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Challenge 33: CleanCut

Sponsors

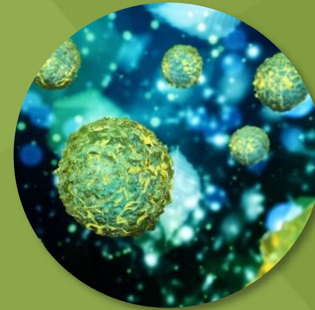
Novartis, Bayer and Takeda

Duration

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

Budget

Phase 1: £100k; Phase 2: £1M



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Challenge 33 “Clean Cut”

Development of an *in vitro* model to replace *in vivo* tumourigenicity studies for safety assessment of genome edited human haematopoietic stem cells

Bayer

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Launch Meeting
11 September 2019

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Monogenic blood diseases

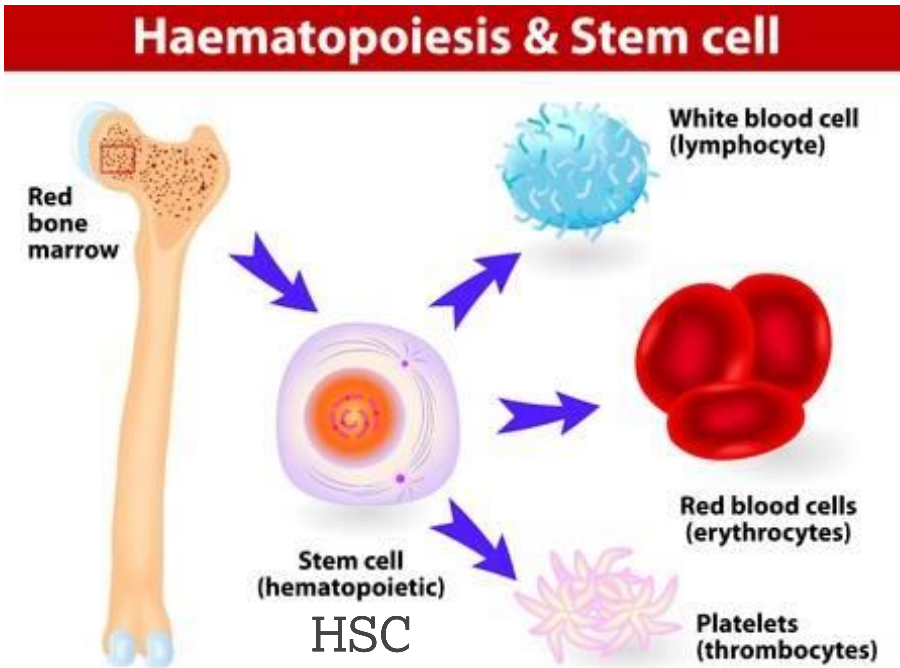

Image from https://www.123rf.com/photo_17878647.html

- Diseases such as SCID, thalassemia and haemophilia result from mutations in single genes affecting the function of hematopoietic stem cells (HSCs) progeny.
- In Europe and East Mediterranean area >2.000 births/year are affected
- The only curative treatment so far is an allogenic HSCs transplant that
  - is expensive
  - requires an appropriate donor and
  - results in lifelong pharmacological immunosuppression

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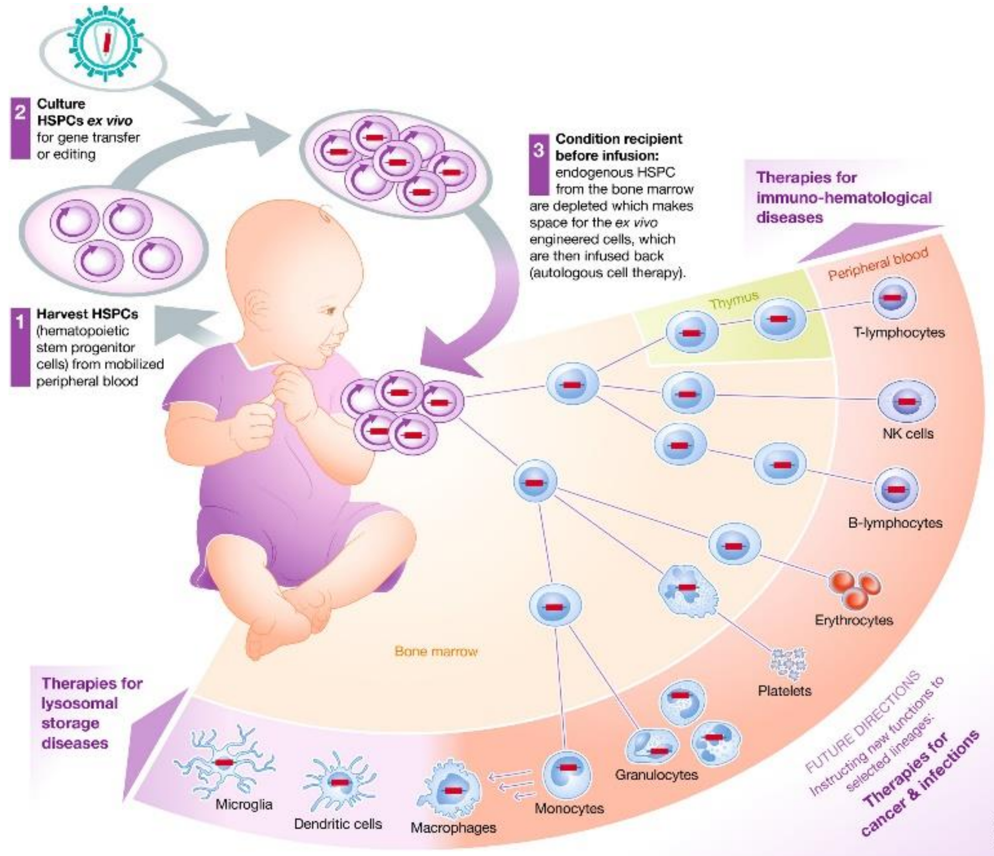
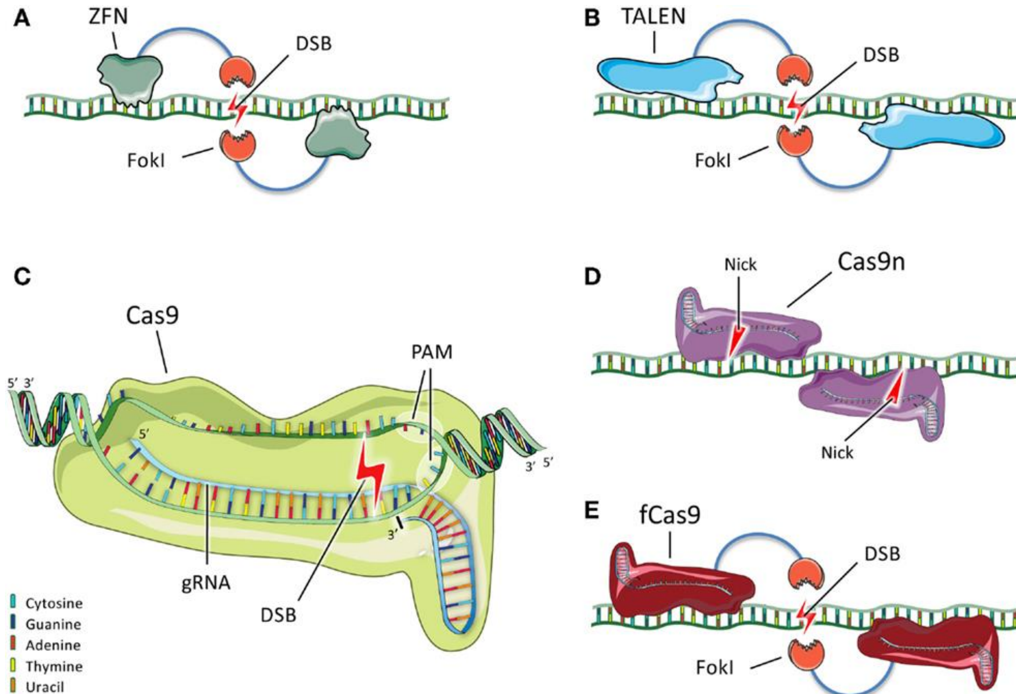
Hematopoietic stem cell gene therapy for monogenic blood diseases



Ex vivo genome modification of hHSCs can be achieved with viral vectors or designer nucleases

EMBO Mol Med, Volume: 11, Issue: 3, First published: 22 January 2019

Designer nucleases can be use to edit the genome of HSCs



https://www.frontiersin.org/articles/10.3389/fimmu.2015.00250/full

- Eight clinical trials based on the use of GE-hHSCs are ongoing (https://clinicaltrials.gov/), and the number is estimated to increase over the next decade.
- Presence of off-targets need to be evaluated to avoid any potential unwanted modification

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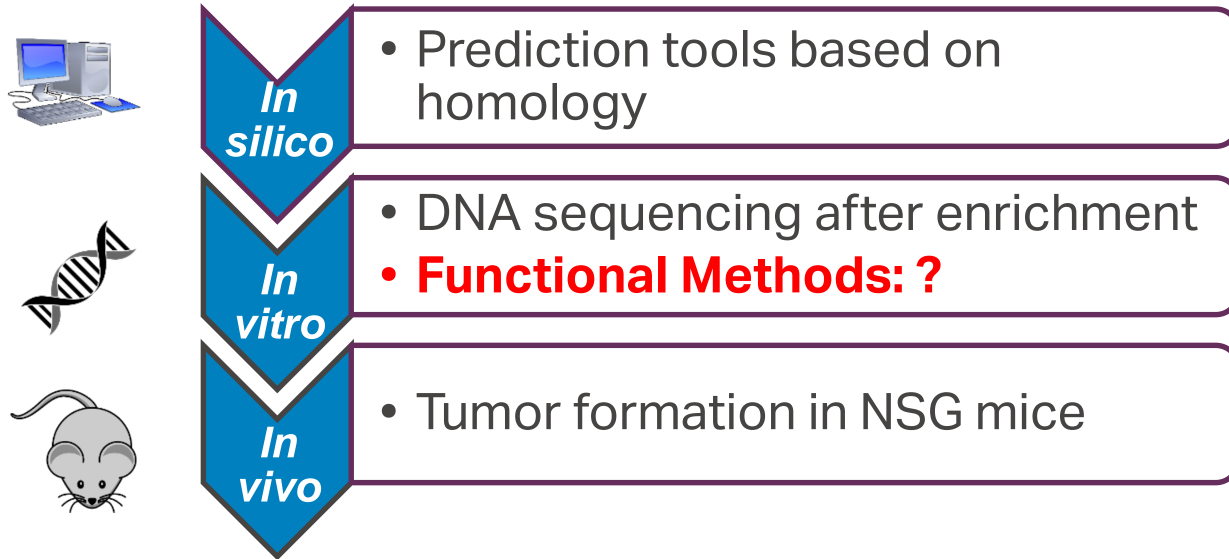
Designer nucleases off-target assessment



Limitations

- *in vitro:* lack of appropriate assays able to monitor **functional consequences** of off-targets
  - Soft-agar is unsuitable for cells in suspension
  - Colony forming unit assay is not optimized for tumourigenicity assessment
- *in vivo*: poor sensitivity of NSG mice studies

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In vivo studies for tumourigenicity assessment of genome edited hHSCs

Experimental design

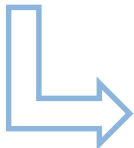
- hHSCs are injected in NSG (NOD-SCID IL2R$\gamma^{-/-}$) mice
- tumour formation is monitored for a minimum of six months.

Limitations:

- Human relevance/predictivity
- Cost
- Time
- Animal discomfort

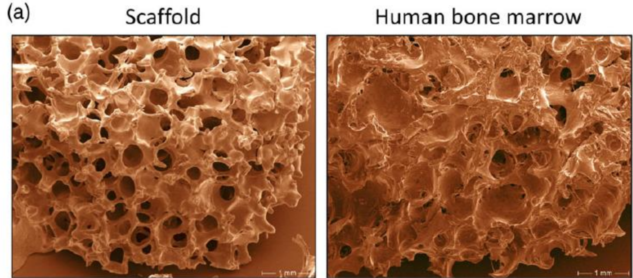
Engraftment rates of primary human hematopoietic malignancies have been improved by:

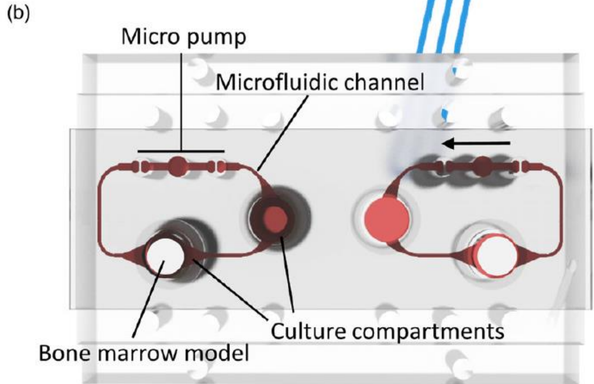
- extending the observation time up to one year
- generating NSG mice expressing human cytokines (e.g. GM-CSF, IL-3, SCF, TPO)
- placing HSCs within subcutaneously implanted humanised ossicles



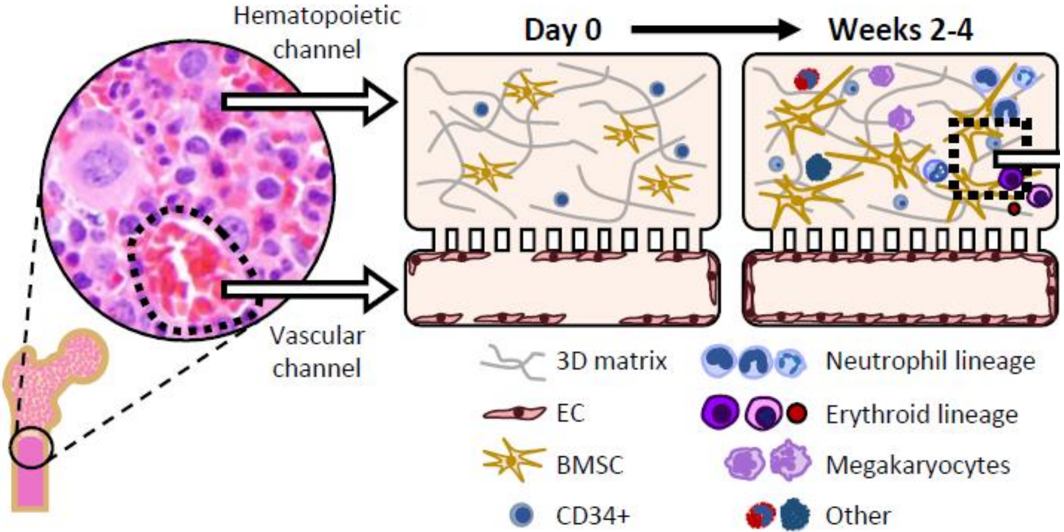
All those procedures **increase** animal stress and use!

3D co-culture systems to generate a humanized HSC niche *in vitro*...





from Sieber S *et al* (2017)



from Chou DB *et al* (2018)

... demonstrated hHSC survival and maintenance of differentiation potential up to four weeks

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Why was this Challenge Developed?

Business

A typical *in vivo* tumourigenicity study requires 200-300 mice and a follow up of at least 24 weeks, with minimum cost of half a million Euros.

Market is expected to reach $8.1 billion by 2025.

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A predictive *in vitro* assay with adequate performance could replace *in vivo* studies for tumourigenicity assessment of GE-hHSCs

Such assay could also be used to assess other gene therapy products or toxicity of new compounds impacting haematopoiesis.

Scientific

Predictivity and relevance of the *in vivo* models currently used is suboptimal

Provide a tool to study bone marrow cross-talk with other organs

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Key deliverables

The model should be able to:

1) Distinguish normal and aberrant tumourigenic behavior of GE-hHSCs,
2) Outperform current *in vivo* and *in vitro* models by overcoming their limitations.

   The model should recapitulate human bone marrow microenvironment and permit human hematopoietic stem cells:
   - survival,
   - proliferation
   - differentiation
   - infiltration of transformed cells into one (or more) target organs

   The model should include measurable readouts to detect aberrant behavior:
   - viability/health, proliferation, differentiation and transformation of hHSCs (e.g. liquid biopsy, real time imaging, "*ex vitro*" analysis)
   - real-time changes of circulating cells and target tissues
   - infiltration into one (or more) target organs

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Deliverables (Phase 1)
Model establishment

- Establish:
  - a human relevant *in vitro* model of the bone marrow
  - a preliminary *in vitro* model of the lymph node (or an alternative organ, if a good rationale is provided).

- Prove the suitability of the models through:
  - Identification of media to support long-term survival (>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.

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Deliverables (Phase 2)
Model performance assessment

Essential (I):

- 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 stability of the system (minimum four weeks, preferentially up to three months) of hHSCs within the bone marrow equivalent.

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Deliverables (Phase 2)
Model performance assessment

Essential (II):

▪ Demonstrate:
- multilineage differentiation potential of HSCs
- the ability of transformed hHSCs to expand within the bone marrow equivalent and invade the target organ to demonstrate predictivity for assessing tumourigenicity.

▪ Identify:
- markers to differentiate normal from tumourigenic hHSCs.
- 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*.

To avoid

Since:

- CRISPR/Cas9 mediated genome editing of iPSCs leads to p53 mutants selection

and

- iPSCs and HSCs differentially respond to DNA damage and to genome editing,

iPSCs ***must not*** be used as a surrogate for hHSCs.

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Sponsor in-kind contribution

- 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

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Thank You!

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