Basic Science for Dummies: Cancer Immunity

Mission (Im)possible?
Act I - The immune response provides effective anti-tumor immunosurveillance.

Act II – The immune response promotes tumor formation and progression.

Act III – The immune response can be harnessed to treat cancer.
Innate Immunity:
• Immediate response, initiated within seconds
• Targets groups of pathogens/antigens
• Diversity: limited # germline encoded receptors
• No memory

Adaptive Immunity:
• Gradual response, generated over 3-4 days
• Targets specific pathogens/antigens
• Highly diverse: TCR & BCR repertoire
  • Memory!

Surface barriers
• Skin
• Mucous membranes

Internal defenses
• Phagocytes
• Fever
• NK cells
• Antimicrobial proteins
• Inflammation

Adaptive defenses

Humoral immunity
• B cells
• Antibodies

Cellular immunity
• T cells
  - CD8 (CTL)
  - CD4 (Th1, Th2, Th17, Treg, Tfh)
The Actors: their balance is critical

The balance of immune cells and their secreted cytokines is critical in determining whether a tumor is suppressed or progresses.

- **Th1** (helper CD4+ T) produces IFN-γ and other cytokines that suppress tumor growth.
- **Th2** (helper CD4+ T) produces IL-4, IL-5, and IL-13, which promote tumor growth.
- **M1** (macrophage) secretes IL-12 and IFN-γ, promoting an anti-tumor response.
- **M2** (macrophage) secretes IL-4 and IL-13, promoting tumor growth.
- **N1** (neutrophil) secretes reactive oxygen species that can kill tumor cells.
- **N2** (neutrophil) secretes pro-inflammatory cytokines that promote tumor growth.
- **DC1** (dendritic cell) presents tumor antigens to T cells.
- **DC2** (dendritic cell) is activated and promotes tumor growth.
- **Tumor Suppression** occurs when Th1 cells and M1 macrophages dominate.
- **Tumor Progression** occurs when Th2 cells and M2 macrophages dominate.

IL-12, IL-17A, IL-17F, and IL-22 are cytokines that play crucial roles in the balance between tumor suppression and progression.

Effector CD8+ (CTL) cells are critical in eliminating tumor cells.

**Symbols:**
- Th = helper CD4+ T
- M = macrophage
- N = neutrophil
- DC = dendritic cell
- MDSC = myeloid suppressor

**Legend:**
- IFN-γ: Interferon-γ
- IL-12: Interleukin-12
- IL-17A: Interleukin-17A
- IL-17F: Interleukin-17F
- IL-22: Interleukin-22
- IgG: Immunoglobulin G
- B cell: B lymphocyte
- Th1 cell: Helper T lymphocyte
- Th2 cell: Helper T lymphocyte
- Tfh cell: Follicular helper T lymphocyte
- Th17 cell: Th17 lymphocyte
- NKT cell: Natural killer T cell
- NK cell: Natural killer cell
- iTreg: Inducible T regulatory cell
- T-bet: T-box expressed in T cells
- CD40: Cell surface receptor
- CD1d: Cell surface receptor
- MDSC: Myeloid suppressor cell
- TGF-β: Transforming growth factor-beta

**Note:**
- The balance of these actors is critical in determining the outcome of tumor suppression vs. progression.
Cancer Immunity
A play in three acts

Act I - The immune response provides effective anti-tumor immunosurveillance.
Elimination: the “good” guys at work

Aberrant cell killing is thought to occur routinely at the single cell level and perhaps also during initial multi-cell stages of premalignancy.

Willard-Gallo, IMPAKT May 2, 2013
Is there evidence that the immune response controls early tumor progression in humans?
Chronic infections induce cancer (≈ 20%)

Evidence exists for an increased risk of cancer due to infection with

<table>
<thead>
<tr>
<th>Infection</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis B virus (HBV)</td>
<td>Sufficient: hepatocellular carcinoma</td>
</tr>
<tr>
<td>Hepatitis C virus (HCV)</td>
<td>Sufficient: hepatocellular carcinoma</td>
</tr>
<tr>
<td>Probable: non-Hodgkin lymphoma</td>
<td></td>
</tr>
<tr>
<td>Helicobacter pylori bacterium (H. Pylori)</td>
<td>Sufficient: stomach carcinoma, non-Hodgkin lymphoma (MALT)</td>
</tr>
<tr>
<td>Human papillomavirus (HPV)</td>
<td>Sufficient: cervix, vulva, other external genitalia</td>
</tr>
<tr>
<td>HIV - Kaposi’s sarcoma associated herpesvirus (KSHV)</td>
<td>Sufficient: Kaposi’s sarcoma</td>
</tr>
<tr>
<td>HIV - Epstein Barr virus (EBV)</td>
<td>Sufficient: non-Hodgkin lymphoma in AIDS</td>
</tr>
<tr>
<td>Epstein Barr virus (EBV)</td>
<td>Sufficient: nasopharyngeal cancer, undifferentiated, Burkitt and other non-Hodgkin lymphoma and Hodgkin’s disease</td>
</tr>
<tr>
<td>HTLV-I (Human T cell leukemia virus)</td>
<td>Sufficient: Adult T cell leukemia</td>
</tr>
</tbody>
</table>

but, at a low incidence in healthy immunocompetent individuals – in immunodeficient individuals (transplant patients, AIDS…) the frequency dramatically increases!

chronic infection = chronic inflammation
Genetic aberrations are detected by IR

The frequency of genetic aberrations in blood parallels the risk of cancer development; however, the immune response routinely does a good job of removing most (but not all) aberrant cells. This immune response weakens with age.

Ex: Philadelphia chromosome BCR/ABL translocation \([t(9;22)(q34;q11)]\) is seen in >99% of CML, 25-30% of ALL and at low frequency in the blood of many healthy donors.

Are these cells indolent leukemia or continually formed and eliminated in healthy individuals?

Schreiber, et al. Immunoediting, various review papers
Equilibrium: a balance of opposing forces

Accumulating advantageous growth and survival mutations slowly change the balance: an equilibrium phase with balanced growth and killing of aberrant cells ensues.

Schreiber, et al. Immunoediting, various review papers
A recurrent 6q deletion in patients with pre-malignant clonal CD3−CD4+ T cell hypereosinophilic syndrome slowly emerges as the malignant subclone

Table II: Evolution of the 6q and 10p deleted clones (percent of total CD3−CD4+ T cells) in successive purified blood samples from P1 and P2

<table>
<thead>
<tr>
<th>Probe used</th>
<th>Chromosome location</th>
<th>Subclone(s) represented in the probe-deleted population</th>
<th>% of probe-deleted nuclei in the total CD3−CD4+ T cell population (a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RP3-429G5</td>
<td>6q21</td>
<td>6q− plus 6q−10p−</td>
<td>77% 80% 91%</td>
</tr>
<tr>
<td>RP11-462L8</td>
<td>10p11.22</td>
<td>6q−10p− plus 10p−</td>
<td>54% 18% &lt; threshold</td>
</tr>
<tr>
<td>RP1-91B17</td>
<td>6q12</td>
<td>6q−10p−</td>
<td>33% 16% &lt; threshold</td>
</tr>
<tr>
<td>RP3-429G5</td>
<td>6q21</td>
<td>6q−</td>
<td>25% 22%</td>
</tr>
</tbody>
</table>

(a) The percentage of probe-deleted cells was normalized to the percentage of CD3−CD4+ T cells.
(b) This sample was 69% CD3−CD4+ T cells.

Willard-Gallo, IMPAKT May 2, 2013

Ravoet et al. 2009 Blood 114:2969
The downside of Equilibrium:

- tumor cells emerge with greater growth/survival capacity;
- slow erosion of effective immune responses as the resulting mutant tumor cells recruit “bad” immune cells;
- tumor-mediated immunosuppression increases.

Schreiber, et al. Immunoediting, various review papers
Act II – The immune response promotes tumor formation and progression.

Wait a minute! Why are we fighting each other? Aren't we all white blood cells?
A myriad of factors (lymphokines, cytokines, chemokines) are known to be produced in the tumor microenvironment (by immune cells, tumor cells, stromal cells, etc.), and therefore:

a) their balance is critical
b) their effect is dynamic
c) tumor heterogeneity = cytokine heterogeneity
d) they or their cellular source may antagonize one another

The constantly changing microenvironment(s)

Different immune, stromal, and tumor cell variants continuously change the balance of immune cells in the tumor microenvironment via the proteins they do/do not produce or express.

All of these activities can be going on simultaneously in different regions of the primary tumor.
Balance between “good” & “bad” is critical

**Th** = helper CD4+ T
**M** = macrophage
**N** = neutrophil
**DC** = dendritic cell
**MDSC** = myeloid suppressor

**NK** cell
**NKT** cell
**Tumor Suppression**
**Tumor Progression**

**M1**
- IFN-γ
- IL-13
- NKT cell

**M2**
- IgG
- Th2
- Gata3
- IL-4
- IL-5
- IL-13
- IL-17A
- IL-17F
- IL-22

**N1**
- IFN-γ
- IL-13

**N2**
- Th17
- RORγt
- IL-17A
- IL-17F
- IL-22

**NKT cell**
- IFN-γ

**Tumor Suppression**

**Tumor Progression**
Act III – The immune system can be harnessed to treat cancer.

“I go home today. They cured me using this new miracle drug. I’m afraid it’ll be years before it’s approved for humans.”
From hybridomas to antibody-based therapeutics

Hybridoma technology discovered: Köhler & Milstein

Hybridoma technology awarded Nobel Prize: Köhler & Milstein

1st murine mAb approved (FDA) for transplant patients; target: CD3

1st chimeric mAb approved (FDA) for cardiac ischemic complications; target: ITGA2B

1st humanized mAb approved (FDA) for kidney transplant rejection; target: CD25/IL-2Rα

1st fully human mAbs approved (FDA) for rheumatoid arthritis; target: TNFα


Fully human mAb approved (FDA) for metastatic breast cancer; target: HER2

1st mAb:drug conjugate approved (FDA) for breast cancer; targets: HER2 + microtubules

Human mAb approved (FDA) for early breast cancer; target: HER2

Murine mAb

Chimeric mAb

Humanized mAb

Human mAb

Decreased immunogenicity

Suffix in antibody name indicates its origin

BC leads the way!

Willard-Gallo, IMPAKT May 2, 2013

M D Pegram, A Lipton, D F Hayes, B L Weber, J M Baselga, D Tripathy, D Baly, S A Baughman, T Twaddell, J A Glaspy and D J Slamon

**Antibody-mediated killing**

**Tumor-associated antigens targeted by monoclonal antibody therapeutics**

<table>
<thead>
<tr>
<th>Antigen category</th>
<th>Examples of antigens</th>
<th>Tumor types expressing antigen</th>
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</thead>
<tbody>
<tr>
<td>Cluster of differentiation (CD) antigens</td>
<td>CD20, non-Hodgkin lymphoma</td>
<td></td>
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<tr>
<td></td>
<td>CD30, Hodgkin lymphoma</td>
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<tr>
<td></td>
<td>CD33, Acute myelogenous leukemia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CD52, Chronic lymphocytic leukemia</td>
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<tr>
<td>Glycoproteins</td>
<td>EpCAM, epithelial tumors (breast, colon, lung)</td>
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<tr>
<td></td>
<td>CEA, epithelial tumors (breast, colon, lung)</td>
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<tr>
<td></td>
<td>gpA33, colorectal carcinoma</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mucins, epithelial tumors (breast, colon, lung, ovarian)</td>
<td></td>
</tr>
<tr>
<td>TAG-72</td>
<td>Epithelial tumors (breast, colon, lung)</td>
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<td>Carbonic anhydrase IX</td>
<td>Renal cell carcinoma</td>
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<td>PSMA</td>
<td>Prostate carcinoma</td>
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<tr>
<td>Folate binding protein</td>
<td>Ovarian tumors</td>
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<tr>
<td>Glycolipids</td>
<td>Gangliosides (e.g., GD2, GD3, GM2) Neuroectodermal tumors, some epithelial tumors</td>
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<tr>
<td>Carbohydrates</td>
<td>Lewis-Y2, epithelial tumors (breast, colon, lung, prostate)</td>
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<tr>
<td>Vascular targets</td>
<td>VEGF, tumor vasculature</td>
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<tr>
<td></td>
<td>VEGFR, epithelium-derived solid tumors</td>
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<td></td>
<td>αVβ3, tumor vasculature</td>
<td></td>
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<tr>
<td></td>
<td>α5β1, tumor vasculature</td>
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<tr>
<td>Growth factors</td>
<td>EMB1/EGER, glioma, lung, breast, colon, head and neck tumors</td>
<td></td>
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<tr>
<td></td>
<td>EMB2/HER2, breast, colon, lung, ovarian, prostate tumors</td>
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<tr>
<td></td>
<td>c-MET, epithelial tumors (breast, ovary, lung)</td>
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<td></td>
<td>IGF1R, lung, breast, and neck, prostate, thyroid, glioma</td>
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<tr>
<td></td>
<td>EphA3, lung, kidney, colon, melanoma, glioma, hematological malignancies</td>
<td></td>
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<tr>
<td></td>
<td>TRAIL-R1, TRAIL-R2, Solid tumors (colon, lung, pancreas) and hematological malignancies</td>
<td></td>
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<td></td>
<td>RANKL, prostate cancer and bone metastases</td>
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<tr>
<td>Stromal and extracellular matrix antigens</td>
<td>FAP, epithelial tumors (colon, breast, lung, head and neck, pancreas)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tenascin, glioma, epithelial tumors (breast, prostate)</td>
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</tbody>
</table>

Effective but still the problem of tumor heterogeneity (ex. HER2+ and HER2− cells) means they are not 100% effective.
Targeting immune checkpoints to relieve tumor-mediated immunosuppression, but is it enough? Are the dangers acceptable?

Adapted from Sharma P. Immune checkpoint strategies (Introduction). Presented at: 2012 ASCO Annual Meeting, Clinical Science Symposium; June 1-5, 2012; Chicago, IL.
Immunological Memory

Memory cells can persist from many years to a lifetime!

*ex. single shot vaccines, lifetime immunity to childhood diseases, etc.*

They may be the only “drug” that can find and kill or suppress the last remaining tumor cell…but has the immune response acquired memory to recent tumor cell mutants/variants?
Can T cell memory be harnessed?

**Effects on progression:**

<table>
<thead>
<tr>
<th>any cancer</th>
<th>human BC</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑↓</td>
<td>↑</td>
</tr>
<tr>
<td>↑↓</td>
<td>↑↓?</td>
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<tr>
<td>↑↓?</td>
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</tbody>
</table>

**Our data**
So, if we could break tolerance/immunosuppression and boost memory to the heterogeneous tumor at diagnosis, would we improve outcome?

Time will tell…
That’s all folks!