CRACK IT

Challenge: InMutaGene

https://www.crackit.org.uk/challenge-21-inmutagene

Launch Meeting
10 September 2015

The Challenge

"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)."

Why is there a need for this Challenge?

Ex vivo GT of haematopoietic stem cells: Unpredictability of Leukaemia development following Insertional Mutagenesis (IM)

X-linked SCID (France & UK)
5 cases of T-cell leukaemia (n>20)
First case 9 months after GT

Wiskott-Aldrich Syndrome (WAS) (Germany)
7 cases of T-cell leukaemia (n=10)
First case 16 months after GT

Chronic Granulomatous Disease (CGD) (Germany)
Myelodysplasia (pre-leukaemic syndrome) in 3/5 patients

Not preceded by clonal expansion?

ADA-SCID
Insertion sites similar? No tumours (n>40; including all trials)

β-Thalassaemia LV Trial
Dominant (myeloid; insertion in HMGA2) clone; but no tumours

Insertional activation of host cellular proto-oncogenes

<u>Enhancer activation</u>

X-SCID trial: *LMO2* or CCND2 proto-oncogenes

WAS: LMO2

CGD: *MDS1/EVI1* proto-oncogene

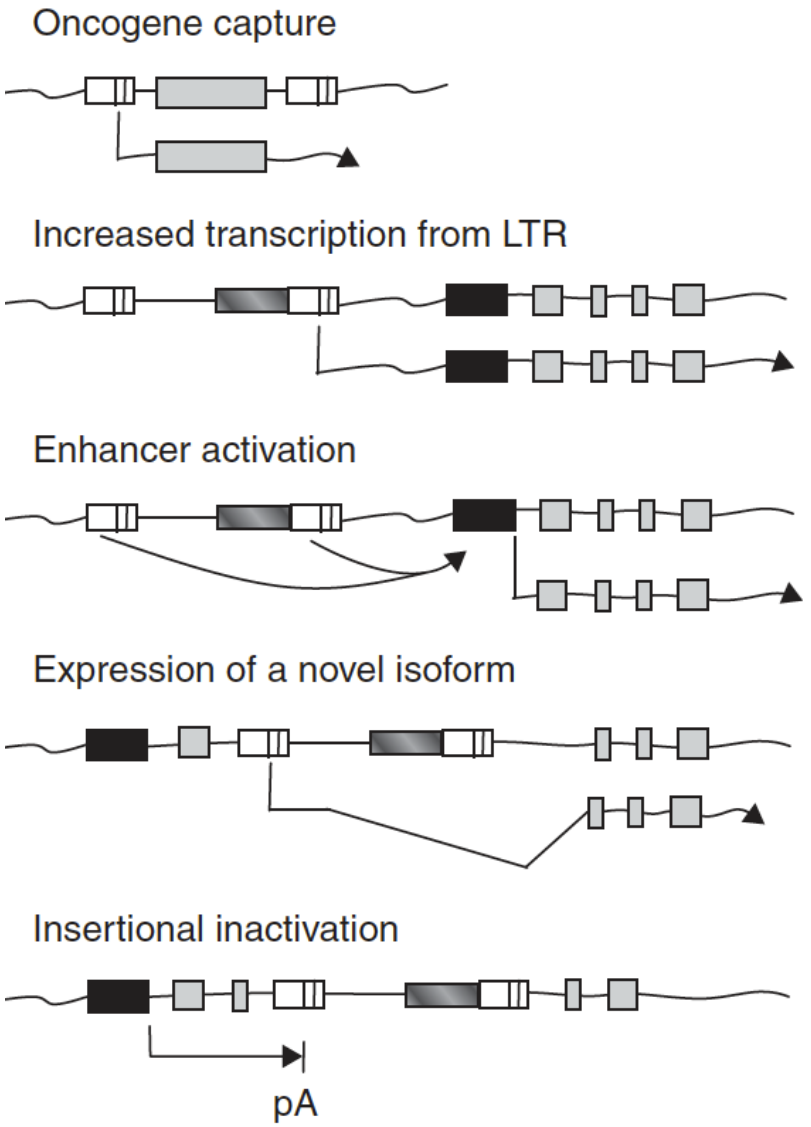


Figure 1. Retroviral mechanisms of oncogenesis. A. Acute-transforming replication-competent retroviruses capture a cellular proto-oncogene that mediates transformation. This mechanism does not occur in replication-incompetent vectors. **B.** The provirus 3′ long terminal repeat (LTR) triggers transcription of a cellular proto-oncogene at increased levels. **C.** Enhancers in the provirus LTRs activate transcription from a nearby cellular proto-oncogene promoter. **D.** Transcription from the provirus 5′ LTR creates a novel truncated isoform of a cellular proto-oncogene via splicing. **E.** A provirus disrupts transcription by causing premature polyadenylation (pA). The integrated provirus is indicated by two LTRs. Cellular proto-oncogene promoter and exons regions are indicated by black and grey boxes respectively.

From Trobridge. Expert Opin. Biol. Ther. (2011): 11(5)

What factors might influence whether insertion at sensitive site leads to eventual neoplasia?

“Disease background” Epigenetics? Other genetic / chromosomal lesions? Increased susceptibility to tumour formation?

Individual patient factors Age? Immune status?

Nature / function of the therapeutic gene product Cell signalling function? (eg γ-chain of IL2 receptor for X-SCID; WAS protein signal transduction function) Over-expression?

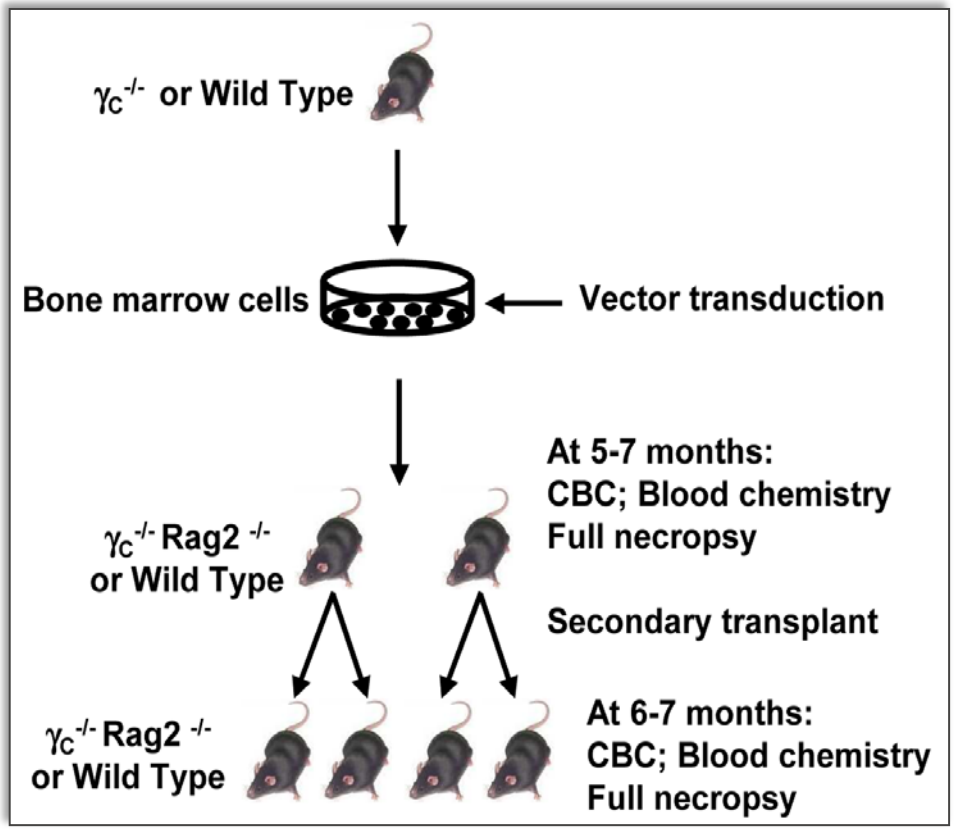
Vector design LTR, insulators

Target cell “Stem-ness”? Lineage?

Transduction protocol Duration of *in vitro* culture? Cytokines? Vector Copy Number / Transduction Efficiency?

Treatment protocol Cell dose? Rate of immune reconstitution?

Example of current *in vivo* models available



Zhou et al. PLOS ONE. Vol 8 (4) e62333(2013).

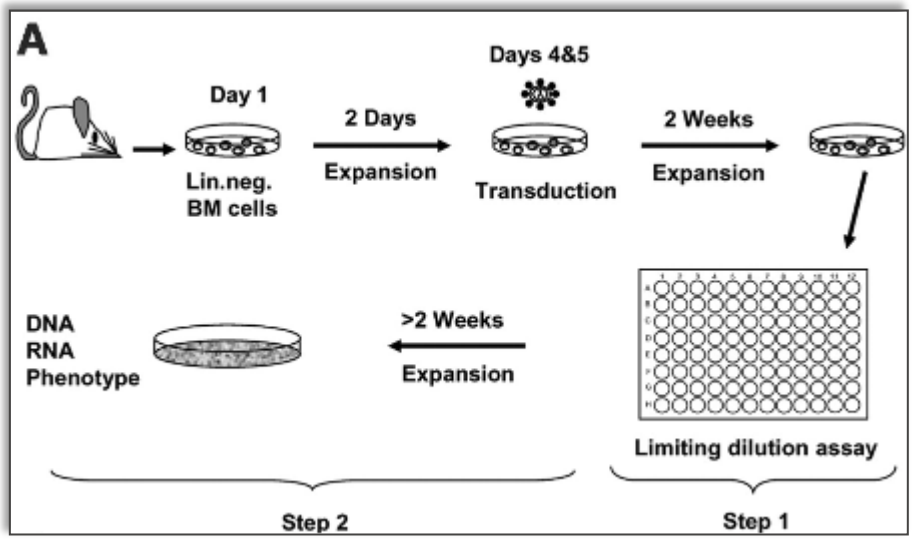
- Can perform on disease background
- Can perform on other genetic backgrounds e.g. tumour-prone model

But:

- Time / cost / animal use
- Severity of procedures
- Uses mouse (or other species) cells not human
- Sensitivity - may need secondary transplant (?relevance)
- Even when "positive" may be through non-relevant mechanism
- Value in quantitative risk assessment?

NC 3Rs gsk NOVARTIS CRACK IT

Example of current *in vitro* models available



*Usually performed with mouse cells
Primary human cells difficult to immortalise
Can use human cells lines

Modlich et al. Blood. Vol 108 (8); pp 2545-2553.

Example: *In vitro* immortalisation (IVIM) assay

Endpoint in a few weeks
Minimal animal use
Useful for determining effects of changes in vector design
Could perform on disease background

Questions:
Effects predominantly seen in myeloid lineage through insertional effects on EVI1 gene. ?relevance for clinical use.

How to use for quantitative risk assessment?

No “gold standard” *in vitro* or *in vivo* model available

Other Questions relevant to this Challenge

Is occurrence of neoplasia secondary to IM only an issue in context of *ex vivo* GT of haematopoietic stem cells?

What are the potential risks (of neoplasia) associated with non-integrating vectors?

What are potential risks (of neoplasia) associated with non-viral vectors?

What are potential risks (of neoplasia) associated with gene editing?

The ideal solution to this Challenge would be able...

..for specific vector / disease, identify & quantify relevant **risk factors** leading to induction of tumour formation

and/or

Provide a predictive **biomarker**

To allow **risk-benefit decisions** to be made more confidently and with measurable **impact on the 3Rs**

Science / Patient / Business Benefits

- Greater understanding of the factors contributing to an elevated tumour risk after Gene Therapy – helping industry to pick the right vector for the right disease
- Identify vectors / diseases at high risk of neoplasia – reduce risk to patients and companies
- Greater confidence in safety of novel vector platforms in the future – reduce risk of the “unexpected”
- Reduced reliance on animal models and reduced costs

3Rs Benefits

Depending on nature of “solution”

Replacement:

- Reduce need for tumour studies (will need regulatory acceptance)

Reduction:

- Predictive endpoints added to *in vivo* studies resulting in reduced animal numbers (because more predictive)

Refinement:

- Non-tumour endpoints on *in vivo* studies – less severe endpoints and possible reduced duration of *in vivo* studies

Deliverables

Phase 1 deliverables

A plausible hypothesis (or hypotheses) – supported by preliminary data: e.g.

- Impact of starting cell composition / heterogeneity, *in vitro* cell manipulation and vector transduction on cell phenotypes.
- 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.

Deliverables

Phase 2 deliverables

Ability to predict which GT protocols are at high risk of inducing oncogenicity in clinical use.

- understanding of the pathways leading from an insertional event to frank neoplasia
- 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; i.e. not just *ex vivo* HSC GT.

Pathogenetic mechanisms should correlate with those seen in the X-SCID and WAS trials.

Nature of In-kind Support

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.

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.

Thank you

The Sponsors are happy to discuss the challenge and potential applications with people in the run up to the submission deadline

Sponsor contacts are:

Jan Klapwijk, Rhiannon Lowe, Gill Stemp and Patrizia Cristofori (GSK)

Hans-Joerg Martus, Peter Ulrich, Silvana Libertini and Timothy MacLachlan (Novartis)

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