“Go CAR-Ts in the Fast Lane”

Raj Rajendra, MD/PhD
Faculty discussant: Brian Till, MD
Outline

- History of Cancer Immunotherapy
- Functioning of T-cells in tumor immunity
- Mechanisms of resistance to T-cell therapies
- CARs- A means to overcome resistance
- Discussion of the paper
- Future directions for CARs
When and How Did It All Start??

*William Coley, considered father cancer immunotherapy*

- Coley had just started as a young surgeon in New York City when he met Elizabeth Dashiell afflicted with sarcoma.
- Her death prompted Coley to compile every sarcoma case for past 15 yrs.
- He came across the case of Fred Stein, who presented in the fall of 1880 with a small red spot on his left cheek.
- The spot grew requiring surgical removal; with multiple recurrences.
- The mass could not be excised as it was attached to the carotid artery leading to a large non-healing ulcer.
- In October, 1884, Stein acquired a *Strep. pyogenes* and had a severe attack of erysipelas, (St. Anthony’s fire) on his face and neck.
- Stein expected to die, miraculously survived and to everyone’s surprise his facial ulcer had developed granulation tissue and was healing.
In February, 1885, he was discharged and had not been heard from.

In 1891 Coley tracked him down and discovered a healthy Fred Stein, his cancer had not returned since 1885.

Subsequently Coley injected streptococcal cultures into cancer patients and observed tumor regression in some cases.

His findings were first published in 1893.

During the next 43 years Coley treated almost 900 cancer patients with his bacterial prep. well known as ‘Coley’s toxin’.

Most of the treated patients had inoperable sarcomas, with the bacterial toxin achieving a cure rate of over 10%.

Coley’s early studies led to the use of BCG for cancer immunotherapy.
MILESTONES IN THE HISTORY OF CANCER IMMUNOTHERAPY

1890s
Paul Ehrlich suggested that molecules within the body might have the ability to fight tumours.

1900
William Coley, a New York surgeon, used live bacteria in the first anti-cancer vaccines.

1957
Interleukin-2 (IL-2) was first discovered.

1965
Burnet suggested that tumour cells may develop in an individual, but that the presence of antigens on the surface of these cells may induce an immune response, destroying the cells without any clinical manifestation.

1970
Morton described marked tumour regression, after injection of BCG vaccine, in melanoma patients.

1975
George Köhler and César Milstein developed the technology for monoclonal antibody generation.
A monoclonal antibody was used to successfully treat B-cell lymphoma. Andrew N. Kolhorn is depicted in the middle of the page. The first monoclonal antibody was approved by the FDA.

1986-2000

Approval for the use of interferons (IFNs) and IL-2 in the treatment of neoplasias was granted.

1988

Dariavach and colleagues cloned the human cytotoxic T-lymphocyte associated antigen-4 (CTLA-4) gene.

1991

Isolation and cloning of the first human tumour-associated antigen (TAA) melanoma antigen (MAGE)-1 from melanoma patients.

1997

FDA approval granted for the use of rituximab as a single agent in non-Hodgkin's lymphoma (NHL).

Phase I-III clinical trials carried out using anti-CTLA-4 antibodies.

2002-2007

Hunder and colleagues infused CD-4+ T-cells into a patient with refractory metastatic melanoma. The patient subsequently showed complete tumour regression.

2008

Bristol-Myers Squibb
Human T-Cell Adoptive Immunotherapy


• 2002: Lymphodepletion + TILs + high dose IL-2 for metastatic melanoma (Dudley et al, *Science* 2002; 298: 850)


• 2002: EBV-specific CTLs for prevention and Rx of EBV-associated PTLs (Heslop et al, *Cancer Res.* 2002;159:123-33)


Paradigms of Adoptive T-cell Therapy

A. Autologous
- TIL isolation
- Preconditioning: irradiation/chemotherapy

B. Allogeneic
- TILs
- Test against tumor and rapid expansion
- Tumor excision
- PBL
- Allorestricted tumor-specific T cells

C. Engineered
- TCR cloning
- Transduction of: PBL HSC
- Viral vector
- Humanized xenogenic donor
Improving T-cell Therapy for Cancer

- Increase enhanced specificity
  --expression of chimeric antigen receptors (CARs)
  --expression of TCR variable α and β chains from TAA–specific T cell clones

- Enhanced survival and proliferation
  --transduce T cells with anti-apoptotic genes to increase survival and hTERT to enhance proliferation and cytokines

- Resistance to inhibitory molecules e.g. TGF-β and Fas/FasL
  --(dnTGF-RII) gene-mod murine T cells preferential tumor infilt. & prolonged persistence in TGF-β+ tumor-bearing mice

- Modification of host environment
  --depletion of IDO and T-reg

CARs: 1st, 2nd and 3rd Generation
# Clinical Trials Using CARs

## Table 2

<table>
<thead>
<tr>
<th>Reference</th>
<th>Type of T cell</th>
<th>CAR construct</th>
<th>Cell Dose</th>
<th>Targeted Cancer/Number of patients</th>
<th>Serious Adverse effects</th>
<th>Persistence</th>
<th>Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kershaw et al. [30]</td>
<td>OKT3 activated T cells (eight patients) Alloantigen activated T cells (six patients)</td>
<td>α-folate receptor CAR retroviral vector with neomycin resistance gene</td>
<td>$3 \times 10^8$ to $5 \times 10^{10}$ (OKT3)</td>
<td>Ovarian cancer/14 patients</td>
<td>None (IL2 effects in cohort receiving high dose IL2)</td>
<td>Up to three weeks in 13 patients</td>
<td>None</td>
</tr>
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<td>Park et al. [56]</td>
<td>OKT3 activated T cells (clones)</td>
<td>CE7R-CAR plasmid with HyTK G250-CAR retroviral vector</td>
<td>$4.0 \times 10^8$ to $1.69 \times 10^{11}$ (alloantigen)</td>
<td>Neuroblastoma/6 patients</td>
<td>None</td>
<td>1-42 days in one patient</td>
<td>One of six with evaluable tumor had a PR</td>
</tr>
<tr>
<td>Lamers et al. [31]</td>
<td>OKT3 activated T cells (clones)</td>
<td>CD20-CAR plasmid with neomycin resistance gene</td>
<td>$0.38$ to $2.13 \times 10^9$</td>
<td>Renal cancer</td>
<td>Grade 2-4 liver toxicity</td>
<td>Up to 53 days</td>
<td>None</td>
</tr>
<tr>
<td>Till et al. [20*]</td>
<td>OKT3 activated T cells (clones in three and lines in four)</td>
<td>CD20-CAR plasmid with neomycin resistance gene</td>
<td>$10^8$ to $3.3 \times 10^9$ cells/m^2</td>
<td>CD20+ low grade B cell lymphoma/7 patients</td>
<td>None</td>
<td>One to three weeks (clones) five to nine weeks (T cell lines and low dose IL2)</td>
<td>Four of five with evaluable disease had stable disease and one a PR</td>
</tr>
<tr>
<td>Pule et al. [34**]</td>
<td>OKT3 activated T cells and EBV-specific CTLs</td>
<td>GD-2-CAR retroviral vector</td>
<td>$2 \times 10^7$ to $2 \times 10^8$ cells/m^2 of each product</td>
<td>Neuroblastoma/11 patients</td>
<td>None</td>
<td>Up to three weeks for the activated T cells and up to six months for CTLs</td>
<td>Four of Eight with evaluable tumor had necrosis or responses with 1 CR</td>
</tr>
</tbody>
</table>

EBV: Epstein Barr virus.  
CTL: Cytotoxic T lymphocyte.  
CR: Complete remission.  
PR: Partial remission.  
HyTK: Hygromycin thymidine k.
BRIEF REPORT

Chimeric Antigen Receptor–Modified T Cells in Chronic Lymphoid Leukemia

David L. Porter, M.D., Bruce L. Levine, Ph.D., Michael Kalos, Ph.D., Adam Bagg, M.D., and Carl H. June, M.D.
Clinical History

- 65 year-old male first diagnosed with CLL in 1996
- After 6 yrs observation, first required Rx for progressive leukocytosis and LAD
- 2002: FR x 2 with normalization of counts and decreased LAD.
- Recurrence in 2006: FR x 4 cycles
- 02/2009 leukocytosis progressed rapidly and LAD recurred. BM extensively infiltrated with CLL, and FISH showed del TP53
- Received BR x 1 cycle and then bendamustine x 3 cycles with transient improvement in lymphocytosis.
- 12/2009 autologous T cells were leukapheresed, and the patient received alemtuzumab for 11 weeks with improved counts and LAD.
- 07/2010 WL enrolled in a phase I clinical trial of chimeric antigen receptor-modified T cells.
Mechanics of the CART19 Manufacturing

![Flowchart of the CART19 manufacturing process]

- **Day 0**: Leukocyte apheresis → CD3/28-positive selection of T cells with anti-CD3/anti-CD28 mAb-coated magnetic beads
- **Day 0-1**: Seed in gas-permeable bags. Transduction with αCD19-41BB;
- **Day 3**: Vector washout. Culture in gas-permeable bags
- **Day 5**: Culture in WAVE bioreactor → Remove beads → Harvest, wash, concentrate → Cryopreserve final product in infusible cryomedia
- **Harvest day (10 ± 2)**
Clinical Protocol

CD19+ B cell malignancy
- FDA-approved therapy
- Monitor for recurrence

Relapse
- Eligibility tumor restaging

Week - 4
- PBMC baseline assays
- Apheresis

Week - 1
- Assess response

Week + 4
- T cell infusion
- PBMC marrow endpoint assays
- Manufacture/cryopreservation

- Monthly observation/monitoring 6 months after infusion
- Quarterly observation/monitoring by year 2
- Roll over to destination protocol for 15 years if for monitoring for delayed AEs related to gene transfer
Study Procedure

• Self-inactivating lentivirus was used to transduce T cells
• 1-5 days prior to infusion patient received chemo
• Total $3 \times 10^8$ T cells, 5% transduced ($1.42 \times 10^7$ total transduced cells)
• $1.46 \times 10^5$ cells/kg split into three consecutive q day IV infusions (10% on D1, 30% D2, and 60% D3)
• No post-infusion cytokines
Tumor Lysis

Day 15: Fevers, chills, rigors and fatigue
Around Day 20: Tmax 39.2 w/rigors, diaphoresis, N and D. Pan Cx. Neg
Day 22: TLS requiring IP admission for fluids and WL was admitted and rasburicase
Day 23: No CLL in marrow, karyotype normal, flow negative for CLL. CR
Around 26: Labs normalized and D/C
Day 28: Adenopathy not palpable

Expansion and Persistence of CART19 in Vivo
Summary

• Extensive marrow involvement and diffuse LAD with 1-3 cm LNs
• Cytogenetics- del17p and Tp53
• Lentiviral vector expressing CD-19 specific CAR coupled with CD-137 (costimulatory receptor) and CD-3ζ (signal transduction)
• $1.5 \times 10^5$ cells/kg of autologous CAR-T reinfused in pt. R/R CLL
• Engraftment ~ 1000x
• Delayed TLS with CR
• Engineered cells persisted for at least 6 months in the blood and bone marrow and continued to express CAR-T
• Toxicity: Delayed TLS and Gr. 3 lymphopenia
• Pt. had a 10 month CR when the paper was published.
T Cells with Chimeric Antigen Receptors Have Potent Antitumor Effects and Can Establish Memory in Patients with Advanced Leukemia

Michael Kalos, Bruce L. Levine, David L. Porter, Sharyn Katz, Stephan A. Grupp, Adam Bagg and Carl H. June1
### Clinical History

<table>
<thead>
<tr>
<th>Subject UPN</th>
<th>Age/sex karyotype</th>
<th>Previous therapies</th>
<th>CLL tumor burden at baseline</th>
<th>Total dose of CART19 (cells/kg)</th>
<th>Response day +30 (duration)</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>65/M normal</td>
<td>Fludarabine × four cycles (2002)</td>
<td>Hypercellular 70% CLL</td>
<td>N/A</td>
<td>6.2 \times 10^{11} to 1.0 \times 10^{12} CLL cells (day −37)</td>
</tr>
<tr>
<td></td>
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<td>Rituximab/fludarabine × four cycles (2005)</td>
<td>2.4 \times 10^{12} CLL cells (day −14)</td>
<td>1.7 \times 10^{12} CLL cells (day −1)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Alemtuzumab × 12 weeks (2006)</td>
<td></td>
<td>2.4 \times 10^{12} CLL cells (day −14)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Rituximab (two courses, 2008 to 2009)</td>
<td></td>
<td>1.7 \times 10^{12} CLL cells (day −1)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>R-CVP × two cycles (2009)</td>
<td></td>
<td>1.7 \times 10^{12} CLL cells (day −1)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Lenalidomide (2009)</td>
<td></td>
<td>1.7 \times 10^{12} CLL cells (day −1)</td>
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<td>PCR × two cycles (5/18/2010 to 6/18/2010)</td>
<td>Hypercellular 70% CLL</td>
<td>N/A</td>
<td>6.2 \times 10^{11} to 1.0 \times 10^{12} CLL cells (day −37)</td>
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<td></td>
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<td>Bendamustine × one cycle (7/31/10 to 8/1/10) pre-CART19</td>
<td>Hypercellular &gt;95% CLL</td>
<td>2.75 \times 10^{11} to 1.2 \times 10^{12} CLL cells (day −24)</td>
<td>5.8 \times 10^{8} (1.0 \times 10^{7}/kg)</td>
</tr>
<tr>
<td>02</td>
<td>77/M del (17)(p13)*</td>
<td>Alemtuzumab × 16 weeks (6/2007)</td>
<td>Hypercellular &gt;95% CLL</td>
<td>2.75 \times 10^{11} to 1.2 \times 10^{12} CLL cells (day −24)</td>
<td>5.8 \times 10^{8} (1.0 \times 10^{7}/kg)</td>
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<td></td>
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<td>Alemtuzumab × 18 weeks (3/2009)</td>
<td>3.2 \times 10^{12} CLL cells (day −47)</td>
<td>2.75 \times 10^{11} to 1.2 \times 10^{12} CLL cells (day −24)</td>
<td>5.8 \times 10^{8} (1.0 \times 10^{7}/kg)</td>
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<tr>
<td></td>
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<td>Bendamustine/rituximab: 7/1/2010 (cycle 1)</td>
<td>Hypercellular &gt;95% CLL</td>
<td>2.75 \times 10^{11} to 1.2 \times 10^{12} CLL cells (day −24)</td>
<td>5.8 \times 10^{8} (1.0 \times 10^{7}/kg)</td>
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<td>7/28/2010 (cycle 2)</td>
<td>Hypercellular &gt;95% CLL</td>
<td>2.75 \times 10^{11} to 1.2 \times 10^{12} CLL cells (day −24)</td>
<td>5.8 \times 10^{8} (1.0 \times 10^{7}/kg)</td>
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<tr>
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<td>8/26/2010 (cycle 3) pre-CART19</td>
<td>Hypercellular &gt;95% CLL</td>
<td>2.75 \times 10^{11} to 1.2 \times 10^{12} CLL cells (day −24)</td>
<td>5.8 \times 10^{8} (1.0 \times 10^{7}/kg)</td>
</tr>
</tbody>
</table>
In Vivo Expansion, Persistence, And BM Trafficking of CART19 Cells

CART19 cells as a fraction of the circulating WBCs

Bulk qPCR analysis of marrow to quantify CART19
Prolonged CART19 Expression and Absent B-cells *in Vivo* in UPN03
Induction of Specific Immune Response In PB and BM after CART19 Infusion

Febrile syndrome with rigors

Febrile syndrome with rigors and TLS
Clinical Responses

Sequential BM biopsies stained for CD20

Sequential CT images demonstrating resolution of LAD
Clinical Responses

UPN 03

Bone Marrow

Conclusions

• 2/3 patients achieved CR.
• UPN02 had a drop in CLL count, however fevers, constitutional symptoms, and transient cardiac dysfunction occurred at day 15 and corticosteroids were initiated. This patient has had a stable partial response and remained asymptomatic.
• The small number of CART 19 T cells infused expanded significantly (1000x) and these cells were estimated to each kill ~1000 CLL (serial killers).
• Analysis suggested that the CART19 T cells had both a central and effector memory phenotype.
• There is concern that the persistence of CAR T cells will lead to long term B cell deficiency
Future Directions

• Reports of early CAR clinical trials for B-cell malignancies are promising
• The incorporation of a suicide gene system into CAR-redirected T cells seems to increase safety
• The novel physiological CAR may avoid immunogenicity
• CAR strategies for B-cell malignancies may provide significant improvements to cancer immunotherapy
References

• Kalos et al. Sci Transl Med 3; 2011
• Parish et al. Immunology and Cell Biology (2003) 81, 106–113
• Mellman et al. Nature; Vol 480; 22; Dec 2011
Questions???
Prolonged Receptor Expression and Establishing Memory CART19 in PB
General Approaches for *Ex Vivo* T-Cell Expansion

[Diagram illustrating the process of ex vivo T-cell expansion starting from T cell repertoire, through antigen-specific stimulation and polyclonal stimulation, leading to functional development and T cell selection.]

CARs- A Really Neat Concept!!!

- CAR-T cells engineered to express the antigen-binding part of monoclonal antibody (single chain Fv) on the surface of the T cells.
- Can recognize antigen on cell surface in a non-MHC-dependent manner, with the affinity and specificity of monoclonal antibodies
- Makes it easier to generate reagents used to treat multiple patients whose cancer cells express target antigens.
- Same CAR can be used to transduce & produce both CD4+ and CD8+ T cells.
- Early studies showed that while CARs could re-direct T cells and allow initial proliferation, the cells did not survive long without additional co-stimulation.
In Vivo Expansion, Persistence, And BM Trafficking of CART19 Cells

Frequency of CART19 cells as average transgene copies

Frequency of total calculated CART19 cells in circulation