Moving T-cell Therapy Forward: Understanding Immune Resistance to Optimize Combination Therapy

Patrick Hwu, MD, Professor and Chairman
Melanoma and Sarcoma Medical Oncology
Leader CCSG Immunotherapy Program
Co-Director Center for Cancer Immunology Research
The University of Texas MD Anderson Cancer Center

SITC 2014 29th Annual Meeting
National Harbor, MD
Sunday, November 9, 2014
Disclosures

• Member of Scientific Advisory Board, Lion Biotechnologies
Clinical Response following Lymphodepletion + T-lymphocyte Infusion
Clinical Response Data from MDACC TIL Clinical Trial

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>CR*</th>
<th>PR*</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>79</td>
<td>4 (5%)</td>
<td>31 (39%)</td>
<td>35 (44%)</td>
</tr>
</tbody>
</table>

*Some patients are still undergoing clinical response

Update to data published in *Clin Cancer Res* 18: 6758-6770, 2012
Radvanyi … Hwu
Objective Tumor Regression in Patients Receiving Autologous TIL Therapy

Fig. 1 Waterfall plot of change in tumor burden in treated patients (n=31). Clinical responses were evaluated using irRC from whole body CT scans. The best overall irRC response is shown for all patients. The patients were treated between August 23, 2007 and October 25, 2010.

Overall Survival After TIL at MD Anderson

Overall survival
(median follow-up 21 months)

N=73

37 months

Median OS ~37 months
(>3 years)
Major Questions

- Does TIL therapy for melanoma work in patients who have failed immune checkpoint blockade?
- How can we increase the throughput for this treatment?
- How do we take T-cell therapy to other cancers?
- What distinguishes responders from non-responders?
- What are the best combinations of therapies?
Patients with Slow to Moderate Growing Melanoma with Good Performance Status

- αPD-1/αPDL1
- αCTLA4
- TIL or High Dose IL-2
- BRAFi + MEKi (in BRAF V600 mutants)
<table>
<thead>
<tr>
<th>No. Patients</th>
<th>Prior anti-CTLA4</th>
<th>Prior anti-PD1</th>
<th>CR (%)</th>
<th>PR (%)</th>
<th>CR + PR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>52</td>
<td>No</td>
<td>No</td>
<td>3</td>
<td>24</td>
<td>27 (52%)</td>
</tr>
<tr>
<td>21(^1)</td>
<td>Yes</td>
<td>No</td>
<td>1</td>
<td>5</td>
<td>6 (29%)</td>
</tr>
<tr>
<td>4(^1)</td>
<td>Yes</td>
<td>Yes</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>No</td>
<td>Yes</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

1 Of the 25 patients treated after anti-CTLA4 therapy, 16 had TIL harvest after anti-CTLA4 (31% response) and 9 had TIL harvest before anti-CTLA4 (22% response).
Major Questions

• Does TIL therapy for melanoma work in patients who have failed immune checkpoint blockade?

• How can we increase the throughput for this treatment?

• How do we take T-cell therapy to other cancers?

• What distinguishes responders from non-responders?

• What are the best combinations of therapies?
Insertion of Genes into Lymphocytes to Enhance Antitumor Properties

Native TCR genes to direct cell specificities against the tumor

Chimeric receptors to enhance T-Cell activation and costimulation

Chemokine receptors to enhance migration of T-cells to tumor

Retroviral vectors can insert novel genes into lymphocytes
Major Questions

• Does TIL therapy for melanoma work in patients who have failed immune checkpoint blockade?
• How can we increase the throughput for this treatment?
• How do we take T-cell therapy to other cancers?
• What distinguishes responders from non-responders?
• What are the best combinations of therapies?
Immune Gene Expression Analysis in FFPE Tissues Using NanoString Probe Assay

mRNA transcript quantification in archival samples using multiplexed, color-coded probes

511 Immune gene Scatter plot matrix

Gene expression in FFPE highly correlated to fresh-frozen tissue

P < 0.0001; r² = 0.977
Differentially-expressed Genes in TIL+ vs. TIL- (595 immune gene probe set)

<table>
<thead>
<tr>
<th>T-cell markers (up):</th>
</tr>
</thead>
<tbody>
<tr>
<td>BTLA</td>
</tr>
<tr>
<td>ICOS</td>
</tr>
<tr>
<td>PDCD1 (PD-1)</td>
</tr>
<tr>
<td>IL-2Rb</td>
</tr>
<tr>
<td>TNFRSF14 (LIGHT)</td>
</tr>
<tr>
<td>TNFRSF4 (OX40)</td>
</tr>
<tr>
<td>CD7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Immune suppression (up):</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDCD1 (PD-1)</td>
</tr>
<tr>
<td>BTLA</td>
</tr>
<tr>
<td>TIGIT</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>APC (up):</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD83</td>
</tr>
<tr>
<td>HLA-DQB</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Innate immunity (down):</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRAF6</td>
</tr>
<tr>
<td>TOLLIP</td>
</tr>
<tr>
<td>TNF</td>
</tr>
<tr>
<td>TNF</td>
</tr>
<tr>
<td>NFKB1</td>
</tr>
</tbody>
</table>

Source: Laszlo Radvanyi/Jie Qing Chen
Major Question

- What are the signaling pathways in the tumor that modulate the immune microenvironment and sensitivity or resistance to immunotherapy?
  - BRAF/MAPK
  - PI3K
  - Aurora Kinase
Combining BRAF(V600E) Inhibition and Immunotherapy

Combination of PLX4720 with Adoptive T-cell Therapy Leads to Enhanced Anti-tumor Activity (B6 nude mice)

Liu C...Hwu P.
Clin Cancer Res 19:393-403, 2013
Administration of PLX4720 Increases Tumor Infiltration of Adoptively Transferred pmel-1 T-cells in vivo

Liu C...Hwu P.

Clin Cancer Res 19:393-403, 2013
Increased T-cell Infiltration may be Mediated by Inhibition of VEGF Production of Melanoma Cells Treated with PLX4720

A

B

Liu C...Hwu P.
Clin Cancer Res 19:393-403, 2013
BRAF Inhibition Downregulates VEGF at the Tumor Site

Liu C...Hwu P.
*Clin Cancer Res* 19:393-403, 2013
Major Question

What are the signaling pathways in the tumor that modulate the immune microenvironment and sensitivity or resistance to immunotherapy?

- BRAF/MAPK
- PI3K
- Aurora Kinase
PI3K Pathway Signaling

Kwong LN and Davies MA. Clin Cancer Res 19:5310-19, 2013
Generation of PTEN-deficient BRAF Mutated Human Tumor Cell Line

A375
hgp100/H2-Db
A375/gp
Pten specific shRNA
A375/shPten-17 or A375/shPten-60
PTEN-specific shRNA Knock Down Induces Resistance of Human Melanoma Cells to T-cell Killing

Weiyi Peng MD, PhD
Decreased Infiltration of Transferred T-cells into PTEN-null Tumor

The intensity of ROI (X105 Photons.S^-1.cm^-2)

- Vehicle
- BRAFi

Control Tu

PTEN knock-down Tu

Weiyi Peng MD, PhD
PTEN-silenced Tumor Poorly Responds to T-cell Therapy

![Graphs showing tumor size and percent survival over days after tumor challenge.](image)

- **Tumor Size (mm²)** over days after tumor challenge for groups: Vehicle (closed circle), T cell (open circle), shNS (closed square), and shPTEN (open square).
- Percent survival graph with days after tumor challenge on the x-axis and percent survival on the y-axis for Control (closed circle) and PTEN-WT Tu (open circle) groups.

![Statistical significance](image)

- *P<0.0001* for tumor size and *P<0.001* for percent survival.
### Increase Percentage of PTEN Loss in Tumors from Melanoma Patients with Failed Initial Expansion of TILs

<table>
<thead>
<tr>
<th></th>
<th>TIL Growth</th>
<th>No TIL Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTEN Absent</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>PTEN Present</td>
<td>72</td>
<td>31</td>
</tr>
<tr>
<td>Percentage without PTEN</td>
<td>11%</td>
<td>26%</td>
</tr>
</tbody>
</table>

P = 0.0405
Less T-cell Infiltration in PTEN-loss Tumor in Stage IIIb/C Melanoma Patients

P < 0.001

CD8+ % at tumor site

PTEN  Absence  Presence
T-cell infiltration to tumor is decreased in melanomas lacking PTEN.
T-cell Infiltration in Tumor from Patients with PTEN Clonal Expression

Case#21

PTEN staining

CD8 staining

Case#39
Decreased Number of Infiltrating T-cells in Patients with Low PTEN Copy Number

- **p-Akt-S473**:
  - High PTEN CN: P=0.039
  - Low PTEN CN: P=0.041

- **p-Akt-T308**:
  - High PTEN CN: P=0.041
  - Low PTEN CN: P=0.041

- **Lck**:
  - High PTEN CN: P=0.002

- **IFNG**:  
  - High PTEN CN: P<0.001

- **GZMB**:  
  - High PTEN CN: P<0.001

**Normal RNA level**
- PTEN copy number (CN) low \(<=0.4\)
- PTEN CN high \(>0.4\)
In Vivo Changes in Chemokine Expression following PTEN Knockdown

B. 

CCL2

P = 0.013

Fold change to GAPDH (X10^4)

shNS shPTEN

CCL21

P = 0.015

Fold change to GAPDH (X10^4)

shNS shPTEN

CXCL1

P = 0.252

Fold change to GAPDH (X10^2)

shNS shPTEN

CXCL10

P = 0.0012

Fold change to GAPDH (X10^2)

shNS shPTEN

VEGF

P = 0.035

Fold change to GAPDH (X10^2)

shNS shPTEN

B. 

P = 0.027

P = 0.165

P = 0.011

P = 0.567

P < 0.001

pg/mg tumor lysis

shNS shPTEN

shNS shPTEN

shNS shPTEN

shNS shPTEN

shNS shPTEN

shNS shPTEN

shNS shPTEN
Hierarchical clustering of gene expression using Nanostring data comparing melanomas from 37 PTEN positive and 10 PTEN negative tumors in patients without systemic treatment for the past 2 months (p<0.05, Mann-Whitney test)

Source: Laszlo Radvanyi/Jie Qing Chen
The Autophagy Pathway

[Diagram showing the autophagy pathway with various molecules and processes involved.]
Decreased ATG16L Expression in PTEN-loss Tumor
Increased T-cell Induced Tumor Apoptosis by Overexpressing Autophagy Related Genes

![Graph showing Capase-3 Cleavage % for different conditions: T cell and LC3B, with A375/GH/shPTEN and A375/GH/shNS as control groups. The graph includes statistical significance indicators (*, **, ***, and ****) to denote differences between conditions.]
Increased T-cell Induced Tumor Apoptosis by Overexpressing Autophagy Related Genes

Nras mutation
Tu 2338

Braf mutation
Tu 2400

Nras and Braf WT
Tu 2549

Caspase-3+ % by ORF only

Comboscore

0.500
1.500
PI3Kβ Inhibitor Improves the Anti-tumor Activity of anti-PD-1 in a Genetically Engineered PTEN Loss Tumor Model

![Graphs showing tumor size and percent survival over days after treatment.](image)

- **Control**
- **PI3Kβ inhibitor**
- **anti-PD-1**
- **PI3Kβ inhibitor+ anti-PD-1**

**Tumor Size (mm²)**

- Days after treatment: 0, 3, 6, 9, 12, 15

**Percent Survival**

- Days after treatment: 0, 10, 20, 30, 40

*P < 0.05*
Summary

- PI3Kα/β/δ
- PTEN
- PTEN loss
- AKT ↑
- mTORC1/2 ↑
- Autophagy ↓
- Response to T-cell induced apoptosis ↓
Major Question

• What are the signaling pathways in the tumor that modulate the immune microenvironment and sensitivity or resistance to immunotherapy?
  – BRAF/MAPK
  – PI3K
  – Aurora Kinase
System to Perform Large Scale Screens Using Autologous Tumor/TIL Pairs and T-cell Mediated Cytotoxicity as a Read Out

Figure 1: Flow cytometry based T cell cytotoxicity assay for high throughput screen. Depiction of the methodology of T cell cytotoxicity assay. The dot plots for gating and flow cytometric analysis are depicted on the right. Briefly, patient derived melanoma tumor cells are co-cultured with reactive autologous T cells, followed by intracellular staining for active Caspase-3. The % cytotoxicity is measured by % active caspase-3 positive tumor cells.
Unbiased Screen #1: Large Scale Drug Screen

Figure 2: Aurora Kinase inhibitors were identified in an unbiased screen to display synergistic effects with T cell mediated anti-tumor cytotoxicity. (A). The comboscores of different bioactive compounds in a representative drug screen using a patient-derived melanoma cell lines. The color bar below is the key for comboscores. (B). Definition of comboscore. The drugs with the highest comboscores i.e. highest synergy potential are indicated by arrows and include Aurora Kinase inhibitors in green (   )

Shruti Malu, Postdoctoral Fellow
Melanoma Medical Oncology - Research

42
Cell Cycle Execution Points and Targets of Aurora A and B Kinases

Synergistic Response of Melanoma Cells Lines to Aurora Kinase Inhibitors

Figure 3: Synergistic response of melanoma cell lines to Aurora Kinase inhibitors with T cell mediated cytotoxicity as determined using Calcusyn™. (A) The curve is depicting combination index for two drugs and areas of synergy and antagonism are shown. (B), Synergy of T cell cytotoxicity with Aurora kinase inhibitor AMG900 and Aurora Kinase B specific inhibitor AZD1152 in melanoma line 2338, (C), in cell line 2400 and, (D) in cell line 2549.

Shruti Malu, Postdoctoral Fellow
Melanoma Medical Oncology - Research
Unbiased Screen #2: ORF Screen

EPIGENOME = 192 ORFs

Number of ORFs

Comboscore

Candidate ORFs that induce resistance to T cell mediated killing: Low Comboscore

Shruti Malu, Postdoctoral Fellow
Melanoma Medical Oncology - Research
Unbiased Screen #3: shRNA Screen

Figure 4: In an unbiased pooled shRNA screen, treatment with shRNA to AURKA results in increased sensitivity to T cell mediated cytotoxicity. In an unbiased pooled shRNA screen, the shRNAs that were deleted on treatment with TILs are depicted in the black box. shRNAs to AURKA were among these depleted from the pooled shRNA expressing cells on treatment with TILs indicating that AURKA is a resistance marker for T cell mediated killing (the individual dots is a single shRNA).
Nanostring™ Analysis of Gene Expression in Tumors from Patients on TIL Therapy

AURKA, CDCA8, TARBP2 have p<0.05
AURKB has p<0.08

Jie Qing, Caitlin Creasy, Sourindra Maiti
Combination of Aurora Kinase B Inhibitor with Immunotherapy (anti CTLA4) is Highly Efficacious in MC38/gp100 Tumor Model

Figure 5: Combination of Aurora Kinase B inhibitor with immunotherapy is highly efficacious in MC38/gp100 tumor model. (A) Mice were inoculated with MC38/gp100 tumor on day 0. On Day 3-6, mice were treated with Aurora Kinase B inhibitor AZD1152 (25mg/kg) and 100µg of anti-CTLA4 antibody on Day 3, 6, 9 and 15. The tumor shrinkage using combination therapy was beyond the response seen for mice treated with either treatments alone, indicating synergy of this combination. *** indicates p<0.005 and * indicates p<0.05. (B) Mouse survival is significantly improved with the combination of AZD1152 and α-CTLA4. ** p-value is < .01.
Significance of Studying Aurora Kinases as Mediators of Resistance to Cancer Immune Therapy
A Four-Screen Hit

Over-expression of Aurora Kinase B in melanoma cells

Inhibition of Aurora Kinases by shRNA in melanoma cells

Inhibition of Aurora Kinases by drugs in melanoma cells

High expression of Aurora Kinase A in melanoma tumors

Decreased T cell mediated cytotoxicity on melanoma cells

Increased T cell mediated cytotoxicity on melanoma cells

Aurora Kinases as mediators of immune resistance

Non-responsiveness to TIL therapy

Shruti Malu, Postdoctoral Fellow
Melanoma Medical Oncology - Research
Major Question

What are the signaling pathways in the tumor that modulate the immune microenvironment and sensitivity or resistance to immunotherapy?

- BRAF/MAPK
- PI3K
- Aurora Kinase
Major Questions

• Does TIL therapy for melanoma work in patients who have failed immune checkpoint blockade?

• How can we increase the throughput for this treatment?

• How do we take T-cell therapy to other cancers?

• What distinguishes responders from non-responders?

• What are the best combinations of therapies?
Acknowledgements

Preclinical Data and Laboratory Endpoints

- Weiyi Peng
- Shruti Malu
- Rina Mbofung
- Jodi McKenzie
- Leila Williams
- Chengwen Liu
- Chunyu Xu
- Zhe Wang
- Donald Sakellariou-Thompson
- Krit Ritthipichai
- Mike Davies
- Jen Wargo
- Zac Cooper
- Tim Heffernan
- Cassian Yee
- Jungsun Park
- Willem Overwijk
- Scott Woodman
- Chantale Bernatchez
  - Cara Haymaker
  - Geok Choo Sim
  - Caitlin Creasy
  - Rene Tavera
- Laszlo Radvanyi
- Luis Vence
- Gordon Mills
- Liz Grimm
- Waun Ki Hong

Peptide Analysis:
- Greg Lizee
- Amjad Talukder
- Jason Roszik
- David Hawke

GI Team:
- Anirban Maitra
- Bob Wolff
- Mike Overman
- Scott Kopetz
- Aaron Schuneman
- Jason Fleming

TIL Lab:
- Marie Andre Forget
  - OJ Fulbright
  - Audrey Gonzalez
  - Valentina Dumitru
  - Arly Wahl
  - Esteban Flores
  - Shawne Thorsen

Clinical Research

Melanoma Medical Oncologists:
- Roda Amaria
- Wen Jen Hwu
- Adi Diab
- Isabella Glitza
- Sapna Patel

Surgeons:
- Jeff E. Lee
- Merrick Ross
- Jeff Gershenwald
- Richard Royal
- Anthony Lucci
- Janice Cormier

Pathologists:
- Victor Prieto
- Carlos Torres Cabala
- Michael Tetzlaff
- Doina Ivan

Research Nurses:
- Anna Vardeleon
- Suzanne Cain
- Portia Velasquez
- Vruti Patel

GMP Lab:
- EJ Shpall
- Enrique Alvarez

IND Office
- Linda Duggan

NCI
GSK
Prometheus
Roche/Genentech
MDACC
Melanoma Moon Shot
Development Office
Ton Schumacher
Zelig Eshhar

Adelson Medical Research Foundation

Peptide Analysis:
- Greg Lizee
- Amjad Talukder
- Jason Roszik
- David Hawke

GI Team:
- Anirban Maitra
- Bob Wolff
- Mike Overman
- Scott Kopetz
- Aaron Schuneman
- Jason Fleming

TIL Lab:
- Marie Andre Forget
  - OJ Fulbright
  - Audrey Gonzalez
  - Valentina Dumitru
  - Arly Wahl
  - Esteban Flores
  - Shawne Thorsen