89Zr-labeled antibodies and fragments for imaging immune cells

Anna M. Wu, Ph.D.
Professor, Department of Molecular and Medical Pharmacology
Co-Associate Director, Crump Institute for Molecular Imaging
David Geffen School of Medicine at UCLA

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Disclosures

Anna M. Wu is a Founder, Board Member, and Consultant to ImaginAb, Inc.

Dr. Wu is also a consultant to Avidity Nanomedicines.
Molecular imaging approaches for imaging immune cells and immune responses

- Metabolic probes (e.g., $^{[18F]}$-fluorodeoxyglucose, FDG; $^{[18F]}$ fluoro-thymidine; FLT, nucleoside analogs and others)
- Pre-labeling cells ($^{111}$In-oxine; $^{89}$Zr-oxine; paramagnetic nanoparticles)
- Reporter genes (optical, PET)
- Direct imaging of cell surface targets using antibodies, nanobodies, etc.

**Double tumor**

**Kidney**

*HSV-tk PET reporter gene; Dubey et al. PNAS 2003*

*Anti-CD8 cys-diabody; Tavaré et al. J Nucl Med 2015*
Molecular imaging approaches for imaging immune cells and immune responses

<table>
<thead>
<tr>
<th></th>
<th>Pros</th>
<th>Cons</th>
<th>Current and future clinical use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic probes</td>
<td>Detect metabolically active, proliferating cells</td>
<td>Relatively non-specific</td>
<td>FDG, FLT and others</td>
</tr>
<tr>
<td>Pre-labeled cells</td>
<td>Low background</td>
<td>Need to remove, modify, reinfuse cells</td>
<td>In-111 oxine</td>
</tr>
<tr>
<td>Reporter genes</td>
<td>Potentially low background</td>
<td>Need to modify cells (in situ or ex vivo)</td>
<td>Non-immunogenic reporter genes (NaI symporter, huTK, etc.)</td>
</tr>
<tr>
<td>Probes for cell surface markers (antibodies, etc)</td>
<td>High specificity</td>
<td>Endogenous antigen sink</td>
<td>Human/humanized probes</td>
</tr>
</tbody>
</table>

Overall challenges for molecular imaging:
- Complexity, cost, and time to develop; regulatory path; lack of financial incentives
- Inability to multiplex
Challenges in immunoPET

Irresistible force vs. Immovable object

F-18 decay curve

$e^{(-x\log(2)/1.8259)}$

$t_{1/2} = 109 \text{ min}$

$t_{1/2} = 2 \text{ wks}$
# Positron-emitting radionuclides for ImmunoPET

<table>
<thead>
<tr>
<th>Radio-nuclide</th>
<th>$T_{1/2}$ (h)</th>
<th>Positron yield (%)</th>
<th>$\beta^+$ max (MeV)</th>
<th>Additional considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{68}$Ga</td>
<td>1.1</td>
<td>89</td>
<td>1.89</td>
<td>Generator-produced</td>
</tr>
<tr>
<td>$^{18}$F</td>
<td>1.8</td>
<td>97</td>
<td>0.63</td>
<td>Common, cyclotron</td>
</tr>
<tr>
<td>$^{64}$Cu</td>
<td>12.7</td>
<td>19</td>
<td>0.66</td>
<td>Also beta, Auger $e^-$</td>
</tr>
<tr>
<td>$^{86}$Y</td>
<td>14.7</td>
<td>33</td>
<td>3.15</td>
<td>Also gamma</td>
</tr>
<tr>
<td>$^{76}$Br</td>
<td>16.2</td>
<td>23</td>
<td>3.98</td>
<td>Also gamma</td>
</tr>
<tr>
<td>$^{89}$Zr</td>
<td>78.5</td>
<td>23</td>
<td>0.90</td>
<td>Also gamma</td>
</tr>
<tr>
<td>$^{124}$I</td>
<td>100.3</td>
<td>23</td>
<td>2.14</td>
<td>Also gamma</td>
</tr>
</tbody>
</table>

Wu, Methods 2014
Engineering antibodies for *in vivo* imaging

**Intact Ab** 150 kDa

**Minibody** 80 kDa

**Diabody** 55 kDa

**scFv** 25 kDa

**Biodistribution of anti-CEA fragments in LS174T xenografted mice**

*From Wu and Senter 2005*

**Minibody and diabody for imaging**

- Bivalent, retain specificity and affinity
- Half-life reduction / accelerated clearance (no FcRn interaction)
- Reduction of immunogenicity (humanized or human)
- Removal of effector functions (no CH2/Fc; not glycosylated)
- Direct clearance to kidneys (< 60 kDa) or liver (>60 kDa)
- Improved diffusion / transport in target tissues
- Site-specific conjugation of imaging moieties
Rapid imaging using $^{124}$I-anti-PSCA engineered antibody fragments

Intact Ab
150 kDa

Minibody
80 kDa

Diabody
55 kDa

LAPC-9 prostate cancer xenografts in SCID mice; microPET/CT; images scaled individually

Minibodies and diabodies as a platform for cell-surface imaging

ImmunoPET at 18-20 h for minibodies; 1-4 h for diabodies

“In vivo immunohistochemistry”

Wu, Methods 2014
Beyond Oncology… Antibodies for Imaging Immunology

- FDG-PET non-specific
- CD antigens as markers of lineage, differentiation, activation
- Applications:
  - Immune responses and inflammation
  - Cancer immunotherapy

Collaborations with Owen Witte, Antoni Ribas, John Timmerman

“The cytotoxic T cell is the drug.”
- Toni Ribas
Imaging CD8 T cell repopulation following HSC transplant

Preclinical hematopoietic stem cell (HSC) transplant model

Bone marrow derived HSCs are injected into lethally irradiated BL/6 mice

Time post-HSC transplantation

2 weeks

4 weeks

8 weeks

89Zr-radiolabeled anti-CD8 169 cys-diabody successfully detects T cell repopulation over time

Imaging CD8 T cell infiltration in tumor immunotherapy

CT26 syngeneic tumor treated with anti-CD137 (4-1BB)

A

Day 0

s.c. 1x10^6
CT26 cells

7

9

11

13

15

16

250 μg i.p. of anti-CD137 Ab

Radiolabeling microPET biodistribution

B

Average tumor Diameter (mm)

0

2

4

6

8

10

12

14

16

CD137

Control

MicroPET imaging using ^89^Zr anti-CD8 169 cys-diabody

Imaging CD8 T cell infiltration in tumor immunotherapy

- CT26 tumor treated with anti-PD-L1
- 25-33% of treated mice respond

EL4 murine lymphoma ± Ova
- Adoptive transfer of OT-I CD8+ T cells

Day 0
CT26 implant

Day 7
Anti-PD-L1 Ab

Day 11
immunoPET Biodistribution

Day 13
Radiolabeling

Injection

Non-responders

Responders

20 % ID/g

0.5 % ID/g

Day -2
900 cGy
Bone marrow

Day 0
EL4/EL4-Ova

Day 5
IL-2

Day 6
OT-I ACT
DC

Day 7

Day 10

Day 11

Day 0

Anti-PD-L1 Ab

Day 7

immunoPET Biodistribution

Radiolabeling

CD8-Block

Imaging **CD4** T cells in lymphoid tissues

**GK1.5 cys-diabody**

<table>
<thead>
<tr>
<th>Time post-HSC transplantation</th>
<th>2 weeks</th>
<th>4 weeks</th>
<th>8 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CLN</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ALN</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sp</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ILN</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PLN</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Li</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>K</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>B</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**89Zr-radiolabeled anti-CD4 GK1.5 cys-diabody detects T cell repopulation following HSC**

*R. Tavaré, J.Nucl. Med. 2015*
$^{18}$F-GA101 cDb imaging in A20-huCD20 metastatic lymphoma in huCD20TM mice


Kirstin Zettlitz, Jeff Salazar, Mike van Dam, unpublished
Challenges to development of radiolabeled antibodies for immunoPET

Complex product:

Biopharmaceutical – time consuming and costly to produce
Radiopharmaceutical
Regulatory path – tox/safety
What is “efficacy” – requirements for approval; indication
Financial incentives/reimbursement
## Selection of radionuclide: Go short or go long?

<table>
<thead>
<tr>
<th>Radionuclide</th>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{68}$Ga 68 min</td>
<td>Generator-produced 89% $\beta+$ Favorable dosimetry; radioactive waste not an issue Need rapidly targeting agent</td>
<td>Every site needs a generator High energy $\beta+$; poorer resolution</td>
</tr>
<tr>
<td>$^{18}$F 109 min</td>
<td>Cyclotron-produced 97% $\beta+$ Favorable dosimetry; radioactive waste not an issue Need rapidly targeting agent</td>
<td>Need cyclotron w/in 2h travel distance</td>
</tr>
<tr>
<td>$^{89}$Zr 3.2 d</td>
<td>Commercially available clinical grade (IBA, NCM, 3D Imaging, PE, etc.) 23% $\beta+$ Can be labeled centrally and shipped (e.g. across US)</td>
<td>Radiation dose (due to mixed emissions and half life)</td>
</tr>
</tbody>
</table>
Clinical development and translation

- Humanized minibodies
- Conjugated with desferrioximine
- $^{89}\text{Zr}$ for immunoPET
  - IAB22M CD8
  - IAB2M PSMA

ImaginAb, Inc.
IAB22M2C for detection and imaging of human CD8 T cells

- Cell surface marker on cytotoxic T cells
- Minibody: Does not contain full Fc; biologically inert (no T cell activation, cytokine release, etc.)
- Preclinical imaging in humanized mouse models

**Diagram:**
- NSG mouse + Human PBMCs → Engraftment → ~4-5 wks
- Graft-versus-Host Disease
Infiltration of Human CD8 T cells into Lungs Can Be Followed in NSG Mice With GVHD

Engraftment of NSG mice with $20 \times 10^6$ hu-PBMCs

1 week - Engraftment
4 weeks - GVHD

huCD8 immunoPET

Lung

Spleen

huCD8 IHC

Olafsen et al. abstract Antibody Engineering 2015; AACR 2016 (ImaginAb, Inc.)

IND Q3 2016
IAB2M anti-PSMA Targets Major Clinical Decision Points in Prostate Cancer

New Diagnosis

PSMA IMAGING

Metastatic Disease

Prostate Removed

↑ PSA (biochemical recurrence)

PSMA IMAGING

Localized Disease (salvage radiotherapy)

Systemic Disease (anti-androgens)

Ongoing Phase 2 study

Next Phase 2 study
First-in-human imaging with $^{89}$Zr-Df-IAb2M anti-PSMA minibody in patients with metastatic prostate cancer: Pharmacokinetics, dosimetry, and lesion uptake

Head-to-head comparison of $^{89}$Zr-Df-IABM PET/CT to $^{111}$In capromab pendetide SPECT/CT scans in the detection of occult prostate cancer in patients undergoing radical prostatectomy (RP) with negative conventional imaging (CI) studies


- 65 Year old male
- Gleason score 8
- PSA (at screening) 25.5 ng/mL
- Negative CI
- Increase $^{89}$Zr-Df-IAB2M in normal size lymph nodes (red arrows)

Bgurek et al., Scottsdale Healthcare abstract, WMIC 2015
Summary and future: Non-invasive Imaging in Immuno-Oncology

• Powerful, specific, and whole-body approaches for monitoring immune cells and immune responses
  – Metabolic probes, pre-labeled cells, reporter genes
  – Engineered antibodies for immunoPET of cell surface markers
• Potential for profiling: immune cell subsets, expansion, trafficking, activation; biomarker microenvironment; potential role in patient selection and treatment monitoring
• Challenges:
  – Sensitivity: lower limit of detection (targets/cell and cells/cc) (AACR 2016)
  – Spatial resolution (macroscopic, not microscopic)
  – Multiplex imaging? Multiple cell types, subsets (e.g. T_{regs})
  – Endogenous vs adoptively transferred cells
  – Complex product, lengthy and expensive clinical development
  – Next targets – what do we need to assess in vivo?

*Imaging can enhance and complement in vitro biomarkers*
“Antibody immunotherapy imaging”

Elisabeth de Vries
Department of Medical Oncology
University Medical Center Groningen
The Netherlands
Disclosures

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  – Roche/Genentech, Amgen, Novartis, Servier
<table>
<thead>
<tr>
<th>radionuclide</th>
<th>SPECT</th>
<th>PET</th>
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<tbody>
<tr>
<td></td>
<td>half-life</td>
<td>residualization</td>
</tr>
<tr>
<td>^{111}\text{In}</td>
<td>67.3 h</td>
<td>+</td>
</tr>
<tr>
<td>^{131}\text{I}</td>
<td>192.5 h</td>
<td>-</td>
</tr>
<tr>
<td>^{123}\text{I}</td>
<td>13.2 h</td>
<td>-</td>
</tr>
<tr>
<td>^{99m}\text{Tc}</td>
<td>6.0 h</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Potential mechanisms of action of trastuzumab

Hudis, New Eng j Med 2007
More lesions with $^{111}$In-trastuzumab-SPECT in patients with HER2+++ metastatic breast cancer compared to conventional imaging.

Overall results: Newly discovered tumor lesions in 13/15 patients.

Perik et al, J Clin Oncol 2006
Limited trastuzumab tumor saturation: \textsuperscript{111}In-trastuzumab

- **Methods:**
  - \textsuperscript{111}In-trastuzumab administered day 1 of cycle 1 and day 15 of cycle 4 trastzumab plus paclitaxel.

- **Results:**
  - 25 tumor lesions in 12 patients visualized on both scintigraphy series
  - Tumor uptake decreased 19.6% ($P = 0.03$)
  - Residence times of normal organs remained similar

*Perik et al, J Clin Oncol 2006
Gaykema et al, Mol Imaging 2014*
$^{89}$Zr-trastuzumab tumor visualization

Dijkers et al, Clin Pharmacol Ther 2011

Day 4
$^{89}$Zr-trastuzumab tumor accumulation dependent on total protein dose

<table>
<thead>
<tr>
<th>Imaging dose</th>
<th>cohort 1</th>
<th>cohort 2</th>
<th>cohort 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{89}$Zr-trastuzumab</td>
<td>1.5 mg</td>
<td>1.5 mg</td>
<td>1.5 mg</td>
</tr>
<tr>
<td>trastuzumab</td>
<td>8.5 mg</td>
<td>48.5 mg</td>
<td>8.5 mg + up to 6 mg/kg therapy</td>
</tr>
</tbody>
</table>

- Cohorts 2 & 3 have better $^{89}$Zr tumor uptake than cohort 1

*Dijkers et al, Clin Pharmacol Ther 2010*
Zephir TDM-1 study

Pre treatment

- Biopsy
- CT
- FDG-PET
- $^{89}$Zr-trastuzumab-PET

Treatment

1. T-DM1 treatment (every 21 days)
2. 2x FDG-PET early and late in treatment

Follow-up until progression

NCT01565200
Despite presence HER2, $^{89}$Zr-trastuzumab does not always reach tumor (PET/CT n=52).

- **39%**: All or most of the tumor load is seen on $^{89}$Zr-trastuzumab PET/CT
- **29%**: Minority of tumor load or no lesions are seen on $^{89}$Zr-trastuzumab PET/CT
- **16%**: Minority of tumor load or no lesions are seen on $^{89}$Zr-trastuzumab PET/CT
- **16%**: Minority of tumor load or no lesions are seen on $^{89}$Zr-trastuzumab PET/CT

*Gebhart et al, ASCO 2015 & Ann Oncol 2016*
89Zr-trastuzumab tumor accumulation associates with T-DM1 time to treatment failure, HER2+ (IHC/FISH) metastatic breast cancer patients.

Heterogeneous $^{89}\text{Zr}$-trastuzumab uptake in tumor lesions

Different signal intensity in different lesions

Dijkers et al, Clin Pharmacol Ther. 2010
Tumor microenvironment

- Macrophage
- Dendritic cell
- T-cell
- Cancer cell
Targets immune checkpoint inhibitors
# Preclinical imaging studies with radiolabeled immune checkpoint inhibiting antibodies

<table>
<thead>
<tr>
<th>Tracer</th>
<th>Target</th>
<th>Origin</th>
<th>Model</th>
<th>Author</th>
<th>Journal</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{111}$In-anti-PD-L1</td>
<td>PD-L1</td>
<td>Humanized anti-human</td>
<td>Human cell lines</td>
<td>Chatterjee et al</td>
<td>Oncotarget, 2016</td>
</tr>
<tr>
<td>$^{111}$In-anti-PD-L1</td>
<td>PD-L1</td>
<td>Hamster anti-mouse</td>
<td>NT2.5: mouse mammary tumor</td>
<td>Josefsson et al</td>
<td>Cancer Res, 2016</td>
</tr>
<tr>
<td>$^{125}$I-anti PD-L1:PRO304397 biodistribution &amp; autoradiography</td>
<td>PD-L1</td>
<td>Chimeric</td>
<td>Mouse</td>
<td>Deng et al</td>
<td>mAbs, 2016</td>
</tr>
<tr>
<td>$^{111}$In-anti-PD-L1</td>
<td>PD-L1</td>
<td>Murine anti-human</td>
<td>Human breast cancer cell lines</td>
<td>Heskamp et al</td>
<td>Cancer Res, 2015</td>
</tr>
<tr>
<td>$^{64}$Cu-anti-PD1</td>
<td>PD1</td>
<td>Hamster anti-mouse</td>
<td>B16F10: mouse melanoma</td>
<td>Natarajan et al</td>
<td>Bioconjug Chem, 2015</td>
</tr>
<tr>
<td>$^{64}$Cu-anti-PD1 ectodomain</td>
<td>PD1</td>
<td>Murine anti-mouse</td>
<td>CT26: mouse colon cancer</td>
<td>Maute et al</td>
<td>PNAS, 2015</td>
</tr>
</tbody>
</table>
Imaging with $^{111}$In-PD-L1-mAb & NIR-PD-L1-mAb in sc CHO xenografts

Chatterjee et al, Oncotarget 2016
$^{111}$In-PD-L1 antibody biodistribution in tumor-bearing transgenic neu-N mice for normal tissues

Coinjected for blocking with excess cold anti–PD-L1 Ab 30× (green) and 100× (yellow)
Autoradiography: distribution of $^{125}$I-antiPD-L1 antibody PRO304397 in murine colorectal MC38 tumors

*Deng et al, mAbs 2016*
PD-L1 expressing tumor cells treated with anti-PD-L1 antibody, show PD-L1 antibody internalization.

Yellow: Anti-PD-L1 antibody: αPD-L1

Heskamp et al, Cancer Res 2015: Chang et al, Cell, 2015
Immunotherapy: 
$^{89}$Zr-labeled PD-L1 antibody

![Diagram of immune cells and cancer cells with PD-L1 interaction]
Immunotherapy:

$^{89}$Zr-labeled PD-L1 antibody
Biopsy provides a snapshot of 1 part of 1 lesions: PD-L1 expression by tumor cells & tumor-infiltrating immune cells (macrophages, dendritic cells & lymphocytes)

Serum pharmacokinetics cycle 1
PD-L1 antibody atezolizumab

Very preliminary PD-L1 antibody imaging results in patients: Primaries
Design trial with $^{89}$Zr-atezolizumab in TNBC, bladder cancer and NSCLC patients

ClinicalTrials.gov Identifier: NCT02453984
$^{89}$Zr-atezolizumab uptake in NSCLC patient over time
$^{89}$Zr-atezolizumab uptake in NSCLC patient day 8
Conclusions PD-L1 antibody imaging

- **Preclinical:**
  - Specific tumor uptake of PD-L1 antibody (radioactive and fluorescent)
  - Biodistribution showed high specific PD-L1 antibody uptake in the spleen
  - PD-L1 antibody internalizes in tumor cells

- **Clinical:**
  - Immunohistochemical PD-L1 staining provides information of 1 part of the tumor at 1 moment
  - Ongoing $^{89}$Zr-atezolizumab trial: currently collecting trial data
Immunotherapy:

$^{89}\text{Zr}$-labeled PD-1 antibody
$^{64}$Cu-anti-mouse antibody (IgG) PD-1 antibody tracer detecting in melanoma (B16F10) tumor bearing mice PD-1 expressing murine TILs

White Arrow = Thymus or lymph nodes,  L = Liver,  T = Tumor,  H = Heart,  S = Spleen.

Natarajan et al, Bioconjug Chem, 2015
Engineering high-affinity PD-1 variants for immuno-PET imaging with $^{64}$Cu after 1 h

Maute et al, Proc Natl Acad Sciences 2016
89Zr-pembrolizumab imaging in melanoma patients

Part A. Imaging dose and schedule finding

- 89Zr-tracer
- 89Zr-scan
- 89Zr-scan
- 89Zr-scan
- Pembrolizumab treatment

Day: 0, 2, 4, 7

Part B. Implementation of imaging

- 89Zr-tracer
- 89Zr-scan
- Biopsy
- Pembrolizumab treatment

Day: 0, 2, 4, 7
Labeled bispecific antibody (construct) tracers
Labeled bispecific T cell engaging antibody targeting CEA
$^{89}$Zr-labeled bispecific T cell engaging antibody construct targeting CEA

Waaijer et al, AACR-NCI-EORTC meeting Abstract # A85, 2015

ClinicalTrials.gov Identifier: NCT02291614
Antibody imaging with radionuclides in the clinic
Optical imaging

- Optical imaging uses light
- High resolution (> PET)
- Non-radioactive
- Limited penetration
  - novel tracers
  - improved detection systems
Intraoperative, endoscopic and hand held systems

- Intraoperative camera
- Optical fiber endoscope
- Optoacoustic handheld system
Dual imaging with
$^{89}\text{Zr}$-bevacizumab & IRDye800CW-bevacizumab

Terwisscha van Scheltinga et al, J Nucl Med 2011
First in human results IV CW800-bevacizumab in 3 small esophageal cancers
Endoscopic mucosal resection specimen including esophageal adenocarcinoma after IV CW800-bevacizumab
Multiplex Advanced Pathology Imaging (MAPI) for bevacizumab-IRDye800CW in breast cancer
Conclusions role molecular imaging

• Antibody imaging for immunotherapy can visualize drug distribution & tumor characteristics
• Provides insight in
  – Heterogeneity in tracer uptake by tumor lesions
  – Pharmacodynamic effects in the tumor
• Insight into localization of the drug in the tumor
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Nathan McKnight
Sandra Sanabria
Simonetta Mocci
Luisa Veronese
Overview of Nuclear Medicine Imaging Capabilities

Michael M. Graham, PhD, MD
Director of Nuclear Medicine
University of Iowa
Major Instruments

Single Photon Emission Computed Tomography (SPECT)

Positron Emission Tomography (PET)

$^{99m}$Tc $^{43}$ $^{99}$Tc

T$\frac{1}{2}$ = 6 hr

T$\frac{1}{2}$ = 200,000 yr

140 Kev gamma ray

511 KeV
Major Instruments

Single Photon Emission Computed Tomography (SPECT)

- Spatial resolution: 12 mm
- Temporal resolution:
  - 10 sec (planar)
  - 10 min (SPECT)
- $^{99m}Tc$, $^{111}$In, $^{123}$I, $^{131}$I

Positron Emission Tomography (PET)

- Spatial resolution: 6 mm
- Temporal resolution: 2 min
- $^{11}$C, $^{13}$N, $^{15}$O, $^{18}$F
- $^{64}$Cu, $^{68}$Ga, $^{89}$Zr, $^{124}$I
SPECT/CT systems

GE Hawkeye

Siemens Symbia
**PET/CT scan protocol**

Spiral CT

Survey

CT

scatter correction

attenuation correction

PET data

FORE OSEM

FUSION

Fused PET/CT

WB PET: 6-40 min

10 mCi; 60 min uptake

PET data

FORE OSEM
Types of studies
(Flow, Metabolism, Receptors, Cell Trafficking)

- **Flow of material**
  - Blood flow (brain, heart)
  - Gastric emptying
  - Lymphatic drainage
  - Bile
  - Urine
  - CSF

- **Metabolism**
  - Bone formation
  - Bile formation
  - Renal tubular function
  - Macrophage activity
    - Liver, spleen, bone marrow
  - Glucose metabolism
  - Fatty acid metabolism
  - Cell membrane synthesis
  - DNA synthesis
  - Protein synthesis
  - Iodine
Types of studies
(Flow, Metabolism, Receptors, Cell Trafficking)

• **Receptor imaging**
  - MIBG
  - Dopamine receptors
  - Somatostatin receptors
  - Prostate specific membrane antigen (PSMA)
  - CD20 (Zevalin)
  - Bombesin
  - Angiogenesis (RGD)
  - Folate receptor
  - CXCR4 (chemokine)

• **Cell Trafficking**
  - Red blood cells
  - White blood cells
  - Platelets
  - Lymphocytes
  - Eosinophils
  - Granulocytes
  - mesenchymal stem cells

• **Hypoxia**
  - FMISO, FAZA, EF-5, HX4

• **Apoptosis**
  - Annexin V, ML-10
Radiolabeled Receptor Ligands

Radio-metal
$^{68}$Ga, $^{64}$Cu, $^{89}$Zr
DOTATOC and DOTATATE

The untapped potential of Gallium 68-PET: The next wave of $^{68}$Ga-agents
D.L. Smith et al. / Applied Radiation and Isotopes 76 (2013) 14–23
- Changes in management in 15 of the 20 patients who had $^{111}$In-Octreoscan®
- Applying for funding to do $^{68}$Ga DOTATATE PET/MR
$^{68}\text{Ga-PSMA}$

**Chemistry ($[^{68}\text{Ga}]-\text{PSMA}$)**

- $^{68}\text{Ga}$ – chelator HBED-CC
- PSMA – binding motif

**μPET**

**Clinical PET**

Glu-NH-CO-NH-Lys-(Ahx)-[${}^{68}\text{Ga(HBED-CC)}$]
Methodology for Labeling Cells

• In vitro (requires separation of specified cell type)
  – $^{111}\text{In}$ oxine
  – $^{99m}\text{Tc}$ HMPAO
  – $^{99m}\text{Tc}$ pertechnetate after incubation with stannous chloride
  – $^{99m}\text{Tc}$ sulfur colloid
  – $^{64}\text{Cu}$ PTSM
  – $^{18}\text{F}$ FDG
  – $^{89}\text{Zr}$-DBN

• In Vivo
  – $^{99m}\text{Tc}$ interleukin-8
  – $^{99m}\text{Tc}$-Fanolesomab (targets CD15)

half-lives
$^{64}\text{Cu}$ 12.7 h
$^{111}\text{In}$ 2.8 d
Figure 11. Infected right knee arthroplasty. On the sagittal images from the simultaneously acquired dual-isotope SPECT-CT, spatially incongruent distribution of activity on $^{111}$In-WBC (top) and marrow (bottom) images can be identified clearly anterior and posterior.

Christopher J. Palestro
Radionuclide Imaging of Osteomyelitis
Seminars in Nuclear Medicine, Volume 45, Issue 1, 2015, 32–46

Figure 4.—Faint linearly increased activity lateral to the right iliac vessels (arrow), which increases in intensity over time, can be appreciated at 5 min after tracer injection. In about half of the patients with appendicitis, the appendix is visualized within 10 min after injection of (courtesy of Frederick L. Weland, M.D.).

Mouse-derived melanoma cells, dendritic cells, and human mesenchymal stem cells were covalently labeled with $^{89}$Zr-DBN via the reaction between the NCS group on $^{89}$Zr-DBN and primary amine groups present on cell surface membrane protein.

$^{89}$Zr half-life of 78.42 h
Griessinger CM et al. $^{64}$Cu antibody-targeting of the T-cell receptor and subsequent internalization enables in vivo tracking of lymphocytes by PET. Proc Natl Acad Sci U S A. 2015; 112:1161-6.

We labeled chicken-ovalbumin-TCR-transgenic TH1 cells (cOVA-TCRtg-TH1) with $^{64}$Cu-DOTA–modified cOVA-TCR–specific mAbs in vitro and investigated the endocytosis-dependent intracellular accumulation of the mAb–TCR complex.
Glucose  Fluorodeoxyglucose (FDG)
Tumors tend to have high levels of glucose transporters (GLUT) and hexokinase.
Fluorothymidine
DNA synthesis

Thymidine

FLT
(3’-deoxy-3’-fluorothymidine)

John R. Grierson and Anthony F. Shields
University of Washington (Seattle) and Wayne State University (Detroit)

Threshold: decrease of >10.9% in SUVmax. PPV & NPV were both 92.9%.
# Types of studies
(Metabolism, Receptors, Cell Trafficking)

<table>
<thead>
<tr>
<th>Metabolism</th>
<th>Receptor imaging</th>
<th>Cell Trafficking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone formation</td>
<td>MIBG</td>
<td>Red blood cells</td>
</tr>
<tr>
<td>Bile formation</td>
<td>Dopamine receptors</td>
<td>White blood cells</td>
</tr>
<tr>
<td>Renal tubular function</td>
<td>Somatostatin receptors</td>
<td>Platelets</td>
</tr>
<tr>
<td>Macrophage activity</td>
<td>PSMA</td>
<td>Lymphocytes</td>
</tr>
<tr>
<td>Glucose metabolism</td>
<td>CD20 (Zevalin)</td>
<td>Eosinophils</td>
</tr>
<tr>
<td>Fatty acid metabolism</td>
<td>Bombesin</td>
<td>Granulocytes</td>
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<tr>
<td>Cell membrane synthesis</td>
<td>Angiogenesis (RGD)</td>
<td>mesenchymal stem cells</td>
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<tr>
<td>DNA synthesis</td>
<td>Folate receptor</td>
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<tr>
<td>Protein synthesis</td>
<td>CXCR4</td>
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<tr>
<td>Iodine</td>
<td>Apoptosis</td>
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</tbody>
</table>

## Goal
Identify specific metabolic pathways, up-regulated receptors, or cell trafficking that can either predict responders or assess response early in the course of therapy.
Bridging the Gap: Funding and Resources at NCI for Molecular Imaging Agents

Paula M. Jacobs, Ph.D.
Associate Director, Division of Cancer Treatment and Diagnosis, NCI
Cancer Imaging Program

NIH NATIONAL CANCER INSTITUTE
Outline

- Grants
  - General NIH funding
  - Specialized imaging funding
  - SBIR/STRR funding

- NCI Experimental Therapeutics Program (NExT)

- Cooperative Group Trials

- Regulatory advice
NIH Grant Funding
General Funding

Funding Opportunities and Notices - NIH & NCI

- [http://www.cancer.gov/researchandfunding/funding/announcements](http://www.cancer.gov/researchandfunding/funding/announcements)

Common types of grant

- Unsolicited – R01, R03, R21
- Request for applications (RFA)
- Program announcement (PA/PAR)
Grant Funding For Imaging

- Early Phase Clinical Trials in Imaging and IGI (R01): PAR-14-166
  - R01 - $500,000 total direct costs over 2 years
  - Supports early phase clinical trials

- Image-guided Drug Delivery in Cancer (R01): PAR-13-185
  - R01 – standard NIH policies
  - Encourages innovative translational research in image-guided drug delivery (IGDD) in cancer.

- Oncology co-clinical QI imaging research resources (U24) PAR 15-266

Grant Funding For Imaging (2)

- **SBIR & STTR**
  - The Small Business Innovation Research (SBIR) [PA-14-071](#); 2.9% set aside
  - Small Business Technology Transfer (STTR) [PA-14-072](#); 0.4% set aside
  - ~$700M annually at NIH; $115 at NCI

- **Not as relevant to imaging immunotherapy**
  - Quantitative Imaging for Evaluation of Responses to Cancer Therapies: [PAR 14-116](#)
    - QIN – U01 – Cooperative Agreement
    - Develop and share quantitative imaging methods to measure tumor response to therapy
  - NCI Informatics (U01, R01, P01, U24): [PAR 12-286-290](#)
But grants don’t get you into the clinic......
Bridging the “Valley of Death”

- Structure-Activity-Relation
- Toxicology studies
- Chemical Process development
NOT A GRANT PROGRAM

- Provides access to NCI resources and expertise – NCI performs the project
- Simple application process
- External expert review
- Internal expert review
- Full team support
- Applicant involved in project
NExT Development Resources

- Multi- and interdisciplinary clinical/translational teams
- Early access to leading-edge translational technologies
- PK/PD modeling and assay development
- Toxicology/Safety Pharmacology
- Formulation & GMP Scale-Up
- Imaging for biodistribution
- Development & validation of pharmacodynamics assays
- Development & validation of clinical assays
- Proof-of-concept or first in human studies
Next Resources Currently Support

- Investigational drugs and biologics
- Investigational imaging agents
- Academic, biotech and pharma projects
- Phase 0, 1 and 2 clinical trials
- HTS, Hit-to-Lead and Lead optimization

**NOT basic research**
Portfolio Stratified by Agent Class (Active Projects)

<table>
<thead>
<tr>
<th>Agent Name</th>
<th>Institution</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choyke, Peter</td>
<td>CCR NCI</td>
<td>A Phase II Study of F-18 DCFBC, a Prostate Specific Membrane Antigen-Target</td>
</tr>
<tr>
<td>Frangioni, John</td>
<td>BIDMC</td>
<td>A NIR Fluorophore for Clinical Translation of Image-Guided Oncologic Surgery</td>
</tr>
<tr>
<td>Griffiths, Gary</td>
<td>CCR NCI</td>
<td>Large Scale Preparation of IR700-Panitumumab for Clinical Use</td>
</tr>
<tr>
<td>Kirsch, David</td>
<td>Duke</td>
<td>Using Molecular Imaging to Detect Microscopic Residual Cancer During Surgery</td>
</tr>
<tr>
<td>Rosenthal, Eben</td>
<td>UAB</td>
<td>Intraoperative Optical Imaging to Guide Surgical Resection of Cancer</td>
</tr>
</tbody>
</table>

http://next.cancer.gov/
Access to NExT

A Unique Partnership with the NCI to Facilitate Oncology Drug Discovery and Development

Who: Researchers in academia, government and industry, nationally and internationally.

http://next.cancer.gov/
Cooperative Group Trials and Regulatory Assistance
NCI Cooperative Groups

- A half-century old national clinical trial system for oncology
- Conduct large scale clinical trials
  - Disease oriented
  - Radiation therapy
  - Surgery
  - Imaging
- Restructured to 1 pediatric & 4 adult groups in 2014
  - COG – pediatrics
  - Alliance – oncology
  - ECOG-ACRIN – oncology, imaging
  - NRG – radiation therapy, surgery, gynecology
  - SWOG – oncology
File IND’s for investigational trials, Group or CCR

- FLT, FES, FMISO, Zr-panitumumab, NaF, DCFBC, ferumoxytol
- Note that filings for Zr-antibody are posted on our web site

Provide cross-file letters to independent PIs

- Between 50 and 60 to date

Provide full SOPs for tracer manufacture

- FLT, FES, FMISO, Zr-panitumumab

Advise on regulatory process

File NDA’s to permit ANDA’s

- $^{18}$F-Sodium Fluoride 2012
- Exploring $^{18}$F Fluoromethylcholine
Thanks for your attention

Imaging.cancer.gov

www.cancer.gov

Jacobsp@mail.nih.gov

www.cancer.gov/espanol
SBIR & STTR: Three-Phase Program

- Proof-of-Concept study
- $150,000 over 6 months (SBIR) or 1 year (STTR)

Direct to Phase II
- Skip Phase I

- Commercialization stage
- Use of non-SBIR/STTR funds

Phase I
- Fast Track Application
- Combined Phase I & II

- Research & Development
- Commercialization plan required
- $1 million over 2 years

Phase II

Phase III
- COMMERCIALIZATION

- Hard caps on award sizes: $225,000 for Phase I; $1.5 million for Phase II
- Certain awards may exceed these caps if covered by topic-specific waivers
- Actual funding may vary by topic
SBIR Eligibility Requirements

- Applicant is a Small Business Concern (SBC), organized for-profit U.S. business
- 500 or fewer employees, including affiliates
- PI’s primary employment (>50%) must be with the SBC at time of award & for duration of project
- > 50% U.S.-owned by individuals and independently operated
  OR
- > 50% owned and controlled by other business concern/s that is/are > 50% owned and controlled by one or more individuals
  OR
- > 50% owned by multiple venture capital operating companies, hedge funds, private equity firms, or any combination of these
STTR Eligibility Requirements

- Applicant is a Small Business Concern (SBC), organized for-profit U.S. business
- Formal cooperative R&D effort
  - Minimum 40% by small business
  - Minimum 30% by US research institution
- US Research Institution: college or university; non-profit research organization; Federally-Funded R&D Center (FFRDC)
- Principal Investigator’s primary employment may be with either the SBC or the research institution
- SBC must have right to IP to carry out follow-on R&D and commercialization
Reasons to seek SBIR/STTR Funding

- Provides seed funding for innovative technology development
- Not a Loan; repayment is not required
- Doesn’t impact stock or shares in any way (i.e., non-dilutive)
- Intellectual property rights retained by the small business
- Provides recognition, verification, and visibility
- Helps provide leverage in attracting additional funding or support (e.g., venture capital, strategic partner)
Orphan Drugs – not NCI, but relevant
Orphan Drugs

- Drugs or biologics (not devices) intended to treat, diagnose, or prevent a rare disease or condition... or,
- A drug that will not be profitable within 7 years following FDA marketing approval (rare)
- Pathway for devices available, but not identical
- Can submit common application to EMA
Is the Disease or Condition Rare?

- The disease or condition prevalence <200,000 in the US
- Acute diseases or conditions: yearly incidence may be used in some cases to estimate the patient population (<200,000 in the US)
- Diagnostics and preventatives: may only be subjected to <200,000 patients in the US annually
- Medically plausible (orphan) subsets of common diseases (e.g. metastatic melanoma)
  - No salami slicing
Medical Plausibility (Orphan) Subsets

- There is some property of the drug such that the use of the drug would be limited to the subset of the disease or condition
- E.g., toxicity profile, mechanism of action
- The drug would not be used in the full complement of the disease
- Regulatory term to delineate persons expected to use the drug
- Not a clinical definition
Benefits of Orphan Designation

- Purely financial in nature:
  - Seven years of market exclusivity
  - Up to 50% of tax credits for clinical research expenses
  - Waiver of marketing application fees

- However...
  - Often the first step in FDA communication
  - OOPD may provide informal guidance
  - May also attract venture capital

- Can apply for FDA grants to support clinical research
Request for Orphan Designation

- Possibly the simplest FDA submission
- The request must be made prior to the submission of a BLA or NDA
- An IND is not required for submission
- May be submitted from sponsors from any country
- May be private citizens, academic institutions, for-profit, non-profit, small biotech, industry, etc.
IMMUNOTHERAPY’S OTHER CHALLENGE: BIOMARKERS AND IMAGING TO DETERMINE WHO WILL BENEFIT?

Elizabeth M. Jaffee, M.D.
Dung Le, M.D.
Lei Zheng, M.D., Ph.D.
Eric Lutz, Ph.D.
Dan Laheru, M.D.
Disclosure Information

Elizabeth M. Jaffee, M.D.

I have the following financial relationships to disclose

I will be discussing the investigational use of:
- GVAX
- Listeria Monocytogenes – mesothelin

Both licensed to Aduro Biotech with potential to receive royalties

Consultation activity: BMS, Adaptive Biotech, MedImmune

Grants: Aduro, BMS, Roche
Immunotherapy has already changed the standard of care for patients with advanced prostate cancer and melanoma and NSCLC.

Current immunotherapies work on up to 30% of all cancers.

Why doesn’t current immunotherapy work on all cancers?
Pancreatic Cancer: A model to study immunotherapy resistant cancers

Still among the deadliest cancers

Microenvironment provides barrier to drug/immune access

Consider non-immunogenic because it lacks effector T cells at diagnosis

Emerging evidence suggest it develops as an inflammatory response to progressive genetic changes
What have we learned from these successes?

- Immune checkpoint agents act on T cells

- Only a minority of tumors have natural T cells
  - 50% of melanomas
  - 20-30% RCCS
  - 10-20% lung and colorectal tumors

- Pancreatic cancers and many other cancers are immunologically quiescent (lack effector T cells)

- For these cancers immune modulation alone is not enough – a T cell generating agent is also needed
Emerging concepts that explain why pancreatic cancers do not respond naturally to immunotherapy
There is an inflammatory response in pancreatic cancer that is a progressive, dynamic process, interrelated with cancer genetics.
Immunobiology of pancreatic carcinoma

- Fibroblasts
- Macrophages
- B cells
- Desmoplastic stroma
- Extracellular matrix
- Immature myeloid cells
- Regulatory T cells

But, minimal infiltration of effector T cells in the TME in most patients
Hypothesis:
It’s not the physical barrier of the stroma but rather an acquired network of oncogene-driven immunosuppression that excludes effector T cells in most of PDA

Implications:
• Checkpoint blockade in PDA will be ineffective clinically

• Without Darwinian-like pressure from T cells, the underlying pancreatic tumor cells remain highly susceptible to T cells…. if these can be provoked
Immunologically “resistant” tumors have inflammation but lack infiltration of effector T cells. 50% of Melanomas have spontaneous infiltration of effector T cells that can respond to checkpoint inhibitors.
Dendritic cells exemplifies the divergent functional polarities of the different inflammatory cell populations.
(Neo)adjuvant PDA vaccine study provides evidence vaccines can recruit T cells that traffic into immune resistant tumors

Cancer Immunology Research, 2014

<table>
<thead>
<tr>
<th>Pre-study Screen/randomization</th>
<th>1st Vaccine</th>
<th>Surgery (PD)</th>
<th>2nd Vaccine</th>
<th>Adjuvant Chemoradiation and Chemotherapy</th>
<th>3rd Vaccine</th>
<th>4th Vaccine</th>
<th>5th Vaccine</th>
<th>6th Vaccine</th>
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<td>-1</td>
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<td>38</td>
<td>42</td>
<td>46</td>
<td>52</td>
<td>54</td>
</tr>
</tbody>
</table>

Week
Lymphoid Aggregates develop in tumors in vaccinated patients 2 weeks after a single vaccine
Lymphoid aggregates in PDAs are composed of organized T and B cell zones and a Germinal Centre-like structure.
Lymphoid Aggregates Are Sites of Immune Activation and Regulation – Not Cytolysis
PD-1/PD-L1 pathway is upregulated in vaccine induced lymphoid aggregates.
Cellular Source of PD-L1 in Lymphoid Aggregates (FFPE samples)

T Tsujikawa, S Kumar, E Lutz, L Coussens, E Jaffee
Multiplex IHC enables detection of 12-different epitopes in a single FFPE section

Tsujikawa T, et al. Manuscript in preparation
Two panels of 12-color multiplex IHC depicted tumor immune infiltrates in pancreatic ductal adenocarcinoma (PDAC) tissues

Human PDAC tissue, neoadjuvant GVAX
Image cytometry enables quantification of 16-different cell lineages
Neoadjuvant GVAX therapy is associated with PD-L1 upregulation in myeloid cell lineages, correlating with prognosis.

Tsujikawa T, et al. Unpublished data
T cells can be found infiltrating between lymphoid aggregates.

**CD8**

- **OS<1.5 yr**
- **OS>3 yr**

**Foxp3**

- **OS<1.5 yr**
- **OS>3 yr**
Neo-Adjuvant Study of Vaccine +/- PD-1 Blockade

Evaluate changes in T cell Activation and infiltration

Evaluate changes in PD-L1 expression on tumors and monocytes

Evaluate immune signatures of response

GVAX Pancreas
Whole-cell tumor vaccine

Nivolumab
Fully human IgG4 antibody against PD-1
What are current challenges?

- Single agent immune modulatory agents work in 30-40% of immune responsive cancers (20% of all cancers)
- Combinations of a T cell inducing agent with immune modulators are likely needed to see responses in most other patients
- Measurable responses are often delayed by weeks to months
- Combinations of immune modulators increase efficacy but also increase toxicity
- Biomarkers and imaging techniques are needed to identify untreated patients who will respond to immunotherapy to avoid toxicity in non-responders
- Ideally biomarkers/imaging are needed to identify relevant checkpoint pathways in different patients to personalize treatment
- Biomarkers/imaging are needed to identify responders during treatment since some patients require months of treatment before exhibiting a radiographic and clinical response
- Biomarkers/imaging are needed to differentiate tumor progression from inflammation
- In vivo imaging of specific pathways are needed to avoid invasive biopsies
How do we distinguish inflammation from cancer recurrence in patients being treated with vaccine and/or immune modulating agents?

An Example
Some vaccinated patients demonstrate recurrent inflammatory reactions not associated with tumor recurrence

- First subject to complete neo-adjuvant and 4 adjuvant vaccines went on to long-term follow-up/boost study
- Boost given every 6 months
- Patient received 1st boost without problems
- Returns for 2nd boost (now at about 21/2 years since diagnosis)
- Patient feels great, no lab abnormalities
- Routine CAT scan evaluation for recurrence shows new mass in tail of pancreas
Resected Lesion: H&E 20X

Chronic Inflammation – no tumor!
Predominantly macrophages

IHC using antiCD68
Pancreatic cancer patients can respond to vaccine + immune checkpoint inhibitors but take up to 6 months and often appear to progress before they regress.

A Few Examples
Phase Ib: Ipilimumab 10 mg/kg Alone or Ipi + Vaccine
Le, et al., J Immunother 2013

**INDUCTION PHASE**

1  2  3  4

1* 4 7* 10 14* 18 22*

**MAINTENANCE PHASE**

34* 46* 58*

**Weeks**

- **Vaccine** = $2.5 \times 10^8$ Panc 6.03 + $2.5 \times 10^8$ Panc 10.05 tumor cells
- *Tumor assessments (TA)*
- Maintenance Phase Dosing And/Or TA q 12 weeks if SD or better at Week 22
Survival Favors GVAX + Ipilimumab Over Ipilimumab

Median OS:
3.6 vs. 5.7 Months

HR: 0.51 (0.23 to 1.08), p = 0.0723

1 year OS: 7% vs. 27%
Radiographic Regressions After 14 Weeks Of Treatment with Ipilimumab (Ipi) + Vaccine
Tumor Marker Kinetics

Arrows: Treatments with GVAX + Ipilimumab
# GVAX/Ipi Frontline Maintenance Study

GVAX Pancreas + Ipilimumab vs. FOLFIRINOX

<table>
<thead>
<tr>
<th>92 Subjects with metastatic pancreatic cancer with stable disease on FOLFIRINOX chemotherapy</th>
<th>1:1 randomization</th>
</tr>
</thead>
</table>

**Arm A, GVAX/Ipilimumab**
- every 3 weeks for 4 doses, then every 8 weeks

**Arm B, FOLFIRINOX**
- one cycle every 14 days

NCT01896869
GVAX/Ipi Frontline Maintenance Study
Delayed Response

Baseline

Week 10

Week 18
PRs are taking Up to 6 months
Patients with metastatic pancreatic cancer; progressing after 1 prior chemotherapy for metastatic disease.

GVAX + CRS-207 Heterologous Prime Boost Vaccination with Programmed Death-1 (PD-1) Blockade

Arm A Vaccine + Anti-PD-1

Arm B Vaccine Alone

1:1 randomization

24 months follow-up

Cy/GVAX

CRS-207

Nivolumab
GVAX + CRS-207 Heterologous Prime Boost Vaccination with Programmed Death-1 (PD-1) Blockade
GVAX + CRS-207 Heterologous Prime Boost Vaccination with Programmed Death-1 (PD-1) Blockade

Baseline

Week 30
Multiplex IHC depicts evidence of T cell reinvigoration with GVAX/CRS207 + nivolumab

Biopsy specimen (STE LLAR Trial)

Tsujikawa T, et al. Unpublished data
What more do we need to learn to effectively treat pancreatic cancers?

- What are the optimal combinations of immune modulators required to induce the most effective and durable immune response?

- Does every patient with a pancreatic cancer have the same immune checkpoint pathways inhibiting immune recognition of their tumors?

- Do patients who respond to inhibitors of PD-1/PD-L1 or CTLA-4 blockade eventually develop immune resistance?

- Are there other T cell regulatory pathways in pancreatic cancers that are inhibiting effective anticancer immunity?
In the future we will likely use repetitive biopsies to personalize each patient's combinations. However, in vivo imaging would provide a less invasive approach to identify combinations of immune modulators and also determine additional modulators needed over time.
Personalizing Immunotherapy to each Patient’s TME

Starting agents

Biopsy or imaging to determine additional checkpoints

Modified from Robert Vonderheide
Scientific Partners

Dan Laheru  
Dung Le  
Eric Lutz  
Lei Zheng  
Todd Armstrong  
Bob Anders  
Sara Solt  
Guanlan Mo  

Chris Wolfgang  
Ralph Hruban  
Joe Herman  
John Cameron  
Carol Judkins  
Rich Schulick  
Barish Edil  
Raka Bhattacharya  
Tianna Dauses  

Immunopathology Lab  
Rajni Sharma  

Lisa Coussens  
Andrew Gunderson  
Takahiro Tsujikawa  

NCI GI Spore  
NCI RO1  
Viragh Pancreatic Cancer Center  
SU2C AACR Lustgarten DREAM TEAM  
Aduro Biotech  
PANCAN AACR
Modernizing Tumor Response Assessment

National Cancer Institute Workshop
Immune Modulation Therapy and Imaging: What can we do now in clinical trials? 2 May 2016

David Leung, MD, PhD
Medical Director for Oncology Imaging
Exploratory Clinical and Translational Research
Bristol-Myers Squibb
Improved Survival Remains a Challenge

5-year Survival in Advanced Cancers (%)\(^1\)

- 5-year survival remains poor for many patients with advanced metastatic solid tumors\(^1\)
- In the US, it is estimated\(^2\) that a total of 589,430 deaths due to cancer will occur in 2015

There is an ongoing need for new treatments and therapeutic modalities for patients with advanced cancers\(^3\)

1. Surveillance, Epidemiology, and End Results (SEER) Stat Fact Sheets
Aspirational Goals of I-O Therapies

Adapted from Sharma and Allison, Cell 2015;161(2):205-214
Immuno-Oncology is an Evolving Treatment Modality

- Immuno-oncology is a fundamentally different approach to fighting cancer that harnesses the body’s own immune system\(^1\)

Through immuno-oncology research, therapies are being investigated in an attempt to utilize the body's own immune system to fight cancer\(^1-3\)

1. Murphy JF. Oncology. 2010;4:67-80
The Long Road in the Development of Immune Therapy for Cancer...

The Discovery of Ipilimumab and Nivolumab

Mouse CTLA4 Cloned¹
CTLA4 Binds to B7²
CTLA-4 is negative regulator of T cell ³⁷
Jim Allison mouse cancer model, Inhibition of CTLA4 as anti-cancer Therapy ⁸
Agreement to develop anti-CTLA4 for clinical use ⁹
Cloning of ipilimumab¹⁰
Ipilimumab FIH
Nivolumab FIH
Nivolumab discovered
PD-1 is a negative signaling molecule¹¹

New generations of IO Agents

Generation 1
- Ipilimumab (Bristol-Myers Squibb)
- Pembrolizumab (Merck)

Generation 2
- T-vec (Amgen)
- Atezolizumab (Genentech/Roche)

Generation 3
- Multiple therapies under development


- Sipuleucel-T (Dendreon, now Valeant Pharmaceuticals)
- Blinatumomab (Amgen)
- CAR-Ts (Novartis)
- Nivolumab (Bristol-Myers Squibb)
- Durvalumab (AstraZeneca)

[Diagram showing timeline with approved and under investigation drugs]

Hoos, A. Nature Review 2016
Tumor flare (growth of existing lesions or appearance of new lesions) may precede antitumor effects resulting in RECIST defined progression and premature discontinuation of therapy. 

Tumor Flare Followed by Durable Response

Screening

Week 12
Swelling and Progression

Week 14
Improved

Week 16
Continued Improvement

Week 72
Complete Remission

Week 108
Complete Remission

Courtesy of Jedd Wolchok. Yervoy patient
Patient with Hodgkin’s Lymphoma on I-O Therapy
Efficacy and Safety of Nivolumab in Patients with Metastatic RCC Who were Treated Beyond Progression in a Randomized, Phase II Dose-ranging Trial

Objective: To evaluate the benefit of continuing nivolumab beyond first RECIST-defined progression in patients with mRCC

Key Criteria
- mRCC with clear-cell component
- ≥1 prior anti-angiogenic agent
- Karnofsky performance status (KPS) ≥70%

Randomize 1:1:1 of nivolumab IV Q3W

0.3, 2, or 10 mg/kg

Endpoints
- Primary: Dose response by PFS
- Key secondary: PFS, ORR, OS, safety

Treat until progression or intolerable toxicity

Treatment beyond progression was permitted if nivolumab was tolerated and clinical benefit was noted

aStratified by Memorial Sloan Kettering Cancer Center (MSKCC) risk group and number of prior therapies in metastatic setting. Motzer, RJ. J Clin Oncol 2015
Overall survival

Median OS, months (95% CI)

<table>
<thead>
<tr>
<th>Treated beyond progression (N = 36)</th>
<th>30.5 (18.1–41.1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not treated beyond progression (N = 92)</td>
<td>15.2 (11.6–23.4)</td>
</tr>
</tbody>
</table>

Patients treated beyond progression

Patients *not* treated beyond progression

Censored

- Patients treated beyond progression: 36 36 36 34 29 26 24 22 21 19 18 16 14 10 3 0
- Patients *not* treated beyond progression: 92 83 69 60 53 47 43 38 35 26 23 23 19 7 2 0
Inclusion of Tumor Shrinkage Metrics Improves Discrimination of Survival Probability in Melanoma Patients

Prognostic Variable Only

$\text{TS}_{\text{max}}^*$

$\text{TS}_{\text{wk8}}^*$

*Prognostic variables: M stage, Sex, ECOG, Albumin, LDH, Weight, Age, Baseline tumor burden.
Why do we measure?

Need to determine relevant early measures of clinical activity predictive of clinical efficacy

- Are there early measurements of clinical activity to identify patients who may benefit from alternative or combination therapy?

- Can we predict long term survival based upon early clinical data allowing for limited sample size and follow-up?
Increasing Complexity in the Future of Immune Modulation

- More immune targets
- More agents
- More combinations
- Immune modulation compared with other treatment modalities
Increasing Complexity in the Future of Immune Modulation

Complex Biology

- IFN-γ-mediated upregulation of tumor PD-L1
- PD-L1/PD-1-mediated inhibition of tumor cell killing
- priming and activation of T cells
- CD8+ cytotoxic T lymphocyte
- M2 macrophage

Larger Tool Box

- Imaging
- Flow Cytometry
- Genomics
- Proteomics
- Pathology

- Predictive Biomarkers
- Resistance mechanisms
- New targets and rational combinations
- Optimal diagnostics

Bristol-Myers Squibb

Immuno-Oncology
Moving Forward – An Evolving Landscape

• Innovative novel therapies
• Comprehensive data analysis – FNIH VolPact, beyond anatomy
• Unified response criteria
• Reliable, robust assessment for optimal patient care
Backup Slides
After first RECIST-defined progression, some patients continuing nivolumab treatment experienced subsequent tumor shrinkage and extended survival.

Each + indicates patient who had at least a 20% increase in target lesions at time of first progression. George S, Motzer RJ et al. ESMO 2015, Vienna.
T Cell Therapies

Lawrence G. Lum, MD, DSc
Director of Cellular Therapy
Scientific Director of BMT
Emily Couric Cancer Center
Professor of Medicine
Department of Medicine
University of Virginia
Charlottesville, VA

NCI Workshop:
Immune Modulation Therapy and Imaging:
What can we do now in clinical trials?

Disclosure: Co-Founder of Transtarget, Inc for Bispecific Antibody armed T cells (BATs)
Bispecific antibody Armed T cells (BATs)

- Can low dose BiAb armed T cells (BATs) be used to target solid tumors?

- Can we avoid CRS related to engaging all T cells with BiAb infusions vivo while inducing long-term immune responses?

- Can we vaccinate with BATs and boost after HDC and SCT to enhance post SCT anti-breast cancer cellular and humoral immunity?

- Can BATs be tracked and imaged on tumors?

- Do BATs work on prostate, pancreatic or liquid tumors?
Targeted Killing by BiAb Armed T cells (BATs)

Anti-CD3 + Anti-Her2 = Anti-CD3 x Anti-Her2

T cell Expansion

BATS (50 ng Her2Bi/10^6 ATC)

Chemical Heterconjugation

T Cell Lysis

Tumor Lysis
Mechanisms for BATs Overcoming Tumor Induced Suppression

**Immunosuppression**

**Progression**
- T regs
- M2 tumor-associated macrophages (TAM):
  - immunosuppression
  - invasion/metastasis
  - vascular remodeling
  - chemoresistance

**Regression**
- BAT-induced Th1 cytokines
- IL-12
- Monocyte
- M1 TAM:
  - T and NK cytotoxicity
  - chemosensitivity
  - regression
- IL-12
- TNFα
- IFNγ
- GM-CSF
- MIP-1α
- TAA
- CD3 xTAABi
- Lum CCR 2015
- Thakur JTM 2013

**Tumor**
- MDSC

**T & NK effectors**
BATS Target “Nil” Expression of Her2 on Sum 1315 Cells

**Fig 2:** Cytotoxicity Remains High in Very Low Her2/neu Expressing Sum 1315. 

*a*) Western blot shows very low (SUM 1315) and high (SK-BR-3) HER2/neu expression in a Western blot; 

*b*) Flow cytometry shows surface expression of Her2/neu on 6.7% of Sum 1315 with very low mean fluorescent intensity (MFI) and 95.1% of SK-BR-3 with a high MFI; and 

*c*) Cytotoxicity remained high in the very low Her2/neu expressing Sum 1315 cell line.
Treatment Schema for Stage IV Breast GM-CSF 250 ug/m²/dose

IL-2 300,000 IU/m²/day

Dose escalation: 5, 10, 20, 40 in standard 3+3 design
# Stage IV Breast Cancer Patients

## Table 1: Patient Characteristics

<table>
<thead>
<tr>
<th></th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 50</td>
<td>14</td>
<td>60.9</td>
</tr>
<tr>
<td>≥ 50</td>
<td>9</td>
<td>39.1</td>
</tr>
<tr>
<td><strong>Cancer Stage</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage IV</td>
<td>23</td>
<td>100</td>
</tr>
<tr>
<td><strong>Performance Status</strong></td>
<td></td>
<td></td>
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<tr>
<td>(ECOG)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>18</td>
<td>78.3</td>
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<tr>
<td>1</td>
<td>5</td>
<td>21.7</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>ER/PR Status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>14</td>
<td>60.9</td>
</tr>
<tr>
<td>Negative</td>
<td>8</td>
<td>34.8</td>
</tr>
<tr>
<td>Unknown</td>
<td>1</td>
<td>4.3</td>
</tr>
<tr>
<td><strong>HER2/neu Status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>10</td>
<td>43.5</td>
</tr>
<tr>
<td>1+</td>
<td>2</td>
<td>8.7</td>
</tr>
<tr>
<td>2+</td>
<td>2</td>
<td>8.7</td>
</tr>
<tr>
<td>3+</td>
<td>8</td>
<td>34.8</td>
</tr>
<tr>
<td>Unknown</td>
<td>1</td>
<td>4.3</td>
</tr>
<tr>
<td><strong>Prior Treatment w/ Herceptin</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>8</td>
<td>26.0</td>
</tr>
<tr>
<td>No</td>
<td>15</td>
<td>74.0</td>
</tr>
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</table>
# Stage IV BrCa Phase I Toxicities

<table>
<thead>
<tr>
<th>Toxicity Grade</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
<th>Total Episodes</th>
<th>% of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chills</td>
<td>0</td>
<td>4</td>
<td>36</td>
<td>0</td>
<td>40</td>
<td>51</td>
</tr>
<tr>
<td>Headache</td>
<td>0</td>
<td>3</td>
<td>14</td>
<td>0</td>
<td>17</td>
<td>22</td>
</tr>
<tr>
<td>N/V</td>
<td>8</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>Fever</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Hypotension</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1.3</td>
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<tr>
<td>SOB</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>14</td>
<td>52</td>
<td>1</td>
<td>77</td>
<td></td>
</tr>
</tbody>
</table>

1 patient died of CHF related to digoxin toxicity after IT was completed. 1 patient developed a subdural hematoma that was evacuated without complications.
Immune Responses to Her2Bi-Armed ATC Infusions and Overall Survival

Stage IV BrCa ($n = 7$)

- IL-12 (pg/ml)

Stage IV BrCa ($n = 9$)

- IL-12 (pg/ml)

Diagrams showing immune responses and overall survival for different infusion types.
### Clinical Responses to Her2Bi-Armed ATC Infusions

<table>
<thead>
<tr>
<th>Response (%)</th>
<th>All Pts #</th>
<th>All Pts %</th>
<th>Dose Level 1</th>
<th>Dose Level 2</th>
<th>Dose Level 3</th>
<th>Dose Level 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR</td>
<td>1</td>
<td>4.3</td>
<td>0</td>
<td>1(100)c</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SD</td>
<td>12</td>
<td>52.2</td>
<td>4(33.3)</td>
<td>2(16.7)</td>
<td>6(50)</td>
<td>0</td>
</tr>
<tr>
<td>PD</td>
<td>8</td>
<td>34.8</td>
<td>4(50)</td>
<td>3(37.5)</td>
<td>1(12.5)</td>
<td>0</td>
</tr>
<tr>
<td>NEb</td>
<td>2</td>
<td>8.7</td>
<td>1(50)</td>
<td>0</td>
<td>1(50)</td>
<td>0</td>
</tr>
</tbody>
</table>

*At one month follow-up after the last infusion and 14.5 weeks after last Tx. bDid not complete infusion schedule or died before 1 month follow-up. cPt received only 80 billion cells due to slow expansion. Evaluation 15 weeks after last chemotherapy/hormone therapy.*

These early results don’t reflect effect on survival; patients Went on to receive dealer’s choice – with prolonged survival; Delayed responses with a pt returning from hospice.
Phase I: Metastatic Breast

Lum 2015 Clin Cancer Research
Her2/neu negative Pt: PR 7 months post IT
Her2 1+: $80 \times 10^9$ Her2 BATS. Sternal biopsy 1 week post treatment

**Shields**

**Figure 1.**

- 111Indium labeled BATs

<table>
<thead>
<tr>
<th>Time</th>
<th>Image</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 HR ANT</td>
<td><img src="image" alt="Image" /></td>
<td><img src="image" alt="Image" /></td>
</tr>
<tr>
<td>ANTERIOR 24HR</td>
<td><img src="image" alt="Image" /></td>
<td><img src="image" alt="Image" /></td>
</tr>
<tr>
<td>ANT 48HR</td>
<td><img src="image" alt="Image" /></td>
<td><img src="image" alt="Image" /></td>
</tr>
<tr>
<td>ANT 4D</td>
<td><img src="image" alt="Image" /></td>
<td><img src="image" alt="Image" /></td>
</tr>
</tbody>
</table>

Scan 1, SUV max = 3.65
Scan 2, SUV max = 5.75

**PreIT**

**PostIT**

Scan 1, SUV max = 3.65
Scan 2, SUV max = 5.75

**Shields**

![Graph](image)
Survival Curves for High Risk Adjuvant BrCa (Her2 0-3+)

Adjuvant Breast Cancer (Her2 0-3+) for High Risk (>10 +nodes)

>10 nodes

Median = 103.5 months), n = 9
ATC Boost with "Immune Cells" after PBSCT for Stage IV Breast Cancer

Vaccinate

aATC infusions

Immune Evaluations

G-CSF Priming

Chemo prep

Pheresis for T cells

Wk1 Wk2 Wk3 Wk4

ATC Boost

Infusions

ASCT

Pheresis for immune T cells

Pheresis for CD34+

M W F M W F M W F

1st 2nd 3rd
wk wk wk

R01 CA 092344

Komen BCTR0707125
Cytotoxicity Directed at SK-BR-3 Pre and Post SCT
### Pancreatic Cancer (Phase I) EGFR BATS: 3/4 infusions and no IL-2 or GM-CSF

<table>
<thead>
<tr>
<th>Pt</th>
<th>Age</th>
<th>Disease</th>
<th>Prior Tx</th>
<th>Dx Date</th>
<th>BATs x 10^9</th>
<th>TTP (days)</th>
<th>OS (days)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>IT20087</td>
<td>58</td>
<td>Mets to liver</td>
<td>Folfirinox</td>
<td>47</td>
<td>186</td>
<td>Dead (409)</td>
<td>13.6 mos</td>
<td>Progressed after Immunotherapy</td>
</tr>
<tr>
<td>IT20091</td>
<td>63</td>
<td>T3 N1Mets to liver. Post Whipple</td>
<td>5FU,Leuk/5FU Folfirinox</td>
<td>1/2012</td>
<td>9.3</td>
<td>CR, 133</td>
<td>Dead (930)</td>
<td>Chemo Induced CR after IT; Treated Twice; Progressing; Folfirinox restarted &amp; responded again</td>
</tr>
<tr>
<td>IT20092</td>
<td>64</td>
<td>T2b Abd Nodes, post Whipple</td>
<td>Gemzar, 5FU, radiation,</td>
<td>2/2012</td>
<td>36</td>
<td>211</td>
<td>Dead (436)</td>
<td>Had chronic diarrhea; Appendicitis From PC tumor with TILs</td>
</tr>
<tr>
<td>IT20102</td>
<td>56</td>
<td>T4, Mets to liver, lungs</td>
<td>Folfirinox</td>
<td>11/2013</td>
<td>74</td>
<td>Stable</td>
<td>Alive (626)</td>
<td>No Treatment; Lesion decrease by 27% at 6 mos; no treatment, progressing chemorestarted, responded</td>
</tr>
<tr>
<td>IT20104</td>
<td>51</td>
<td>T4, Abd Nodes</td>
<td>FOLFOX stable 1 yr then Xeloda</td>
<td>9/2012</td>
<td>72</td>
<td>71, CR</td>
<td>Alive (577)</td>
<td>Chemo Induced CR after IT; On Xeloda</td>
</tr>
</tbody>
</table>

Updated 3-14-16; median OS ~19 mos from ~6 mos
Summary of Clinical Trials using BATs

1. Hormone Refractory Prostate Cancer – BATs induce 1 of 7 PR and 2 minor responses in PSA and bone pain decreased by >80% in pts. Vaishampayan Pros Cancer 2015

2. High risk (>10 nodes) adjuvant breast cancer (Her2 0-3+) treated with HER2 BATs with 5 of 9 pts alive and NED 14 years later (Lum, unpublished).

3. Encouraging results in High risk NHL and Multiple Myeloma using CD20Bi BATs (Lum BMT 2013 and Lum 2013 BBMT).

4. Phase 2 in heavily pretreated (Her2 negative) MBC in 31 evaluable pts with median OS of 19 mos (Lum unpublished)
Model: NeuT transgenic mice develop spontaneous tumors over time
Ab4 is a murine antibody raised against Neu antigen
$^{64}$Cu ($t_{1/2} \sim 12.7$ d) is labeled onto Ab4 using NOTA as chelator

Note: Numbers on the images reflect the tumor uptake of $^{64}$Cu-Ab4.

*Unpublished data. Courtesy of Nerissa Viola-Villegas
$^{89}$Zr-Ab4

- $^{89}$Zr ($t_{1/2}$ ~ 3.27 d) is labeled onto Ab4 using DFO as chelator
- Mouse injected with 4 micrograms=133 ng/ml

* Unpublished data.

Courtesy of Nerissa Viola-Villegas
Thanks to Those who made it Happen!

- **BMT Team and Leukemia**: R Rathore (RWMC), A Deol, L Ayash, M Abidi (UCD) Z Al-Kadhimi (Emory), V Ratanatharathorn (KCI), J Uberti (KCI), and J Zonder (KCI)

- **Breast Cancer Team**: A Thakur (Uva), R Rathore (RWMC), F Cummings, Z Nahleh (TTU), E Gartner (SG), L Choi (KCI), A Weise, M Simon, L. Flaherty (KCI)

- **Neuroblastoma Team**: M. Yankelevich (CHOM), S. Modak (MSKCC), NK Cheung (MSKCC)

- **GI and Imaging Team**: A Shields (KCI), M Choi (Stony Brook), N Viola-Villegas (KCI)

- **GU Team**: U Vaishampayan, E Heath (KCI)

- **Immune Evaluations**: A Thakur (Uva), V Kondadasula (KCI)

- **Lab Staff**: C Pray, Y Gall, P Davol, C Sorenson, E Tomaszewski (KCI), D Schalk (Uva), H Yano (U of Pittsburgh)

- **Nursing Staff**: W Young, L Hall, A Olson, P Steele, K Meyers, K Fields, M Dufresne, BMT and IV infusion nurses at RWMC, KCI

*Work supported by*: R01 CA 092344, R01 CA 140314, R01 CA 182526, LLS TRP Awards #6092-09 and #6066-06, Komen, Michigan Life Science Grant, Gateway For Cancer Research G-15-800 and G-15-1600, DOD, RWMC and KCI startup funding.
Imaging with chemokine receptors and small molecules

Sridhar Nimmagadda, Ph.D.
Opportunities

• Imaging immune cells (metabolic tracers, CD8, PD-1, PD-L1, chemokine receptors, chemokines?)

• Imaging the immunosuppressive tumor microenvironment (IDO, A2AR)
Deoxyguanosine kinase (dGK)

- dGK is a mitochondrial protein
- dGK activity is found in most tissues (liver, lymphoid tissues such as B and T cells, spleen, skin, and brain)
- dGK phosphorylates deoxyguanosine and exhibits broad substrate specificity (cladribine, fludarabine, cytarabine (Ara-C), gemcitabine, nelarabine (AraG) and clofarabine)
- Guanine-β-d-arabinofuranoside (AraG) has a specific toxicity for T lymphocytes
Imaging T-cell metabolism with $[^{18}\text{F}]\text{F-AraG}$

VisAcT: $[^{18}\text{F}]\text{F-AraG}$ is Fluorine 18 labeled analog of an FDA approved drug AraG – ArabinoFuranosyl Guanine

Mechanism of Action
- Activated T Cells overexpress dGK
- Tracer phosphorylated and trapped in cells with high levels of dGK
- Detected with existing PET scanners

Courtesy: CellSight Technologies
Visualizing activated T cells with $[^{18}F]F$-AraG

- Pan T cells isolated from spleen & lymph nodes of mice
- Cell group A treated with CD3/CD28 to activate T cells
  - Cell group B untreated
- 48 hrs post CD3/CD28 exposure both cell groups incubated with VisAcT
- Cells implanted subcutaneously
  - Left shoulder - naïve T cells
  - Right shoulder - activated T cells
- PET scanned

Courtesy: CellSight Technologies
[\textsuperscript{18}F]F-AraG in a Healthy Male

Images from 4 time points post VisAcT injection show ideal imaging characteristics with hepatobiliary and renal clearance.

Courtesy: CellSight Technologies
PD-L1 imaging with a humanized antibody
$^{64}$Cu-MPDL3280A-PET @ 24h

Human and mouse cross-reactive mAb

Chatterjee et al., Oncotarget, 2016
Chatterjee et al., unpublished
Chemokines/Chemokine receptors

- 48 chemokines and 20 chemokine receptors
- Involved in immune cell migration
- CXCL9, CXCL10, CCL5 and CXCL12 are emerging as important chemokines in the tumor microenvironment
Chemokines and immunotherapy response

All indications  Melanoma  NSCLC  RCC

CXCL9

## Chemokine Receptors
### Expression and Function

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Ligand</th>
<th>Expression</th>
<th>Function</th>
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</thead>
<tbody>
<tr>
<td>CXCR3</td>
<td>CXCL9</td>
<td>Th1, CD8+ TCM and TEM, NK, NKT, pDC, B cell, Treg, Tfh</td>
<td>Th1-type adaptive immunity; Th1, CD8, NK trafficking</td>
</tr>
<tr>
<td></td>
<td>CXCL10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CXCL11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CXCR4</td>
<td>CXCL12</td>
<td>Most leukocytes</td>
<td>Hematopoiesis, organogenesis, bone marrow homing</td>
</tr>
<tr>
<td>CCR5</td>
<td>CCL5</td>
<td>Monocyte, macrophage, Th1, NK, Treg, CD8+ T, DC, neutrophil</td>
<td>Type 1 adaptive immunity; Macrophage and NK cell migration; T cell–DC interactions</td>
</tr>
</tbody>
</table>
Imaging Ova induced immune response with a CXCR4 imaging agent $[^{64}\text{Cu}]\text{AMD3100}$

Imaging agents in the clinic (CPCR4-2)

What does change in tumor CXCR4 expression correlate with?

Plerixafor IC$_{50}$: 651 ± 37nM
Indoleamine 2,3-dioxygenase (IDO1)

- Tumor induced tolerance is both acquired and active
- IDO catabolizes tryptophan
- Tryptophan metabolites blunt tumor immunity
- Deregulated in many cancers
- Small molecule inhibitors in clinical trials

![Diagram of tryptophan and kynurenine metabolism]

*effector T-cells

Nat. Med. 9 (10): 1269–74. doi:10.1038/nm934
Imaging agents for IDO1

Boc-D-Trp-OEt → 1) $^{11}$C-CH$_3$/NaOH/DMSO, 80°C, 5 min

2) 2M HCl, 100°C, 5 min → $^{11}$C-D-1MTwrp

$^{11}$C-D-1MTwrp

Scientific Reports 5, Article number: 16417 (2015)
doi:10.1038/srep16417
Imaging agents for IDO1

INCB024360
IC50=10 nM
High selectivity for IDO1

J. Label Compd. Radiopharm 2015, 58 156–162
Challenges

- A reliable biomarker for immunotherapy efficacy
- Dynamic nature of immune-tumor cell interactions → molecularly targeted imaging agent
- Most of the known targets are cell surface proteins
- Changes in target expression may not always correlate with changes in TME
- How to detect non-functional immune responses?
- Readily translatable
- Should we focus on two or more imaging markers?
Acknowledgements

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DOD BCRP Idea Award
JHU Career Catalyst Award
Hopkins-Allegheny Cancer Research Fund
Univ. of Wisconsin team for Cu-64 production

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Pravin Bhansali, Ph.D.
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Ravindra De Silva, Ph.D.

Martin Pomper, M.D., Ph.D.
Zaver Bhujwala, Ph.D.
Ronnie Mease, Ph.D.
Leisha Emens, M.D., Ph.D.
[\textsuperscript{18}F]F-AraG accumulation in cells is dGK dependent
Activities and Opportunities in Cancer Immunotherapy at the NCI

Elad Sharon, M.D., M.P.H.

May 2, 2016
Clinical Translational Research and Cancer Biology: Bedside to Bench and Back

Clinical observations:
- Clinical response
- PK
- Functional imaging
- Tumor and normal tissue PD markers
- CTCs, CECs
- Tumor-initiating cells

Patients eligible for early phase clinical trials

Analysis of tumor and other tissues for pathway activation or biomarker

Patient assigned to trial based on molecular characterization of tumor

Patient monitoring

Patient monitoring: Post-treatment molecular re-analysis for response/resistance

Non-clinical models for targets

Translational research with clinical models
- Sequencing
- Methylation
- FISH
- IHC
- Expression array
Rationale:

- Precision Medicine Initiative (PMI) - Oncology - 4 parts
  - Clinical trials to advance precision oncology
    - Advanced sequencing for NCI-MATCH
    - Pediatric MATCH
    - Expand immunotherapy trials—combinations, molecular characterization, reagents
  - Develop better pre-clinical models for cancer treatment
  - Overcome therapeutic resistance in the clinic
  - Knowledge system for precision oncology
Definition of “Immunotherapy” used in this inventory –

- Agents with the **primary** MOA mediated through modulation of cancer immunity and effected through the immune system/cells (e.g. cytokines, check point inhibitors, vaccines, adoptive cell therapy)

- Antibodies or agents directed at tumor cell targets/angiogenesis, with the primary MOA uncertain, or mediated through signal transduction or cytotoxic payload were **NOT** included in this analysis (e.g. bevacizumab, trastuzumab, immunotoxin, radioimmunotherapy)
### Single-project grants (# of grants)

<table>
<thead>
<tr>
<th>Division</th>
<th>All grants</th>
<th>Grants related to Immunotherapy</th>
<th>% for immunotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DCB (Division of Cancer Biology)</strong></td>
<td>1894</td>
<td>114</td>
<td>6%</td>
</tr>
<tr>
<td>- <em>Mostly basic science</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>DCTD (Division of Cancer Treatment and Diagnosis)</strong></td>
<td>1486</td>
<td>196</td>
<td>13%</td>
</tr>
<tr>
<td>- <em>Translational and clinical</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SBIR (Small Business Innovation Research Program)</strong></td>
<td>171</td>
<td>20</td>
<td>12%</td>
</tr>
<tr>
<td><strong>CCT (Center for Cancer Training)</strong></td>
<td>977</td>
<td>79</td>
<td>8%</td>
</tr>
<tr>
<td>- <em>Training and Career Development Awards</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>DCP (Division of Cancer Prevention)</strong></td>
<td>391</td>
<td>4</td>
<td>1%</td>
</tr>
</tbody>
</table>

1. Not included in this Table: Type 3’s
2. Not included in this table – Multi-project grants - P01, P20, P30, P50, U19, U54, U10, UG1, UM1
3. Primary IC=CA
## NCI Extramural Funding for Immunotherapy – A list of projects funded in FY 2014

### Multi-project grants or funding mechanisms

<table>
<thead>
<tr>
<th>Grant Type</th>
<th>All grants/subprojects</th>
<th>Immunotherapy</th>
<th>% for ImmunoRx</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SPORE (P50)</strong></td>
<td>52 grants</td>
<td>26 with ImmunoRx</td>
<td>50%</td>
</tr>
<tr>
<td></td>
<td>209 subprojects</td>
<td>49 for ImmunoRx</td>
<td>23%</td>
</tr>
<tr>
<td><strong>Program Project Grant (P01)</strong></td>
<td>109 grants</td>
<td>24 with ImmunoRx</td>
<td>22%</td>
</tr>
<tr>
<td></td>
<td>708 subprojects</td>
<td>66 with ImmunoRx</td>
<td>9%</td>
</tr>
<tr>
<td><strong>CTEP Clinical Trial Network</strong></td>
<td>170 Trials (Phase 3: 47 trials)</td>
<td>37 for ImmunoRx (Phase 3: 7 trials)</td>
<td>22% (15%)</td>
</tr>
<tr>
<td><strong>New trials opened in 2014-2015</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*SPORE grants are based on FY 2015*
168 of 739 (23%) Intramural Research Projects (IRPs) were identified as being relevant to immunotherapy.
## Immunotherapy Trials in CTEP Clinical Trial Networks

**CTEP Clinical trial network:**
- NCTN (Cooperative Groups)
- CITN Cancer Immunotherapy Trials Network
- ETCTN (Early clinical trials)
- Disease specific consortia (ABTC, PBTC)

<table>
<thead>
<tr>
<th></th>
<th>ImmunoRx</th>
<th>% of ImmunoRx</th>
</tr>
</thead>
<tbody>
<tr>
<td>All CTEP trials</td>
<td># of clinical trials</td>
<td>1274</td>
</tr>
<tr>
<td></td>
<td>(Phase 3)</td>
<td>(128)</td>
</tr>
<tr>
<td>Before 2000</td>
<td># of clinical trials</td>
<td>1002</td>
</tr>
<tr>
<td></td>
<td>(Phase 3)</td>
<td>(111)</td>
</tr>
<tr>
<td>Activated between 2000-2009</td>
<td># of clinical trials</td>
<td>184</td>
</tr>
<tr>
<td></td>
<td>(Phase 3)</td>
<td>(10)</td>
</tr>
<tr>
<td>Activated between 2010-2013</td>
<td># of clinical trials</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>(Phase 3)</td>
<td>(2)</td>
</tr>
<tr>
<td>Activated between 2014-2015</td>
<td># of clinical trials</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>(Phase 3)</td>
<td>(7)</td>
</tr>
</tbody>
</table>

*Trial without therapeutic interventions are excluded from the analysis.*
Recent NCI-Supported Immunotherapy Trials

Between 2010 -2015

- **88** Phase I-III immunotherapy trials were activated in the DCTD Clinical Trial Network (NCTN, ETCTN, CITN, and PBTC)
- **9** Phase III trials, **14** Randomized Phase 2 trials
- **Clinical settings:** common, rare tumors; neoadjuvant, adjuvant and metastatic disease
- **Study regimens include single agent and novel combinations**

*Most randomized trials have mandatory collection of baseline tissues/blood
*Many early clinical trials include serial biopsies
Check point inhibitors:
- Anti- CTLA-4 (Ipilimumab)
- Anti-PD-1 Nivolumab, Anti-PD-1 Pembrolizumab
- Anti-PD-L1 (MEDI4736 and MPDL3280A)

Cytokines:
- IL-15
- IL-12
- Others:

T-cell engaging bispecific antibody:
- CD19 BiTE (Blinatumomab)

Vaccines:
- CDX1401 (against NY-ESO-1)
- PSA PROSTVAC/TRICOM
- CEA TRICOM/PANVAC
- Other: peptide (gp100, HPV, RAS, P53, MART and others)

Other immune modulators:
- IDO (INDB0243360) ~ 2 trials
- Lenalidomide, Pomalidomide: - not counted in the analysis FLT3 ligands
- Anti-CD27 mAb (CellDex)

Types of trials sponsored by CTEP:
- Rare indications
- Special populations (Pediatric, HIV)
- Novel combinations
- Phase III and registration trials
- Biomarkers as the primary endpoints
High Priority Targets and DCTD/CTEP Agents

- EGF-R
- VEGF-R
- bevacizumab
- ziv-aflibercept
- other receptors
- Surface antigens
  - SGN 35 (CD30)
  - HA 22 (CD22)
  - CDX-011
- IGF-1R
  - ganitumab
  - Cixutumumab
  - linsonitib
- HER2
  - Lapatinib
  - Pertuzumab
  - trastuzumab
- c-Kit
  - imatinib
  - sunitinib
  - sorafenib
- Met
  - tivantinib
  - AMG337
  - cabozantinib
  - pilrotumub
- PDGF
  - sunitinib
  - pazopanib
  - cediranib
- CDKs
  - dinaciclib
  - Microtubules
  - bexotumimab
  - vedotin
- CHK1
  - SCH 900776
- Aurora kinase A
  - MLN 8237
  - Wee1 kinase
  - MK-1775
- PD1
  - pembrolizumab
  - nivolumab
- HDAC
  - belinostat
  - entinostat
  - vorinostat
- Topoisomerases
  - LMP400/776
- Alkylating
  - Dimethane
- Sulfonylate
- Methylation inhibitors
  - FdCyd/THU
  - TRC102

- Stem cell signaling
  - PARP
    - velparib
    - BMN673
    - olaparib
- DNA repair
  - epigenetics
CTEP by the Numbers

- CTEP sponsors over 120 INDs
- Approximately 18,000 registered investigators at 3,300 institutions in the US and internationally
- Over 900 active protocol
  - 140 new protocols per year
  - Approximately 33,000 patients accrued per year
- Largest sponsor of cancer-related combination studies
  - Two-thirds of all combination studies in clinicaltrials.gov are CTEP-sponsored
- Over 100 collaborative agreements (CRADAs, CTAs, agent-CRADAs, and CSAs) with pharmaceutical companies
Cooperative Group Sites in US

3,300 Institutions

ECOG - ACRIN
SWOG
Alliance
NRG
COG

close collaboration with NCIC

Accrual Distribution:
Phase 3: 83.4%
Phase 2: 15.1%
Phase 1/Pilot: 1.5%
NCI Early Phase Drug Development Program: ETCTN
Other Significant CTEP-Affiliated Groups

- NCI Intramural Research Program (IRP)
- Adult Brain Tumor Consortium (ABTC)
- Pediatric Brain Tumor Consortium (PBTC)
- Cancer Immunotherapy Trials Network (CITN)
- AIDS Malignancy Consortium (AMC)
- Agreements with France (INCa), South Korea, Japan, and Taiwan
  - NCI CGH has been instrumental in developing and maintaining these relationships
Immunotherapy: A Rapidly Growing Part of NCI portfolio

- New agents being added to portfolio regularly
- Engaged with critical industry and academic stakeholders at the forefront of the field
- Efficient use of taxpayer dollars, with major results coming from small trials, shifting our understanding of immunotherapy
- Judicious use of Phase 3 trial resources to focus on critical unmet medical needs, which industry cannot or will not address on its own
Biomarkers are critical to further development of cancer immunotherapy

- Immunotherapy has remarkable activity in many tumor types, but for most only a minority of patients benefit
  - Response rate to anti-PD1: Melanoma (30%); Renal cancer (20%), Lung ca (15-20%)
  - Even with ipilimumab-nivolumab in melanoma: ORR was 57%
  - Highest response rate thus far seen in Merkel Cell Cancer (NCI-sponsored trial, published in NEJM)
- Some tumors do not respond (pancreatic cancer, microsatellite stable colon cancer, myeloma) Mechanisms of intrinsic resistance poorly understood
- Combination is a potential strategy to improve outcome, however:
  - Which combination should be given to which patients to induce synergistic effect?
  - What is the optimal dose, sequence and schedule?
  - Understanding of pattern of immune receptors, tumor microenvironment and molecular characteristic of the tumor is critical to develop rational combinations.

Potential role of immune biomarker studies in the context of clinical trials:
- Explore and validate predictive markers of response and toxicity
- Reveal mechanisms of actions/resistance of individual agents, and guide selection of partners for combination regimens
- Enhance the understanding of cancer immunobiology
Small trials with a big impact

- ETCTN Trial of ipilimumab in patients who had already failed bone marrow transplant
  - First evidence of in patients with AML or in the post-transplant setting, including 5 complete responses

Figure 1. Clinical and histopathologic responses after 13 weeks of ipilimumab therapy in a patient with leukemia cutis.
Small trials with a big impact

- CITN Trial Merkel Cell Cancer (published online in NEJM on 4/19/16) had highest rate of response of any solid tumor
  - Responses in Merkel Cell Cancers that were virally-mediated as well as those that were non-viral
Large Trial Efforts: NCTN Trials with Immunotherapy

• Blinatumomab is a bispecific T-cell engaging (BiTE) antibody, now approved in relapsed Acute Lymphoblastic Leukemia (ALL)
  – BiTE technology acts as an off-the-shelf version of adoptive cell transfer: BiTEs form a link between T cells and tumor cells and exerts an effect independent of the presence of MHC I or co-stimulatory molecules
  – Three trials, including two registration trials in NCTN
    • Up-front use in conjunction with chemotherapy for adults
    • Children with ALL and high-risk features

• Variety of Adjuvant Trials now underway or planned in bladder cancer, lung cancer, melanoma, head and neck cancer, renal cell cancer, and brain cancer in Phase 1, 2, and 3 settings in NCTN
  – Developing immunotherapy arms for MATCH and cognate trials for unmatched, but sequenced patients
Examples of Phase 2/3 Trials in Melanoma

- Ipilimumab and bevacizumab randomized trial exploring angiogenesis/immunotherapy
- Nivolumab/Ipilimumab +/- GMCSF, following up on evidence of cytokine augmentation of checkpoint inhibitor effect
- Sequencing trial exploring BRAF/MEK inhibitor therapy versus combination nivolumab-ipilimumab
- Adjuvant trial of pembrolizumab versus ipilimumab or high-dose interferon for resected Stage 3 or 4 patients
A Phase I clinical trial of ipilimumab was conducted in pediatric patients with advanced solid tumors
  - First time a checkpoint inhibitor was studied in children
  - Safety shown
  - Results: No objective responses were seen using this monotherapy but subjects with immune-related toxicities had an increased overall survival compared with those who showed no evidence of breaking tolerance
  - Future studies: combination immunotherapy strategies in pediatric patients
Correlating Imaging with Biopsies

- Trials available in all CTEP networks
- NCI Intramural Program and ETCTN are both funded for obtaining biopsies, and a variety of immunotherapy trials are currently planned and ongoing
- ABTC/PBTC have immunotherapy trials focused on gliomas, which can be biopsy-driven
- Variety of industry and academic partners, interested in utilizing our expertise and networks
MPACT: Precision Medicine in ETCTN

- Randomized trial of treatment of patients according to molecular mutations vs. a more traditional approach
- Now undergoing expansion across all centers in ETCTN
- Immunotherapy arm being contemplated, which would treat screened patients without molecular targets
- Perfect timing to discuss a potential CTEP/CIP collaboration
Immunology Overview and Imaging’s Current Role

Immune Modulation Therapy and Imaging:
What can we do in clinical trials now?
Monday May 2, 2016: 8:00 am – 5:30 pm
National Cancer Institute Shady Grove
Disclosures

Consulting

• Genentech-Roche, Bristol-Myers, Astra-Zeneca/Medimmune, Pfizer, Novartis, Kyowa-Kirin, Immune Design, Prometheus, Nektar, Pierre-Fabre, Lilly, Merck, Alexion, Theravance, Biodesix, Vaccinex, Janssen/Johnson and Johnson

Scientific Advisory Board (paid)

• Symphogen, Lion Biotechnologies, Amphivena (Stock options only), Adaptive Biotechnologies (stock options only), Intensity (stock options only), Lycera, Adaptimmune
Cancer Cell Antigens:
- Mutations
- Aberrant expression of developmental proteins
- Tissue differentiation proteins
- Stem cell ‘drivers’

Professional antigen presenting cells:
- Dendritic cells (DC)

DC process proteins to peptides
- Peptides bind to MHC molecules
- Peptide-MHC complex presentation of antigen to T-cells

T-cells ‘find tumor’, kill cells or secrete cytokines to create anti-tumor inflammatory response

T-cell activation, proliferation
1. Co-stimulation via CD28 ligation transduces T cell activating signals

2. CTLA-4 ligation on activated T cells down-regulates T cell responses

3. T cell function in tissue is subject to feedback inhibition
<table>
<thead>
<tr>
<th>Antigen Presenting Cell or Tumor</th>
<th>T-lymphocyte</th>
<th>Function (excluding Treg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptide-MHC</td>
<td>T cell receptor</td>
<td>Signal 1</td>
</tr>
<tr>
<td>CD80/CD86 (B7.1, B7.2)</td>
<td>CD28/CTLA-4</td>
<td>Stimulatory/inhibitory</td>
</tr>
<tr>
<td>CEACAM-1</td>
<td>CEACAM-1</td>
<td>inhibitory</td>
</tr>
<tr>
<td>CD70</td>
<td>CD27</td>
<td>stimulatory</td>
</tr>
<tr>
<td>LIGHT</td>
<td>HVEM</td>
<td>stimulatory</td>
</tr>
<tr>
<td>HVEM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD-L1 (B7-H1)</td>
<td>PD-1 and CD80</td>
<td>Inhibitory (Th1)</td>
</tr>
<tr>
<td>PD-L2 (B7-DC)</td>
<td>PD1 and ?</td>
<td>Inhibitory (Th2) or stimulatory</td>
</tr>
<tr>
<td>OX40L</td>
<td>OX40</td>
<td>stimulatory</td>
</tr>
<tr>
<td>4-1BBL</td>
<td>CD137</td>
<td>stimulatory</td>
</tr>
<tr>
<td>CD40</td>
<td>CD40L</td>
<td>Stimulatory to DC/APC</td>
</tr>
<tr>
<td>B7-H3</td>
<td>?</td>
<td>Inhibitory or stimulatory</td>
</tr>
<tr>
<td>B7-H4</td>
<td>?</td>
<td>inhibitory</td>
</tr>
<tr>
<td>PD-1H (Vista)</td>
<td>?</td>
<td>inhibitory</td>
</tr>
<tr>
<td>GAL9</td>
<td>TIM-3</td>
<td>inhibitory</td>
</tr>
<tr>
<td>MHC class II</td>
<td>LAG-3</td>
<td>inhibitory</td>
</tr>
<tr>
<td>B7RP1</td>
<td>ICOS</td>
<td>stimulatory</td>
</tr>
<tr>
<td>MHC class I</td>
<td>KIR</td>
<td>Inhibitory or stimulatory</td>
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<tr>
<td>GITRL</td>
<td>GITR</td>
<td>stimulatory</td>
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<tr>
<td>CD48</td>
<td>2B4 (CD244)</td>
<td>inhibitory</td>
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<tr>
<td>HLA-G, HLA-E</td>
<td>ILT2, ILT4; NKG2a</td>
<td>inhibitory</td>
</tr>
<tr>
<td>MICA/B, ULBP-1, -2, -3, and -4+</td>
<td>NKG2D</td>
<td>Inhibitory or stimulatory</td>
</tr>
<tr>
<td>CD200</td>
<td>CD200R</td>
<td>inhibitory</td>
</tr>
<tr>
<td>CD155</td>
<td>TIGIT/CD226</td>
<td>Inhibitory/stimulatory</td>
</tr>
</tbody>
</table>

Other Inhibitory Factors
- IDO
- Treg
- MDSC
- Macrophages
- TGF-beta
- IL-10?
- VEGF
**Presence of PD-L1 or TILs**

**Table 2.** Correlation of B7-H1 expression by melanocytes with the presence of immune cell infiltration.

<table>
<thead>
<tr>
<th>Histology</th>
<th>Total</th>
<th>B7-H1+</th>
<th>B7-H1+</th>
<th>B7-H1+</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TIL+</td>
<td>TIL−</td>
<td>TIL+</td>
<td></td>
</tr>
<tr>
<td>Benign nevi</td>
<td>40</td>
<td>14/14 (100)</td>
<td>0/14 (0)</td>
<td>4/26 (15)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Primary melanomas (in situ or invasive)</td>
<td>54</td>
<td>19/19 (100)</td>
<td>0/19 (0)</td>
<td>15/35 (43)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Metastases</td>
<td>56</td>
<td>23/24 (96)</td>
<td>1/24 (4)</td>
<td>7/32 (22)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>All</td>
<td>150</td>
<td>56/57 (98)</td>
<td>1/57 (2)</td>
<td>26/93 (28)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*Fisher's exact test, two-sided, was conducted on the 2 x 2 matrix defined by B7-H1 (±) expression and TIL (±) for each lesion type. Including mild, moderate, and severe lymphocyte infiltrates and their associated histiocytes/macrophages.*

*More than 5% melanocytes with membranous expression on HEC.*

Schalper and Rimm, Yale University
taube et al
Melanoma TIL – Expression of Co-inhibitory and Co-stimulatory Receptors (Gros et al)

The Journal of Clinical Investigation  http://www.jci.org  Volume 124  Number 5  May 2014
Provided by Kavita Dhodapkar, Yale University
Phenotypic comparison of CD8 and CD4 T cells infiltrating into tumor, normal tissue, and peripheral blood in the same patient.


©2009 by American Society of Hematology
Cytokine Production in TIL vs PBL in Metastatic Melanoma

Figure shows Th1, Th2 and Th17 cytokines that were secreted by peripheral blood lymphocytes (n=15) and tumor tissue (n=41), when incubated with anti-CD3/28 beads. Bar graph shows mean and standard error of mean (*; p<0.05)

Data provided by Kavita Dhodapkar, Yale University
Tumor antigen–specific CD8 T cells infiltrating the tumor express high levels of PD-1 and are functionally impaired

(Blood. 2009;114:1537-1544)

Mojgan Ahmadzadeh, Laura A. Johnson, Bianca Heemskerk, John R. Wunderlich, Mark E. Dudley, Donald E. White, and Steven A. Rosenberg

Figure 6. PD-1 expression on tumor-infiltrating T cells correlates with impaired effector function. Tumor digests and peripheral blood sample from patients with metastatic melanoma were thawed and immediately stimulated with PMA/I for 6 to 8 hours in the presence of monensin. Cells were subsequently stained with anti-CD3, anti-CD8, and anti–PD-1 mAb along with anti–IL-2 and anti–IFN-γ mAbs. (A) Dot plots were gated on CD3+ T cells. The numbers represent the percentages of T cells in each quadrant and the value in parentheses represents the MFI for each quadrant. (B) The percentage of CD3+CD8+ T cells that were IFN-γ+ is depicted for PD-1+ and PD-1−CD8 TILs. (C) The MFI for IFN-γ+ CD3+CD8+ T cells are depicted for PD-1+ and PD-1−CD8 TILs. (D) The percentage of CD3+CD8+ T cell that were IL-2+ is depicted for PD-1+ and PD-1−CD8 TILs for 6 patients. P values are calculated based on the paired t test. (E) IFN-γ production by MART-1 tetramer+ CD8 T cells in tumor digests versus peripheral blood (PBL) from the same patient is shown. The percentage values represent the fraction of MART-1 tetramer+ CD8 T cells that produced IFN-γ.
Tumor-specific T cells are contained in the PD-1+ TIL population and are functional after in vitro culture.
Options for Immune Intervention in Cancer

• Vaccines (induce immune response against presumed cancer antigen)
  • Defined antigen and delivery method
  • Promote Ag presentation in vivo

• Cytokines to promote T-cell activation, proliferation and function

• Provide T cell co-stimulatory signals

• Block T cell inhibitory signals

• Modulate tumor signaling pathways that affect immune infiltration (STING, beta-catenin, VEGF, others)

• Adoptively transfer antigen-specific T cells

• Give antibodies that kill by CDC or ADCC

• Activate NK cell function to kill tumor cells
Host genetics → Lifetime environmental exposures → TCR repertoire

Patient Presenting for Treatment

Tumor evolution
- Metastases
- Evolution of Tumor-Host immune relationship

Carcinogenesis:
- Mutations
- Altered gene expression
- Chronic inflammation

Tumor microenvironment and Host Anti-tumor immune response

T-cells
- How many?
- What type?
- Recognize tumor antigens?
- Breadth of antigen recognition (one, a few, many)
- Affinity of TCR for peptide-MHC complex
- Functional state
- Differentiated state
- Expression of inhibitory receptors
- Metabolic state and access to glucose
- Where located?

Tumor
- Mutations/Antigens/neo-antigens
- Density of peptide/MHC complexes
- Expression of inhibitory ligands
- Expression of stimulatory ligands
- Production of inhibitory cytokines
- Production of other inhibitory substances
- Expression of chemokines
- Signaling pathway activation/inhibition
- Innate resistance to lytic mechanisms

Stroma/Other Immune Cells
- Treg
- MDSC
- Monocytes/macrophages/APC
- B-cells
- NK and NKT cells
- Tumor Vasculature
- Fibroblasts
- Metabolic Milieu
  - Oxygen
  - Glucose

Immune Intervention → Outcome

Host genetics

Carcinogenesis:

Tumor evolution

Tumor microenvironment and Host Anti-tumor immune response

Immune Intervention

Outcome
1970
- Autologous and allogeneic tumor cell cancer vaccines
- Intratumoral BCG

1980
- Interferon-alfa
- IL-2
- IL-2 and LAK cells
- Other cytokines (TNF, IFN-γ)
- IL-2 and TILs

1990
- Gene-transfected tumor cell vaccines
- Defined antigen vaccines, viral vectors, and DCs

2000
- Blockade of T-cell activation checkpoints (CTLA-4)
- Lymphocyte ablation + TIL
- T-cell and DC co-stimulatory antibodies
- Blockade of tumor immune suppressive mechanisms (PD-1)
- Gene (CAR, TCR, cytokine) modified lymphocytes for ACT

2010
- Combination of immune checkpoint inhibitors (CTLA-4, PD-1)

ACT = adoptive cell transfer; BCG = Bacillus Calmette-Guérin; CAR = chimeric antigen receptor; CTLA-4 = cytotoxic T-lymphocyte-associated protein 4; DC = dendritic cell; IL-2 = interleukin-2; INF-γ = interferon-gamma; LAK = lymphokine-activated killer cell; PD-1 = programmed cell death protein 1; TCR = T cell receptor; TILs = tumor infiltrating lymphocytes; TNF = tumor necrosis factor.
- Enhances T cell proliferation
- Increases T cell repertoire
- Causes ‘resistance’ of T-effectors to Treg suppression
- ‘killing’ of intratumoral Treg
- Causes tumor T cell infiltration
- Increases PD-1+ T cells

- Requires blockade on both CD4+ and CD8+
- Interaction with CTLA-4 on both effectors and Treg
- Isotype dependent in animal models (ADCC-dependent)
Key Aspects of Anti-CTLA4 Therapy

- Can be associated with autoimmune adverse events
  - Any organ, but rash, colitis, hepatitis and endocrinopathies are most common
  - May require steroids +/- additional immunosuppressive agents
- Unique kinetics of response in some patients
  - SD with slow, steady decline in total tumor volume
  - Response after initial increase in total tumor volume
  - Response in index plus new lesions at or after the appearance of new lesions
  - Continued benefit after Rx of discordant progressing lesions
- Possibility of second response with re-induction after PD
The PD-L1/PD-1 Pathway

Slide courtesy of Lieping Chen
Nivolumab (anti-PD-1) versus DTIC – OSS and PFS

Atkinson et al, SMR 2015
Nivolumab versus DTIC- Duration of Response

- **NIVO**
  - On Treatment
  - Off Treatment
  - First Tumor Response
  - Ongoing Response

- **DTIC**
  - On Treatment
  - Off Treatment

**Weeks**

<table>
<thead>
<tr>
<th>NIVO</th>
<th>DTIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of response, median (range), mo</td>
<td>NR</td>
</tr>
<tr>
<td>Ongoing response among responders*</td>
<td>73/90 (81%)</td>
</tr>
</tbody>
</table>

*At the time of the last follow-up
mo = month; NR = not reached
## Anti-PD-1 (Pembrolizumab) Versus Ipilimumab: Treatment of Advanced Disease

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>ORR</th>
<th>Median PFS</th>
<th>OS at 12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pembrolizumab 10 mg/kg Q2W</td>
<td>279</td>
<td>33.7</td>
<td>5.5</td>
<td>74.1%</td>
</tr>
<tr>
<td>Pembrolizumab 10 mg/kg Q3W</td>
<td>277</td>
<td>32.9</td>
<td>4.1</td>
<td>68.4%</td>
</tr>
<tr>
<td>Ipilimumab 3 mg/kg Q3W × 4</td>
<td>278</td>
<td>11.9</td>
<td>2.8</td>
<td>58.2%</td>
</tr>
</tbody>
</table>

### Progression-Free Survival (PFS)

<table>
<thead>
<tr>
<th></th>
<th>Month</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pembrolizumab, Q2W</td>
<td>279</td>
<td>231</td>
<td>147</td>
<td>98</td>
<td>49</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Pembrolizumab, Q3W</td>
<td>277</td>
<td>235</td>
<td>133</td>
<td>95</td>
<td>53</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Ipilimumab</td>
<td>278</td>
<td>186</td>
<td>88</td>
<td>42</td>
<td>18</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

### Overall Survival (OS)

<table>
<thead>
<tr>
<th></th>
<th>Month</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
<th>16</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pembrolizumab, Q2W</td>
<td>279</td>
<td>268</td>
<td>248</td>
<td>233</td>
<td>219</td>
<td>212</td>
<td>177</td>
<td>67</td>
<td>19</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Pembrolizumab, Q3W</td>
<td>277</td>
<td>266</td>
<td>251</td>
<td>238</td>
<td>215</td>
<td>202</td>
<td>158</td>
<td>71</td>
<td>18</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Ipilimumab</td>
<td>278</td>
<td>242</td>
<td>212</td>
<td>188</td>
<td>169</td>
<td>157</td>
<td>117</td>
<td>51</td>
<td>17</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Randomized phase III trials of nivolumab vs. docetaxel in NSCLC

**Trial 17: Squamous Cell Carcinoma**

- Nivolumab: 135 patients
- OS: 9.2 months (95% CI: 7.3, 13.3)
- Event: 86
- HR: 0.59 (95% CI: 0.44, 0.79), P = 0.00025

**Trial 57: Non-Squamous Cell Carcinoma**

- Nivolumab: 292 patients
- OS: 12.2 months
- DOC: 290 patients
- OS: 9.4 months
- HR: 0.73 (95% CI: 0.59, 0.89), P = 0.0015
Nivolumab Improves Overall Survival in mRCC

Spectrum of PD-1/PD-L1 Antagonist Activity

Active

- Melanoma
- Renal cancer (clear cell and non-clear cell)
- NSCLC – adenocarcinoma and squamous cell
- Small cell lung cancer
- Head and neck cancer
  - Gastric and gastroesophageal junction
- MMR-repair deficient tumors (colon, cholangiocarcinoma)
- Bladder
- Triple negative breast cancer
  - Ovarian
  - Hepatocellular carcinoma
  - Thymoma
  - Mesothelioma
  - Cervical
- Hodgkin lymphoma
  - Diffuse large cell lymphoma
  - Follicular lymphoma
  - T-cell lymphoma (cutaneous T-cell lymphomas, peripheral T-cell lymphoma)
- Merkel cell

Minimal to no activity

- Prostate cancer
- MMR+ (MSS) colon cancer
- Myeloma
- Pancreatic cancer

Major PD-1/PD-L1 antagonists

- Nivolumab (anti-PD-1)
- Pembrolizumab (anti-PD-1)
- Atezolizumab (MPDL3280, anti-PD-L1)
- Durvalumab (anti-PD-L1)
- Avelumab (anti-PD-L1)
Synergistic Activity with Anti-PD-1 and Anti-CTLA-4 Antibodies

Combination of Non-Efficacious Doses of anti-PD1 and anti-CTLA-4 Antibodies is Efficacious in Mouse Model

Different roles in T cell Differentiation -
Compensatory upregulation
Anti-CTLA4 elimination of tumor Treg
Anti-CTLA4 induced tumor T cell infiltration

Provided by Alan Korman, BMS
**Study Design**

### Figure 1: Study CA209-004 concurrent cohorts

Previously treated or untreated advanced melanoma

<table>
<thead>
<tr>
<th>Cohort 1</th>
<th>NIVO 0.3 + IPI 3</th>
<th>Q3W x 4</th>
<th>NIVO 0.3</th>
<th>Q3W x 4</th>
<th>NIVO 0.3 + IPI 3</th>
<th>Q12W x 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 14)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cohort 2</th>
<th>NIVO 1 + IPI 3</th>
<th>Q3W x 4</th>
<th>NIVO 1</th>
<th>Q3W x 4</th>
<th>NIVO 1 + IPI 3</th>
<th>Q12W x 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 17)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cohort 3</th>
<th>NIVO 3 + IPI 3</th>
<th>Q3W x 4</th>
<th>NIVO 3</th>
<th>Q3W x 4</th>
<th>NIVO 3 + IPI 3</th>
<th>Q12W x 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cohort 2a</th>
<th>NIVO 3 + IPI 1</th>
<th>Q3W x 4</th>
<th>NIVO 3</th>
<th>Q3W x 4</th>
<th>NIVO 3 + IPI 1</th>
<th>Q12W x 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 16)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cohort 8a</th>
<th>NIVO 1 + IPI 3</th>
<th>Q3W x 4</th>
<th>NIVO 3</th>
<th>Q2W x ≤48</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 41)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

All units are mg/kg. Results from Cohorts 6 and 7 (sequenced treatment cohorts – IPI followed by NIVO) were reported previously.

*FDA approved regimen.

IPI = ipilimumab; NIVO = nivolumab; Q2W = every 2 weeks; Q3W = every 3 weeks; Q12W = every 12 weeks.
CA209-067: Ipi/Nivo vs. Nivolumab vs. Ipilimumab: Objective Response Rate


### ORR (Patients)

<table>
<thead>
<tr>
<th>Total population</th>
<th>NIVO + IPI</th>
<th>NIVO</th>
<th>Unweighted ORR difference vs IPI (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>57.6% (314)</td>
<td></td>
<td></td>
<td>38.6% (31.3–45.2)</td>
</tr>
<tr>
<td>43.7% (316)</td>
<td></td>
<td></td>
<td>24.6% (17.5–31.4)</td>
</tr>
</tbody>
</table>

### BRAF

<table>
<thead>
<tr>
<th>Wild-type</th>
<th>NIVO + IPI</th>
<th>NIVO</th>
<th>Unweighted ORR difference vs IPI (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>53.3% (212)</td>
<td></td>
<td></td>
<td>35.6% (26.8–43.6)</td>
</tr>
<tr>
<td>46.8% (218)</td>
<td></td>
<td></td>
<td>29.1% (20.5–37.1)</td>
</tr>
<tr>
<td>Mutant</td>
<td></td>
<td></td>
<td>44.7% (31.5–55.6)</td>
</tr>
<tr>
<td>36.7% (98)</td>
<td></td>
<td></td>
<td>14.7% (2.0–26.8)</td>
</tr>
</tbody>
</table>

### M Stage

<table>
<thead>
<tr>
<th>M1c</th>
<th>NIVO + IPI</th>
<th>NIVO</th>
<th>Unweighted ORR difference vs IPI (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>51.4% (185)</td>
<td></td>
<td></td>
<td>37.1% (27.9–45.4)</td>
</tr>
<tr>
<td>38.9% (185)</td>
<td></td>
<td></td>
<td>24.6% (15.8–33.0)</td>
</tr>
</tbody>
</table>

### Baseline LDH

<table>
<thead>
<tr>
<th>≤ULN</th>
<th>NIVO + IPI</th>
<th>NIVO</th>
<th>Unweighted ORR difference vs IPI (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>65.3% (199)</td>
<td></td>
<td></td>
<td>40.6% (31.1–48.9)</td>
</tr>
<tr>
<td>51.5% (196)</td>
<td></td>
<td></td>
<td>26.8% (17.3–35.6)</td>
</tr>
<tr>
<td>&gt;ULN</td>
<td></td>
<td></td>
<td>35.2% (24.1–45.2)</td>
</tr>
<tr>
<td>44.7% (114)</td>
<td></td>
<td></td>
<td>20.8% (10.5–30.7)</td>
</tr>
<tr>
<td>&gt;2x ULN</td>
<td></td>
<td></td>
<td>37.8% (20.0–53.9)</td>
</tr>
<tr>
<td>30.4% (112)</td>
<td></td>
<td></td>
<td>21.6% (6.3–37.2)</td>
</tr>
</tbody>
</table>

### Age (yr)

<table>
<thead>
<tr>
<th>≥65 and &lt;75</th>
<th>NIVO + IPI</th>
<th>NIVO</th>
<th>Unweighted ORR difference vs IPI (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>57.4% (94)</td>
<td></td>
<td></td>
<td>39.5% (25.8–51.0)</td>
</tr>
<tr>
<td>48.1% (79)</td>
<td></td>
<td></td>
<td>30.1% (16.0–42.8)</td>
</tr>
<tr>
<td>≥75</td>
<td></td>
<td></td>
<td>27.0% (5.3–45.8)</td>
</tr>
<tr>
<td>54.3% (35)</td>
<td></td>
<td></td>
<td>16.3% (-4.1–35.2)</td>
</tr>
</tbody>
</table>

### PD-L1 Expression Level

<table>
<thead>
<tr>
<th>&lt;5%</th>
<th>NIVO + IPI</th>
<th>NIVO</th>
<th>Unweighted ORR difference vs IPI (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>54.8% (210)</td>
<td></td>
<td></td>
<td>36.9% (28.0–45.0)</td>
</tr>
<tr>
<td>41.3% (208)</td>
<td></td>
<td></td>
<td>23.5% (14.8–31.8)</td>
</tr>
<tr>
<td>≥5%</td>
<td></td>
<td></td>
<td>50.7% (35.0–62.8)</td>
</tr>
<tr>
<td>72.1% (68)</td>
<td></td>
<td></td>
<td>36.2% (21.0–49.0)</td>
</tr>
</tbody>
</table>
Updated Survival CA209-004, Iplimumab + Nivolumab in Metastatic Melanoma

Sznol et al, SMR 2015
• 30/47 (64%) of patients randomized to IPI crossed over to receive any systemic therapy at progression

Postow et al, AACR 2015
### Table 3. Adverse Events.

<table>
<thead>
<tr>
<th>Event</th>
<th>Nivolumab (N=313)</th>
<th>Nivolumab plus ipilimumab (N=313)</th>
<th>Ipilimumab (N=311)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any adverse event</td>
<td>311 (99.4)</td>
<td>312 (99.7)</td>
<td>308 (99.0)</td>
</tr>
<tr>
<td>Any grade 3 or 4</td>
<td>136 (43.5)</td>
<td>215 (68.7)</td>
<td>173 (55.6)</td>
</tr>
<tr>
<td>Treatment-related adverse event†</td>
<td>257 (82.1)</td>
<td>299 (95.5)</td>
<td>268 (86.2)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>60 (19.2)</td>
<td>138 (44.1)</td>
<td>103 (33.1)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>107 (34.2)</td>
<td>110 (35.1)</td>
<td>87 (28.0)</td>
</tr>
<tr>
<td>Pruritus</td>
<td>59 (18.8)</td>
<td>104 (33.2)</td>
<td>110 (35.4)</td>
</tr>
<tr>
<td>Rash</td>
<td>81 (25.9)</td>
<td>126 (40.3)</td>
<td>102 (32.8)</td>
</tr>
<tr>
<td>Nausea</td>
<td>41 (13.1)</td>
<td>81 (25.9)</td>
<td>50 (16.1)</td>
</tr>
<tr>
<td>Pyrexia</td>
<td>18 (5.8)</td>
<td>58 (18.5)</td>
<td>21 (6.8)</td>
</tr>
<tr>
<td>Decreased appetite</td>
<td>34 (10.9)</td>
<td>56 (17.9)</td>
<td>39 (12.5)</td>
</tr>
<tr>
<td>Increase in alanine aminotransferase level</td>
<td>12 (3.8)</td>
<td>55 (17.6)</td>
<td>12 (3.9)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>20 (6.4)</td>
<td>48 (15.3)</td>
<td>23 (7.4)</td>
</tr>
<tr>
<td>Increase in aspartate aminotransferase level</td>
<td>12 (3.8)</td>
<td>48 (15.3)</td>
<td>11 (3.5)</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>27 (8.6)</td>
<td>47 (15.0)</td>
<td>13 (4.2)</td>
</tr>
<tr>
<td>Colitis</td>
<td>4 (1.3)</td>
<td>37 (11.8)</td>
<td>36 (11.6)</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>24 (7.7)</td>
<td>33 (10.5)</td>
<td>19 (6.1)</td>
</tr>
<tr>
<td>Headache</td>
<td>23 (7.3)</td>
<td>32 (10.2)</td>
<td>24 (7.7)</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>14 (4.5)</td>
<td>32 (10.2)</td>
<td>13 (4.2)</td>
</tr>
<tr>
<td>Treatment-related adverse event leading to discontinuation</td>
<td>24 (7.7)</td>
<td>114 (36.4)</td>
<td>46 (14.8)</td>
</tr>
</tbody>
</table>

* The safety population included all the patients who received at least one dose of study drug. The severity of adverse events was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.

† The treatment-related adverse events listed here were those reported in at least 10% of the patients in any of the three study groups.
Nivolumab versus DTIC- OSS by PD-L1 Status

Atkinson et al, SMR 2015
PFS by PD-L1 Expression Level (1%)

**PD-L1 ≥1%**

<table>
<thead>
<tr>
<th>No. at risk</th>
<th>Months</th>
<th>NIVO + IPI</th>
<th>NIVO</th>
<th>IPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIVO + IPI</td>
<td>155</td>
<td>113</td>
<td>91</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>171</td>
<td>115</td>
<td>97</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>164</td>
<td>83</td>
<td>47</td>
<td>36</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. at risk</th>
<th>Months</th>
<th>NIVO + IPI</th>
<th>NIVO</th>
<th>IPI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
<td>32</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**PD-L1 <1%**

<table>
<thead>
<tr>
<th>No. at risk</th>
<th>Months</th>
<th>NIVO + IPI</th>
<th>NIVO</th>
<th>IPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIVO + IPI</td>
<td>123</td>
<td>82</td>
<td>65</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>117</td>
<td>50</td>
<td>42</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>113</td>
<td>39</td>
<td>19</td>
<td>12</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. at risk</th>
<th>Months</th>
<th>NIVO + IPI</th>
<th>NIVO</th>
<th>IPI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6</td>
<td>26</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>13</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>mPFS</th>
<th>HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIVO + IPI</td>
<td>12.4</td>
</tr>
<tr>
<td>NIVO</td>
<td>12.4</td>
</tr>
<tr>
<td>IPI</td>
<td>3.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>mPFS</th>
<th>HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIVO + IPI</td>
<td>11.2</td>
</tr>
<tr>
<td>NIVO</td>
<td>2.8</td>
</tr>
<tr>
<td>IPI</td>
<td>2.8</td>
</tr>
</tbody>
</table>

*Per validated PD-L1 immunohistochemical assay with expression defined as ≥1% of tumor cells showing PD-L1 staining in a section of at least 100 evaluable tumor cells.*
• Reduces Tumor bulk – Improves T-cell: tumor target ratio
• Separate mechanism of kill – ‘synergize’ with T-cell mechanism of killing
• Reduces T-cell inhibitory substances produced by tumor
• Alters tumor barriers (vasculature/pressure) to T-cell penetration
• Kills tumor cells in a manner that increases their recognition by T-cells and APC (vaccination)
• Alters T-cell signaling/gene expression to produce T-cell attractants
Imaging and immune therapy

• Predictive of Response
  • T-cell infiltration (extent, location, function, and type)
  • Other immune cells (MDSC, Treg?)
  • Expression of antibody targets (CD47, CD73, PD-L1, PD-1, TIM-3, PD-1H, etc)
  • Metabolisms/metabolic state (hypoxia, glucose consumption, other)

• Tumor response in the absence of regression
  • Differentiate scar from residual tumor versus persistent inflammation without tumor
    • When to stop therapy?

• Differentiate pseudo-progression from true regression

• Biodistribution and pharmacodynamic endpoints
  • Receptor saturation
  • Tumor T-cell activation, T-cell infiltration, change in T cell ratios, cytokine production
Quantitative immunohistochemical analysis of ITI and PTI by CD4+ and CD8+ cells.

Huang R R et al. Clin Cancer Res 2011;17:4101-4109
Figure 7. CD8 Staining in the Tumors of Patients With RCC After Treatment With MPDL3280A + Bevacizumab

- The increase in CD8+ cells was greatly enhanced in patients after treatment with MPDL3280A + bevacizumab.

Figure 8. Chemokine Expression in the Tumors of Patients With RCC After Treatment With MPDL3280A + Bevacizumab

- CCL2 is generally produced by tissue injury or infection and serves as a chemoattractant for monocytes, T cells and dendritic cells.
- CCL5 is a chemoattractant for T cells, eosinophils and basophils.
- CCR5 is the receptor for CCL5.
- CX3CL1 is a potent chemoattractant for T cells and monocytes and is primarily expressed in endothelial cells.
- CCR7 is a chemoattractant for T cells and stimulates dendritic cell maturation.
- CXCL10 is secreted by monocytes, endothelial cells and fibroblasts in response to IFNγ and serves as a chemoattractant for immune cells.
A single dose of NKTR-214 leads to ~500-fold greater tumor exposure compared to an equivalent dose of aldesleukin.

- 62-fold higher tumor exposure compared to aldesleukin for 8-fold lower IL-2 equivalent dose
NKTR-214 Tips the Balance in Favor of Tumor Killing T-Cells Within the Tumor Microenvironment

Immune Cell Populations Isolated from Mouse Tumors

**CD8+ Memory Effector T-Cells Increased After NKTR-214 Treatment**

- **Vehicle**
- **Aldesleukin**
- **NKTR-214**

\[ \text{Cells/Tumor mm}^{-2} \]

- 300-10^4
- 250-10^4
- 200-10^4
- 150-10^4
- 100-10^4
- 50-10^3
- 0

*P=0.0233 two tailed t test (NKTR-214 vs. aldesleukin)*

Day 7 - Absolute Number Tumor Derived CD8+ Memory Effector T-Cells Per Tumor Volume Identified as CD8+CD122+CD44hi (N=3)

**CD4+ Regulatory T-Cells Reduced After NKTR-214 Treatment**

- **Vehicle**
- **Aldesleukin**
- **NKTR-214**

\[ \text{Cells/Tumor mm}^{-2} \]

- 200
- 150
- 100
- 50
- 0

***P=0.0002 two tailed t test (NKTR-214 vs. aldesleukin)*

Day 7 - Absolute Number Tumor Derived Regulatory T-Cells Per Tumor Volume Identified as CD4+CD25+FoxP3+ (N=3)
Summary of responses to MPDL3280A in paired biopsies

<table>
<thead>
<tr>
<th>Maximum SLD decrease</th>
<th>Increase in PD-L1 (TC) (no. (%))</th>
<th>Increase in PD-L1 (IC) (no. (%))</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;30% reduction</td>
<td>3/6 (50)</td>
<td>5/6 (83)</td>
</tr>
<tr>
<td>0%-30% reduction</td>
<td>3/8 (37)</td>
<td>2/8 (25)</td>
</tr>
<tr>
<td>0%-20% increase</td>
<td>2/9 (22)</td>
<td>1/9 (11)</td>
</tr>
<tr>
<td>&gt;20% increase</td>
<td>0/3 (0)</td>
<td>1/3 (33)</td>
</tr>
<tr>
<td>Unevaluable SLD</td>
<td>1/1 (100)</td>
<td>1/1 (100)</td>
</tr>
</tbody>
</table>

Objective response per RECIST v1.1

| Best response of PR                  | 3/5 (60)                           | 4/5 (80)                           |
| Best response of SD                  | 5/12 (42)                          | 2/12 (17)                          |
| Best response of PD                  | 1/11 (9)                           | 4/11 (36)                          |

b) Immunological ignorance

Pre-treatment

On treatment (week 9)

Non-functional immune response

Pre-treatment

On treatment (week 6)

Excluded infiltrate

Pre-treatment

On treatment (week 6)
Is the dose of anti-PD-1 optimal in the combination?
Response to Ipilimumab 10 mg/kg x 2 doses

2 baseline brain mets regressed also:
No disease progression 8+ years
Left untreated, brain lesions grew slightly at week 19 and began to regress at week 25.
After ipi/nivo x 4, nivo x one year:
- Marked clinical improvement, normalized LDH
- MRI flair activity lateral left globe
- New FDG avid Left hilar node
- Increasing FDG avid Left adrenal
- Increasing avidity in right inguinal mass
- Improvement in hepatic lesions but still several with increased FDG uptake

- RAI plaque left lateral eye lesion
- Resected right inguinal mass
- Re-induction ipi/nivo

- Adrenal decreased in size and FDG uptake
- Left hilar node resolved
- Decrease FDG uptake in liver lesions
Ipi + Nivo x 4, Nivo q2w x 2 years, marked regression of most lesions (lung, LN, mesenteric and RP implants)

Persistent, slowly shrinking right middle lobe lesion, mildly FDG-avid

3 mesenteric nodules resected, 2/3 with active melanoma

Multiple other small residual lesions, not FDG-avid

Resect all FDG avid lesions?
Continue anti-Pd-1?
Re-induce with Ipi/nivo?
Imaging and immune therapy

High levels of clinical activity for immune therapy, complex biology, mechanisms of action and resistance poorly understood, complex patterns of clinical response, and innumerable agents and trials
HELP!!!!

• Predictive of Response
  • T-cell infiltration (extent, location, function, type)
  • Other immune cells (MDSC, Treg?)
  • Expression of antibody targets (CD47, CD73, PD-L1, PD-1, TIM-3, PD-1H, etc)
  • Metabolisms/metabolic state (hypoxia, glucose consumption, other)

• Tumor response in the absence of regression

• Differentiate scar from residual tumor versus persistent inflammation without tumor
  • When to stop therapy?

• Differentiate pseudo-progression from true regression

• Biodistribution and pharmacodynamic endpoints
  • Receptor saturation
  • Tumor T-cell activation, T-cell infiltration, change in T cell ratios, cytokine production
Imaging Inflammation with FDG, FLT and Beyond

Annick D. Van den Abbeele, MD
Chief, Department of Imaging
Founding Director, Center for Biomedical Imaging in Oncology
Co-Director, Tumor Imaging Metrics Core

Center for Biomedical Imaging in Oncology
Disclosures for
Annick D. Van den Abbeele, MD

• Research funding support to the Dana-Farber Cancer Institute from Novartis, Pfizer, Bayer, GSK, BMS
Immune Checkpoint Therapy: “A Game-Changer”

• Radical and disruptive change in cancer therapy:
  – Drugs are not designed to target the tumor cell, i.e., tissue of origin is becoming less relevant
  – Goal is to remove inhibitory pathways that block effective antitumor T cell responses

• Knowledge of the tumor microenvironment is becoming more important

Sharma et al Science 348:6230, April 2015
Immune response is dynamic and changes rapidly.

A single biomarker may not be enough to predict response as with molecularly-targeted therapy.

Must be able to assess the effectiveness of an evolving immune response and define the response that contributes to clinical benefit.

Sharma et al Science 348:6230, April 2015
Therapies that Might Affect the Cancer-Immunity Cycle

1. Release of cancer cell antigens
   - Chemotherapy
   - Radiation therapy
   - Targeted therapy

2. Cancer antigen presentation
   - Vaccines
   - IFN-α
   - GM-CSF
   - Anti-CD40 (agonist)
   - TLR agonists

3. Priming and activation
   - Anti-CTLA4
   - Anti-CD137 (agonist)
   - Anti-OX40 (agonist)
   - Anti-CD27 (agonist)
   - IL-2
   - IL-12

4. Trafficking of T cells to tumors

5. Infiltration of T cells into tumors
   - Anti-VEGF

6. Recognition of cancer cells by T cells
   - CARs

7. Killing of cancer cells
   - Anti-PD-L1
   - Anti-PD-1
   - IDO inhibitors

Blood vessel
Lymph node
Tumor
Is this an immune-related adverse event or a sign of qualitative and quantitative "immunocompetency" in spleen and draining lymph nodes?
• Diagnosis
• Tumor characterization (prognostic value)
• Staging
• Restaging
• Assessment of response (predictive value)
• Tumor heterogeneity
• Guide biopsy to relevant tissue
Evaluation of PD-L1 expression in metachronous tumor samples and FDG-PET as a predictive biomarker in Ph2 study (FIR) of atezolizumab (MPDL3280A)

Jamie Chaff,^1^ Bo Chao,^2^ Wallace Akerley,^3^ Michael S. Gordon,^4^ Scott J. Antonia,^5^ Jason Callahan,^6^ Alan Sandler,^7^ Roel Funke,^7^ Larry Leon,^7^ Jill Fredrickson,^7^ Marcin Kowanetz,^7^ Scott Gettinger^8^

^1^Memorial Sloan-Kettering Cancer Center, New York, NY; ^2^Ohio State University, Wexner Medical Center, Columbus, OH; ^3^Huntsman Cancer Institute, Salt Lake City, UT; ^4^Pinnacle Oncology Hematology, Scottsdale, AZ; ^5^Moffit Cancer Center, Tampa, FL; ^6^Peter MacCallum Cancer Center, East Melbourne, Australia; ^7^Genentech Inc., South San Francisco, CA; ^8^Yale School of Medicine, New Haven, CT
FDG-PET metrics and examples of response

Patient 1: Metabolic response
- Metabolic response determined by EORTC criteria based on 5 target lesions
- Whole body analyses: metrics for metabolic tumor burden derived from automated volume of interest
  - Percentage Injected Dose (%ID): reflects both metabolic volume and intensity of FDG uptake
- Patients with metabolic response by EORTC criteria on week 6 scans had higher ORR by RECIST 1.1 than metabolic non-responders (71% [15/21] vs 4% [3/67])

Patient 2: Metabolic disease progression
- Week 6: 100% reduction in target lesion uptake, but new lesions

Courtesy of Bernard M. Fine, MD PhD
Conclusions

- Baseline metabolic tumor burden was a significant negative prognostic marker for OS
- Early metabolic response (week 6) was a significant predictor of OS

Courtesy of Bernard M. Fine, MD PhD
FDG and Immune-Adverse Events (IAEs)
## Ipilimumab Potential Side Effects

<table>
<thead>
<tr>
<th>Condition</th>
<th>Any Grade</th>
<th>&gt;Grade 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dermatitis</td>
<td>40%</td>
<td>3%</td>
</tr>
<tr>
<td>Diarrhea/Colitis</td>
<td>30%</td>
<td>8%</td>
</tr>
<tr>
<td>Hypophysitis/Thyroiditis</td>
<td>6%</td>
<td>1%</td>
</tr>
<tr>
<td>Hepatitis and Pancreatitis</td>
<td>9%</td>
<td>6%</td>
</tr>
<tr>
<td>Other</td>
<td>6%</td>
<td>2%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>– Nephritis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>– Uveitis or Episcleritis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>– Neuritis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>70%</td>
<td>20%</td>
</tr>
</tbody>
</table>

*IRAEs can be waxing and waning*

Courtesy of Steve Hodi, MD
### Nivolumab Adverse Events

<table>
<thead>
<tr>
<th>Drug-Related Adverse Event</th>
<th>All Grades</th>
<th>Grades 3-4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tot Pop*</td>
<td>MEL</td>
</tr>
<tr>
<td><strong>No. (%) of Patients, All Doses</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any adverse event</td>
<td>207 (70)</td>
<td>82 (79)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>72 (24)</td>
<td>30 (29)</td>
</tr>
<tr>
<td>Rash</td>
<td>36 (12)</td>
<td>21 (20)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>33 (11)</td>
<td>18 (17)</td>
</tr>
<tr>
<td>Pruritus</td>
<td>28 (9)</td>
<td>15 (14)</td>
</tr>
<tr>
<td>Nausea</td>
<td>24 (8)</td>
<td>9 (9)</td>
</tr>
<tr>
<td>Appetite ↓</td>
<td>24 (8)</td>
<td>7 (7)</td>
</tr>
<tr>
<td>Hemoglobin ↓</td>
<td>19 (6)</td>
<td>7 (7)</td>
</tr>
<tr>
<td>Pyrexia</td>
<td>16 (5)</td>
<td>5 (5)</td>
</tr>
</tbody>
</table>

*AEs occurring in ≥5% of the total population.

†Common grade 3-4 AEs also included lymphopenia (3 pts) and abdominal pain and lipase increased (2 each). An additional 27 grade 3-4-related AEs were observed and one or more occurred in a single patient.

Courtesy of Steve Hodi, MD
## Treatment-Related Select Adverse Events Occurring in ≥1 Patient (ipilimumab and nivolumimab combination)

<table>
<thead>
<tr>
<th>Select Adverse Event</th>
<th>All Cohorts (n=53)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All Gr</td>
<td>Gr 3-4</td>
<td></td>
</tr>
<tr>
<td>Pulmonary</td>
<td>3 (6)</td>
<td>1 (2)</td>
<td></td>
</tr>
<tr>
<td>Renal</td>
<td>3 (6)</td>
<td>3 (6)</td>
<td></td>
</tr>
<tr>
<td>Endocrinopathies</td>
<td>7 (13)</td>
<td>1 (2)</td>
<td></td>
</tr>
<tr>
<td>Uveitis</td>
<td>3 (6)</td>
<td>2 (4)</td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>37 (70)</td>
<td>2 (4)</td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>20 (38)</td>
<td>5 (9)</td>
<td></td>
</tr>
<tr>
<td>Hepatic</td>
<td>12 (23)</td>
<td>8 (15)</td>
<td></td>
</tr>
<tr>
<td>Infusion reaction</td>
<td>1 (2)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Lipase</td>
<td>10 (19)</td>
<td>7 (13)</td>
<td></td>
</tr>
<tr>
<td>Amylase</td>
<td>8 (15)</td>
<td>3 (6)</td>
<td></td>
</tr>
</tbody>
</table>

Courtesy of Steve Hodi, MD
Immune-related adverse events

Immune-related adverse events

9/5/14
2/3/15
4/6/15
8/10/15

3/13/15
8/19/15
Immune-related adverse events

1 month after ipilumimab cycle 3

1 month after corticosteroids, started on infliximab

Follow-up

Immune-related adverse events

Immune-related adverse events

Role of FDG in Immune-adverse events

- Clinically relevant
- May be seen months prior to symptom development
- Timely initiation of corticosteroid therapy may alleviate serious complications and life-long dependency on hormonal therapy
Clinical activity in patients with non–small-cell lung cancer (NSCLC) receiving nivolumab

Scott N. Gettinger et al. JCO doi:10.1200/JCO.2014.58.3708

©2015 by American Society of Clinical Oncology
Evaluation of Treatment Response

- Cancer vaccines and immunomodulatory monoclonal antibodies have demonstrated:
  - delayed response to treatment when compared to cytotoxic chemotherapy

Do we need to wait that long to assess response?

- In the longest follow-up study after ipilimumab treatment for metastatic melanoma, the average time to achieve response in complete responders was 30 months.

Imaging Assessment Criteria are evolving

- WHO
- **RECIST 1.0 and 1.1**
- Volumetric Assessment
- Choi
- Cheson (Original and Revised)
- EORTC (European Organization for Research and Treatment of Cancer)
- PERCIST (PET Response Criteria in Solid Tumors)
- irRC (Immune-Related Response Criteria)
- Macdonald criteria (diagnostic criteria for multiple sclerosis)
- RANO (Revised Assessment in NeuroOncology) and iRANO
- PCWG for prostate cancer (Prostate Cancer Working Group)
- EBMT for myeloma (European Group for Blood and Marrow Transplantation)

Moertel et al Cancer 1976;38:388-394
Eisenhauer et al. Eur J Cancer 2009;45:228
Nishino et al. AJR 2010;195:281

Polman et al Annals of Neurology 2011; 69:292
Wen et al JCO 2010;28:1963
Cheson et al, J Clin Onc. 2007;45:579
Young et al Eur J Cancer 1999; 35:1773
Scher et al JCO 2008; 26:1148
Durie et al Leukemia 2006; 20: 1467
Okada et al Lancet Oncology 2015
Tumor Imaging Metrics Core at the Dana-Farber/Harvard Cancer Center

Currently deployed at 5 NCI Comprehensive Cancer Centers
Imaging Assessment Criteria are evolving

- **WHO**
- **RECIST 1.0 and 1.1**, new criteria forthcoming for IT (ASCO 2016),
- Volumetric Assessment
- **Choi**
- **Cheson (Original and Revised)**, new criteria forthcoming for IT
- **EORTC** (European Organization for Research and Treatment of Cancer)
- **PERCIST** (PET Response Criteria in Solid Tumors)
- **irRC** (Immune-Related Response Criteria)
- **Macdonald criteria** (diagnostic criteria for multiple sclerosis)
- **RANO** (Revised Assessment in NeuroOncology) and **iRANO**
- **PCWG** for prostate cancer (Prostate Cancer Working Group)
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Young et al Eur J Cancer 1999; 35:1773
Scher et al JCO 2008; 26:1148
Durie et al Leukemia 2006; 20: 1467
Okada et al Lancet Oncology 2015
Decoding the Tumor Phenotype: Radiomics
Radiomics

- Data are designed to be extracted from standard-of-care images
- Extraction and analysis of large amounts of advanced quantitative imaging features with high throughput from medical images obtained with CT, PET or MRI
Radiomics Process and Challenges
Beyond anatomy?
Molecular Imaging Explores the Hallmarks of Cancer Biology

Add Specificity

Immunotherapy
“Burning” Questions from Investigators
(Gordon Freeman, PhD, Steven Hodi, MD)

- Can we use imaging to:
  - characterize the tumor for the presence of inflammation (CD8 T cells) prior to treatment
  - determine if these CD8 T cells are activated (? is there a marker for CD107a?)
  - determine if CD69 T cells present?
  - evaluate PD1/PD-L1 axis expression (Zr-89-labeled PD-1, PD-L1, …) and compare it to IHC
  - Differentiate inflammatory response from tumor progression (new response criteria)
CD8+ T cells Before and during pembrolizumab treatment
Co-localization of PD-L1 and infiltrating T cells in melanoma

Slide provided by Lieping Chen, courtesy of Steve Hodi, MD
Ipilimumab and local radiotherapy result in an abscopal response

Abscopal: *ab-scopus*, “away from the target”

©2013 by American Association for Cancer Research
Enhanced tumor-infiltrating lymphocytes in an abscopal lesion (left supraclavicular node)
Regressing tumours during treatment are associated with proliferating CD8+ T cells that localize to the tumour parenchyma.

Sample obtained during tumour regression shows double-positive T cells localized to the tumour parenchyma.

Red line separates the invasive margin (above line) and tumour (below line).

Representative single-positive quiescent CD8+ brown cells (no Ki67 labelling) from the invasive margin.

Representative double-positive cells (red, labelled Ki67 nucleus; brown, labelled CD8 membrane) with characteristic chromatin patterns associated with sub-phases of mitosis.
\(^{18}\)F-FDG and \(^{18}\)F-FLT PET/CT scans in patient with metastatic melanoma with objective tumor response to tremelimumab (human IgG2 anti-CTLA4 monoclonal antibody)
Advanced Melanoma patients: received Tremelimumab (CTLA4 blockade)

PET Imaging of spleen at 1-2 months post-treatment

SUV measurements:
- Statistically significant difference in FLT uptake ($\text{SUV}_{\text{mean}}$ and $\text{SUV}_{\text{max}}$)
- Variable response observed (2/9 had decreases)
- No significant changes in FDG uptake

FLT-PET was therefore able to detect cell activation in most patients (variable response)


Courtesy of Anne Goodbody, PhD and John Valliant, PhD
Early identification of antigen-specific immune responses in vivo by [18F]-labeled 3′-fluoro-3′-deoxy-thymidine ([18F]FLT) PET imaging

Potential applications of FLT-PET in Tumour Vaccination

- Sensitive tool to examine kinetics and localization of activation
  - Longitudinal monitoring by non-invasive imaging
  - Measurement of *in vivo* cell functionality to determine subsequent individualized treatment

*Aarntzen et al. PNAS 2011; 108: 18396*

Courtesy of Anne Goodbody and John Valliant
FLT-PET: Autoimmune Rheumatoid Arthritis Preclinical Imaging

6 days post-induction of arthritis

\( ^{18} \text{F-FLT PET} \) scans in arthritic (n = 5-7) and healthy ankles (n = 3-6). **p < 0.05

- Correspondence with Ki-67 expression
- Potential to detect sub-clinical arthritis, enabling early treatment, especially where classification criteria of RA are only partially met

Courtesy of Anne Goodbody, PhD and John Valliant, PhD

Exploratory Clinical Investigation of (4S)-4-(3-18F- Fluoropropyl)-L-Glutamate PET of Inflammatory and Infectious Lesions

Sun Young Chae1, Chang-Min Choi2, Tae Sun Shim2, Yangsoon Park3, Chan-Sik Park3, Hyo Sang Lee1, Sang Ju Lee1, Seung Jun Oh1, Seog-Young Kim4, Sora Baek5, Norman Koglin6, Andrew W. Stephens6, Ludger M. Dinkelborg6, and Dae Hyuk Moon1

FIGURE 1. A 53-y-old man with sarcoidosis (patient 5). ¹⁸F-FDG (A) and ¹⁸F-FSPG (B) present similar major uptake involving pleura, supraclavicular lymph nodes, and thoracic lymph nodes. The only differences are normal physiologic uptake in brain, pancreas, and kidney. On immunohistochemistry evaluation (×400), proportion of inflammatory cells positive for xCT (C), CD44 (D), CD68 (E), and CD163 (F) was 80%, 80%, 80%, and 1%, respectively.
Table 3. Imaging agents for antitumor immune function in published preclinical studies.

<table>
<thead>
<tr>
<th>IMAGING AGENT</th>
<th>TARGETING CONCEPT</th>
<th>IMAGING TECHNOLOGY</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{18}$F-$^{64}$Cu anti-CD11b or MHC-II$^{81}$</td>
<td>Labeled antibody fragments binding to CD11b or MHC II on tumor macrophage or myeloid cells</td>
<td>PET</td>
</tr>
<tr>
<td>$^{64}$Cu-anti-CD8$^{76}$</td>
<td>Labeled antibody fragments binding to CD8 on tumor infiltrating cytotoxic T lymphocytes</td>
<td>PET</td>
</tr>
<tr>
<td>$^{89}$Zr-anti-CD8$^{78}$</td>
<td>Labeled antibody fragments binding to CD8 on tumor infiltrating cytotoxic T lymphocytes</td>
<td>PET</td>
</tr>
<tr>
<td>$^{18}$F-FEAU$^{101}$</td>
<td>Labeled ligand identifies viral transgene in activated CAR-T that are present in tumor</td>
<td>PET</td>
</tr>
<tr>
<td>$^{111}$I-anti-PD-L1$^{84}$</td>
<td>Labeled monoclonal antibody binds to PD-L1 expressed on macrophage and tumor cells</td>
<td>SPECT</td>
</tr>
<tr>
<td>$^{89}$Zr-anti-CD47$^{83}$</td>
<td>Labeled monoclonal antibody binds to CD47 expressed on cells within tumor</td>
<td>PET</td>
</tr>
<tr>
<td>$^{64}$Cu-Anti-CTLA-4$^{80}$</td>
<td>Labeled monoclonal antibody binds to CTLA-4 expressed on cytotoxic T lymphocytes within tumor</td>
<td>PET</td>
</tr>
<tr>
<td>MB-anti-B7-H3$^{85}$</td>
<td>Ultrasound microbubbles labeled with monoclonal antibody against B7-H3. Identifies cells expressing B7-H3 on macrophage and tumor cells</td>
<td>US</td>
</tr>
<tr>
<td>$^{64}$Cu-SPION$^{102}$</td>
<td>CAR-T cells loaded with $^{64}$Cu-SPION (iron nanoparticles). Image accumulation of therapeutic CAR-T</td>
<td>PET</td>
</tr>
<tr>
<td>DiR labeled T cells$^{103}$</td>
<td>DiR fluorophore, activated by near-Infrared light, is used to label T cells. T cells that located in tumor are imaged</td>
<td>Fluorescence imaging</td>
</tr>
</tbody>
</table>

and many others…

Juergens et a Biomarkers in CanCer 2016:8(s2)
Targeted imaging probe for immunotherapy

$^{18}$F-VHH7 (anti-mouse class II MHC)

$^{18}$F-VHHDC13 (anti-mouse CD11b)

Courtesy of Quang-Dé Nguyen, PhD
Table 2. Imaging agents in current immunotherapy trials.

<table>
<thead>
<tr>
<th>AGENT</th>
<th>CANCER</th>
<th>IMMUNOTHERAPY (IT) AND IMAGING TARGET</th>
<th>NATIONAL CLINICAL TRIALS (NCT) NUMBER</th>
<th>IMAGING TECHNOLOGY</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{18}$F-FDG$^{99,100}$</td>
<td>Melanoma, renal cell, lung</td>
<td>IT: anti-CTLA-4, anti-PD-1 Target: tumor metabolism</td>
<td>NCT01666353</td>
<td>PET/CT</td>
</tr>
<tr>
<td>$^{18}$F-FDG</td>
<td>Cervical, squamous cell</td>
<td>IT: anti-CTLA-4 Target: tumor metabolism</td>
<td>NCT01711515</td>
<td>PET/CT</td>
</tr>
<tr>
<td>$^{18}$F-FDG</td>
<td>Multiple Ca</td>
<td>IT: CAR-T, anti-CTLA-4, IL-2 Target: tumor metabolism</td>
<td>NCT02070406</td>
<td>PET</td>
</tr>
<tr>
<td>$^{18}$F-FDG</td>
<td>Renal cell</td>
<td>IT: IL-2 (plus chemo) Target: tumor metabolism</td>
<td>NCT01038778</td>
<td>PET/CT</td>
</tr>
<tr>
<td>$^{18}$F-FDG</td>
<td>Multiple Ca</td>
<td>IT: CAR-T, IL-2, DC vaccine Target: tumor metabolism</td>
<td>NCT01697527</td>
<td>PET</td>
</tr>
<tr>
<td>$^{18}$F-FDG or Na$^{18}$F</td>
<td>Prostate</td>
<td>IT: DC vaccine with GM-CSF Target: tumor metabolism</td>
<td>NCT02042053</td>
<td>PET/CT PET/MRI</td>
</tr>
<tr>
<td>$^{18}$F-FET</td>
<td>Brain melanoma metastases</td>
<td>IT: anti-PD-1, anti-CTLA-4 Target: tumor metabolism</td>
<td>NCT02374242</td>
<td>PET/MRI</td>
</tr>
<tr>
<td>$^{11}$C-PBR28a</td>
<td>Brain</td>
<td>IT: various IT treatments, Target: tumor benzodiazepine receptor</td>
<td>NCT02431572</td>
<td>PET</td>
</tr>
<tr>
<td>$^{18}$F-HBG$^{71}$</td>
<td>Glioma</td>
<td>IT: CAR-T, IL-2 Target: CAR-T cells</td>
<td>NCT01082926</td>
<td>PET</td>
</tr>
<tr>
<td>$^{89}$Zr-MPDL3280A$^{74}$</td>
<td>Multiple cancers</td>
<td>IT: anti-PD-L1 Target: PD-L1 on tumor or other cells</td>
<td>NCT02453984</td>
<td>PET</td>
</tr>
<tr>
<td>$^{99}$Tc-IL-2$^{88}$</td>
<td>Melanoma</td>
<td>IT: anti-CTLA-4, anti-PD-1, IL-2 Target: TIL expressing IL-2 receptor</td>
<td>NCT01789827</td>
<td>SPECT</td>
</tr>
<tr>
<td>$^{18}$F-L-FAC$^{70}$</td>
<td>Healthy volunteers and multiple cancers</td>
<td>IT: various immunotherapies Target: activated T-cells in tumor</td>
<td>NCT01808688</td>
<td>PET</td>
</tr>
<tr>
<td>$^{89}$Zr-GC1008$^{72}$</td>
<td>Brain glioma</td>
<td>IT: anti-TGF-β Target: TGF-β</td>
<td>NCT01472731</td>
<td>PET</td>
</tr>
<tr>
<td>Ferumoxytol$^{73}$ (iron nanoparticles)</td>
<td>Brain</td>
<td>IT: various immunotherapies Target: macrophage in tumors</td>
<td>NCT02452216</td>
<td>MRI</td>
</tr>
<tr>
<td>$^{18}$F-F-AraG$^{69}$</td>
<td>Healthy subjects</td>
<td>IT: prior to various cancer IT trials Target: activated T-cells</td>
<td>NCT02323893</td>
<td>PET</td>
</tr>
</tbody>
</table>

and many others…
Adding More Specificity to Characterize the Immune Response

- Precision medicine era
- Will help:
  - characterize the tumor (prognostic value)
  - stratify patients
  - enrich patient population in clinical trials
  - define and validate biomarker(s) (predictive value)
  - to be relevant to the disease, immune process, immune therapy
- May become a clinically actionable test
MORE Than Meets the EYE
Going Forward

• Response criteria will continue to evolve
• Pay particular attention to:
  o disease assessment time points to align with the natural course of the disease, the treatment and the response to the treatment
  o defining progression and duration of follow-up
  o immune-adverse events
  o discontinuation criteria
• Test Radiomics
Going Forward

• Imaging needs to be relevant to the immune system
• Co-develop novel immunotherapeutic drugs with companion diagnostics (PET probes specific to relevant mechanism of action and immune features) and co-validate them in prospective trials
All hands on deck!

- Learn from ongoing work in other specialties: neurodegeneration, atherosclerosis, CAD, vasculitis, myocardial inflammation, sarcoidosis, rheumatoid arthritis, infection or inflammation
- Create multidisciplinary teams of basic scientists, immunologists, infectious disease, oncologists, imaging, radiation therapy, surgeons…
- Think globally
Outlook for cancer patients has never been better

“Much to celebrate, but even more to do”
(Nancy E Davison, MD)