T cells as a Drug for the Personalized Immunotherapy of Cancer

BSA/NCAB Meeting

June 26, 2018

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Surgery Branch, National Cancer Institute
Major Challenge Confronting Cancer
Immunotherapy

The development of effective immunotherapies for patients with metastatic epithelial solid cancers that result in over 80% of cancer deaths.
ADVANTAGES OF CELL TRANSFER THERAPY

1. Administer large numbers of highly selected cells with high avidity for tumor antigens.

2. Administer cells activated ex-vivo to exhibit anti-tumor effector function.

3. Potentially identify exact cell subpopulations and effector functions required for cancer regression in vivo.

4. Manipulate host prior to cell transfer to provide altered environment for transferred cells.
Adoptive cell therapy (ACT) using tumor-infiltrating lymphocytes (TIL)
Summary of Cell Transfer Protocols for the Treatment of Patients with Metastatic Melanoma* (median f/u 6.3 years)

<table>
<thead>
<tr>
<th>Total number of patients (duration in months)</th>
<th>PR (31%)</th>
<th>CR (24%)</th>
<th>OR (55%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>194</td>
<td>60</td>
<td>46</td>
<td>106</td>
</tr>
</tbody>
</table>

- 84, 71+,70+,63+,58+,55+,51+,37, 152+,142+,137+,136+,136+
- 36, 28, 25, 22, 21, 19, 19+,14, 134+,132+,123+,121+,118+
- 14, 14, 14, 13, 12+,11, 11, 11, 114+,113+,113+,112+,110+
- 10, 10, 9, 9, 9, 9, 8, 8, 110+,108+,64+,63+,63+
- 7, 7, 7, 7, 6, 6, 6, 63+,62+,62+,62+,62+
- 6, 6, 5, 5, 5, 5, 5, 1+61+,61+,60+,60+,60+
- 4, 4, 4, 4, 4, 3, 3, 60+,59+,58+,57+,54+
- 3, 3, 3, 2, 53+,53+,50+,45+,45+
- 1+,14+,43+,39+,38+,27,19

*from four trials (5 groups) using different lymphodepleting regimens

(44 of 46 Complete Responders ongoing from 14 to 152 months)
(44 of 46 Complete Responders received a single treatment)
Overall Survival of Patients with Metastatic Melanoma Treated with Autologous Tumor Infiltrating Lymphocytes and IL-2
(NMA+/TBI 93 Sequential + 101 Randomized - Deaths due to melanoma)

(ACT appears able to eliminate the last melanoma cell)

(Median followup: 6.3 years)
Question

What do TIL recognize that enables the in vivo destruction of the last melanoma cell?

(Specific cancer regression in the absence of off-tumor on-target, toxicities in patients led us to explore the role of specific cancer mutations as the targets of TIL.)
For a mutation to be a cancer antigen it has to:

1) be processed intracellularly into a 9-11 amino acid peptide

2) the peptide must fit and be presented in the groove of one of the patient's surface MHC molecules

Thus, only rare mutations will be antigenic.
Antigen recognition by CD4$^+$ and CD8$^+$ T lymphocytes
Blueprint for the generation of mutation-reactive T-cells in common cancers

1. Isolate genomic DNA and RNA
2. Whole exome and transcriptome sequencing to identify mutations
3. Synthesize mutated tandem minigenes (TMGs) that encode 25mers containing all mutations and/or peptides
4. Expand TIL (IL-2)

Tandem minigene (TMG):

String of minigenes encoding the mutated AA flanked by 12 AA

\[
\begin{array}{c}
\text{RVLKGGGSVRKLR} \\
\text{mutation} \\
\text{H} \\
\text{AKQLVLELGEEA}
\end{array}
\]
Blueprint for the generation of mutation-reactive T-cells in common cancers

1. Isolate genomic DNA and RNA
2. Whole exome and transcriptome sequencing to identify mutations
3. Synthesize mutated tandem minigenes (TMGs) that encode 25mers containing all mutations and/or peptides
4. Introduce TMGs into APCs
5. Pulse peptides onto APCs
6. Expand TIL (IL-2)
7. 1) IFN-γ ELISPOT
8. 2) Flow cytometry for 4-1BB/OX40 upregulation

Autologous antigen presenting cells (APC), e.g., dendritic cells
Tandem minigene (TMG):
String of minigenes encoding the mutated AA flanked by 12 AA

Advantages of this approach:

No need to predict peptide binding to MHC.

All candidate peptides and all MHC loci are included in the screen.

No tumor cell line necessary.

Minigene approach: J. bulk TILs recognize tandem minigene RJ-1

71 non-synonymous mutations
12 tandem minigene constructs
HLA-A*0205
Kinesin family member 2C (KIF2C) also known as mitotic centromere-associated Kinesin (MCAK)
### Immunogenic Mutations in Patients with Melanoma

<table>
<thead>
<tr>
<th>Patients evaluated (number)</th>
<th>Median (number of mutations)</th>
<th>Total</th>
<th>Screened</th>
<th>Immunogenic neoepitopes</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>318</td>
<td>13664</td>
<td>3938</td>
<td>54</td>
</tr>
</tbody>
</table>

Patients with mutation reactive T cells in TIL: 
\[ \frac{18}{22} = 82\% \]

Immunogenic mutations of number screened: 
\[ \frac{54}{3938} = 1.4\% \]

- 6% CD4
- 94% CD8

All neoantigens were unique, none shared.

(updated 6/18)
63% of Patients with Melanoma Recognized Two or More Immunogenic Mutations

<table>
<thead>
<tr>
<th># neoantigens per patient</th>
<th># patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4 (18%)</td>
</tr>
<tr>
<td>1</td>
<td>4 (18%)</td>
</tr>
<tr>
<td>2</td>
<td>6 (27%)</td>
</tr>
<tr>
<td>3</td>
<td>4 (18%)</td>
</tr>
<tr>
<td>&gt;3</td>
<td>4 (18%)</td>
</tr>
</tbody>
</table>

Immunogenic mutations 54/3938 = 1.4%

(updated 6/18)
Preliminary Conclusion

Adoptive cell therapy mediates complete, durable, and likely curative, regressions of metastatic melanoma based on the recognition of immunogenic cancer mutations.

Can this insight be used to develop a “blueprint” for the treatment of common epithelial cancers?
Immunotherapy for Cancer Using Checkpoint Modulators

(Alexandrov et al, Nature 2013)
The common epithelial cancers such as those arising in the colon, liver, stomach, pancreas, prostate, ovary, etc very rarely respond to current immunotherapies and account for over 80% of cancer deaths.

Are there antigens on the common epithelial human cancers that can be targeted by cell-based immunotherapy?
Patient M.B.

45 y.o. female with metastatic cholangiocarcinoma

12/2009  Right hepatectomy for cholangiocarcinoma

4/2010  Multiple lung and liver metastases
        Received cisplatin and gemcitabine: PD

5/2011  Taxotere chemotherapy: PD in lung and liver

3/2012  Unselected TIL from resected lung lesion infused; PD

10/2013  TMG approach to target unique cancer mutations (26)

Ongoing response and living normally 56 months later.
(Tran et al, Science 344:641-5, 2014)
Isolation of ERBB2IP reactive cells

Use enriched ERBB2IP autologous lymphocytes for treatment

Objective response of lung and liver metastases ongoing for 53 months

(Tran et al, Science 344:641-5, 2014)
Response to Pembrolizumab after ACT Targeting of ERB2IP; Ongoing overall response at 4 years

Pre-Treatment

6 Months
## Immunogenic Mutations in Patients with Gastrointestinal Cancers

<table>
<thead>
<tr>
<th>Patients evaluated (number)</th>
<th>Median (number of mutations)</th>
<th>Total</th>
<th>Screened</th>
<th>Immunogenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>72</td>
<td>113</td>
<td>10261</td>
<td>7496</td>
<td>120</td>
</tr>
</tbody>
</table>

Immunogenic mutations of number screened: $\frac{120}{7496} = 1.6\%$

49% CD8  
51% CD4

All neoantigens were unique except 2 patients shared the same KRAS mutation.

(updated 6/18)
50% of Patients with GI Cancers Recognized Two or More Immunogenic Mutations

Immunogenic mutations 120/7496 = 1.6%

<table>
<thead>
<tr>
<th># neoantigens per patient</th>
<th># patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>13 (19%)</td>
</tr>
<tr>
<td>1</td>
<td>22 (31%)</td>
</tr>
<tr>
<td>2</td>
<td>22 (31%)</td>
</tr>
<tr>
<td>3</td>
<td>9 (13%)</td>
</tr>
<tr>
<td>&gt;3</td>
<td>6 (9%)</td>
</tr>
</tbody>
</table>
# Mutated Antigens Recognized by TIL from 99 Patients with Epithelial Cancers

<table>
<thead>
<tr>
<th>Cancer</th>
<th># of patients screened</th>
<th># of patients with neoantigen reactivity</th>
<th>Total # of neoantigens recognized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorectal</td>
<td>45</td>
<td>39 (87%)</td>
<td>95</td>
</tr>
<tr>
<td>Cholangiocarcinoma</td>
<td>12</td>
<td>9 (75%)</td>
<td>20</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>6</td>
<td>5 (83%)</td>
<td>7</td>
</tr>
<tr>
<td>Esophageal</td>
<td>2</td>
<td>2 (100%)</td>
<td>3</td>
</tr>
<tr>
<td>Endometrial</td>
<td>3</td>
<td>3 (100%)</td>
<td>4</td>
</tr>
<tr>
<td>Breast</td>
<td>10</td>
<td>7 (70%)</td>
<td>22</td>
</tr>
<tr>
<td>NSCLC</td>
<td>11</td>
<td>8 (73%)</td>
<td>34</td>
</tr>
<tr>
<td>Ovarian</td>
<td>7</td>
<td>6 (86%)</td>
<td>16</td>
</tr>
<tr>
<td>Stomach</td>
<td>3</td>
<td>2 (67%)</td>
<td>5</td>
</tr>
</tbody>
</table>

| TOTAL              | 99                     | 81 (81.8%)                              | 197                              |

All neoantigens were unique except for 2 KRAS antigens.
Recognition of random somatic mutations is the “final common pathway” explaining cancer regression from most immunotherapies for solid cancers.

IL-2  
anti-CTLA4  
anti-PD1  
anti-CD40  
Tumor infiltrating lymphocytes
J.A. 51 year old female with metastatic breast cancer

- 2003: Localized Ductal Carcinoma in Situ; underwent mastectomy
- Aug. 2013: ER+, PR+ invasive breast cancer metastatic to multiple nodal groups, chest wall, bone
- Sept. 2013: Pacitaxel chemotherapy
- Feb. 2014: Arimidex
- Sept. 2014: Xeloda chemotherapy
- Oct. 2014: Navelbine chemotherapy
- Nov. 2014: Taxotere, Adriamycin, Cytoxan chemotherapy
- Jan. 2015: Lucitanib (TKI inhibitor)
- Sept. 2015: Everolimus (mTOR inhibitor)
- Dec. 2015: NCI for cell transfer immunotherapy targeting mutations expressed by her cancer (62 mutations)
  Received 80e9 cells plus 7 doses of IL-2 and 4 doses of Pembrolizumab
  She is now in an ongoing complete response of multiple nodal, chest wall, and liver metastases 30 months after treatment

Mutations Targeted in Patient with Metastatic Breast Cancer

SL3A2: 4F2 cell-surface heavy chain
   Function: Required for the function of light chain amino-acid transporters

KIA0368: Proteasome-associated protein ECM29 homolog
   Function: Adapter/scaffolding protein that binds to the 26S proteasome

CADPS2: Calcium-dependent secretion aviator 2 Calcium-binding protein
   Function: Involved in exocytosis of vesicles filled with neurotransmitters and neuropeptides

CTSB: Cathepsin B. Thiol protease
   Function: Which is believed to participate in intracellular degradation and turnover of proteins

23% of infused cells contained neoantigen reactivity. All 8 neoantigen TCR present in PBL at 6 weeks.
J.A.: ACT using autologous lymphocytes targeting somatic mutations
Isolation and in vivo Persistence of Mutation-reactive Cells in a Patient with Breast Cancer
Patient S.S.

36 y.o. female with metastatic cervical cancer

10/2011  Presented with fungating cervical mass, lung and intraperitoneal metastases

11/29/11  Radiation therapy and cisplatin chemotherapy

10/06/12  Cancer progressed. She underwent hysterectomy and excision of both ovaries

11/2012 to 1/2013  Developed liver, lymph node, intra-abdominal metastases and urinary tract obstruction requiring a stent

3/15/13  At NCI/Surgery Branch treated with cell transfer immunotherapy (75 billion of her own tumor infiltrating lymphocytes and IL-2)

Experienced complete regression of all disease including relief of urinary obstruction and remains disease-free 4 years later.

Patient S.S. with metastatic cervical cancer treated with cell transfer immunotherapy

Baseline

11 months
Patient C.R.

49 y.o. female with metastatic colon cancer

9/5/13  Sigmoid colectomy, partial cystectomy
         Multiple lung metastases

5/14/14  Radiotherapy to bladder suture line

9/13/14  FOLFOX chemotherapy: PD

3/29/15  Two lung metastases resected for TIL

7/1/15   TMG approach to target unique cancer mutations
         (61 somatic mutations including KRAS-G12D)
Patient CR (4095) with treatment refractory metastatic colorectal cancer

- Whole-exome and transcriptome sequencing performed on lung lesions
  - 61 putative mutations identified
  - 5 TMGs constructed

Rx:
- $1.48 \times 10^{11}$ cells, ~75% KRAS$^{G12D}$-reactive
- 5 doses IL-2

(Tran et al, New Engl J Med, 375, 2016)
Response after infusion with KRAS$^{G12D}$-reactive TIL

- 6/7 lesions regressed at 9 months post ACT
- 1 lesion (#3) progressed at 9 months; excised; patient NED 35 months after treatment
Progressing lesion exhibited copy number neutral loss of heterozygosity (LOH) at chromosome 6, which contains the HLA alleles.
Four different KRAS\textsuperscript{G12D}-reactive TCRs in patient infusion TIL

Specificity and Sensitivity of KRAS\textsuperscript{G12D}-Specific TCRs

(A) 50%  
(B) 19%  
(C) 7%  
(D) 0.1%

Frequency of TCR (%)  
Days relative to cell transfer

[Peptide] (nM)

Days relative to cell transfer

Four different KRAS\textsuperscript{G12D}-reactive TCRs in patient infusion TIL
Blueprint for Cancer Immunotherapy Directed Against the Common Epithelial Cancers

Target the immunogenic somatic mutations unique to the autologous patient’s cancer.

Raise a library of T-cell receptors against shared cancer mutations (e.g. Kras, p53)
Any intracellular protein can potentially be a “cancer antigen” if mutated and processed intracellularly to a peptide that can bind to the autologous MHC.

(About 1 in 70 mutated neoepitopes are neoantigens.)

Bad news: Treatment will be highly individualized and thus complex. 
Good news: Virtually all cancer patients are potentially eligible.
Adoptive cell transfer for patients with cancer

(Alexandrov et al, Nature 2013)
Potential improvements in targeting of somatic mutations in epithelial cancers

- Improve methods to identify multiple mutation targets expressed by tumor (robotics)
- Develop rapid methods for identifying mutation-reactive TCRs (robotics)
- Transduce mutation-reactive TCRs into naïve or CM cells (FDA approval 11-1-17 to use a GMP 293GP line to produce transient vectors with minimal testing)
- Add anti-PD-1 (reexpressed by infused cells in vivo) or other CPM
- Knockout CISH or PD-1 (or other inhibitory molecules) on transferred cells
- Vaccinate with mutations recognized by transferred cells
- Obtain mutation-reactive TCRs from circulating lymphocytes and mutations from paraffin sections or liquid DNA) – eliminate need for tumor resection
Cancer Antigens

1. Unique somatic mutations in an intracellular protein

2. Mutations in driver oncogenes or tumor suppressor genes that can be shared among patients.
   e.g.: Kras
   p53
   PIK3CA

3. Non-mutated proteins on the surface of cells that are not essential for survival and can be recognized by antibodies or specific ligands
   e.g.: CD19 (CAR: αCD19 antibody)
   CD70 (CAR: CD27 ligand)
KRAS Mutations and Human Cancers

KRAS protein is a GTPase essential for normal tissue signaling

Activating mutations are essential steps in the development of many cancers.

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Frequencies of KRAS mutation</th>
<th>% of All KRAS Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G12A</td>
<td>G12D</td>
</tr>
<tr>
<td>Pancreatic CA</td>
<td>70%</td>
<td>2</td>
</tr>
<tr>
<td>Colorectal</td>
<td>36%</td>
<td>7</td>
</tr>
<tr>
<td>Lung Adeno CA</td>
<td>20%</td>
<td>7</td>
</tr>
<tr>
<td>Endometrial</td>
<td>18%</td>
<td>11</td>
</tr>
<tr>
<td>Ovarian (EOC)</td>
<td>14%</td>
<td>4</td>
</tr>
<tr>
<td>Prostate</td>
<td>7%</td>
<td>2</td>
</tr>
</tbody>
</table>

Modified from Cosmic database by James Yang
Anti-Kras T-cell Receptors Isolated from Patients with Metastatic Cancer

<table>
<thead>
<tr>
<th>KRAS Mutation</th>
<th>Patient #</th>
<th>Cancer Diagnosis</th>
<th>CD4/8</th>
<th>HLA-restriction</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>G12D</td>
<td>4095</td>
<td>Colon</td>
<td>CD8</td>
<td>C<em>08:02 (8%)</em></td>
<td>TMG</td>
</tr>
<tr>
<td></td>
<td>4238</td>
<td>Colon</td>
<td>CD4</td>
<td>DR3*02 (16%)</td>
<td>IVS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CD4</td>
<td>DRB1*08:02 (5%)</td>
<td>IVS</td>
</tr>
<tr>
<td>G12V</td>
<td>4148</td>
<td>Endometrial</td>
<td>CD4</td>
<td>DRB1*07:01 (25%)</td>
<td>TMG</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CD8</td>
<td>A*11:01 (14%)</td>
<td>IVS</td>
</tr>
<tr>
<td>G12C</td>
<td>4173</td>
<td>Ovarian</td>
<td>CD4</td>
<td>DRB1*11.01 (10%)</td>
<td>IVS</td>
</tr>
<tr>
<td>G12R</td>
<td>4268</td>
<td>Colon</td>
<td>CD4</td>
<td>CLASS II</td>
<td>TMG</td>
</tr>
</tbody>
</table>

*allele frequency

(Gal Cafri, Rami Yoseph, submitted)
P53 Mutations in Human Cancer

Tumor suppressor gene

50% of human cancers contain a p53 mutation

Most frequently mutated gene in human cancers

Mutations occur throughout the gene but there are up to 10 major “hot spots”
A novel method to screen for T cell responses to p53 “hotspot” mutations

Synthesize one TMG (ten 25mers) encoding the top ten p53 “hotspot” mutations.

Synthesize the top 10 25mer peptides.

Coculture patient TIL with TMGs and peptides.
### 50% of p53 “hotspot” Mutations are Immunogenic

<table>
<thead>
<tr>
<th>Mutation</th>
<th># Pt</th>
<th># tested</th>
<th># immunogenic</th>
<th>% reactive of screened</th>
</tr>
</thead>
<tbody>
<tr>
<td>R175H</td>
<td>5</td>
<td>4</td>
<td>2</td>
<td>50.0%</td>
</tr>
<tr>
<td>Y220C</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>66.7%</td>
</tr>
<tr>
<td>G245D</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>G245S</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>100.0%</td>
</tr>
<tr>
<td>R248Q</td>
<td>6</td>
<td>6</td>
<td>3</td>
<td>50.0%</td>
</tr>
<tr>
<td>R248W</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>75.0%</td>
</tr>
<tr>
<td>R249S</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>R273C</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>R273H</td>
<td>5</td>
<td>4</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>R282W</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>66.7%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>32</strong></td>
<td><strong>26</strong></td>
<td><strong>13</strong></td>
<td><strong>50.0%</strong></td>
</tr>
</tbody>
</table>

T-cell receptors against common p53 “hotspot” mutations can potentially be used to treat multiple patients whose cancers express these mutations.

(P.Malekzadeh, submitted)
<table>
<thead>
<tr>
<th>Patient</th>
<th>Age / Sex</th>
<th>Cancer Type</th>
<th>TP53 mutation</th>
<th>T cell type</th>
<th>HLA restriction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>52M</td>
<td>Colon</td>
<td>R175H</td>
<td>CD8</td>
<td>A*02:01</td>
</tr>
<tr>
<td>2</td>
<td>36M</td>
<td>Colon</td>
<td>R175H</td>
<td>CD8</td>
<td>A*02:01</td>
</tr>
<tr>
<td>3</td>
<td>55M</td>
<td>Colon</td>
<td>R175H</td>
<td>CD4</td>
<td>Class-II</td>
</tr>
<tr>
<td>4</td>
<td>46M</td>
<td>Colon</td>
<td>R175H</td>
<td>CD4</td>
<td>DRB1*13:01</td>
</tr>
<tr>
<td>5</td>
<td>44F</td>
<td>Colon</td>
<td>Y220C</td>
<td>CD4</td>
<td>DRB1*04:01</td>
</tr>
<tr>
<td>6</td>
<td>39F</td>
<td>Ovary</td>
<td>Y220C</td>
<td>CD4</td>
<td>DRB3*02:02</td>
</tr>
<tr>
<td>7</td>
<td>58F</td>
<td>Ovary</td>
<td>G245S</td>
<td>CD4</td>
<td>DRB3*02:02</td>
</tr>
<tr>
<td>8</td>
<td>62M</td>
<td>Colon</td>
<td>R248Q</td>
<td>CD8</td>
<td>Class-I</td>
</tr>
<tr>
<td>9</td>
<td>69F</td>
<td>Colon</td>
<td>R248Q</td>
<td>CD4 and CD8</td>
<td>Class-I and -II</td>
</tr>
<tr>
<td>10</td>
<td>41F</td>
<td>Colon</td>
<td>R248W</td>
<td>CD8</td>
<td>A*68:01</td>
</tr>
<tr>
<td>11</td>
<td>49M</td>
<td>Rectal</td>
<td>R248W</td>
<td>CD4</td>
<td>DPB1*02:01</td>
</tr>
<tr>
<td>12</td>
<td>66F</td>
<td>Pancreas</td>
<td>R282W</td>
<td>CD4</td>
<td>Class-II</td>
</tr>
</tbody>
</table>
Cancer Antigens

1. Unique somatic mutations in an intracellular protein

2. Mutations in oncogenes or tumor suppressor genes that can be shared among patients.
   e.g.: Kras
   p53

3. Non-mutated proteins on the surface of cells that are not essential for survival and can be recognized by antibodies or specific ligands
   e.g.: CD19 (CAR: αCD19 antibody)
   CD70 (CAR: CD27 ligand)
Conclusions

Cell transfer therapy can mediate durable regressions in patients with metastatic cancer refractory to other treatments.

T-cells that recognize unique somatic mutations can be found in TIL and PBL in patients with common epithelial cancers.

Identification and targeting of mutations unique to each cancer or shared mutations such as KRAS or p53 has the potential to extend cell therapy to patients with common epithelial cancers.
# Treatment of Patients with Diffuse Large B-cell Lymphoma

<table>
<thead>
<tr>
<th></th>
<th>Objective Response (%)</th>
<th>Complete Response Total</th>
<th>Complete Response Ongoing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgery Branch</td>
<td>73%</td>
<td>47%</td>
<td>42%</td>
</tr>
<tr>
<td>Kite Pharma</td>
<td>82%</td>
<td>54%</td>
<td>40%</td>
</tr>
</tbody>
</table>

(FDA approval October, 2017)
Blueprint for Cancer Immunotherapy Directed Against the Common Epithelial Cancers

Target the immunogenic somatic mutations unique to the autologous patient’s cancer.

Raise a library of T-cell receptors against shared cancer mutations (e.g. Kras, p53)
## Mutation specific TCRs in Peripheral Blood Lymphocytes are Present in the Memory Subpopulations

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Patient</th>
<th>$T_N$</th>
<th>$T_{CM}$</th>
<th>$T_{EM}$</th>
<th>$T_{EMRA}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI</td>
<td>4213</td>
<td>None</td>
<td>None</td>
<td>SMAD5, (0.000812)</td>
<td>None</td>
</tr>
<tr>
<td>Ovarian</td>
<td>4097</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>4046</td>
<td>None</td>
<td>USPX, (0.001392)</td>
<td>USPX, (0.000776)</td>
<td>None</td>
</tr>
<tr>
<td>NSCLC</td>
<td>4014</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>4134</td>
<td>None</td>
<td>None</td>
<td>GRB7, (0.023095)</td>
<td>None</td>
</tr>
</tbody>
</table>
Isolation of KRAS mutation-reactive TCRs from cancer patients’ blood samples using IVS

- Approach overview

**imDC generation**

- PBMCs (day -5)
- Plastic adherent cells
- Immature DC’s + long peptides or TMG
- Antigen loaded immature DC’s

**Day 0: start of co-culture (IL-21)**

- Expand and add cytokine mixtures: IL2 and IL21 for memory, IL7 and IL15 for Naive

**Day 10: Re-stimulation with DC’s and enrichment for OX40+ and/or 41BB+ cells**

- If needed

**Screen against individual peptides or TMG’s**

**T cell subsets sort**

➢ Approach overview
The development of effective immunotherapies for the 80% of patients with metastatic epithelial solid cancers that cannot be cured by any available treatment.

Are there antigens on solid human cancers that can be targeted by cell-based immunotherapy?
Recognition of colon cancer by T-cells expressing a natural p53 mutation

**p53^{R248W}-specific T cells**

- no tumor cell line (TC)
- Autologous (auto.) TC
- auto. TC + αClass-I
- auto. TC + αClass-II
- auto. TC + peptide

<table>
<thead>
<tr>
<th>Condition</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>no tumor cell line (TC)</td>
<td>0.04%</td>
</tr>
<tr>
<td>Autologous (auto.) TC</td>
<td>51.9%</td>
</tr>
<tr>
<td>auto. TC + αClass-I</td>
<td>16.0%</td>
</tr>
<tr>
<td>auto. TC + αClass-II</td>
<td>51.4%</td>
</tr>
<tr>
<td>auto. TC + peptide</td>
<td>99.2%</td>
</tr>
</tbody>
</table>

41BB
Limiting dilution culturing of sorted TILs for enhanced detection of neoantigen-reactive cells
## Summary of neoantigen-reactive T cells identified by limiting dilution

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Age/Sex</th>
<th>Tumor histology</th>
<th>No. of mutation assessed</th>
<th>Reactivities found in TIL fragments screen†</th>
<th>T cell type</th>
<th>Reactivities found in LD cultures‡</th>
<th>T cell type</th>
<th>No. of reactive TCRs found using LD §</th>
</tr>
</thead>
<tbody>
<tr>
<td>4078</td>
<td>48/M</td>
<td>Gastroesophageal junction adenocarcinoma</td>
<td>104</td>
<td>None</td>
<td>CD4</td>
<td>GBAS[^{G202X}^]</td>
<td>CD8</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PLXNB3[^{W609G}^]</td>
<td>CD4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>DLAT[^{I928L}^]</td>
<td>CD4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TMPRSS5[^{I933I}^]</td>
<td>CD4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PSMD1[^{G544A}^]</td>
<td>CD4</td>
<td>1</td>
</tr>
<tr>
<td>4097</td>
<td>59/F</td>
<td>Ovarian</td>
<td>317</td>
<td>HIST1H1B[^{A74D}^]</td>
<td>CD4</td>
<td>HIST1H1B[^{E71D}^]</td>
<td>CD4</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>INPP5K[^{L157V}^]</td>
<td>CD4</td>
<td>HYAL4[^{G94S}^]</td>
<td>CD4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HSPG2[^{A95E}^]</td>
<td>CD4</td>
<td>1</td>
</tr>
<tr>
<td>4148</td>
<td>68/F</td>
<td>Endometrial</td>
<td>108</td>
<td>None</td>
<td>CD4</td>
<td>KRAS[^{Q61V}^]</td>
<td>CD4</td>
<td>1</td>
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<tr>
<td>4217</td>
<td>49/M</td>
<td>Colon</td>
<td>176</td>
<td>MAP3K2[^{I152F}^]</td>
<td>CD4</td>
<td>MAP3K2[^{I152F}^]</td>
<td>CD4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>UEVLD-1/2[^{F91V}^]</td>
<td>CD4</td>
<td>UEVLD-1/2[^{F91V}^]</td>
<td>CD4</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>RAD51B[^{D202X}^]</td>
<td>CD4</td>
<td>RAD51B[^{D202X}^]</td>
<td>CD4</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MUC4[^{A54355}^]</td>
<td>CD8</td>
<td>TBCK[^{R17S}^]</td>
<td>CD4</td>
<td>1</td>
</tr>
<tr>
<td>4227</td>
<td>58/F</td>
<td>Ovarian</td>
<td>180</td>
<td>TP53[^{G244S}^]</td>
<td>CD4</td>
<td>TP53[^{G244S}^]</td>
<td>CD4</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HIST1H2BM[^{T77V}^]</td>
<td>CD4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GORASP2[^{245R}^]</td>
<td>CD4</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TUBA1B[^{E227T}^]</td>
<td>CD4</td>
<td>1</td>
</tr>
<tr>
<td>4166</td>
<td>40/M</td>
<td>Pancreatic</td>
<td>156</td>
<td>NPLOC4[^{D12V}^]</td>
<td>CD8</td>
<td>ZNF727[^{W1830}^]</td>
<td>CD4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TNC[^{P1229}^]</td>
<td>CD4</td>
<td>4</td>
</tr>
</tbody>
</table>

| Total:     | 8       | CD8: 2                           | 19                        | CD8: 1                                     |
|           |        | CD4: 6                           |                           | CD4: 18                                    |

* At the time of admission  
† Neoantigen specificity was determined by testing against WT peptides  
‡ TCR that were constructed and tested are bolded  
§ FS Frame shift mutation  

- We were able to rediscover most of the neo-antigen reactivities found in fragment screenings  
- We identified substantial additional number of new reactivities that were missed when TIL fragments were screened  
- Limiting dilution cultures are clonal or highly oligoclonal, that makes the isolation faster and more reliable using scPCR techniques
Tumor biopsies represent the main source for the isolation of tumor-reactive and mutation-specific lymphocytes

PD-1 expression in the fresh tumor can guide the identification of tumor-reactive cells (Gros et al. JCI 2014)
Enrichment of tumor and mutation-reactive cells by sorting for CD8+PD1+ cells

$P < 0.005$

PBMC vs. 3713mel

IFN-γ spots/2e4 cells

% 4-1BB+ cells (of CD8+)

CD8+PD-1+ cells isolated from peripheral blood of Pt#3784 recognize three unique mutated antigens

FLNAmut: Filamin A (cytoskeleton remodeling)  
VVISQSEIGDASCVRVSGQGLHEGH

KIF16Bmut: Kinesin-like protein (intracellular trafficking)  
REKQQREALERAPARLERRHSALQR

SONmut: DNA binding protein (promotes pre-mRNA splicing)  
RKTVRARSTPSCRSRSHTPSRRR
Can mutation-specific cells be identified in peripheral blood of patients with gastrointestinal cancers?

Metastatic gastric cancer: 175 mutations

<table>
<thead>
<tr>
<th>Populations sorted from Pheresis:</th>
<th>Populations sorted from FrTu:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Isotype</strong></td>
<td><strong>Anti-PD-1 (AMP514)</strong></td>
</tr>
<tr>
<td>CD3+CD8+</td>
<td><img src="image1" alt="CD3+CD8+" /></td>
</tr>
<tr>
<td>CD3+CD4+</td>
<td><img src="image3" alt="CD3+CD4+" /></td>
</tr>
</tbody>
</table>

1) CD8+
2) CD8+PD-1-
3) CD8+PD-1+
4) CD8+PD-1hi
5) CD4+
6) CD4+PD-1-
7) CD4+PD-1+
8) CD4+PD-1hi

Evaluated recognition of 175 total putative mutations
Summary of neoantigen reactivities detected in the blood and tumor subsets derived from Pt. 4078

<table>
<thead>
<tr>
<th>Patient</th>
<th>Tumor</th>
<th>mutations evaluated</th>
<th>Pheresis</th>
<th>FrTu</th>
<th>TIL fragments</th>
</tr>
</thead>
<tbody>
<tr>
<td>4078</td>
<td>GE</td>
<td>175</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CD8+PD-1+/hi</td>
<td>CD8+PD-1+</td>
<td>CD4+PD-1+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 CD8+ (DLAT, GBAS)</td>
<td>3 CD4+ (TMPRSS4, TCF25, PSMD2)</td>
<td>2 CD8+ (DLAT, GBAS)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>High background</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CD4+</td>
</tr>
</tbody>
</table>

Circulating CD8+PD-1+ cells AND intratumoral CD8+PD-1+ cells recognized DLAT and GBAS but only circulating lymphocytes recognized TM PRSS4, TCF25, PSMD2.

Thus improved techniques have the potential to identify increased T cells reactive with mutated antigens.
NetMHCpan3.0 ranked immunogenic 25mers (Melanoma)

3 of 59 (5%)

12 of 59 (20%)
NetMHCpan3.0 ranked immunogenic 25mers (Epithelial cancers)

Top 10% of predicted epitopes included 14 of 187 (7%) identified immunogens.
Top 25% of predicted epitopes included 33 of 187 (18%) identified immunogens.
A novel method to screen for T cell responses to p53 “hotspot” mutations

Whole-exome and transcriptome sequencing to identify TP53 “hotspot” mutations

Isolate DNA and RNA

Tumor sample

T cells from tumor or blood

Tumor infiltrating lymphocytes

Co-Cultured with autologous antigen presenting cells

1. TMG-IRR
2. p53wtTMG
3. p53mutTMG

1. DMSO
2. p53 LP wt
3. p53 LP mut

Readout

Y= reactive, interferon-gamma ELISPOT and/or 41BB expression higher than controls.

N= no reactivity, interferon-gamma ELISPOT and/or 41BB expression similar to or lower than controls.

YKQSQHMTEVVRHCPHHERCSDDSEGNLRLGRNFEVHVCA PCGRDRRTEYMCN SSMGNNRPILTITLDS
R175H R273H R248L
FEVRVCACGRDRWTEEENLRKGESEGNLRLGRNFEVHVCA PCGRDRRTEHYNYM CNSSCMGNNMNRRPILTITL
R282W R273C G245S
YMCNSSCMGNNMNRRPILTITLDSHYNYM CNSSCMGNNMNRRPILTITLDSGNNLRLGRNFEVHVCA PCGRDRRTE
R248Q G245D R273L
YMCNSSCMGNNMNRRPILTITLDSRNFTRRVSVPCEPPEVGSSTDCTTIYM CNSSCMGNNMNRRPILTITLDS
R248W Y220C R249S

4-1BB or OX40

CD4 or CD8
“Blueprint” for Identification of Neoantigen-reactive T cells from Patients with Cancer

1. Isolate DNA and RNA
2. Whole-exome and transcriptome sequencing to identify somatic mutations
   - Wild type: GAAACTGAGCAGTT
   - Mutant: GAAACTGAGCAGTT

3. TMGs containing all mutations
   - ...GAAACTGGGCAGTT...
   - Minigene
   - Variable number of minigenes
   - Tandem minigene
   - Plasmid or in-vitro-transcribed RNA
   - Synthetic long mutant peptides
     - Mutation
     - TSFLSINSKEETGHLENNGKYPNLE
     - QNADSYWELPQAEERAMENQYSP
     - RVLKGGSRKLRHAKQLVLELGEEA
     - Etc.

4. Co-culture
5. Transfect TMGs or pulse long peptides into autologous APCs
6. Assay for T cell activation:
   - Cytokine ELISPOT or ELISA
   - Analysis of activation molecules by flow cytometry
   - 4-1BB or OX40
   - CD4 or CD8
Cancer Antigens

1. Unique somatic mutations in an intracellular protein

2. Mutations in oncogenes or tumor suppressor genes that can be shared among patients.  
   e.g.: Kras  
   p53

3. Non-mutated proteins on the surface of cells that are not essential for survival and can be recognized by antibodies or specific ligands  
   e.g.: CD19 (CAR: αCD19 antibody)  
   CD70 (CAR: CD27 ligand)
Progress on generating a TCR library targeting p53 mutations

<table>
<thead>
<tr>
<th>p53 mutation</th>
<th>HLA</th>
<th>TCRs</th>
</tr>
</thead>
<tbody>
<tr>
<td>R175H</td>
<td>A<em>02:01, DRB1</em>13:01</td>
<td>3, 1</td>
</tr>
<tr>
<td>Y220C</td>
<td>A<em>02:01, DRB1</em>04:01, DRB3*02:02</td>
<td>pending, 1, 1</td>
</tr>
<tr>
<td>G245S</td>
<td>DRB3*02:02</td>
<td>4</td>
</tr>
<tr>
<td>R248Q</td>
<td>pending</td>
<td>pending</td>
</tr>
<tr>
<td>R248W</td>
<td>A<em>68:01, DPB1</em>02:01</td>
<td>3, pending</td>
</tr>
<tr>
<td>R273H</td>
<td>pending</td>
<td>pending</td>
</tr>
<tr>
<td>R282W</td>
<td>pending, DRB4*01:01</td>
<td>1, Pending</td>
</tr>
</tbody>
</table>
Summary - Human TCRs targeting $KRAS^{G12}$ mutations identified in the Surgery Branch

<table>
<thead>
<tr>
<th>Mutation</th>
<th>CD4/CD8</th>
<th>HLA restriction (*)</th>
<th>Minimal epitopes</th>
</tr>
</thead>
<tbody>
<tr>
<td>$KRAS \text{ p.G12V}$</td>
<td>CD4</td>
<td>HLA-DRB1*07:01 (25%)</td>
<td>VVGAVGVGK</td>
</tr>
<tr>
<td></td>
<td>CD8</td>
<td>HLA-A*11:01 (14%)</td>
<td>VVVGAGGVGK</td>
</tr>
<tr>
<td>$KRAS \text{ p.G12D}$</td>
<td>CD4</td>
<td>HLA-DR3*02 (16% allele freq.)</td>
<td>GADGVGKSA (3 TCRs)</td>
</tr>
<tr>
<td></td>
<td>CD8 - 4 TCRs (E. Tran)</td>
<td>HLA-C*08:02 (11.7%)</td>
<td>GADGVGKSA (1 TCR)</td>
</tr>
<tr>
<td></td>
<td>CD4</td>
<td>HLA-DRB1*08:01 (~4.5%)</td>
<td></td>
</tr>
<tr>
<td>$KRAS \text{ p.G12C}$</td>
<td>CD4 (G. Cafri)</td>
<td>HLA-DRB1*11:01 (10%)</td>
<td></td>
</tr>
<tr>
<td>$KRAS \text{ p.G12R}$</td>
<td>CD4 (M. Parkurst, A Sachs)</td>
<td>TDB</td>
<td></td>
</tr>
</tbody>
</table>

* % US Caucasians individuals that have the allele in - Allelefrequencies.net

Identify additional TCRs targeting $KRAS$ mutations from other potential candidates
Dr. James Yang laboratory has isolated G12V and G12D HLA-A11 restricted KRAS TCRs.
Limitations of Current Immunotherapies

Treatment using checkpoint modulators or adoptive cell therapy with unselected TIL can be effective in treating tumors with high mutation rates (melanoma, smoking-induced lung cancer, some bladder cancers).

The common epithelial cancers such as those arising in the colon, liver, stomach, pancreas, prostate, ovary, etc rarely respond to current immunotherapies.

Are there antigens on the common epithelial human cancers that can be targeted by cell-based immunotherapy?
The development of effective immunotherapies for the 80% of patients with metastatic epithelial solid cancers that cannot be cured by any available treatment.
Estimated Cancer Deaths in 2017 in the U.S.

<table>
<thead>
<tr>
<th></th>
<th>New Cases</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>1,688,780</td>
<td>600,920</td>
</tr>
<tr>
<td>Solid cancers</td>
<td>1,515,870</td>
<td>542,620</td>
</tr>
<tr>
<td>Hematologic</td>
<td>172,910</td>
<td>58,300</td>
</tr>
</tbody>
</table>

(American Cancer Society, 2017)
## Systemic Treatments that Can Cure Metastatic Solid Cancers

<table>
<thead>
<tr>
<th>Year</th>
<th>Treatment Description</th>
<th>Researcher/Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1958</td>
<td>methotrexate for choriocarcinoma</td>
<td>Min Chiu Li, NCI</td>
</tr>
<tr>
<td>1977</td>
<td>cis-platin combination chemotherapy for germ-cell testicular cancers</td>
<td>Lawrence Einhorn, U. Indiana</td>
</tr>
<tr>
<td>1985</td>
<td>interleukin-2 for melanoma and renal cancer</td>
<td></td>
</tr>
</tbody>
</table>
Potential improvements in targeting of somatic mutations in epithelial cancers

Enrich cancer mutation-reactive cells
- PD1+ cells in tumor and circulating lymphocytes
- 41BB+ after antigen stimulation

Improve methods to identify multiple mutation targets expressed by tumor (robotics)

Develop rapid methods for identifying mutation-reactive TCRs (robotics)

Transduce mutation-reactive TCRs into naïve or CM cells (FDA approval 11-1-17)

Add anti-PD-1 (reexpressed by infused cells in vivo) or other CPM

Knockout CISH or PD-1 (or other inhibitory molecules) on transferred cells

Vaccinate with mutations recognized by transferred cells

Obtain mutation-reactive TCRs from circulating lymphocytes and mutations from paraffin sections or liquid DNA) – eliminate need for tumor resection
Potential improvements in targeting of somatic mutations in epithelial cancers

Improve methods to identify multiple mutation targets expressed by tumor (robotics)

Develop rapid methods for identifying mutation-reactive TCRs (robotics)

Transduce mutation-reactive TCRs into naïve or CM cells (FDA approval 11-1-17 to use a GMP 293GP line to produce transient vectors with minimal testing)

Add anti-PD-1 (reexpressed by infused cells in vivo) or other CPM

Knockout CISH or PD-1 (or other inhibitory molecules) on transferred cells

Vaccinate with mutations recognized by transferred cells

Raise libraries of TCRs reactive with shared KRAS or P53 mutations (?other driver genes)

Obtain mutation-reactive TCRs from circulating lymphocytes and mutations from paraffin sections or liquid DNA) – eliminate need for tumor resection
NCI/Kite-Gilead CRADA

NCI Principal Investigator – Steven Rosenberg, M.D., Ph.D.
Kite-Gilead Principal Investigator – Alessandro Riva, M.D.

Cooperative Research and Development Agreement for the Development of T cell Therapy Using Neoantigen Reactive T Cell Receptors Retrovirally Transduced into Autologous Peripheral Blood Lymphocytes

Experimental Plan
1. To generate clinical proof-of-concept data for personalized neoAg TCR-engineered T-cell therapy using retroviral insertion.

2. To develop a streamlined, high-throughput process for neoAgTCR isolation from peripheral blood lymphocytes (PBL).

3. To evaluate the safety and efficacy of KRAS mutation targeting TCR therapy
Conclusions

Peripheral blood CD8+PD-1+ lymphocytes represent a small subset of all the circulating CD8+ cells.

The CD8+PD-1+ lymphocyte subset in peripheral blood can contain multiple mutation-specific cells capable of recognizing tumor.

Each immunogenic mutation detected was unique to the autologous tumor.

Circulating CD8+PD-1+ lymphocytes from melanoma patients also contain T cells recognizing shared tumor antigens.
<table>
<thead>
<tr>
<th>Tumor</th>
<th>Antigen</th>
<th>AA change</th>
<th>HLA RE</th>
<th>Tumor</th>
<th>Antigen</th>
<th>AA change</th>
<th>HLA RE</th>
<th>Tumor</th>
<th>Antigen</th>
<th>AA change</th>
<th>HLA RE</th>
</tr>
</thead>
<tbody>
<tr>
<td>164</td>
<td>ARCT1</td>
<td>altORF</td>
<td>DRB1*0101</td>
<td>3466</td>
<td>COL18A1</td>
<td>p.S306F</td>
<td>A*02:01</td>
<td>3784</td>
<td>FLNA</td>
<td>p.R2049C</td>
<td>B*07:02</td>
</tr>
<tr>
<td>1359</td>
<td>CDC27</td>
<td>p.S711L</td>
<td>DRB1*0401</td>
<td></td>
<td>ERBB2</td>
<td>p.H458Y</td>
<td>A*02:01</td>
<td>3795</td>
<td>KIF16B</td>
<td>p.L1020P</td>
<td>B*07:02</td>
</tr>
<tr>
<td>1363</td>
<td>LDLR-FUT</td>
<td>gene fusion</td>
<td>DRB1*0101</td>
<td></td>
<td>PXMP4</td>
<td>p.S176C</td>
<td>B*39:01</td>
<td>3868</td>
<td>GANAB</td>
<td>p.S320F</td>
<td>A*02:01</td>
</tr>
</tbody>
</table>

78 neoepitopes identified as targets of autologous TIL from 34 patients with melanoma. 31 of 34 (91%) expressed neoantigens. All were unique. (unpublished)
Identification of neoantigen-reactive T cells from patients with cancer

1. Tumor sample
2. Isolate DNA and RNA
3. Whole-exome and transcriptome sequencing to identify somatic mutations
   - Wild type: GAACTGAGCCTTG
   - Mutant: GAAACTGGGCCTTG
4. TMGs containing all mutations
5. Mutation
6. Minigene
7. Variable number of minigenes
8. Tandem minigene
9. Plasmid or in-vitro-transcribed RNA
10. Synthetic long mutant peptides
11. Mutation
12. Transfect TMGs or pulse long peptides into autologous APCs
13. Assay for T cell activation: Cytokine ELISPOT or ELISA, analysis of activation molecules by flow cytometry
Conclusions

Cell transfer therapy can mediate durable regressions in patients with metastatic cancer refractory to other treatments.

T-cells that recognize unique somatic mutations can be found in TIL and PBL.

Identification and targeting of mutations unique to each cancer has the potential to extend cell therapy to patients with common epithelial cancers.
Cancer Antigens

1. Unique somatic mutations in an intracellular protein

2. Mutations in oncogenes or tumor suppressor genes that can thus be shared among patients.
   e.g.: Kras
   p53

3. Non-mutated proteins on the surface of cells that are not essential for survival and can be recognized by antibodies or specific ligands
   e.g.: CD19 (CAR: αCD19 antibody)
Construction of T-cell Receptors (TCR) and Chimeric Antigen Receptors (CAR)

TCR Vector (eg, MART1, NY-ESO)

- LTR
- SD
- TCRα
- 2A
- TCRβ
- SA
- LTR

TCR receptor

CD3ζ, γ, ε, δ

CAR Vector (eg, CD19)

- LTR
- SD
- Anti-tumor scFv
- CD28
- CD3ζ
- SA
- LTR

CAR receptor

scFv

V_i

V_L

V_H

CD28

CD3ζ
Patient E.K.

48 year old male with follicular non-Hodgkin lymphoma

Aug. 2002  diagnosed with stage IV lymphoma
7 cycles PACE chemotherapy (cisplatin,
doxorubicin, cyclophosphamide, etoposide)

April 2004  idiotypic/KLH vaccine (5 doses)

Sept. 2007  ipilimumab

Nov. 2007  6 cycles EPOCH-R chemotherapy
(etoposide, predisone, vincristine,
cyclophosphamine, rituximab)

May 2009  To NCI for treatment with autologous anti-CD19
CAR transduced T cells

In ongoing progression-free regression as of February, 2014 (57+ months).

E.K.

Follicular lymphoma

June 2, 2009

March 14, 2012
Bone marrow biopsies showed extensive CLL before treatment and nearly absent B-lineage cells after treatment.
In Patient 8, normal blood B cells were eliminated after CAR-transduced T cell infusion. In contrast, T and NK cell counts rapidly recovered after treatment.
Rapid Identification of the TCR Sequence from a Polyclonal Population Using Single Cell RNA/Seq

Co-culture of a polyclonal TIL population with antigen-presenting cells

Automatic single-cell sorting & RNA-seq sample preparation by Fluidigm C1 system

Deep sequencing by Illumina MiSeq

Bioinformatics analysis

Quantitation of T cell activation markers (e.g. IFN-γ, IL-2)

Assembly of full-length TCRα/β chains

Illumina MiSeq sequence reads

Variable region (V) CDR3 Constant region (C)

(William Lu, Anna Pasetto)
Rapid Identification of the TCR Sequence from a Polyclonal Population Using Single Cell RNA/Seq

A

B

C

<table>
<thead>
<tr>
<th>TCR variable region</th>
<th>CDR3 (nucleotide sequence)</th>
<th>CDR3 (amino acid sequence)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AV38-1</td>
<td>TGTGCTTTTCAATGGGGGATTAGTCAGAAATTITGCTTT</td>
<td>CAFMWGLGQNFVF</td>
</tr>
<tr>
<td>BV28</td>
<td>TGTGCCAGCAGTGTGGAGGACGGAGAACACCCGGGAGCTTTTTTT</td>
<td>CASSVERENTGELFF</td>
</tr>
</tbody>
</table>
Four different KRAS\(^{G12D}\)-reactive TCRs in patient infusion TIL

**Specificity and Sensitivity of KRAS\(^{G12D}\)-Specific TCRs**

- **A)** 50%
- **B)** 19%
- **C)** 7%
- **D)** 0.1%

**Frequency of TCR (%)**

- **R \times 1**
- **Blood**
- **Tu-1**
- **Tu-2A**
- **Tu-2B**

**Days relative to cell transfer**

- **(1)**
- **(2)**
- **(3)**
- **(4)**
- **(5)**
- **(6)**
- **(7)**

Why the differences in the persistence in blood? T-cell differentiation state? Avidity for antigen?
Half of Patients with Melanoma Recognize Three or More Immunogenic Mutations

Immunogenic mutations 56/3961 = 1.4%

<table>
<thead>
<tr>
<th># neoantigens per patient</th>
<th># patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>1</td>
<td>4 (22%)</td>
</tr>
<tr>
<td>2</td>
<td>4 (22%)</td>
</tr>
<tr>
<td>3</td>
<td>4 (22%)</td>
</tr>
<tr>
<td>4</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>&gt;4</td>
<td>4 (22%)</td>
</tr>
</tbody>
</table>
Minigene approach: J. bulk TILs recognize tandem minigene RJ-1

71 non-synonymous mutations
12 tandem minigene constructs
HLA-A*0205
Kinesin family member 2C (KIF2C) also known as mitotic centromere-associated Kinesin (MCAK)

Rapid Identification of the TCR Sequence from a Polyclonal Population Using Single Cell RNA/Seq

A

\[ \text{IFN-\(\gamma\) (FPKM)} \]

\[ \text{IL-2 (FPKM)} \]

B

\[ \text{IFN-\(\gamma\) (FPKM)} \]

\[ \text{IL-2 (FPKM)} \]

C

<table>
<thead>
<tr>
<th>TCR variable region</th>
<th>CDR3 (nucleotide sequence)</th>
<th>CDR3 (amino acid sequence)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AV4</td>
<td>TGCCTCGTGGGGTACATGGGACCAGGAGGAACTGCTCTGATCTTTT</td>
<td>CLVGMDQAGTALIF</td>
</tr>
<tr>
<td>BV5-6</td>
<td>TGTGCCAGCAGCGTGGGGAGGGCAAGCAATCAGCCCAGCATTTT</td>
<td>CASSLGRASNPQHF</td>
</tr>
</tbody>
</table>
Mutated antigen KIF2C (kinesin family member 2C) recognized by R. J. TILs
A.H.: N-M cell transfer
Potential improvements being explored to improve targeting of somatic mutations in epithelial cancers

- Purify tumor reactive cells
  - PD1+ cells in tumor and circulating lymphocytes
  - 41BB+ after antigen stimulation

- Identify multiple mutation targets expressed by tumor

- Add anti-PD-1 (reexpressed by infused cells in vivo)

- Transduce mutation-reactive TCRs into naïve or CM cells

- Knockout CISH or PD-1 on transferred cells

- Vaccinate with mutations recognized by transferred cells
### Rapid Selection and Identification of TCRs Recognizing Cancer Mutations

1. 4112 TIL fragments were screened against TMG library, and Fragment 5 recognized TMG-9

2. Fragment 5 T cells co-cultured with TMG-9-pulsed DCs for 4 hr and single-cell RNAseq performed on 41BB positive cells

<table>
<thead>
<tr>
<th>Single-cell ID</th>
<th>Total reads (R1)</th>
<th>IFNg reads</th>
<th>% of IFNg reads</th>
<th>TCR variable region</th>
<th>CDR3 (nucleotide)</th>
<th>CDR3 (amino acid)</th>
<th># of reads</th>
<th>TCR variable region</th>
<th>CDR3 (nucleotide)</th>
<th>CDR3 (amino acid)</th>
<th># of reads</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>163002</td>
<td>4829</td>
<td>2.96</td>
<td>TRBV28</td>
<td>TGTGCCAGCATGTTGGAG</td>
<td>CASSVERENTGELFF</td>
<td>125</td>
<td>TRBV28</td>
<td>TGTGCCAGCATGTTGGAG</td>
<td>CASSVERENTGELFF</td>
<td>98</td>
</tr>
<tr>
<td>62</td>
<td>176886</td>
<td>52</td>
<td>0.029</td>
<td>TRBV19</td>
<td>TGTGCCAGCATGTTGGAG</td>
<td>CASSLTFPSEYYEQYF</td>
<td>53</td>
<td>TRBV19</td>
<td>TGTGCCAGCATGTTGGAG</td>
<td>CASSLTFPSEYYEQYF</td>
<td>46</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TRAV24</td>
<td>TGTGCCCTTTATGGACAGA</td>
<td>CAFMDRRDKIIF</td>
<td>19</td>
<td>TRAV24</td>
<td>TGTGCCCTTTATGGACAGA</td>
<td>CAFMDRRDKIIF</td>
<td>14</td>
</tr>
</tbody>
</table>

% of IFNg reads (IFNg reads / Total reads)

>2% IFNg reads: 23 single-cells with identical TCR(BV28) sequences
1~2% IFNg reads: 6 single-cells with identical TCR(BV28) sequences
6 sample without any TCR sequences

All single-cell samples
The whole-transcriptome of 4112 F5 after 4 hr co-culture with TMG-9 pulsed DC

<table>
<thead>
<tr>
<th>Single-cell ID</th>
<th>Sequence</th>
<th>IFNg</th>
<th>TNF</th>
<th>TNFRSF9 (4-1BB)</th>
<th>PDCD1 (PD-1)</th>
<th>LAG3</th>
<th>CD244 (2B4)</th>
<th>CD160</th>
<th>HAVCR2 (Tim-3)</th>
<th>CD4</th>
<th>CD8A</th>
<th>CD8B</th>
<th>GAPDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>MiSeq</td>
<td>15736</td>
<td>1093</td>
<td>536</td>
<td>0</td>
<td>223</td>
<td>0</td>
<td>376</td>
<td>0</td>
<td>0</td>
<td>34</td>
<td>0</td>
<td>3517</td>
</tr>
<tr>
<td>26</td>
<td>HiSeq</td>
<td>34685</td>
<td>2011</td>
<td>221</td>
<td>0</td>
<td>388</td>
<td>0</td>
<td>434</td>
<td>0</td>
<td>0</td>
<td>71</td>
<td>0</td>
<td>8327</td>
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<tr>
<td>62</td>
<td>MiSeq</td>
<td>150</td>
<td>40</td>
<td>81</td>
<td>0</td>
<td>0</td>
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<td>33</td>
<td>87</td>
<td>3353</td>
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<td>62</td>
<td>HiSeq</td>
<td>305</td>
<td>102</td>
<td>230</td>
<td>0</td>
<td>21</td>
<td>0</td>
<td>266</td>
<td>0</td>
<td>0</td>
<td>76</td>
<td>89</td>
<td>7441</td>
</tr>
</tbody>
</table>

Unit: FPKM (Fragments Per Kilobase of transcript per Million mapped reads)

Sequenced by
(1) MiSeq v3: 2 X 250 b.p. X ~200,000 reads / single-cell sample
(2) HiSeq 2500 rapid mode: 2 X 100 b.p. X ~1,500,000 reads / single-cell sample

Analyzed by Partek Flow
Potential improvements being explored to improve targeting of somatic mutations in epithelial cancers

- Purify tumor reactive cells
  - PD1+ cells in tumor and circulating lymphocytes
  - 41BB+ after antigen stimulation

- Identify multiple mutation targets expressed by tumor

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Categories of antigens to target using cell therapy

1. Mutations unique to each individual cancer

2. Antigens expressed on cancers and on non-essential normal tissues (CD19, thyroglobulin)

3. Shared antigens unique to cancer (cancer-testes antigens)
Construction of T-cell Receptors (TCR) and Chimeric Antigen Receptors (CAR)

**TCR Vector (eg, MART1, NY-ESO)**

TCR receptor

**CAR Vector (eg, CD19)**

CAR receptor
Patient E.K.

48 year old male with follicular non-Hodgkin lymphoma

<table>
<thead>
<tr>
<th>Date</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aug. 2002</td>
<td>diagnosed with stage IV lymphoma</td>
</tr>
<tr>
<td></td>
<td>7 cycles PACE chemotherapy (cisplatin, doxorubicin, cyclophosphamide, etoposide)</td>
</tr>
<tr>
<td>April 2004</td>
<td>idiotypic/KLH vaccine (5 doses)</td>
</tr>
<tr>
<td>Sept. 2007</td>
<td>ipilimumab</td>
</tr>
<tr>
<td>Nov. 2007</td>
<td>6 cycles EPOCH-R chemotherapy (etoposide, predisone, vincristine, cyclophosphamide, rituximab)</td>
</tr>
<tr>
<td>May 2009</td>
<td>To NCI for treatment with autologous anti-CD19 CAR transduced T cells</td>
</tr>
</tbody>
</table>

In ongoing progression-free regression as of October, 2017 (101+ months).

Bone marrow biopsies showed extensive CLL before treatment and nearly absent B-lineage cells after treatment.
In Patient 8, normal blood B cells were eliminated after CAR-transduced T cell infusion.

In contrast, T and NK cell counts rapidly recovered after treatment.
Patients with Refractory Lymphomas Treated with Anti-CD19 CAR in the Surgery Branch, NCI

Lymphodepleting chemotherapy:
300-500 mg/m² cyclophosphamide qd x 3
30mg/m² fludarabine qd x 3

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>CR</th>
<th>PR</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>(number, duration months)</td>
<td></td>
<td>(number, duration months)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DLBCL</td>
<td>19</td>
<td>9 (47%)</td>
<td>5 (26%)</td>
<td>14 (73%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(20+, 15+, 11+, 9+, 8+, 7+, 7+, 6+, 6+)</td>
<td>(14, 13+, 7, 3**, 1)</td>
<td></td>
</tr>
<tr>
<td>Follicular</td>
<td>2</td>
<td>2</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(19, 8+)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mantle cell</td>
<td>1</td>
<td>1</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(13+)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*LTFU
** allotransplant in PR
Patient with DLBCL after anti-CD19 T-cell infusion

Before treatment                               24 weeks after treatment
Patient with DLBCL after infusion of anti-CD19 CAR T cells

Before treatment

14 weeks after treatment
Complete remission of chemo-refractory primary mediastinal B-cell lymphoma ongoing 13 months after treatment

Before treatment

9 months after treatment
Categories of antigens to target using cell therapy

1. Mutations unique to each individual cancer

2. Antigens expressed on cancers and on non-essential normal tissues (CD19, thyroglobulin)

3. Shared antigens unique to cancer (cancer-testes antigens)
Cancer/Testes Antigens - Shared Tumor Specific Antigens

Expressed during fetal development

Restricted in their expression in adult normal tissues to germ cells

Up-regulated in 10-80% of cancers from multiple tissues

NY-ESO-1 Family

Small family of X-linked genes that includes NY-ESO-1 and LAGE-1

MAGE Family

Family of ~ 45 X-linked genes
### Responses to Therapy with NY-ESO-1 TCR

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>PR</th>
<th>CR</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>number of patients (duration in months)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Melanoma</strong></td>
<td>19</td>
<td>6 (32%)</td>
<td>4 (21%)</td>
<td>10 (53%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(10**, 28, 8, 6+, 3, 3)</td>
<td>(58+, 54+, 28, 40+**)</td>
<td></td>
</tr>
<tr>
<td><strong>Synovial Cell Sarcoma</strong></td>
<td>15</td>
<td>9 (60%)</td>
<td>1 (7%)</td>
<td>10 (67%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(47**, 18*, 12**, 10, 8, 7, 5, 4, 3**)</td>
<td>(20+)</td>
<td></td>
</tr>
</tbody>
</table>

* treated twice

** plus ALVAC vaccine

A.R.
Synovial sarcoma
NY-ESO-1
TCR
Conclusions

Cell transfer therapy can mediate durable regressions in patients with metastatic cancer refractory to other treatments.

T-cells that recognize unique somatic mutations can be found in TIL and PBL.

Identification and targeting of mutations unique to each cancer has the potential to extend cell therapy to patients with common epithelial cancers.

Autologous lymphocytes genetically engineered to express TCRs or CARs can mediate the regression of metastatic cancers.