Monitoring for Insertional Oncogenesis and Clonality of Hematopoiesis after Gene Therapy

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Monitoring for Insertional Oncogenesis and Clonality of Hematopoiesis after Gene Therapy

- Theoretical risk of insertional oncogenesis due to:

  RCR

  Random or pseudo-random insertional mutagenesis

- Insertional mutagenesis could activate proto-oncogenes, inactivate tumor suppressor genes, or activate genes that could lead to cellular dysregulation, e.g, generation of an autocrine signaling loop.

- One subject (Subject #4) of ten successfully treated in Paris with retroviral gene therapy for X-SCID has developed a malignant proliferation of T lymphoid cells, in which the retroviral vector appears to have transactivated the LMO2 proto-oncogene.

Nosology of the SAE

- Immunophenotype dissimilar to typical pediatric T-ALL, which are not usually TCR-γ/δ leukemias.

- Gene activation by retroviral insertion is not a common etiology of human leukemias.

- Therefore, phrases other than “leukemia” have been used to describe SAE, e.g., “leukemoid”, “leukemia-like”, “lymphoproliferation”.

Characteristics of Leukemia

- √ Clonal proliferation of lymphohematopoietic cells
- √ Genetic alteration(s)
- √ Malignant
  - √ Population growth
  - √ Impairment of normal hematopoiesis
  - √ Infiltration of peripheral organs
- √ Genomic instability

Goals of Monitoring Protocol

1) provide adequate monitoring so that determination of the need for therapeutic intervention is made as expeditiously as possible;

2) assure that subjects are not exposed to either physical or psychological harm from unnecessary interventions;

3) characterize any leukemias occurring in gene therapy trials;

4) determine whether the leukemia resulted from insertional mutagenesis;

5) characterize the clonality of hematopoiesis after retroviral gene therapy of HSC.

Features of Monitoring Protocol

1) Routine prospective monitoring of clonality of integrants in lymphohematopoietic cells by LAM-PCR.

2) Routine monitoring for clinical signs of abnormally growing populations of cells.

3) Detailed characterization of peripheral and/or marrow populations if indicated by increasing predominance of retroviral integrant; evidence of abnormal hematopoiesis (clinical or laboratory).

4) Decision to intervene based on clinical and laboratory evidence of leukemia.

5) Lifetime monitoring.

Monitoring of Clonality

1) LAM-PCR Q 6months for vector integrants.

2) Increasing oligoclonality or monoclonality: 2-fold increase over two successive timepoints to >20% of total transduced cells.

3) If increasing oligoclonality or monoclonality: initiate clinical evaluation, including blood counts, chemistries, evaluation for lymphoproliferation.

4) If no evidence of leukemia, then repeat LAM-PCR Q 3months for two years until evidence of stabilization or leukemia.

5) Lifetime routine monitoring.

Techniques for Characterization of Increasingly Prevalent or Abnormal Clones

1) Sequencing of LAM-PCR product to determine site of integration.

2) If abnormal hematopoietic population, then analyze immunophenotype to assign lineage.

3) If lymphoid cells, then appropriate immunoreceptor gene analyses to determine and characterize clonality, e.g., BCR or TCR.

4) Cytogenetic analyses.

5) Characterization of peripheral invasion and interference with hematopoiesis, i.e., marrow sample as indicated.

Diagnostic Questions re Increasingly Prevalent or Abnormal Clones

Is clone malignant?

Clinical behavior

- Population growth
- Impairment of normal hematopoiesis
- Infiltration of peripheral organs

Molecular and cellular abnormalities

- Cytogenetic abnormality
- Inference from integration site
- Abnormal differentiation

Diagnostic Decisions re Increasingly Prevalent or Abnormal Clones

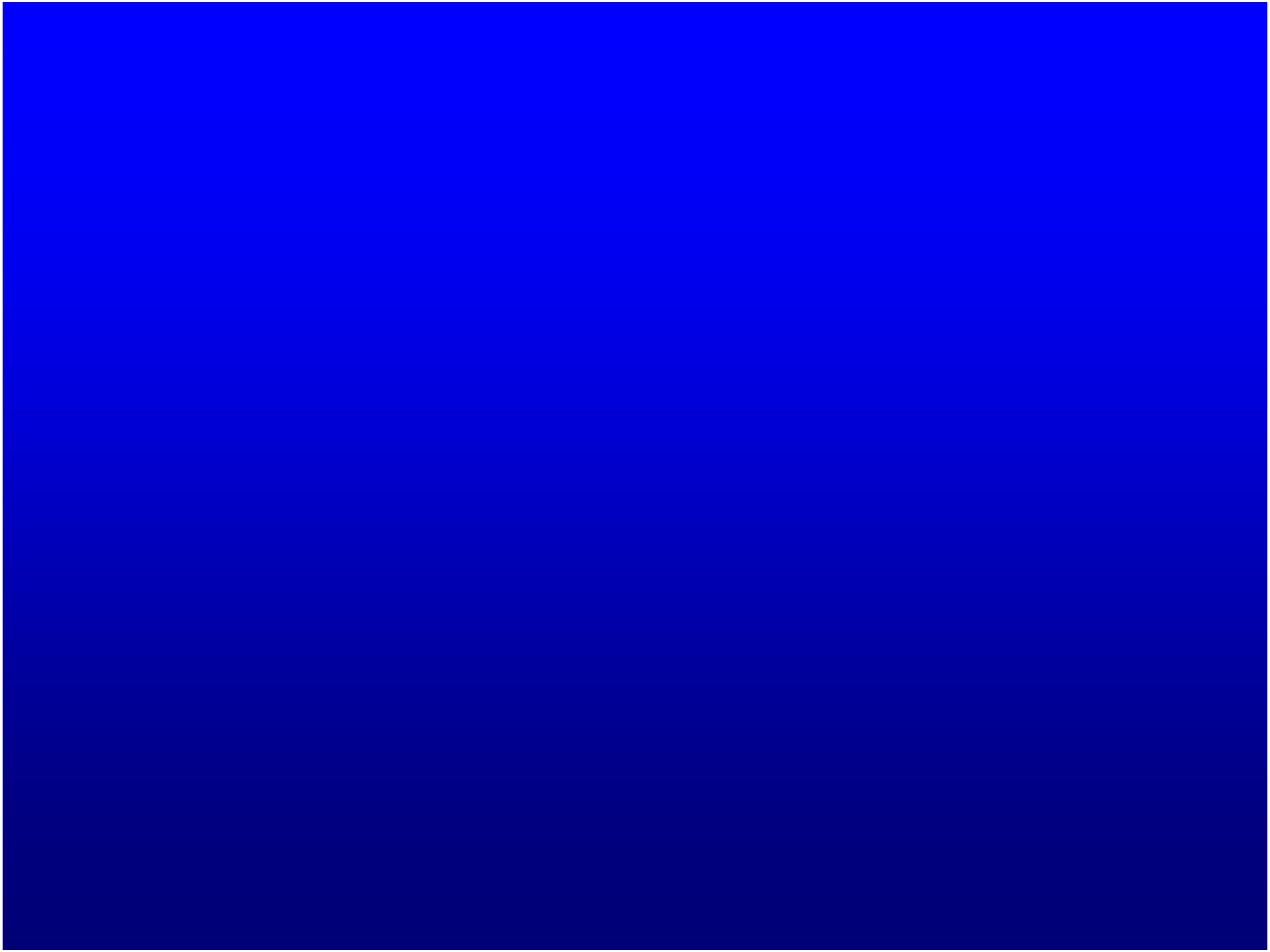
Is clone differentiating normally?

Clinical behavior

- Population growth stable
- Normal hematopoiesis
- No evidence of block in differentiation

Molecular and cellular abnormalities

- Integrant present in cells of multiple lineages
- Diversity of TCR, BCR rearrangements



Insertional Oncogenesis and Clonality of Hematopoiesis after Gene Therapy - IC Issues

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Considerations in IC Discussion of Risk of Leukemia

Context

- **The subjects of the SCID gene therapy protocols are at risk for a number of potentially fatal complications of their disease or its treatment.**
- **Frank discussion of these risks is part of the consent process for both gene therapy and non-gene therapy alternatives.**
- **For example, discussion of a complex malignant disease, EBV-lymphoproliferative disease (EBV-LPD), is a standard part of the IC process for haploidentical BMT.**
- **Discussion of the risk of leukemia in SCID gene therapy protocols is not substantially different from other subjects that are addressed during discussions between families and investigators regarding therapeutic options.**

Considerations in IC Discussion of Risk of Leukemia

Terminology

- **The SAE in Subject 4 is a *leukemia*, albeit one that has a mechanism that reflects complex interactions of novel iatrogenic as well as genetic and environmental causes.**
- **Besides being scientifically accurate, “leukemia” is a term understood by general public as a serious, potentially fatal, cancer of the blood system.**

Considerations in IC Discussion of Risk of Leukemia

Risk level (Incidence)

- **Discussion of incidence of leukemia would be desirable as means of presenting likely risk.**
- **However,**

  1. The actual incidence of leukemia in gene therapy studies is unknown.

  2. It is not known what the relevant denominator for such a statement would be: the number of subjects in HSC gene therapy trials, the number in SCID trials, the number in X-SCID trials?

- **Quantitative statement re risk would not be based on fact and could be coercive or misleading.**

Considerations in IC Discussion of Risk of Leukemia

Causality

- The evidence indicates a causal role for activation of the LMO2 gene in inducing leukemia.
- There may be other factors that may be contributing to the development of leukemia in the subject, e.g, genetic susceptibility, varicella infection.
- The statement that leukemia could occur as a result of the treatment is correct.

Considerations in IC Discussion of Risk of Leukemia

Explanation of mechanism(s)

- **The parents of the children who are eligible for the gene therapy trials are generally sophisticated in their knowledge of medicine, particularly as it pertains to their child's disease.**
- **Discussion of RCR-related malignancy has already been part of the IC discussion.**
- **The discussion of the risk of leukemia needs to provide information on how gene therapy could cause leukemia.**