FUNCTIONAL ASSESSMENT OF IMMUNE RESPONSES AND IDENTIFICATION OF NEW ANTIGENS

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Disclosure slide

• I have no disclosures
Chen et al. Immunity 2013

1. Release of cancer cell antigens (cancer cell death)

2. Cancer antigen presentation (dendritic cells/ APCs)

3. Priming and activation (APCs & T cells)

4. Trafficking of T cells to tumors (CTLs)

5. Infiltration of T cells into tumors (CTLs, endothelial cells)

6. Recognition of cancer cells by T cells (CTLs, cancer cells)

7. Killing of cancer cells (Immune and cancer cells)
**Neoantigens and tumor mutational load**

**Immunodynamics, biomarkers**

**a** Antigens: high tumour specificity
- Mutation: Most tumours
- Tumour-specific expression: Many tumours

(b) Antigens: low tumour specificity
- Tissue-specific expression: Melanomas
- Overexpression: Some tumours

**Normal cell**
- Nucleus
  - Demethylation
  - No protein

**Tumour cell**
- MHC
  - Demethylation
  - No protein

- Spermatocytes
- Spermatogonia
- Trophoblasts

- Other normal cells
- Melanocytes
- Other normal cells

Coulie et al. Nat Rev Cancer 2014
NSCLC cohort treated with pembrolizumab
Chen et al. Immunity 2013

1. Release of cancer cell antigens (cancer cell death)
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3. Priming and activation (APCs & T cells)
4. Trafficking of T cells to tumors (CTLs)
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Antigen presentation and T-cell priming

Dendritic cells and cross-presentation of cancer antigens

CDP or pre-cDC

IRF4

IRF8

IRF8

CD8α+ cDC

IRF8

BATF3

CD4+ cDC

• IL-23 production
• MHC class II-mediated antigen presentation
• Cross-presentation
• Early IL-12 production

pDC

• IFN production

Murphy et al. Nat Rev Immunol 2013
Intratumoral T-cell infiltration

T-CELL INFLAMMED

TYPE I/II IFN +

Gajewski et al. Nat Immunol 2013

Immunodynamics, biomarkers

NON T-CELL INFLAMMED

TYPE I/II IFN -

Gajewski et al. Nat Immunol 2013
**Intratumoral T-cell infiltration**

**Immunodynamics, biomarkers**

*(anti-PD-1/L1)*

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**Tumeh et al. Nature 2014**

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**Herbst et al. Nature 2014**
¿How can we predict CD8 T-cell reactivity against cancer antigens?

- Measuring CD8-PD1+ TILs
- Analyzing TCR-CDR3 clonality in TILs
- Detecting neoantigen-specific TILs
Intratumoral T-cell infiltration/T-cell inflamed

Tumor reactive TILs: CD8-PD-1+


Immunodynamics, biomarkers
Intratumoral T-cell infiltration/T-cell inflammed
Tumor reactive circulating CD8-PD-1+ T cells
Intratumoral T-cell infiltration/T-cell inflammed
Tumor reactive TILs: TCR clonaltity

Immunodynamics, biomarkers
(anti-PD-1/L1)

Tumeh et al. Nature 2014
Intratumoral T-cell infiltration/T-cell inflammed
Tumor reactive TILs: TCR clonality

Immunodynamics, biomarkers
(anti-PD-1/L1)

Tumeh et al. Nature 2014
TIL clones could be detected expanding in the blood at 3 weeks posttreatment
Is the combination of pre-treatment TIL and blood clonality predictive of clinical benefit? (n=24)

<table>
<thead>
<tr>
<th>Pre-Treatment Clonality Status*</th>
<th>N</th>
<th>Survival &gt; 1 YR (n,%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High TIL Clonality AND Low Blood Clonality</td>
<td>6</td>
<td>6 (100%)</td>
</tr>
<tr>
<td>EITHER High TIL Clonality OR Low Blood Clonality</td>
<td>12</td>
<td>(33%)</td>
</tr>
<tr>
<td>NEITHER High TIL Clonality NOR Low Blood Clonality</td>
<td>6</td>
<td>2 (33%)</td>
</tr>
</tbody>
</table>

*high/low cutoff based on median values
Intratumoral T-cell infiltration/T-cell inflamed

Neoantigen specific TILs

Immunodynamics, biomarkers

Schumacher et al. Science 2015
Intratumoral T-cell infiltration/T-cell inflamed
Neoantigen specific circulating T-cells

Immunodynamics, biomarkers (anti-PD1/L1)
Intratumoral T-cell infiltration

**T-CELL INFLAMMED**

- PD-L1
- Cytokines
- CD8+ CTL
- CXCL9, CXCL10
- DC, Mφ, IDO
- Anergy
- Low CD80, CD86
- Tryptophan depletion

**TYPE I/II IFN +**

**NON T-CELL INFLAMMED**

- Foxp3+ Treg cell
- PD-1
- Mφ
- MDSC
- Arginase
- Arginine depletion

**TYPE I/II IFN -**
Intratumoral T-cell infiltration/Non T-cell inflammed

¿Which mechanisms drive T-cell exclusion?

- Endothelial barrier
- Neoantigen burden (?)
- Ineffective T-cell priming
- Immunosuppressive molecules (secreted by tumor cells, myeloid-derived cells, Tregs...)

Overlapping mechanisms
Intratumoral T-cell infiltration/Non T-cell inflammed

b) Neoantigen burden

Immunodynamics, biomarkers

Charoentong et al. Cell Rep 2017
Intratumoral T-cell infiltration/Non T-cell inflammed

b) Neoantigen burden

[Graphs showing data on neoantigen burden and binding scores for T-cell high and low groups.

Immunodynamics, biomarkers

Spranger et al. PNAS 2016]
Intratumoral T-cell infiltration/Non T-cell inflammed

c) Priming defects

Immunodynamics, biomarkers

Spranger et al. PNAS 2016
Intratumoral T-cell infiltration

Non T-cell inflamed

β-catenin and priming defects

Immunodynamics, biomarkers

Spranger et al. Nature 2015
Tumor types with excess β-catenin pathway activation in the non-T cell-inflamed subset

<table>
<thead>
<tr>
<th>Cancer Abbrev</th>
<th>Cancer Type</th>
<th>Non-T cell-inflamed</th>
<th>T cell-inflamed</th>
</tr>
</thead>
<tbody>
<tr>
<td>COAD</td>
<td>Colon adenocarcinoma</td>
<td>67.39%</td>
<td>45.83%</td>
</tr>
<tr>
<td>SKCMmets</td>
<td>Skin Cutaneous Melanoma (metastatic)</td>
<td>36.11%</td>
<td>12.93%</td>
</tr>
<tr>
<td>LGG</td>
<td>Brain Lower Grade Glioma</td>
<td>25.41%</td>
<td>0.00%</td>
</tr>
<tr>
<td>TGCT</td>
<td>Testicular Germ Cell Tumors</td>
<td>25.00%</td>
<td>5.19%</td>
</tr>
<tr>
<td>LUSC</td>
<td>Lung squamous cell carcinoma</td>
<td>24.00%</td>
<td>7.21%</td>
</tr>
<tr>
<td>SARC</td>
<td>Sarcoma</td>
<td>19.12%</td>
<td>4.82%</td>
</tr>
<tr>
<td>LUAD</td>
<td>Lung adenocarcinoma</td>
<td>18.18%</td>
<td>8.57%</td>
</tr>
<tr>
<td>UVM</td>
<td>Uveal Melanoma</td>
<td>16.36%</td>
<td>0.00%</td>
</tr>
<tr>
<td>LIHC</td>
<td>Liver hepatocellular carcinoma</td>
<td>13.79%</td>
<td>8.33%</td>
</tr>
<tr>
<td>KIRP</td>
<td>Kidney renal papillary cell carcinoma</td>
<td>13.64%</td>
<td>3.51%</td>
</tr>
<tr>
<td>UCS</td>
<td>Uterine Carinosarcoma</td>
<td>10.53%</td>
<td>0.00%</td>
</tr>
<tr>
<td>ESCA</td>
<td>Esophageal carcinoma</td>
<td>9.71%</td>
<td>5.56%</td>
</tr>
<tr>
<td>KICH</td>
<td>Kidney Chromophobe</td>
<td>9.30%</td>
<td>0.00%</td>
</tr>
</tbody>
</table>
**Intratumoral T-cell infiltration**/Non T-cell inflammed

d) Immunosuppressive microenvironment

Immunodynamics, biomarkers
(anti-PD-1/L1)
Intratumoral T-cell infiltration/Non T-cell inflammed
d) Metabolic reprogramming, antioxidant signature

Immunodynamics, biomarkers (anti-PD1/L1)

A

![Heatmap showing gene expression levels with different color coding for Metabolism, molecular transport, Immune functions, and Other functions.]

B

![Graphs showing positive tumor cells in UGT1A6-Negative and UGT1A6-Positive RCC. The graph compares responders (n=4) to nonresponders (n=8) with a p-value of 0.036.]
Some T-cell immunosuppressive molecules (or surrogates of T-cell suppressive states) can be measured in blood and potentially used to monitor or predict outcome of checkpoint inhibitors

- IL-1, IL-6, IL-8, IL-17, CXCL1, VEGF, CRP
- Neutrophil/lymphocyte count
- Myeloid-derived suppressor cell counts
- LDH
IL-8 is a chemokine secreted by malignant cells and has different protumoral actions:

- **Increase proliferation and survival of tumour cells**
  - CXCR-1/2
  - Brew R, Cytokine, 2000

- **Chemoattraction of myeloid cells**
  - Baggiolini M, J Clin Invest, 1989

- **Promote angiogenesis**
  - Koch E, Science, 1992
  - Strieter RM, J Biol Chem, 1995

- **Favour cancer stem cells**
  - Infanger DW, Cancer Res, 2013
  - Singh JK, Breast Cancer Res, 2013
Changes in serum IL-8 levels reflect tumor response to anti-PD-1 mAbs in melanoma patients

Sanmamed et al. ESMO 2016
Immunodynamics, biomarkers (anti-PD1/L1)

Serum IL8 levels correctly reflected true response in a patient presenting pseudoprogression

![Graph showing serum IL8 levels over time with pseudoprogression and partial response milestones](image)

Sanmamed et al. ESMO 2016
Changes in serum IL-8 levels are associated with OS in cancer patients treated with anti-PD-1 mAbs
Recognition and killing of cancer cells

Adaptive vs. innate PD-L1 expression

Adaptive

Innate

T cell

PD1

IFNγ

PDL1

Macrophage

Tumour cell

Activated oncogene

Recognition and killing of cancer cells

Immunodynamics, biomarkers
(anti-PD1/L1)

T-cell effector markers

Herbst et al. Nature 2014
Fehrenbacher et al. The Lancet 2016
Advanced microscopy techniques to evaluate cancer immunotherapies

Molecular dynamics of cytotoxic cell polarization, degranulation, and target cell apoptosis
Morphological features of apoptosis in target cells
Dynamic interactions and persistence of cytotoxic cells with target cells.
High-resolution confocal imaging of fixed specimens
Intravital microscopy
Live-cell microscopy
Tissue infiltration and localization of immune cell subsets.
Cell motility and tissue infiltration of immune cells
Molecular/morphological features of killing synapse
CONCLUSIONS

• The efficacy of many immune therapies in cancer (particularly anti-PD-1/L1 drugs) relies on pre-existing immunity, in particular on the presence or generation of tumor reactive T-cells.

• Surrogate dynamic molecular markers of the presence or absence of adaptive T-cell immune activation (or T-cell immune suppression) can be analyzed in tumor or blood as predictive markers for therapy response or treatment monitoring with anti-PD-1/L1 drugs.

• On the pillar of PD-1 pathway blockade, many combinatorial strategies in clinical development seek to induce T-cell infiltration and/or reverse other immune suppressive mechanisms in the tumor microenvironment.

• Serial pre- and post treatment biopsies and blood collections are paramount in new immune therapy development (early immune-based trials)

• Other tools to measure dynamic changes in the immune and tumor microenvironment might be helpful for translational research
¿Cómo seleccionar pacientes candidatos a anti-PD-1/L1?
3. Intratumoral T-cell infiltration

**Tumor reactive TILs: CD8-PD-1**

Gajewski et al. Nat Immunol 2013
Intratumoral T-cell infiltration/Non T-cell inflammed

b) Neoantigen burden

**Immunodynamics, biomarkers**

![Gene expression score graph](image)

**A**

- Paraganglioma
- Glioma (LG)
- Uveal Melanoma
- Kidney (Chromophobe)
- Prostate
- Glioblastoma
- Uterine Carcinoma
- Adenocortical
- Rectum
- Uterine Carcinosarcoma
- Colon
- Thyroid
- Bladder
- Osteosarcoma
- Uterine Carcinosarcoma
- Cervix
- Kidney (papillary)
- Head and Neck
- Breast
- Stomach
- Lung (Squamous)
- Pancreatic
- Mesothelioma
- Kidney (cc)

**B**

- Paraganglioma
- Glioma (LO)
- Uveal Melanoma
- Kidney (Chromophobe)
- Prostate
- Glioblastoma
- Uterine Carcinoma
- Adenocortical
- Rectum
- Uterine Carcinosarcoma
- Colon
- Thyroid
- Bladder
- Osteosarcoma
- Cervix
- Kidney (papillary)
- Head and Neck
- Breast
- Stomach
- Lung (Squamous)
- Pancreatic
- Mesothelioma
- Kidney (cc)

**Spranger et al. PNAS 2016**
REVIEW

Immunodynamics: a cancer immunotherapy trials network review of immune monitoring in immuno-oncology clinical trials

Intratumoral T-cell infiltration/T-cell inflamed

Immunodynamics, biomarkers
(anti-PD-1/L1)

Fehrenbacher et al. The Lancet 2016
Intratumoral T-cell infiltration/Non T-cell inflammed

a) Endothelial barrier

The Endothelial Barrier Hypothesis

Endothelin B receptor mediates the endothelial barrier to T cell homing to tumors and disables immune therapy

Tumor endothelium FasL establishes a selective immune barrier promoting tolerance in tumors

Coukos, ESMO 2016
Early changes in serum IL-8 levels predict responses to anti-PD-1 mAbs in melanoma patients

Identification Melanoma cohort

ROC curve

Serum IL-8 levels

% change

Baseline 2-3 weeks

% of change serum IL-8 levels

Responders Non-Responders

AUC: 0.91 (95% CI: 0.78-1.04)
P = .0002

Sanmamed et al. ESMO 2016
Neoantigens and tumor mutational load

Immunodynamics, biomarkers

Schumacher et al. Science 2015