Academic Cell and Gene Therapy Development and Manufacturing

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T-cell Manufacturing Process circa 2000

Washed Apheresis Product

T cell Activation
OKT-3 + IL-2

14 Days
2-5 Cycles
14 Days each

1 x 10^8 MNC (Feeders)
20 x 10^6 LCL
30 ng/ml OKT-3
37.5 IU IL-2
200 ml Media
200 µg/ml Hygromycin

200 µg/ml Linearized CD19R DNA

Bulk Population
Of Exhausted T-Cells
40-80% CAR+

Cryopreserved Product Intermediate

T-Cell Expansion/Selection

Stanford Medicine
Fast forward 2016 - Bi-Specific CAR-T cells

• Process Challenges
  • Closed system processing of Apheresis products
  • CD4/CD8 T-cell enrichment required
  • Lentiviral Transduction in closed system
  • Bioreactor culture and harvest in closed system

• Analytical Challenges
  • Demonstrating simultaneous expression of both CARs
  • Deciding on in process metrics
  • Rapid reliable assay for RCL detection
Closed and (Semi) Automated Blood Cell Culture Systems

7 Day Process on Prodigy
- Apheresis Washing
- Magnetic Bead Labelling
- Target Cell Enrichment
- Cell Growth Formulation
- Cell Stimulation
- Viral Transduction
- Cell Expansion
- Cell Harvest
- Release Testing

CAR T-cells
Phenotypic Analysis of CD19/CD22 bi-Specific CAR-T

Anti-CD19 idotypic antibody to CD19 CAR (Laurence Cooper)

rhSiglec 2/Fc Chimera (CD22 on Ig stalk – R&D Systems)
CAR-T Production Metrics

**ViaB-Hls**

- % Viable Cells
- Percent
- % Viable Cells

**Cell Counts Transduction**

- % CAR+ (of Total)
- Average copies/cell
Production summary

• Pros
  • Reproducible production of CAR-T cells
  • All cells passed all release testing
  • Semi-Closed system for production
    • Still open harvest/formulation steps performed in BSC

• Cons
  • Sole source provider for all reagents/tubing sets
  • Multiple device failures (valves, motors, tubing sets)
  • Expensive incubator for 6/7 days
    • Limits productivity in GMP suite
qPCR for detection of RCL

- Using VSV-G as a target
  - All lentiviral constructs contain VSV-G envelop sequences
  - Env is on a helper plasmid and should not be contained in transduced T-cells
  - RCL could be formed in VSV-G env is transferred to T-cells

- Determine Quality of the assay (MIQE)
  - Carefully quantified plasmid DNA as Control
  - Linearity, slope are as predicted by dilutions
  - Specificity and Limit of detection is established
    - Measure frequency of false positive and false negative

Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE)
Linearity and slope of qPCR of VSV-G Plasmid

N=7 independent experiments

A  pDNA diluted in water

B  pDNA Diluted in gDNA

y = -3.2846x + 38.975
R² = 0.997

y = -3.2703x + 38.733
R² = 0.992

Water diluent
DNA diluent

p = 0.0041
Inclusion of 3% DMSO Improves Detection of Low Copy Number DNA

Limit of Detection in DNA with 3% DMSO

<table>
<thead>
<tr>
<th>Copies/reaction</th>
<th>Water Diluent</th>
<th>DNA diluent</th>
<th>DNA diluent + 3% DMSO</th>
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</thead>
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<tr>
<td>10 copies</td>
<td>100.00%</td>
<td>83.33%</td>
<td>97.92%</td>
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<tr>
<td>8 copies</td>
<td>83.33%</td>
<td>75.00%</td>
<td>83.33%</td>
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<td>6 copies</td>
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<td>66.67%</td>
<td>79.17%</td>
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<td>5 copies</td>
<td>76.19%</td>
<td>61.90%</td>
<td>68.75%</td>
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<td>4 copies</td>
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<td>58.33%</td>
<td>68.75%</td>
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<td>3 copies</td>
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<td>33.33%</td>
<td>52.08%</td>
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<tr>
<td>NTC</td>
<td>0.00%</td>
<td>0.00%</td>
<td>2.08%</td>
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N = 16 independent experiments in triplicate
Pre-Clinical Qualification Runs and First 7 patient samples

A

Non-transduced Control
Transduced CAR T Cells
10 copy positive control

B

Sample DNA
Sample DNA + 10 copies VSV-G
10 copy positive control
Analytical Summary

• Pros
  • Simultaneous Detection of expression of both CAR proteins
  • Reproducible, sensitive RCL assay
    – Reliable detection of 10 copies of VSV-G
    – False Negative rate <3%
    – False Positive rate ~2%
    – Low Inter-operator variability
  • All testing completed and reviewed on day of product harvest (12 hours)

• Cons
  • Requires clean, dedicated space for assays
  • Requires carefully quantified source of control DNA
  • Approximately 1/40 products may be falsely considered positive for RCL
  • Approximately 1/33 products may miss 10 copies of RCL
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Thank You for Your Attention!