Non-Invasive Quantification of Immune Checkpoint Blockade at the Tumor

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Advanced squamous-cell NSCLC

Advanced urothelial carcinoma

Advanced melanoma

Status of Immune Checkpoint Therapeutics

Combination clinical trials
PD-1/PD-L1

Number of approved biomarkers to guide therapy: 2

N Engl J Med 2017; 376:1015-1026
N Engl J Med 2017; 377:1345-1356

**Positron Emission Tomography (PET)**

*Target mapping* (expression levels, enzyme activity)
*Probe disposition in the tumor and other tissues (PK)*
*Changes in target expression* (receptor density etc.)
*Modulation of the biochemical pathway (effector pathways)*
*Desired biological effect*
*Clinical response*

**Tracer doses**

$[^{11}C]$carfentanil (m opioid receptor ligand)

$5.5 \times 10^{-6}$ mg/kg mass dose

Dose for adult male elephants (1 Ton) ~ 10 mg

Molecularly targeted high affinity probe

**The Cancer Immunity Cycle & Imaging Targets**

Chen and Mellman, Immunity, Volume 39, Issue 1, Pages 1-10

- PD-L1
- CD8
- Granzyme B
Programmed Cell Death Ligand 1 (PD-L1)

A target for imaging immune checkpoint blockade

- Expressed in a variety of cancers
- Associated with aggressive disease
- Poor prognosis and poor survival.
- Three FDA approved antibody therapeutics
- PD-L1 IHC is a companion diagnostic
- Imperfect biomarker?
  - 50% of NSCLC are PD-L1 +ve
  - ~8% of PD-L1 -ve patients show response

Imaging PD-L1 using Antibodies

SPECT and PET Imaging with Atezolizumab

PD-L1 Detection with a Peptide

$[^{64}\text{Cu}]\text{WL12}$
PD-L1 Imaging Agents with Improved Image Contrast for Clinical Translation, [^{18}F]DK222

**TNBC models**

MDA-MB231 xenografts (PD-L1 +ve)  
SUM159 (PD-L1 –ve)

High contrast PD-L1 specific images within 60 min

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**Imaging Functional Activity of T-cells using Granzyme B**

- Lymphocyte granule protease
- Essential for cytolytic activity of T-cells
- Increased GrB activity at the disease site

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**^{68}Ga-GZP PET Imaging Quantitatively Detects Response to I-O Therapy**

- Ga-GZP PET Imaging
- Quantitative detection of response to I-O therapy
- Treated Responder
- Treated Non-Responder
- Vehicle

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[Image of tumor model with PET imaging results]

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[Graph showing tumor/muscle and tumor/blood ratios over time]
Success of Anti-cancer Therapies

• Cancer and immune cell heterogeneity (Target expression, mutational burden, gene rearrangements, TCR clonality etc.)

• Drug activity (affinity, selectivity, ADME, drug access, target engagement, biological activity)

Success of Anti-cancer Therapies

Cancer and immune cell heterogeneity
• Programmed death-ligand 1 (PD-L1)
• MSI-high
• Tumor mutation burden
• CD8+ cells
• Neoantigen burden
• T-cell clonal diversity
• Multiplex IHC
• Microbiome

Drug activity at the tumor

Dose-Exposure-Response Relationships

Dose

Exposure

Response
Status of Immune Checkpoint Therapeutics

Agents in development

PD-1/PD-L1

Antibody Therapeutics

FDA approved antibodies ~79
Oncology focused ~35
mAbs, ADCs, bi-specific antibodies, fusion proteins

Advantages
• Long half-lives
• High therapeutic index

Disadvantages
• Large size
• Development of anti-therapeutic antibodies
• Poor tissue penetration-solid tumors
• Expensive treatments

Peripheral T cell vs. Tumor Target Occupancy

Because peripheral-blood T cells express PD-L1, it is possible to assess in vivo receptor occupancy by anti-PD-L1 antibody as a pharmacodynamic measure. Median receptor occupancy was more than 65% for the doses tested. Although these studies provide a direct assessment and evidence of target engagement in patients receiving anti-PD-L1 antibody, relationships between receptor occupancy in peripheral blood and the tumor microenvironment remain poorly understood.
Drug-Target Engagement

Drug + Protein → Protein-Drug Complex → Biological effect

Measurements in Cells and Tissues
The Cellular Thermal Shift Assay
Mass Spectrometry
Fluorescence Anisotropy Imaging

No real-time information, multiple measurements across multiple lesions are difficult

Structural analysis indicates an overlap between mAb and peptide interaction surfaces on PD-L1

PD-1
WL12

Atezolizumab
Avelumab
Durvalumab
BMS936559
KM035 (nanobody)

PD-L1

Structural analysis indicates an overlap between mAb and peptide interaction surfaces on PD-L1

Target Engagement by Antibodies

- Free fraction PD-L1 levels
- Effect of combination therapy on PD-L1 levels
- Dose optimization
- Target potency of different antibodies
- Dose-Exposure-Response relationships
- Drug development and evaluation - Biosimilars

Antibody vs. peptide<1 nM vs. ~ 20 nM
Slow vs. Fast PK

Courtesy: Sports Illustrated
Free Fraction PD-L1 Levels Quantified using [\(^{64}\text{Cu}\)]WL12 after Atezolizumab Treatment

\([\text{PET Imaging}}\]

\([\text{Ex Vivo Biodistribution}}\]

\([\text{PD-L1 IHC}}\]

\([^{64}\text{Cu}]WL12\)-PET: A common denominator to Quantify PD-L1 Therapeutic Activity at the Tumor

\([\text{PET Imaging}}\]

\([\text{Biodistribution}}\]

\([\text{PD-L1 IHC}}\]

Summary and future directions...

- High affinity and high contrast imaging agents for quantification of target expression and dynamics
- A PD-L1 specific imaging agent to determine mAb kinetics at the tumor
- Dose selection, optimization and intensification
- Quantitative relationships between dose \(\rightarrow\) tumor and tissue exposure \(\rightarrow\) response and toxicity
- Drug development and evaluation
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