Intratumoral IL-12 Therapy in HNSCC: Can in-situ vaccination solve the problem of anti-PD-1 non-response?

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1. Anti-PD-1 responder/non-responder phenotype = great unmet medical need
2. In-situ vaccination as a potential solution for poorly immunogenic tumors
3. Intratumoral electroporation-mediated gene therapy with IL-12 as an example of in situ vaccination
4. Clinical data in melanoma, MCC and preliminary data in SCCHN → combination with PD1 blockade
PD-1 Non-Response: The Scope of the Unmet Medical Need

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Anti-PD1/PDL1 mAb Non-Response</th>
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</thead>
<tbody>
<tr>
<td>Melanoma</td>
<td>~ 60 – 80%</td>
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<tr>
<td>Triple Negative Breast (TNBC)</td>
<td>~ 70% – 80% &lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Renal Cell Carcinoma (RCC)</td>
<td>~ 71%</td>
</tr>
<tr>
<td>Lung Carcinoma (NSCLC)</td>
<td>~ 79 – 83%</td>
</tr>
<tr>
<td>Head and Neck (H&amp;N)</td>
<td>~ 80%&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bladder</td>
<td>~ 84%&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Anti-PD-1 non-responders constitute the majority of patients, even in “immune therapy tractable tumors” like melanoma and RCC

<sup>1</sup> PD-L1 selected patients; 18.5% (5/27) ORR using Merck 22C3 assay and pembrolizumab; 33% (3/9) using Genentech’s PCD4989g assay and MPL3280A

<sup>2</sup> Patients were preselected by Merck PD-L1 IHC assay

<sup>3</sup> 11% in PD-L1 (Roche) negative; 43% in PD-L1 + population
‘Adaptive Immune Resistance’ Predicts Response to Anti-PD-1 in Melanoma

Low TIL/poorly immunogenic tumors represent the major non-responder phenotype

HPV+ Squamous Cell Carcinoma of the Tonsil with PD-1/PD-L1 Suggestive of ‘Adaptive Immune Resistance’

Model of TIL-Induced PD-L1-Mediated "Adaptive Immune Resistance"
Frequency of ‘Partially Exhausted’ PD-1+CTLA-4+ CD8+ TIL Correlates with Response to Pembrolizumab in Melanoma

Expression of PD-1+ CTLA-4+ on CD8+ TILs differentiates anti-PD1 mAb responders from non-responders

Intracellular cytokine staining after ex vivo stimulation
Fraction of PD-1+ CTLA-4+ CD8+ ‘Partially Exhausted’ TILs Predicts Response to Pembrolizumab in Melanoma

<20% ➔ 12/12 Non-responders

20%
SCCHN Gene Expression Signature Analyses in Pre-Treatment Samples

• **IFNγ 6-gene signature**
  - **CXCL9, CXCL10, IDO1, IFNG, HLA-DRA, STAT1**
  - Discovered in melanoma\(^1\)
  - Confirmed predictive of response in gastric cancer and initial HNSCC cohort\(^2\)

• **FFPE-extracted RNA levels counted using Nanostring nCounter Analysis System**\(^*\)

• **Composite score calculated by averaging normalized\(^†\) values for each gene**

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\(^*\)Nanostring Technologies, Seattle, WA. \(^†\)Normalization based on 11 house-keeping genes.

Association of IFNγ Signature and PFS in SCCHN Patients Treated with Pembrolizumab

Separation into three groups:

1. **IR-group: Inflamed – Responders**
   - Gamma-IFN Inflamed
   - Benefitting from anti-PD1 therapy

2. **INR-group: Inflamed – NonResponders**
   - Gamma-IFN Inflamed
   - Not Benefitting from anti-PD1 therapy
   - Given biologic signal - Can these patients be converted into responders e.g. via combinations, vaccine etc.

3. **NI-group: Non-Inflamed**
   - Very high negative predictive value (95%)
   - Clinically potentially useful: Identify patients who should NOT receive PD-1 therapy
   - Unclear whether non-inflamed phenotype can be converted into inflamed phenotype

No significant difference in response between HPV\textsuperscript{neg} and HPV\textsuperscript{pos} patients! 

Adapted from: Tanguy Seiwert, ASCO 2015 (Keynote 12, cohort B)
Anti-PD-1 Non-Responders: Rationale for Therapies Directed at Enhancing Immunogenicity

Adapted from Pardoll-DM, Nature Reviews Cancer 252 V12, April 2012

The Treatment of Malignant Tumors by Bacterial Toxins as Developed by the Late William B. Coley, M.D., Reviewed in the Light of Modern Research

Helen Coley Nauts, Walker E. Swift, M.D., and Bradley L. Coley, M.D.

(Received for publication October 15, 1945)
The Intratumoral “In-Situ Vaccine” Paradigm

- Induction of adaptive immunity requires vaccination with Immunogenic antigen + appropriate adjuvant “Danger Signal”
- Conversion of immunosuppressive APC/microenvironment to immunogenic APC/context
- Central role of IL-12 and IFNg in specifying Th1-biased cell-mediated immune response, effective against intracellular pathogens and cancer
- In situ vaccination → immunogenic cell death, exposing the immune system to all tumor-associated antigens (TAAs), including neoantigens
- No requirement for a priori selection of vaccine antigen(s)
What Makes a “Good” Tumor Antigen?

Tumor Associated Antigens (TAAs):

- **Cancer-testis (CT) Antigens:** MAGE, NY-ESO-1, SSX, […] ➔ Not normally expressed in somatic tissue (restricted to testes, sometimes, ovary or trophoblast); ectopically expressed by some tumors.

- **Differentiation Antigens:** GP-100, Melan-A/Mart-1, Tyrosinase, PSA, CEA, […] ➔ Normal expression restricted to specific cell lineages.

- **Overexpressed Antigens:** HER-2, p53, CSPG4, survivin, […] ➔ Ubiquitous, but low-level normal expression; increased in some tumors.

- **Mutation-derived neoantigens**

- **Virus-encoded “neo”-antigens:** HPV, MCPyV, EBV, etc.
Intratumoral Electroporation-mediated Gene Therapy with Plasmid IL-12 (IT-pIL12-EP)

- 1500V/CM, using 6 pulses of 100 microsecond duration with a six needle array
- Plasmid encoding IL-12 (CMV-p35-IRES-p40)
- Clinical electroporation parameters were empirically determined based on intratumoral B16F10 tumors, treated 3X; 70% of “cured” mice were subsequently resistant to re-challenged with B16F10 (Lucas-ML, Mol Ther. 2002 Jun;5(6):668-75)
- B16.F10 is a poorly-immunogenic, low-TIL, PD-1 non-responsive tumor model
IT-pIL12-EP Results in Local Necrosis, TIL Infiltration and Complete Regression of Treated B16F10 Tumors

H&E (low power)  H&E (high power)

3 Treatments:
Day 1, 5, 8
Complete response in >95% of treated B16.F10 tumors
IT-pIL12-EP Leads to Systemic Anti-tumor Responses in a Subset of Untreated Contralateral Tumors

**Pathologic Assessment:**
- Day 11 (5/5 mice with no significant pathologic changes);
- Day 18 (3/5 mice with TIL infiltrate and histologic changes c/w regression);
- Day 22 (1/5 mice with pathologic complete response; 4/5 with no significant pathologic changes)
IT-pIL12-EP Increases TILs and Leads to ‘Adaptive Immune Resistance’ in Untreated Contralateral B16.F10 Tumors

![Graph showing the average fold over baseline (NanoString™) for CD8, PD-1, and PD-L1 on Day 0, Day 11, and Day 18.](image1)

![Graph showing the average fold over baseline (NanoString™) for various proteins on Day 0, Day 11, and Day 18.](image2)
IT-pIL12-EP Inhibition of Contralateral Tumor Growth is Associated with Systemic Expansion of TAA-specific CD8s

Note: Single EP treatment with IL-12 at 350 V/CM (10 millisecond x 8 pulses); 6 mice per time point harvested for flow cytometric analysis

Spleen

Untreated

CD44+ SIINFEKL+ 0.13

CD44 SIINFEKL+ 8.23

Untreated IT-pIL12-EP

% SIINFEKL tetramer

0 2 4 6 8 10

0 1 2 3 4 5 6 7 8 9 10 11
IT-pIL12-EP leads to necrosis and generation of TILs in treated tumors: Phase 1 study in metastatic melanoma.

- >20% necrosis present in 76% of treated lesions
- No measurable systemic IL-12 or IFNγ (ELISA)
Phase 1 in Advanced Melanoma: Complete Responses and Distant Lesion Regressions after One Cycle of Local Treatment

Only the numbered lesions on the chest were injected and electroporated.

No lesions on the back were injected or electroporated.

Residual pigmentation in macrophages.

Seborrheic Keratosis (non-cancerous pigmentation).

Daud-A, et al. JCO 2008
Phase 2: IT-pIL12-EP Demonstrates Anti-tumor Activity in Advanced Melanoma

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients (N = 30)</th>
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<tbody>
<tr>
<td>Gender (male)</td>
<td>16 (53.3%)</td>
</tr>
<tr>
<td>Age</td>
<td>Median 67 yr (44-88 yr)</td>
</tr>
<tr>
<td>AJCC Stage</td>
<td></td>
</tr>
<tr>
<td>IIIIB/C</td>
<td>19 (63.3%)</td>
</tr>
<tr>
<td>IVA</td>
<td>8 (26.7%)</td>
</tr>
<tr>
<td>IVB</td>
<td>2 (6.7%)</td>
</tr>
<tr>
<td>IVC</td>
<td>1 (3.3%)</td>
</tr>
<tr>
<td>Prior Immunotherapy* (Yes)</td>
<td>16/29** (55.2%)</td>
</tr>
</tbody>
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**Modified Skin RECIST**

- Measurable Disease: >0.3 CM in the longest dimension for lesions measured by clinical exam.
- New Lesions: New lesions on skin permitted if sum total <30% from nadir.

<table>
<thead>
<tr>
<th>Response Category*</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete Response (CR)</td>
<td>4 (14%)</td>
</tr>
<tr>
<td>Partial Response (PR)</td>
<td>5 (17%)</td>
</tr>
<tr>
<td><strong>Stable Disease (SD)</strong></td>
<td>5 (17%)</td>
</tr>
<tr>
<td>Progressive Disease (PD)</td>
<td>15 (52%)</td>
</tr>
<tr>
<td>Overall Response Rate (CR + PR)</td>
<td>9 (31%)</td>
</tr>
<tr>
<td>Disease Control Rate (CR + PR + SD)</td>
<td>14 (48%)</td>
</tr>
</tbody>
</table>

*by Modified “Skin” RECIST
**SD required to last for at least 90 days

Evaluable for Objective Response, N=29

**Abscopal Response Rate:**
13/26 (50%) of evaluable patients had objective regression (>30% decrease in longest diameter) in at least 1 anatomically-distinct non-treated lesion
IT-pIL12-EP Drives Tumor-antigen Specific CD8 TILs in a Patient with Metastatic Merkel Cell Carcinoma

- Enrichment of MCPyV-specific CD8+ T-cells in TILs of treated and distant tumors
- Increased neo-antigen CD8 population correlated with timing of deep (75% reduction in target lesions) partial response (PR)
IT-pIL12-EP May Improve Subsequent Response to PD1 Blockade: Retrospective Analysis of 14 Advanced Melanoma Patients

<table>
<thead>
<tr>
<th>Best Overall Response</th>
<th>Without Intervening Therapy N=8</th>
<th>With Intervening Therapy N=6</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>4 (50%)</td>
<td>1 (17%)</td>
</tr>
<tr>
<td>PR</td>
<td>2 (25%)</td>
<td>2 (33%)</td>
</tr>
<tr>
<td>SD</td>
<td>1 (12.5%)</td>
<td>1 (17%)</td>
</tr>
<tr>
<td>PD</td>
<td>1 (12.5%)</td>
<td>2 (33%)</td>
</tr>
</tbody>
</table>
Clinical Response Profile in a Melanoma Patient who Progressed on IT-pIL12-EP and Responded to Subsequent Pembrolizumab

- Patient only received one cycle of IT-pIL12-EP with PD (clinical progression) at D90
- Complete response of treated lesion at Day 90
- 49% CTLA4+/PD1+ in non-IT-pIL12-EP treated tumor observed prior to initiating Pembrolizumab (CD45+CD3+CD8+ gate)
- Ongoing complete response (1 year+) to pembrolizumab despite overall non-response to IT-pIL12-EP
IT-pIL12-EP Leads to Increased CD8s in Treated Tumor and TAA-reactive T Cells in Circulation

CD8 IHC on IT-pIL12-EP treated tumor:

~ 2.5X in total CD8 TILs after IT-pIL12-EP
IT-pIL12-EP Can Increase the Inflammatory/Suppressive Balance in the Tumor Microenvironment

**Multispectral Fluorescent IHC**

<table>
<thead>
<tr>
<th>Pre-IT-pIL12-EP</th>
<th>Immune Ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimal change in lesion size from baseline to Day 39</td>
<td>CD8:PD-L1 = 0.13</td>
</tr>
<tr>
<td></td>
<td>CD8:FoxP3 = 1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Day 11</th>
<th>Immune Ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CD8:PD-L1 = 0.014</td>
</tr>
<tr>
<td></td>
<td>CD8:FoxP3 = 13</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Day 39</th>
<th>Immune Ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CD8:PD-L1 = 0.32</td>
</tr>
<tr>
<td></td>
<td>CD8:FoxP3 = 41</td>
</tr>
</tbody>
</table>

41X increase in CD8:FoxP3 ratio
2.5X increase in CD8:PD-L1
Rationale for Combination of IT-pIL12-EP and Anti-PD-1 Blockade

Multicenter Phase II Trial of Intratumoral pIL-12 Electroporation in SCCHN: Preliminary Findings

Indication:
- Treatment-refractory metastatic and unresectable squamous cell carcinoma of the head and neck (HNSCC): NCT02345330

Treatment:
- Each 6-week cycle of pIL-12 EP consists of three treatment days (e.g. 1, 5, 8).
- Patients may receive up to nine cycles of treatment.
- 4 patients treated to date: 3 at UCSF (Algazzi); 1 U Chicago (Sewiert)
- Study open, not currently recruiting

No treatment-related Severe Adverse Events (SAEs) reported

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Institution</th>
<th>Age</th>
<th>Sex</th>
<th>Race</th>
</tr>
</thead>
<tbody>
<tr>
<td>001-001</td>
<td>UCSF</td>
<td>47</td>
<td>Male</td>
<td>White</td>
</tr>
<tr>
<td>001-002</td>
<td>UCSF</td>
<td>58</td>
<td>Female</td>
<td>Other</td>
</tr>
<tr>
<td>001-003</td>
<td>UCSF</td>
<td>53</td>
<td>Male</td>
<td>White</td>
</tr>
<tr>
<td>002-001</td>
<td>U. Chicago</td>
<td>53</td>
<td>Male</td>
<td>White</td>
</tr>
</tbody>
</table>
IT-pIL12-EP in SCCHN Can Increase IL-12 and IFN\(\gamma\)-Associated Gene Signature
Only 2 cases were evaluable by Multi-Spectral Analysis.

On each slide, 3 ROIs (regions of interest) are captured for spectral-deconvolution-based analysis.

ROIs were selected for tumor/stroma interface with high immune cell (CD3+CD8) infiltrates “hot spots”.
Multi-spectral Imaging-based “Hot Spot” Analysis Suggests that IT-pIL12-EP Increases CD8 TILs

MSI DECONVOLUTION

- PD-L1 – Cy5
- CD8 – Cy3
- FoxP3 – FITC
- CD3 – Alexa 594
- CD163 (Mcp-hg) – Cy5.5
- Cytokeratin– Coumarin
- DAPI

Number of CD8/mm²

![Graph showing number of CD8 TILs](image)
Radiologic Response and Increase in CD8+ TILs in a SCCHN Patient Treated with 3 cycles of IT-pIL12-EP and Subsequent Pembrolizumab

Cells/mm²
CD3: 173
CD8: 299
FOXP3: 57
PD-L1: 135

CD8/FOXP3 = 0.9X
CD8/PD-L1 = 4.9X

Cells/mm²
CD3: 1666
CD8: 2350
FOXP3: 496
PD-L1: 215

CD8 = 7.9X
Conclusions

**Intratumoral IL-12 therapy in HNSCC: Can in-situ vaccination solve the problem of anti-PD-1 non-response?**

1. **PD-1 nonresponse is a great unmet medical need in oncology**
   - Need to find new therapies to increase immunogenicity and increase TILs
   - In-situ vaccination can “reboot” the immune system and do this!

2. **Intratumoral electroporation-mediated gene therapy with IL-12**
   - Functions as an in-situ vaccination → TAA-specific TILs and abscopal effects
   - Monotherapy ORRs in melanoma and MCC
   - Tantalizing preliminary findings in 4 SCCHN patients suggests that therapeutic IL-12 biology is similar and promising in other solid tumors
   - Strong rationale for combination with anti-PD1/PDL1 agents

3. **Intratumoral therapies in general & in-situ vaccines in particular will play a key role in combinatorial immuno-oncology regimens in the future**
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