1st ADVANCED AIRB COURSE IN RADIOBIOLOGY
BRESCIA MEETINGS IN RADIATION ONCOLOGY – 2015 EDITION
THE POWER OF BIOLOGY

Exploiting new evidences of drug-interactions in clinics. *Immunology, Immunotherapy and Radiotherapy*

Marco Trovò

Brescia, October 9th 2015

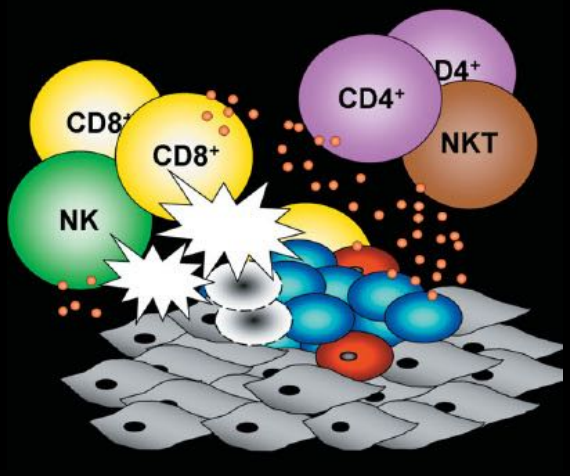
Outline

- Cancer and Immune System
- Radiation and Immune System
- Preclinical Combinations of Radiation and Immunotherapy
- Clinical Combinations of Radiation and Immunotherapy

Cancer and Immune System

Tissue changes during neoplastic transformation are sensed by *innate immune system*

Elimination phase

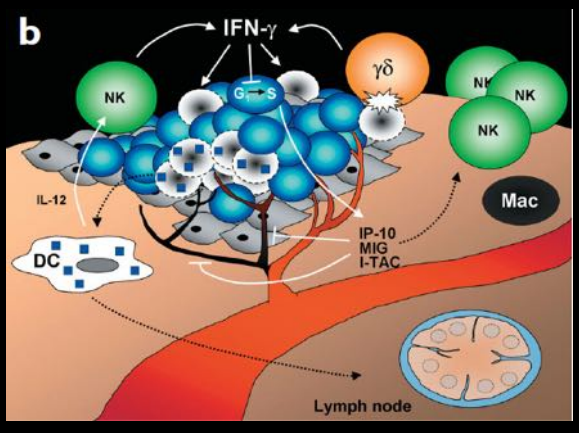


Interferon γ – a mediator of immunosorvellance against tumors - is produced by natural killer to promote cytotoxic activity of macrophages.

Cancer and Immune System

Tissue changes during neoplastic transformation are sensed by *innate immune system*

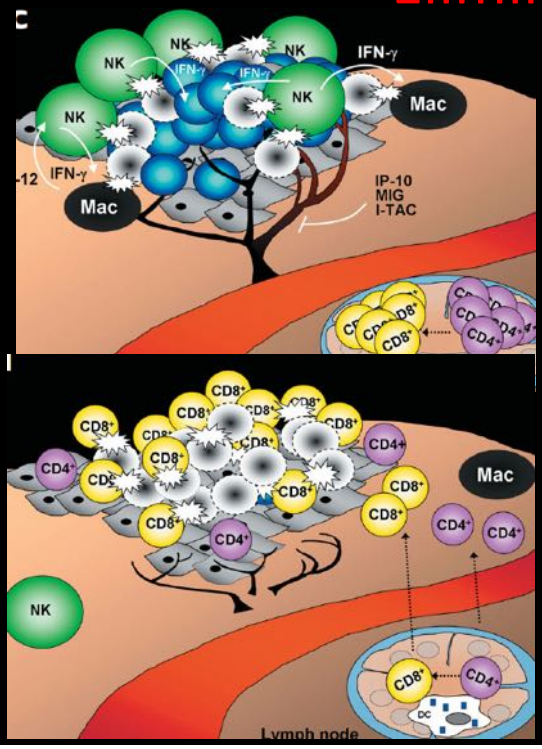
Elimination phase



The cytocidal activity of innate immune cells leads to the release of TAA for cross presentation to DC

Cancer and Immune System

Elimination phase



DC process the antigens into peptides that can be recognised by CD8 and CD4 T cells : → activation of tumor-specific T cells to perform antitumor activities

Cancer and Immune System

Equilibrium phase

between proliferation and killing of tumor cells by T cells.

Vol 450|6 December 2007|doi:10.1038/nature06309 nature

LETTERS

Adaptive immunity maintains occult cancer in an equilibrium state

Catherine M. Koebel[1], William Vermi[1,2], Jeremy B. Swann[3,4], Nadeen Zerafa[3], Scott J. Rodig[5], Lloyd J. Old[6], Mark J. Smyth[3,4]* & Robert D. Schreiber[1]*

Cancer and Immune System

Escape phase

Tumor cells continue to engage the immune system, which can still slow down the tumor progression

Oncogene (2010) 29, 1093–1102

www.nature.com/onc

REVIEW

Immune infiltration in human tumors: a prognostic factor that should not be ignored

F Pagès[1,2,3,4], J Galon[1,3,4], M-C Dieu-Nosjean[3,4,5], E Tartour[2], C Sautès-Fridman[3,4,5] and W-H Fridman[2,3,4,5]

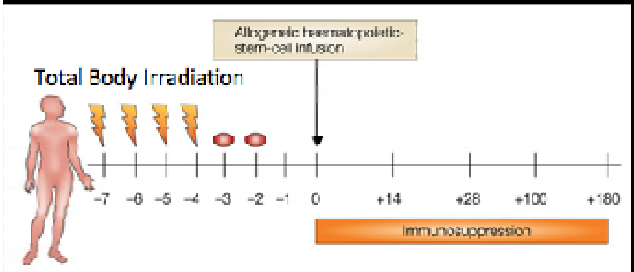
Cancer and Immune System

Opportunity to recover effective immune reactivity:

- Radiotherapy may promote the release of tumor neoantigens in an immunogenic way
- Strategies to overcome dominant immonosoppressive pathways

Radiation and Immune System

Low-doses systemic RT



Bleakley & Riddell, Nature Rev Canc 2004

Total Body Irradiation
- Myeloablative
- Immunosuppressive

High-doses local RT

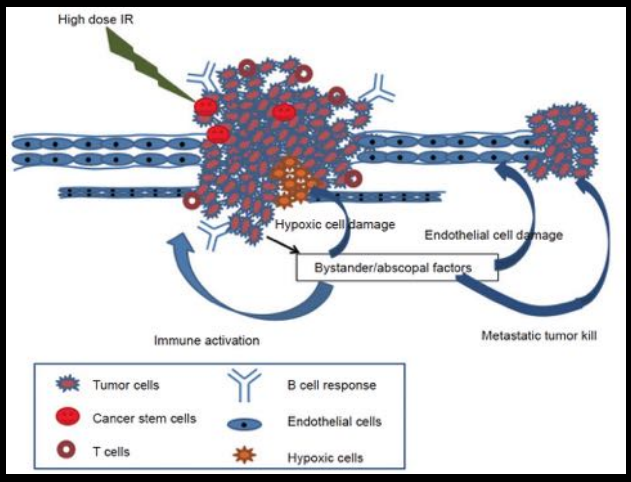
Editorial

Int Journal of Radiat Oncol Biol Phys 2012

Radiation Therapy to Convert the Tumor Into an In Situ Vaccine

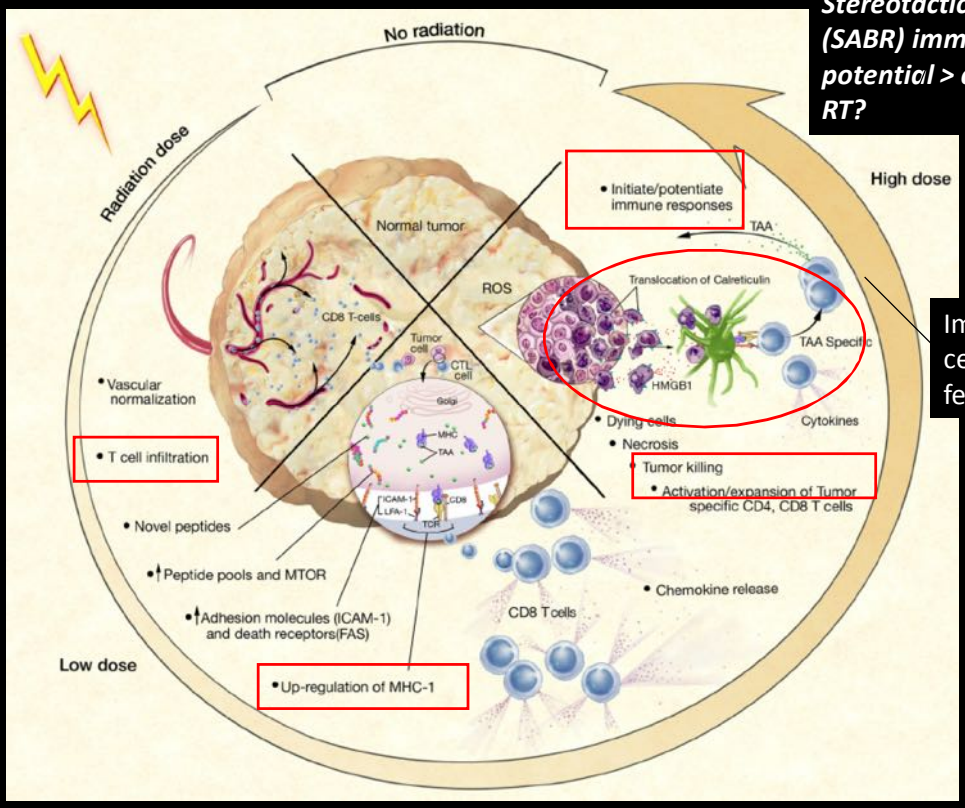
Silvia C. Formenti, MD,* and Sandra Demaria, MD†

*Departments of *Radiation Oncology, and †Pathology, New York University School of Medicine and NYU Langone Medical Center, New York, New York*



Radiation and Immune System

Stereotactic Ablative RT (SABR) immunogenic potential > conventional RT?



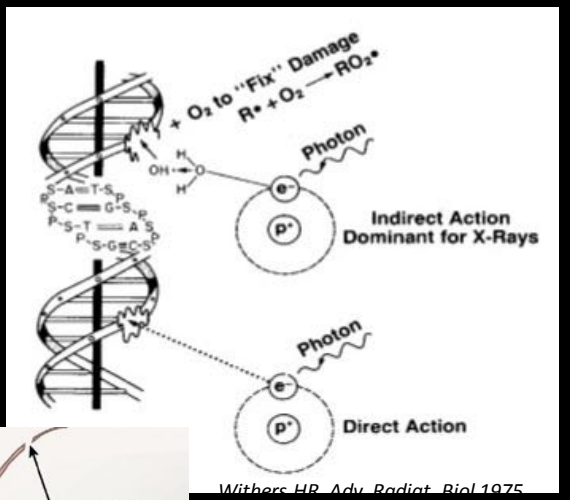
Immunogenic cell death (ICD) features

Tumor Rejection by the immune system as the "5th R" of radiobiology

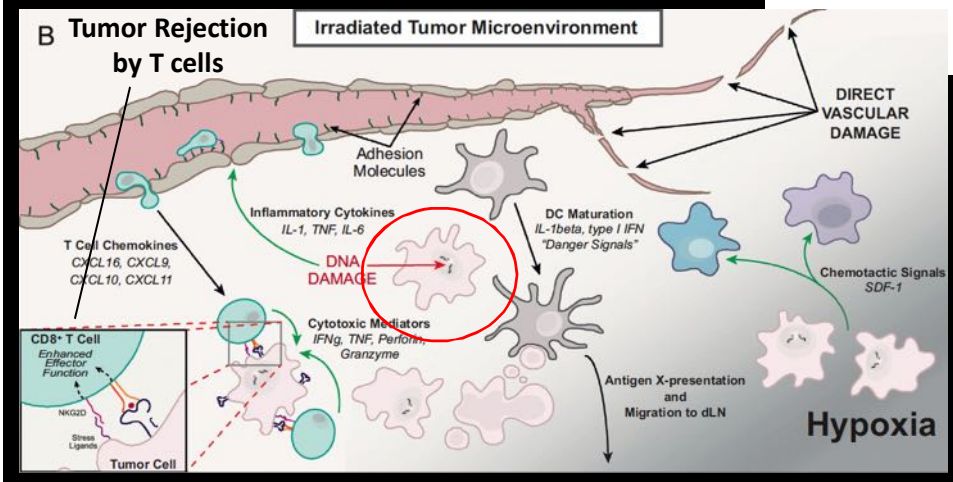
The "4 R's" of radiobiology
1. Redestribution of cells into radiosensitive phases of the cell cycle (G2/M)
2. Reoxygenation of hypoxic cells in a tumor core
3. Repair of sublethal damage
4. accellerated Repopulation of cells due to proliferation

\+ intrinsic Radiosensitivity

Effects caused:
- directly with a damage on DNA of tumor cells
- indirectly after the induction of free radicals

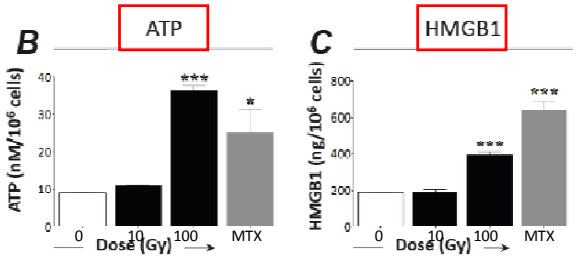


Withers HR. Adv. Radiat. Biol 1975



Active crosstalk between tumor microenvironment and immune system

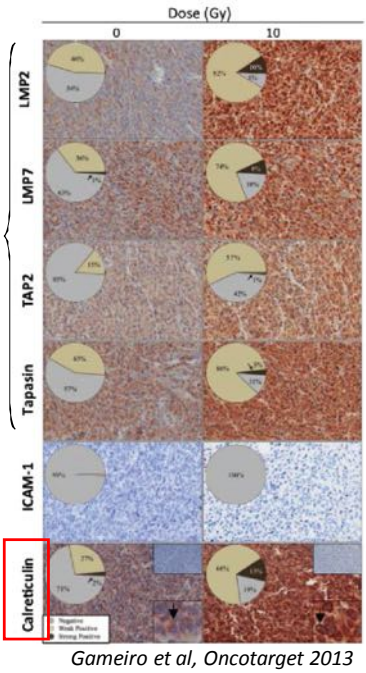
Radiation induces *in vitro* the hallmarks of immunogenic cell death



modified from Gameiro et al, Oncotarget 2013

- radiation-induced immunogenic modulation of tumor enhances antigen-processing and calreticulin exposure, resulting in enhanced T-cell killing

Nude mice bearing prostate xenografts



Antigen-processing machinery components

Immunogenic cell death (ICD) features

Calreticulin

Gameiro et al, Oncotarget 2013

Radiation and Immune System

Immune Response Background

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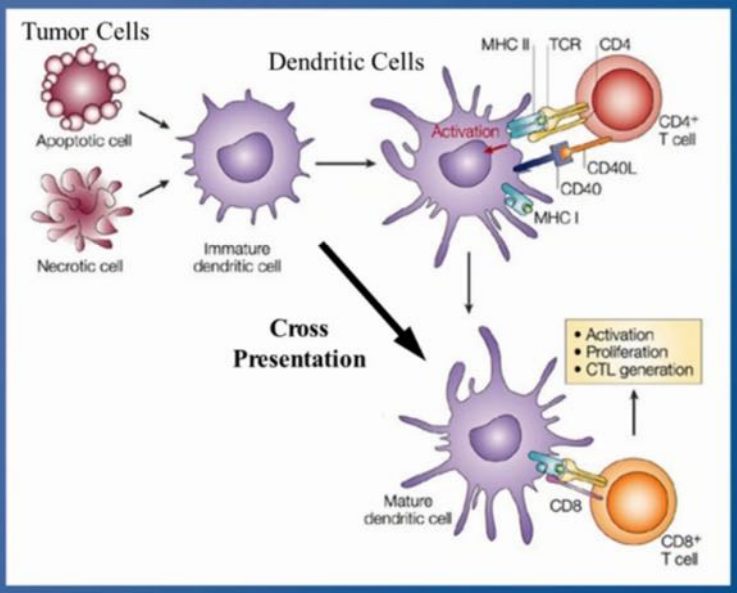

CD4 Helper T-cells

CD8 Cytotoxic T-cells

Nature Reviews Immunology

Radiation and Immune System

Radiation and Immune Responses

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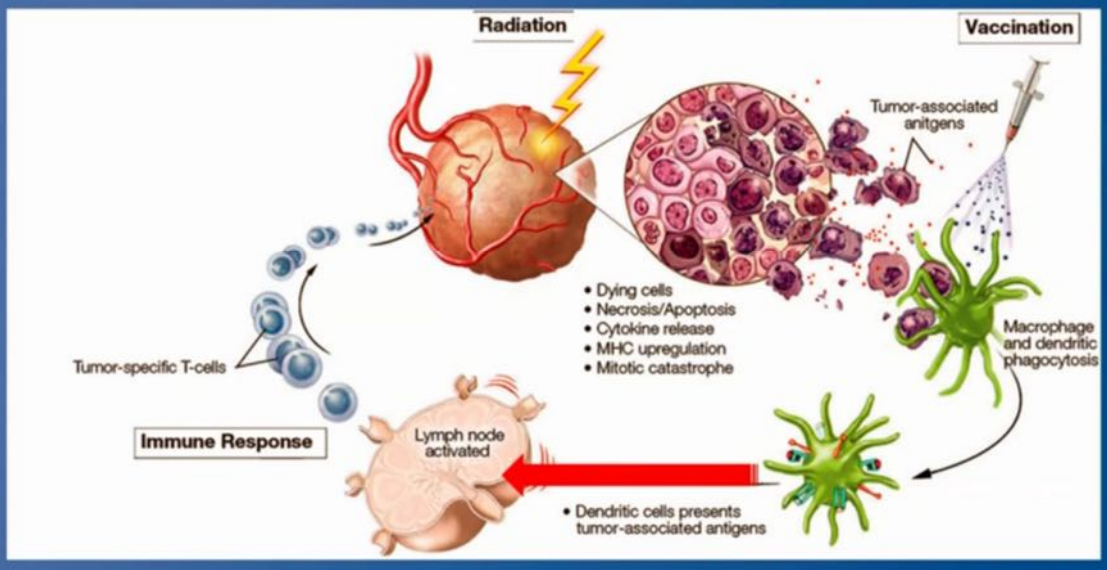


Kamrava M., Hodge JW., et al. Mol. BioSyst., 2009 - Adapted with Permission

Radiation and Immune System

Radiation-induced tumor cell death
<–>
priming of antitumor T-cell response

1. Cell death is an efficient process to transfer antigens from tumor cells to DC
→ activation of tumor-specific T cells

2. Signals for achieving an "immonogenic cell death":
   - Cell surface translocation of calreticulin
   - Extracellular release of high mobility group protein B1 (HMGB1)
   - Release of ATP

Radiation and Immune System

The relationship between Radiotherapy and the Immune system can explain 3 clinical scenarios:

1. Effect of local control on survival

2. Success of concurrent chemo-radiation vs. sequential chemotherapy and radiation

3. Abscopal effect

Radiation and Immune System

1. Effect of local control on survival.

Two metaanalyses of randomized trials in breast cancer demonstrated a direct contribution of adjuvant radiotherapy to patients' long-term survival.

→Successful immunization against the primary tumor once residual microscopic disease at the tumor bed and involved nodes is irradiated.

→Immune memory would reject early systemic recurrences

→Improved recurrence-free survival could reflect a return to the equilibrium phase between tumor and immune system of the host.

Radiation and Immune System

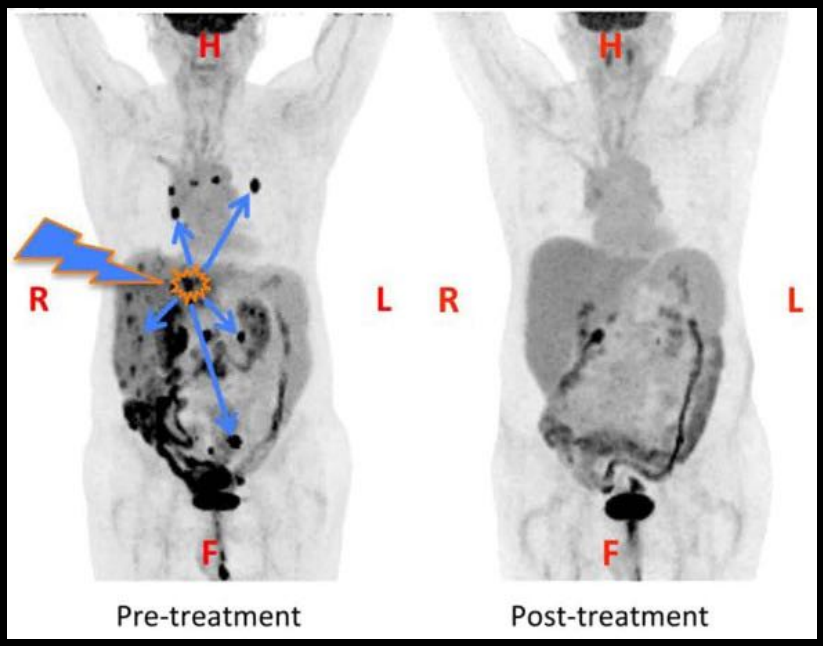
2. Success of concurrent chemo-radiation vs. sequential chemotherapy and radiation

→ Both local control AND systemic control are higher with concurrent treatments.

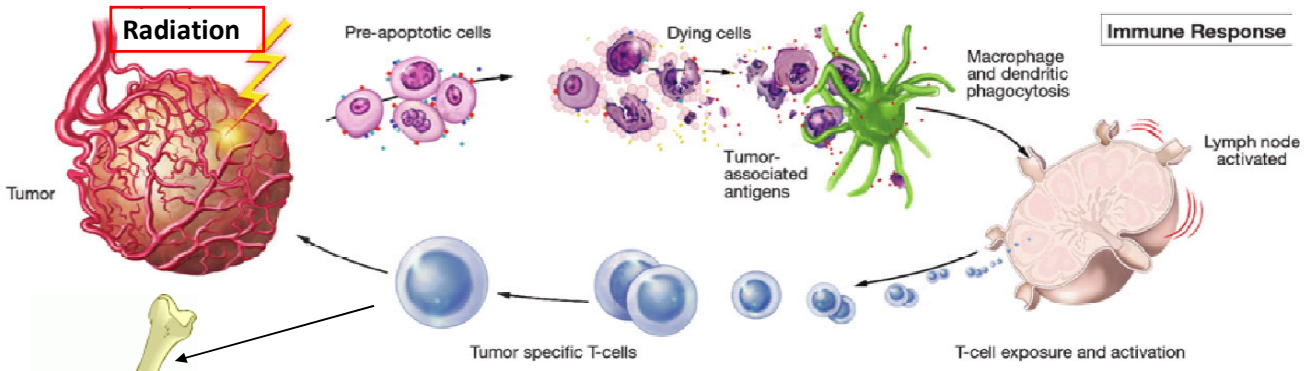
→ Radiation and chemical agents may complement each other in fulfilling the requirements for each of the three molecular signals of immunogenic cell death.

Radiation and Immune System

3. Abscopal effect.

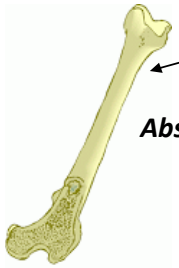


The "vaccine role" of RT may induce the abscopal effect



Abscopal effect?

Modified from Kamrava et al, Molecular BioSystems 2009



Bone metastases

Why Abscopal effect is so rare?

1. A highly suppressive tumor microenvironment
2. "Escaping phase" : the more immonogenic antigens are alreadey lost.
3. Cancer cells release immunosuppressive cytokines (TGF-β)
4. Cancer cells express surface receptors with inhibitory function for T cells: programmed death ligand-1
5. CD4 T cells with regulatory funtion (Treg) inhibit tumor rejection by direct contact with effector t cells and by secretion of immunosuppressive cytokines.
6. Radiation has been shown to promote :
   - ↑ TGF-β
   - Treg are more radio resistant and can increase after radiation

Abscopal effect.

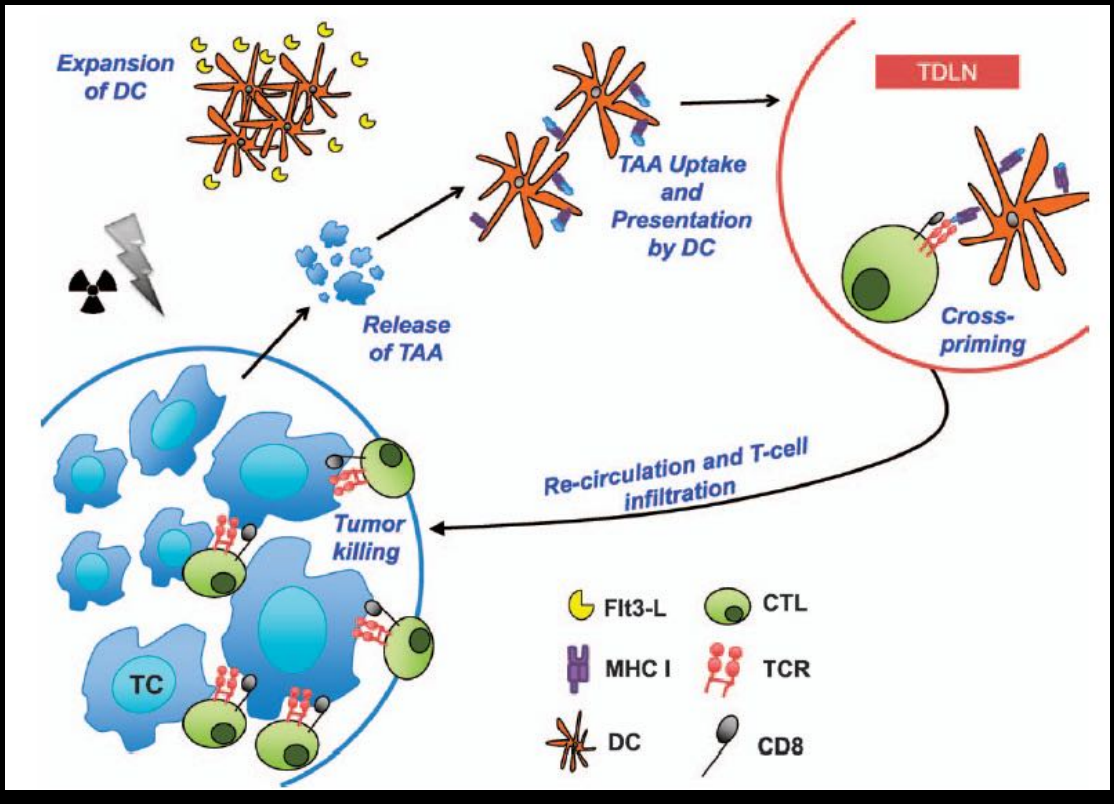
Radiotherapy may induce an immune-mediated abscopal effect only when has the ability to alter the preexisting immunosuppressive tumor environment with pro-immunogenic effects prevailing over immunosuppressive effects.

Preclinical Combinations of Radiation and Immunotherapy

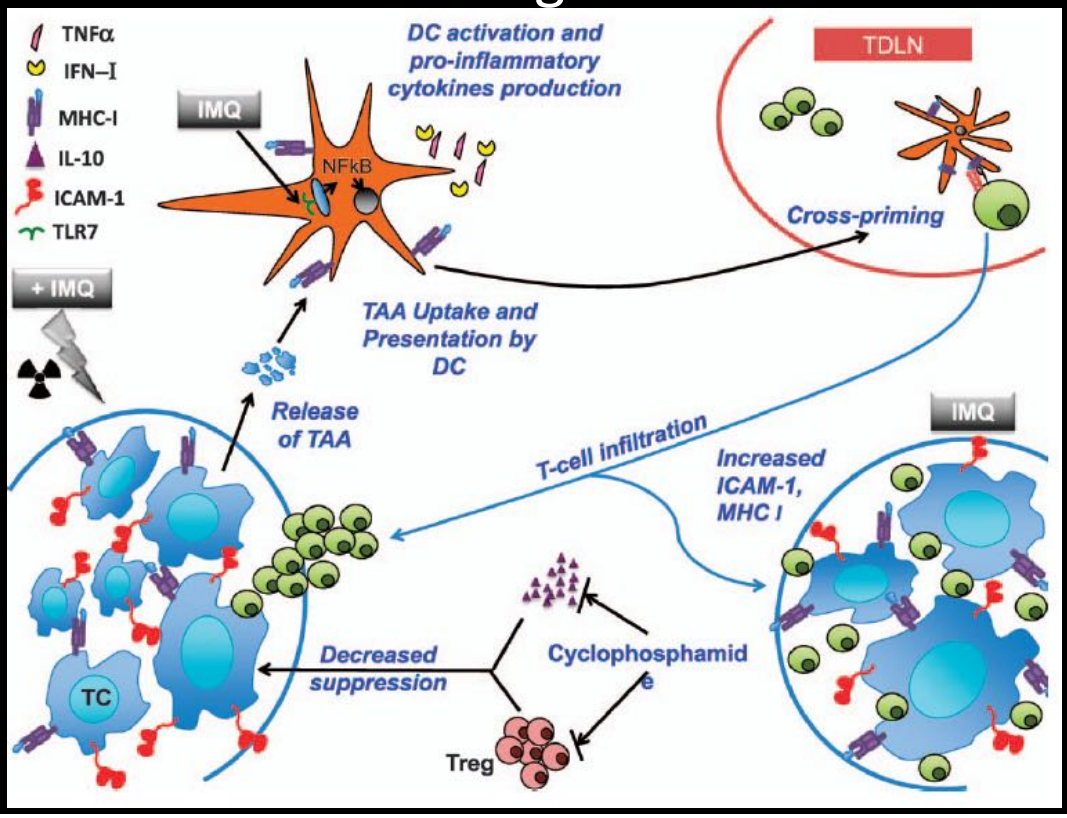
Table 1. Combinations of immunotherapy and local radiotherapy tested in preclinical tumor models*

Immunotherapy	Schedule of administration	Radiation effect relevant to the immune system	Detected immunomodulation
Flt3-ligand	Postradiation	Release of tumor antigens	Induction of antitumor T cells
Exogenous DCs, s.c. or i.v.	Postradiation	Recruitment of DCs and release of tumor antigens	Induction of antitumor T cells
Exogenous DCs, i.t.	Postradiation	Release of tumor antigens	Induction of antitumor T cells
CpG, s.c. peritumorally, and i.t.	Pre- and postradiation	Release of tumor antigens	Induction of antitumor T cells
Synthetic modified TLR-9 agonist, s.c.	Concomitant with and postradiation	Release of tumor antigens	Recruitment and activation of NKDCs
ECI301 (CCL3 variant), i.v.	Postradiation	Release of tumor antigens	Induction of antitumor T cells
Anti-CTLA-4 antibody, i.p.	Postradiation	Release of tumor antigens	Induction of ant-tumor T cells
		Induction of CXCL16 release	Improved recruitment of CCXR6+ effector CD8 T cells
		Induction of NKG2D ligand expression on tumor cells	Stable interaction between NKG2D+ effector CD8 T and tumor cells
Anti-CD137 antibody, i.v or i.p.	Postradiation	Release of tumor antigens and/or MHC class 1 induction on tumor cells	Induction of antitumor T cells.
Anti-CD137 and anti-PD-1 antibodies, i.p.	Concomitant with and postradiation	Release of tumor antigens	Induction of antitumor T cells
Adoptive T-cell transfer	Postradiation	Induction of Fas/CD95 on tumor cells Upregulation of MHC class 1 on tumor cells	Improved killing of tumor cells by adoptively transferred effector CD8 T cells
Vaccinia and avipox recombinants expressing CEA and T-cell costimula tory molecules	Pre- and postradiation	Induction of Fas/CD95 on tumor cells	Improved killing of tumor cells by vaccine-induced T cells, induction of antigenic cascade
Autologous tumor cell vaccine expressing GM-CSF	Postradiation	Upregulation of MHC class 1 on tumor cells	Improved killing of tumor cells by vaccine-induced T cells

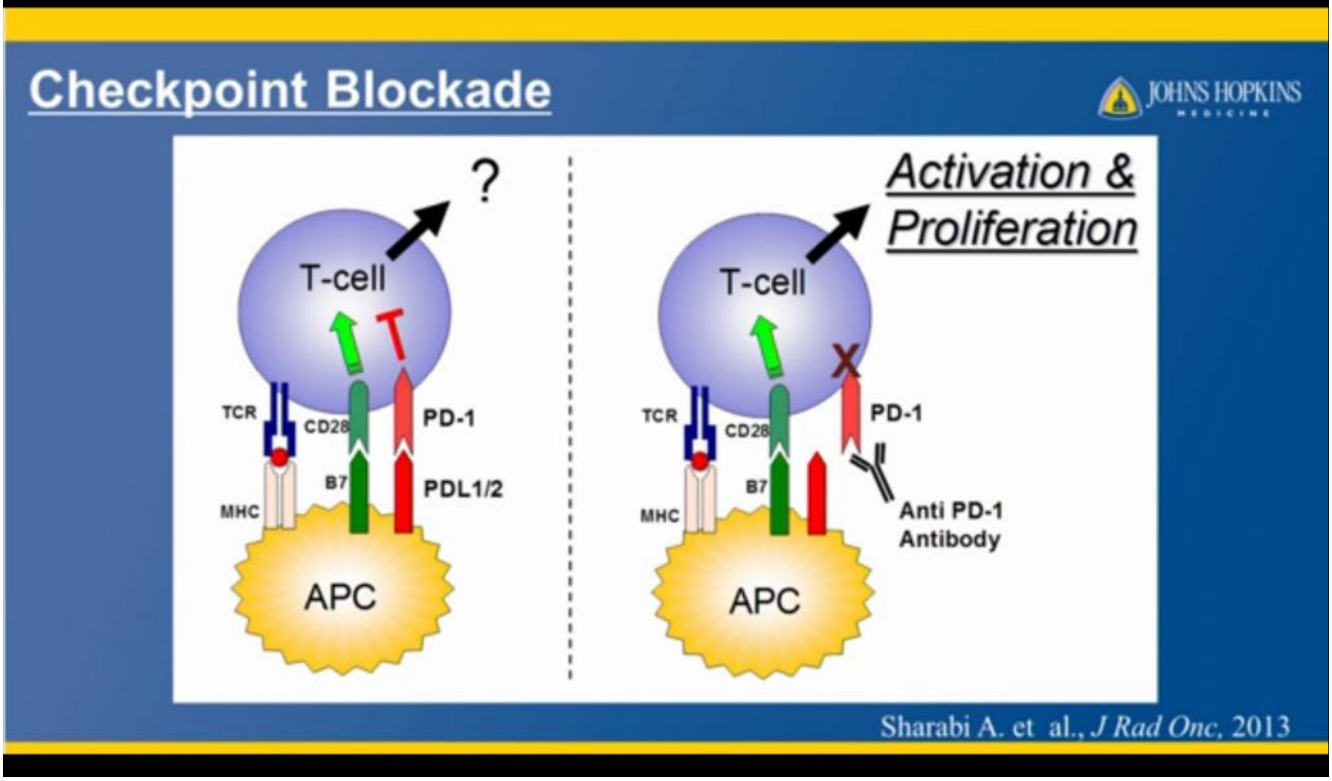
Combination of Local Radiotherapy with Dendritic Cell Growth Factor



Combination of Local Radiotherapy and TLR7 agonist



Combination of Local Radiotherapy with anti-Programmed-Dead Receptor 1



Combination of Local Radiotherapy with anti-Programmed-Dead Receptor 1

Radiation induces tumor infiltrating lymphocytes (TIL)

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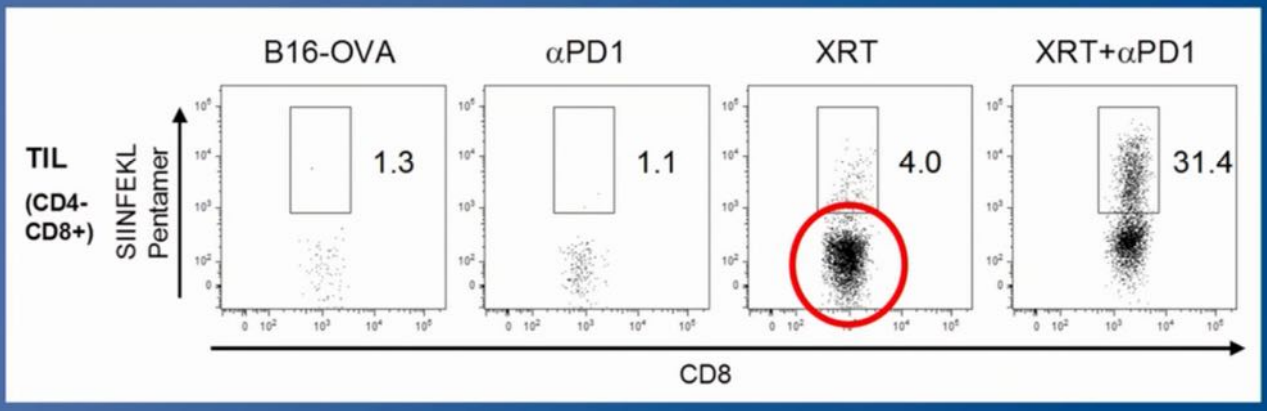

Sharabi et al.
Under Review

Preclinical Combinations of Radiation and Immunotherapy

Radiation combined with anti-PD-1 immunotherapy improves local tumor control

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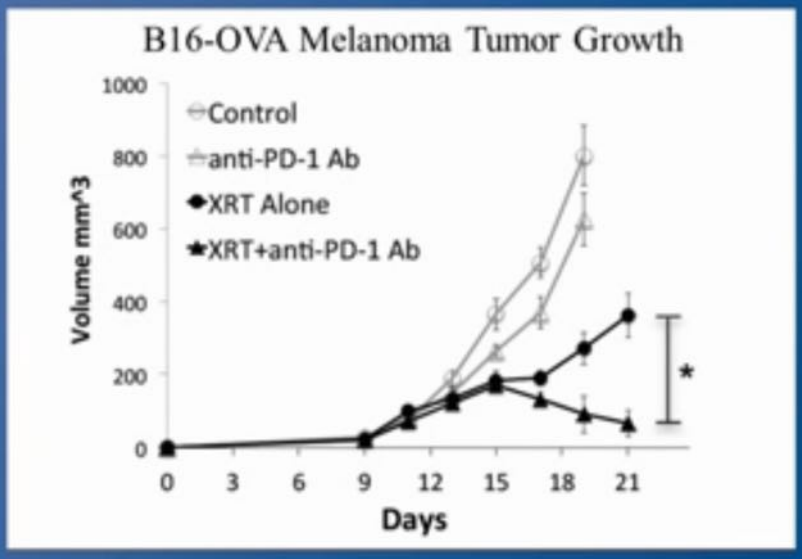
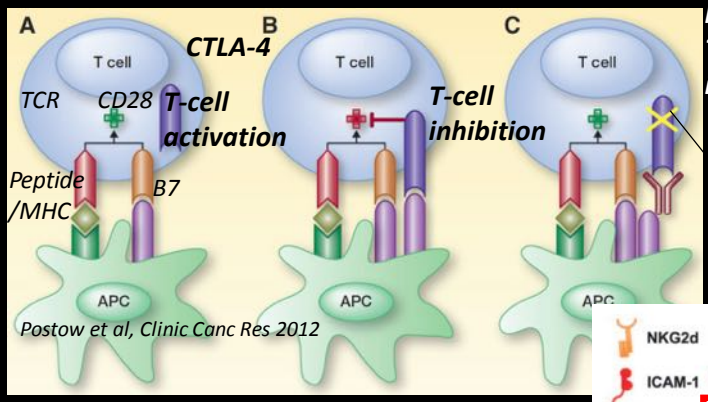
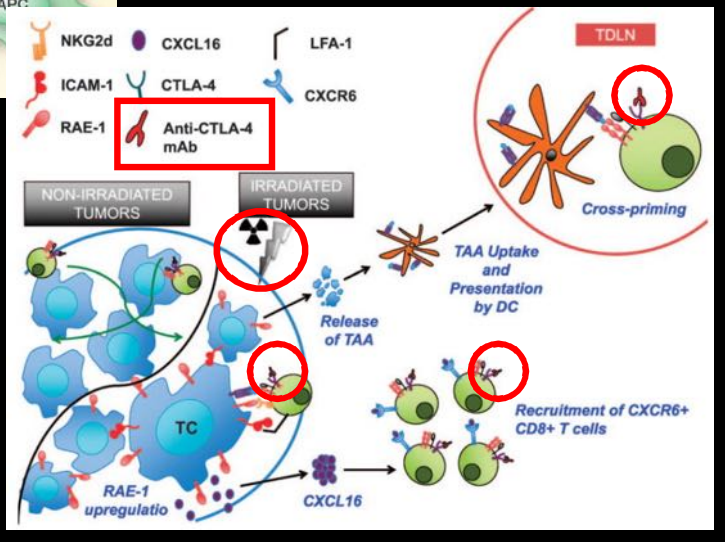


Sharabi et al.
Under Review

Combination of Local Radiotherapy with checkpoint receptor blockade



• Ipilimumab blocks cytotoxic T-lymphocytes antigen 4 (CTLA-4) releasing T cells from this immunologic checkpoint to exert their full antitumor effect



• The association **RT+Ipilimumab** has a synergistic effect:
1. increased of immune responses against tumor-associated antigens
2. clinical response

Clinical Combinations of Radiation and Immunotherapy

Table 1 Examples of successful immune-mediated tumor rejection after treatment with radiation therapy and CTLA-4 checkpoint blockade: Treatment and target specifications

Reference	Radiation regimen	CTLA-4 antibody dose	Tumor type	Targeted site	Setting
Dewan et al (8)	6 Gy × 5 and 8 Gy × 3	10 mg/kg (9H-10)	Breast cancer	Primary tumor	Preclinical
Hiniker et al (9)	18 Gy × 3	3 mg/kg	Melanoma	Liver metastases	Clinical
Postow et al (4)	9.5 Gy × 3	10 mg/kg	Melanoma	Paraspinal metastasis	Clinical
Golden et al (5)	6 Gy × 5	3 mg/kg	Lung cancer	Liver metastasis	Clinical

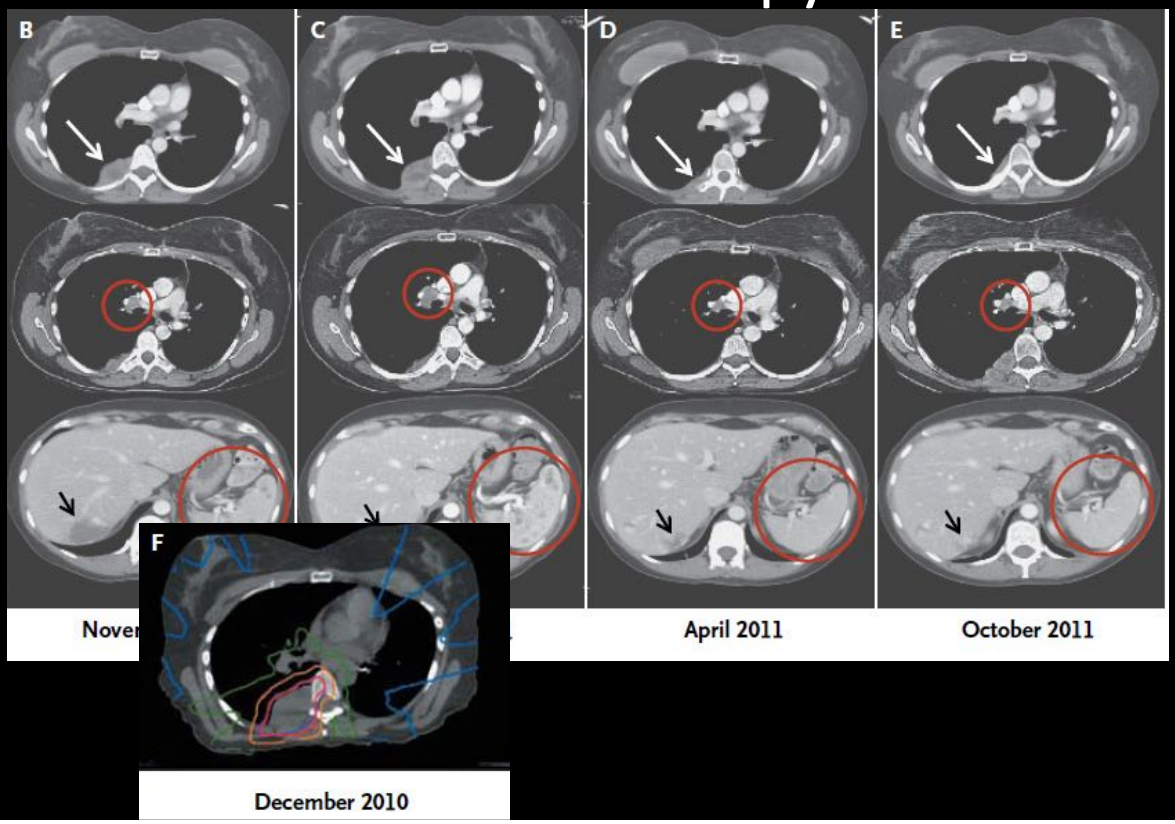
The NEW ENGLAND JOURNAL of MEDICINE

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 Gnjatic, Ph.D., and Jedd D. Wolchok, M.D., Ph.D.

Clinical Combinations of Radiation and Immunotherapy

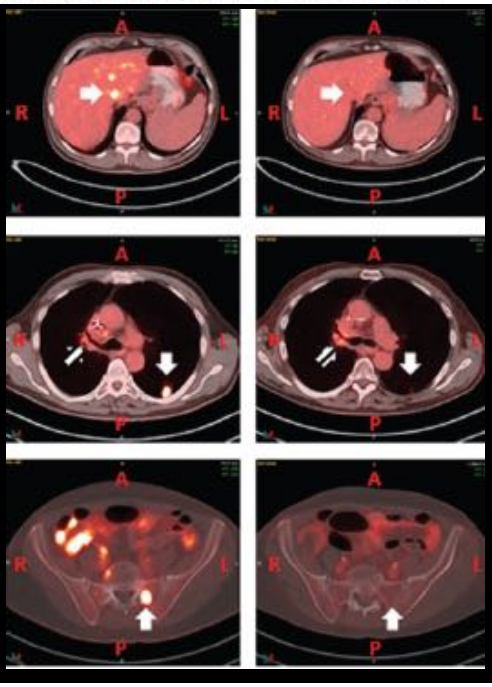


Cancer Immunology Miniatures

Cancer Immunology Research

An Abscopal Response to Radiation and Ipilimumab in a Patient with Metastatic Non–Small Cell Lung Cancer

Encouse B. Golden[1], Sandra Demaria[1,2], Peter B. Schiff[1], Abraham Chachoua[3], and Silvia C. Formenti[1]



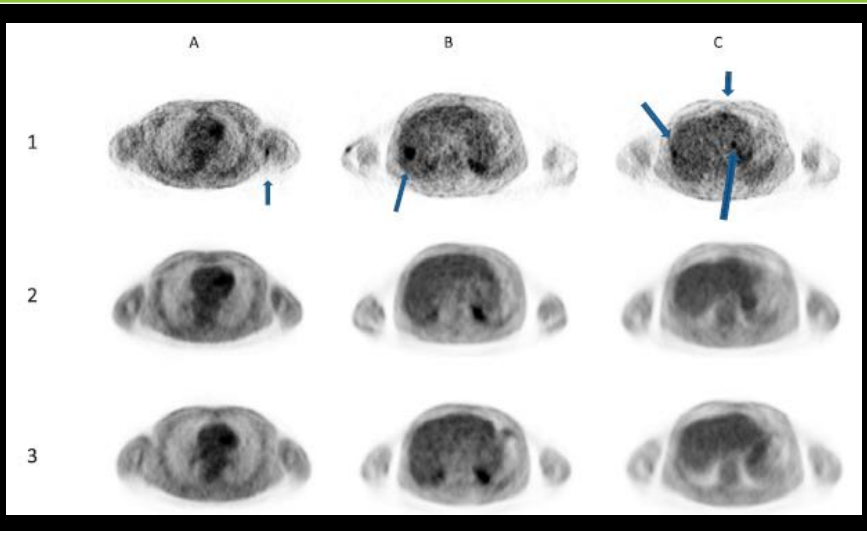
Translational Oncology
www.transonc.com

Volume 5 Number 6 December 2012 pp. 404–407 404

A Systemic Complete Response of Metastatic Melanoma to Local Radiation and Immunotherapy

Susan M. Hiniker*, Daniel S. Chen†, Sunil Reddy†, Daniel T. Chang*, Jennifer C. Jones*, Joseph A. Mollick†, Susan M. Swetter‡ and Susan J. Knox*

*Department of Radiation Oncology, Stanford University School of Medicine, Stanford, CA; †Department of Medical Oncology, Stanford University School of Medicine, Stanford, CA; ‡Department of Dermatology, Stanford University School of Medicine, Stanford, CA



Clinical Combinations of Radiation and Immunotherapy

RT combination with:	Trial/ tumor site	accrual
Flt3L *(Demaria et al., Int J Radiat Oncol Biol Phys, 2004)*	Proof of principle abscopal trial (met disease all sites) NYU 02-58	37/37
anti-CTLA-4 *(Demaria et al., Clin Cancer Res 2005; Matsumura et al., J Immunol 2008; Pilones et al., Clin Cancer Res 2009; Dewan et al., Clin Cancer Res 2009; Ruocco et al., J Clin Invest 2012)*	Ipilimumab –RT randomized Met melanoma S12-02746	12/48
	Ipilimumab -RT Met NSCLC trial S14-00208	18/29
TLR7-agonist *(Dewan et al. Clin Cancer Res 2012)*	Imiquimod-RT trial NCT01421017	14/29
anti-TGFβ *(Bouquet et al Clin Cancer Res 2012)*	Fresolimumab-RT Randomized NCT01421017	24/24

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Ipilimumab versus placebo after radiotherapy in patients with metastatic castration-resistant prostate cancer that had progressed after docetaxel chemotherapy (CA184-043): a multicentre, randomised, double-blind, phase 3 trial

*Eugene D Kwon, Charles G Drake, Howard I Scher, Karim Fizazi, Alberto Bossi, Alfons J M van den Eertwegh, Michael Krainer, Nadine Houede, Ricardo Santos, Hakim Mahammedi, Siobhan Ng, Michele Maio, Fabio A Franke, Santhanam Sundar, Neeraj Agarwal, Andries M Bergman, Tudor E Ciuleanu, Ernesto Korbenfeld, Lisa Sengeløv, Steinbjorn Hansen, Christopher Logothetis, Tomasz M Beer, M Brent McHenry, Paul Gagnier, David Liu, Winald R Gerritsen, for the CA184-043 Investigators**

All patients received a single dose of radiotherapy of 8 Gy for at least one, and up to five, bone fields, at the investigator's discretion. This single-administered radiation dose (8 Gy in one treatment fraction) was previously shown to be therapeutically equivalent to a fractionated regimen (30 Gy in ten treatment fractions over 2 weeks) with respect to pain palliation.[33] Radiotherapy was done some time within the 2 days before initiation of the study drug regimen, and palliative radiotherapy was allowed for any bone lesion while on study. Sites of radiotherapy included the arm, leg, pelvis, spine, rib, and skull. We did not assess the efficacy of the radiotherapy with respect to pain palliation or lesional regression as part of the study, because it was given to stimulate immune response. Until database lock, investigators assessing disease progression (including by radiographic assessment) remained masked to treatment allocation.

Site: bone mets

Dose : 8 Gy, single fraction

Time: RT within 2 days from IPI, then anytime during IPI

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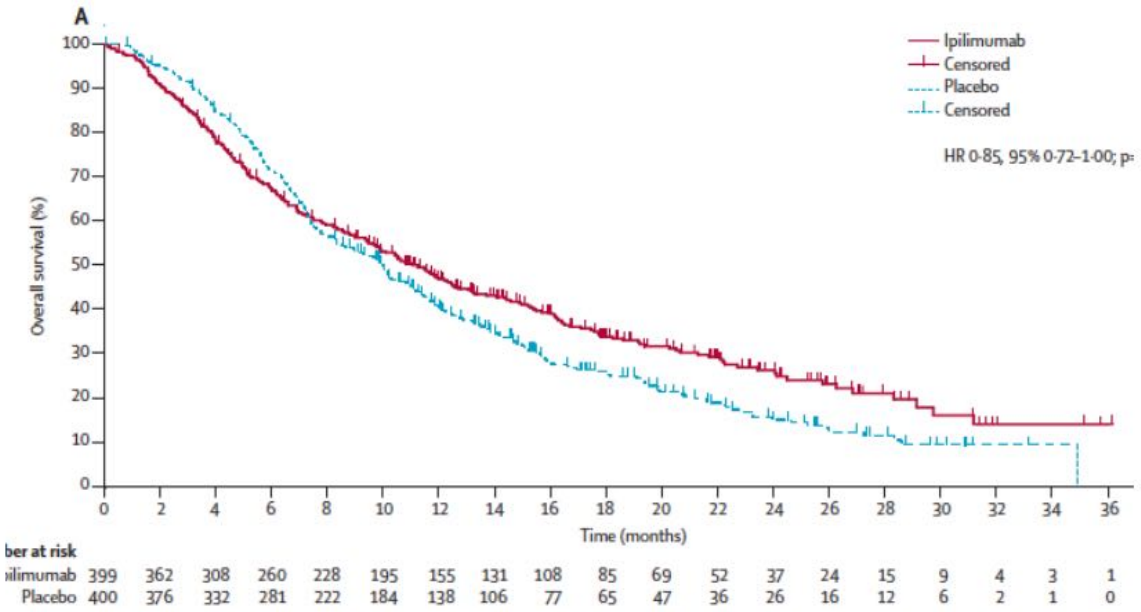
Study failed to meet its main endpoint

Study powered to detect a 4 month difference in median overall survival (15.8 versus 12 months)



Curves split after 6 months

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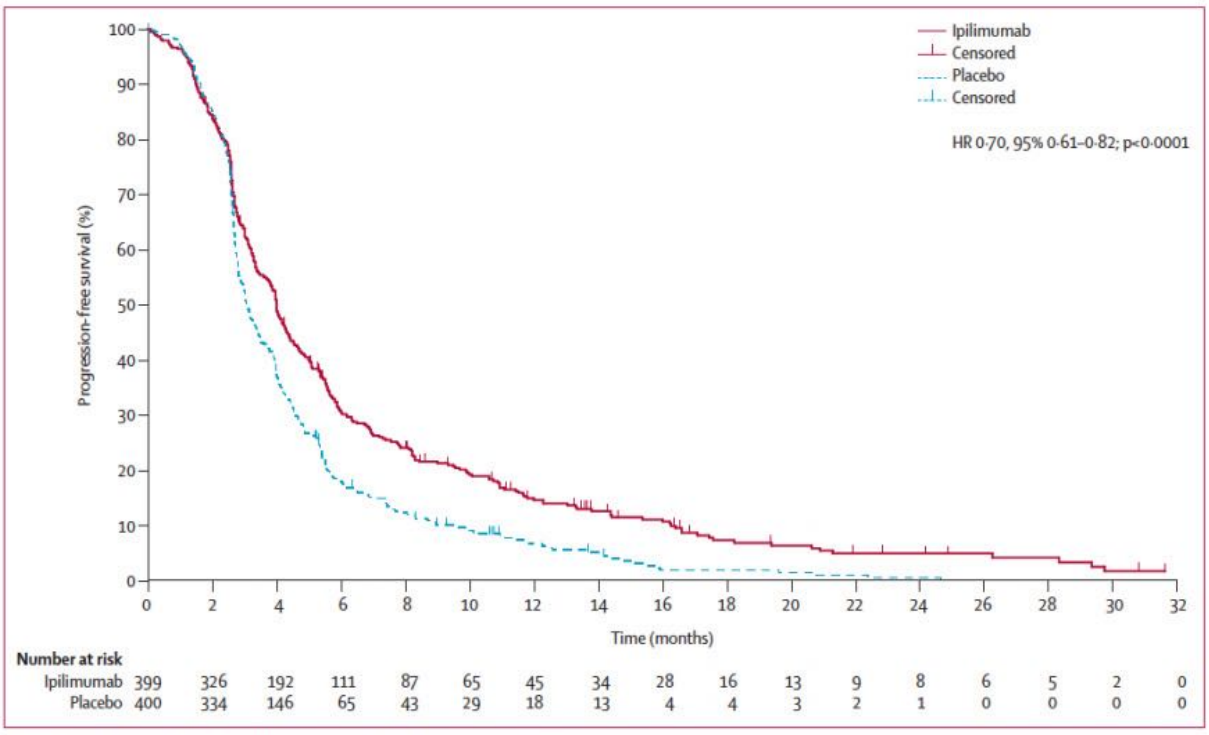


Figure 4: Progression-free survival in the intention-to-treat population

At 6 months 30.7% versus 18.1%

Clinical Combinations of Radiation and Immunotherapy

Many questions remain:

- Optimal site to irradiate in metastatic disease
- Patient selection
- Sequencing of RT/Immunotherapy
- RT dose and fractionation
- Best combination

Potential predictive role of immune biomarkers in SABR treatment?

ClinicalTrials.gov
A service of the U.S. National Institutes of Health

Monitoring Anti-Prostate Cancer Immunity Following Stereotactic Body Radiotherapy (SBRT)

This study is currently recruiting participants. (see Contacts and Locations)
Verified January 2013 by Mayo Clinic

Sponsor:
Mayo Clinic

Information provided by (Responsible Party):
Sean S. Park, Mayo Clinic

ClinicalTrials.gov Identifier:
NCT01777802

First received: January 24, 2013
Last updated: January 28, 2013
Last verified: January 2013
History of Changes

CRO Aviano

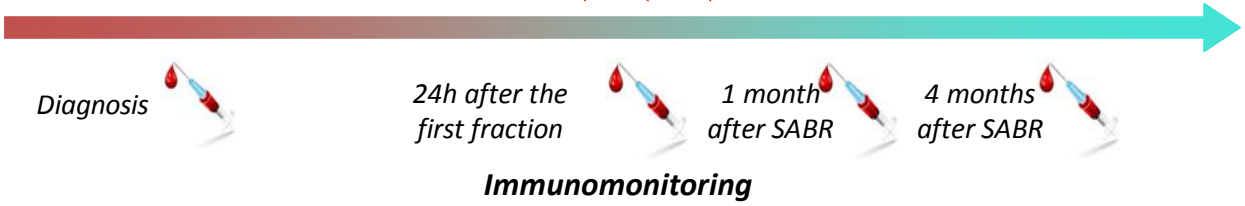
- Phase II clinical trial employing **SABR** for **oligometastatic breast cancer patients**
- To evaluate SABR effects on anti-tumor immune response

Study design

Patients enrollment
- ≤ 6 metastatic lesions (diagnosis torugh FDG-PET/CT)
- controlled loco-regional disease
- no brain metastases

SABR treatment
- 30 Gy / each lesion
- 3 daily fractions (10 Gy/fraction)

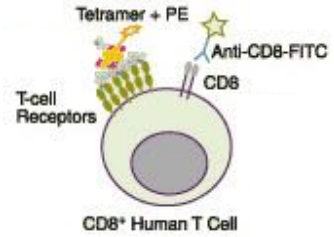
Follow up
Possible concurrent treatments:
- hormonal treatment and/or
- chemotherapy
- steroids
- **Trastuzumab**

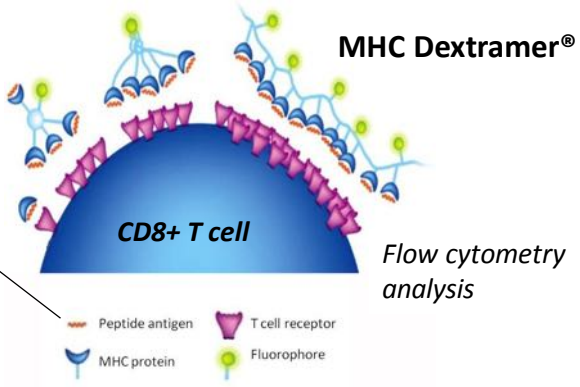


Immunomonitoring

- October 2012-February 2014: 10 evaluable patients with tumor control 6 months after SABR

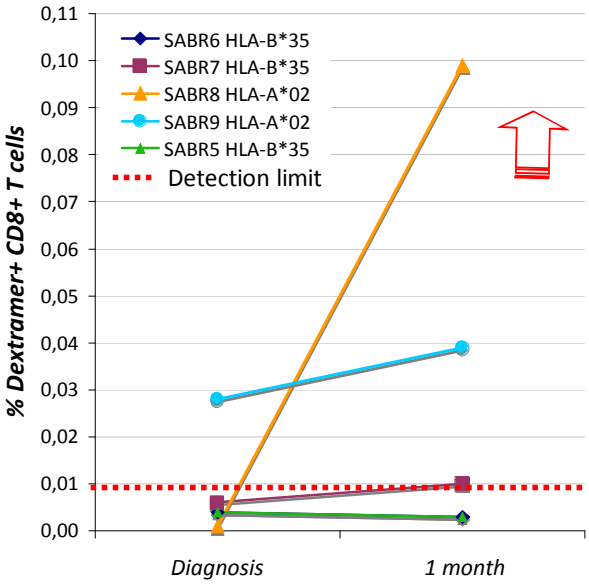
Enhanced survivin-specific CD8+ T-cell responses 1 month after SABR



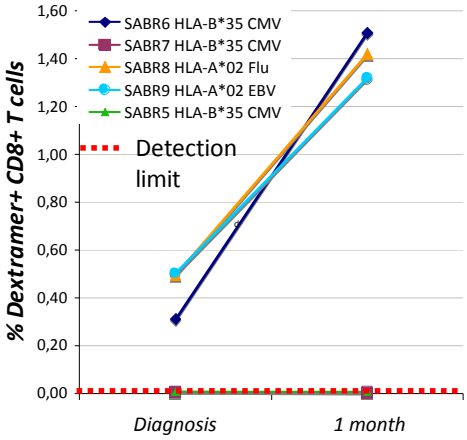


Flow cytometry analysis

CD8+ T lymphocytes specific for survivin-derived epitopes

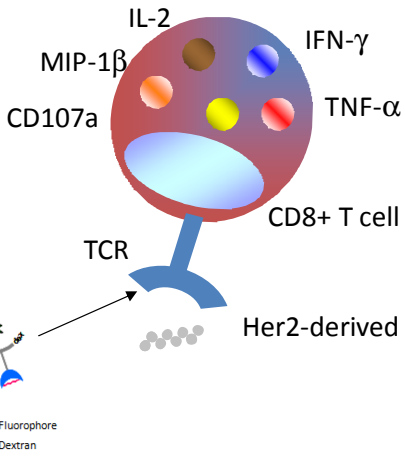


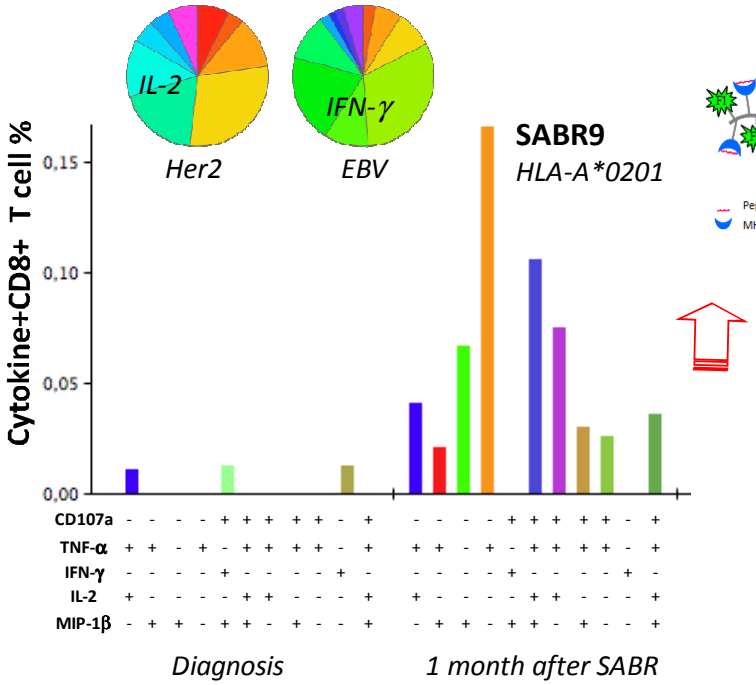
CD8+ T lymphocytes specific for viral antigen-derived epitopes



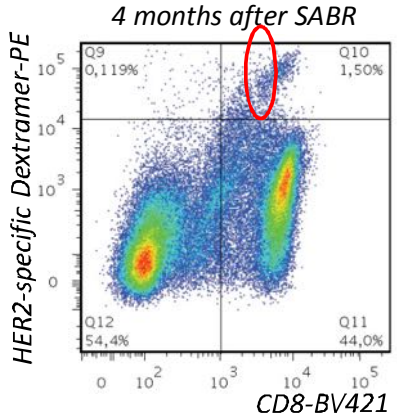
Induction of polyfunctional HER2-specific CD8+ T cell responses after SABR

• *In vitro prestimulation of patients' lymphocytes with HLA-matched epitopes derived from BC-associated antigens (survivin, mammaglobin-A, HER2) for 12 days. Then, flow cytometry characterization of antigen-specific CD8+ T cells*





4 months after SABR

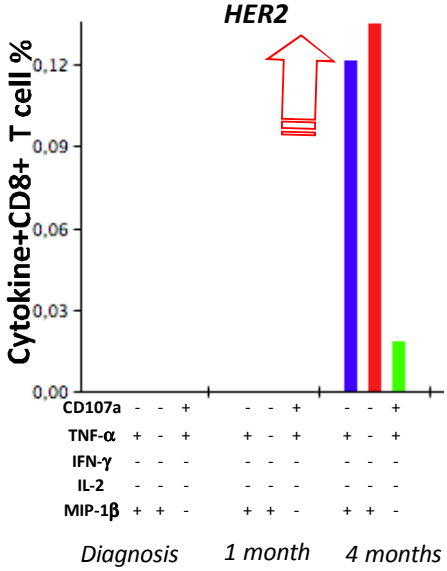


Differential modulation of T-cell responses after SABR

SABR5

*HLA-A*2402*

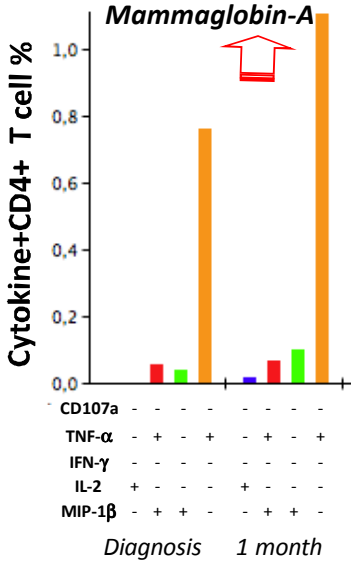
HER2



SABR12

*HLA-DRB1*03*

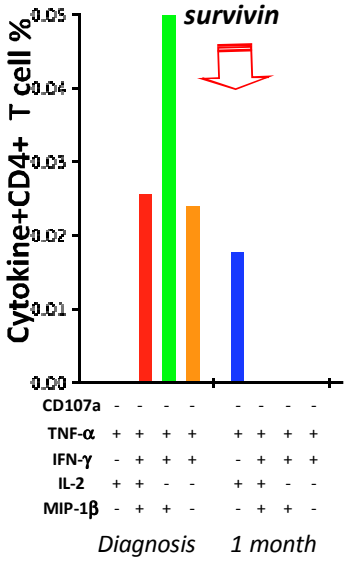
Mammaglobin-A



SABR1

*HLA-DRB1*03*

survivin



- 5/10 patients showed the enhancement or even the appearance of anti-tumor polyfunctional T cells

Possible correlation with clinical response?

Thank you for your attention!

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