Testing CAR-T Cell Products

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CAR-T Vector Production

CAR T cells are genetically modified, often using viral vectors, these require extensive testing in their own right
In-Process Testing

• To evaluate changes to manufacturing procedure
• Test at critical control points
  • Where there are Go/No Go criteria
  • Where there may be risk to cells
• To validate final manufacturing process

Release Testing Assays

• Microbiology (Sterility)
• Mycoplasma
• Identity
• Purity
• Viability
• Potency
• Dose

Guidance for FDA Reviewers and Sponsors
Release Testing - Microbiology

- 21 CFR Part 610.12 - approved method
- Rapid methods e.g. Bactec/BacT ALERT – more readily approved +/- validation
- 14 day incubation
- Often extended for fungal cultures
- Gram stain additional for fresh products

Release Testing - Mycoplasma

- FDA approved Culture assay – USP >63 need not be validated if followed directly
- PCR – Roche MycoTool Mycoplasma Detection Kit, Approved 2012
- Rapid Test – MycoAlert (Validation)
Release Testing – Identity/Purity

• Flow Cytometry
  • T cell markers
  • CAR Expression
  • Contaminating cells

Release Testing – Purity

• Removal of manufacturing reagents
  • Animal sera
  • Antibiotics
  • DMSO etc.

• Calculate residual reagent by dilution factor
• Perform assay for residual reagent
Release Testing – Viability

• Manual: e.g. Trypan Blue exclusion
• Automated: Flow cytometry – e.g. 7AAD staining +/- apoptosis markers
  • Pre-freeze
  • Post-thaw?
• Used also for stability testing

Potency - Choice of Assays

No single test adequately predicts clinical efficacy

• Provide “substantial evidence” that the product will have the effect it purports to have under the conditions of use prescribed
• Obtained from “adequate and well-controlled investigations conducted with a consistently manufactured product
Release Testing – Potency

• Not “formally” required before Phase 3 trial
• Should correlate with in vivo activity
• Use Phase 1 & 2 to refine and validate the potency assay

Why Assay Potency in Phase 1?

• To show activity, quality & consistency during development
• Generate data to define specifications for lot release
• To assess effects of manufacturing changes
• To evaluate product stability
• To detect problems
• Evaluate a variety of assays and their correlation
Release Testing - Potency

Potency for CAR-T

• Cytotoxicity against cells line bearing the specific antigen e.g. $^{51}$Cr release

• ELISpot assay

• Proliferation assay

An Innovative in Vitro Potency Assay Designed to Predict the Fate of Chimeric Antigen Receptors Modified T Cell Therapy Post Infusion in ALL

Junxia Wang, Mark Dudley, Therese Choquette, Margit Jeschke, Erik Rutjens and Sadik Kassim

Blood 2016 128:5830;

CAR T Cell - Potency Assays

<table>
<thead>
<tr>
<th>CD19 CAR-T cell therapies in the clinic.</th>
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<td><strong>Center</strong></td>
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<tr>
<td>Kite ZUMA-1</td>
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<td>Novartis CTL019</td>
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</tbody>
</table>

4 Cytotoxicity: 75% Responders
6 IFN-γ: 53% Responders

Kassim S. H., BioInsights 2017
Future Testing Methods

From meeting of Cell Manufacturing Technologies Consortium at Georgia Institute of Technology, November 2018

Release Testing – Potency

Guidance for Industry

Potency Tests for Cellular and Gene Therapy Products

Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD), HFM-40, 1401 Rockville Pike, Suite 200N, Rockville, MD 20852-1444, or by calling 1-800-835-4709 or 301-827-1800, or email ocod@fda.hhs.gov, or from the Internet at http://www.fda.gov/BiologicsBloodVaccines/ComplianceEnforcement/RegulatoryInformation/Guida
recommendation.htm.

For questions on the content of this guidance, contact OCOD at the phone numbers listed above.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
January 2011
Release Testing – Additional

• Replication-competent virus
  • Send for Testing or Obtain results before release

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.

NIH is issuing this guidance for immediate implementation in accordance with D. CFR 101.369(c)(6). Submission comments on this guidance in the Federal Register by the Division of Student Health and Human Services, 4012 N. 18th St., Denver, CO 80205. You should submit comments to the title of this guidance.

Additional copies and information about the Office of Communication, Testing and Mandate Human (http://www.cdc.gov) and 462-2801-0019 or email at cdcinfo@cdc.gov. For inquiries on the content of this guidance, contact the Office of Cellular, Tissue, and Gene Therapy at NCI 4151-3340.

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

Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication Competent Retrovirus During Product Manufacture and Patient Follow-up

Draft Guidance for Industry

This guidance document is for current purposes only.

Figures are available on the website of the National Institutes of Health (NIH) and the FDA. Copies of the guidance are available at http://www.fda.gov/cber/guidance.

For questions and comments on the content of this guidance, contact CDC at 800-315-4657 or email at ccdirectors@cdc.gov.

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

Release Testing – Additional

• Replication-competent virus – expensive to establish, validate & perform

Replication-competent virus testing – RCR / RCL

RCR/RCL testing

- Ideally most sensitive assay employed
- Cell-based assay:
  - Amplification in supernatant-inoculated cells for at least 21 days (USFDA)
  - End-point virus detection in naïve cells (marker, p24, PERT, VSV-G protein)
  - Length of procedure
- Direct PCR-based assay
  - Less sensitive, quicker.


Paul-Ehrlich-Institut
Certificate of Analysis

- Used for product release
- Details
  - Tests performed & assay used
  - Assay sensitivity
  - Lab. performing assay
  - Assay criteria for release
  - Results obtained
- Reviewed and signed by Quality Unit representative

Conclusions

- Release assays for CAR-T cells follow the usual criteria for other cellular therapy products
  - Microbiology (Sterility)
  - Mycoplasma
  - Identity
  - Purity
  - Viability
  - Potency – most problematic
  - Dose
- Addition of replication-competence assay
Thank you