Adoptive Cell Therapy

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Assistant Professor, Ludwig Cancer Lausanne Branch

Adjunct Assistant Professor, University of Pennsylvania
How does immunotherapy work?
The Ultimate Goal
A Tumor Assassin

T lymphocyte (T cell)

Tumor cell

We need More T cells
We need them functional (Armed and Activated)
We need them Powerful and Persistent
We want them to reach tumor site
We need them targeted and specific
Intratumoral T Cells, Recurrence, and Survival in Epithelial Ovarian Cancer

Lin Zhang, M.D., Jose R. Conejo-Garcia, M.D., Ph.D., Dionyssios Katsaros, M.D., Ph.D., Phyllis A. Gimotty, Ph.D., Marco Massobrio, M.D., Giorgia Regnani, M.D., Antonis Makrigiannakis, M.D., Ph.D., Heidi Gray, M.D., Katia Schlienger, M.D., Ph.D., Michael N. Liebman, Ph.D., Stephen C. Rubin, M.D., and George Coukos, M.D., Ph.D.

CD3⁺

Stroma

Islet

TIL Present 55%

TIL Absent 40%

After CR with chemotherapy, only patients with TILs survive or are in remission long-term.

The analysis performed in 186 frozen specimens from advanced-stage EOCs showed that the presence of CD3+ TILs was associated with a significant improvement in median PFS (22.4 vs 5.8 months) and OS (50.3 vs 18 months).

The association of immune cell infiltrate with prognosis in various types of cancer

OPTION 1: Adoptive Immunotherapy Using Natural T Cells
TILS are powerful: Compelling Results in Late Stage Disease
TILs: Regressions in Late-Stage Disease
Success of Adoptive Therapy Using TILs in Melanoma

Durable Responses in advanced melanoma patients
19 of 20 complete responders are ongoing to >10 years

Rosenberg S A et al. *Clin Cancer Res* 2011
Effect of HPV targeted Tumor infiltrating T Cells on Cervical Cancer

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Histology</th>
<th>HPV Type</th>
<th>Sites of Disease</th>
<th>Prior RT</th>
<th>Prior Systemic Treatment</th>
<th>Cells (x 10^9)</th>
<th>Within CD3+ (%)</th>
<th>CD4+</th>
<th>CD8+</th>
<th>No. of IL-2 Doses</th>
<th>Response</th>
<th>Duration or TTP (months)</th>
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<td>18</td>
<td>Iliac lymph nodes, lung, lung hilum, retroperitoneum, vaginal cuff</td>
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<td>Cisplatin</td>
<td>101.4</td>
<td>29</td>
<td>72</td>
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<td>7</td>
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<tr>
<td>2</td>
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<td>18</td>
<td>Bone, liver, lung, lung hilum, mediastinum, pelvis</td>
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<td>22+</td>
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<td>4</td>
<td>55</td>
<td>SCC</td>
<td>16</td>
<td>Axilla, breast, liver, omentum, pleura, soft tissue</td>
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<td>Cisplatin, carboplatin, paclitaxel, fluorouracil, irinotecan, doxitinib, pemetrexed</td>
<td>80.1</td>
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<td>5</td>
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<td>36</td>
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<td>9</td>
<td>37</td>
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<td>18</td>
<td>Axilla, bone, lung, mediastinum, pelvis, retroperitoneum</td>
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<td>59</td>
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<td>1</td>
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</table>

Abbreviations: AC, adenocarcinoma; ASC, adenosquamous cell carcinoma; CR, complete response; HPV, human papillomavirus; IL-2, interleukin-2; PD, progressive disease; PR, partial response; RT, radiotherapy; SCC, squamous cell carcinoma; TTP, time to progression.

DO/CTE/TPF | Lausanne TIL – Culture et expansion des TILs

Premier Étap

Culture & expansion avec IL-2
2 à 5 semaines

Décongélation & Culture avec IL-2
Jour 0

15 pré-Rep

Deuxième Étap

2 REP – 150 x 10⁹ Cellules
1 REP - Juin

2 REP - 150 x 10⁹ Cellules
2 semaines
Challenges of TIL Therapy

• Patients have to undergo surgery- Tissue needs to be stored PROPERLY

• T cells are functionally ‘exhausted’

• TILs are of unknown antigen specificity

• The immune system tolerates self-proteins (TCR may not be of optimal affinity)

• Need for IL-2 and lymphodepletion (toxic)
Patient had previously been treated with 30 billion conventional nontransduced TILs, plus 7 doses of IL2 (720,000 IU/kg) and tumors progressed.

Using TILs expanded from the same original culture, the patient was retreated with a culture of 3 x 100 million NFAT.IL12 gene-modified TILs and has an ongoing complete regression at 38 months of disease metastatic to lung and lymph nodes.
Overcoming the Challenges

A “blueprint” for the treatment of patients with T cells recognizing tumor-specific mutations.
Cancer Immunotherapy Based on Mutation-Specific CD4+ T Cells in a Patient with Epithelial Cancer

Eric Tran, Simon Turcotte, Alena Gros, Paul F. Robbins, Yong-Chen Lu, Mark E. Dudley, John R. Wunderlich, Robert P. Somerville, Katherine Hogan, Christian S. Hinrichs, María R. Parkhurst, James C. Yang, Steven A. Rosenberg

Patient with metastatic cholangiocarcinoma

Identified a T cell contained (CD4+ T helper 1 (TH1) cells) recognizing a mutation in erbb2 interacting protein (ERBB2IP) expressed by the cancer.

ACT of TIL containing about 25% of the mutation-specific T-cells

the patient achieved a decrease in target lesions with prolonged stabilization of disease.

The patient was retreated with a >95% pure population of mutation-reactive T cells
Naturally-occurring neo-epitope specific CD8+ T cells ARE detected in Ovarian Cancer Patients

19 Patients
1300 non-synonymous somatic mutations
Average of 69 somatic substitutions/patient
776 (9mer or 10mer) peptide neoepitopes were predicted to bind w/ high affinity to HLA-1

one-third (6/19)

Sara Bobisse
Alex Harari
George Coukos

Kandalaft et al, submitted
Ovarian TILs recognize tumor neo-epitopes

<table>
<thead>
<tr>
<th>patient</th>
<th>somatic mutations</th>
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<td></td>
<td></td>
<td>HLA-C07:02</td>
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<tr>
<td>total</td>
<td>10</td>
<td></td>
<td>16</td>
<td></td>
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</tbody>
</table>

% IFN-γ+ cells (gated on CD8+)

Annalisa Roberti
Brian Stevenson
Christian Iseli
Sara Bobisse
Alex Harari
George Coukios
Tumor-specific T cell therapy
Lung cancer

Extraction of TILs

Identification and selection of mutation-specific TILs

Expansion

Transfer

Solange Peters
Tu Nguyen
Angela Orcurto
Krisztian Homicsko
Lana Kandalaft
Alex Harari
Urania Dafni
George Coukos
OPTION 2: Adoptive T cell Therapy with Genetically Engineered Peripheral Blood Lymphocytes.
TCRs are composed of one α chain and one β chain, and they recognize antigens that have been processed and presented by one of the patient’s own MHC molecules.
CARs are artificial receptors that can be constructed by linking the variable regions of the antibody heavy and light chains to intracellular signaling chains (such as CD3-zeta, CD28, 41BB) alone or in combination with other signaling moieties.

CARs recognize antigens that do not need to be MHC-restricted, but they must be presented on the tumor cell surface.
Generations of CARs

First generation
- CD8
- CD3ζ
- CD28

Second generation
- TRAF1
- CD137

Third generation
- PI3K
- GRB2
- VAV
- LAT
- SLP76
- PLCγ
- mTOR
- IκB

Signal intermediates
- ASK1
- MKK
- MAPK
- RAS
- CAN
- PKCα

Transcription factors
- ATF2
- NFAT
- NF-κB

Proliferation, survival and cytokine production

Nature Reviews | Cancer
Genetically Modified 'Serial Killer' T-Cells Obliterate Tumors in Leukemia Patients

ScienceDaily (Aug. 10, 2011) — In a cancer treatment breakthrough 20 years in the making, researchers from the University of Pennsylvania's Abramson Cancer Center and Perelman School of Medicine have shown sustained remissions of up to a year among a small group of advanced chronic lymphocytic leukemia (CLL) patients treated with genetically engineered versions of their own T cells. The protocol, which involves removing patients' cells and modifying them in Penn's vaccine production facility, then infusing the new cells back into the patient's body following chemotherapy, provides a tumor-attack roadmap for the treatment of other cancers including those of the lung and ovaries and myeloma and melanoma.

The findings, published simultaneously in the New England Journal of Medicine and Science Translational Medicine on August 10, are the first demonstration of the use of gene transfer therapy to create "serial killer" T cells aimed at cancerous tumors.

"Within three weeks, the tumors had been blown away, in a way that was much more violent than we ever expected," said senior author Carl June, MD, director of Translational Research and a professor of Pathology and Laboratory Medicine in the Abramson Cancer Center, who led the work. "It worked much better than we thought it would."

The results of the pilot trial of three patients are a stark contrast to existing therapies for CLL. The patients involved in the new study had few other treatment options. The only potential curative therapy would have involved a bone marrow transplant, a procedure which requires a lengthy hospitalization and carries at least a 20 percent mortality risk -- and even then offers only about a 50 percent chance of a cure, at best.
CAR and TCR Cancer Clinical Trials in the US 1994 - 2014

The CD19 CAR T Cell Success Story for relapsed ALL and CLL

- Complete remission and long-term responses in up to 90% of acute lymphoblastic leukemia (ALL) patients (both adult and pediatric)
- And in > 50% of chronic lymphocytic leukemia (CLL) patients.
- On target side effects include B cell aplasia and cytokine release syndrome.

Emily Whitehead

Maud et al, NEJM 2015 & Blood 2015
Tumor Therapy with Engineered T Cells

Porter *NEJM* 2011
Adoptive transfer of TCR-transferred T cells

NYESO-1 TCR
Sarcoma Patient

2017

Robbins JCO 2011
Melanoma Regression in Patients after Transfer of Genetically Engineered Lymphocytes (TCR)

A

B

C

D

E

F

Science 314, 126-129, 2006
Treatment of Patients with Metastatic Synovial Cell Sarcoma with Autologous T Cells Expressing TCRs Specific for NY-ESO-1

Treatment of Patient with Unresectable Pulmonary Mass

<table>
<thead>
<tr>
<th>Baseline</th>
<th>1 month post</th>
<th>2 months post</th>
<th>4.5 months post</th>
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<td>11/20/14</td>
<td>12/19/14</td>
<td>1/28/15</td>
<td>4/20/15</td>
</tr>
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Mass Remains Nearly Undetectable ~1.5 year Since Treatment

Images kindly provided by Crystal L. Mackall MD, Director Cancer Immunology and Immunotherapy Program, Stanford Cancer Institute
A phase I clinical trial of adoptive transfer of folate receptor-alpha redirected autologous T cells for recurrent ovarian cancer

Lana E Kandalaf, Daniel J Powell Jr and George Coukos

LIMITATIONS??
Challenges for CAR Therapy

- Limitation: Antigen targets with surface expression are often not specific
- More specific targets are intracellular (NYESO-1)
- Heterogeneous expression of tumor antigen in solid tumors
- Suppressive tumor microenvironment factors
- Highly TOXIC
- Expensive (personalized) and infrastructure is required
Future Approaches

• Armored CARs
  – IL-12 secreting

• Combination Therapy
  – PD-1/PDL-1

• Suicide Switch : Abrogate CRS

• Finding cleaner Targets
  – To avoid on target toxicities
June 2015 - 11 month old Layla with ALL has been treated in London for the first time with “off the shelf” allogenic TALEN gene-edited CD19 CAR T cells from Cellectis (UCART19: TCR expression is disrupted and CD52 is targeted so that the donor cells are insensitive to Alemtuzumab)

Second baby gets Cellectis "designer" cells to clear leukaemia

A second baby with aggressive leukaemia has been treated in London with "designer immune cells" developed by Cellectis and, six months after treatment, remains in remission, the French biotech firm said.

Cellectis shares jumped 14 percent following Friday's news.

More time is needed to see whether the therapy has cured the disease, or simply slowed its progression. But the fact that Layla is still doing well 11 months after her injection and the second case has so far been successful is encouraging.
Chimeric Antigen Receptor– and TCR-Modified T Cells Enter Main Street and Wall Street

David M. Barrett,* Stephan A. Grupp,*† and Carl H. June†‡

- University of Pennsylvania with Novartis
- Baylor College of Medicine with Bluebird Bio and Celgene
- Memorial Sloan Kettering Cancer Center and the Fred Hutchinson Cancer Research Center with Juno Therapeutics
- the National Cancer Institute with Kite Pharma
- the Cellular Biomedicine Group with the Chinese PLA General Hospital
THANK YOU
T cell-based Clinical Trials in the USA versus the EU

<table>
<thead>
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<th>Table 1 Ongoing clinical trials with TCR- or CAR-modified T cells</th>
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<td><strong>Targeted antigens</strong></td>
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<tr>
<td>TCR based ((n=13))</td>
</tr>
<tr>
<td>NY-ESO-1 ((n=6)); MAGE-A3 ((n=2));</td>
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<tr>
<td>WT-1 ((n=2)); MART-1 ((n=1)); miscellaneous ((n=2))</td>
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<td>CAR based ((n=52))</td>
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<td>CD19 ((n=27)); GD2 ((n=4)); mesothelin</td>
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<td>HER2 ((n=3)); miscellaneous ((n=14))</td>
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de Witte et al, Cancer Immunol Immunother 2015
Novel Two-Step Immunotherapy Shows Promise in Early-Stage Ovarian Cancer Study

Anna Azvolinsky, PhD | July 09, 2013
Whole Tumor Antigen Dendritic Cell Vaccine Study

1. Debulking
2. Tumor cells
3. HOCL Lysate
4. Apheresis
5. Monos
6. GM-CSF + IL-4
7. Immature DC
8. Pulsing with Whole Tumor Antigen
9. LPS + IFN-9
10. DC Vaccinations
Adoptive T-cell Study

1. Dendritic cell vaccinations administered to patient
2. Apheresis
3. T Cell expansion
4. 3 months later

Kandalaft et al, OncolImmun. 2013
Patients’ Tumor Reactive T Cells Correlate with Clinical Outcome

Kandalaft et al, OncoImmun. 2013