Radiotherapy with TGFβ Blockade Jump-Starts Tumor-Specific T Cells and Immune Checkpoint Inhibitors Keep Them Going

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The following relationships exist related to this presentation:

Honoraria/Advisory: 

Sanofi
Radiotherapy can generate an in situ tumor vaccine

Demaria & Formenti, Front Oncol 2012
Radiotherapy generates a suboptimal tumor vaccine

(Barcellos-Hoff et al., J Clin Invest 1994)

Formenti & Demaria, J Natl Cancer Inst 2013
TGFβ is key immunosuppressive cytokine
Hypothesis: TGFβ activation by radiation hinders priming of anti-tumor T cells

- **Activation of latent TGFβ**
- **Tumor antigen release in the presence of high levels of active TGFβ**
- **Anti-TGFβ (1D11)**
- **TAA Uptake by DC**
  - Inhibition of DC activation
- **Inhibition of T cell effector function**
- **Tolerance / suboptimal activation of anti-tumor T cells**

TDLN
Hypothesis: Blocking active TGFβ improves radiation-induced priming of anti-tumor T cells

Activation of latent TGFβ

Tumor antigen release in the presence of anti-TGFβ mAb

TAA Uptake by DC

Improved DC activation

Improved activation of anti-tumor T cells

Restored T cell effector function
Monitor responses to endogenous tumor antigens

- gp70
- twist
- survivin
- OVA
Preclinical models of metastatic breast cancer

**4T1**: spontaneous systemic metastasis

**TSA**: two synchronous tumors

Lungs
Metastases

« primary » tumor

« secondary » tumor

Abscopal effect: tumor response outside of the irradiation field

« primary » tumor
**TGFβ signaling in T cells**

**Diagram:**
- Smad 7
- Smurf 1/2
- Smad 2/3
- Smad 4
- TGFβR I
- TGFβR II
- Co-Transcription Factors
- Ski/SnoN
- TGFβ Target Genes
- Cytoplasm
- Nucleus

**Graphs:**
- Gated on CD4+ T cells
- Gated on CD8+ T cells

**Legend:**
- Control
- α-TGFβ
- RT alone
- α-TGFβ+RT

**PhosphoSmad 2/3**

- Red curve = Control; n=3 per group; triplicate

**Statistical Tests:**
- t Test – *p<0.05; **p<0.005; ***p<0.0005
Radiation-induced activation of tumor-infiltrating DC is enhanced by TGFβ blockade.
Priming of tumor-specific CD8 T cells by radiation requires TGFβ blockade

Claire Vanpouille-Box
Therapeutic synergy of radiation and TGFβ blockade
Therapeutic synergy of radiation and TGFβ blockade
Upregulation of immune system activation pathways

- Control
- α-TGFβ
- RT alone
- RT + α-TGFβ

Bar chart showing changes in gene expression levels with different treatments.
Increased TILs in irradiated and non-irradiated tumors by radiation and TGFβ blockade treatment

CD8+ TILs

Sham + Isotype

Sham + 1D11

RT+Isotype

RT+1D11

4T1 irradiated tumor

TSA non-irradiated tumor

**Positive cells/field ± SD**
**Therapeutic effect of radiation and TGFβ blockade is mediated by T cells**

The figure illustrates the therapeutic effect of radiation and TGFβ blockade on tumor growth and lung metastases. The diagram shows the timeline of tumor growth/ regression with immunotherapy treatments.

- **Sham + Isotype**
- **RT + Isotype**
- **RT + 1D11**
- **RT + 1D11 + CD4 depletion**
- **RT + 1D11 + CD8 depletion**

**Tumor volume (mm^3) ± SEM**

- Days post tumor cells injection:
  - 12, 14, 16, 18, 20, 22, 24, 26, 28

- **Lung metastases**

- **Sham**
- **RT**
- **Isotype**
- **1D11**
- **CD4 depletion**
- **CD8 depletion**

Therapeutic effect of radiation and TGFβ blockade is mediated by T cells.
Conclusions:

- TGFβ blockade is required for radiation-induced priming of T cells specific for endogenous tumor antigens.

- Elicited anti-tumor T cells inhibit growth of the irradiated tumor and non-irradiated metastases.

- TGFβ is a master regulator of radiotherapy ability to generate an in situ tumor vaccine.
Fresolimumab 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)

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Increase in tumor-specific CD8 T cells in patients treated with radiotherapy + fresolimumumab
Is adaptive immune resistance limiting tumor response to radiotherapy + TGFβ blockade?
Increased PD-1 expression on TILs by radiation and TGFβ blockade
Increased PDL-1 and PDL-2 expression on tumor and myeloid cells by radiation and TGFβ blockade
PD-1 blockade extends survival in mice treated with radiation and TGFβ blockade

![Graph showing tumor volume and survival](image)

- **Days post tumor cells injection**
  - Tumor volume (mm³) ± SEM
  - % Survival
  - N=19/group
Lessons and Take Home Messages

• TGFβ neutralization during radiotherapy is required to generate an in situ vaccine in two mouse models of metastatic breast cancer.

• Early data in patients with metastatic breast cancer suggest a survival benefit of TGFβ neutralization during radiotherapy.

• Adaptive immune resistance limits the effectiveness of the anti-tumor immune response elicited by radiation + TGFβ blockade.

• Addition of anti-PD-1 delays tumor recurrence and extends survival.
Lessons and Take Home Messages

• Anti-TGFβ and anti-PD-1 mAbs target different immunosuppressive pathways but did not have any effect alone or when combined in the absence of radiotherapy in the poorly immunogenic 4T1 breast cancer model.

• Radiotherapy is a suitable partner for combination with multiple immunotherapies.
Thank you!