Replication competent lentivirus (RCL) and replication competent retrovirus (RCR) testing of drug product

Kenneth Cornetta MD
Indiana University
Outline

• Risk from Exposure
• Risk of Exposure
• Clinical findings of RCR/RCL testing
• Interpretation of data
• Comments on Draft Guidance
Retroviruses and cancer

• Majority of retroviral vectors are derived from murine gamma retroviruses which are oncogenic

• These viruses do not carry oncogenes but cause dysregulation of cellular gene(s)
  • Dysregulation involves the LTR enhancer in many cases
  • The enhancer is important is vector transgene expression so is retain in many vectors
  • The Moloney murine leukemia virus is associated with murine lymphoma
Risks from RCR Exposure

• RCR from MoMLV-based vectors:
  • Cause lymphomas in neonatal mice
  • Cause lymphoma in immuno-deficient non-human primates

• Replication defective retroviral vectors can cause malignancy in a manner similar to that of RCR

• Therefore, inadvertent exposure to RCR is predicted to greatly increase the risk of treatment-related malignancy
Lentiviral Risk is different

• While insertion of lentiviral vectors can lead to growth dysregulation, the risk appears to be significantly lower and through a different mechanism

• HIV-1 is not oncogenic

• Lentiviral vectors are stripped of accessory regions important in HIV-1 growth and pathogenesis

• The lack of accessory genes in HIV-1 suggests any RCL would have a low risk of immunodeficiency
Risk of Exposure

• First clinical use of retroviral gene transfer conducted in 1989
• Retroviral vectors (RV) generally designed to be replication defective
• Many of the early packaging cell lines developed replication competent retrovirus (RCR) through recombination:
  • between homologous regions of vector and packaging sequences
  • endogenous retrovirus sequences in the packaging cells
• Redesign to decrease homology decreases the chance of recombination
• Replication competent lentivirus (RCL) from current packaging systems has not been detected and remains theoretical.
indicated by the standard assay but cannot be compared relative titer. Helper virus was
the S^+L^- assay as previously described

RESULTS

cell line expressing MoMLV gag and pol
FLV gag-pol expression construct de-
.. introduced into NIH 3T3 TK^- cells by
g the herpes simplex virus thymidine
selectable marker, as previously described
selectable marker plasmid to gag-pol
was 1:20 or 1:100. Seventeen TK^-

would not be restricted by the presence of the same
already in the cells. Clone designations all begin w
(packaging cells having a GαLV pseudotype), follow
number from 1 to 49 for clones transfected with d
from 50 to 99 for clones transfected with hpt.

About 20 clones made with each marker were tes
testing for packaging function by measuring transient virus pro-
2 days after transfection with the retrovirus vector 1
pLN (Fig. 1) as described previously (19). Virus was h
by using HeLa cells as targets for infection. The LN

TABLE 1. Reverse transcriptase production by cells tran


Advancing knowledge, awareness, and education of gene and cell therapy
## Manufacturing and Risk of RCR/RCL

<table>
<thead>
<tr>
<th><strong>Producer Cell</strong></th>
<th><strong>Transient</strong></th>
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<tbody>
<tr>
<td>Retroviral producer cell lines typically contain all the components needed to generate RCR. May or may not have all components for generating replicating virus.</td>
<td>Production occurs over a few days, minimizing risk of recombination.</td>
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<tr>
<td>Producer cell lines often cloned from single cells, to Master Cell Bank, then expanded to high density for production. Protracted expansion increases risk of recombination.</td>
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<td>Some producer cells suppress gene expression until harvest, may decrease risk.</td>
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<tr>
<td>If vector pseudotype is unable to infect producer cell, risk for RCR may be decreased.</td>
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Guidance for Industry

Supplemental Guidance on Testing for Replication Competent Retrovirus in Retroviral Vector Based Gene Therapy Products and During Follow-up of Patients in Clinical Trials Using Retroviral Vectors

This guidance is for immediate implementation.

FDA is issuing this guidance for immediate implementation in accordance with 21 CFR 10.115(g)(4)(i). Submit written comments on this guidance at any time to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. Additional copies of this guidance are available from the Office of Communication, Training and Manufacturers Assistance (HFM-40), 1401 Rockville Pike, Suite 200N, Rockville, MD 20852. Call the toll-free number 1-800-827-1800, or from the Internet at http://www.fda.gov/cber/guidelines.htm.

For questions on the content of this guidance, contact the Office of Cellular, Tissue, and Gene Therapies at 301-827-5102.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
November 2006

Monitoring Burden for Integrating Vectors

Vector Product
- Replication Competent Viruses Testing
  - Biologic Assays

Cell Product
- Replication Competent Viruses if cultured for > 4 days
  - Biologic Assays

Patients
- Monitor multiple timepoint, continue if vector persists
  - Serology or Molecular Assays (qPCR)
Detecting RCR/RCL in Vector Products

- Vector and RCR/RCL particles generally share structural proteins – no specific protein-based assay for RCR/RCL in vector products
- Vector products (and particles) contain cellular and/or plasmid DNA
  - Molecular assays often false positive in vector products
- Biologic assays significantly more sensitive than molecular assays
Replication Competent Virus Testing - Biologic Assay Example

Amplification Phase (3 weeks)

- RCR - Mus dunni (Ampho) or HEK293 (GALV, RD114)
- RCL - C8166

Indicator Assay

- RCR - S+/L-
- RCL - p24, PERT

Vector
- 5% of vector product
- 1% of cells up to $10^8$ EOP cells

Cell Products
- If cells cultured 4 or more days
- 1% of cells up to $10^8$ EOP cells
Lessons learned - vector products

• Retroviral vectors
  • Indiana University experience - over 30 vector products generated in PG13 cell line RCR free
  • Baylor, Memorial Sloan Kettering and NIH all have similar experience

• Lentiviral vectors
  • In 2011, Cornetta et al. reported no RCL detected in
    • 16 Vector Products, 17 EOP Cells, 7 cell lines
    • Material from 6 different GMP facilities (20% IU)
    • $1.3 \times 10^{14}$ virions, $1.8 \times 10^9$ cells

Currently, no report of RCL detected in clinical lots
Screening Clinical Cell Products for Replication Competent Retrovirus: The National Gene Vector Biorepository Experience


- **Transduced cell products (n=282) screened for RCR from 14 clinical trials, all negative for RCR.**

- **241 of the clinical trial participants were also screened for RCR by analyzing peripheral blood at least 1 month after infusion, all of which were also negative for RCR**

Molecular Therapy Methods and Clinical Development (2018)
[https://doi.org/10.1016/j.omtm.2018.08.006](https://doi.org/10.1016/j.omtm.2018.08.006)

- **An additional 95 cell products were negative at NGVB**
Screening Clinical Cell Products for Replication Competent Retrovirus: Baylor College of Medicine Experience

- Transduced cell products (n=266) screened for RCR from 17 clinical trials, all negative for RCR.
- 220 clinical trial participants received these products (some received more than 1 product) were also screened for RCR by analyzing peripheral blood at least 1 month after infusion, all of which were also negative for RCR.

Courtesy of Dr. Helen Heslop
Absence of Replication Competent Lentivirus in the Clinic: Analysis of Infused T Cell Products.

  - T cell products screened from 26 clinical trials, 6 centers
  - 460 cell products from 375 patients
  - 275 patients subsequently screened by qPCR for VSV-G env at least one month post T cell infusion
  - All negative

Molecular Therapy 26: 280-288, 2018
Retroviral and Lentiviral Safety Analysis of Gene-Modified T Cell Products and Infused HIV and Oncology Patients


- 17 vector lots, 375 manufactured, T cell products, and 308 patients post-infusion across both HIV and oncology indications, showing no evidence of RCR/L

- Poisson probability model estimates that a single patient, or a group of patients, would need to be followed for at least 52.8 years to observe one positive RCR/L event

Molecular Therapy 26: 269-279, 2018
Testing Ex Vivo Products

• Little scientific rationale or data indicating it protects patients from RCR/RCL exposure
• Biologic assays the gold standard but are cumbersome and expensive
• Alternatives methods may not detect RCR/RCL or lead to false positives
No evidence of RCR/RCL post-infusion of cell products

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<thead>
<tr>
<th></th>
<th>GALV</th>
<th>VSVG</th>
<th>RD114</th>
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<tbody>
<tr>
<td>NGVB</td>
<td>1735</td>
<td>461</td>
<td>10</td>
</tr>
<tr>
<td>Non-NGVB</td>
<td>717</td>
<td>764</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>2452</td>
<td>1225</td>
<td>10</td>
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Multiplex qPCR analysis
LOD 10 copies in 0.2 ug DNA
run in triplicate
Preliminary Timepoint Analysis (NGVB Samples)

- **GALV**
  - 64 studies
  - Baylor
  - NIH
  - MSKCC
  - UCLA
  - Harvard
  - Stanford
  - Fred Hutch
  - Roger Williams
  - Cinci Child

- **VSVG**
  - 12 studies
  - UCLA
  - Grand Ormond
  - Harvard
  - NIH
  - MSKCC
  - Fred Hutch

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Summary

• In the commonly used production systems, RCR and RCL has not been reported.

Vector Product
Cell Product
Patients

Risk of Exposure is Low
Interpretation of Data

• The risk from and of an RCR exposure is predicted to be higher than RCL

• For well-characterized systems, testing of transduced cell products does not add value and should no longer be a requirement.

• Recommend RCL testing post-infusion only in case of adverse event
The guidance does provide for limiting RCR and RCL testing.

- Agree with different levels of testing for novel versus established vector systems
- Current specifics vague

Testing for products cultured less than 4 days is not supported by scientific rationale

Does not differentiate vector systems based on risk of virus development or risk of exposure

Guidelines for RCR/RCL testing assays (appendices) are problematic in terms of implementation
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