Towards Optimized DC-based Cancer Therapies:
Targeting the Induction and Effector Phase of Cancer Immunity

Pawel Kalinski, MD, PhD
Surgery, ImmunoTransplantation Center
University of Pittsburgh Cancer Institute

No Financial Disclosures
aDC1s, one of the DC types discussed in this presentation, is a subject to issued and pending patent applications
Preventive versus Therapeutic “Vaccination”

Prevention
- T naive
  - Signal 1
  - Signal 2
  - +/- Signal 3
- T memory
  - Signal 1
  - Signal 2
  - Signal 3
- T effector
  - GrB+,
  - CCR5+
  - CXCR3+
- IL-12
- IFNα
- TLR-Ls
- IFNs
- TNF
- Chemokines
  - CCR1,2,5
  - CXCL9,10,11
- Tissue

Cancer Treatment
- T naive
  - Signal 1
  - Signal 2
  - Signal 3
- Signal 1
  - Signal 2
  - Signal 3
- Treg
- Tumor
- MDSC
- IL-10
- PGE2
- IDO
- Chemokines
  - CCR1,2,5
  - CXCL9,10,11
- IL-12
- (?)
Targeting the Induction and Effector Phases of Immunity in Cancer Immunotherapy

Suppressive environment of cancers a) promotes the development of inappropriate forms of immune response, and b) excludes the desirable killer cells and suppresses their antitumor function by preferential attraction and activation of suppressive cells

1. **Instruct** dendritic cells (DC) to induce killer-type immune cells (type-1 immunity; CTLs, TH1 and NK cells)

2. **Condition** cancer microenvironment to become permissive to immune attack
   a. Promote the attraction of CTLs, Th1 and NK cells
   b. Counteract the accumulation & function of Tregs and MDSCs
\(\alpha\text{DC1}:\) High-IL-12-producing Mature DCs Induced by Mediators of Anti-Viral Immunity

TNF\(\alpha\) / IL1\(\beta\)
IL6 / PGE2
("Standard" DC)

TNF\(\alpha\) / IL1\(\beta\)
pl:C / IFN\(\alpha\) / IFN\(\gamma\)
(\(\alpha\text{DC1/Polarized DC}\))

- IL-12 producing DC needed for **activation of NK cells** (Gustafsson K., Canc. Res. 2008)
- IL-12 producing DC needed for **Th1 cell induction** (Kalinski P., JI 1997, Wesa A. J.It 2007)
- DC-produced IL-12 **predicts prolonged TTP in cancer patients** (Okada H., JCO 2011)

Polarized DC1s Induce GrB^{high} Effector CTLs

**Conditions of DC maturation**

- **Polarized DCs**
  - TNFα+PGE₂
  - LPS+PGE₂
  - TNFα+IL1β+IL6+PGE₂
    ("standard" DCs)
  - TNFα+IFNγ
  - LPS+IFNγ
  - TNFα+IL1β+IFNγ+IFNα+pl: C
    (αDC1s)

- **Non-Polarized DCs**

**T cell expansion (x10^5)**

**T cell expression of Granzyme B**
(fold increase MFI)

*Watchmaker, Berk et al. 2010; J. Immunol. 184: 591-597*

*Berk, Watchmaker, et al. in preparation*
αDC1 Vaccines: Preferential Interaction with Naïve, Effector and Memory Cells, but not Tregs

Chemotaxis of blood-isolated CD4+ T cells

(-) sDC supers αDC1 supers

FOXP3 expression in migrated T cells

CD4 cells migrated to:

Total CD4

Medium

αDC1

sDC

*-fullyCCL22-dependent
αDC1-induced CTLs Express High Levels of CCR5 & CXCR3

Priming of naïve CD8+ T Cells (d6)

CTL activity (% killing)

0 10 20 30
1:1 3:1 10:1 30:1

Primed by αDC1
Primed by sDC

Expansion
CTL function & FACS

IVS of total CD8+ T Cells (d14)

Sensitized by αDC1
Sensitized by sDC

Gated on tumor-specific T cells

Berk, Watchmaker, et al. in preparation
**UPCI 05-115 (H. Okada): Phase I/II Study in Recurrent High-Grade Glioma (22 patients)**

- i.n. injections of aDC1s (1 or 3 x 10^7 per dose) loaded with IL-13Ra2345-353:1A9V, gp100209-217:2M, EphA2883-891 and YKL-40201-210
- i.m. injections of poly-ICLC (20 mcg/kg; Twice/week)

---

**Vaccines (Q2W)**
- Poly-ICLC (twice/week)

**Booster Vaccine Phase 1 (Q4W)**
- Poly-ICLC (twice/week)

**Booster Vaccines Phase 2 (Q3M)**
- Poly-ICLC (once/week)

<table>
<thead>
<tr>
<th>Weeks</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>7</th>
<th>13</th>
<th>17</th>
<th>21</th>
<th>25</th>
<th>29</th>
<th>Up to 3 Years</th>
</tr>
</thead>
</table>

Recurrent High Grade Glioma (WHO III or IV) HLA-A2+
αDC1-produced IL-12 Predicts Delayed Time to Progression in αDC1-Vaccinated Patients

<table>
<thead>
<tr>
<th>Patient #</th>
<th>TTP (Months)</th>
<th>IL-12 (pg/10^5 cells/24h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>&lt;2</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>4</td>
<td>&lt;2</td>
<td>&lt;10</td>
</tr>
<tr>
<td>5</td>
<td>&lt;2</td>
<td>26</td>
</tr>
<tr>
<td>6</td>
<td>&gt;30</td>
<td>27</td>
</tr>
<tr>
<td>7</td>
<td>25</td>
<td>919</td>
</tr>
<tr>
<td>8</td>
<td>16</td>
<td>480</td>
</tr>
<tr>
<td>9</td>
<td>&lt;2</td>
<td>24</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>&lt;10</td>
</tr>
<tr>
<td>11</td>
<td>&lt;2</td>
<td>38</td>
</tr>
<tr>
<td>12</td>
<td>13</td>
<td>111</td>
</tr>
<tr>
<td>13</td>
<td>&lt;2</td>
<td>151</td>
</tr>
<tr>
<td>14</td>
<td>18</td>
<td>35</td>
</tr>
<tr>
<td>15</td>
<td>4</td>
<td>35</td>
</tr>
<tr>
<td>16</td>
<td>&gt;14</td>
<td>985</td>
</tr>
<tr>
<td>17</td>
<td>&lt;2</td>
<td>123</td>
</tr>
<tr>
<td>18</td>
<td>&lt;2</td>
<td>125</td>
</tr>
<tr>
<td>19</td>
<td>&gt;13</td>
<td>199</td>
</tr>
<tr>
<td>20</td>
<td>&gt;13</td>
<td>287</td>
</tr>
<tr>
<td>21</td>
<td>&lt;2</td>
<td>27</td>
</tr>
<tr>
<td>22</td>
<td>&gt;12</td>
<td>779</td>
</tr>
</tbody>
</table>

P = 0.025

Directing CTLs to Tumors: Intra-Tumoral CXCL10 & CCL5 Levels in Metastatic CRC Correlate with CTL Infiltration

N=72

CCL10/IP-10

R=0.60
P<0.0001

CXCL10/IP-10

R=0.42
P<0.0003

CD8

R=0.88
P<0.0001

GZMB

R=0.79
P<0.0001

Muthuswamy R. 2012; Canc.Res. 272:3735 + POSTER 205
Variable Response of Different Cancer Lesions to Individual Factors & Uniform Response to Combination Treatment
COX Inhibition Helps to Optimize the Chemokine Production Patterns in Tumor Tissues

B.

- CCL5 (ng/ml)
- CXCL10 (ng/ml)
- CCL22 (ng/ml)

N = 3

(-)

IFN\(\alpha\)+pL:C

Indo+IFN\(\alpha\)+pL:C

#### CCL5 (ng/ml)

#### CXCL10 (ng/ml)

#### CCL22 (ng/ml)
**Combination** of IFNα, Poly-I:C & COX2 Blockade **Uniformly** Induces CTL-attracting CCL5 & CXCL10, Blocks $T_{reg}$-attracting CCL22 in Tumor Lesions

<table>
<thead>
<tr>
<th>CCL5/RANTES (Teff-attracting chemokine)</th>
<th>CXCL10/IP10 (Teff-attracting chemokine)</th>
<th>CCL22 (Treg-attracting chemokine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>Treated</td>
<td>Untreated</td>
</tr>
<tr>
<td>Patient 1</td>
<td>Patient 2</td>
<td>Patient 3</td>
</tr>
</tbody>
</table>
“Conditioned” Tumors **Selectively** Attract \( \alpha \)DC1-induced CTLs and Spontaneously Arising TILs and, but Not Tregs

**A.**

*Ex vivo* generated (\( \alpha \)DC1-induced) effector CTLs

- Untreated tumors
- Treated tumors

**B.**

Tumor isolated TILs

- Untreated tumors
- Treated tumors

---

- CRC tumor sups
- CRC tumor sups
- CRC tumor sups

FOXP3 mRNA

- UNDETECTABLE

- N=3
Tumor-**selectivity** of the Pharmacologic Induction of Teff-attracting Chemokines

N = 6 Tumor Explants

![Graph showing CXCL10 levels in untreated and treated samples](image)

- Marginal
- Tumor

P=0.2

P<0.05
Tumor-selectivity of the Pharmacologic Modulation of Teff-attracting Chemokines: Role of NF-κB

A. Untreated Treated

Margin

Tumor

NF-κB, DRAQ5

B. CXCL10 (ng/ml)

N=3

P<0.001

(-) (-) (PIC)

Treated (PIC)

CAY

Margin Tumor

See Poster 205: STAT-1/IRF1 vs PKA/pCREB pathways in the regulation of Teff/Treg chemokines
Phenotype and CTL-Suppressive Function of OvCa-isolated CD11b+ MDSCs

Natasa Obermajer, PhD
PGE$_2$ is Needed *Both* for **CXCR4 Expression** by MDSCs and for **Local Production of CXCL12** in Cancer Tissues.
Positive Feedback between COX2 & PGE$_2$ Is Required for Functional Stability of MDSCs in Ovarian Cancer

Natasa Obermajer, PhD
Directing Vaccination-Induced T Cells to Tumors

- Type-1 DC polarization can be used to preferentially activate naïve, effector and memory T cells ($T_{\text{EFF}}$ and $T_{\text{MEM}}$), rather than $T_{\text{reg}}$ cells.
- αDC1s loaded with peptide- or cell-associated Ags induce 20-70 fold more tumor-specific CTLs than non-polarized DCs.
- αDC1s induce CTL functions and responsiveness to tumor-produced chemokines (CCR5- and CXCR3 ligands) in naïve and memory CD8$^+$ T cells.
- Combination of IFNs with TLR-ligands and COX2 blockers selectively enhances the production of $T_{\text{EFF}}$-attracting chemokines in tumor lesions and counteracts $T_{\text{reg}}$/MDSC attraction and function.
- Ongoing and upcoming combination trials in colorectal cancer (UPCI 10-131 and UPCI 11-123) and ovarian cancer (UPCI 11-128).
Conditioning Tumor Microenvironment for Effective Immune Attack

Supported by: NIH (1PO1132714) and DOD (W81XWH-09-2-0051)