Cancer Immunotherapy: 
Toward adoptive T-cell therapy against cancer mutations 

The Cholangiocarcinoma Foundation Webinar 
Oct. 21, 2014 

Eric Tran, PhD 
Steven A. Rosenberg, MD, PhD
Adoptive cell therapy (ACT)

- A process by which immune cells are removed from a patient, grown/manipulated in the lab, and then reintroduced back into a patient.

- In adoptive cell therapy for cancer, this ideally involves the transfer of activated immune cells, usually T cells, that can target the cancer.

- Two major types of adoptive cell therapy:
  1. ACT using tumor-infiltrating lymphocytes (TIL)
  2. ACT using T cells derived from blood that have been genetically modified with anti-tumor receptors.
Adoptive cell therapy (ACT) using tumor-infiltrating lymphocytes (TIL)

Adoptive transfer of TIL can cure some patients with metastatic melanoma
Adoptive cell therapy (ACT) with gene-modified T cells

Targeting B-cell leukemias and lymphomas with T cells genetically modified with receptors that target CD19

Adoptive transfer of T cells genetically modified with receptors targeting CD19 can mediate regression in patients with B-cell cancers

Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19
Kochenderfer et al., 2010 Nov 18, 116, 20
First report demonstrating efficacy on a patient with follicular lymphoma

Chemotherapy-Refractory Diffuse Large B-Cell Lymphoma and Indolent B-Cell Malignancies Can Be Effectively Treated With Autologous T Cells Expressing an Anti-CD19 Chimeric Antigen Receptor
Kochenderfer et al., 2014 Aug 25. pii: JCO.2014.56.2025
Response rate: 13/15 (8 complete, 4 partial, 1 stable)

Chimeric Antigen Receptor T Cells for Sustained Remissions in Leukemia
Maude et al., 2014 Oct 16;371(16):1507-17
27/30 patients with ALL achieved a complete response

Efficacy has also been demonstrated in chronic lymphocytic leukemia (CLL): e.g., Kochenderfer et al., Blood, 22 March 2012, 119; 12, 2709; and Porter et al., N Engl J Med, August 25, 2011; 365:725-733
Why can’t we use a similar approach to treat common solid cancers?

• Unlike B-cell leukemias and lymphomas where almost all these cancers express the CD19 molecule, so far we have been unable to find a suitable target that is frequently expressed in a large percentage of solid cancers.

• Some solid cancers over-express certain proteins (e.g., carcinoembryonic antigen, CEA, in colorectal cancers)
  • Can we use T cells genetically engineered with receptors that target these over-expressed molecules to successfully treat cancer patients?
Why can’t we use a similar approach to treat common solid cancers?

T Cells Targeting Carcinoembryonic Antigen Can Mediate Regression of Metastatic Colorectal Cancer but Induce Severe Transient Colitis

Colonoscopies prior to and after transfer of T cells targeting CEA

• T cells can destroy normal cells/tissues if they express the targeted molecule

• The current lack of common targets that are expressed by most solid tumors, and not on normal essential tissues, limits the use of genetically modified T cells to treat these cancers

• Are there other options?
Adoptive transfer of TIL can cure some patients with metastatic melanoma

Can TIL therapy be effective in other more common solid cancers?
The gastrointestinal (GI) cancer TIL clinical trial (NCT01174121)

• A phase II study using short-term cultured autologous tumor-infiltrating lymphocytes following a lymphodepleting regimen in metastatic digestive tract cancers (ClinicalTrials.gov identifier: NCT01174121)

• **Basic eligibility requirements**
  - 18-66 years of age with metastatic cancer originating from the digestive tract (esophagus, stomach, pancreas, colon, rectum, liver or bile ducts)
  - Normal basic laboratory values
  - Refractory to approved standard systemic therapy
  - Good performance status (ECOG 0 or 1)

• **AIM:** To determine if autologous TIL infused in conjunction with high dose IL-2 following lymphodepletion can mediate tumor regression in patients with metastatic digestive tract adenocarcinomas

• (Note: Protocol was recently revised to include patients with metastatic breast, ovarian/endometrial, and urothelial cancers)
Trial overview

The gastrointestinal (GI) cancer TIL clinical trial (NCT01174121)

-40 to -30

Surgery
TIL harvest

-7

CTX: qd x 2
Flu: qd x 5

0

TIL growth

TIL infusion

IL-2 (q8h), max 15

28+

Post treatment evaluation

CTX: cyclophosphamide
Flu: fludarabine
Conventional TIL therapy is largely ineffective against metastatic GI cancers

<table>
<thead>
<tr>
<th>Patient</th>
<th>Primary</th>
<th>Cells ($x10^9$)</th>
<th>IL-2 doses</th>
<th>Response</th>
<th>PD: progressive disease</th>
<th>SD: stable disease</th>
<th>PR: partial response</th>
</tr>
</thead>
<tbody>
<tr>
<td>3454</td>
<td>Colorectal</td>
<td>18.5</td>
<td>8</td>
<td>PD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3596</td>
<td>Colon</td>
<td>32.1</td>
<td>10</td>
<td>PD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3610</td>
<td>Rectal</td>
<td>20.0</td>
<td>3</td>
<td>PD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3671</td>
<td>Colon</td>
<td>30.3</td>
<td>3</td>
<td>PD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3674</td>
<td>Colorectal</td>
<td>69.5</td>
<td>1</td>
<td>PD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3690</td>
<td>Colon</td>
<td>50.0</td>
<td>7</td>
<td>PD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3717</td>
<td>Gastric</td>
<td>68.8</td>
<td>0</td>
<td>PD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3737</td>
<td>Cholangio</td>
<td>42.4</td>
<td>4</td>
<td>PD (13 mo SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3788</td>
<td>GE junction</td>
<td>98.1</td>
<td>3</td>
<td>PD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3812</td>
<td>Cholangio</td>
<td>45.2</td>
<td>3</td>
<td>PD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3894</td>
<td>Colon</td>
<td>67.8</td>
<td>3</td>
<td>PD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3942</td>
<td>Rectal</td>
<td>68.3</td>
<td>2</td>
<td>PD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3948</td>
<td>Esophageal</td>
<td>97.3</td>
<td>2</td>
<td>PR (unofficial)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3970</td>
<td>Colon (Lynch)</td>
<td>90</td>
<td>2</td>
<td>not evaluable</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3971</td>
<td>Colon</td>
<td>40.8</td>
<td>4</td>
<td>PD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3978</td>
<td>Cholangio</td>
<td>78.5</td>
<td>4</td>
<td>PD</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Metastatic cholangiocarcinoma (liver, lung)
- Prior Tx: cisplatin + gemcitibine, gemcitibine, taxotere
- T-cell therapy with 42.4 billion TIL, 4 doses of IL-2
- Max. tumor regression: 30% (for one month), stable disease for ~13 months

- Did the TIL contribute to disease stabilization?
- What could the T cells be targeting?
T cells recognizing cancer mutations may play a major role in the efficacy of T-cell therapy in melanoma patients

Efficient Identification of Mutated Cancer Antigens Recognized by T Cells Associated with Durable Tumor Regressions

Mining exomic sequencing data to identify mutated antigens recognized by adoptively transferred tumor-reactive T cells
Robbins et al. NATURE MEDICINE VOLUME 19 | NUMBER 6 | JUNE 2013

Mutated PPP1R3B Is Recognized by T Cells Used To Treat a Melanoma Patient Who Experienced a Durable Complete Tumor Regression
Lu et al. The Journal of Immunology 2013, 190: 6034–6042

What is a mutation?
Mutation: a change in the DNA sequence in the genome of a cell

All cancers contain mutations or other genetic alterations

Normal cells

Cancer cells

Could TIL from patient 3737 recognize tumor mutations?
Assessing T-cell reactivity against mutated antigens

1. Isolate DNA from the tumor.
2. Whole exome sequencing to identify mutations.
3. Make gene constructs (tandem minigenes, TMGs) that contain all the mutations.
4. Introduce TMGs into antigen presenting cells (APCs).
5. Expand TIL cells.
6. Co-culture TIL with TMG-loaded APCs.
7. Assess T-cell activation.

Autologous APC + TIL = Assess T-cell activation.
**Whole exome sequencing identifies 26 mutations in a lung metastasis**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutation Position</th>
<th>Nucleotide (genomic)</th>
<th>Amino Acid (protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALK</td>
<td>chr2_29996620-29996620_C_T</td>
<td></td>
<td>137R&gt;H</td>
</tr>
<tr>
<td>AR</td>
<td>chrX_66858483-66858483_C</td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>CD93</td>
<td>chr20_23012929-23012929_C_T</td>
<td></td>
<td>634R&gt;Q</td>
</tr>
<tr>
<td>DIP2C</td>
<td>chr10_365545-365545_C_T</td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>ERBB2IP</td>
<td>chr5_65385316-65385316_A_G</td>
<td></td>
<td>805E&gt;G</td>
</tr>
<tr>
<td>FCER1A</td>
<td>chr1_157544227-157544227_G_C</td>
<td></td>
<td>219D&gt;H</td>
</tr>
<tr>
<td>GRXCR1</td>
<td>chr4_42590102-42590102_C_T</td>
<td></td>
<td>21A&gt;V</td>
</tr>
<tr>
<td>HLA-DOA</td>
<td>chr6_33085209-33085209_C_T</td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>KIF9</td>
<td>chr3_47287859-47287859_T_C</td>
<td></td>
<td>155T&gt;A</td>
</tr>
<tr>
<td>KLHL6</td>
<td>chr3_184692410-184692413_CAGA_</td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>LHX9</td>
<td>chr1_196164923-196164923_A</td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>LONRF3</td>
<td>chrX_118007666-118007666_A_C</td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>NAGS</td>
<td>chr17_39440355-39440355_G_A</td>
<td></td>
<td>412R&gt;H</td>
</tr>
<tr>
<td>NLRP2</td>
<td>chr19_60186650-60186650_G_T</td>
<td></td>
<td>591S&gt;I</td>
</tr>
<tr>
<td>PDZD2</td>
<td>chr5_32124833-32124833_A</td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>POU5F2</td>
<td>chr5_93102847-93102847_A_C</td>
<td></td>
<td>60V&gt;G</td>
</tr>
<tr>
<td>RAC3</td>
<td>chr17_77584690-77584690_C_A</td>
<td></td>
<td>125T&gt;N</td>
</tr>
<tr>
<td>RAP1GDS1</td>
<td>chr4_99532209-99532209_C_A</td>
<td></td>
<td>198L&gt;I</td>
</tr>
<tr>
<td>RASA1</td>
<td>chr5_86703757-86703757_C_T</td>
<td></td>
<td>589R&gt;C</td>
</tr>
<tr>
<td>RETSAT</td>
<td>chr2_85424308-85424308_C_T</td>
<td></td>
<td>553R&gt;K</td>
</tr>
<tr>
<td>SEC24D</td>
<td>chr4_119872085-119872085_A_G</td>
<td></td>
<td>901M&gt;T</td>
</tr>
<tr>
<td>SENP3</td>
<td>chr17_7408824-7408824_A_G</td>
<td></td>
<td>292M&gt;V</td>
</tr>
<tr>
<td>SLIT1</td>
<td>chr10_98753840-98753840_G_C</td>
<td></td>
<td>1280N&gt;K</td>
</tr>
<tr>
<td>TARBP1</td>
<td>chr1_232649342-232649342_C_A</td>
<td></td>
<td>655G&gt;V</td>
</tr>
<tr>
<td>TGM6</td>
<td>chr20_2332325-2332325_G_A</td>
<td></td>
<td>398D&gt;N</td>
</tr>
<tr>
<td>TTC39C</td>
<td>chr18_19966475-19966475_A_C</td>
<td></td>
<td>503N&gt;T</td>
</tr>
</tbody>
</table>

Three tandem minigenes (TMGs) were made containing all mutations

<table>
<thead>
<tr>
<th>TMG-1</th>
<th>TMG-2</th>
<th>TMG-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALK</td>
<td>RAP1GDS1</td>
<td>SENP3</td>
</tr>
<tr>
<td>CD93</td>
<td>RASA1</td>
<td>LHX9</td>
</tr>
<tr>
<td>ERBB2IP</td>
<td>RETSAT</td>
<td>KLHL6</td>
</tr>
<tr>
<td>FCER1A</td>
<td>SEC24D</td>
<td>AR</td>
</tr>
<tr>
<td>GRXCR1</td>
<td>SLIT1</td>
<td>PDZD2</td>
</tr>
<tr>
<td>KIF9</td>
<td>TARBP1</td>
<td>HLA-DOA</td>
</tr>
<tr>
<td>NAGS</td>
<td>TGM6</td>
<td>LONRF3</td>
</tr>
<tr>
<td>NLRP2</td>
<td>TTC39C</td>
<td></td>
</tr>
<tr>
<td>RAC3</td>
<td>POU5F2</td>
<td></td>
</tr>
</tbody>
</table>

**Mutation**

- **12 AA**
  - **QNAADSYSWPE**
  - **AESRAMENQYSP**

**Minigene**

- Tandem minigene (variable # of minigenes)

**Assess T-cell activation**

- **T cells used for treatment**
  - Co-culture
  - Autologous APC

**Three tandem minigenes (TMGs) were made containing all mutations**

- TMG-1: ALK, CD93, ERBB2IP, FCER1A, GRXCR1, KIF9, NAGS, NLRP2, RAC3
- TMG-2: RAP1GDS1, RASA1, RETSAT, SEC24D, SLIT1, TARBP1, TGM6, TTC39C, POU5F2
- TMG-3: SENP3, LHX9, KLHL6, AR, PDZD2, HLA-DOA, LONRF3

**Tandem minigene (variable # of minigenes)**

**Mutation**

- **12 AA**
  - **QNAADSYSWPE**
  - **AESRAMENQYSP**

**Minigene**

- Tandem minigene (variable # of minigenes)

**Assess T-cell activation**

- **T cells used for treatment**
  - Co-culture
  - Autologous APC
Only TMG-1 is recognized by T cells in 3737 infusion bag

Co-culture exp’’t (transfected DCs + Infusion bag T cells):
A) IFN-γ ELISPOT assay

A

Mock | TMG-1 | TMG-2 | TMG-3

IFN-γ ELISPOT: Bachini infusion bag co-culture with dendritic cells electroporated with indicated
Mock | TMG-1 | TMG-2 | TMG-3

0 100 200 300
est. 50K DC per well

IFN-γ spots/1e3 cells

T-cell activation
Only mutated ERBB2IP is recognized by T cells in 3737 infusion bag

Co-culture exp’t (DCs expressing TMGs + Infusion bag): IFN-γ ELISPOT assay

TMG-1 (mutated genes)
ALK
CD93
ERBB2IP
FCER1A
GRXCR1
KIF9
NAGS
NLRP2
RAC3

~25% of the infusion bag T cells were reactive against mutated ERBB2IP
These T cells do not react against wild-type (normal) ERBB2IP

Gene reverted to wt

wt = wild-type (normal sequence)
Disease progression after about 1 year after first T-cell treatment

Cell Infusion
(42.4 billion cells; ~25% mutation-reactive T cells)

Can we do better?
Re-treatment with a highly pure population of mutation-reactive T cells

- 1st treatment infusion bag T cells were made up of 5 different T-cell cultures
  - F1, F2, F3, PF1, G-Macs

- Approximate % of ERBB2IP mutation-reactive T cells prior to expansion
  - F1: 0.7%
  - **F2: 89%**
  - F3: 0.6%
  - PF1: 2.8%
  - G-Macs: 4.4%

This T-cell culture was exclusively expanded for re-treatment

After expansion, ~95% of the cells were reactive to mutated ERBB2IP

2nd treatment: 126 billion T cells, ~95% (120 billion) ERBB2IP mutation reactive
Tumor regression after treatment with ERBB2IP-mutation-reactive T cells

Official Partial Response (first, and only, responder on our trial)
<table>
<thead>
<tr>
<th>Patient</th>
<th>Cancer</th>
<th># of mutations assessed</th>
<th>Mutation Reactive T cells detected?</th>
<th>Mutated gene recognized</th>
<th>T cell</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>3737</td>
<td>Cholangio</td>
<td>25</td>
<td>Y</td>
<td>ERBB2IP</td>
<td>CD4</td>
<td>Multiple clonotypes; TCRs isolated</td>
</tr>
<tr>
<td>3812</td>
<td>Cholangio</td>
<td>179</td>
<td>N</td>
<td>NUP98</td>
<td>CD8</td>
<td>High background in TIL</td>
</tr>
<tr>
<td>3942</td>
<td>Rectal</td>
<td>140</td>
<td>Y</td>
<td>NUP98, KARS, GPD2</td>
<td>CD8, CD8</td>
<td>TCRs isolated</td>
</tr>
<tr>
<td>3948</td>
<td>Esophageal</td>
<td>210</td>
<td>Y</td>
<td>PLEC, ASTN2</td>
<td>CD4, CD4</td>
<td></td>
</tr>
<tr>
<td>3971</td>
<td>Colon</td>
<td>119</td>
<td>Y</td>
<td>CASP8</td>
<td>CD8</td>
<td>TCR isolated</td>
</tr>
<tr>
<td>3978</td>
<td>Cholangio</td>
<td>37</td>
<td>Y</td>
<td>ITGB4</td>
<td>CD4</td>
<td></td>
</tr>
<tr>
<td>4007</td>
<td>Colon</td>
<td>265</td>
<td>Y</td>
<td>SKIV2L, H3F3B</td>
<td>CD8, CD8</td>
<td>Two clonotypes for SKIV2L; TCRs isolated; potential low freq. CD4</td>
</tr>
</tbody>
</table>

In most cases, the frequency of mutation-reactive cells in expanded TIL is relatively low
Adoptive cell therapy with TIL can cure some patients (~20%) with metastatic melanoma.

Adoptive cell therapy using T cells engineered with receptors targeting CD19 can be highly effective against B-cell leukemias and lymphomas.

However, most solid cancers do not express a similar/suitable molecule that we can target with gene-engineered T cells. This limits the use of this approach for these cancers.

Unlike melanoma, conventional TIL therapy is not very effective against metastatic gastrointestinal cancers.

In a patient with cholangiocarcinoma, the transfer of a highly pure population of T cells targeting a unique mutation expressed by a patient’s tumors resulted in tumor regression.

Conclusions
Most patients with metastatic gastrointestinal cancers seem to mount a T-cell response against at least one mutation expressed by their tumors, although the overall frequency of mutation-reactive T cells appears to be relatively low.

**Conjecture**

The identification and specific targeting of mutations unique to each patient’s tumors may be a way to extend T-cell therapy to common epithelial cancers.
The future of T-cell therapy for common solid cancers?
Using Patients’ Own Immune System to Knock-out Cancer:

Adoptive Cell Therapy

Presenters:
Steven A. Rosenberg, M.D., Ph.D., National Cancer Institute
Eric Tran, Ph.D., National Institutes of Health Surgery Branch, Tumor Immunology Section
Melinda Bachini, CCF Patient Advocate and NIH Research Study Participant