InMutaGene: Development of a technology to address the risks of insertional mutagenesis and oncogenesis and to improve translational research in gene therapy

Background

Gene therapy (GT) is now emerging as a medical reality, with clinical efficacy demonstrated in a number of GT trials for rare diseases. Over 1800 GT clinical trials have now been initiated or approved worldwide, and whilst most are phase I/II, an increasing number of products are entering phase II and III trials each year (Ginn *et al,* 2013). Based on this progress and the success of Glybera, the first licensed GT product in Europe, further GT initiatives are underway in a number of genetic disorders, malignancies and neurodegenerative disorders.

A wide variety of GT products are being developed to replace or disrupt existing genes. Generally the therapeutic DNA sequence is delivered via a vector system. Some vectors integrate permanently into the genome to provide *ex vivo* or *in vivo* gene addition/correction and represent a promising tool for the long-term treatment of inherited and acquired disorders. The integrated sequences are then transmitted to the cell progeny during cell replication. Insertional mutagenesis leading to oncogenesis is a recognised GT safety concern, which has manifested in a number of trials involving *ex vivo* transduction of haematopoietic stem cells with gamma-retroviral vectors (Howe *et al,* 2008; Hacein-Bey-Abina *et al,* 2008; Braun *et al,* 2014). However, the extent of this risk following other vector types, target cells and/or *in vivo* methods of administration is not clear. Understanding the risk factors associated with insertional mutagenesis of diverse gene transfer systems is a crucial, outstanding issue for the whole GT field. The nature of the target cells, the vector design, the mode of delivery and the disease background can affect insertional properties of the vector and, potentially, the likelihood of oncogenesis (Rothe M. *et al,* 2014; Chaungfen W. *et al,* 2011). Vector integration and clonality assays (which measure the diversity of such integrations in a cell population) are standard tools in the safety evaluation of GT products in both non-clinical and clinical studies. However, recently, the need to evaluate other factors (for example, epigenetic factors) that might influence the transition from a mutagenic to an oncogenic event has been highlighted.

As GT becomes a clinical reality, there is an increasing demand for non-clinical tests to assess the safety of the therapeutic strategy for the patient. A robust *in vitro/ in silico* tool to evaluate the risk of insertional mutagenesis/oncogenesis will support risk assessment while limiting the use of animals. A number of non-clinical assays (both *in vitro* and *in vivo*) for the assessment of mutagenicity and oncogenicity of GT approaches have been developed. Approaches include the *in vitro* immortalisation assay and the Jurkat/LMO2 cell line model. Although these are sensitive assays, they are limited to specific oncogenes, cell lineages and/or mechanisms of genotoxicity. Hence, they are more useful for screening of novel vectors for inherent genotoxicity by specific mechanisms rather than providing an estimate of risk for oncogenicity.

GT based on direct local or systemic administration of the transgene vector are mainly non-clinically supported by *in vivo* studies in wildtype rodents or in non-rodent species provided they are relevant with respect to transgene insertion, function and biodistribution. In some cases KO mice deficient for the transgene function may be of value. Therapies applying *ex vivo* genetic modification of autologous or heterologous cells require the use of immunocompromised animals, disease models or tumour-prone animals (Montini, 2006; Modlich, 2009; Montini, 2012). All models currently available have limitations in their ability to predict oncogenic risk. To run GT studies, particular animal strains (for example, immune-deficient NSG mice or specific disease models) are used that are expensive, low throughput and require special controlled barrier areas. In addition, in those cases where *ex vivo* GT is being evaluated, transplantation procedures are complex (requiring bone marrow sampling, cell purification and *ex vivo* transduction) and many animal “donors” are used to collect hematopoietic stem cells (HSCs). In addition, serial transplants are sometimes requested by regulatory authorities, requiring further use of recipient animals. Whilst these non-clinical studies are able to address safety issues related to vector design, the biological relevance of the disease environment and the impact of

the target tissue, cell type / lineage and differentiation status on potential genotoxicity is only partially explored (Aiuti. *et al,* 2013).

There are also considerations surrounding efficacy. For a GT product to be efficacious the gene-corrected cells must be present at high enough levels to enable a therapeutic effect. Information regarding the dynamics of clonal dominance amongst gene-corrected cells in non-clinical models and patients is currently scarce. A thorough evaluation of the target cells insertional profile and a comprehensive tracking of the clonal dynamics of gene-corrected clones in treated individuals would increase the predictive value for the clinical outcome.

3Rs benefits

Animal testing is generally required by regulatory authorities for GT products prior to initiation of human trials and *in vivo* studies are generally performed on each individual GT product. However, the unique biological properties of individual integrating vectors means that careful consideration is required to define appropriate non-clinical studies relevant for the prediction of clinical outcome. A tailored, fit for purpose, non-clinical development programme is expected by regulators based on sound science and risk assessment (European Medicine Agency, 2009; Narayanan G. *et al,* 2014). For some GT products, where there is a great deal of experience (non-clinical and/or clinical), it may be possible to use information from the literature to waive *in vivo* studies. However, in all cases and particularly for novel vectors, new *in vitro/in silico* approaches, validated against existing non-clinical and clinical data, would provide strong rational to reduce the need for animal studies.

- As an example, for *ex vivo* GT, a typical study might involve the transplantation of control and transduced cells from tumour prone rodents, or a disease animal model, into lethally irradiated recipient animals and group sizes can often exceed 30 with a typical study involving a minimum of two groups (Montini E. *et al,* 2012). Up to 100 rodents may be used and costs are in the range of approximately £100K to 300K. However, there is no standard study design and a large number of animals may also be required to provide donor cells or in the process of generating a disease animal model.
- Procedures used during such tumourigenicity studies are often of moderate to severe severity under EU animal protection legislation and may include:
    - Isolation of cells from tumour prone donors.
    - Irradiation of and transplantation of cells into, recipient mice.
    - Blood sampling of recipients to explore engraftment efficiency.
    - Tumour analysis and vector copy number analysis from euthanized mice.

If successfully validated, an *in vitro/ in silico* platform to characterise early the safety profile potentially associated with insertional mutagenesis could be used to screen vectors and to provide early go/no-go decisions, avoiding the need for *in vivo* tumourigenicity and genotoxicity studies. The impact on animal use will be greatest if it was widely applicable to future novel vector types / serotypes and for *in vivo* as well as *ex vivo* gene therapy approaches (O'Reilly M. *et al,* 2012). In addition, it is anticipated that the development and application of such an *in vitro/in silico* platform could be extended to assess the safety profile of emerging technologies, such as gene editing, which also carries the potential risk of insertional mutagenesis.

Need for collaboration

To solve this Challenge collaboration between scientists from different disciplines will be required including expertise in:

- GT products including vector and target cell properties (*in vitro* and *in vivo*).
- Vector design and production or access to vector batches.
- Techniques for integration site retrieval, sequencing and bioinformatics.
- Statistical and mathematical models for data elaboration and interpretation.

- Applied risk assessment strategies for GT products.

Overall aim

To develop *in vitro / in silico* assay(s) that can be used singly or in combination to improve risk assessment for GT products. These assays should be applicable to the assessment of a wide range of vector types (in addition to the 'first generation' gamma-retroviral vectors and autologous bone marrow-derived stem cells), different target tissues and modes of delivery and emerging technologies (e.g. gene editing).

Key deliverables

A platform that can be used to evaluate factors that might influence the transition from a mutagenic to an oncogenic event, such as the vector insertion profile, the presence of elements favouring insertional activation or inactivation, the biology of the transgene, of the specific target cell/tissue, and other predisposing factors. The platform(s) developed will need to provide a comprehensive characterisation of oncogenicity potential resulting in reduced animal studies overall and increased predictivity of animal studies when they are still needed.

A comprehensive characterisation tool for GT products would need to be able to:

- Evaluate the impact of diverse target cell lineages, level of differentiation, disease background and *in vitro* manipulation and expansion on vector integration profiles.
- Provide information to evaluate the predictive value of the non-clinical test developed.

Phase 1 deliverables

Phase 1 should deliver preliminary data in support of a plausible hypothesis (or hypotheses) explaining which factors are important and predictive for the conversion of an insertional event(s) to eventual oncogenicity. This may include:

- Development of a method(s) to evaluate and analyse starting cell composition in terms of heterogeneity (e.g. cytofluorimetric assays and cell sorting strategies).
- Preliminary results of the impact of *in vitro* cell manipulation and vector transduction on cell phenotypes.
- Preliminary results, for example using a tool vector(s), of the impact of changes in the above on early indicators of clonal expansion.
- A proposal for Phase 2 based on the preliminary results which includes identifying endpoints, reference vectors/cells/ conditions for *in vitro* cell manipulation and vector transduction.

Data for Phase 1 may be based on a single cell / tissue type.

Phase 2 deliverables

Required

Development and validation of a solution that is able to predict which GT protocols are at high risk of inducing oncogenicity in clinical use. This should include:

- Identification and a better understanding of the pathways leading from an insertional event to frank neoplasia, including the impact of, for instance, *in vitro* manipulation.
- A better understanding of the links between insertion sites, clonal dynamics / dominance and neoplasia.
- A comprehensive list of investigations on which to base the evaluation of the risk of insertional mutagenesis / oncogenesis for the clinical setting.
- A set of criteria, thresholds or algorithms to allow GT products to be ranked into high, medium or low risk for oncogenicity.
- Evidence and data which demonstrates applicability of the suggested approach to multiple different cell / tissue types.

Desirable

- Validation against known insertional oncogenicity events e.g X-linked severe combined immunodeficiency (X-SCID) and Wiskott-Aldrich Syndrome (WAS) trials.

It is important to note that the CRACK IT Challenges competition is designed to support the development of new 3Rs technologies and approaches, which will improve business processes and/or lead to new marketable products. The applications for both Phases must include a plan to commercialise the results into a product or service. This should be taken into consideration when completing your application.

Sponsor in-kind contributions

Phase 1

- Intellectual input in hypotheses development and industry perspective on relevant factors for conversion of insertional mutagenesis to oncogenicity.

Phase 2

- Access to data, plasmids and / or vectors where available.
- Access to non-clinical and clinical samples where available.
- Advice and recommendations to maximise predictive value of these investigations when translating results into the clinical setting (e.g. experimental study design, method validation, regulatory expectations).
- Expertise and advice on applied risk assessment.
- Access to facilities and industry experience, where appropriate and agreed in advance.

Duration

Phase 1: six months. Phase 2: up to three years.

Budget

Phase 1: up to 3 awards of up to £100k. Phase 2: up to £0.7million.

Sponsors

GlaxoSmithKline, Novartis.

References

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