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Challenge 33: CleanCut Surgery Q&As

Q. There is a clear emphasis that the approach developed should contain the lymph node as the primary target organ to assess the tumourigenic potential of modified human haematopoietic stem cells (hHSCs)- are the Sponsors open to other organs?

A. The lymph node is preferred, but the Sponsors will consider other organs as long as a good rationale is provided.

Q. Can induced pluripotent stem cells (iPSCs) be used to generate the model?

A. Since genetic manipulation of iPSC cells often leads to selection of p53 mutants (Ihry *et al*, 2018), iPSCs derived HSCs must not be used as a surrogate for hHSCs. However, iPSCs could be used to generate the other tissues required in the model (e.g. liver, lymph node etc) and you will need to demonstrate that the derived cells are functional.

Q. What level of characterisation of the hHSCs is required in Phase 1?

A. For Phase 1, the Sponsors require phenotypic hHSCs that demonstrate multipotency after three weeks of culture.

Q. What are the Sponsors preferred readouts for measuring multipotency of hHSCs?

A. The Sponsors are open to suggestions, but the colony forming unit (CFU) assay or flow cytometry of cell surface markers are approaches that could be used.

Q. Is there an existing positive control, a genome edited hHSC line that shows tumourigenicity, which can be tested in the model?

The Sponsors are not aware of any hHSC line that currently produces tumours. However, recent papers have shown the use of designer nucleases to generate tumourigenic HSCs (Reimer J *et al*, 2017; Tothova Z *et al*, 2017; Schneidawind C *et al*, 2018). Sponsors will be happy to discuss with the applicants the generation of those positive controls.

The model developed for this Challenge may also rely on markers other than demonstrable tumour formation. For example, primed hHSCs may have a preference to differentiate into a particular cell type or may spend longer in the lymph node. Regression models based on CFSE fluorescence dilution factor, as suggested by Griessinger E *et al*, 2017, maybe also be considered.

Q. Is reproducibility of the model important?

A. Yes, it is very important to demonstrate that the model is reproducible.

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Q. What level of throughput do the Sponsors require?

A. Low to medium.

Q. What is the end goal of the Challenge?

A. To have a robust system to allow the identification of tumorigenic potential of genome engineered hHSCs.

Q. What support is offered by the Sponsors?

A. The Sponsors in-kind contributions to help solve the Challenge are detailed in the Challenge brief. In-kind contributions from the Sponsors are typically provided in Phase 2.

Q. Six months for Phase 1 is a very short time. What do the Sponsors expect to achieve in this time?

A. Sponsors are expecting proof-of-concept after six months as outlined in the deliverables; proof that the approach has the potential to deliver Phase 2 and data to demonstrate that. Solving this Challenge is likely to require a multidisciplinary approach involving expertise in complex *in vitro* models, high content imaging or quantitative biosensing, *in vitro* haematopoiesis/leukemogenesis, genome editing and tumourigenicity assessment.

Q. I have expertise in certain areas, but not in all areas that are required to solve the Challenge. How can I find other expertise?

A. Speak to the NC3Rs office (crackitenquiries@nc3rs.org.uk) and we will do our best to help connect you with the expertise you are seeking. You can also make use of the Challenge-specific LinkedIn pages that have been established.

Q. Who should we email with questions?

A. General questions can be sent to the NC3Rs. Questions regarding a specific Challenge can be sent to the Sponsors, but enquiries should be sent to ALL Sponsor parties for a particular Challenge. If preferred, please email the NC3Rs to introduce you to the Sponsors at crackitenquiries@nc3rs.org.uk

References:

Griessinger E *et al.* (2018) Acute myeloid leukemia xenograft success prediction: Saving time. Experimental Hematology 59:66-71.

Ihry RJ *et al.* (2018). p53 inhibits CRISPR-Cas9 engineering in human pluripotent stem cells. *Nat Med* 24(7):939-946.

Reimer J *et al.* (2017). CRISPR-Cas9-induced t(11;19)/MLL-ENL translocations initiate leukemia in human hematopoietic progenitor cells *in vivo*. *Haematologica* 102(9): 1558-1566.

Schneidawind C *et al.* (2018) MLL leukemia induction by t(9;11) chromosomal translocation in human hematopoietic stem cells using genome editing. *Blood Advances* 2(8):832-845.

Tothova Z *et al.* (2017). Multiplex CRISPR/Cas9-Based Genome Editing in Human Hematopoietic Stem Cells Models Clonal Hematopoiesis and Myeloid Neoplasia. *Cell Stem Cell* 21(4):547-555.