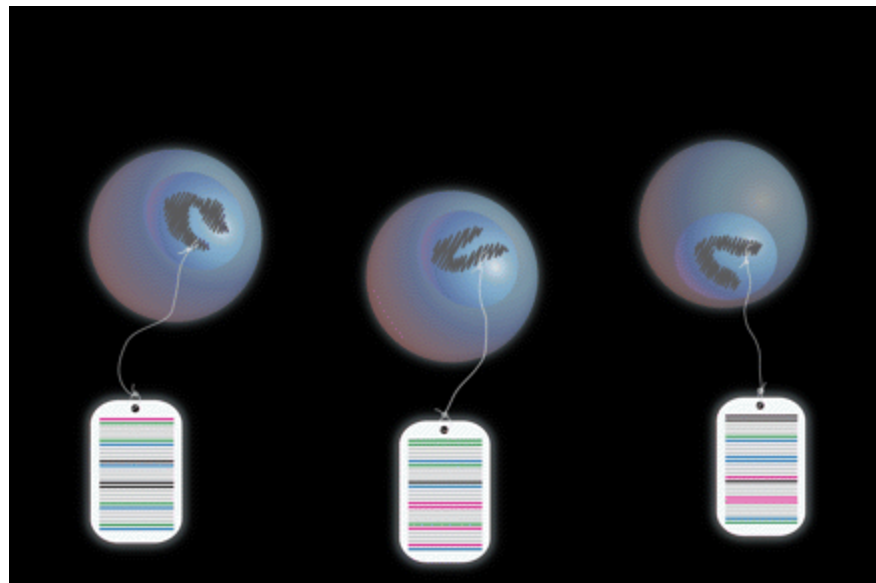


# Genotoxicity Considerations in Clinical Trials Utilizing Integrating Vectors

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*From Gerrits et al, Blood, 2010*



AMERICAN SOCIETY OF GENE & CELL THERAPY  
13<sup>TH</sup> ANNUAL MEETING | Washington, DC USA May 19-22, 2010

Cynthia Dunbar

**The following relationships exist related to this presentation:**

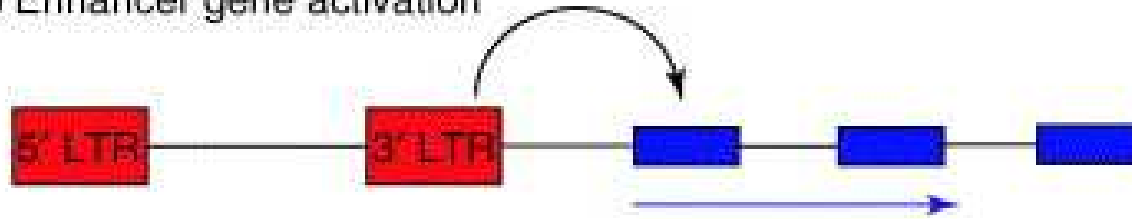
**No Relationships to Disclose**

# Where to Look for Advice

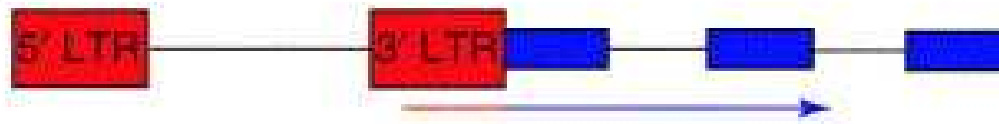
- FDA non-binding guidance document dated 2006, resulting in part from 2004 workshop at ASGT
  - <http://www.fda.gov/BiologicsBloodVaccines/Guidance/ComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/ucm072957.h>
- Evolving knowledge regarding vector integration site selection, vector enhancer activity, target cells, and host factors
- Common sense

# Integrating Vector Perturbations

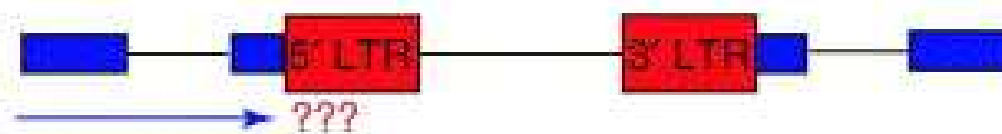
(a) Enhancer gene activation



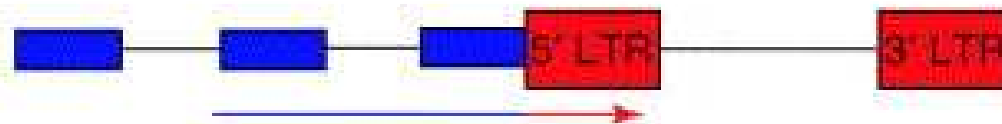
(b) Promoter fusion



(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

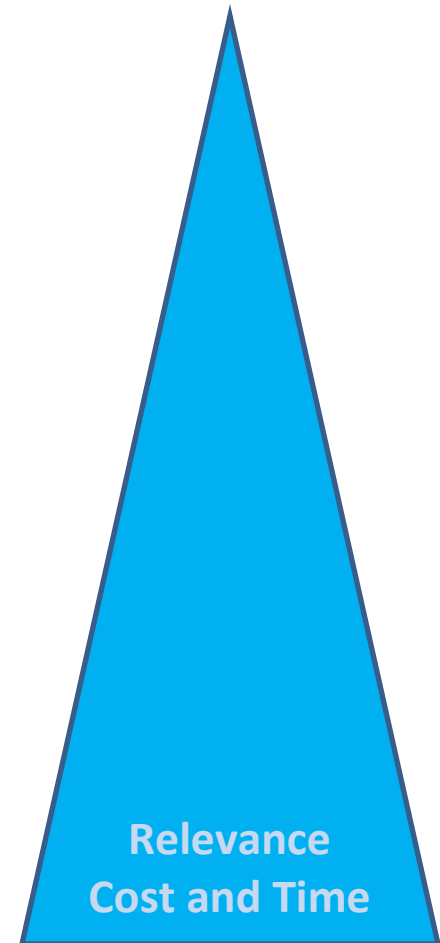
– Newrzela et al, *Blood* 2008

# 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

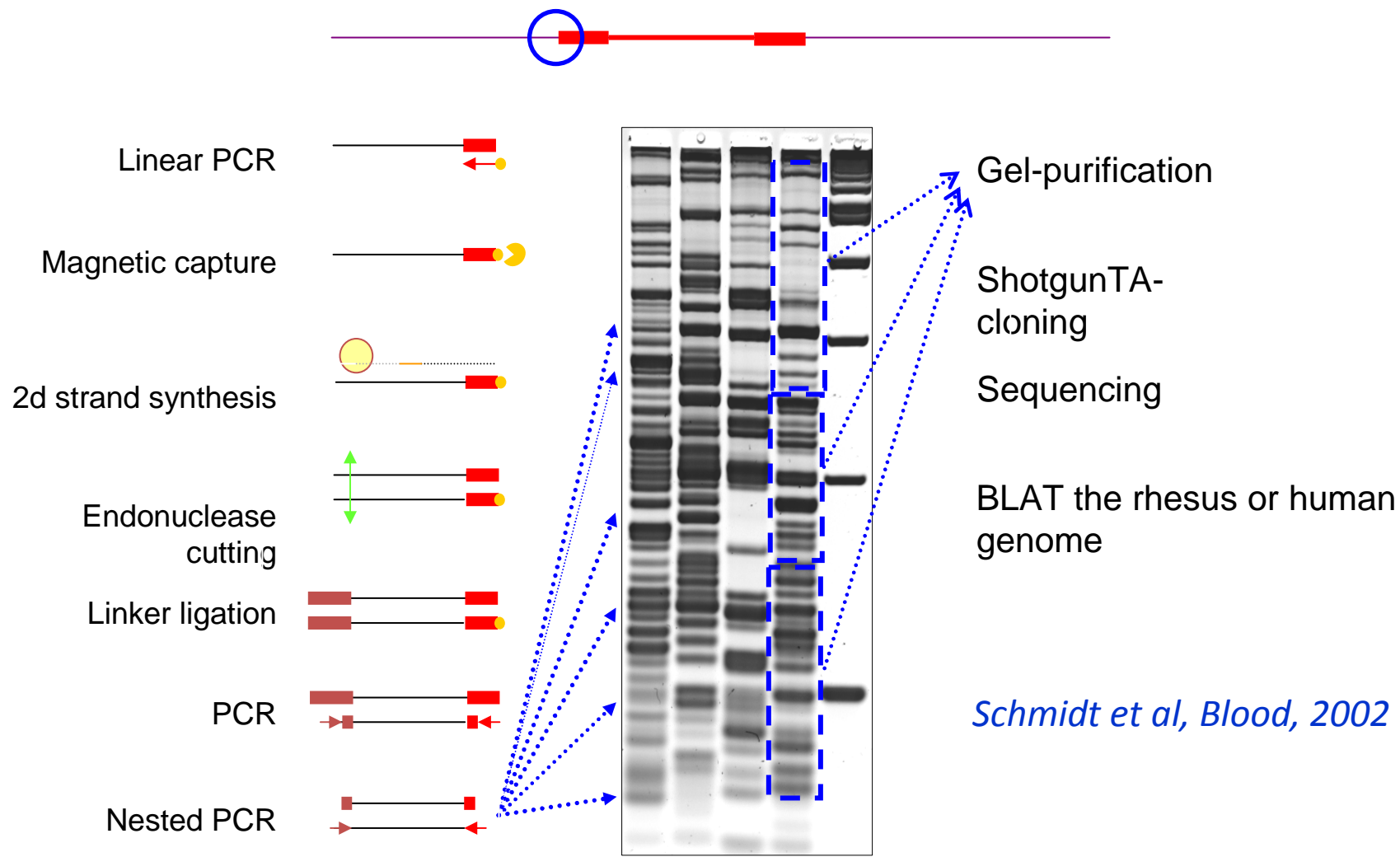
# Type of Samples to be Collected

- 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



# Alternative Methods

- Inverse PCR
- Spinkurette 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