Emerging Concepts of Cancer Immunotherapy

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Center for Cancer Research
National Cancer Institute, NIH
Immune Cell Infiltrate in Primary Tumors as a Predictor of Prognosis in Patients with Colorectal Cancer

Galon, et al. Type, Density, and Location of Immune Cells Within Human Colorectal Tumors Predict Clinical Outcome. Science 2006;313:1960-4
# Studies of Tumor Infiltrating Immune Cells and Prognosis in Colorectal Carcinoma

<table>
<thead>
<tr>
<th>Reference</th>
<th>Principal findings</th>
<th>n</th>
<th>P value</th>
<th>Stage of carcinoma</th>
<th>Methods</th>
<th>Definition of immune cells</th>
<th>Follow-up period (years)</th>
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</thead>
<tbody>
<tr>
<td>7</td>
<td>Positive correlation for CD8+ and CD45RO+ T-cells.</td>
<td>602</td>
<td>&lt;0.0001</td>
<td>I-II</td>
<td>RT-PCR, TMA, IHC</td>
<td>CD8+ and CD45RO+ lymphoid infiltrates in tumors / invasive margin.</td>
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<tr>
<td>4</td>
<td>Positive correlation for CD45RO+ T-cells.</td>
<td>490</td>
<td>&lt;0.05</td>
<td>I-IV</td>
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<td>CD3+, CD8+, GrB+, and CD45RO+ lymphoid infiltrates in tumors / invasive margin.</td>
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<td>6</td>
<td>Positive correlation for CD8+ and CD45RO+ T-cells.</td>
<td>142</td>
<td>&lt;0.05</td>
<td>Metastatic/ non-metastatic</td>
<td>IHC, RT-PCR, FACS</td>
<td>CD3+, CD5+, CD8+, TCR+, CD1a+, Ki67+, CD68+, FoxP3+, and cytoDEATH+ tumor infiltrating cells.</td>
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<td>Positive correlation for lymphocytes.</td>
<td>710</td>
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<td>I-IV</td>
<td>H, LM</td>
<td>Lymphocytic infiltration.</td>
<td>&gt;10</td>
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<td>8</td>
<td>Positive correlation for lymphocytes.</td>
<td>843</td>
<td>&lt;0.01</td>
<td>I-IV</td>
<td>H, LM, RT-PCR</td>
<td>Lymphocytes on top of tumor cells.</td>
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<td>Positive correlation for lymphocytes.</td>
<td>276</td>
<td>&lt;0.001</td>
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<td>H, LM</td>
<td>Lymphocytic infiltration in the center and periphery of tumors.</td>
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<td>Positive correlation for CD8+ T-cells.</td>
<td>109</td>
<td>&lt;0.001</td>
<td>II-III</td>
<td>H, IHC</td>
<td>CD3+, CD8+, and GrB+ tumor infiltrating cells.</td>
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<td>Positive correlation for CD8+ T-cells.</td>
<td>119</td>
<td>&lt;0.001</td>
<td>I-IV</td>
<td>TMA, IHC</td>
<td>CD8+ cells in tumor tissue.</td>
<td>&gt;10</td>
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<tr>
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<td>Positive correlation for CD8+ and CD57+ cells.</td>
<td>93</td>
<td>&lt;0.05</td>
<td>II-III</td>
<td>H, IHC</td>
<td>CD4+, CD8+, CD56+, and CD57+ intraepithelial cells.</td>
<td>18</td>
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<td>14</td>
<td>Positive correlation for CD8+ T-cells, negative for CD4+ T-cells.</td>
<td>41</td>
<td>&lt;0.05</td>
<td>I-IV</td>
<td>FACS</td>
<td>CD3+, CD8+, and CD4+ tumor infiltrating cells.</td>
<td>5</td>
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<td>Positive correlation for CD8+ T-cells, negative for CD4+ T-cells.</td>
<td>162</td>
<td>&lt;0.001</td>
<td>IV</td>
<td>H, IHC, TMA</td>
<td>CD3+, CD4+, CD8+ and CD45RO+ tumor infiltrating cells.</td>
<td>&gt;10</td>
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<tr>
<td>17</td>
<td>Positive correlation for T-reg in tumor, negative in normal mucosa.</td>
<td>967</td>
<td>&lt;0.001</td>
<td>II-III</td>
<td>IHC, TMA</td>
<td>CD8+, CD45RO+, and FoxP3+ tumor infiltrating cells.</td>
<td>6</td>
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<tr>
<td>18</td>
<td>Positive correlation for T-reg.</td>
<td>57</td>
<td>&lt;0.001</td>
<td>IV</td>
<td>IHC</td>
<td>CD4+, CD8+, and FoxP3+ T-cells in stroma adjacent to neoplastic glands.</td>
<td>1.25</td>
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<tr>
<td>19</td>
<td>Negative correlation for T-reg, positive for CD3+ T-cells.</td>
<td>160</td>
<td>&lt;0.05</td>
<td>II-III</td>
<td>IHC, LM</td>
<td>CD4+, CD8+, CD25+, and FoxP3+ T-cells.</td>
<td>8</td>
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<tr>
<td>20</td>
<td>Negative correlation for dendritic cells.</td>
<td>104</td>
<td>&lt;0.05</td>
<td>II-III</td>
<td>H, IHC</td>
<td>Tumor infiltrating S-100+, HLA class II+, CD208+, and CD1a+ dendritic cells.</td>
<td>15</td>
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<tr>
<td>22</td>
<td>Positive correlation for lymphocytes.</td>
<td>361</td>
<td>&lt;0.001</td>
<td>I-III</td>
<td>H, LM</td>
<td>Lymphocytic infiltration.</td>
<td>10</td>
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<tr>
<td>23</td>
<td>Positive correlation for CD8+ T-cells.</td>
<td>131</td>
<td>0.016</td>
<td>I-IV</td>
<td>H, IHC</td>
<td>CD8+ and GrB+ tumor infiltrating cells.</td>
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<td>Positive correlation for CD8+ T-cells.</td>
<td>371</td>
<td>&lt;0.0001</td>
<td>I-IV</td>
<td>H, IHC</td>
<td>CD8+ T-cells within cancer cell nests.</td>
<td>10</td>
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<tr>
<td>27</td>
<td>Positive correlation for CD3+ T-cells in node-negative CRC.</td>
<td>286</td>
<td>&lt;0.01</td>
<td>III</td>
<td>IHC</td>
<td>CD3+ cells at the invasive margin.</td>
<td>6</td>
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</tbody>
</table>


Modes of Immunotherapy

**Vaccine Therapy**
- Antigen does not need to be on cell surface
  - CD8<sup>+</sup> — cytolytic T cell (CTL)
  - CD4<sup>+</sup> — helper T cell
  - NK — other cell type

**Checkpoint Inhibitor Monoclonal Antibodies**
- Reduce/eliminate negative inhibitory signals on tumor
- Reduce/eliminate negative inhibitory signals on T cells

**Cytokines**
- IL-2, GM-CSF, etc.

**T-Cell Adoptive Transfer**

**Monoclonal Antibody (MAb)**
- Antigen must be on cell surface
  - MAb — toxin, drug
  - MAb — radionuclide
  - MAb — non-conjugated
## Immunotherapy vs. Conventional Therapy

<table>
<thead>
<tr>
<th></th>
<th>Conventional Therapy</th>
<th>Therapeutic Vaccines</th>
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</thead>
<tbody>
<tr>
<td><strong>Target</strong></td>
<td>Tumor or its microenvironment</td>
<td>Immune system</td>
</tr>
<tr>
<td><strong>Pharmacodynamics</strong></td>
<td>Often immediate action</td>
<td>Delayed</td>
</tr>
<tr>
<td><strong>Memory Response</strong></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Limitations</strong></td>
<td>Toxicity</td>
<td>Requires adequate immune system function (both systemically and at tumor site)</td>
</tr>
</tbody>
</table>
Immunotherapy May Impact Tumor Growth Rate

Stein WD, Clin Cancer Res, 2010
Madan RA, Semin Oncol, 2012
Tumor Associated Antigens (TAAs) are, by definition, either weakly immunogenic or functionally non-immunogenic.

Strategies must be developed in which the presentation of TAAs to the immune system results in far greater activation of T cells than is being achieved naturally in the host.

— recombinant vectors
— T-cell costimulation
— combination therapies
— immune modulation
CEA/MUC-1/TRICOM: PANVAC V/F (CV-301)
Vaccination of Patients with Metastatic Colorectal Cancer (Liver or Lung) Post-Metastasectomy

- Primary endpoint: Relapse-free survival
- Multicenter trial (n = 74)
- M. Morse and K. Lyerly, PIs, Duke University
- PANVAC vs. PANVAC-infected DC
  - no statistical difference between the 2 arms
- Comparison with Concurrent Control Group treated at Duke

Multiple randomized/multicenter trials have demonstrated no change in TTP, yet improved survival

- Sipuleucel-T (Phase III – prostate)
- Ipilimumab (Phase III – melanoma)
- Sipuleucel-T (Phase III – prostate)
- CEA-MUC1-TRICOM (Phase II – colorectal)
- PSA-TRICOM (Phase II – prostate)
- Anti-PD1 (melanoma)
Modes of Immunotherapy

Vaccine Therapy
- Antigen does not need to be on cell surface
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  - CD4$^+$ — helper T cell
  - NK — other cell type

Checkpoint Inhibitor Monoclonal Antibodies
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- Antigen must be on cell surface
  - MAb — toxin, drug
  - MAb — radionuclide
  - MAb — non-conjugated
mAb Mechanisms of Action
Direct Effect on Receptor Function

Ribas. NEJM. 366; 26, 2012.
mAb Mechanisms of Action
Direct Effect on Receptor Function

Ribas. NEJM. 366; 26, 2012.
Effect of Vaccination on Tumor PD-L1 Expression

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**Reasons for immune evasion**

<table>
<thead>
<tr>
<th>Lack of innate immune activation</th>
<th>Expression of inhibitory factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lack of chemokines</td>
<td>T cell anergy</td>
</tr>
<tr>
<td>Dense stroma</td>
<td>Presence of regulatory immune cells</td>
</tr>
</tbody>
</table>

**Therapeutic interventions**

<table>
<thead>
<tr>
<th>Innate immune activation</th>
<th>α-PD-1/PD-L1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stroma disruption</td>
<td>Treg depletion</td>
</tr>
<tr>
<td>Manipulation of oncogene singaling pathway</td>
<td>IDO inhibition</td>
</tr>
<tr>
<td></td>
<td>Homeostatic cytokines</td>
</tr>
</tbody>
</table>

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**Similar results with LLC lung carcinoma cells**

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*Gajewski T et al. Current Opinion in Immunology, 25, 1-9 2013*
Cancer Vaccine Therapy

- Antigen does not need to be on cell surface for T-cell recognition
  - Potential for targeting of transcription factor(s) - Brachyury
- Antigens are processed and presented on the cell surface as short peptides in the context of MHC molecules
Targeting of EMT: an Opportunity for Interventions Against Tumor Progression

**HYPOTHESIS**

Immunological strategies targeting essential transcriptional regulators of the EMT process may be able to interfere with metastasis and therapeutic resistance.
BRACHYURY OVER-EXPRESSION INDUCES EMT IN EPITHELIAL CANCER CELLS

<table>
<thead>
<tr>
<th>Epithelial</th>
<th>Mesenchymal</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-cadherin</td>
<td>Plakoglobin</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>Vimentin</td>
</tr>
</tbody>
</table>

PANC-1-pcDNA
BRACHYURY LOW

PANC-1-pBrachyury
BRACHYURY HIGH

Fernando...Palena. J Clin Invest. 2010
Expression of Brachyury Protein in Colon Tissues (Immunohistochemistry)

ANOVA: F(3;76) = 39.045; p < 0.0001
Increased Expression of Brachyury with Severity of Colon Dysplasia

ANOVA: F(2;7) = 70.7; p=0.00002
Patients Who Have Generated T-Cell Responses to Brachyury Post-Vaccination

<table>
<thead>
<tr>
<th>Pt</th>
<th>Vaccine</th>
<th>Metastatic Tumor Type</th>
<th>PSA/CEA</th>
<th>ELISPOT Brachyury</th>
<th>HIV</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>PSA-TRICOM + αCTLA-4</td>
<td>Prostate Ca.</td>
<td>Pre</td>
<td>&lt;1/200,000</td>
<td>&lt;1/200,000</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Post</td>
<td>1/150,000</td>
<td>1/46,000</td>
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<tr>
<td>2</td>
<td>PSA-TRICOM + αCTLA-4</td>
<td>Prostate Ca.</td>
<td>Pre</td>
<td>&lt;1/200,000</td>
<td>&lt;1/200,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Post</td>
<td>1/40,000</td>
<td>1/41,000</td>
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<tr>
<td>3</td>
<td>Yeast-CEA</td>
<td>Medullary Thyroid Ca.</td>
<td>Pre</td>
<td>&lt;1/200,000</td>
<td>&lt;1/200,000</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Post</td>
<td>1/9,677</td>
<td>1/12,766</td>
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<tr>
<td>4</td>
<td>Yeast-CEA</td>
<td>Colorectal Ca.</td>
<td>Pre</td>
<td>&lt;1/200,000</td>
<td>&lt;1/200,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Post</td>
<td>1/200,000</td>
<td>1/60,000</td>
</tr>
</tbody>
</table>

- No autoimmune thyroid events or thyroid function changes in patients 1, 2, and 4. Patient 3 had prior thyroidectomy.

- ANA remained negative for patients 1 and 2 and TSH within normal limits. No autoimmune work-up was performed or indicated for patients 3 and 4.
LTIB is focused on the development of vector-based cancer vaccines
Interaction of “Non-Immune” Therapies with Immune-Based Therapies

• Prior studies:
  – inverse correlation between the number of prior chemotherapies and immune response to immunotherapy

• Rationale for the concomitant use of “non-immune” and immune-based therapies:
  – cisplatin/vinorelbine in patients with lung cancer
  – docetaxel in patients with prostate cancer
Certain chemotherapeutics when given post-immunotherapy will lyse populations of tumor cells acting as a boost for the initial immunotherapy.

Certain chemotherapeutic agents and/or radiation can alter the phenotype of tumor cells rendering them more susceptible to T-cell–mediated lysis.
Attacking Tumor Cells That Survive Therapy: Exploiting Immunogenic Modulation

- **T-cell induction/expansion**
  - Vaccine
  - Anti-CTLA-4
  - Anti-PDL-1

- **Immune Checkpoints**
  - Anti-CTLA-4
  - Anti-PDL-1

- **Tumor Targets**
  - Anti-Her2
  - Anti-EGFR
  - Anti-CD20

**Death**
- Tolerogenic Cell Death
  - Rapid plasma membrane rupture
  - Loss of intracellular contents such as TAA
  - Inefficient activation of innate immune response

**Autophagy**
- Immature dendritic cell

**Immunogenic Cell Death**
- Calreticulin/ERp57 translocation
- HMGB1 secretion
- ATP secretion

**Activated dendritic cell**

**Immunogenic Modulation**
- Phenotype changes: Upregulation of MHC I, adhesion molecules, tumor antigen(s) and mAb Targets
- Downregulation of anti-apoptotic/pro-survival genes
- Antigen processing machinery modulation
- Calreticulin translocation
- Modulation of immune-responsive genes

**Surviving cell fraction**
- Endogenous T cell induction

**Increased immune mediated tumor lysis**

**Immunotherapy**

**Death**
- Tumor cells
### Flow Cytometry Analysis of PBMC Immune Subsets

**Immune cell subsets analyzed:** 30 markers, 127 subsets

1. **CD4:** Helper T lymphocytes *(32 subsets)*
2. **CD8:** Cytotoxic T lymphocytes *(29 subsets)*
3. **Markers of PD1 pathway and T cell activation:**
   - EOMES: activation
   - TCR-αβ: activation
   - Tbet: activation
   - BATF: activation/exhaustion
4. **Maturation status of T lymphocytes:**
   - Naïve: CD45RA+ CCR7+
   - Central Memory: CD45RA- CCR7+
   - Effector Memory: CD45RA+ CCR7-
   - Terminal (EMRA): CD45RA+ CCR7-
5. **T lymphocyte markers:**
   - CTLA4: inhibition
   - PD1: activation/inhibition
   - PDL1: activation/cross-inhibition
   - TIM3: inhibition
   - ICOS: activation (only on CD4)
6. **Tregs:** Regulatory T lymphocytes *(CD4+ CD25+ FoxP3+ CD127+) *(7 subsets)*
7. **Treg markers:**
   - CD45RA: Tregs highly expandable *in vitro*
   - CTLA4: Treg suppression
   - CD49d: contaminating effector lymphocytes
   - ICOS: Treg suppression
   - PD1: inhibition
   - PDL1: cross-inhibition
8. **MDSCs:** Myeloid-derived suppressor cells *(CD11b+ HLA-DRlow/ CD33+) *(20 subsets)*
9. **Markers of MDSCs:**
   - CD14: Common Myeloid Marker (high in monocytes, dim in granulocytes)
   - CD15: Granulocyte marker
   - CD16: most immature monocytic MDSCs
   - PD1: inhibition
   - PDL1: cross-inhibition
10. **NK:** Natural killer cells *(CD56+ CD3-) *(20 subsets)*
11. **NK markers:**
   - CD16+ CD56br: Functional intermediate, lytic and cytokine production
   - CD16+ CD56dim: Mature NK, cytokine production
   - CD16- CD56br: Immature, abundant in human placenta
   - CD16- CD56dim: non-lytic, non-cytokine production
   - Tim3: activation
   - PD1: inhibition
   - PDL1: cross-inhibition
12. **NK-T:** CD56+ CD3+ *(4 subsets)*
   - Tim3: activation
   - PD1: inhibition
   - PDL1: cross-inhibition
13. **DCs:** Dendritic cells *(CD1c+ CD303-) *(10 subsets)*
   - CD1c+ CD303-: conventional DCs (cDCs)
   - CD1c- CD303+: plasmacytoid DCs (pDCs)
   - CD83: activation
   - Tim3: inhibition
   - PD1: inhibition
   - PDL1: cross-inhibition
14. **B lymphocytes:** CD19+ *(5 subsets)*
   - CTLA4: inhibition
   - TIM3: inhibition
   - PD1: Marker of inhibition
   - PDL1: Marker of cross-inhibition
## Analyses of Immune Cell Subsets in PBMCs of Colorectal Cancer Patients Pre- and Post-Therapy with Folfox and Bevacizumab

<table>
<thead>
<tr>
<th>Immune cell subset</th>
<th>Median frequency Pre</th>
<th>Median frequency Post 1</th>
<th>Median frequency Post 2</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>% CD4 of live cells</td>
<td>28.5</td>
<td>30.0</td>
<td>27.3</td>
<td>0.956</td>
</tr>
<tr>
<td>% ICOS^+ CD4</td>
<td>23.5</td>
<td>21.1</td>
<td>22.5</td>
<td>0.740</td>
</tr>
<tr>
<td>% CD8 of live cells</td>
<td>12.9</td>
<td>16.1</td>
<td>12.1</td>
<td>0.956</td>
</tr>
<tr>
<td>% Treg of live cells</td>
<td>0.9</td>
<td>0.7</td>
<td>0.8</td>
<td>0.740</td>
</tr>
<tr>
<td>Ratio CD4:CD8</td>
<td>2.3</td>
<td>2.5</td>
<td>2.1</td>
<td>0.956</td>
</tr>
<tr>
<td>Ratio CD4:Treg</td>
<td>29.3</td>
<td>39.5</td>
<td>37.6</td>
<td>0.956</td>
</tr>
<tr>
<td>Ratio CD8:Treg</td>
<td>11.8</td>
<td>16.2</td>
<td>17.7</td>
<td>0.956</td>
</tr>
<tr>
<td>% CTLA4^+ Treg</td>
<td>8.0</td>
<td>7.1</td>
<td>10.1</td>
<td>0.430</td>
</tr>
<tr>
<td>% ICOS^+ Treg</td>
<td>63.7</td>
<td>56.0</td>
<td>65.1</td>
<td>0.029</td>
</tr>
<tr>
<td>% B-cells of live cells</td>
<td>8.0</td>
<td>8.4</td>
<td>7.4</td>
<td>0.052</td>
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<tr>
<td>% MDSC of live cells</td>
<td>15.0</td>
<td>11.3</td>
<td>14.9</td>
<td>1.000</td>
</tr>
<tr>
<td>% Monocytic MDSC of live cells</td>
<td>11.1</td>
<td>8.0</td>
<td>12.9</td>
<td>0.956</td>
</tr>
<tr>
<td>% Granulocytic MDSC of live cells</td>
<td>0.8</td>
<td>0.9</td>
<td>0.4</td>
<td>0.184</td>
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<tr>
<td>% Non-lineage MDSC of live cells</td>
<td>2.5</td>
<td>1.5</td>
<td>1.7</td>
<td>0.430</td>
</tr>
<tr>
<td>% NK of live cells</td>
<td>8.8</td>
<td>11.6</td>
<td>16.3</td>
<td>0.571</td>
</tr>
<tr>
<td>% CD16^+ NK</td>
<td>84.1</td>
<td>77.7</td>
<td>79.9</td>
<td>0.430</td>
</tr>
<tr>
<td>% CD56^{br} NK</td>
<td>20.0</td>
<td>18.9</td>
<td>21.4</td>
<td>0.571</td>
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<tr>
<td>% NKT of live cells</td>
<td>0.5</td>
<td>0.7</td>
<td>0.6</td>
<td>0.252</td>
</tr>
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</table>

FACS analysis of 6 patients with CRC treated with Folfox and Bevacizumab
Analyzed approximately Days 0, 8 and 15
Immunotherapy as a Form of “Personalized Medicine”

- Antigen cascade/Antigen spreading

- Immune-mediated cancer cell destruction releases tumor antigens

- These antigens can be acquired by the immune system and become *new immunologic targets*

- May have delayed effects relative to standard cytotoxic therapies

- May have impact beyond the period of administration – subsequent therapies
Unique Properties of Immunotherapy

- Minimal toxicity

- Effect on the host immune system
  - indirect effect on the tumor
  - anti-tumor effects may be delayed

- Overall survival vs RECIST or time to progression as the appropriate primary endpoint

- Induction of host immunity is a dynamic process that can persist post-immunotherapy

- Potential for an enhanced effect on concomitant or subsequent therapies