Limited benefit

It's complex!

16,000' perspective

THE VIEWS AND OPINIONS PRESENTED ARE OF THE PRESENTER AND NOT REFLECT THE OPINIONS OR POLICY OF NIH OR NCI.
1. The immune system is extraordinarily complex and activation and de-activation are tightly regulated.

2. In cancer immunotherapy there are many targets: tumor cells, innate immune response cells, adaptive immune response cells, stroma & microenvironmental factors (metabolites), normal epithelial cells, endothelial cells and surrounding normal tissue (including lymph nodes).

3. Processes occur over time- not an “all at once” event, although primary event to stimulate response may be key.

4. So, logically “one size doesn’t fit all” to harness the immune response. (Maybe we’ll be lucky and there is one “best” radiation dose, schedule and target volume.)

5. Experiments need good biomarkers and critical thinking so that “null” or disappointing results teach us something.

1. With the excitement and potential of immunotherapy there are many publications (10,000’s) and clinical trials: clinicaltrials.gov listed 112 (not including many industry trials). So many targets- molecular, cellular, interactions.

2. The multi-step process involves cells and signals throughout the body. What volumes should be irradiated, not irradiated or unknown needs to be defined… and also when to Rx.

3. The clinical results involve mostly melanoma, renal cell cancer, non-small cell lung cancer and some lymphomas. Response criteria need to be adapted for the “pseudoprogression”.

4. Many patients do not yet benefit.

5. Radiation goes where one aims it and tissues know they’ve been hit.

6. Good opportunities for models and analytical minds.

Cancer Immunotherapy Development

http://www.fightcancerwithimmunotherapy.com
Checkpoint Inhibitors
Left unchecked, immune responses can be so powerful that they will destroy healthy tissue. Thus, specialized immune cells called T cells must pass several biological checkpoints before achieving full strength. Cancer cells often act on these checkpoints in a way that prevents the immune system from attacking the tumor. New drugs—called checkpoint inhibitors—disable the cancer cells’ immunedampening signals, allowing the immune system to do its job.

Dendritic Cell Vaccine
Dendritic cells normally patrol the body looking for bits of proteins called antigens that look unfamiliar. They present the off ending antigens to other immune defenders, known as CD4+ and CD8+ T cells. The T cells then attack any other cells that bear the targeted antigen. By choosing antigens found on cancer cells but not on healthy ones and mixing the antigens with a patient’s own dendritic cells outside the body, researchers create a kind of vaccine that will seek out and destroy those same cancer cells for years to come.

CAR-T Cells
Chimeric antigen receptor (CAR) T cells combine attributes of two types of immune defenders: T cells and B cells. Molecules called receptors found on a CAR-T cell look like a hybrid of receptors on B cells and T cells. The CAR protein allows this unusual cell to both latch onto select antigens and destroy any cells that bear the target antigen. This mishmash eliminates intermediate steps typically taken by B and T cells, making CAR-T cells virtually unstoppable.

General classes of immunotherapy

Tumor-Immune Interaction

Effective Therapeutic immune-balance

Adapted from SciAm, April 2016, p48
Induction of immune response

Inhibition of suppression

Effective Therapeutic immune-balance

Co-inhibitory Molecules
There are similar families of co-stimulatory molecules

Molecular mechanisms of T cell co-inhibition and co-stimulation
Liping Chen & Dallas B. Flies
Nature Reviews Immunology 13, 227-242 (April 2013)
Co-inhibitory Molecules


PD-1/PD-L1 Engagement Suppresses Effector T-cells

PD-1/PD-L1 Engagement Suppresses Effector T cells

MDSC
Combinational Immunotherapy

- **Vaccines**
- **Immune Modulators**
  - Immune Agonists
    - Stimulatory cytokines (IL-2, IL-12, IL-15, TLR etc.)
  - Co-stimulatory molecules (OX-40, GITR, 4-1BB)
  - Immune inhibitors
    - Check point inhibitors (CTLA4, PD1/PDL1, LAG3, TIM3, IDO)
    - Inhibitory cytokines/factors (IL-10, TGFb)
- **Standard Therapy**
  - Chemotherapy
  - Radiation Therapy
- **Small Molecules**
- **Chimeric Antigen Receptors**

### Table 1: Final Rankings of Agents with High Potential for Use in Tumour Cases

<table>
<thead>
<tr>
<th>Rank</th>
<th>Agent</th>
<th>Target</th>
<th>Source</th>
<th>Status</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Anti-Programmed Cell Death 1 (PD1) &amp; anti-B7-R (PD1)</td>
<td>T-Cell Checkpoint Blockade Inhibitor</td>
<td><strong>Cell Line</strong></td>
<td><strong>Preclinical</strong></td>
<td><strong>Cell Line</strong></td>
</tr>
<tr>
<td>2</td>
<td>Anti-IL-12</td>
<td>T-Cell Growth Factor</td>
<td><strong>Cell Line</strong></td>
<td><strong>Preclinical</strong></td>
<td><strong>Cell Line</strong></td>
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<tr>
<td>3</td>
<td>Anti-IL-7</td>
<td>T-Cell Growth Factor</td>
<td><strong>Cell Line</strong></td>
<td><strong>Preclinical</strong></td>
<td><strong>Cell Line</strong></td>
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<tr>
<td>4</td>
<td>Anti-CD40</td>
<td>Antigen-Presenting Cell Stimulator</td>
<td><strong>Cell Line</strong></td>
<td><strong>Preclinical</strong></td>
<td><strong>Cell Line</strong></td>
</tr>
<tr>
<td>5</td>
<td>Anti-CD40</td>
<td>Antigen-Presenting Cell Stimulator</td>
<td><strong>Cell Line</strong></td>
<td><strong>Preclinical</strong></td>
<td><strong>Cell Line</strong></td>
</tr>
</tbody>
</table>

NCI Immunotherapy Agent Workshop Proceedings
Reason for enthusiasm is obvious!

<table>
<thead>
<tr>
<th>Drug</th>
<th>Trade name</th>
<th>Dosage</th>
<th>Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nivolumab</td>
<td>Opdivo</td>
<td>3 mg/kg IV over 60 min every 2 weeks</td>
<td>Melanoma, NSCLC, Renal, Hodgkin's</td>
</tr>
<tr>
<td>Pembrolizumab</td>
<td>Keytruda</td>
<td>2 mg/kg IV over 30 min every 3 weeks</td>
<td>Melanoma, NSCLC</td>
</tr>
</tbody>
</table>

Checkpoints inhibitors - 3

Reason for enthusiasm is obvious!

#1: Tumor-associated antigens (TAAs) are released by irradiated dying cancer cells. TAAs and cell debris are engulfed in the tumor microenvironment by phagocytes such as macrophages, neutrophils, and dendritic cells for antigen processing and presentation.

#2: RT-induced cell death releases danger signals including heat shock proteins (Hsp), HMGB1, and calreticulin (eat-me signal for phagocytes).

#3: RT induces increased expression of tumor antigens and MHC class I molecules on tumor cells.

#4: RT-induced T-cell activation increases expression of negative stimulatory molecules such as CTLA-4.

#5: Certain radiation doses may increase tumor production/secretion of immunosuppressive cytokines such as IL-10 and TGF-β.

#6: Activated APCs migrate to the draining lymph node, further mature upon encountering T helper cells, release interferons (IFNs) and IL-12/18 to stimulate Th1 responses that support the differentiation and proliferation of antigen-specific CTLs. Activated antigen-specific CTLs traffic systematically from the draining lymph node to infiltrate and lyse primary and distal tumors.

A Schematic view of RT-induced immune modulations

Table 3 Proposed ISABR studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>ARM</th>
<th>Treatment</th>
<th>Tumor</th>
<th>Histology</th>
<th>Score</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td></td>
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<td>3</td>
<td>+</td>
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</tbody>
</table>


Fig. 1 Patients treated with ipilimumab (IPI) before radiation therapy had higher 6- and 12-month response rates, as well as increased response duration, compared to those who received ipilimumab after radiation therapy. Abbreviations: CI = confidence interval.

Fig. 2 Patients receiving ablative radiation therapy had numerically higher 6- and 12-month overall survival (OS) rates versus patients treated with non-ablative radiation therapy. Abbreviation: CI = confidence interval.

Rosie Qin, Adam Olson, Shweta Singh, Griffin Sin, Adam Olson.
Safety and Efficacy of Radiation Therapy in Advanced Melanoma Patients Treated With Ipilimumab
International Journal of Radiation Oncology*Biology*Physics, 2016
http://dx.doi.org/10.1016/j.ijrobp.2016.04.017
Radiation can
- Impact both innate and adaptive immunity
- Provide a source of robust tumor antigens
- Induce cytokines that can help to alter the profile and function of immune infiltrates
- Remodels the stromal and angiogenic compartments of the tumor microenvironment

More importantly
Surviving tumor cells after radiation therapy are more sensitive to immune-mediated killing

LETTER

Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer

The Where, the When, and the How of Immune Monitoring for Cancer Immunotherapies in the Era of Checkpoint Inhibition

Priti S. Hegde and Stefan Evers

Clin Cancer Res; 22(8); 1865–74. 2016 AACR.
The "cancer immunogram"
Christian U. Blank1, John B. Haanen, Antoni Ribas, Ton N. Schumacher
Netherlands Cancer Institute and UCLA
Science 352: 658, (May 2) 2016

The cancer immunogram.
The radar plot depicts the seven parameters that characterize aspects of tumor-immune interactions for which biomarkers have been identified or are plausible. Potential biomarkers for the different parameters are shown in italics. Desirable states are located in blue; progressively undesirable states are shown in the red gradient. The black line connecting the data values for each parameter represents a plot for a single hypothetical patient. In the case shown, it may be argued that single-agent PD-L1 blockade, rather than combined PD-L1 and CTLA-4 blockade, could be a first treatment of choice. For details on this case and other hypothetical patient cases, see (2).

- Few trials testing RT parameters in combination with checkpoint blockade
  - One trial testing low-dose ultrafractionated radiation (<1 Gy per fraction) with PD-L1.
    - Low-dose radiation was an effective inducer of tumor infiltrating T-cells in mouse models (Klug et al. Cancer Cell 2013).
    - Low-dose RT allows exploration of synergistic effects on local control.
  - Only one trial explicitly evaluating RT dose (40 patients, 5 histology, multiple timings), not using combined checkpoint blockade
The Lewis lung carcinoma 1 (LLC1), a mouse cancer cell was used to develop syngenic tumors in C57BL/6 mice.

Tumors were initiated by subcutaneous injection of 2 x 10^6 LLC1 cells into the flanks of both left and right hind legs of each mouse.

Tumors were allowed to grow to an area of 5 mm x 5 mm before irradiation and sorted within 10% differences in the intra and inter-tumor volumes.

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**MOUSE MODEL AND SCHEMES**

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**Single fraction, high-dose LRT significantly delayed growth of both local and distant tumors**

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**Immune responses in LLC xenograft tumor model: Lattice high-dose radiation induces increased secretion of inflammatory cytokines**

There was a significant decrease in both IL-4 and IL-10 in serum obtained from one 50% lattice vertex at both time points. Treatment with two 10% lattice vertices significantly reduced IL-4 and IL-10 secretion in serum although IL-10 levels returned to normal on day 7.
FIG. 1. Inflection point kinetics of immune genes in multifractionated treated PC3 and DU145 cells as assessed by real-time RT-PCR. PC3 and DU145 cells were exposed to 1–10 Gy of radiation delivered as 1 Gy fractions. Fold change in MFI27, MX1, CXCL11, BST2, HSH2D and IL1A expression 24 h after cells received 1–10 Gy of radiation was determined by RT-PCR. Data shown are fold change (AV ± SEM) of 3 biologically distinct experiments. *P < 0.05, **P < 0.01.

List of Immune Genes Modulated Greater than Twofold in PC3 Cells 24 h after Single-Dose or Multifractionated Treatment

Networks and Associated Functional Categories Identified by IPA for PC3, DU 145 and LNCaP Cells 24 h after Exposure to 10 Gy of Fractionation Administered as a Single-Dose or Multifractionated Regimen (1 Gy × 10)

Fractionation can induce an adaptive response—immune response genes upregulated in tumors and endothelial cells:
Dose, Fx and volume matter
May even vary dose and schedule?
Differential expression of stress and immune response pathway transcripts and miRNAs in normal human endothelial cells subjected to fractionated or single-dose radiation.

Palayoor ST. Mol Cancer Res. 12(7):1002-15; 2014

Challenges

**Immune-modulation of tumor microenvironment and tumor cells by radiation**

- Quality of radiation (high versus low-LET), dose, size, fractionation (low-dose versus high-dose fractionation) and dose-rate (high-dose rate versus low-dose rate), and schedule (hypofractionation versus multifractionation)

- Irradiation of complete tumor volume or partial volume adequate for effective modulation of tumor immune microenvironment
  - Gross tumor volume (GTV) or GTV plus LN
  - Evoking it by irradiating normal tissue

- HLA class I loss or low TCR diversity or checkpoint expression or TILs.

- Balance between radio-induction of immune suppressive cytokines and radio-induction of immune activating cytokines

**Effective combinations of radiation and immunotherapy**

- Relevant pre-clinical models (NSG-PDX, GEMMs and canine) or from clinical trials (reverse translational)

- Radiation effect on normal tissues and its impact on the efficacy of radiation + checkpoint blockade therapy
  - Efficacy area versus safety area
    - Use of traditional endpoints for safety and efficacy

- Significant challenges in the selection of opportune biomarkers of immunogenicity when radiation is combined with immunotherapy

- Remember tumor cell heterogeneity - many clones to start with and there is ongoing evolution.

- Combinations with molecular targeted agents will add complexity of pathway activation/suppression, tumor adaptation and impact on normal tissues!
A Phase II Trial of Durvalumab and Tremelimumab Alone or in Combination with High or Low-dose Radiation in Metastatic Colorectal and NSCLC

PD-L1 Project Team
Jonathan Schoenfeld, Arta Monjazeb, Mansoor Ahmed, Stephen Hodi

Questions asked by this trial

- This clinical trial is designed to answer novel and critical questions which are unlikely to be answered by existing trials
  - Does RT synergize with dual check-point blockade (Saint-Victor et al Nature 2015)
  - What is the influence of RT dose (Chandra et al Oncoimmunology 2015)
  - Can RT overcome resistance to T-cell exclusion mediated resistance to dual checkpoint blockade (Spranger et al Nature 2015)
  - Does RT increase intratumoral TCR diversity and how does this correlate with response to RT + dual checkpoint blockade (Saint-Victor et al Nature 2015)
  - Does RT increase tumor antigenic load and how does this correlate with response to RT + dual checkpoint blockade (Rizvi et al Science 2015)
Study Hypotheses

Overall Hypothesis:
Due to complimentary immunologic effects (increasing the magnitude and diversity of the T-cell response in the tumor microenvironment), radiation will synergize with dual checkpoint blockade.

- Two cohorts:
  - Metastatic NSCLC (~15-20% response to anti-PD-1 therapy)
    - Hypothesis: The addition of low or high dose radiation to combined blockade of PD-L1 (durvalumab) and CTLA-4 (tremelimumab) will increase response rate
  - Metastatic colorectal cancer, microsatellite stable (few to no responders to anti-PD-1 therapy)
    - Hypothesis: Low or high dose radiation combined with PD-L1/CTLA-4 blockade will lead to a measurable response rate

Trial Schema – NSCLC Cohort

Mathematical modeling of cancer immunotherapy and its synergy with radiotherapy
P. Serre1, S. Benzsky2, L. Padovan3, C. Mellot4, N. Andrè5, 6, J. Ciccolini1, F. Barlesi1, 7, X. Muracciole∗3, D. Barbolosi∗1
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[2] Inria Bordeaux Sud-Ouest, team MONC, Institut de Mathématiques de Bordeaux, Bordeaux, France
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[6] Centre Émile Ehrmann, INSERM U911, Marseille, France
[7] Multidisciplinary Oncology & Therapeutic Innovations Unit, AP-HP, Marseille, France

Mathematical model of radiation and immunology
The model is described by 5 discrete-time equations:
- Tumor dynamics (equation 1)
- Antigen dynamics (equation 2)
- Lymphocytes (or immune effectors) dynamics (equation 3)
- Primary immune response (equation 4)
- Secondary (or memory) immune response (equation 5)
Dose, timing, fractionation, combined modality - many parameters to understand so avoid the (cute for sure) herd mentality.

...or important potential clinical benefit for our patients may lead to inappropriate “ruin” of what our “focused biology” can accomplish!

Entering a new era for which great opportunities exist, based on critical science (much yet to be learned).