Genotoxicity Considerations in Clinical Trials Utilizing Integrating Vectors

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From Gerrits et al, Blood, 2010
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The following relationships exist related to this presentation:

No Relationships to Disclose
Where to Look for Advice

• FDA non-binding guidance document dated 2006, resulting in part from 2004 workshop at ASGT

• Evolving knowledge regarding vector integration site selection, vector enhancer activity, target cells, and host factors

• Common sense
Integrating Vector Perturbations

(a) Enhancer gene activation
(b) Promoter fusion
(c) Insertional inactivation
(d) Hybrid transcript with altered splicing
Assessing the Risk of Genotoxicity to Determine Appropriate Pre-Clinical Data, Product Screening and Long-Term Patient Follow-up
Vector Characteristics

• Integrating? **Higher** risk
• Pattern of integration of vector
  – Random vs non-random (could be **higher** or **lower** risk)
  – Targeted or limited number of sites (depending on site, **lower** risk)
• Enhancers, constitutive promoters, splicing elements in the vector (**higher** risk)
• Insulators: potential for **lower** risk
• Copy number-**higher** risk if require multiple copies per cell for efficacy
Transgene Characteristics

• Is the therapeutic expression window narrow? High

• A potential proto-oncogene if expression is too high or dysregulated? VERY HIGH

• Any known interaction with activated cellular genes (IL2R and LMO2 possible synergism) High
Target Cell Characteristics

• Hematopoietic stem cells very high risk

• Impact of cell dose-??????

• Other stem cell populations (endothelial progenitors, iPS or ESC-derived cells). ?????

• Lymphocytes lower risk despite high proliferative potential and self-renewal abilities

  — Newrzela et al, Blood 2008
Patient Characteristics

• If life expectancy of patient is short, then can tolerate higher genotoxicity risk

• Rapid expansion into an “empty” cellular compartment
  HIGH

• Impact of conditioning therapy

• Role of immunodeficiency, ability to tolerate future chemotherapy possible factors in risk assessment.
Preclinical Risk Assessment Models

- No “gold” standard
- Factor-dependent cell line conversion to factor-independence model
- Murine bone marrow immortalization assay
- Tumor-prone mouse model
- Long-term murine or serial transplant model
- Large animal model
- NONE VALIDATED with clinical outcomes
Integration Profiling of Cell Therapy Products

• Does this approach make sense?
  – NOT if highly polyclonal and a tiny fraction of cells delivered engraft long-term (CD34+ cells, lymphocytes)
  – POSSIBLY if administering a single or a few genetically-modified clones
Long-term Follow-up for Genotoxicity

What to do when, on which samples???????
FDA Guidelines for Integrating or Higher-Risk Vectors

• Suggest 15 years, or explanation of why a shorter time period

• First 5 years, directed detection of adverse events, minimum interval of one year, then yearly contact for another 10 years

• Case history focusing on exposure to mutagens, and other potential interacting events

• Search for and record new malignancies, and hematologic disorders, as well as neurologic and autoimmune disorders
Type of Samples to be Collected

• Peripheral blood, separated prior to freezing into myeloid and lymphoid cell types (at least via density gradient separation). Freeze for DNA and RNA, possibly viably for later flow sorting

• Bone marrow very useful for later colony studies if frozen viably. Obtain at long intervals

• Serum
Routine Screening of Samples

• Copy number of vector at least twice a year until undetectable or 5 years

• Expression of transgene

• FDA suggests “...perform assays to assess the pattern of vector integration sites in relevant surrogate cells” for instance blood cells following HSC gene therapy
  – If marking level > 1% of cells
Identification of Insertion Sites via LAM-PCR

- Linear PCR
- Magnetic capture
- 2d strand synthesis
- Endonuclease cutting
- Linker ligation
- PCR
- Nested PCR
- Gel-purification
- ShotgunTA-cloning
- Sequencing
- BLAT the rhesus or human genome

Schmidt et al, Blood, 2002
Alternative Methods

• Inverse PCR

• Spinkertette PCR

• Modifications not requiring restriction enzyme cutting

• Need for direct “deep sequencing” instead of shotgun cloning
How to Comply?

How to Discover Interesting Biology?

• DO NOT simply perform LAM-PCR, run it out on a gel and stop when see multiple bands
  – Must clone and validate the reliability of your assay in terms of bands being real inserts and not artifacts

• Low level marking results in lower insert/artifact ratio

• Validate inserts of interest via allele-specific PCR

• Validate approaches to quantitation
If Suspicious for Monoclonal Outgrowth

• May require multiple restriction enzymes to capture all relevant insertions

• Not the situation to utilize “deep sequencing”

• Save cells for RNA and protein analyses