Building Synthetic Immunity to Cancer Using Chimeric Antigen Receptors

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ASFA
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Disclosure Information

Michael C. Milone

- Speaker and members of his laboratory, and the University of Pennsylvania have financial interest due to IP that is licensed to Novartis for CTL019 and related CAR technology.

- Funding support for clinical trials: ACGT, LLS, NCI, Novartis

- COI managed in accordance with University of Pennsylvania policy and oversight
Cancer vaccines have clinical activity
- Sipuleucel-T for prostate cancer
- gp100 peptide vaccine + Ipilimumab for melanoma

Checkpoint blockade can trigger dramatic clinical responses even without vaccines
- Melanoma and NSCLC
- Melanoma
Somatic mutation frequencies are highly variable across tumors...

Checkpoint Blockade works
Additional Barriers to Successful Immunotherapy for Cancer

- **Immune tolerance**
  - Many additional immune tolerance mechanisms exist beyond PD-1 and Tregs
    - Lag3, myeloid suppressor cells, inhibitory KIRs, etc..

- **Cancer-induced immunosuppression is worsened by the chemotherapy used to treat cancer**

- **Tumors acquire local properties that further help them evade immunity**
  - Alterations in presentation of tumor antigens (e.g. loss of MHC)
  - Disruption of normal immune cell migration and activation
    - Altered chemokine expression and possibly distribution
    - Abnormal presentation of other cellular guidance cues (e.g. semaphorins)
  - Creation of an adverse environment for immune cells (e.g. hypoxia)
  - Physical barriers (e.g. poor vascularity, dense stroma, etc)
Synthetic Biology

• the design and fabrication of biological components and systems that do not already exist in the natural world
• the re-design and fabrication of existing biological systems.

Up to now we are working on the descriptive phase of molecular biology. ... But the real challenge will start when we enter the synthetic biology phase of research in our field. We will then devise new control elements and add these new modules to the existing genomes or build up wholly new genomes. This would be a field with the unlimited expansion potential and hardly any limitations to building new better control circuits.
Synthetic Approaches to Engineering Anti-Tumor T-cell Immunity

TCR approach can target most proteins expressed by cell

CAR approach is limited to targets (protein and non-protein) expressed on the cell surface
Bead-Based T cell Expansion with Lentiviral Gene Transfer

αCD3/αCD28 Coated 4.5 μM Microbeads

T cells

αCD19-ζ
αCD19-BB-ζ
αCD19-28-ζ
αCD19-28-BB-ζ

CD4+ T cells
Mock

CD8+ T cells

scFv surface expression

T-body expressing Lentiviral Vector

3rd generation SIN vector

Fold Expansion
0 1 2 3 4 5 6 7 8 9

Days
Clinical Grade T cell expansion

Adoptive Immunotherapy Approach to Treating Cancer

1. T cell collection by apheresis
2. Cell Fractionation and Gene transfer
3. Ex vivo expansion
4. Antigen-specific or polyclonal expansion
5. Cells re-infused into patient

~10 day process

10^9

>10^{11}
CD19: An Ideal Target for a CAR

- CD19 expression is generally restricted to B cells and B cell precursors\(^1\)
  - CD19 is not expressed on hematopoietic stem cells

- CD19 is expressed by most B-cell malignancies
  - CLL, B-ALL, DLBCL, FL, MCL

- Antibodies against CD19 inhibit tumor cell growth

![Diagram of B cell development and CD19 expression](image)

T cells require multiple signals for optimal function
What is the best CAR for B-cell leukemia?

αCD19-Δζ

αCD19-ζ

αCD19-BB-ζ

αCD19-28-ζ

αCD19-28-BB-ζ

Population Doublings

Days in Culture

Mock
αCD19-ζ
αCD19-BB-ζ
αCD19-28-ζ
αCD19-28-BB-ζ

3 weeks post-T cell infusion

Milone, et al. Mol Ther 2009

Penn Medicine
CTL019 CLL Study Overview*

1. Leukapheresis
2. T-cell activation/transduction
3. Modified T-cell expansion
4. Chemotherapy
5. Modified T-cell infusion

5-10d


* UPCC04409, ClinicalTrials.gov NCT01029366
CTL019 for r/r Chronic Lymphocytic Leukemia: Generalities on First 3 Treated Patients

- All 3 patients had Chronic Lymphocytic Leukemia (CLL)
  - Late stage incurable leukemia
  - 3.5-7.7 pounds of tumor/patient
- Each infused CAR T cell or its progeny killed more than 1000 tumor cells
- Remissions durable to date
- Sustained antibody delivery with a single infusion of reprogrammed T cells (beyond 4+ yrs)

CTL019 Phase I Trial for r/r CLL: Summary of patient baseline characteristics

- N= 14 patients, protocol 04409 (NCT01029366)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Statistics, N(%)</th>
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<tr>
<td>N</td>
<td>14</td>
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<tr>
<td>Age at infusion in years</td>
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<tr>
<td>Mean (SD)</td>
<td>66.9 (8.1)</td>
</tr>
<tr>
<td>Median (range)</td>
<td>66 (51-78)</td>
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<tr>
<td>Gender</td>
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<tr>
<td>Male</td>
<td>12 (85%)</td>
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<tr>
<td>Female</td>
<td>2 (14%)</td>
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<tr>
<td>Number of prior therapies</td>
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<tr>
<td>Mean (SD)</td>
<td>5.3 (2.8)</td>
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<tr>
<td>Median (range)</td>
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<td>P53 or 17p deletion</td>
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<td>8 (57%)</td>
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<td>Yes</td>
<td>6 (43%)</td>
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<td>IGHV mutation</td>
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<td>9 (64%)</td>
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<tr>
<td>Yes</td>
<td>4 (29%)</td>
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<tr>
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<td>1 (7%)</td>
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</table>

- Overall response rate: 57%
- CR 4/14 (28%)
- PR 4/14 (28%)
- NR 6/14 (43%)

Long term persistence and expression of CTL019 in CLL patients with durable remission

- Persistence for first year after infusion

![Graph showing persistence and expression of CTL019 over months after infusion for different patients.

IGH deep sequencing analysis: eradication of malignant CLL clone

<table>
<thead>
<tr>
<th>Subject number</th>
<th>Source</th>
<th>Timepoint</th>
<th>Total reads of IgH</th>
<th>Total unique IgH reads</th>
<th>Tumor clone reads</th>
<th>CLL clone % of total</th>
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<tbody>
<tr>
<td></td>
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<td>408,579</td>
<td>48</td>
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<td>01</td>
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<td></td>
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<tr>
<td></td>
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<td></td>
<td>PB</td>
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<tr>
<td></td>
<td>BM</td>
<td>mo 1</td>
<td>179</td>
<td>3</td>
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<tr>
<td></td>
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<td>BM</td>
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<td></td>
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<td>1,385,340</td>
<td>4544</td>
<td>1,285,862</td>
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<tr>
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<td>PB</td>
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<td>8</td>
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<tr>
<td></td>
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<td>2</td>
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<tr>
<td></td>
<td>BM</td>
<td>yr 2</td>
<td>601</td>
<td>25</td>
<td>0</td>
<td>0.00%</td>
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</table>
Persisting CTL019 cells remain functional for > 2 years after infusion

- 1.5% CAR cells
- Mostly CD8+

CTL019 function 1039 days after infusion:

- PBMC reisolated from subject 02. Cells expressing CAR identified with anti-idiotype
- Stimulated for 6 hours with CD19+ or control tumor cells. Cytokine induction and degranulation

Jos Melenhorst
Simon Lacey
Summary of CTL019 Efficacy in ALL
Case Mix on phase I: Pediatric and Adult

>180 patients with CLL, ALL, NHL, MM have gotten CTL019
73 at CHOP (5 with CTL119)

- 48 r/r pediatric ALL pts: 45 in CR at 1 mo (94%)
- 3/180 treatment-related mortality
- 5 went to subsequent transplant
- 6-month DOR: 76%
- No relapses past 1 year

Maude, et al, NEJM 2014 results updated to July 1, 2015
CTL019 Toxicities

- **B cell aplasia**
  - observed in all responding patients to date
  - managed with replacement therapy

- **Tumor lysis syndrome (TLS)**
  - may be delayed for 20 to 50 days post infusion

- **Cytokine release syndrome (CRS)**
  - reversible, on-target toxicity
  - Severity related to tumor burden: Treat MRD as outpatient?

- **Macrophage activation syndrome (HLH / MAS)**
  - elevated serum ferritin (>500,000 ng/ml), CRP, D-dimer
  - elevated cytokines: IL-6, IFN-gamma
  - Reversed with tocilizumab
Disease burden is well-correlated with grade 4 CRS (r/r pre-B cell ALL)
Tocilizumab Anti-Cytokine Therapy for Cytokine Release Syndrome

CLL Pt 04409-09
ALL: Mechanisms of Resistance to CART19

• In pediatric and adult ALL, there is a >90% CR at 1 month
• To date, there have been 15 relapses in the first 50 patients given CART19:
  • No patient has relapsed beyond 1 year
  • 15 patients have relapsed, and 10 of these patients relapsed with CD19 negative leukemia
• What are the mechanisms of CD19 negative relapse in ALL?
Mechanism of resistance in ALL: CD19 escape

PREDICTED PROTEIN PRODUCTS FOR ISOFORMS

- CD19 escape appears due to a combination of mutations and shifts in splicing that favor retaining some CD19 protein
- DNA mutations can be subclonal, and may include splice factors sites
Determine Efficacy and Safety of CTL019 in Pediatric Patients With Relapsed and Refractory B-cell ALL (ELIANA)

US sites
- Children’s Hospital of Philadelphia
- Cincinnati Children’s Hospital
- University of Wisconsin
- Children’s Medical Center of Dallas
- Children’s Mercy Kansas University
- Oregon Heath & Science University
- Stanford University
- University of Minnesota
- Children’s Hospital Los Angeles
- University of Michigan
- Duke University

Ex-US
(Canada, Australia, EU)
- Royal Children’s Hospital (Australia)
- Hospital St. Justine (Canada)
- Ghent University (Belgium)
- Oslo Univ. Hospital (Norway)

Clinicaltrials.gov NCT02435849
Single Arm, Open-Label, Multi-Center, Phase II Study of Efficacy and Safety of CTL019 in Adult DLBCL Patients (JULIET)

US sites
• Emory Winship Cancer Institute
• University of Chicago
• University of Kansas
• University of Michigan
• University of Minnesota
• Duke University
• Ohio State James Cancer Hospital
• Oregon Health Sciences University
• MD Anderson Cancer Center

Ex- US
(Canada, Japan, EU)
• Montreal
• Sapporo
• Oslo

Clinicaltrials.gov NCT02445248
Published clinical trials employing CAR T cells targeting CD19+ B cell malignancies

<table>
<thead>
<tr>
<th>CAR</th>
<th>Signaling</th>
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</thead>
<tbody>
<tr>
<td>Brentjens R, et al. Mol Ther. 2010</td>
<td>CD19</td>
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<tr>
<td>Jensen MC, et al. BBMT. 2010</td>
<td>CD19/CD20</td>
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<tr>
<td>Kalos et al. Sci Transl Med. 2011</td>
<td>CD19</td>
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<tr>
<td>Cruz et al. Blood. 2013</td>
<td>CD19</td>
</tr>
<tr>
<td>Lee DW et al. Lancet. 2014</td>
<td>CD19</td>
</tr>
</tbody>
</table>
CART19 (CTL019) shows significant anti-leukemic activity...

What about solid tumors?
# CARs in Development Worldwide

## Commercial CARs: Celgene, Juno, Kite, Novartis, Cellectis/Pfizer

<table>
<thead>
<tr>
<th>Institute (US)</th>
<th>Target(s)</th>
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<tr>
<td>Fred Hutchinson Cancer Center</td>
<td>CD20, CD19</td>
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<tr>
<td>Baylor College of Medicine</td>
<td>GD-2, Her2, CD30, kappa Ig</td>
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<tr>
<td>National Cancer Institute (NCI)</td>
<td>CD19, EGFRvIII, mesothelin</td>
</tr>
<tr>
<td>Roger Williams Medical Center (RI)</td>
<td>CEA, PSMA</td>
</tr>
<tr>
<td>University of Pennsylvania</td>
<td>CD19, mesothelin, EGFRvIII</td>
</tr>
<tr>
<td>Memorial Sloan Kettering Cancer Center</td>
<td>CD19, PSMA</td>
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<tr>
<td>Children's Mercy Hospital Kansas City</td>
<td>GD-2</td>
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## Institute (non-US)

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<tr>
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<th>Target(s)</th>
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<tbody>
<tr>
<td>Chinese PLA General Hospital</td>
<td>CD19, CD20, CD33, CD138, HER2</td>
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<td>Christie Hospital NHS Foundation Trust</td>
<td>CD19</td>
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<tr>
<td>Peter MacCallum Cancer Centre, Australia</td>
<td>LewisY</td>
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<tr>
<td>University of Zurich</td>
<td>FAP</td>
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</table>
Target Selection is Important

Treatment of Metastatic Renal Cell Carcinoma With Autologous T-Lymphocytes Genetically Retargeted Against Carbonic Anhydrase IX: First Clinical Experience
Cor H.J. Lammers, Stefan Sleijfer, Arnold G. Vulto, Wim H.J. Kruit, Mike Kliffen, Reno Debets, Jan W. Gratama, and Gerrit Stoter

Case Report of a Serious Adverse Event Following the Administration of T Cells Transduced With a Chimeric Antigen Receptor Recognizing ERBB2
Richard A Morgan¹, James C Yang¹, Mio Kitano¹, Mark E Dudley¹, Carolyn M Laurencot¹ and Steven A Rosenberg¹

T Cells Targeting Carcinoembryonic Antigen Can Mediate Regression of Metastatic Colorectal Cancer but Induce Severe Transient Colitis
Maria R Parkhurst¹, James C Yang¹, Russell C Langan¹, Mark E Dudley¹, Debbie-Ann N Nathan¹, Steven A Feldman¹, Jeremy L Davis¹, Richard A Morgan¹, Maria J Merino², Richard M Sherry¹, Marybeth S Hughes¹, Uday S Kammula¹, Giao Q Phan¹, Ramona M Lim³, Stephen A Wank², Nicholas P Restifo¹, Paul F Robbins¹, Carolyn M Laurencot¹ and Steven A Rosenberg¹
Mesothelin-specific CARTs show in vivo cytotoxic activity toward solid tumors

Mesothelin negative

Carpenito C, Milone MC et al. PNAS 2009
Mesothelin is also expressed by normal tissues that may pose challenges to using it as a CAR.

Carpenito C, Milone MC et al. PNAS 2009
Lessons learned to date from CARTmeso

3x10^7/m2 CARTmeso, no Cytoxan

3x10^7/m2 CARTmeso, Cytoxan

- Pharmacokinetics of CARTmeso: Cytoxan major effect on PK
- Murine CAR immunogenic
- Modest antitumor effects to date: 2 PR (metastatic pancreatic and mesothelioma)
- hCARTmeso is currently planned
Mesothelioma can show significant resistance to CART therapy in pre-clinical xenograft models

CAR T cells Rapidly Lose Function In Vivo

2x10^6 EM-meso cells SQ

Tumor growth to ~200 mm^3

Meso-CAR T cells IV

TIL isolation

d0

- How do we overcome tumor-induced T cell hypofunction to improve CAR therapy in solid tumor models?

Pilot Trial Testing CD19 CARs for Chemotherapy-Resistant/Refractory Leukemia: Status

<200 patients treated to date. How do we treat 1000s per year?

First Patient Dosed: 7/31/2010
Health Care Challenges

Issues
- Patient specific “n of 1”
- Blood bank model?
- Central manufacturing?

Chris Mason et al, Regen Med. 2011
Levine and June, Nature. 2013
A Lesson From History
“Outscaling” for BMT

For Immediate Release
Jan. 30, 2013

1 millionth blood stem cell transplant marks major medical milestone

International cooperation among physicians, scientists credited for landmark achievement

Bern, Switzerland, Jan. 30, 2013—The collaborative work of medical scientists and
Robots and Automation: Lessons from Detroit

Performance characteristics of assembly systems following different assembly principles (Heilala, J. Modular Reconfigurable Flexible Final Assembly Systems, Assembly Automation, 21/1: 20–28, 2001)
Colleagues and Collaborators

Milone Lab
Enxiu Wang
Chune Zhang
Roddy O’Connor
Ai Wang
Vijay Bhoj
ACC TRP
Carl June
Anne Chew
Gabriela Plesa
John Scholler
Carmine Carpenito
John Scholler
TCSL
Michael Kalos
Jos Melenhorst
Simon Lacey
Irina Kulikovskaya

Steve Albelda Lab
Steve Albelda
Liang-Chuan Wang
Edmund Moon

CVPF
Bruce Levine
Zoe Zheng
Alexey Bersenev
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Hima Patel
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Lynn Schuchter
Martin Carroll
Gregory Beatty
Robert Vonderheide
Adam Bagg
Don Siegel
Sharyn Katz
Ran Reshef
Sunita Nasta
Saar Gill
Alison Rager
Jacob Svoboda

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David Barrett
Christine Strait
Bernd Hauck
Fraser Wright

MD Anderson
Lawrence Cooper
Bipulendu Jena

Novartis
Usman Azam
Jennifer Brogdon
Glenn Dranoff

And many others at Penn... it takes a village!
Special thanks to Dr. Porter’s Adult Leukemia Patients
...And Dr Grupp’s Pediatric Leukemia Patients
Questions??