CAR-T Clinical Trials: New Directions in Biomarkers

Matthew A. Spear, MD
Chief Medical Officer
What is the Future of CAR-T?

• Initial CAR-T outcomes have been revolutionary (e.g., high 3-5yr disease-free survival rates)
  – But, the field is nascent and there is extraordinary remaining potential
  – And, products are complex- in addition to the differences in constructs and methods, each product is a living drug liable to intra-product variability in patient (e.g., source T-cells, host environment and tumor) & manufacturing

• Advances in genetic engineering and manufacturing techniques provide markedly greater potential for rationale improvement than in classical drug development

• What is being done to advance the field?
  – Novel binding domains and multi-CARs
  – Vectors- viral and non-viral (transposons)
  – Safety switches
  – Selection
  – Editing (KOs)
  – Biomarkers- inform all above
    • For CAR-T cells
    • For patients (host and apheresis)
    • For disease
    • For in vivo activity
Biomarkers

• Predictive biomarkers are well established in oncology, particularly when a drug specifically targets an oncogenic driver

• CAR-T cells are a nascent and far more complex field

• Although CAR-T cells target tumor selective antigens, correlations between antigen levels and efficacy have been poor

• Each patient may not only have a unique tumor genotype / phenotype, but a unique drug (T cell) genotype / phenotype and host immunologic milieu

• Thus, benefit in assessing each tumor, CAR-T and host
T stem-cell memory / central memory (Tscm/Tcm)

**Tscm** May Be Key to Safe, Potent and Durable Responses

- “The extreme longevity, the robust proliferative potential and the capacity to reconstitute a wide-ranging diversity of the T cell compartment make the Tscm cell type an ideal cell population to employ in adoptive immunotherapy”

- Correlates with CAR-T clinical response
  - Melenhorst J. et al., UPenn (2017) 20th ASGCT
  - Basu et al., Adaptimmune (2017) CAR-TCR Summit
  - Tcm: Larson, Juno (2018) AACR
  - Tcm: Fraietta J. et al., UPenn (2018) TET2 Disruption, PMID: 29849141
PiggyBac® DNA Transposon System

PiggyBac® is a Superior DNA Delivery System for Developing CAR-T and Other Gene Therapy products

- **Unprecedented cargo capacity (>30X lentivirus)** – three-in-one transgene and possibility of multiple CAR or TCR molecules
- **Non-viral delivery system** – non-oncogenic and non-mutagenic
- **High insertion efficiency** and stable transgene expression
- **Faster** to clinic with **lower cost** than viral methods
- **Substantial IP portfolio** with no dominant or competing IP
- Creates products with highly desirable **T Stem Cell Memory (Tscm) Phenotype**

![Diagram of PiggyBac® Transposon System]
piggyBac® Preferentially Transposed *Early T<sub>SCM</sub> Cells*; Lentivirus Transduced *More Differentiated T-Cells* In Preclinical Studies

We purified donor cells to these T-cell subsets and then performed optimized piggyBac or optimized lentivirus manufacturing on each subset. Percentage transposed (% GFP+) data are displayed for CD4+ T cells (CD3+CD4+CD8-) or CD8+ T cells (CD3+CD4+CD8+) within the final cell product.
Not All T-Cells are Equal: The Importance of Stem Cell Memory Cells

Stem Cell Memory
- $T_{SCM}$ Cell
  - Self renewing
  - Long lived
  - Multipotent

Central Memory
- $T_{CM}$ Cell

Effector Memory
- $T_{EM}$ Cell

Effector T-Cell
- $T_{EFF}$ Cell

LESS DIFFERENTIATED

MORE DIFFERENTIATED

THE POTENTIAL BENEFITS
Products with High % of $T_{SCM}$ Cells:
- More gradual tumor killing with less toxicity
- Better duration of response and potential for re-response
- Key to CAR-T success in solid tumors

POSEIDA THERAPEUTICS
P-BCMA-101/P-PSMA-101: Three-In-One Transgene CAR-T products

1. **CAR-T MOLECULE**
   - Superior binding molecule
     - Centyrin binder with high-specificity binding to target
     - Fully human and not susceptible to tonic signaling

2. **POSITIVE SELECTION**
   - Drug resistance gene permits positive selection
     - ~100% of T-cells in final product express the CAR molecule
     - Predicted to result in better therapeutic index

3. **SAFETY SWITCH**
   - Incorporates proprietary safety switch
     - Rapid, dose-dependent elimination of engineered T-cells if needed
     - Management of Cytokine Release Syndrome (CRS) or other AEs
Stem Cell Memory T\textsubscript{SCM} Phenotype in Poseida’s Product Candidates

Our product more closely matches a T\textsubscript{SCM} phenotype when we do extensive cell surface markers and even intracellular markers.

Adapted from Gattinoni et al. (2017) Nat. Med.
P-BCMA-101 Eliminated Tumors in Aggressive MM Cancer Model

- **Fast initial response and durability** at a high and low dose
- **Spontaneous relapse control** without re-administration of P-BCMA-101

Days Post P-BCMA-101 Administration

Tumor Burden (Total Flux)

P-BCMA-101 admin

- Tumor only
- P-BCMA-101 (4 x 10^6 cells)
- P-BCMA-101 (12 x 10^6 cells)
High % of $T_{SCM}$ Cells: Unlocking Potential of CAR-T to Successfully Treat Solid Tumor Indications

Conventional Experience and Perception

• Poor CAR-T responses in solid tumors to date
• Rare instances with complete response (GBM, HCC) have occurred only after multiple administrations
• CAR-T can cause complete responses in solid tumors, but numerous waves of more differentiated cells are required

Our Approach

Our product candidates are comprised of a high percentage of $T_{SCM}$ cells, which we believe hold the potential to engraft, self renew and create wave after wave of more differentiated effector cells with one administration
P-PSMA-101 Observed Potent In Vivo Activity

Efficacy of P-PSMA-101 in Prostate Cancer Model (LNCaP.luc)

Imaging

Caliper Measurement

Tumor Elimination in 100% of Animals at Standard and Low Doses After ~ 2 Weeks

Data presented at SITC 2017. One animal in the low dose cohort relapsed later in the study.
A Population of P-PSMA-101 $T_{SCM}$ Persists

P-PSMA-101: Solid tumor (LNCaP) SC implantation in NSG mice

- P-PSMA-101 ($T_{SCM}/T_{CM}$) give rise to CAR Tyrin+ $T_{CM}$, $T_{EM}$, and Teff to attack solid tumor
- After solid tumor elimination, a population of P-PSMA-101 $T_{SCM}$ persists

No tumor by caliper (Day 20-25)
Phase 1/2 Relapsed/Refractory Multiple Myeloma Clinical Trial (PRIME)

Phase 1 Trial Design
- Open Label, 3+3 Design, Single Ascending Dose Study
- 30 mg/m2 fludarabine + 300 mg/m2 cyclophosphamide x 3d lymphodepletion regimen
- P-BCMA-101 administered intravenously
  - Allowance for multiple doses and retreatment after other CAR-Ts
  - Outpatient administration allowed
- Up to 80 subjects

Phase 2 Trial Design
- Same schema as Phase 1
- P-BCMA-101 administered intravenously at 6-15 x 10^6 cells/kg
- 100 subjects

Clinical Trial Sites
- Colorado Blood Cancer Institute- Tara Gregory, M.D.
- Hackensack University Medical Center- David Siegel, M.D.
- Johns Hopkins- Syed Abbas Ali, M.D.
- Karmanos Cancer Institute- Abhinav Deol, M.D.
- MD Anderson Cancer Center- Krina Patel, M.D.
- Swedish Cancer Institute- William Bensinger, M.D.
- Tennessee Oncology- Jesus G. Berdeja, M.D.
- UC San Diego Moores Cancer Center- Caitlin Costello, M.D.
- UC San Francisco- Nina Shah, M.D.
- University of Chicago- Andrzej Jakubowiak, M.D.
- University of Kansas Cancer Center- Siddhartha Ganguly, M.D.
- University of Maryland- Aaron Rapoport, M.D.
- University of Pennsylvania- Adam Cohen, M.D.
High Response Rates

Tumor Response in Evaluable Patients by Dose

### Objective Response Rate, %

<table>
<thead>
<tr>
<th>Mean Dose</th>
<th>Median follow-up (min, max), d</th>
<th>ORR= 50% (n=2)</th>
<th>ORR= 71% (n=7)</th>
<th>ORR= 50% (n=8)</th>
<th>ORR= 100% (n=3)</th>
<th>ORR= 67% (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>52 x 10^6</td>
<td>302 (259, 345)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>152 x 10^6</td>
<td>241 (191, 297)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>459 x 10^6</td>
<td>143 (17, 190)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>857 x 10^6</td>
<td>122 (122, 129)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1194 x 10^6</td>
<td>64 (44, 66)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data cutoff: January 31st, 2019. ORR, objective response rate, attaining sCR (inc. MRD-), CR, VGPR or PR, including confirmed and unconfirmed responses. Evaluable patients: evaluable first response assessment by IMWG m-protein criteria or PD/death.

Patient 105-002 PET

Data: Jan 26th, 2018 (4 weeks post-P-BCMA-101)

Catheter / injection site (FDG)

Oligosecretory disease, M-protein, SPEP, UPEP, FLC not measurable/within normal limits.
%Tscm Correlates with Response in Patients Treated with P-BCMA-101

**Analysis of Maximum Likelihood Estimates**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>D</th>
<th>F</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>Wald Chi-Square</th>
<th>Pr &gt; ChiSq P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1</td>
<td></td>
<td>-0.8267</td>
<td>0.8603</td>
<td>0.9233</td>
<td>0.3366</td>
</tr>
<tr>
<td>TscmFP4S</td>
<td>1</td>
<td></td>
<td>12.3798</td>
<td>6.5225</td>
<td>3.6025</td>
<td><strong>0.0577</strong></td>
</tr>
</tbody>
</table>
Adverse Events of Interest

Treatment-Emergent Adverse Events (n=26)

<table>
<thead>
<tr>
<th>TEAE, n (%)</th>
<th>Overall</th>
<th>≥ Grade 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose Limiting Toxicity (DLT)a</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cytokine Release Syndromea</td>
<td>5 (19.2%)</td>
<td>0</td>
</tr>
<tr>
<td>Neurotoxicitya</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 2 CRES with Grade 3 confusion (1 pt)</td>
<td>1 (3.8%)</td>
<td>1 (3.8%)</td>
</tr>
<tr>
<td>Neutropenia/Neutrophil count decreasedb</td>
<td>17 (65.4%)</td>
<td>16 (61.5%)</td>
</tr>
<tr>
<td>Thrombocytopenia/Platelet count decreasedb</td>
<td>11 (42.3%)</td>
<td>8 (30.8%)</td>
</tr>
<tr>
<td>Anemia</td>
<td>11 (42.3%)</td>
<td>9 (34.6%)</td>
</tr>
<tr>
<td>Infectionc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>9 (34.6%)</td>
<td>4 (15.4%)</td>
</tr>
<tr>
<td>First month</td>
<td>6 (23.1%)</td>
<td>2 (7.7%)</td>
</tr>
</tbody>
</table>

Data cutoff: January 31, 2019

a by investigator assessment
CRES based on confusion reported in a patient with baseline mental status decrement, tabulated in CRS & Neurotoxicity not including orthostatic dizziness or peripheral neuropathy/tremor
b subject counted once for either term
c includes events in the SOC Infections and Infestations. Subject counted once for any PT within the SOC.
Cytokine Release Syndrome Minimal, IL-6 Low but Correlates

Cytokine Release Syndrome Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dosed Patients (n=26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with a CRS event, n</td>
<td>5 (19.2%)</td>
</tr>
<tr>
<td>Maximum CRS grade</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>21 (80.8%)</td>
</tr>
<tr>
<td>1</td>
<td>3 (11.5%)</td>
</tr>
<tr>
<td>2</td>
<td>2 (7.7%)</td>
</tr>
<tr>
<td>Median time to onset, d</td>
<td>8</td>
</tr>
<tr>
<td>Median duration, d</td>
<td>4</td>
</tr>
</tbody>
</table>

Peak IL-6 Levels After P-BCMA-101

Levels generally reported for patients with severe CRS\(^1\)

- Orange dots: Grade 2 CRS assessed
- Brown dots: Grade 1 CRS assessed
- Black dots: No CRS assessed

\(^1\) Maude et al., 2014
Ali et al., 2016
P-BCMA-101 CAR-T Cells in PB: Gradual Expansion

- Many CAR-T products show peak expansion between 5-14 days
  - Peak expansion of CAR-Ts often associated with CRS

- P-BCMA-101 shows peak expansion between 14-21 days
  - P-BCMA-101 reaches peak expansion gradually without CRS
Correlations with Cmax/AUC and Outcome

Analysis of Maximum Likelihood Estimates

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</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1</td>
<td>-4.0596</td>
<td>2.8251</td>
<td>2.0649</td>
<td>0.1507</td>
</tr>
<tr>
<td>LCmax</td>
<td>1</td>
<td>1.1008</td>
<td>0.6063</td>
<td>3.2967</td>
<td>0.0694</td>
</tr>
</tbody>
</table>

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</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1</td>
<td>-26.2121</td>
<td>14.2531</td>
<td>3.3821</td>
<td>0.0659</td>
</tr>
<tr>
<td>LqPCR_AUC</td>
<td>1</td>
<td>4.7476</td>
<td>2.5183</td>
<td>3.5541</td>
<td>0.0594</td>
</tr>
</tbody>
</table>
New Disease Markers in MM/CAR-T: BCMA Correlations?

**MM cells**
- IHC
- Flow
- Transcription

**Soluble**
- ELIZA
- Luminex

- Statistically significant correlation between decrease in sBCMA in the first 4 weeks and response.
- sBCMA tracks with FLC kinetics

![Analysis of Maximum Likelihood Estimates](image)

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<th>Estimate</th>
<th>Standard Error</th>
<th>Pr &gt; ChiSq</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCMA Day 0 to 28</td>
<td>-0.0779</td>
<td>0.0362</td>
<td>0.0315</td>
</tr>
</tbody>
</table>

bb2121: Raje et al. ASCO 2018
New Disease Markers in MM: MRD

- Assessment for residual MM cells in bone marrow
- Increase sensitivity over standard measures of disease burden after treatment (m-protein, FLC, BMPC)
  - Studies indicate complete response of these markers correlate with survival outcomes
  - Most patients relapse in spite of a complete response in these markers

- Methods (bone marrow sample)
  - multiparametric flow cytometry for myeloma-associated markers (MFC) (1:10e5)
  - allele-specific oligonucleotide for IGH rearrangements (ASO)-qPCR (1:10e5)
  - next-generation sequencing of VDJ sequences for rearrangements (NGS) (1:10e6)
  - CTD?

<table>
<thead>
<tr>
<th>Disease status and treatment</th>
<th>N (total)*</th>
<th>MIB-negative patients</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Puig et al**</td>
<td>GEM2000* and GEMO5* trials</td>
<td>103 (170)</td>
<td>47%</td>
</tr>
<tr>
<td>Korthals et al**</td>
<td>Induction: 2–4 cycles of idarubicin and dexamethasone followed by ASCT</td>
<td>53 (70)</td>
<td>49%</td>
</tr>
<tr>
<td>Putkonen et al**</td>
<td>Patients with multiple myeloma who had achieved a complete response after ASCT or SCT</td>
<td>30 (37)</td>
<td>57%</td>
</tr>
<tr>
<td>Martinez-Sanchez et al**</td>
<td>Patients enrolled in the GEM2000* protocol</td>
<td>33 (88)</td>
<td>53%</td>
</tr>
<tr>
<td>Ladello et al**</td>
<td>Four cycles of bortezomib, thalidomide, and dexamethasone consolidation after ASCT</td>
<td>39 (112)</td>
<td>18%</td>
</tr>
<tr>
<td>Saracino et al**</td>
<td>Patients with multiple myeloma who had achieved a complete response after transplantation</td>
<td>24 (32)</td>
<td>29%</td>
</tr>
<tr>
<td>Martinelli et al**</td>
<td>Patients who achieved a complete response following ASCT or SCT</td>
<td>44 (50)</td>
<td>27%</td>
</tr>
</tbody>
</table>

Correlations with long-term outcomes:

Kumar, 2016
New Disease Markers in MM/CAR-T: MRD Correlations?


bb2121: Raje et al. NEJM 2019
Immunosuppressive tumor microenvironment likely decreases efficacy especially in solid tumors

- PD-L1, TGFβ, IL6, IL10, etc...
- Tregs, MDSC, TAM, etc...
- poor CAR-T durability
Gene Expression Analysis using Nanostring in CAR-T Cells

Nanostring CAR-T Panel Measures Eight Essential Components of CAR-T Biology

- Optimize CAR-T method development
- Create manufacturing acceptance criteria
- Measure metabolic fitness and persistence
- Monitor post-infusion exhaustion and toxicity

Advanced Analysis Modules available for CAR-T Characterization:

- TCR Diversity Score (coming soon)
- Normalization
- Quality Control
- Pathway Analysis
- Cell Profiling
- Differential Expression
- Gene Set Analysis
- Built-in compatibility for Panel-Plus and Protein analysis

From: www.nanostring.com
Gene Expression Analysis using Nanostring in DLBCL

High ImmunoSigne21 was Associated with Objective Response

Rossi et al, AACR 2018
Summary

New Methods are Continually Being Introduced to Evolve CAR-T cells

The field is nascent with extraordinary results, and advances in genetic engineering and manufacturing techniques allow for extraordinary potential in rationale design to improve CAR-T cells.

P-BCMA-101 incorporates a number of these advances and has been assessed in a clinical trial where it induced high response rates and deep responses in a heavily pretreated r/r MM population, with an excellent safety profile.

In Poseida’s clinical trial of P-BCMA-101, %Tscm was strongly correlated with efficacy; proliferative capacity (Cmax and AUM) also correlated with efficacy and durability and strongly support the Tscm hypothesis.

There are significant opportunities in novel biomarker methods to help guide the evolution of the field.