Three key elements for a significant acceleration in ATMP development - San Raffaele Telethon Institute for Gene Therapy (SR-Tiget) position paper

The present paper reflects the official position of SR-Tiget and Fondazione Telethon. SR-Tiget is directed by Luigi Naldini; Fondazione Telethon Director for Research & Development is Lucia Faccio.
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Among the challenges that IMI2 is addressing in order to boost the development of advanced therapies in Europe, we suggest 3 topics which could provide substantial advances:

- Improving preclinical studies of Advanced Therapy Medical Products (ATMPs), and more specifically:
    a. Developing new approaches based on targeted gene editing
    b. Improving the reproducibility of preclinical studies
- Novel approaches for clinical study of ATMPs, and more specifically:
    c. Investigating the safety of ATMPs.

On one hand, the impressive speed at which genome editing is developing and its wide use in basic scientific research pave the way to a major change in the development of ATMPs for clinical application. The new technologies for genome editing (and especially CRISPR/Cas9) generate excitement among scientists, industry, investors and patients, which see great promise in these advances. However, there are still several hurdles to address before entering the clinical stage and a public-private European-wide initiative in the field could really make the difference.
On the other hand, considering the ATMPs which are in a more advanced stage of preclinical or clinical development, two mayor bottlenecks we identified are the lack of standards in preclinical studies and in safety studies of gene therapy approaches employing integrative vectors. As mentioned by the European Medicines Agency (EMA) "Reflection paper on management of clinical risks deriving from insertional mutagenesis" (2013), "In the clinical setting, integration studies may be required for the monitoring of potential safety issues identified in nonclinical studies. Such studies could complement the overall safety monitoring of patients and aid the assessment as to whether or not a particular integration profile is more or less prone to cause adverse events." Setting standards in this field is therefore pivotal for a faster development of gene therapy clinical applications.

a) Developing new approaches based on targeted gene editing

Current gene therapy strategies for inherited monogenic diseases are based on the correction of the genetic defect through the integration of the therapeutic gene into affected cells, using viral vectors to ensure maximal efficacy of gene delivery. Gene therapy thus bears the promise of a long-term clinical benefit with a single administration of the treatment. However, gamma-retroviral and lentiviral vectors integrate the therapeutic gene in semi-random positions of the genome of target cells, therefore precluding any real control on gene expression. In addition, several cases of leukemia occurring in X-linked severe combined immunodeficiency (SCID-X1), Chronic Granulomatous Disease (CGD) and Wiskott-Aldrich

Syndrome (WAS) patients treated by gene therapy using gamma-retroviral vectors have highlighted the risks associated with insertional mutagenesis.
The development of more precise gene editing strategies based on targeted genome editing could instead allow to stringently reconstitute gene function and expression control by *in situ* gene correction and to abrogate any residual risk of insertional mutagenesis by targeted integration.
We envision to focus initial application of gene editing on diseases for which gene correction is associated with growth advantage (e.g. SCID-X1, CD40LG deficiency, RAG-1 deficiency), in order to compensate for the current low efficiency of gene editing in Hematopoietic Stem Cells (HSC). In parallel, we think it is crucial to increase the efficiency and specificity of targeted gene editing and the purity and yield of edited HSC in order to support safe and effective application also when there is no growth advantage associated with gene correction.
Overall, these studies may position gene editing as new golden standard for precise HSC engineering.

b) Improving the reproducibility of preclinical studies

A rigorous preclinical evaluation represents the foundation stone for the progression to clinical trials of conventional pharmaceutical products as well as ATMPs. Confidence in the quality of preclinical data, irrespective of where they have been produced (academia, industry, contract laboratory), is indeed a necessary condition to evaluate the safety and efficacy of new medicines intended for therapeutic use in humans.
Good Laboratory Practice (GLP) compliance provides such confidence, as it ensures that:

- preclinical studies are designed and conducted by experts;
- standardized and validated test methods as well as appropriately calibrated test devices and instruments are employed;
- all data, including raw laboratory records, are stored and available for independent review.

The regulatory authorities recognize that the biological and technological complexity of ATMPs represents a challenge and have issued several guidelines, which however acknowledge that there is no single right way to perform preclinical evaluations of ATMPs and are open to assess novel evaluation strategies. In order to face the hurdles of the preclinical development of the ATMPs being developed at SR-Tiget, a GLP compliant Test Facility has been established within the Institute. This represents to our knowledge the first GLP certified facility able to perform preclinical gene and cell therapy studies embedded within an academic Institution. Also, we are working on the creation within our Institute of a Good Scientific Practice (GSP) laboratory, for the development of innovative protocols for vector production, gene editing and *ex vivo* cell manipulation in a context of Quality by Design, as well as of a Good Clinical Laboratory Practice (GCLP) laboratory, to ensure integrity and reliability of data obtained by processing and analyzing samples from clinical trials.
We are indeed convinced that Good Practice (GXP) is crucial along the advanced stages of development of a new medicinal product and that promoting the definition and implementation of GXP standards across Europe would be tremendously valuable.

c) Investigating the safety of ATMPs

Focus on: the importance of safety testing in gene therapy applications with integrative vectors

Thanks to the clinical benefit demonstrated in a number of trials for rare diseases, gene therapy is now emerging as a medical reality and an increasing number of gene therapy approaches for the long-term treatment of several genetic diseases are being pursued. Consequently, there is a growing demand for

assessing the safety and the efficacy of such therapeutic strategies in both preclinical and clinical studies. In particular, as anticipated above, the assessment of mutagenicity and oncogenic potential of semi-random vector insertions in the genome is a crucial issue for the whole gene therapy field. Indeed, the benefit derived by the stable integration of genetic material into millions of target cells is also associated with the risk of activation of cellular oncogenes and tumorigenesis. Although the more advanced designs of lentiviral vectors have greatly reduced the concerns related to insertional mutagenesis, we and others have shown that even these improved vectors are not entirely neutral upon integration. Therefore, fundamental to address the safety of integrating vectors is the study of vector integration sites in the genome of cells / tissues from preclinical studies in animal models and from patients which have been treated with gene therapy. Indeed, this approach allows to identify which genes are targeted by vector insertions, to detect or exclude sustained clonal expansions (a worrisome preluding sign of genotoxicity), and in general to monitor the clonal composition and the dynamics of hematopoietic reconstitution in HSC gene therapy applications.
Because of the crucial information that integration studies provide, regulatory authorities have started to require them, and with an increasing level of detail and accuracy, for the evaluation of gene therapy products. However, performing these studies properly is challenging, since it requires the setup of state-of-the-art techniques for integration site retrieval, standardized bioinformatics pipelines and the use of Laboratory Information Management Software (LIMS) for the tracking of preclinical and clinical samples in pharmacovigilance studies.
Given the complexity of these studies and in view of the expected expansion in gene therapy initiatives, we strongly believe there is an urgent need to standardize procedures for the analysis of vector integration sites and to increase the capability to study a significantly larger number of samples from both from preclinical and clinical studies.

We hope that our considerations contribute to a fruitful discussion.

The institute

The San Raffaele Telethon Institute for Gene Therapy (SR-Tiget) was created in 1995 in Milan as a joint-venture between the Telethon Foundation and the San Raffaele Scientific Institute (SRSI), with the mission to perform cutting edge research on gene and cell therapy and to translate its results into therapeutic advances for genetic diseases.
Over the years, the Institute has contributed to the field with relevant discoveries in vector design, gene transfer strategies, targeted gene editing, stem cell biology, identity and mechanism of actions of pro-angiogenic monocytes and of regulatory T cells in immune responses, and with the implementation of successful gene therapy clinical trials. Notably, in May 2016 the European Commission granted marketing authorization for Stimvelis for the treatment of Adenosine Deaminase Severe Combined Immunodeficiency (ADA-SCID). Strimvelis, which is the first approved *ex vivo* gene therapy worldwide, was developed at SR-Tiget and brought to the market under a strategic alliance with the pharmaceutical company GlaxoSmithKline. Moreover, two first-in-human pivotal clinical trials of HSC gene therapy with lentiviral vectors, for Wiskott-Aldrich Syndrome (WAS) and Metachromatic Leukodystrophy (MLD), both initiated in 2010, have completed the treatment phase and are showing evidence of persistent and clear therapeutic benefits; a third HSC gene therapy clinical trial with lentiviral vectors, for beta-thalassemia, started in 2015 and is showing promising preliminary results. In addition, a first-in-human clinical trial of HSC gene therapy with lentiviral vectors for Mucopolysaccharidosis type I (MPS-I) is planned to start in 2018.