Melanoma Vaccines: New targets and combination therapy

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1. Melanoma and the anti-tumor immune responses: a dynamic system

**Activation**
- Cytotoxic CD8+ Lymphocytes
- CD4+ T helper lymphocytes
- B lymphocytes (antibodies)

**Suppression**
- T regulatory (Treg) cells
- Dysfunctional myeloid cells (MDSC, iDC...)
- T and B “exhausted” or anergic lymphocytes

(inflammation /chronic immunostimulation)
Examples in the clinic
Density and distribution of TIL is a positive independent prognostic factor in primary melanoma

Azimi F et al. JCO 2012;30:2678-2683

Histopathologic photomicrographs of various tumor-infiltrating lymphocyte (TIL) grades. (A) TIL grade 0 infiltrate: lymphocytes at the base of the melanoma, but they do not infiltrate the tumor. Such lymphocytes are not designated as TILs. (B) TIL grade 1 infiltrate: mild infiltrate of lymphocytes focally interspersed among the melanoma cells. (C) TIL grade 2 infiltrate: dense multifocal infiltrate of lymphocytes interspersed among the melanoma cells. (D) TIL grade 3 infiltrate: dense infiltrate of lymphocytes illustrated in the figure was present diffusely throughout the tumor.
Accumulation of immunoregulatory cells in the blood is associated with the stage of disease (Rivoltini L, Parmiani G)

**Myeloid suppressor cells**
(CD11b+CD14+HLA-DRneg)

<table>
<thead>
<tr>
<th>Stage (HD - IV)</th>
<th>% Positive Cells</th>
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</thead>
<tbody>
<tr>
<td>HD</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td></td>
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<tr>
<td>III</td>
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<td>IV</td>
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**T regulatory cells**
(CD4+CD25highFoxp3+)

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Melanoma patients

Prostate Carcinoma patients
## Immune escape mechanisms: A long list

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Immune system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lack of antigens or down-regulation of HLA</td>
<td>Immune anergy or ignorance</td>
</tr>
<tr>
<td>Dysfunction of antigen presentation</td>
<td>Lack of tissue homing molecules</td>
</tr>
<tr>
<td>Release of immune-suppressive factors (IL-10, TGFβ, VEGF, PGE2)</td>
<td>T-cell receptor dysfunction</td>
</tr>
<tr>
<td>Tumour counterattack (Fas/FasL)</td>
<td>Inactivation of T-cells within the tumor environment</td>
</tr>
<tr>
<td>IDO, SPARC, osteopontin</td>
<td><strong>T-regulatory cells, NKcells</strong></td>
</tr>
<tr>
<td>Expression of FoxP3, CTLA4</td>
<td>MDSC, iNKT</td>
</tr>
<tr>
<td>Tumor ER stress</td>
<td><strong>Epithelial/mesenchimal transition</strong></td>
</tr>
<tr>
<td>Tie2+ Monocytes</td>
<td>Galectin-1, 3</td>
</tr>
</tbody>
</table>
How to down-regulate Tregs in melanoma patients?

- Anti-CD25 (e.g. Daclizumab) to reprogram T regs; anti–CTLA4 antibody
- ONTAK (CD25/Diftitox)
- Cyclophosphamide (?) * and other drugs (e.g. paclitaxel)
- Anti-IDO drugs (1-methyl-triptophan)
- Vaccination

*(Camisaschi et al., 2013; Cancer Immunol Immunother)
How to down-regulate MDSCs in melanoma patients?

- Targeting STAT3 in the myeloid compartment
- ATRA (All Transretinoic Acids)
- TGFβ blockade
- NKT cell activation
- IFN-α/CTLA4
- Chemo (Paclitaxel-carboplatin)
- PD-1, CSF1R (mouse) (Zhu et al., Cancer Res 2014)
- Bone Morphogenic Protein 4

- Vaccination
1. Conclusions

- We need to know which suppressive factor(s) are activated in the tumor microenvironment and/or in the blood according to site of tumor growth, of its histology and stage of disease.

- A detailed map of suppressive factors?
Use of cell- and peptide-based vaccines involving self differentiation or cancer testis MAAs *available at that time* and molecularly characterized

**Examples:** MAGE-3 or MelanA/MART1 peptides in metastatic melanoma (*T. Boon, S.A. Rosenberg*)
The issue of shared self MAAs used in early trials

- Normal subjects and melanoma patients show some form of tolerance to “self” MAAs (immune ignorance, peripheral or central tolerance, low frequency of T cells, etc.).

- Tolerance needs to be broken in order to induce a T cell immune response against “self” MAAs.

- Thus, these MAAs are considered to be “weak antigens”
The impact of vaccination modalities in metastatic melanoma

- Using MAGE-3.A1:
  - DC better than peptides better than ALVAC minigene

- 24 out of 99 pts showed evidence of tumor regression but only 6 had CR or PR

- (Connerotte T, Coulié PG et al., 2008; Cancer Res)
Results of first generation (1998-2008) of self peptide-based vaccination of metastatic melanoma patients (Phase I-II studies)

<table>
<thead>
<tr>
<th>Type of MAA peptide</th>
<th>N. of patients</th>
<th>Clinical response (CR+PR) (mean %)</th>
<th>Immune response (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lineage related (e.g. Melan-A)</td>
<td>159</td>
<td>14</td>
<td>20-65</td>
</tr>
<tr>
<td>Cancer/Testis (e.g. MAGE)</td>
<td>92</td>
<td>17</td>
<td>30-50</td>
</tr>
<tr>
<td>DC peptides</td>
<td>124</td>
<td>16</td>
<td>56</td>
</tr>
<tr>
<td>DC lysates</td>
<td>106</td>
<td>18</td>
<td>46</td>
</tr>
</tbody>
</table>

Slingluff et al. (2008) reported 100% immune response and survival benefit in melanoma patients vaccinated with 12 peptides.
Vaccination of metastatic melanoma patients with the MAGE-3.A1 peptide

From M. Marchand et al., Int. J. Cancer 1999
Outcome of self peptide-based early melanoma vaccines

- Clinical responses (both CR and PR) and immune responses were seen but in a minority of patients
- Such responses were late appearing and long lasting but the lack of biomarkers of the response prevented a routine clinical use of these vaccines.

**Pessimism was high in our scientific community**
3. Thanks to the progress of knowledge in immunology and tumor biology, however, subsequent clinical studies provided better results for cancer vaccines

- Examples:
## Evidence for clinical activity of cancer vaccines

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Tumor</th>
<th>Phase</th>
<th>N. patients</th>
<th>Stage</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAGE-3</td>
<td>NSCLC</td>
<td>II R</td>
<td>182</td>
<td>IB-II</td>
<td>Trend</td>
</tr>
<tr>
<td>MUC-1 L-BLP25</td>
<td>NSCLC</td>
<td>II</td>
<td>34</td>
<td>IIIB</td>
<td>P=0.016</td>
</tr>
<tr>
<td>IL2+/-gp100</td>
<td>Melanoma</td>
<td>III</td>
<td>185</td>
<td>IV</td>
<td>P&lt;0.02</td>
</tr>
<tr>
<td><strong>Provenge DC</strong></td>
<td>Prostate Cancer</td>
<td>III</td>
<td>341/171</td>
<td>HR</td>
<td>P&lt;0.03</td>
</tr>
<tr>
<td>E75/Her2/neu</td>
<td>Breast cancer</td>
<td>IIR</td>
<td>101/75</td>
<td>IV</td>
<td>P&lt;0.04</td>
</tr>
<tr>
<td>DC/NHL</td>
<td>NHL</td>
<td>II</td>
<td>177</td>
<td>III</td>
<td>P=0.047</td>
</tr>
<tr>
<td>BiovaxID GM-CSF</td>
<td>Follicular Lymphoma</td>
<td>III</td>
<td>76/41</td>
<td>II</td>
<td>P&lt;0.04</td>
</tr>
<tr>
<td>IMA901+Cyclo</td>
<td>RCC</td>
<td>IIR</td>
<td>96</td>
<td>iii</td>
<td>P&lt;0.023</td>
</tr>
</tbody>
</table>
4. New melanoma vaccines (2010-2014)

- A systemic review and meta-analysis
  - (Chi and Dudek, 2011; Melanoma Res)

- Different vaccine strategies
- 56 studies, 4375 patients
- Overall disease control 25%
- Tumor-specific immune response associated with prolonged OS compared with lack of response
- No evidence that anti-melanoma vax provides better OS as compared to other treatments
4. New melanoma vaccines (2010-2014)

- Phase III study of gp100 peptide vaccine and IL-2 in melanoma (Schwartzentruber et al., NEJM 2011)

- A phase III study comparing ipilimumab or gp100 vaccine and the combination in patients with stage III or IV melanoma (O’Day et al., 2010)

- Dendritic cells-based vaccination in metastatic melanoma patients: phase II clinical trial. (Oshita et al., 2012, Oncol Rep) (24 pts, gp100, tyrosinase, MAGE, MART1, KLH) (75% immune response; significant OS prolongation)

- *Melanoma microenvironment and T cells* (Gajewsky T et al., 2008)
New melanoma vaccines (2010-2014) (cont.)

- A randomized phase II trial of multiepitope vaccination with melanoma peptides for cytotoxic T cells and helper T cells for patients with metastatic melanoma. *(Slingluff et al, Clin Cancer Res, 2013) (association of survival and immune response to 6MHP)*

- Phase II trial of multipepitopes DC-based melanoma vaccine. *(gp100, MAGE-A1, -A2, -A3, MART-1, Tyrosinase, KLH) in metastatic melanoma; survival was 21.9 mos for CTL responders and 8.1 mos for non-responders and was longer for immunized vs. non-immunized pts (13.6 vs. 7.3 mos)* *(Oshita et al., 2012)*
A phase III study of the MAGE-A3 vaccine in adjuvant setting

- DERMA trial: Melanoma pts (N=1349) resected of stage IIIB-C did not meet a co-primary point (prolongation of DFS) but a gene signature was obtained that might predict response to this MAGE-A3 immunotherapy
- Adjuvant QS21
- (Brichard V et al., 2014; Clinicaltrials. Gov 2014)
The issue of MAAs used as vaccines in melanoma trials
Melanoma antigens recognized by T cells

Tissue antigens (Mart-1, Tyrosinase, Gp100)

CT and Mutated antigens (MAGE, NY-ESO1, neo)

Neoplastic transformation-associated antigens (BRAF, survivin, telomerase...)

4.1 Melanoma antigens recognized by T cells
5. A reverse immunology approach to go from mutated cancer genes to neo-peptide antigens and specific T cell immunity.

Massive sequencing of CAN genes -> Somatically mutated TAAs

Bioinformatic epitope prediction (peptides)

Investigation of autologous T cell responses *in vitro/ex vivo*

Clinical trial vaccination

(Snyder et al., NEJM, 2014)
The antigenomics

- Given the great progress of genome sequencing (NGS) technology during the last few years, the possibility of identifying new somatic mutation-derived tumor-specific epitopes/antigens for each patient provides an opportunity to construct individualized therapeutic melanoma vaccines.

- Mainly in tumors with strong response to CTLA4 blockade. (Snyder et al., 2014; NEJM)
6 Crucial issues for melanoma vaccines

- *Self shared* differentiation vs. mutated antigens
- Monospecific vs. poly-specific vaccines
- Combination of vaccines with chemotherapy or with new immunotherapeutic approaches (Immune check-point inhibitors like anti-CTLA4, anti-PD1, -PDL1)
- Combination with molecular targeting agents (e.g. Vemurafenib) that can modulate the immune system
7. The new scenario in the immunotherapy of melanoma

- The therapeutic role of immune check-point inhibitors has changed the strategies of metastatic melanoma treatment.

- Several presentations during the meeting will critically discuss this issue
The new scenario in the immunotherapy of melanoma (cont.)

- However, studies of the mechanism(s) of the activity of immune check-points blockade are necessary.
- Which effector cells are involved? which melanoma antigens are targeted? Which biomarkers?
- Answer these questions is crucial for improving the clinical outcome of metastatic melanoma.

(see Van Roij et al., JCO, 2013; Snyder et al., NEJM, 2014; Kvistborg et al. Sci Transl Med 2014; Simeone et al., Cancer Immunol Immunother, 2014)
8. Monitoring of immunological correlates of clinical outcome in Ipilimumab (Expanded Access Program)-treated patients

- Metastatic melanoma patients were treated with Ipilimumab: 3 or 10 mg/Kg (every 3 weeks x 4 doses).

approved by regulatory Italian authorities

(Maccalli C, Di Giacomo AM, Parmiani G, Maio M. in preparation)
Conclusions

- Ipi treatment helps in triggering a possible clinically effective MAA-specific response of melanoma patients targeting **self/shared** and/or **mutated neoantigens**
Melanoma vaccines: still promising?

- Yes, because: 
  a) clinical responses were obtained even in the early studies though with a low frequency; 
  b) more recently, a phase III trial (gp100+IL-2) showed benefit for melanoma patients; 
  c) the combination with immune checkpoint inhibitors and target agents is promising; 
  d) new immunogenic and specific mutated MAA will become available.

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