Convergence of radiation and immunogenic signaling pathways

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DISCLOSURES

Consultant/Speaker:
Bristol Myers Squibb, Varian, Elekta, Janssen, Regeneron, GlaxoSmithKline, Eisai, Dynavax, Astra Zeneca

Principal Investigator:

NCI R01CA161891-01
*Immunomodulation of breast cancer via TLR7 agonist IMQ and RT*

DOD BC100481 / W81XWH-11-1-0530
Multi-Team Award (MTA)
*Radiation-Induced Vaccination to Breast Cancer*

13-A0-00-001870-01
Breast Cancer Research Foundation
*Targeting key inhibitory pathways to improve radiation-induced vaccination in breast cancer*

NIH 1 S10 RR027619-01 Preclinical Research Irradiator
Radiation-induced immunogenic cell death and cross-priming

Need for sufficient naïve T cells
Radiotherapy and immune-mediated rejection: a balancing act

- TGFβ
- ActivinA (PS3-63)
- Adenosine
- PDL-1
- Tregs

NEGATIVE EFFECTS

POSITIVE EFFECTS

Tumor Reaction

IFN-I
ATP
NKG2D-Ligands
Teff chemokines
Radiation as a generator of T cells: overcoming immunosuppression in the TME (adenosine, TGFβ)

Role of the PD-1/PDL-1 pathway in resistance to treatment with RT and TGFβ neutralization

RT re-positioning of anti-CTLA-4 in NSCLC: dose fractionation and technique for the abscopal effect
In vitro assay for RT-induced ICD

CRT

HMGB1

ATP

Encouse Golden

Oncoimmunology, 2014
Blockade of adenosine generation improves recruitment and maturation of DCs and tumor response to SBRT
Does adenosine blockade improve radiation-induced anti-tumor immunity?

**PHARMACOLOGICAL ADENOSINE BLOCKADE**

- **BALB/c WT**
  - Tumor inoculation (right flank, s.c.)
  - TSA or MCA38
  - Day: 0
  - 20 Gy RT
  - Day: 11, 12, 14
  - Anti-CD73 mAb (TY/23), i.p. injections or A2AR-inhibitor (SCH58261, daily i.p. Injections)
  - Day: 17, 18, 20
  - Tumor progression and survival

**A2AR-DEFICIENT MICE**

- **C57BL/6 WT / Adora2A**
  - Tumor inoculation (right flank, s.c. MCA38)
  - Day: 0
  - 20 Gy RT
  - Day: 12
  - Isolation and phenotyping of intratumoral immune cells
  - Peptide re-stimulation of tumor-draining lymph node cells
  - Day: 18
  - Tumor progression and survival
Adenosine-blockade promotes intratumoral infiltration of CD8α+ activated DCs

**Anti-CD73 Ab (TY/23)**

**A2AR inhibitor (SCH58261)**

**A2AR KO mice (ADORA2A⁻/⁻)**
CD73-blockade reduces radiation mediated Treg infiltration while promoting CD8+ T cell infiltration

**Anti-CD73 Ab (TY/23)**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Tregs (% of CD4+ T cells)</th>
<th>CD8+ T cells (% of T cells)</th>
</tr>
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<tbody>
<tr>
<td>IgG2a</td>
<td>20 Gy + IgG2a</td>
<td>20 Gy + 2A3</td>
</tr>
<tr>
<td>anti-CD73</td>
<td>20 Gy + anti-CD73</td>
<td>20 Gy + TY/23</td>
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*P < 0.05
Combined RT and anti-CD73 treatment delays tumor progression and prolongs survival

Modulation of adenosine generation and/or uptake in the TME following tumor tx may facilitate the immunogenic effect of radiation
TGFβ activation by radiation-induced ROS hinders priming of anti-tumor T cells

Inhibition of DC activation

Inhibition of T cell effector function
Therapeutic synergy of radiation and TGFβ blockade

Tumor volume (mm³) ± SEM

Sham + Iso
Sham + 1D11
RT + Iso
RT + 1D11

Lung metastases

Days post tumor cells injection

***

1D11
Fresolimumumab and Radiotherapy in Metastatic Breast Cancer

- Fresolimumab: 1 or 10 mg/kg IV
- RT: 7.5 Gy x 3, weeks 2 and 7
- Blood: weeks 0, 2, 5 and 15
- Response assessed at week 15 (PET/CT)

NCT01401062, supported by DOD Breast Cancer Research Program- Multi-Team Award (PI S. Formenti)
Increase in tumor-specific CD8 T cells in patients treated with radiotherapy + fresolimumumab

5/9 HLA-A2+ patients showed increase/induction of survivin-specific CD8 T cells by tetramer analysis
RESULTS

Anti-TGFbeta + RT:
22 patients, <10% ORR

59 F with metastatic Triple Negative Breast Cancer

4th line therapy 18 months after diagnosis: RT+ Fresolimumab
Comparison of OS and PFS based on fresolimumab dose

Accrued 22 patients: 11 per arm
fresolimumab dose (arm A=10mg, arm B=1 mg)

Overall Survival

Progression Free Survival

16 out of 22 (73%) patients died. (7/11 in arm A, 9/11 in arm B)
Hazard Ratio = 2.174 (95% CI: 0.753 – 6.272)
BACK TO THE MOUSE

Is adaptive immune resistance limiting tumor response to radiotherapy + TGFβ blockade?
Increased PDL-1 and PDL-2 expression on tumor and myeloid cells by RT and TGFβ blockade

Vanpouille-Box, Cancer Research 2015
PD-1 blockade extends survival in mice treated with radiation and TGFβ blockade.
Local RT + multiple ITs may be required in established tumors.
Elevated baseline levels of PD-1 and PD-L1 expression and reduced TCR signaling in breast cancer patients (SCNP)

Single cell network profiling at baseline: 7 healthy donors and 15 BC patients (antiTGFβ/RT trial)
Reduced TCR signaling in PD-1+ CD4+ and CD8+ T cells vs PD-1− T cells

TCR \rightarrow p\text{-}ERK

PD-1 :

CD4+ CD8+

- + - +

Healthy donors

TCR \rightarrow p\text{-}AKT

PD-1 :

CD4+ CD8+

- + - +

Breast cancer patients

Colors represent different donors

time point

○ Null
× week0
+ week5
□ week15
In vitro anti-PD-1 (pembrolizumab) partially restores TCR→p-ERK /p-AKT.

▶ Basis for in vitro PD-1 patient selection marker for combination therapies.
Radiation as a generator of T cells: overcoming immunosuppression in the TME (adenosine, TGFβ)

Role of the PD-1/PDL-1 pathway in resistance to treatment with RT and TGFβ neutralization

RT re-positioning of anti-CTLA-4 in NSCLC: dose fractionation and technique for the abscopal effect
Generation of anti-tumor T cell responses requires tumor irradiation + CTLA-4 blockade

AH1

IFN

α-CTLA4 - + - +
RT - - + +

Demaria et al., Clin Cancer Res 2005
RT and anti-CTLA-4 drive oligoclonal expansion of CD8 TILs

CDR3 region of B chain = unique identifier

Pilones et al., AACR 2015
Clinical study design to test for abscopal responses

- Either a prospective randomized trial (IT+ RT versus IT)
- Or a trial of radiation with an immunotherapy proven ineffective when used alone
Limited objective response rate to CTLA-4 Blockade (without and with chemo) in NSCLC

<table>
<thead>
<tr>
<th>Reference</th>
<th>Stage</th>
<th>Study Design</th>
<th># PTS</th>
<th>OR</th>
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</thead>
<tbody>
<tr>
<td>Zatloukal et al</td>
<td>LOCALLY ADV/METS</td>
<td>-TREMELIMUMAB (15 mg/kg) VERSUS BSC</td>
<td>87</td>
<td>4.5% (2 PRs)</td>
</tr>
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<td>ASCO 2009</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Lynch et al</td>
<td>Stage III/IV</td>
<td>Carbo/Taxol vs Carbo/T with Ipi (10mg/kg) Carbo/T and Ipi sequential (10mg/kg)</td>
<td>204</td>
<td>NS PFS</td>
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<td>JCO 2012</td>
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No CRs in either studies
Patient with Refractory Metastatic NSCLC

Progressing after 3 lines of chemo and chest RT: Multiple lung, bone and liver metastasis

RT to one liver met 6 Gy X 5 (TD 30 GY)
Ipilimumab, 3 mg/Kg, after first RT q3 weeks, X 4 cycles

Golden et al Cancer Immunology Research, 2014
Same patient, response to RT+ ipilimumab
Clinical and radiological CR at one year: currently NED at 36 m
Ipilimumab and localized RT in chemo-refractory metastatic NSCLC

39 patients, Response rates (CR + PR):
Intent to treat = 18%
Pts completing 4 Ipi = 33%

Median follow-up: 12 months
Median survival: CR/PR/SD = not reached
Log-rank test: p = 0.0161
HR = 9.174
PD = 9 months
TCR repertoire changes in PBMC  (baseline versus day 22)

**Adaptive Biotechnologies ImmunoSEQ platform**

NYU S14-00208
Ipilimumab and localized RT in chemo-refractory metastatic NSCLC
Same patient: PDL-1 up-regulation as a marker for the induction of an effective anti-tumor T cell response

CD8 (brown) Ki67 (red)

12.1% of CD8 T cells are Ki67+

Demaria and Stack, (PerkinElmer)
Radiation Dose, Fractionation Technique
Fractionated but not single dose RT elicits an abscopal response in combination with anti-CTLA-4

Differentially expressed Immune Response genes in at least one of 4 comparisons (>2-fold, Paired T-test p-value<0.05) are displayed as normalized to 0Gy control within each set of three samples.

Claire Vanpouille-Box

N. Coleman & M. Aryankalayil
NIH Radiation Oncology Branch
IFN-I pathway activation in irradiated tumors

Haller et al. 2007.
Cytokine & Growth Factor Reviews 18 (2007) 425–433
The delicate balance of cross-presentation

Need for sufficient naïve T cells
The Etiology of Treatment-related Lymphopenia in Patients with Malignant Gliomas: Modeling Radiation Dose to Circulating Lymphocytes Explains Clinical Observations and Suggests Methods of Modifying the Impact of Radiation on Immune Cells

Susannah Yovino¹, Lawrence Kleinborg¹, Stuart A. Grossman², Manisha Narayanan³, and Eric Ford¹

¹Department of Radiation Oncology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

8-cm tumor, 60 Gy/30 fractions modeled with Pinnacle™ radiation planning system

Radiation doses to circulating cells (DCC) analyzed using MatLab™

Circulating lymphocytes: 

\[
\begin{align*}
D_{10} &= 3 \text{ Gy} \\
D_{50} &= \sim 2 \text{ Gy} \\
D_{90} &= \sim 0.5 \text{ Gy}
\end{align*}
\]

A single radiation fraction delivered 0.5 Gy to 5% of circulating cells, after 30 fractions 99% of circulating blood had received \( \geq 0.5 \text{ Gy} \)

Naïve T cells are the most radiosensitive
Impact of Number of fractions, Dose rate, Target Size

High dose rate
Small, superficial fields
Hypo-fractionated RT

Yovino et al Cancer Invest. 2013
Conclusions

• RT-induced signaling interacts with multiple immunological pathways, including adenosine, TGF-β, PD-1 etc.

• Success of combination of anti-CTLA-4 and radiation in metastatic NSCLC was independent from PD-L1 expression/blockade. Conversely, effectiveness of blocking TGFβ likely depends on overcoming PD-L1 expression.

• Hypo-fractionated, short courses of RT to a small target to avoid lymphopenia may be key to the success of RT and immunotherapy.
Radiation and Immunity Research Team

Our patients