

Is There a Role for Radiation Therapy and Immunotherapy?

Gilda G. Hillman, PhD

Professor

Karmanos Cancer Institute

Presentation prepared in collaboration with

Stephen Shiao MD, PhD

Department of Radiation Oncology

Cedars-Sinai Medical Center

Disclosures

- 💧 No relevant financial relationships to disclose

A two-way winner: Radiation to Enhance Immunotherapy Or Immunotherapy to Enhance Radiation

Radiation limitations: Considerable tumor debulking achieved by radiotherapy for solid cancers; however tumors recur locally due to radioresistance resulting in cancer progression.

Immunotherapy limitations: Is not effective in patients with large tumor burdens and compromised by tumor-induced immunosuppression.

Strategy: Radiotherapy combined with immunotherapy/cancer vaccine to enhance a local and systemic anti-tumor immune response.

Rationale: To reduce large tumor burdens localized in the primary tumor by radiation and eradicate local residual tumor and metastases by inducing a specific and non-specific anti-tumor immune response with cytokine gene therapy, cancer vaccines, or targeting immune checkpoints.

Outline

- Radiation induces immune events in tumors and their microenvironment
- Radiation combined with immunotherapy approaches to augment anti-tumor immune response
- Radiation combined with immunotherapy to target immune suppression and enhance anti-tumor immune response

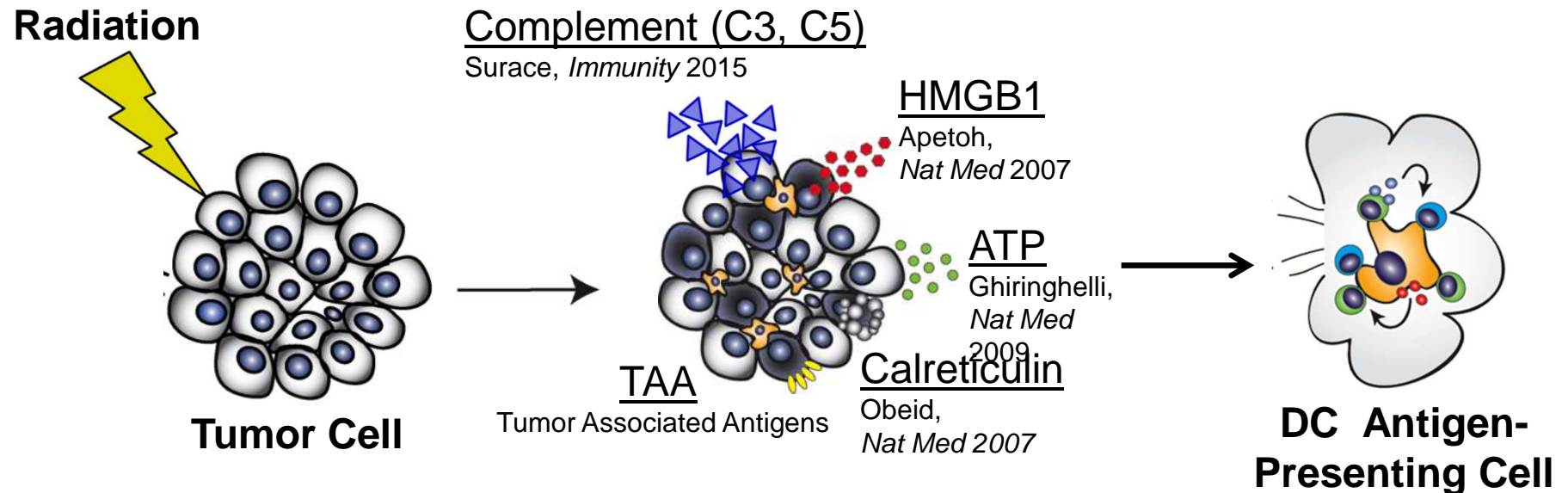
Radiation Kills Tumor Cells, and is also Immunomodulatory

Radiation induced tumor cell killing: Traditional research in radiation has focused on mechanisms of cell killing: direct and indirect DNA damage , ROS production, tumor cell kill/survival.

Radiation induced modulation of tumor micro-environment (TME): Recent research focuses on inflammatory responses induced by radiation destruction of tumor cells, disruption of stroma and vasculature in TME and their role at favoring or suppressing immune responses against tumor.

Immune Stimulation by Radiation-Induced Tumor Cell Killing: Activation of Dendritic Cells (DC) for TAA presentation

Immunogenic Cell Death: Factors released from dying tumor cells activate TAA presentation for induction of anti-tumor immune response



Complement (C3, C5): upregulated by RT, binds to receptor on DC and causes DC maturation

HMGB1: binds to toll-like receptor TLR-4 on DC

ATP: triggers DC secretion of IL-1 and IL-6

Calreticulin: uptake of tumor cells by DC

TAA: uptake of TAA by DC released from dying tumor

Radiation Enhances Multiple Inflammatory Pathways: *In Situ* Vaccination

Initiation of Local Inflammation

Increased cytokines:

IL-1, TNF α , Type I Interferons

Hallahan, *PNAS* 1995; Deng, *Immunity* 2014

DC Migration to LN
Enhanced Cross
Presentation

Reits, *J Exp Med* 2006

Increased IFN γ production

Lugade, *J Immunol* 2008

Tumor Destruction

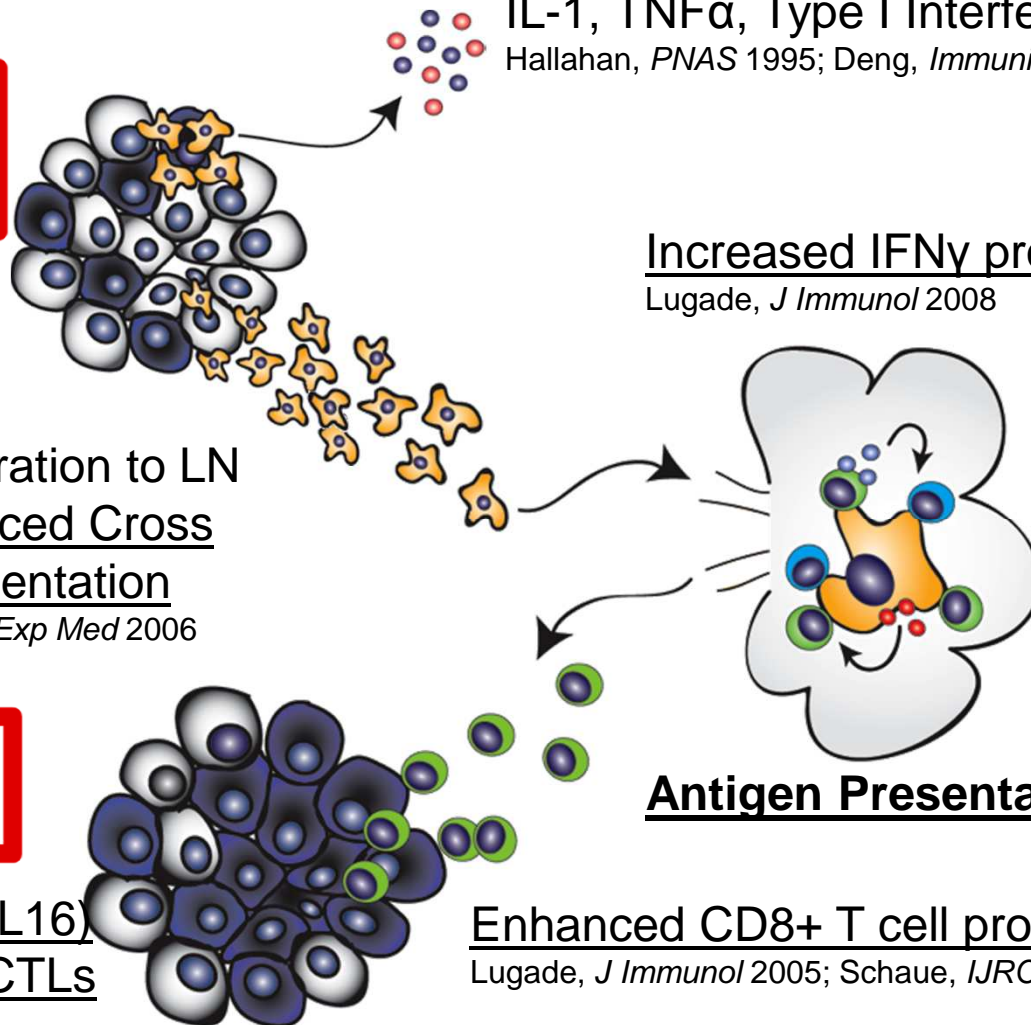
Increased chemokine (CXCL16)
production to attract CD8+ CTLs

Matsumura, *J Immunol* 2012

Antigen Presentation

Enhanced CD8+ T cell production

Lugade, *J Immunol* 2005; Schaue, *IJROBP* 2012



Radiation Can Also Promote Immunosuppressive Mechanisms

Resolution of Radiation-Induced Inflammatory Response

M2 macrophages
secrete IL-10, $\text{TGF}\beta$

Promote tumor growth

Tsai *IJROBP* 2007; Chiang *Front Oncol* 2012

Increased regulatory T cells

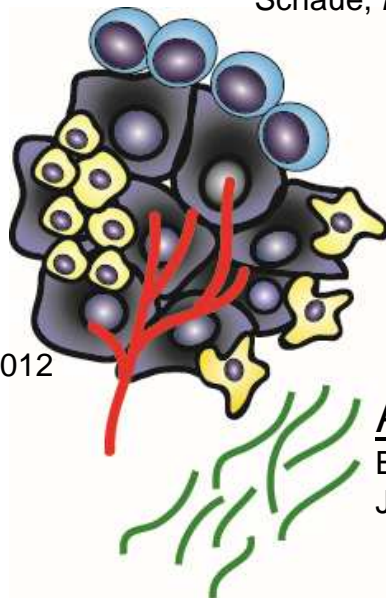
Schaue, *IJROBP* 2012; *Front Oncol* 2012;
Kachikwu *IJROBP* 2011

Upregulation of PD-L1

Deng, *J Clin Invest* 2014; *Oncoimmunol* 2014

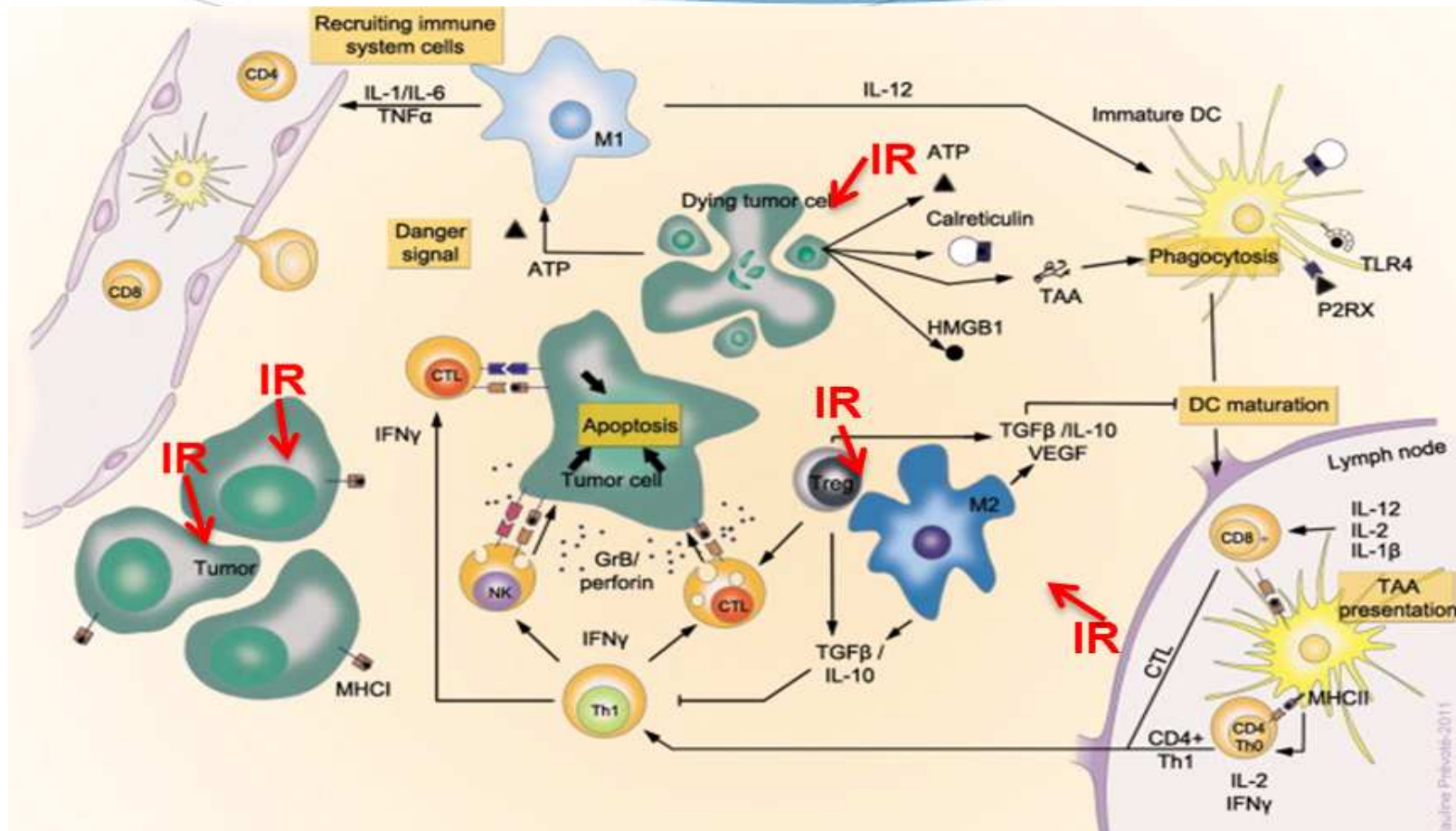
Activation of $\text{TGF}\beta$

Barcellos-Hoff *J Clin Invest.* 1994;
Jobling *Radiat Res.* 2006



Like with any immune response, the immune system regulates and controls RT-induced inflammation

Immunological Effects of Ionizing Radiation Summary



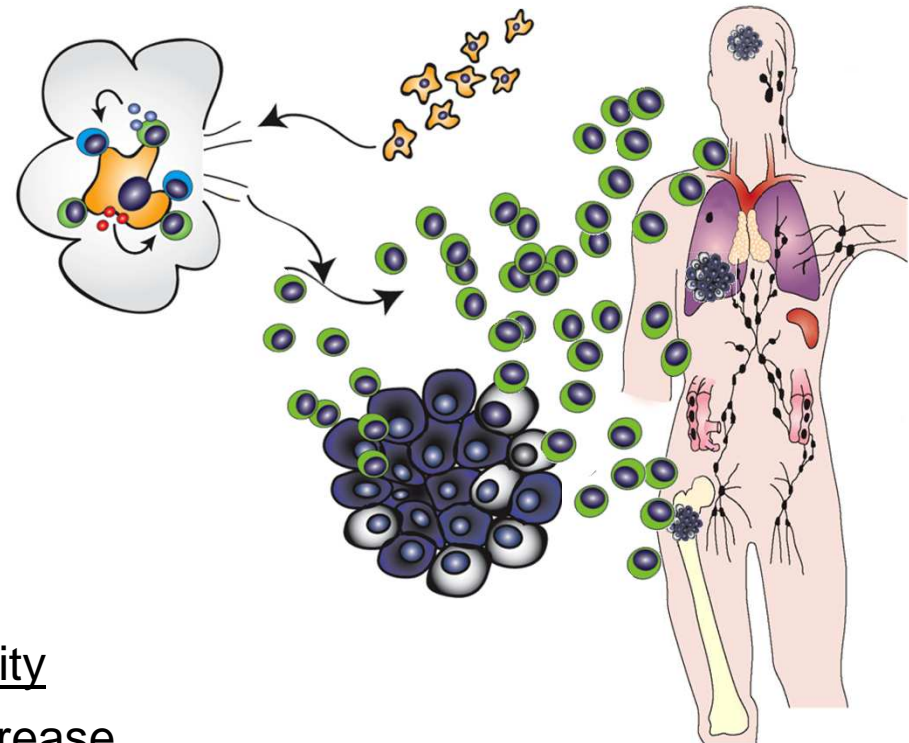
1. **Factors released from tumor:** ATP, calreticulin, HMGB1, TAA activate cell-mediated immunity.
2. **Activated tumor associated macrophages (TAM)-M1** secrete IL-1 and IL-6 and activate DC that migrate to the lymph nodes to expose the TAA to CD4+ and CD8+ T cells.
3. **Anti-tumor immune response:** NK cells and CTL, mature DC can activate a strong Th1 immune response that produces IFN-g.
4. **T-regulatory cells (Tregs) and TAM-M2** may **repress** immune response by secretion of cytokines IL-10, TGF-b

Radiation can also induce distant effects: “The Abscopal Effect”

- Abscopal effect: Rare clinical cases of regression at distant metastatic sites after tumor irradiation.
- Indicates some degree of anti-tumor immune response by radiation but not sufficient to achieve immune mediated tumor rejection.

Induction of Systemic Anti-tumor Immunity

- Addition of immunotherapy could increase abscopal effects by inducing systemic anti-tumor immunity such as strategies to improve cross-priming of anti-tumor T cells.



Demaria, *IJROBP* 2004; Sharabi, *Cancer Immunol Res* 2014
Formenti, *JNCI* 2013

Summary

- Radiation is immunomodulatory, produces a targeted *in situ* vaccination by triggering immunogenic cell death that could enhance an anti-tumor immune response.
- However, no specific and lasting anti-tumor immune response is caused by radiation alone, probably due to tumor-mediated immunosuppression and radiation-induced immunosuppression.

Outline

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Key Question

How do we enhance the immune response induced by radiation?

1. Augment the anti-tumor immune response induced by RT using immunotherapy approaches
2. Prevent the innate tumor and radiation-induced suppression of the anti-tumor immune response by blocking immuno-suppressive events

Augmenting the immune response: Pre-clinical data

- By ***increasing*** the immune response, multiple groups have shown that immunotherapy can enhance the radiation-induced anti-tumor immune response. Some examples from murine models showing improved efficacy with RT include:
 - **Adjuvants** – CpG (TLR9 agonist), Imiquimod (TLR7 agonist)
 - **Cytokines** – IL-2, IL-12, GM-CSF, Flt-3L
 - **T cell Costimulation** – OX-40L Ab, 4-1BBL Ab, ICOSL Ab
 - **Antigen Presentation** – DC vaccines, Viral vaccines

Tumor Irradiation Enhances Systemic Cytokine and Intratumoral Gene Immunotherapy

Hillman Lab Pioneer Studies 1990-2005

Synergy of local tumor irradiation with systemic IL-2 therapy and gene-mediated therapy

Demonstrated in renal, lung, prostate and bone pre-clinical tumor models

Mechanisms:

- DNA damaging and tissue-debulking effects that decrease tumor burden to be targeted by immunotherapy.
- Radiation could enhance the permeability of the tumor allowing a greater influx of activated immune cells inside of the nodules.
- Radiation increases gene transduction efficiency and duration of expression thus increases efficiency of in situ gene modification leading to immune response.

Key points for translation to clinical issues:

- Sequence of radiation followed by gene therapy is more effective and takes advantage of:

(a) Presence of inflammatory cells in the vicinity of the tumor mobilized by radiation participating in cytokine/gene therapy triggered immune response.

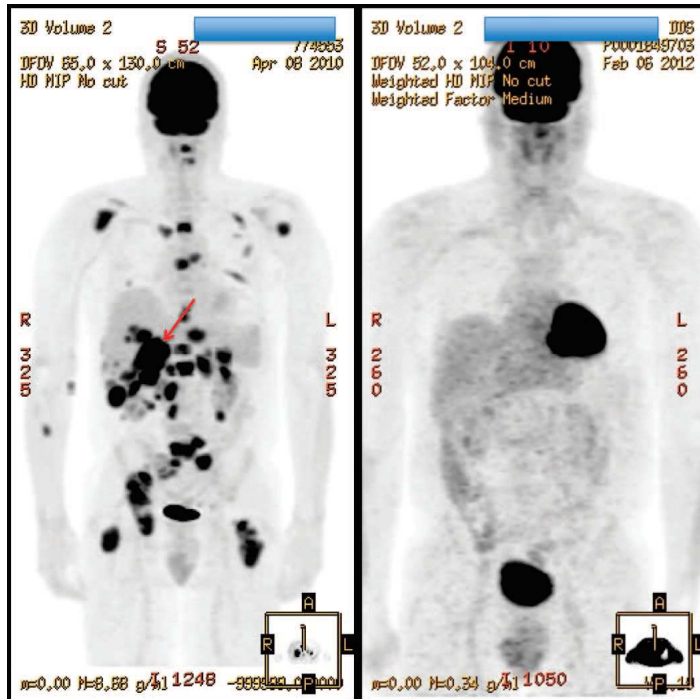
(b) Pool of tumor proteins and peptides generated from radiation-induced tumor apoptosis.

- Higher dose of radiation to tumor prior to gene therapy is more effective

Hillman et al., Cellular Immunol, 1995, Clin Cancer Res, 1996, 2001; IJROPB, 2003, Human Gene Therapy, 2003; Cancer Gene Therapy, 2004; Human Gene Therapy, 2005

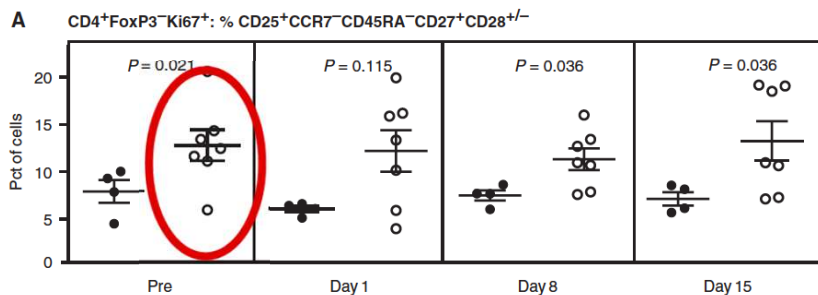
Augmenting the immune response: Clinical examples

Metastatic Melanoma PET imaging
before and after SBRT to liver lesions+ IL-2



IL-2 and RT (Sueng SK *Sci Transl Med* 2012)

- IL-2 is a cytokine stimulating proliferation and differentiation of CD4+ and CD8+ T-cells and has shown synergy when given after tumor irradiation in pre-clinical models of kidney, lung, prostate, bone (Hillman 1992-2005)
- IL-2 + SBRT (20 Gy x 1,2 or 3 fractions) in metastatic RCC / melanoma showed CR in 8/12 pts
- Higher frequency of CD4+ T cells with an early activated memory phenotype in responders



Augmenting the immune response: Clinical examples

DC Vaccination and Fractionated RT (Finkelstein *Immunotherapy* 2012)

- High risk prostate cancer pts tx with ADT +EBRT 45Gy (25 fractions of 1.8 Gy) + 3 x DC injections into prostate after RT fraction 5,15,25
- Autologous DCs were cultured in vitro and reintroduced directly into the prostate
- Serial bx show tumor cell apoptosis and prostate specific CD8+ T cells in the peripheral blood, but limited increase in tumor-

Table 3. Quantitation of therapy-related changes and apoptosis among visible tumor cells within the biopsy and CD4⁺ or CD8⁺ infiltrates among 22 evaluable specimens.

Time point	Subject 1		Subject 2		Subject 3		Subject 4		Subject 5	
	CD4	CD8	CD4	CD8	CD4	CD8	CD4	CD8	CD4	CD8
Baseline biopsy	1+	1+	1+	1+	1+	1+	1+	1+	0	1+
At fraction 5	1+	1+	1+	1+	1+	1+	0	1+	0	1+
At fraction 15	1+	1+	1+	1+	0	0	ND	ND	0	1+
At fraction 25	1+	1+	0	1+	1+	1+	ND	ND	1+	1+
>3 months later	1+	1+	0	0	1+	1+	ND	ND	ND	ND

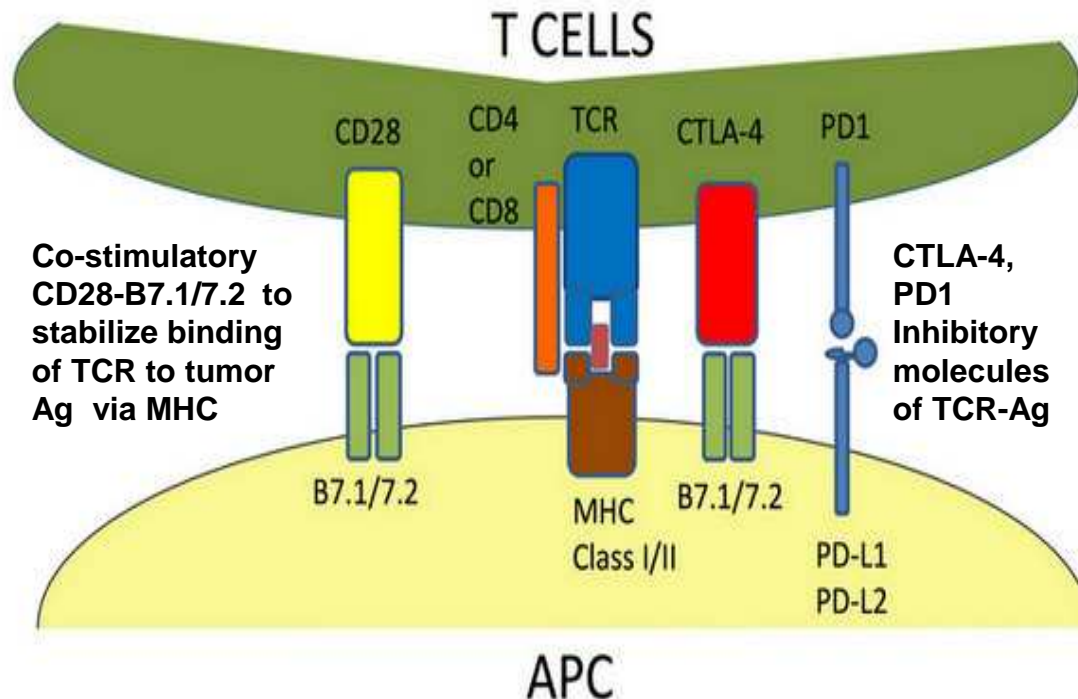
CD4, CD8 infiltrates: 0/1+/2+/3+.
ND: No data.

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Targeting Immune Checkpoints: Immune inhibitory receptors from tumor cells and stroma in tumor microenvironment

Antigen presentation to TCR (T cell receptor) via peptide-MHC complex in CD4 Th and CD8 CTL



Immune Checkpoints:

- **CTLA-4:** Cytotoxic T-Lymphocyte Antigen 4 (CD152), expressed on T cells, inhibitory competitor for CD80 (B7-1) and CD86 (B7-2) co-stimulation of T cells through CD28 resulting in blocking T cell activation.

Ipilimumab (Yervoy): Anti-CTLA-4 in melanoma clinical trials, approved by FDA (2011)

PD-1: Programmed Cell Death: expressed on activated T cells, B cells and myeloid cells. T cell Inhibition and apoptosis if **PD-1 binds** to its ligands **PD-L1 or-2** expressed on tumor cells/APC.

To inhibit this pathway, antibodies and soluble PD-1 ligands are used.

CTLA-4 and PD-1 Dual blockade approach and cancer vaccines: under consideration based on pre-clinical studies (Cancer Res 2013).

Preventing tumor immune suppression

Checkpoint inhibitors – Anti-CTLA-4

Preventing inhibition of the immune response has also been shown not only to enhance RT, but also potentiate a systemic response

- **Pre-Clinical:** Anti-CTLA-4 (Dewan MZ, CCR 2009)
 - CTLA-4 blocking Ab is synergistic with RT to produce greater tumor inhibition in irradiated s.c. tumor and an **abscopal** response in secondary non-irradiated s.c. tumors of murine cancer models.
 - Specific doses are critical: 8 Gy x 3 is more effective than 20 Gy x 1 or 6 Gy x 5 in mouse models with anti-CTLA-4 therapy
- **Clinical:** anti-CTLA-4 Ipilimumab: CTLA-4 inhibition allows increased proliferation and function of activated T-cells, including tumor-specific CTLs
 - Several retrospective studies showed improved survival treating brain mets with SRS and ipilimumab with limited toxicity except at the highest doses of RT (Barker and Postow. *IJROBP* 2014)
 - Multiple phase I/II trials are currently enrolling testing the safety and efficacy of RT + Ipilimumab



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BRIEF REPORT

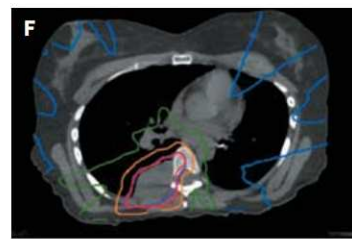
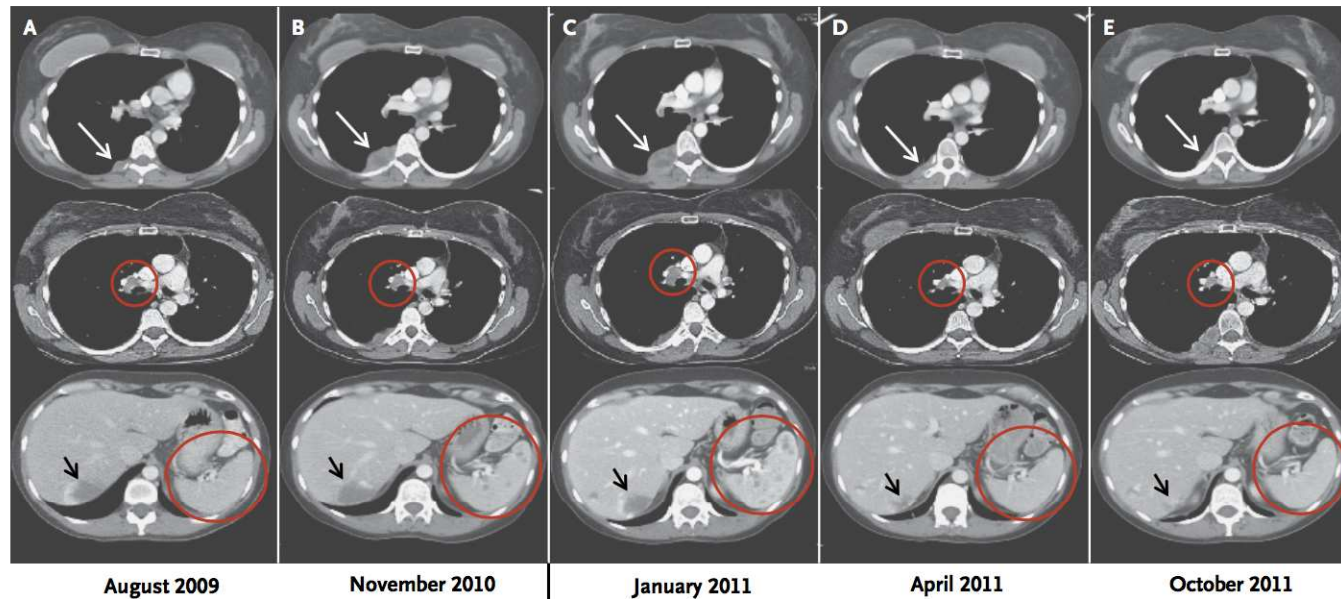
Immunologic Correlates of the Abscopal Effect in a Patient with Melanoma

Michael A. Postow, M.D., Margaret K. Callahan, M.D., Ph.D.,
Christopher A. Barker, M.D., Yoshiya Yamada, M.D., Jianda Yuan, M.D., Ph.D.,
Shigehisa Kitano, M.D., Ph.D., Zhenyu Mu, M.D., Teresa Rasalan, B.S.,
Matthew Adamow, B.S., Erika Ritter, B.S., Christine Sedrak, B.S.,
Achim A. Jungbluth, M.D., Ramon Chua, B.S., Arvin S. Yang, M.D., Ph.D.,
Ruth-Ann Roman, R.N., Samuel Rosner, Brenna Benson, James P. Allison, Ph.D.,
Alexander M. Lesokhin, M.D., Sacha Gnjjatic, Ph.D.,
and Jedd D. Wolchok, M.D., Ph.D.

Radiotherapy
+ Ipilimumab

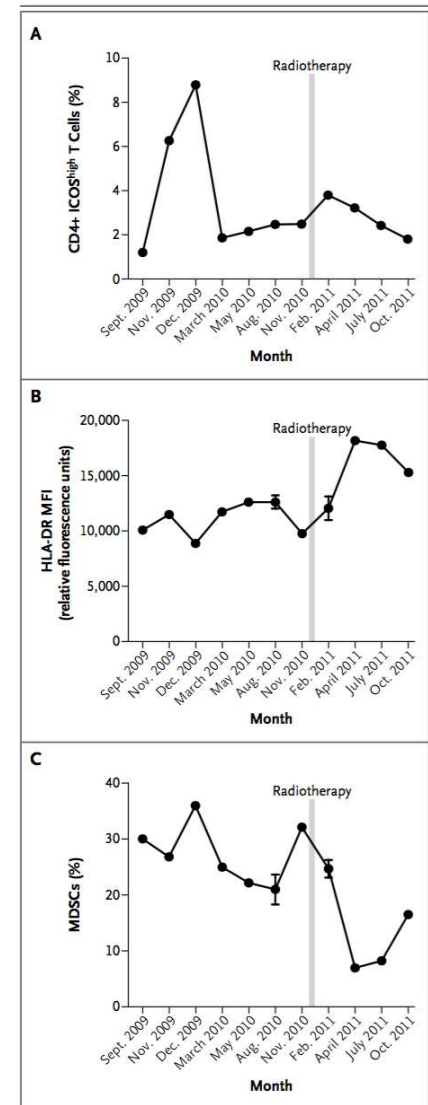
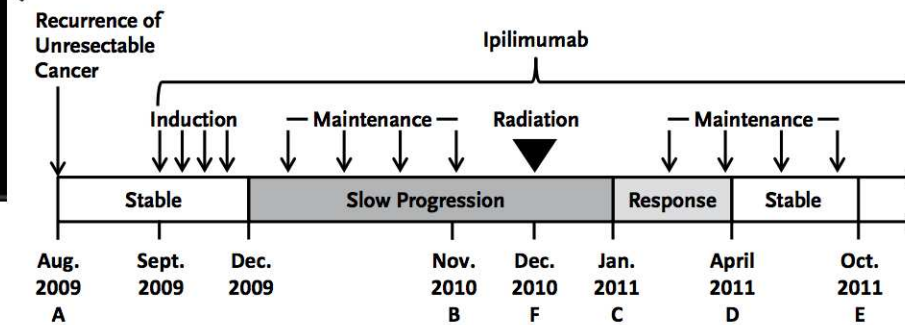
N Engl J Med Volume 366(10):925-931 March 8,
2012

RT/Ipi can induce distant immune-mediated tumor regression



December 2010

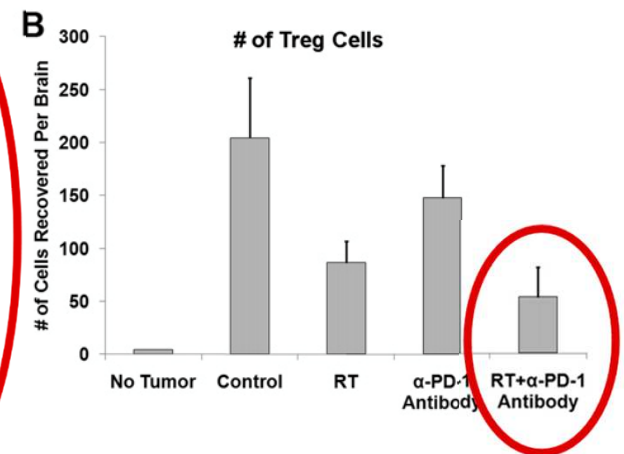
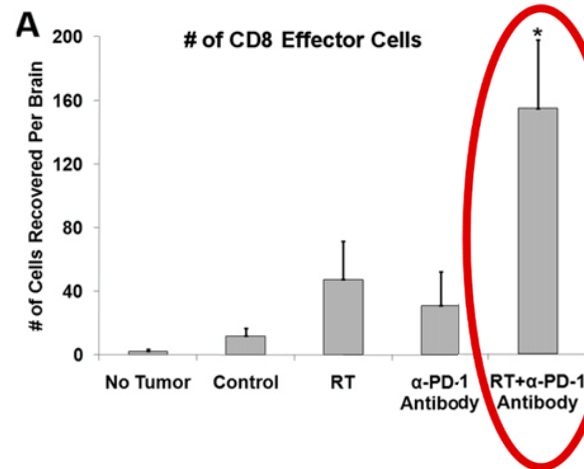
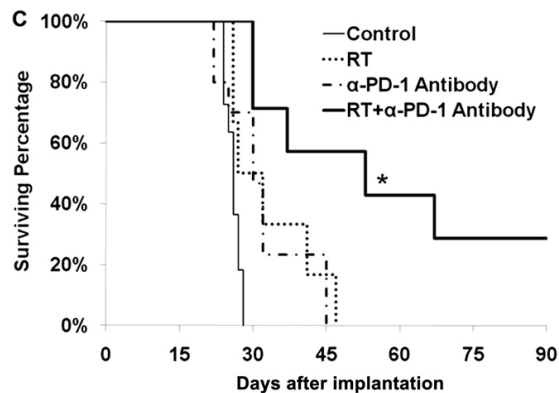
9.5 Gy *
3 fractions



Preventing tumor immune suppression: Checkpoint inhibitors – anti-PD-1

Single high dose RT + anti-PD-1 (Zeng J, *IJROBP* 2013)

- Glioma model
- 10 Gy RT+ 3 injections of anti-PD-1 treatment **increased survival** and **tumor infiltration by cytotoxic T cells** (CD8+/IFN- γ +/TNF- α +) and **decreased regulatory T cells** (CD4+/FOXP3+)

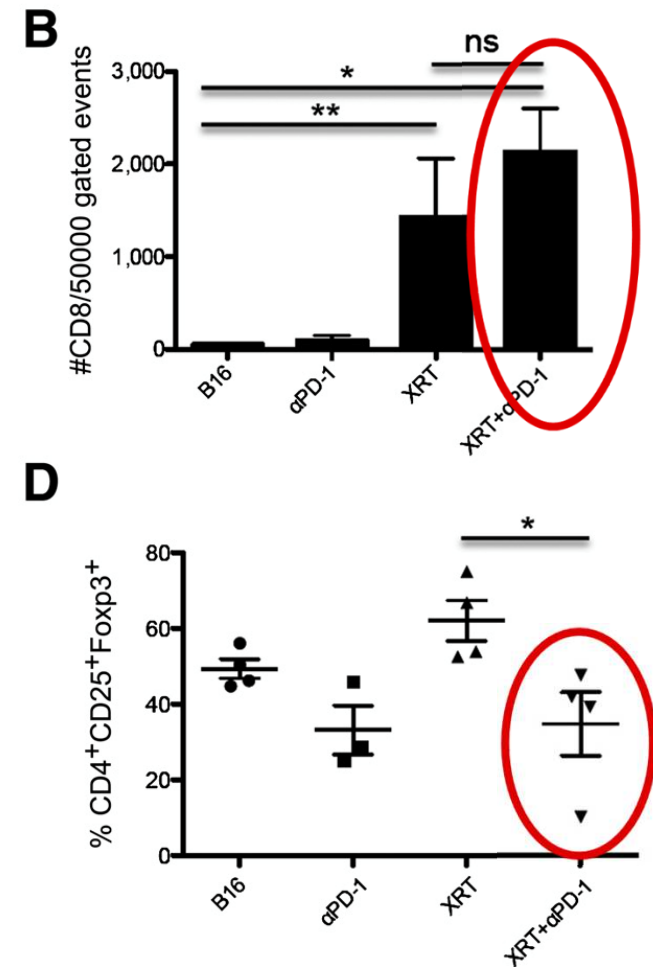


Preventing tumor immune suppression: Checkpoint inhibitors – anti-PD-1

Single high dose RT + PD-1 blockade

(Sharabi A et al, *Cancer Immunol Research* 2014)

- Melanoma tumor model
- 12 Gy RT and 3 x anti-PD-1 immunotherapy decreased percentages of CD4+CD25+FOXP3+ Tregs but increased CD8+ T-cell infiltration into tumors and presentation of tumor antigen



Role for Radiation and Cancer Immunotherapy:

Recent Developments: The years 2000...

Major Points from recent studies supporting previous hypotheses:

- Radiation modulates tumor immunity in tumor microenvironment, and could have distant abscopal effects mediated by inflammatory cytokines TNF, IL-1, IL-6 increased by radiation ??
- Cell death induced by radiation generates increased pool of tumor-associated antigens and uptake by tumor associated macrophages and dendritic cells triggering immune response.
- Radiation enhances immune cell trafficking via chemokines and vascular damage in tumor facilitating activated immune cell infiltration.
- Neutralizing the immunosuppressive effects of the tumor microenvironment can lead to enhanced responses locally and systemically

Potential Impact on the field:

- Over 35 trials are currently open throughout the nation studying combinations of RT and immunotherapy including Cancer Vaccines and Checkpoint Inhibitors (CTLA-4, PD-1, PD-L1)

Lessons Learned:

- Combinations of RT with immunotherapy have been shown in pre-clinical and clinical studies to be synergistic with the most promising combinations thus far being with checkpoint inhibitors

Levy A. et al., *Critical reviews in Oncology Hematology*, 2012

“Radiation Therapy and Immunotherapy: Implications for a combined cancer treatment”

Formenti et al., *J. National.Cancer Inst*, 2013

“Combining Radiotherapy and Cancer Immunotherapy: a paradigm shift “.

How to Improve Efficacy of Radiation and Immunotherapy

Critical Clinical Issues: To consider when combining radiotherapy and immunotherapy

- **Sequence** of both modalities and frequency of administration of immunotherapy
- **Radiation dose and schedule**, high doses versus fractionated radiation
- **Route of Administration** of immunotherapy involving cancer vaccines, gene therapy or antibody therapy:

Intratumoral versus Systemic