy and cell therapy provide an opportunity for a curative, single treatment for many of these devastating diseases, eliminating the need for chronic treatment. This topic aims to accelerate the research and development of Advanced Therapy Medicinal Products (ATMPs) by filling gaps in our knowledge-base in, and tools for, gene and cell therapy. This will provide medicines developers and regulators with the information they need to more swiftly move these potentially transformative medicines forward so that they can benefit patients in need.

The goal of gene and cell therapy is to provide, with a single treatment, sustained therapeutic levels of transgene expression or cell activity, with potentially life long duration. Challenges to this goal are immunological and non-immunological factors that may impact persistent expression and the eligibility for treatment [1][3]. Patients with pre-existing neutralizing antibodies (nAbs) due to natural viral infections that result in cross-reactive antibodies, or perhaps due to prior exposure to viral gene therapy capsids, are typically excluded from treatment [4][5]. After treatment patients are also excluded from redosing due to the high titer nAb response to the dose of vector [6]. Additionally, some patients, when treated systemically with gene/cell therapy, mount an immune response to transduced cells that have resulted, in some instances, in damage of targeted liver and muscle tissues [7]. Molecular features, such as concatemer state and integration mechanism, may influence persistence which in turn may be impacted by age and tissue target [8]. Consequently, the potential dilutional impact of tissue division and growth on persistence requires deeper molecular understanding to develop efficacious and long-lasting gene/cell therapy products.

Conventional medicinal product characterisation, clinical safety/efficacy, and regulatory requirements already pose challenges to developing treatments for rare monogenic diseases. These challenges are amplified for gene and cell therapies due to knowledge gaps in our understanding of these ATMPs. By addressing these existing knowledge gaps, we hope to accelerate and improve the feasibility of product development and decrease development time and costs to bring effective new advanced therapies to patients. For many aspects of ATMP biology and safety, regulatory agencies have to consider theoretical concerns in this

emerging field, largely due to a lack of supporting data and evidence. This can be a major burden for the efficient development of ATMPs.

To streamline regulatory requirements, it would be highly beneficial to continue to build a greater understanding and evidence-base of essential performance parameters needed in the field of gene/cell therapy. Those parameters include: persistence of gene/cell therapy efficacy, potential for re-treatment, impact of host immunology on patient inclusion and product efficacy, molecular characterisation of common features of each delivery modality and the possibility of creation of “plug-and-play” platform approaches, and delineation of the right balance between the standards for product characterisation, safety, and the value of the medicine.

## Need and opportunity for public-private collaborative research

The collaboration between public and private partners is essential and will enable directed research to solve challenges posed in this topic, provide learning opportunities for the next generation of scientists in the ATMP area, and foster open scientific interaction in the public domain. Much of the expertise in gene and cell therapy lies in academia, however, clear data important for ATMP development regarding host responses, persistence of efficacy, redosing, and safety is lacking. Working together in this public-private partnership, combining the deep expertise and innovation in vector design, adeno-associated virus (AAV) biology, cell biology, and immunology that resides in academia with growing industry ATMP development expertise and data emerging from clinical trials, and regulatory expertise lying in regulatory agencies, will synergise to enable the building of a data-driven consensus around ATMP biology, immunology, and persistence. This in turn will support the development of guidance by regulators on the development of ATMPs.

## Scope

The main focus of this topic is to develop a product characterisation framework (rather than delineating the methodologies for achieving such a framework) thus limiting the scope to the pre-competitive space. This topic intends to utilise both therapeutically relevant systems, as well as model systems that rely on the use of marker transgenes. In order to develop such a framework, there is a need for a coordinated and substantive effort to acquire and analyse the currently available data and then design preclinical and clinical studies to fill the knowledge gaps. This information will help to address gene/cell therapy risks and also guide product developers and regulators to determine and implement an appropriate and effective characterisation framework to enable efficient and safe development of gene/cell therapies.

The main objectives of the topic, intended to address existing knowledge/data and tools gaps focused on viral-mediated gene therapy and cell therapy, are:

- 1) Develop better, standardised models for predicting product immunogenicity in humans;
- 2) Build our understanding of gene/cell therapy drug metabolism inside a host and explore any loss of efficacy (persistence), particularly with non-integrating viral vectors or cell therapy;
- 3) Understand the clinical factors around pre-existing immunity limiting patient access to ATMP therapy, and adaptive immune responses affecting product safety, efficacy and persistence, including for integrating vectors-based therapies;
- 4) Engage regulators to ensure that the models and data generated through the funded action will provide the necessary information to support regulatory filings and to address regulatory and safety concerns.

Specifically, the scope of the project is expected to address:

- **Develop better, standardised models for predicting ATMP immunogenicity in humans:** some aspects of human immunology are not adequately captured in current pre-clinical models. Improving such models would enable development regimens for modulating humoral and cellular immune responses to cell and gene/cell therapy products. Specific areas to address for each ATMP type are:
  - Gene Therapy: Predictive tools for testing immunogenic properties of viral or liposomal delivery systems to enable design of vectors that will evade immune recognition in order to: 1) treat a higher proportion of patients; 2) achieve successful transfer of the therapeutic gene enzyme to the target cells; and 3) mitigate the risk of immunotoxicity on target organs;
  - Somatic cell therapy: Expand on current paradigms in transplantation immunology using innovative ex-vivo and in vivo systems. Aim for deeper understanding of mechanisms that influence acute immune responses at the site of implantation and how the nature of disease affects long term immunity against therapies using autologous, allogeneic, or xenogeneic non-germline cells;
  - Tissue engineered products: Develop new models to investigate the innate and adaptive immunity that contribute to the inflammatory response to natural and artificial scaffold structures.
  
- **Characterise host, tissue, and target cell metabolic responses to gene/cell therapy vectors and transgene products to understand persistence:** as many rare genetic diseases manifest in childhood and the cells in the target organs in young patients continue to divide, it is of interest to characterise the dilution of the therapeutic effect. Specifically, it needs to be investigated whether there is a dilution effect in children and/or in specific organs or tissues. It is of interest to characterise the metabolism of the vector genome in different cell types to understand whether rates of degradation, episomal maintenance, or integration vary from cell to cell, and to define strategies to improve the persistence of vector genomes. Prospective paediatric samples may be obtained to explore how the levels of expression are affected by growth. Finally, strategies to generate more efficient hybrid vectors to accommodate transgenes that exceed the packaging capacity of adeno-associated virus (AAV) will be investigated.
  
- **Understand the clinical factors around pre-existing immunity limiting patient access to ATMP therapy, and adaptive immune responses to gene/cell therapy drug substance and product:** in order to address challenges of potential immunologic barriers, the funded action is expected to:
  - Develop novel protocols for modulation of immune responses to capsid and transgene products, or induction of tolerance to expressed transgene products;
  - Develop cohesive metrics for immunological characterisation applicable in gene and cell therapies, for both patient inclusion and post-treatment monitoring phases;
  - Develop standardised pathways for characterisation of pre-existing immunity to gene/cell therapy products, including memory T-cells and neutralizing and binding antibodies;
  - Establish a geographically diverse prospective biobank from treated and untreated donors with matching cell, serum, and plasma samples to enable evaluation of the pre-existing and adaptive immunity, assuring that an appropriate informed consent is obtained, and privacy maintained;
  - Develop and standardise innovative characterisation/functional assessments methods for gene/cell therapy drug substances and products;

- Evaluate the safety risk of administering viral vectors in the presence of humoral and/or cellular immunity;
  - Evaluate novel approaches to allow for vector re-administration in order to re-establish therapeutic protein levels.
- **Engage with regulators to ensure the results of the funded action will support regulatory filings and address regulatory and safety concerns:** specifically, concerns such as insertional mutagenesis/carcinogenicity, vector shedding, viral clearance, safety, and persistence, need to be addressed. In addition, since large amounts of data are generated across the field it is important to explore, jointly with regulators, how to bring this information together in a meaningful way to potentially address issues across a class of products. It is expected that the models and data generated through this funded action will provide necessary information to support the alignment efforts and development of harmonized guidance through International Council for Harmonisation (ICH), and optimise the risk-benefit of the ATMPs covered in this initiative. Therefore, the funded action is expected to:
- Gather examples, develop criteria and evaluate options to standardise differences in regulatory requirements across countries;
  - Identify and address scientific gaps in current knowledge and generate new evidence/systems to support the development of improved standards for safety, while enabling accelerated product development;
  - Identify mechanisms for unified regulatory approaches to key issues in gene/cell therapy development, including environmental assessments, characterisation of replication competent viruses, viral clearance/shedding, patient screening criteria, and long-term follow up for persistence and delayed adverse events;
  - Explore, and where feasible, enable developments that effectively and appropriately allow new developments to benefit from and utilize existing regulatory analogies or frameworks;
  - Conduct a comprehensive review of clinical data and prepare a package (or white paper) aimed at evaluating theoretical risk of insertional mutagenesis and formulating recommendations to the regulatory agencies.

## Expected key deliverables

The expected key deliverables to be achieved during the duration of the funded project:

- In vitro, ex vivo, and animal models with better translatability of the immune responses to gene/cell therapy; once in place these models should be sustainable;
- Deep understanding of how host cells and tissues metabolise gene/cell drug products and how this affects persistence;
- Identification of immunogenicity hurdles and potential solutions, for de-immunisation or immunomodulation that can improve overall efficacy and minimise patient risk along with a standardised vector characterisation platform;
- During the first year, the consortium is expected to develop a plan for which issues will benefit the most from a comprehensive database(s) to address regulatory needs;
- A sustainable, beyond the timeframe of the action, prospective biobank of samples obtained with appropriate informed consent and privacy from healthy volunteers and patients treated with gene or cell therapies;

- Optimised and validated specific methods and models, which will increase regulatory acceptance and thereby facilitate regulatory success of future gene therapy projects.

## Expected impact

Primarily, the action funded under this topic will fill gaps in our knowledge base around gene/cell therapy host responses which will allow for a data-driven development of a product characterisation framework to aid researchers, developers and regulators to more rapidly move effective and safe gene/cell therapies forward.

Understanding the host immune responses and the prevalence of pre-existing immunity in humans in broad geographic areas will be instrumental for finding the best immune-modulating regimens, thus increasing patients' access to advanced medicines. Understanding the determinants of immunogenicity may enable re-dosing with gene/cell therapy products, while studying the mechanisms of persistence will help to define the optimal age for gene/cell therapy intervention.

Finally, joint efforts across pharma, biotech, academia, and regulatory functions will inform patient inclusion criteria, limit subtherapeutic dosing, and define the impact of the pre-existing and adaptive immunity on efficacy and persistence of gene/cell therapy. This broad understanding will help to focus industry resources on actual (not theoretical) risks and will facilitate harmonisation of regulatory requirements; these improvements will, in turn, enable accelerated cures for rare diseases *via* a defined regulatory framework.

Applicants should also indicate how their proposal will impact the competitiveness and industrial leadership of Europe by, for example engaging suitable Small and Medium-sized Enterprises (SMEs).

## Potential synergies with existing Consortia

Applicants should take into consideration, while preparing their short proposal, relevant national, European (both research projects as well as research infrastructure initiatives), and non-European initiatives. Synergies and complementarities should be considered in order to incorporate past achievements, available data and lessons learnt where possible, thus avoiding unnecessary overlap and duplication of efforts and funding.

## Industry Consortium

The industry consortium will contribute the following expertise and assets:

- Anonymised existing or prospective data from clinical trial cohorts from industry partners supplementing the academic cohorts;
- Personnel with in-depth knowledge in the fields of experimental and clinical immunology, cell and in vivo biology, virology/vectorology, histology, genetic toxicology, omics, chemistry manufacturing and controls (CMC) analysis, medical affairs, statistics, regulatory, bioethics, epidemiology and non-clinical development;
- Know-how and means to support the establishment of the federated database including legal advice, setting up the database, and making analysis feasible, accessible and sustainable over time;
- Limited supplementary funding for supporting the derivation of a novel methodology for modulation of immune responses to capsid and transgene product, and autologous or allogeneic gene-modified or unmodified transplanted tissues and cells. Similarly, develop protocols to induce tolerance to expressed transgene products or to autologous or allogeneic gene-modified or unmodified cell products. Also, for the design of improved hybrid vectors that have a higher efficiency of

concatemerisation, and full-length vector genome reconstitution, and to accommodate transgenes that exceed the packaging capacity of AAV.

## Indicative duration of the action

The indicative duration of the action is 60 months.

## Applicant consortium

The applicant consortium will be selected on the basis of the submitted short proposals. The applicant consortium is expected to address all the research objectives and make key contributions to the defined deliverables in synergy with the industry consortium, which will join the selected applicant consortium in preparation of the full proposal. Therefore, the consortium should mobilise all relevant expertise, skillsets and stakeholders to implement proposed activities in order to achieve the objectives of the topic. This may require mobilising, as appropriate the following:

- Groups with experience and relevant skillsets in research and development and regulation of gene and cell therapy ATMPs, including experience with AAV biology and production, cell biology and production, transgenic animals, immunology, virology/vectorology, histology, omics, and in vivo experimentation;
- State-of-the-art experience and expertise in the establishment of databases, data harmonisation, database management and data security;
- Experience in translating and conveying data for regulatory purposes;
- Access to clinical cohorts and samples from patients dosed with gene or cell therapies.

The applicant consortium should engage with relevant patient organisations and incorporate patient input and active involvement into the project.

In addition to academic groups, relevant Small and Medium-sized Enterprises (SMEs) are encouraged to participate in the applicant consortium;

The size of the consortium should be proportionate to the objectives of the project.

## Suggested architecture of the full proposal

The applicant consortium should submit a short proposal, which includes their suggestions for creating a full proposal architecture, taking into consideration the industry participation including their contributions and expertise provided above and below.

In the spirit of the partnership, and to reflect how IMI2 JU call topics are built on identified scientific priorities agreed together with EFPIA beneficiaries/large industrial beneficiaries, these beneficiaries intend to significantly contribute to the programme and project leadership as well as project financial management. The final architecture of the full proposal will be defined by the participants in compliance with the IMI2 JU rules and with a view to the achievement of the project objectives. The allocation of a leading role within the consortium will be discussed in the course of the drafting of the full proposal to be submitted at stage 2. To facilitate the formation of the final consortium, until the roles are formally appointed through the consortium agreement, the proposed project leader from among EFPIA beneficiaries/large industrial beneficiaries shall facilitate an efficient negotiation of project content and required agreements. All beneficiaries are encouraged to discuss the project architecture and governance and the weighting of responsibilities and priorities therein.



The consortium is expected to have a strategy on the translation of the relevant project outputs into regulatory and clinical practice. A plan for interactions with regulatory agencies / health technology assessment bodies with relevant milestones and resources allocated should be proposed.

A plan for aspects related to sustainability, facilitating continuation beyond the duration of the project should also be proposed.

The architecture outlined below for the full proposal is a suggestion. The architecture of the full proposal should be designed to fulfil the objectives and key deliverables within the scope of this proposal.

### **Work package 1 – Management, coordination, and dissemination**

The goals of this work package will be as follows:

- General oversight and coordination;
- Dissemination of research results and data amongst the consortium and into the public domain via workshops, publications, and presentations.

Expected applicant consortium contribution: project management including coordination of work package deliverables, periodic reporting and budget administration, dissemination of scientific results.

EFPIA consortium contribution: overall leadership of project goals, communication, and dissemination of project results.

### **Work package 2 – Develop better, standardized models for predicting product immunogenicity in humans**

The goals of this work package will be as follows:

- Develop models for evaluation of the impact of pre-existing immunity or of adaptive immunity on product efficacy and/or safety using *in vitro* cell-based assays and/or various routes of administration in relevant animal species;
- Expand on current mechanistic understanding of innate immune response during initial ATMP exposure, the priming of adaptive response, and the maturation of the immune response against targeted tissues that can provide a basis for rational design of immunomodulation protocols that can be evaluated in work package 4 for clinical application.

Expected applicant consortium contribution:

- Innovative models of interactions between immune cells and target cells;
- Next generation technologies for assessing immunity in those models across a breadth of immune cells and receptor repertoires;
- Identification of cellular and/or protein biomarkers that could contribute to potential stratification of patients in order to reduce the risk of deleterious immune responses;
- Application of the most relevant models (e.g. humanized rodent, non-human primates) already in use or under development;
- Strategies for investigating the role of patient genotype on the anti-ATMP response, with consideration on how to mitigate for small numbers of subjects;
- Translation of mechanisms learned from *in vivo* and *in vitro* systems to clinical approaches for immunomodulation or immunosuppression of the response to ATMP (in alignment with WP4);

- Using the knowledge and patient samples from work package 4, develop methods to determine the predictive value of *in vivo* and *in vitro* models.

EFPIA consortium contribution:

- Selection and prioritization of models with an emphasis on those dealing with cellular immune responses;
- Models, including *in vitro* and *in vivo* for evaluation;
- Expertise in cellular immune assays including assay development, validation, and data interpretation;
- Scientific input for innovative approaches to develop additional models;
- Data management/Bioinformatics infrastructure.

**Work package 3 – Build our understanding of gene/cell therapy drug metabolism inside a host and explore any loss of efficacy (persistence), particularly with non-integrating viral vectors or cell therapy**

The goals of this work package will be as follows:

- WP3 broadly aims to understand the molecular stability and metabolism of AAV derived therapeutic vector genomes, both wild type size and oversized, in target tissues. Characterisation of the effect of vector genome dilution, as a consequence of target tissue growth, and thereby therapeutic potential, will be addressed. Additionally, characterisation of the metabolism of the therapeutic vector genome in different cell types will be explored. Finally, strategies to improve the persistence of vector genomes as well as to generate hybrid vectors to accommodate transgenes that exceed the packaging capacity of AAV will be investigated;
- Identify strategies to mitigate loss of vector genomes and explore the idea of stabilising, non - integrated AAV vector genomes within the target cell;
- Characterise the metabolism of the vector genome in different cell types to understand whether rates of degradation, episomal maintenance, or integration vary from cell to cell;
- Design improved hybrid vectors that have a higher efficiency of concatemerisation, and full-length vector genome reconstitution.

Expected applicant consortium contribution:

- Small and large animal models of disease. Focus on central nervous system (CNS), muscle and liver;
- Development and utilisation of technology to measure vector copy number, vector genomic structure, monomers, concatemers, epigenetic status of vectors over time in relevant tissues;
- Development of and utilisation of tools to analyse the cellular milieu to identify factors which govern vector stability and genomic structure.

EFPIA consortium contribution:

- Disease relevant animal models;
- Registry of results from pre-clinical data;
- Prospective pediatric patient data and samples.



**Work package 4 – Understand the clinical factors around pre-existing immunity limiting patient access to ATMP therapy, and adaptive immune responses affecting product safety, efficacy and persistence, including for integrating vector-based therapies.**

Objective: perform translational and clinical research with the intent of standardising existing analytics based on biobanked samples, and development of the new immune-modulatory protocols.

The goals of this work package will be as follows:

- Establish a geographically diverse prospective biobank from treated and untreated donors with matching cell, serum, and plasma samples to enable evaluation of the pre-existing and adaptive immunity; assure that informed consent is properly obtained and strict adherence to privacy is maintained;
- Develop standardised pathways for characterisation of pre-existing immunity to gene/cell therapy products, including macrophages, natural killer (NK) cells, memory T-cells, and other cells, and neutralising and binding antibodies;
- Develop cohesive metrics for immunological characterisation, applicable for gene and cell therapies, for both patient inclusion and post-treatment monitoring;
- Standardise assays for use in safety and persistence biomarker monitoring;
- Develop and standardise innovative characterization/functional assessment methods for gene/cell therapy drug substance and product;
- Establish novel protocols for modulation of immune responses to capsid and transgene products, and autologous or allogeneic gene-modified or unmodified transplanted tissues and cells. Similarly, develop protocols to induce tolerance to expressed transgene products or to autologous or allogeneic gene-modified or unmodified cell products;
- Evaluate safety risks when dosing viral gene therapies in the background of humoral and/or cellular immunity against the virus.

Expected applicant consortium contribution:

- Organisation of biobanking from healthy volunteers and recipients of cell and gene therapies from broad geographic areas;
- Characterisation of the relationship between binding antibodies and neutralising antibodies. Define the interplay between humoral immunity, complement activation, and cell-mediated immunity. Establish models to allow prediction of innate immune responses. Discern mechanisms of activation of memory T-cell and NK-cell activation and their role in loss of transgene expression. Expand knowledge regarding non-antibody mediated neutralisation;
- Define metrics for immunological characterisation, applicable for gene and cell therapies, for both patient inclusion and post-treatment monitoring;
- Evaluation of innovative characterisation/functional assessments methods for gene/cell therapy drug substance and products in cell-based assays and in vivo models from WP2;
- Use animal models developed in WP2 to access modulatory/intervention strategies. The learning and knowledge derived from WP2 will be used to inform this goal of developing novel animal models and establish novel protocols for modulation of immune responses to capsid and transgene or cell products, as well as induction of tolerance to vectors, expressed transgene products, and autologous or allogeneic gene modified or unmodified cell products;
- Conduct nonclinical studies to identify potential adverse events when dosing the presence of viral vector immunity.

EFPIA consortium contribution:

- Prospective data from clinical samples;
- Validation of immunosuppressive protocols in animal models.

**Work package 5 – Engage regulators to ensure that the models and data generated through this project will provide the necessary information to support regulatory filings and to address regulatory and safety concerns**

The goals of this work package will be as follows:

- Enable data-driven regulatory requirements. Identify and address scientific gaps in current knowledge in order to generate improved and data-driven standards for safety while enabling accelerated product development. This may include key issues in gene/cell therapy development, including environmental assessments, characterisation of replication competent virus, viral clearance in the manufacturing process, genetically-modified organism (GMO) issues such as viral shedding after administration, patient screening criteria, and long-term follow up for persistence and delayed adverse events such as those related to insertional mutagenesis. This will enable a move from theoretical concerns to data driven risk assessments that can be used to update regulatory requirements;
- Identify opportunities for regulatory harmonisation. Conduct a landscape analysis of regulatory requirements and gather examples, develop criteria and evaluate options to standardise differences in regulatory requirements across countries. Utilise project efforts to guide the development of ATMP specific ICH guidelines;
- Perform a landscape analysis of regulatory requirements and identify differences in existing requirements in order to develop recommendations for regulatory harmonisation;
- Publish a white paper(s) outlining the results of the data analysis and regulatory landscape analysis with specific recommendations for updated regulatory requirements;
- Participation in meetings or workshops with regulators to drive acceptance of consortium-recommended regulatory harmonisation;
- Create predictable regulatory pathways for innovation. Work with regulators to develop a more predictable path to implementing innovative systems and technology such as the qualification of novel biomarkers (e.g. transgene expression) for use as endpoints in clinical trials, the use of standardized manufacturing platforms, improved comparability strategies, and utilization of predictive immunogenicity strategies, engage with Health Authorities, take advantage of regulatory tools and procedures such as Innovation Task Force (ITF); the European Medicines Agency (EMA) (including committee on Advanced Therapies) Scientific Advice (SA) and qualification advice as well as national Scientific Advice (SA).

Expected applicant consortium contribution:

- Based on the plan generated, develop a prospective database where non-competitive data can be collected such as replication competent virus testing, vector shedding, and long-term follow up. The database should be set up to ensure patient confidentiality and protect corporate competitive intelligence. Compile data and perform cross-sectional analysis to determine actual experience related to the unique risks of cell and gene therapy to enable a move from theoretical to data-driven recommendations for regulatory requirements.

#### EFPIA consortium contribution:

- Share non-competitive data related to regulatory requirements such as replication competent virus testing, vector shedding, and long-term follow up to allow for a cross-sectional analysis of data to enable a move from theoretical to data-driven recommendations for regulatory requirements.
- Contribute to landscape analysis of regulatory requirements and develop recommendations for regulatory harmonisation.

## References

- [1] Vandamme, C., O. Adjali, and F. Mingozzi, *Unraveling the Complex Story of Immune Responses to AAV Vectors Trial After Trial*. Hum Gene Ther, 2017. **28**(11): p. 1061-1074.
- [2] Colella, P., G. Ronzitti, and F. Mingozzi, *Emerging Issues in AAV-Mediated In Vivo Gene Therapy*. Mol Ther Methods Clin Dev, 2018. **8**: p. 87-104.
- [3] Manno, C.S., et al., *Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response*. Nat Med, 2006. **12**(3): p. 342-7.
- [4] Boutin, S., et al., *Prevalence of serum IgG and neutralizing factors against adeno-associated virus (AAV) types 1, 2, 5, 6, 8, and 9 in the healthy population: implications for gene therapy using AAV vectors*. Hum Gene Ther, 2010. **21**(6): p. 704-12.
- [5] Masat, E., G. Pavani, and F. Mingozzi, *Humoral immunity to AAV vectors in gene therapy: challenges and potential solutions*. Discov Med, 2013. **15**(85): p. 379-89.
- [6] Nathwani, A.C., et al., *Adenovirus-associated virus vector-mediated gene transfer in hemophilia B*. N Engl J Med, 2011. **365**(25): p. 2357-65.
- [7] Mingozzi, F., et al., *CD8(+) T-cell responses to adeno-associated virus capsid in humans*. Nat Med, 2007. **13**(4): p. 419-22.
- [8] Cunningham, S.C., et al., *Gene Delivery to the Juvenile Mouse Liver Using AAV2/8 Vectors*. Mol Ther, 2008. **16**(6): p. 1081-1088.