Session II
Review of Strategies to Promote Persistence of T cells

GENE DELIVERY CONSIDERATIONS
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Session begins 2:30 PM
Gene Delivery Systems for CAR-Based T-cell Immunotherapy: An overview of what’s being deployed in the clinic

Retroviral/Lentiviral

Transposase/Transposon
# CAR CD19-specific Trials

<table>
<thead>
<tr>
<th>Institute</th>
<th>Clinical trial.gov identifier</th>
<th>Vector for T-cell modification</th>
<th>Env</th>
<th>CAR Design</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCM</td>
<td>NCT00586391, NCT00840853, NCT00709033</td>
<td>Gammaretrovirus (MoMLV)</td>
<td>GALV (PG13)</td>
<td>FMC63 CD19scFv-IgG1 Fc- CD28-CD3z</td>
<td>Savoldo et al., JCI 2011</td>
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<tr>
<td>FHCRC</td>
<td>NCT01475058</td>
<td>Lentivirus (SIN-hEf1a)</td>
<td>VSV.G</td>
<td>FMC63 CD19scFv-IgG4 Fc- CD28-CD3z-T2A-huEGFRt</td>
<td>Wang et al., Blood, 2011</td>
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<tr>
<td>MDACC</td>
<td>NCT00968760, NCT01497184, NCT01362452</td>
<td>Plasmid (SB hEf1a Transposon/Transposase)</td>
<td>N/A</td>
<td>FMC63-CD19scFv-IgG4 Fc-CD28-CD3z</td>
<td>Singh et al., PLOS One, 2013</td>
</tr>
<tr>
<td>MSKCC</td>
<td>NCT01430390, NCT00466531, NCT01416974, NCT01044069</td>
<td>Gammaretrovirus (MoMLV)</td>
<td>GALV (PG13)</td>
<td>SJ25C1 CD19scFv-CD8a-CD28-CD3z</td>
<td>Brentjens et al., CCR, 2007</td>
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<td>NCI</td>
<td>NCT00924326, NCT01087294, NCT01593696</td>
<td>Gammaretrovirus (MSCV)</td>
<td>RD114 (293-GP)</td>
<td>FMC63 CD19scFv-CD28(114-153)-CD28-CD3z</td>
<td>Kochenderfer et al., 2009 J of Immunotherapy</td>
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<tr>
<td>UPENN</td>
<td>NCT01029366, NCT01626495</td>
<td>Lentivirus (SIN-hEf1a)</td>
<td>VSV.G?</td>
<td>FMC63 CD19 scFv-CD8a-CD8aTM-4-1BB-CD3z</td>
<td>Porter et al., 2011, NEJM</td>
</tr>
</tbody>
</table>

MoMLV - Maloney murine leukemia virus  
MSCV - Mouse stem cell virus  
huEGFRt - human EGFR truncated  
GALV - Gibbon Ape Leukemia Virus  
VSV.G = Vesicular Stomatitis Virus glycoprotein  
RD114 - Endogenous Feline Virus glycoprotein  
Retroviral and lentiviral constructs are flanked by Long Terminal Repeats (LTRs). Transposon constructs are flanked by IR/DR-type inverted repeats.
General Comparison of viral versus transposon based gene delivery systems

Retroviral/Lentiviral

**Advantages**
- Efficient gene delivery
- High expression of transgene
- Well characterized systems
- Flexibility of pseudotyping env genes of choice to maximize transduction efficiency

**Disadvantages**
- Potentially hazardous – need to demonstrate lack of replication competent virions in each prep
- Requirement for packaging cell line (labor intensive)
- Expense of production/release of clinical-grade material
- Concern over integration at sites of active transcription
- May activate oncogenes
- Requires T-cell activation for transduction (including lentivirus)

Sleeping Beauty

**Transposase/Transposon**

**Advantages**
- More efficient than “naked” DNA
- High level of expression
- Plasmid DNA is not cost prohibitive and not hazardous to produce (allows for testing of multiple constructs)
- Does not appear to integrate at sites of active transcription (not shown to activate oncogenes)
- Can electroporate whole resting PBMC and then numerically expand T cells

**Disadvantages**
- Low to mid/variable transfection efficiency
- Ensure that the transposase does not integrate (separate non-integrating expression plasmid is used or in vitro-transcribed mRNA) to prevent introduced transposon from “jumping”
Retroviral and Lentiviral Gene Delivery

Viruses offer the flexibility of pseudotyping with multiple env proteins but are encoded by infectious genomes.

**Lentivirus**

It is required to separate the gag/pol, env and LTR flanked transgene into separate plasmids for transfection to limit the chance of the generation of replication competent virions. Must test for p35 expression as from HIV in cell after transduction. (Larger cargo load).

**Retrovirus**

Engineering of permanent viral producing cells (e.g. PG13 co-expressing gag/pol/GALV cells) is acceptable.

<table>
<thead>
<tr>
<th>Envelope Protein</th>
<th>Receptor</th>
<th>Cell type transduced</th>
<th>Toxicity</th>
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<tbody>
<tr>
<td>GALV</td>
<td>sodium-dependent phosphate transporter protein (Pit-1)</td>
<td>Lymphocytes and hematopoietic cells</td>
<td>Low</td>
</tr>
<tr>
<td>RD114</td>
<td>neutral amino acid transporter SLC1A5</td>
<td>Stems cells and hematopoietic cells</td>
<td>Low</td>
</tr>
<tr>
<td>VSV.G</td>
<td>LDL receptor and its family members</td>
<td>Pantropic</td>
<td>High</td>
</tr>
</tbody>
</table>

**Note**

To express GALV or RD114 on lentivirus, a chimeric env protein consisting of the TM/cyt domains of the Amphotropic Murine Leukemia Virus env and the ectodomain of GALV or RD114 must be used (GALV/TR , RD114/TR)
Retroviral and Lentiviral Gene Delivery

- Requires the production of genomic-integrating replication-deficient virus
- Employs helper plasmids and packaging cells
- Production is complex as it needs manufacturing components for safety.
  - Lentiviral production is dependent on transient transfection of packaging cells.
  - Retroviral production can be based on “stable” packaging cell lines.
Retroviral and Lentiviral Transduction

Advantages
- High transduction efficiency and level of transgene expression

Considerations
- Gene silencing via LTR methylation: more prominent in MoMLV- than MSCV- (from MoMLV, but less prone to LTR methylation) based retroviruses
- Use of self inactivating (SIN) viruses with mutated 3’ LTRs and internal promoters offer flexibility of internal promoter choice (cellular compatibility and decreased promoter methylation)
- All lentiviruses used for gene therapy are SIN

Institutions using Lentivirus
- University of Pennsylvania (VSV.G)
- Fred Hutchinson Cancer Research Center (VSV.G)

Institutions using Retrovirus
- National Cancer Institute (MSCV; RD114)
- Memorial Sloan-Kettering Cancer Center (MoMLV; GALV)
- Baylor College of Medicine (MoMLV; GALV)
The *Sleeping Beauty* Transposon/Transposase System

**Encode CAR between IR/DR-type inverted repeats**

**Encode Transposase in standard expression vector**

Co-electroporate the transposon and transposase plasmids into PBMC or T cells

Each T cell prep is checked for integration of transposase via RT-PCR. None has been observed. Nevertheless, we are moving towards using mRNA

Transposon integrates at TA rich sites in the genome. T cells expressing CAR can be selected on artificial APC expressing CD19
Vector systems to express CAR transgenes used in clinical trials

Sleeping Beauty transposition

Transposon DNA Plasmid

CD19RCD28

Transposase

SB11

Transposase DNA Plasmid

T-cell genome
Potential Risk in using plasmid DNA as a source for SB11

SB11 from plasmid DNA

Potential risk of integration into host genome

Re-excision of integrated transgene, paste into another site (transgene “hopping”)

mRNA for Transposase
The Sleeping Beauty Transposon integrates into the T cell genome in a random fashion and has not been observed to activate oncogenes.

- **Human reference genome**
  - 3% Exons
  - 14% Introns
  - 50% Repeats

- **Gene Expressions**
  - 7,436,108 Raw Reads
  - 33 samples

- **Chromosomal Locations**
  - 100,000 Non-Redundant Loci

- **Chromatin States**
  - 95% AT rich region

- **Sleeping Beauty Transposition Sites**
  - 39% intragenic

- **Gene Names**
  - Sleeping Beauty

- **Non-obvious hotspots**
  - No insertion bias towards potentially dangerous loci (e.g. oncogenes [LMO2], tumor suppressors, miRNAs etc.).

- **No insertions in constitutively heterochromatic centromeric regions of chr 1, 9, 16**

- **No insertions in constitutively heterochromatic regions of acrocentric chr 13, 14, 15, 21, 22**

- **Conformationally open sites favored**

- **TSS associated with quiescent T cells favored**

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Thank You

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