From basic to tumour immunology for oncologists

Andrea Anichini
Human Tumors Immunobiology Unit,
Dept. of Experimental Oncology and Molecular Medicine
Fondazione IRCCS Istituto Nazionale dei Tumori, Milan
1. Development and regulation of anti-tumor immunity: some of the main cellular subsets

- Dendritic cell
  - MHC Class I
  - TCR
  - CD8
  - "naive" T cell
  - TCR
  - CD4
  - "mature" CD8
  - CTL
- B cell
  - Plasmacell
  - TH2 (IL-4)
  - MHC Class II
  - Co-stimulatory molecules and inhibitory receptors
  - cytokines
  - IL-12
  - Antigen
  - Gene
- Tumor cell
  - Antigen

- NK cell
  - CD16
  - KIR
- CTLA-4
  - PD-1
- CD80
  - CD86
  - PD-L1
  - PD-L2
- CD80
  - PD-L1
  - PD-L2
  - PD-L1
  - PD-L2
  - CD80
  - CD86
  - PD-L1
  - SLAMF7
- TH1 (IFN-γ)
- Tregs
  - (CD4+ CD25+ FOXP3+ CD39+)
- (-)
  - CTLa-4
  - MDSCs
  - (HLA-DR- CD14+ CD33+)
  - (HLA-DR- CD15+ CD33+)
- Immunosuppressive and pro-tumoral subsets
  - M2 macrophages
  - CD163/CD204
  - CAFs: cancer-associated fibroblasts
2. How tumors evade the immune system.

- Production of immunosuppressive factors (IL-10, TGF-β1, VEGF-A, ...)
- Reduced expression and/or constitutive expression of HLA molecules
- Loss of MHC class I and II (HLA-I, HLA-II)
- Loss of TAA by dedifferentiation
- Intra-tumor TAA heterogeneity (branched evolution)
- Constitutive expression of immunoregulatory genes (CD80, CD86, PD-L1, PD-L2)
- Active recruitment, activation/differentiation of immunosuppressive and pro-tumoral subsets
- Immunosuppressive and pro-tumoral subsets (M2 macrophages, Treg (CD4+ CD25+ FOXP3+ CD39+), MDSCs (HLA-DR CD14+ CD33+), ...)
- Th1 (IFN-γ) skewing towards Th2 profile
- Production of immunosuppressive factors (IL-10, TGF-β1, VEGF-A, ...)
- Reduced expression of ligands for activatory receptors of NK cells
- Reduced expression of ligands for activatory receptors of T cells
- Upregulation of ligands of inhibitory receptors
- Loss of expression of HLA molecules
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- Upregulation of ligands of inhibitory receptors
- Loss of expression of HLA molecules
3. Main immunotherapy strategies

- Dendritic cell
  - MHC Class I
  - TCR
  - CD8
  - "naive" T cell
- B cell
- "mature" CD8 T cell
- NK cell
- Plasmacell
- B cell
- TH2 (IL-4)
- IL-12

**Gene Vaccines**

- Adoptive T cell transfer
- CAR-T cells
- Tumor targeting mAbs

**Immune checkpoint Blockade**

- CTLA-4
- PD-1
- PD-L1
- PD-L2
- CD80
- CD86

**Tumor cell Antigen**

- Antigen release

**Targeting suppressive cells**

- Tregs (CD4+CD25+ FOXP3+ CD39+)
- M2 macrophages (CD163/CD204)
- MDSCs (HLA-DR+ CD14+ CD33+)
- (HLA-DR+ CD15+ CD33+)

**Targeting immunoregulatory genes**

- Promoting immunogenic cell death.
- Inducing tumor cell death by oncolytic viruses.
- Promoting Type I IFN response by DNMT inhibitors.
- Targeting immunoregulatory genes.

**Co-stimulatory molecules and inhibitory receptors**

- CD80
- CD86
- PD-L1
- PD-L2
- PD-1
- CTLA-4
- SLAMF7
- CD137

**Cytokines**

- IL-12
The relevance of the spontaneous anti-tumor response and why it is generated only in a subset of patients
Immune reaction to melanocytic lesions


Tumor-Infiltrating Lymphocytes Predict Sentinel Lymph Node Positivity in Patients With Cutaneous Melanoma
Rebecca C. Taylor, Anu Patel, Katherine S. Panasewicz, Elena J. Baum, and Mary S. Brady

Immune profile and mitotic index of metastatic melanoma lesions enhance clinical staging in predicting patient survival
Douglas Bogunovic1, David W. O'Neill2, Elena Bolotskaya-Lovy3, Vladimir Yacik4, Yi-Luo Yu4, Sylva Adams4, Farzad Davoodi4, Russell Bermeer5, Richard Shapiro5, Anna C. Pau1ick5, Stefano Lonardi6, Jith Ravindra7,8, Inman Osenar9, and Nina Hardwicke10,11


PD-1 blockade induces responses by inhibiting adaptive immune resistance
Paul C. Tumeha, Christina L. Harviek2, Jennifer H. Yeung1, L. Peter Shimakura1, Emma M. Taylor1, cellphonea, Bartosz Chmielnicka2,3, Marko Spanica4, Gina Henry1, Voka Calama1, Ahlena N. Wolf1, Marcella Carmona1, Christine Kirovka1, Elizabeth Segui1, G. B., Mandar1,2, Agustoni F, G. M., Giordani1,2, Enrico R. Grigioni1,4, Christiane Meroni, Lenna Sentens1,4, Olga A. Glape3,4, Krysta O. Engwicht1, Harlan Robb1, Robert H. Herbst1, David S. Efird1,4, Carinna Robert1, and Alessio Ribatti1,4

Genomic classification of cutaneous melanoma: four genomic subtypes and three gene expression subsets.
Expression clustering identifies four different types of bladder cancer.
Four molecular subtypes of pancreatic cancer
"T-cell-inflamed tumors" vs "non-T-cell-inflamed tumors" in bladder cancer

MHC-II+ melanomas express immune-related signatures ('PD-1 signalling', 'allograft rejection' and 'T-cell receptor signalling')
MITF/AXL alternative gene programs: role in shaping the tumor microenvironment

Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq

Tirosh I et al. Science 352.189, 2016
Some molecular subsets of NSCLC are associated with inflammatory reaction

- Simultaneous PD-L1 and JAK2 amplification in 5/94 (5.3%) NSCLC surgical specimens.

- ADC or SCC cell lines with distinct mutations (NRAS, KRAS, BRAF, PIK3CA, EML4-ALK, RET) or FGFR1 amplification have active AKT/mTOR pathway driving constitutive PD-L1 expression.

- EGFR activation by EGF stimulation, exon-19 deletions, and L858R mutation induces PD-L1 in cell lines. - PD-L1 expression in ADC surgical specimens with EGFR Del-19 and L858R mutations.

- PD-L1 expression in cell lines and surgical specimens positive for ALK-EML4 fusion gene.

- "KP" subset (KRAS-mut p53-mut) ADC show higher levels of somatic mutations, inflammatory markers, immune checkpoint effector molecules, and improved relapse-free survival.

- PD-1 and PD-L1, TIM-3, BTLA, CTLA-4, LAG-3, CD3+ TIL, CD80, CD86, OX40L, 4-1BB, CD127, IFN-γ-induced genes, CD4+Foxp3+ Tregs in ADC with a “mesenchymal” profile, not associated with mutational lad.
1. Higher mutational load, more immunogenic tumors
2. Frequent PD-L1 expression

Constitutive PD-L1 expression?

High frequency of patients in the “KP” subset in this study?
The “arrow” of tumor immunity and response to immunotherapy

Neoantigens \( \rightarrow \) T cell response \( \rightarrow \) immune-related upregulation of PD-L1 \( \rightarrow \) response to ICB

**Future Oncol.** (2015) 11(9), 1307–1326
The “arrow” of tumor immunity

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Evidence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanoma</td>
<td>A neoantigen landscape present in tumors with a strong response to CTLA-4 blockade.</td>
<td>Snyder, NEJM 2014</td>
</tr>
<tr>
<td>Melanoma</td>
<td>Overall mutational load, neoantigen load, and expression of cytolytic markers in the immune microenvironment were significantly associated with clinical benefit.</td>
<td>Van Allen, Science 2015</td>
</tr>
<tr>
<td>NSCLC</td>
<td>higher nonsynonymous mutation burden in tumors associated with improved objective response.</td>
<td>Rizvi, Science 2015</td>
</tr>
<tr>
<td>MSI-CRC</td>
<td>Higher response rate to anti-PD-1 in mismatch-repair deficient tumors</td>
<td>Le, NEJM, 2015</td>
</tr>
</tbody>
</table>

Colorectal cancer


Melanoma, Stage III

Madore, Clin Cancer Res 2016
“Hypermutant tumors”: good candidates for spontaneous development of tumor immunity?

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Evidence</th>
<th>Implication</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glioblastoma resulting from biallelic mismatch repair deficiency (bMMRD) syndrome</td>
<td>Neoantigen loads 7-16 times higher than in immunoresponsive melanomas or lung cancers</td>
<td>Highly responsive to anti-PD-1</td>
<td>Bouffet, JCO 2016</td>
</tr>
<tr>
<td>Colorectal cancer with mismatch-repair deficiency</td>
<td>Mean of 1782 somatic mutations /tumor in mismatch repair–deficient tumors, vs 73/tumor in mismatch repair–proficient tumors</td>
<td>Response to anti-PD-1: immune-related ORR 40% in mismatch repair–deficient colorectal cancers vs 0% for mismatch repair–proficient tumors</td>
<td>Le, NEJM 2015</td>
</tr>
<tr>
<td>BRCA1/2-mutated high grade serous ovarian cancer</td>
<td>-Higher predicted neoantigen load in BRCA1/2-mutated tumors compared to tumors without alterations in homologous recombination (HR) genes. -Better prognosis in BRCA1/2-mutated tumors with high number of TILs</td>
<td>BRCA1/2-mutated HGSOCs may be more sensitive to PD-1/PD-L1 inhibitors compared to HR-proficient HGSOCs.</td>
<td>Strickland, Oncotarget 2016</td>
</tr>
<tr>
<td>Higher PD-1+ T infiltration</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Which antigens are relevant?

Antigens encoded by non mutated genes (tissue-specific TAA or cancer-testis antigens)

Neoantigens resulting from nonsynonymous somatic mutations

**Vitiligo is an independent favourable prognostic factor in stage III and IV metastatic melanoma patients: results from a single-institution hospital-based observational cohort study**

*Annals of Oncology* 21: 400-414, 2010

P. Quaglini1, F. Mancin1, S. Osella-Abate1, N. Cappello1, M. Ortoncelli1, B. Salomone1, M. T. Pier1, P. Savoini1 & M. G. Berembi1

**Prognostic Significance of Autoimmunity during Treatment of Melanoma with Interferon**


Helen Gogas, M.D., John Ioannou, M.D., Uramia Dafni, S.C.D., Catherine Sutropoulos-Grotzes, M.D., Konstantina Frangia, M.D., Dimosthenis Tsiatou, M.D., Petros Papadopoulos, M.D., Aristidis Polyzos, M.D., Othonas Papadopoulos, M.D., Alexandros Stratigos, M.D., Christos Markopoulos, M.D., Dimitris Bafaloukos, M.D., Dimitrios Pectasides, M.D., George Fountzilas, M.D., and John M. Kirkwood, M.D.

**Integrated NY-ESO-1 antibody and CD8+ T-cell responses correlate with clinical benefit in advanced melanoma patients treated with ipilimumab**

*PNAS* October 4, 2011 vol. 108 no. 40 16725

Jiandong Yuan1, Matteo Adamov1, Brian A. Ginsberg1, Teresa S. Rasalan1, Enika Ritter1, Humiliation F. Gafford1, Yinian Hu2, Eveline Popple1, Stephanie L. Terek1, Deborah Kuhl1, Katherine S. Parpeaux1, Geri Ritter1, Mario Smol1, Ruth Helman1, Achim A. Jungbluth2, James P. Allison1, Lloyd J. Old1, Jed D. Wolchok1, and Sacha Grivennik1

**Functional T Cells Targeting NY-ESO-1 or Melan-A Are Predictive for Survival of Patients With Distant Melanoma Metastasis**


Adoptive transfer of MART1-specific CTLs generated by priming with peptide-pulsed dendritic cells in the presence of interleukin-21, followed by anti-CTLA-4 therapy.
In addition ..., we should not forget the relevance of response to antigens encoded by viral genomes.

HPV+ HNSCC

Table 1. PD-L1 Expression in head and neck cancers

<table>
<thead>
<tr>
<th></th>
<th>HPV+ (N = 20)</th>
<th>HPV- (N = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>PD-L1 expression</td>
<td>14/20 (70%)</td>
<td>6/20 (30%)</td>
</tr>
<tr>
<td>Membranous staining</td>
<td>14/14 (100%)</td>
<td>—</td>
</tr>
<tr>
<td>Tumor periphery</td>
<td>13/14 (93%)</td>
<td>—</td>
</tr>
<tr>
<td>Diffuse within tumor</td>
<td>1/14 (7%)</td>
<td>—</td>
</tr>
<tr>
<td>Presence of TILs</td>
<td>3/6 (50%)</td>
<td>2/5 (40%)</td>
</tr>
</tbody>
</table>

PD-1 in Advanced Merkel-Cell Carcinoma

Cancer Res; 73(6) March 15, 2013
DOI: 10.1056/NEJMoal603702

positive tumors. The response rate was 62% among patients with MCPyV-positive tumors (10 of 16 patients) and 44% among those with virus-negative tumors (4 of 9 patients). Drug-related grade 3 or 4 adverse events occurred in 15% of the patients.

Lancet Oncol. 2016
A high neoantigen load is not enough: relevance of the clonal architecture analysis

Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade

Cite as: N. McGranahan et al., Science 10.1126/science.aaf490 (2016).
Genes and genetic alterations associated with suppression of tumor immunity

Loss of PTEN Promotes Resistance to T Cell-Mediated Immunotherapy

B

Change from baseline after PD-1 therapy (%)

PTEN absent
PTEN present

CD8+ % at tumor site

PTEN
Absent
Present

P < 0.001

BRFA^V600E PTEN-null inducible melanoma model

C

Tumor size (mm^3)

Days after treatment

0

50

100

150

Control
GSK2636771
Anti–PD-1
GSK2636771+anti–PD-1

D

Percent survival

Days after treatment

0

20

40

Control
GSK2636771
Anti–PD-1
GSK2636771+anti–PD-1

The emerging role of master immunoregulatory genes

Melanoma-intrinsic β-catenin signalling prevents anti-tumour immunity

Stefan Spranger¹, Riyue Mao², and Thomas F. Gajewski³

9 July 2015 | Vol 523 | Nature | 231
COX-2 suppresses development of tumor immunity

Cyclooxygenase-Dependent Tumor Growth through Evasion of Immunity

Santiago Zelenay, Annemarthe G. van der Veen, Jan P. Böttcher, Kathryn J. Snelgrove, Neil Rogers, Sophie E. Acton, Probir Chakravarty, Maria Romina Girotti, Richard Marais, Sergio A. Quezada, Erik Sahai, and Caetano Reis e Sousa

Cell 162, 1257–1270, September 10, 2015

A

Graph showing tumor diameter and percentage rejection over time. The graph compares untreated, anti-PD-1, aspirin, and aspirin + anti-PD-1 treatment groups.
Targeting melanoma dedifferentiation

Perotti, Oncogene, 2016
Pre-existing immune response associated with response to immunotherapy

Responding patients

Non-responding patients

Tumor

Invasive margin

CD8$^+$ density (cells mm$^{-2}$)

Density (cells mm$^{-2}$)

PD-1$^+$

PD-L1$^+$

Response

Progression

Proximity of PD-1/PD-L1 expression

NATURE | VOL 515 | 27 NOVEMBER 2014
Relevance of immune-related signatures in pre-therapy lesions

### NSCLC, anti-PD-L1

<table>
<thead>
<tr>
<th></th>
<th>n (%)</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD-L1 median</td>
<td>112 (59)</td>
<td>1.10 (0.83-1.48)</td>
</tr>
<tr>
<td>PD-L1 &lt; median</td>
<td>112 (59)</td>
<td>0.94 (0.68-1.27)</td>
</tr>
<tr>
<td>PD-L2 median</td>
<td>112 (59)</td>
<td>1.01 (0.68-1.49)</td>
</tr>
<tr>
<td>PD-L2 &lt; median</td>
<td>112 (59)</td>
<td>0.95 (0.68-1.33)</td>
</tr>
<tr>
<td>PD-L1 median</td>
<td>112 (59)</td>
<td>0.81 (0.54-1.20)</td>
</tr>
<tr>
<td>PD-L1 &lt; median</td>
<td>112 (59)</td>
<td>0.67 (0.41-1.09)</td>
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<td>PD-L1 median</td>
<td>112 (59)</td>
<td>0.71 (0.52-0.95)</td>
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<tr>
<td>PD-L1 &lt; median</td>
<td>112 (59)</td>
<td>0.73 (0.54-0.99)</td>
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### Urothelial ca., anti-PD-L1

**PD-L1 on immune cells**

- **A** T-effector gene expression vs IC score
- **B** T-effector gene expression vs response
- **C** CD8 infiltration vs IC score
- **D** CD8 infiltration vs response

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www.thelancet.com Published online March 9, 2016 | http://dx.doi.org/10.1016/S0140-6736(16)00587-0
Multispectral immunohistochemical analysis of neoplastic sections. A multiparametric approach to the tumor immune contexture

Nghiem et al NEJM 2016

Orange: Merkel carcinoma cells
Yellow: CD8+ T cells
Red: CD68+ macrophages
White: PD-1
Green: PD-L1
Blue: nuclear DNA

Archival Biopsy Specimen of Primary Merkel-Cell Carcinoma
Post-treatment Biopsy Specimen of Subcutaneous Metastasis
High nonsynonymous mutational load
⇒ high, but “clonal” neoantigen load

Increased likelihood of spontaneous activation of anti-tumor response (= T cell infiltration in pre-therapy lesions)

The microenvironment will likely express PD-1 ligands on tumor and/or on immune cells

Increased likelihood of response to immunotherapy targeting the PD-1/PD-L1 axis by re-activation of functionally impaired tumor-specific T cells

Immunosuppressive mechanisms, fostered by gene programs in neoplastic cells, and immune escape processes, limit efficacy of spontaneous and therapy-rescued immunity

Translational approaches for improving immunotherapy: targeting the PD-1/PD-L1 axis as an example

Success vs failure in targeting the PD-1/PD-L1 axis: a working model

1. If:

A
1. Identify “hypermutant tumors”.
2. Whole exome sequencing of matched tumor/normal tissue.
3. Identification and validation of candidate neoantigens.
4. Clonal architecture analysis.

B
1. (Multispectral) immunohistochemical analysis.
2. TH1-, Teff- and IFN-related gene signatures.

C
1. Immunohistochemistry for PD-1 ligands.
2. Oncogene-dependent PD-L1 expression
3. PD-L1 genetic alterations.

D
1. Transcriptional signatures of resistance/response.
2. Expression of master immunoregulatory genes.
3. Genetic changes with immunoregulatory effects.
4. Immune contexture of the lesions for Tregs, MDSCs, TAM.
5. Analysis for main immune escape mechanisms.

On pre/post-therapy lesions

Predict response by
A + B + C
<table>
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<tr>
<td>22 major cancer types</td>
<td>Copy number alterations. Tumors with PD-L1 gains harbored significantly higher mutational load compared to non-amplified cases.</td>
<td>Budczies, Genes, Chrom. Cancer, 2016</td>
</tr>
<tr>
<td>Several tumors, including adult T-cell leukaemia/lymphoma, diffuse large B-cell lymphoma, and stomach adenocarcinoma.</td>
<td>Structural variations (SVs) commonly disrupting the 3’ region of the PD-L1 gene leading to a marked elevation of aberrant PD-L1 transcripts.</td>
<td>Kataoka, Nature 2016</td>
</tr>
<tr>
<td>Squamous Cell Carcinomas of the Cervix and Vulva.</td>
<td>Co-gain or co-amplification of CD274 and PDCD1LG2 in 32/48 cervical SCCs and 10/23 vulvar SCCs.</td>
<td>Howitt, JAMA Oncol 2016</td>
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