CleanCut: animal free tumourigenicity assessment of genome edited human haematopoietic stem cells

Overall aim

This Challenge aims to develop an *in vitro* model to replace *in vivo* tumourigenicity studies for safety assessment of genome edited human haematopoietic stem cells (hHSCs).

Duration

Phase 1: six months, Phase 2: Up to three years

Budget

Phase 1: Up to £100k, Phase 2: Up to £1 million

Sponsor(s)

Novartis, Bayer and Takeda

Background

Diseases such as sickle cell anaemia, haemophilia, thalassemia and severe combined immunodeficiency result from modifications in a single gene occurring throughout the cells of the body. In the European and East Mediterranean area alone, at least 2,000 births a year are affected [1,2,3]. The only curative treatment is an allogenic haematopoietic stem cell (HSC) transplant, which involves transferring HSCs from a healthy donor to the patient's body after high-intensity chemotherapy or radiation. These treatments are expensive, (the cost of the transplant is ~ $275,000) [4], require patients to undergo lifelong pharmacological immunosuppression (costs ~ $12,000/year/patient [5], and appropriate donors need to be identified to prevent adverse immune responses.

A number of blood-related monogenic diseases have the potential to be cured with genome edited human HSCs (GE-hHSCs) that have been modified by inserting, deleting, modifying or replacing genetic material. This has created a significant amount of research and development to penetrate a market expected to reach $8.1 billion by 2025 [6]. Eight clinical trials based on the use of GE-hHSCs are ongoing (https://clinicaltrials.gov/), and the number is expected to increase over the next decade.

The e*x vivo* genome modification of hHSCs can be achieved using vectors to integrate genetic material into cells and the method is currently under development using designer nucleases [7]. Designer nucleases, including but not limited to zinc finger nucleases, TALENs, and the CRISPR/Cas9 system, target one specific DNA sequence and create a double-stranded DNA break. Repair by non-homologous end joining can lead to mutations or, if a donor template is made available, gene replacement by homology directed repair. Designer nucleases offer a high level of precision but can cleave undesired genomic regions, causing off-target modifications, which could generate cells with functional impairment, altered fitness, or oncogenic potential [8]. Recent studies have shown that human induced pluripotent stem cells (iPSCs) that survive CRISPR/Cas9-mediated editing present concomitant mutations in the P53 gene [9]. Although the selection of p53 mutants does not appear to happen in hHSCs [10], other unexpected modifications could occur, and genome edited cells need to be broadly assessed for such safety liabilities prior to their use in the clinic.

Current assessment of potential off-target effects includes *in silico* prediction tools, extensive DNA analysis of *in vitro* edited cells/DNA (using GUIDE-seq, Digenome-seq, CIRCLE-seq, SITE-seq,

BLESS/BLISS, HTGTS, IDLV capture and others) and *in vivo* carcinogenicity/tumourigenicity studies [8,11].

In silico analysis can predict, and DNA-based analysis can confirm, the presence of off-targets, but they cannot anticipate the functional consequences of those mutations. *In vivo* carcinogenicity studies, most often using NSG (NOD-SCID IL2Rγ−/−) mice, are lengthy, costly, require large numbers of animals and are poorly predictive of human safety, often due to poor engraftment of human cells in the animals [12,13,14,15]. Extending the study observation time has been shown to increase engraftment of cells, but extended studies present increased welfare burdens on the animals and costs [16]. Engraftment rates have improved by generating NSG mice that express human cytokines [17,18,19,20] or by placing hHSCs within subcutaneously implanted humanised synthetic ossicles. While promising, these approaches still require animals and their use for tumourigenicity assessment of GE-hHSC has not been evaluated [21,22].

In vitro models to assess tumourigenicity are available but are currently not fit-for-purpose for the assessment of GE-hHSCs. They include:

- A soft agar colony forming assay has been shown to be more sensitive and predictive for assessing the tumourigenicity of iPSCs when compared to *in vivo* studies [23] (and unpublished data from HESI CT-TRACS). However, it is not appropriate for cells in suspension, such as hHSCs, that are intrinsically anchorage independent.
- A haematopoietic specific colony forming unit assay which is frequently used to evaluate toxic effects on HSCs is not optimised for tumourigenicity assessment.
- 3D co-culture systems that generate a humanised HSC niche exclusively *in vitro*, have been published [24,25,26,27]. In these studies, only a single organ is present and the parameters to distinguish between normal and malignant hHSCs are not identified.

These emerging techniques provide a strong basis for the development of a more complex system suitable for animal free assessment of tumourigenic potential of hHSCs.

3Rs benefits

In vivo studies to assess the tumourigenicity of genome edited products are a regulatory requirement [28,29]. These studies are lengthy, costly, require the use of large numbers of animals and are not always predictive of the risk to human safety [30].

Novartis currently runs at least one study per year on GE-hHSCs. A typical *in vivo* experiment requires a minimum of 200 to 300 immunocompromised mice and a follow up of at least 20 to 24 weeks. Mice are often irradiated, then dosed *via* an intravenous infusion, followed by repeated peripheral blood sampling to confirm engraftment which can cause stress and discomfort in the animals, and assessment for potential tumour formation. Engraftment is not always successful which means that many animals are dosed but do not provide useful data, potentially requiring repeat studies to be performed.

An *in vitro* assay developed through this Challenge could potenially replace these *in vivo* tumourigenicity studies. There is also significant potential for the assay to be used for the assessment of other gene therapy products such as viral-mediated gene transfer or antisense oligonucleotides, replacing the use of thousands of rodents in tumourigenicity assessment. The assay could also be used to identify appropriate dosing regimens and assess toxicity of new compounds impacting haematopoiesis.

Key deliverables

The model developed through this Challenge should be able to assess the normal and aberrant tumourigenic behaviour of hHSCs *in vitro*, providing increased predictivity over the current *in vivo* and *in vitro* models.

The approach could be based on an organ-on-chip device (bone marrow together with other appropriate organ/s), but the Sponsors will consider alternative approaches.

The model must:

- Recapitulate the human bone marrow microenvironment.
- Permit the survival, proliferation and differentiation of hHSCs.
- Permit measurable readouts to monitor health, proliferation, differentiation and transformation of hHSCs (e.g. liquid biopsy, real time imaging).
- Permit the monitoring of real-time changes in the physiology of both circulating cells and target tissues (e.g. high content imaging).

The approach developed must be able to assess infiltration into one or more target organs (preferably the lymph node, though others will be considered) to permit the assessment of the tumourigenic potential of modified hHSCs.

iPSCs and HSCs differentially respond to DNA damage and to genome editing, therefore iPSCs ***must not*** be used as a surrogate for hHSCs.

Sponsors welcome suggestions from applicants for cells and materials to develop the scaffolds and tissue micro-environment.

Phase 1 deliverables

- Establish a human relevant *in vitro* model of the bone marrow.
- Establish a preliminary *in vitro* model of the lymph node. Alternatives to the lymph node will be considered if a good rationale is provided.
- Evidence the suitability of the models through:
    - Identification of media to support long-term survival (at least four weeks) and function of both systems.
    - Identification of markers to monitor the viability of differentiated cell types within the two tissues.
    - Demonstration of the survival of hHSCs in the system for at least three weeks.

Phase 2 deliverables

Essential:

- Establish a connected dual system model that includes bone marrow and a second organ (lymph node or suitable alternative) and permits the circulation of hHSCs.
- Demonstrate survival and circulation of an established oncogenic (leukaemic) cell line within the system.
    - Measure leukaemic cell proliferation within the bone marrow equivalent and infiltration into the target organ, including any perturbation of the normal physiology/structure of the

infiltrated organ. Human tumour cell lines, which engraft and proliferate faster than primary hHSCs, could be used to accelerate development of the dual-system model.

- Achieve long-term engraftment (at least four weeks (essential), but up to three months (desirable)) of hHSCs within the bone marrow equivalent.
- Demonstrate the multilineage differentiation potential of HSCs, for example, by colony forming unit assays on cells directly isolated from the system.
- Demonstrate that hHSCs with engineered oncogenic mutations expand within the bone marrow equivalent and invade the target organ to demonstrate predictivity for assessing tumourigenicity.
- Identify markers (such as membrane antigens, secreted factors, proliferation index, cellular average permanence time in a given organ) for the differentiation of normal HSCs from tumourigenic hHSCs.
- Identify the minimum time required for a reliable discrimination between normal and transformed cells.

Desirable:

- Include a third organ, preferentially liver, spleen, brain or lung to further investigate potential metastasis *in vivo*.

Sponsor in-kind contributions

The Sponsors will provide:

- Scientific advice and support.
- Data collected from *in vivo* experiments to be used for identification of proper markers and/or validation of the proposed *in vitro* model.
- Genome edited human cells in Phase 2.
- Bioinformatics analysis/support in Phase 2.
- In-house testing of the model in Phase 2.

The provision of certain in-kind contributions may be subject to applicable legal and compliance requirements and may require prior execution of agreements.

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