XXVI Congresso Nazionale AIRO
XXX Congresso Nazionale AIRB
IX Congresso Nazionale AIRO GIOVANI
Rimini, Palazzo dei Congressi, 30 settembre – 2 ottobre 2016

Radioterapia e Modulazione della Risposta Immunitaria Antitumorale

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DICHIARAZIONE

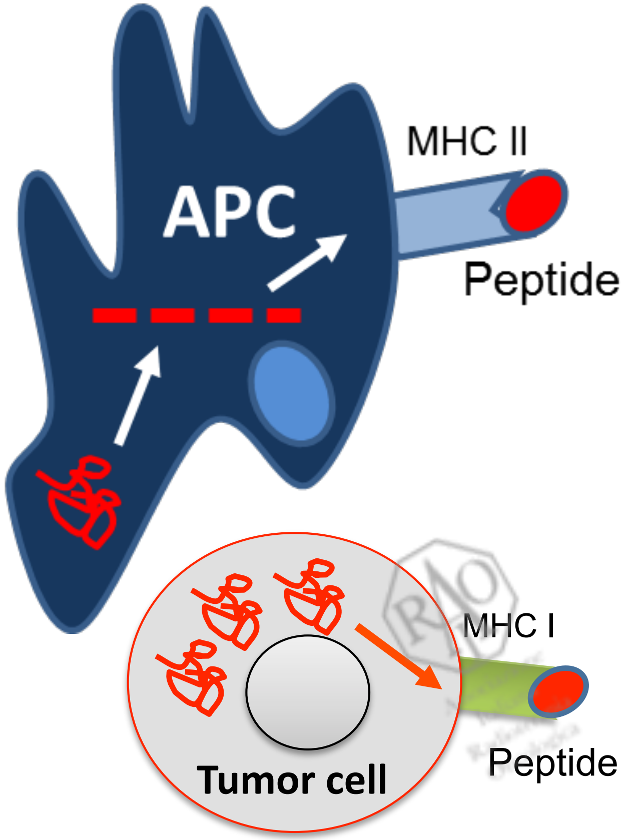
Relatore: Simone NEGRINI

Come da nuova regolamentazione della Commissione Nazionale per la Formazione Continua del Ministero della Salute, è richiesta la trasparenza delle fonti di finanziamento e dei rapporti con soggetti portatori di interessi commerciali in campo sanitario.

- Posizione di dipendente in aziende con interessi commerciali in campo sanitario **NIENTE DA DICHIARARE**
- **Consulenza ad aziende con interessi commerciali in campo sanitario GLAXO**
- Fondi per la ricerca da aziende con interessi commerciali in campo sanitario **NIENTE DA DICHIARARE**
- Partecipazione ad Advisory Board **NIENTE DA DICHIARARE**
- Titolarietà di brevetti in compartecipazione ad aziende con interessi commerciali in campo sanitario **NIENTE DA DICHIARARE**
- Partecipazioni azionarie in aziende con interessi commerciali in campo sanitario **NIENTE DA DICHIARARE**
- Altro **NIENTE DA DICHIARARE**

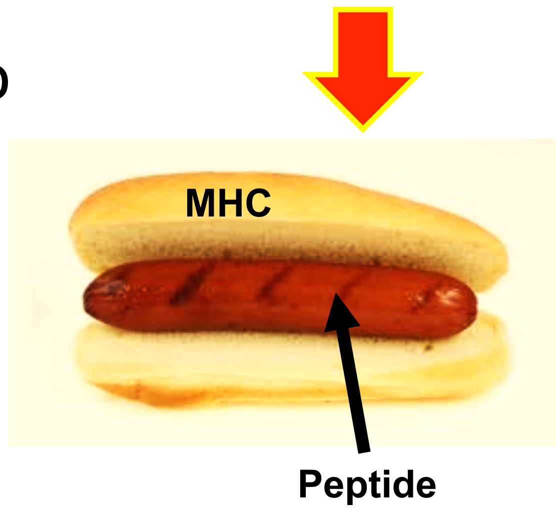
- Basic immunology
- Cancer immunotherapy
- Immunomodulatory properties of RT
- RT-immunotherapy combination trials
- Logistical challenges in associating IT and RT

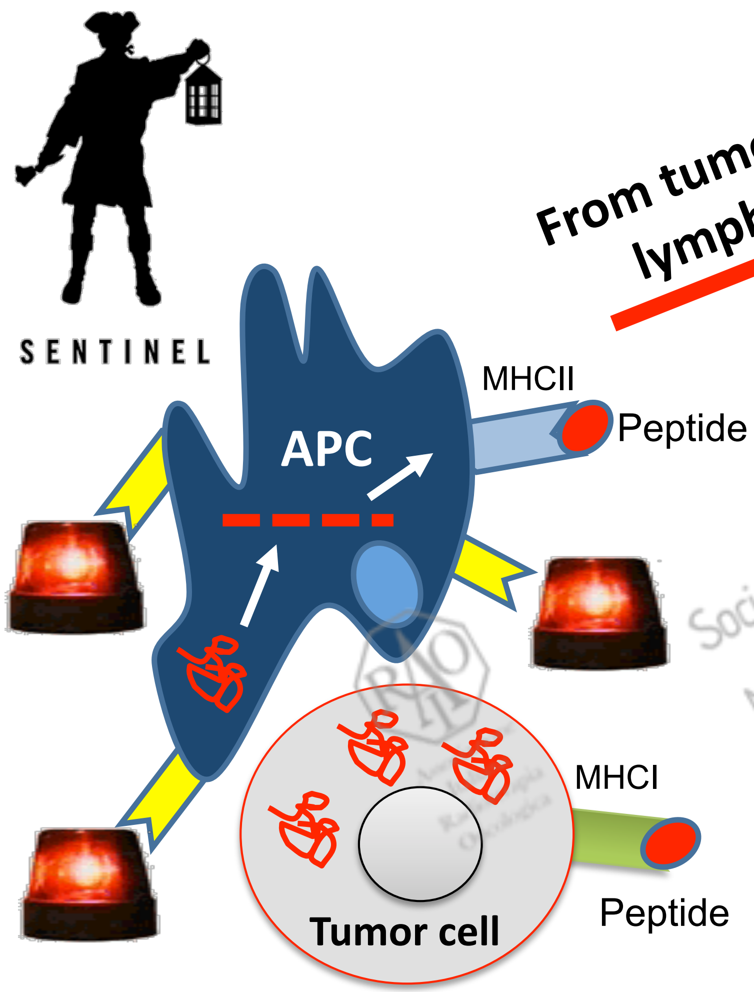
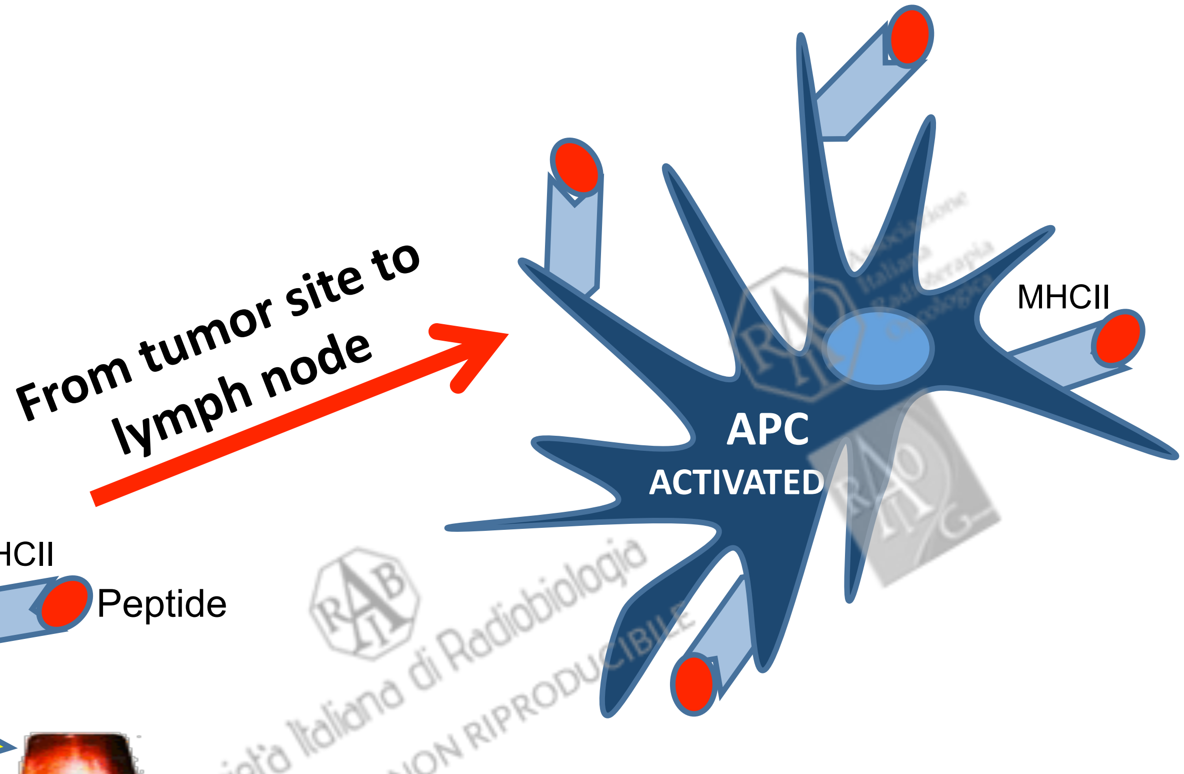
Basic immunology "The anti-tumor immune response"



Antigen presenting cells (e.g. Dendritic Cell) internalize and process "exogenous" antigen for presentation in the MHC II

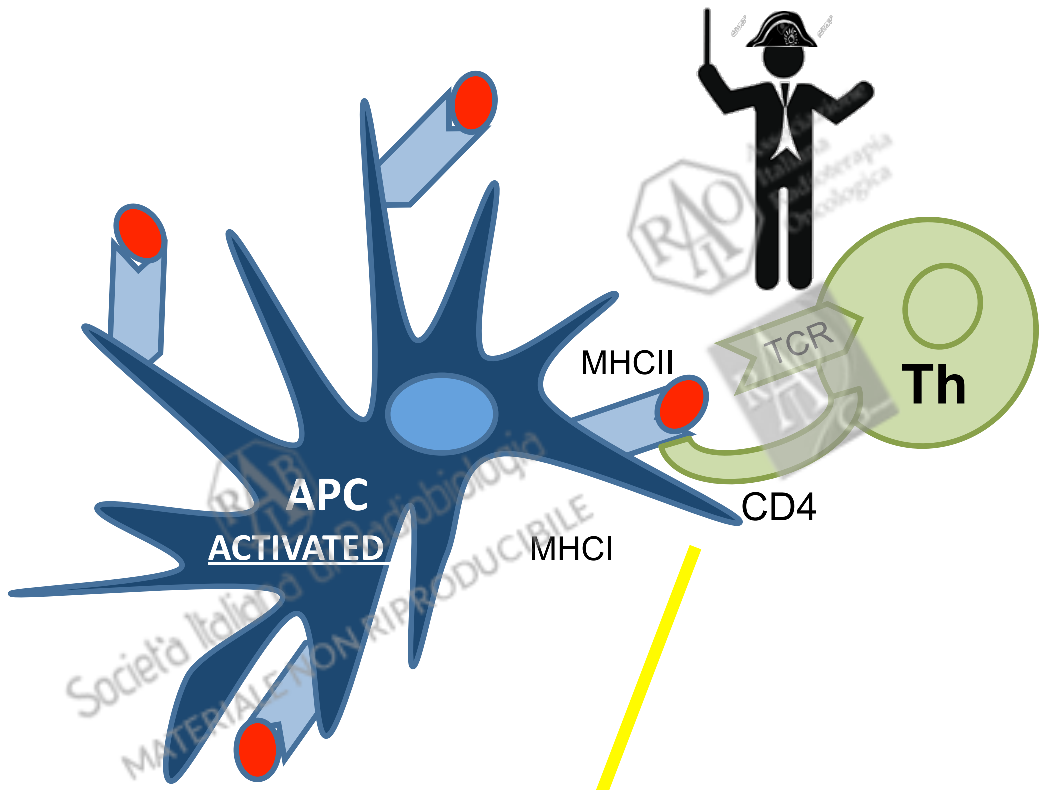
All **nucleated cells** process "endogenous" antigen (fragment the antigen into small peptides) for presentation through the "Major Histocompatibility Complex" class I

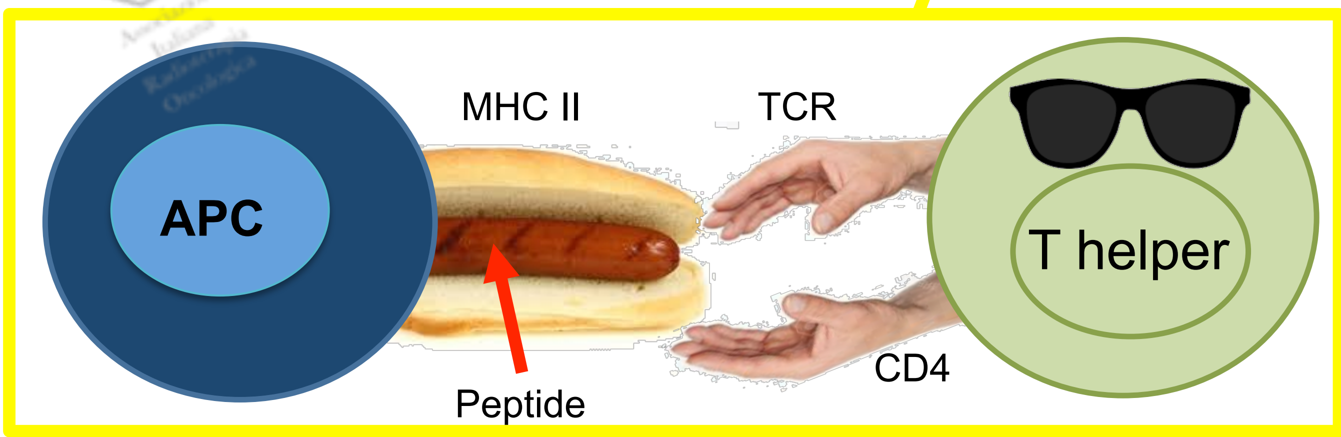


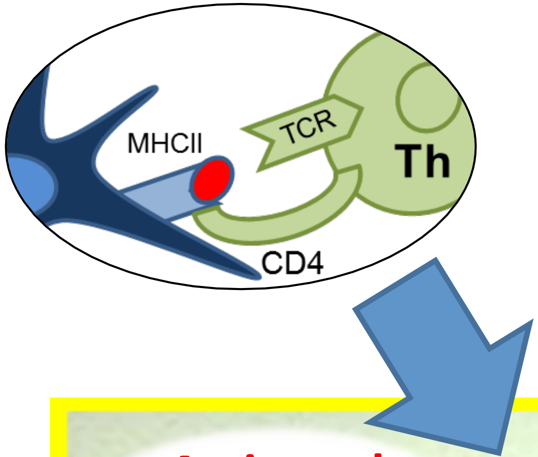



If "**danger signals**" are present, APC becomes activated (↑ MHC, ↑ co-stimulatory molecules, cytokines secretion)

APC presents the antigenic peptide (via MHC II) to CD4+ T helper

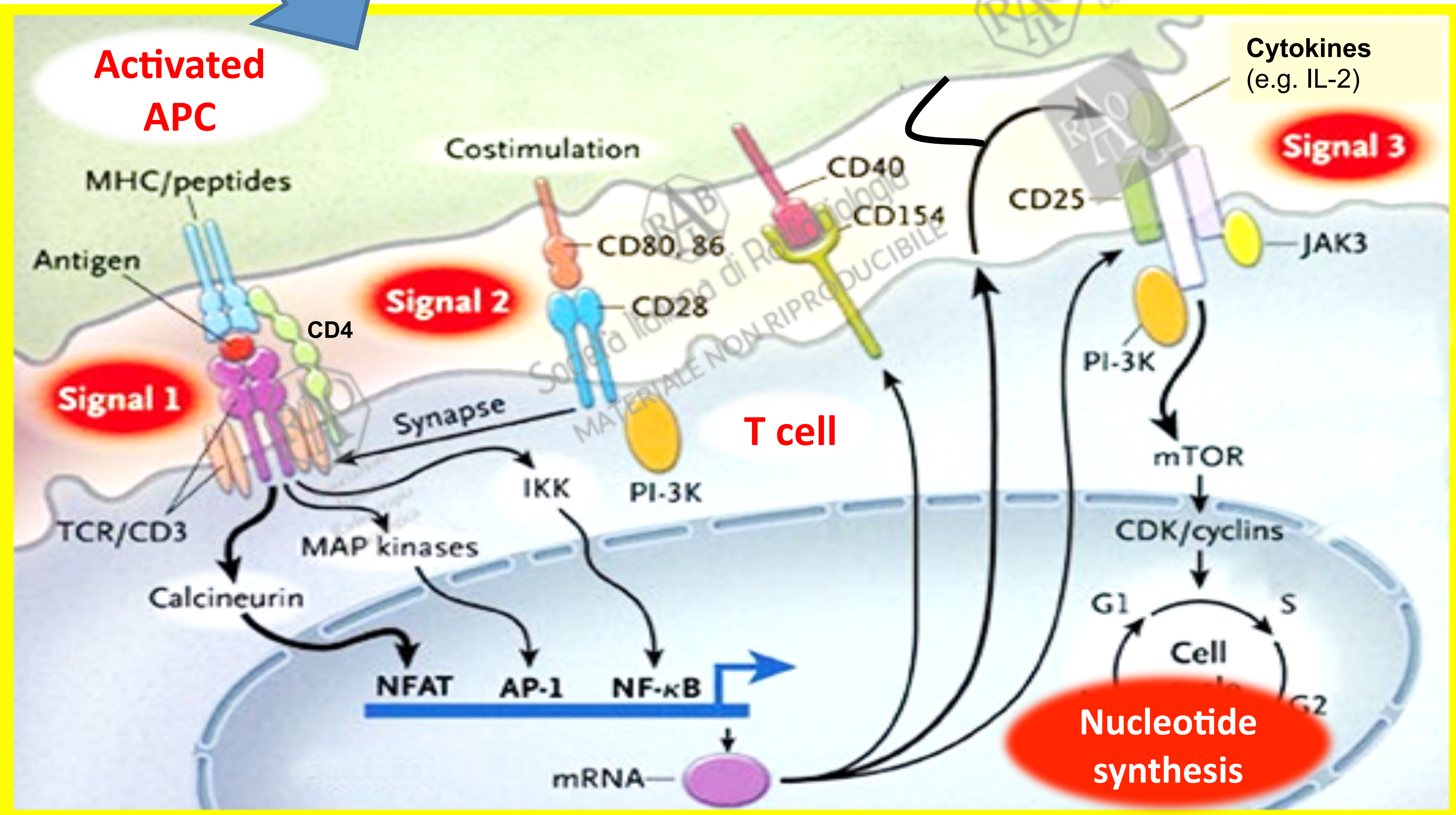






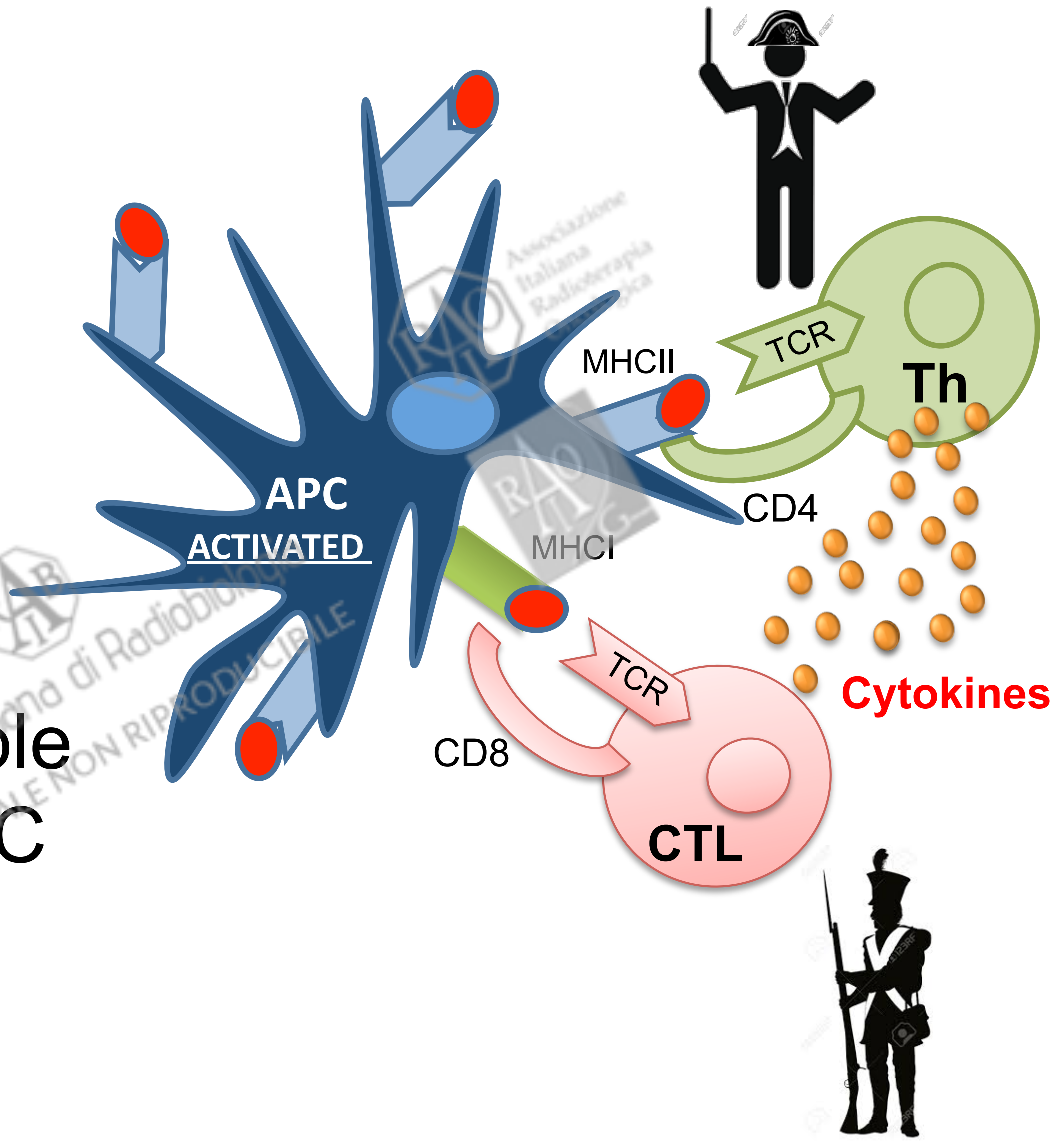
T cell activation

APC presents antigen to CD4+ T helper and **gives co-stimulatory signals**



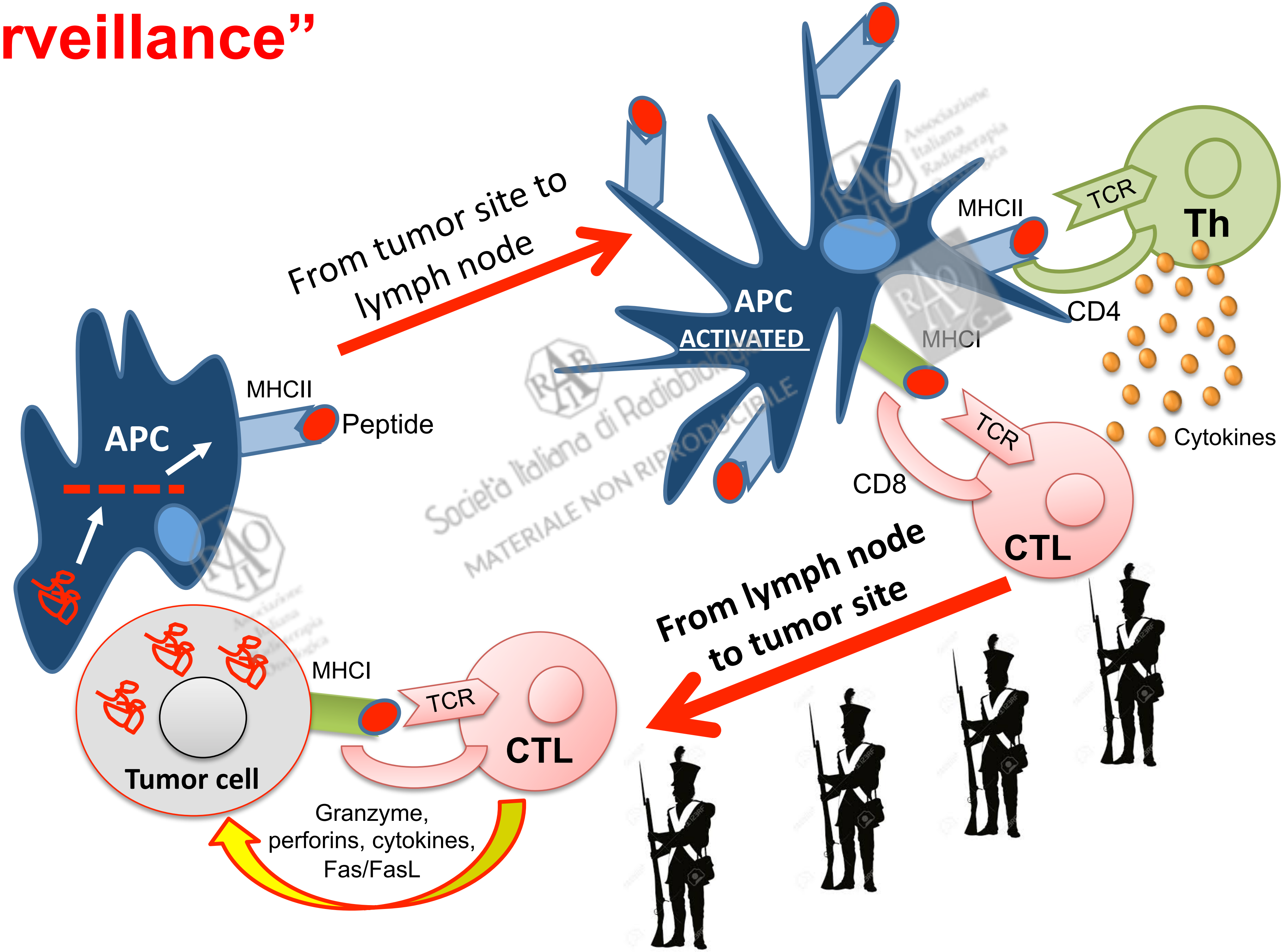
Adapted from: Halloran PF. N Engl J Med 2004;351:2715-2729.

Activated CD4 T helper, providing **cytokines**, activate CD8 T cell

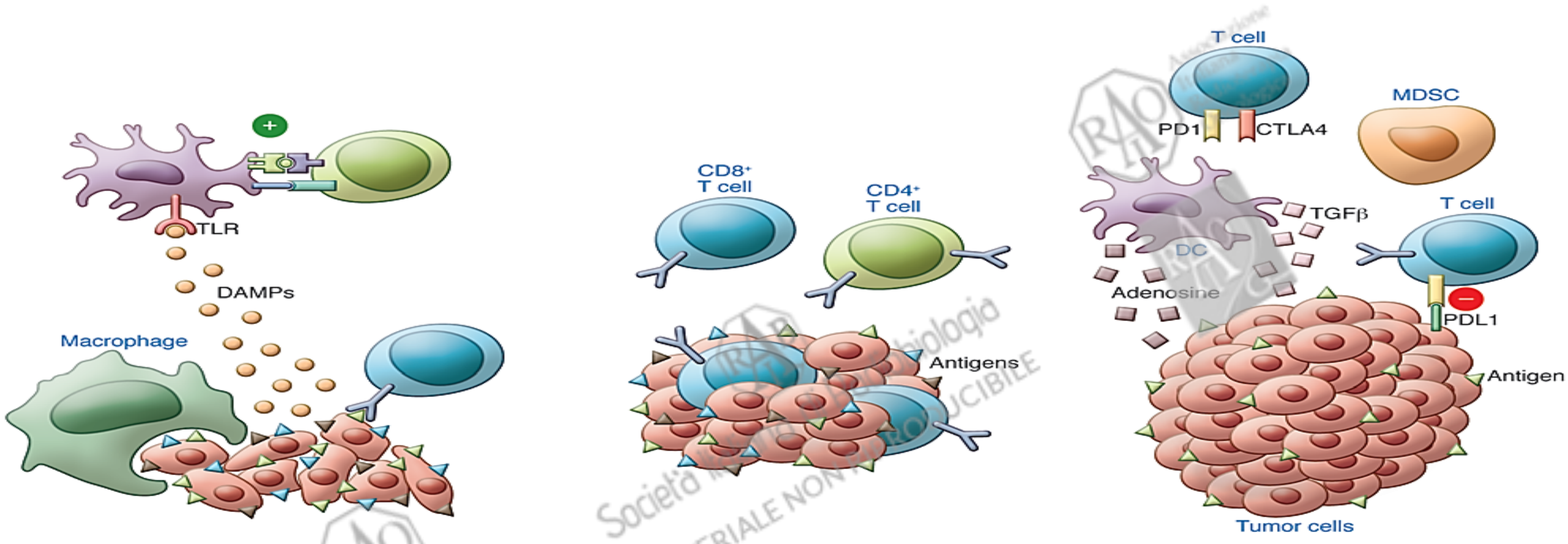


Some DC are also capable to present antigen in MHC class I to CD8 T cell (**cross-priming**) thus enhancing CD8 T cell activation

The concept of "immuno-surveillance"



The "**cancer immunoediting**" hypothesis (tumor-host immune system relationship)



Elimination ⟷ **Equilibrium** ⟷ **Escape**

Immune surveillance

Tumor cells can **decrease their intrinsic immunogenicity**

and

Tumor can **induce tolerance** through interactions with the immune system

Dunn GP, et al. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol. 2002;3(11):991–998.

- Basic immunology
- **Cancer immunotherapy**
- Immunomodulatory properties of RT
- RT-immunotherapy combination trials
- Logistical challenges in associating IT and RT

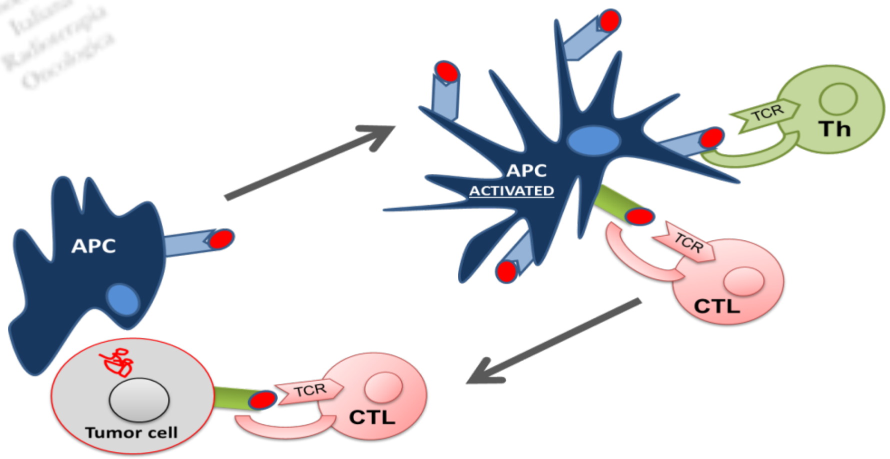
Cancer immunotherapy

A cancer treatment that boosts the patient's own immune system and/or uses "man-made" versions of the components of the immune system

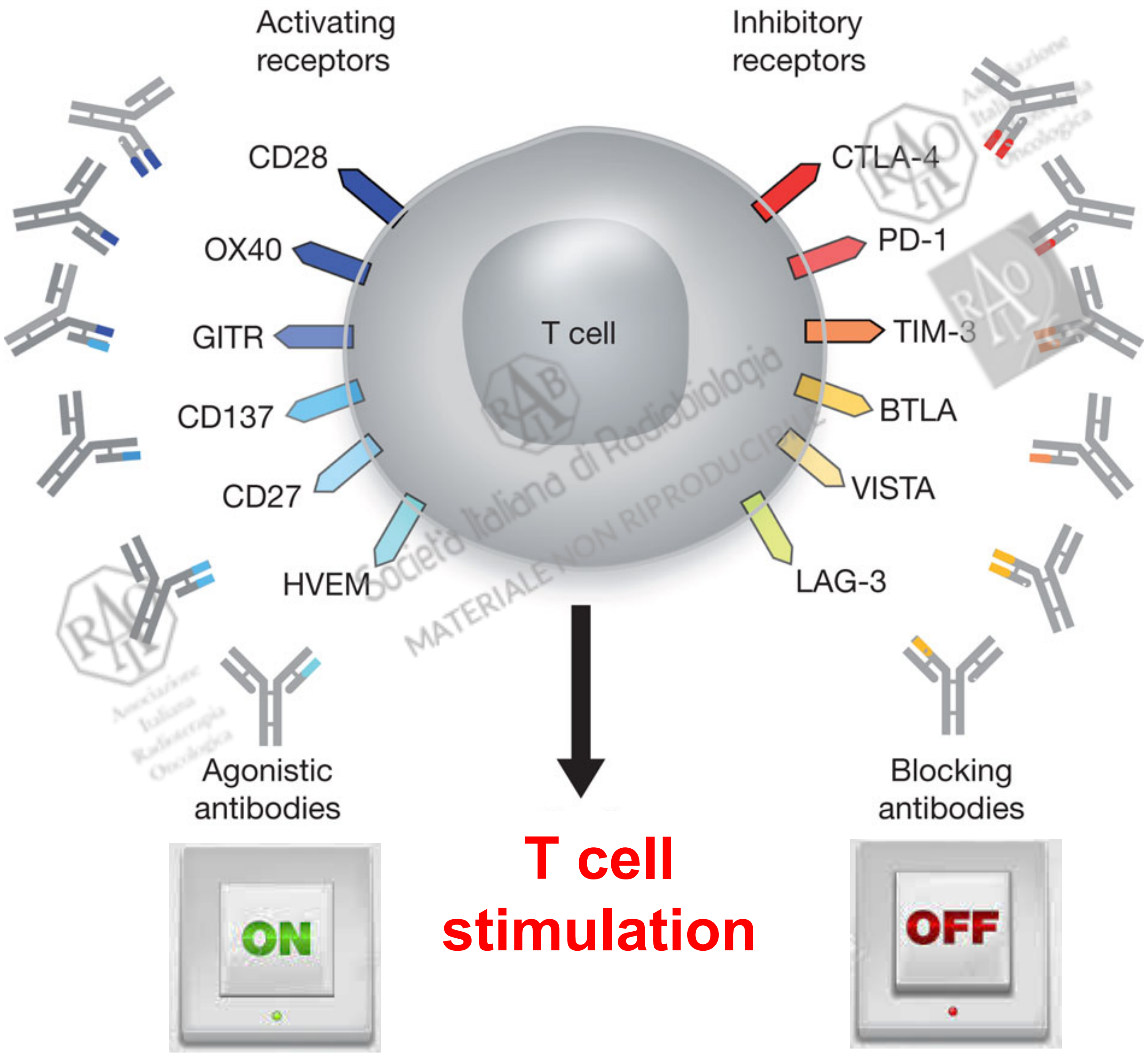
Over the past two decades, scientific and technological advances in immunotherapy have contributed to its role as one of the more promising cancer treatments

Cancer immunotherapy strategies (alone or in combination):

- Monoclonal antibodies **targeting tumor cells** (alone, conjugated)
- **Cytokines** (to increase the immune response)
- **Vaccine-based strategies** → indirectly amplify the effector component of immunity (antigen, APC)
- **Delivering effector cells** → directly contributes an effector population (also "engineered" cells)
- Manipulation of "**co-stimulatory – checkpoints**" signals

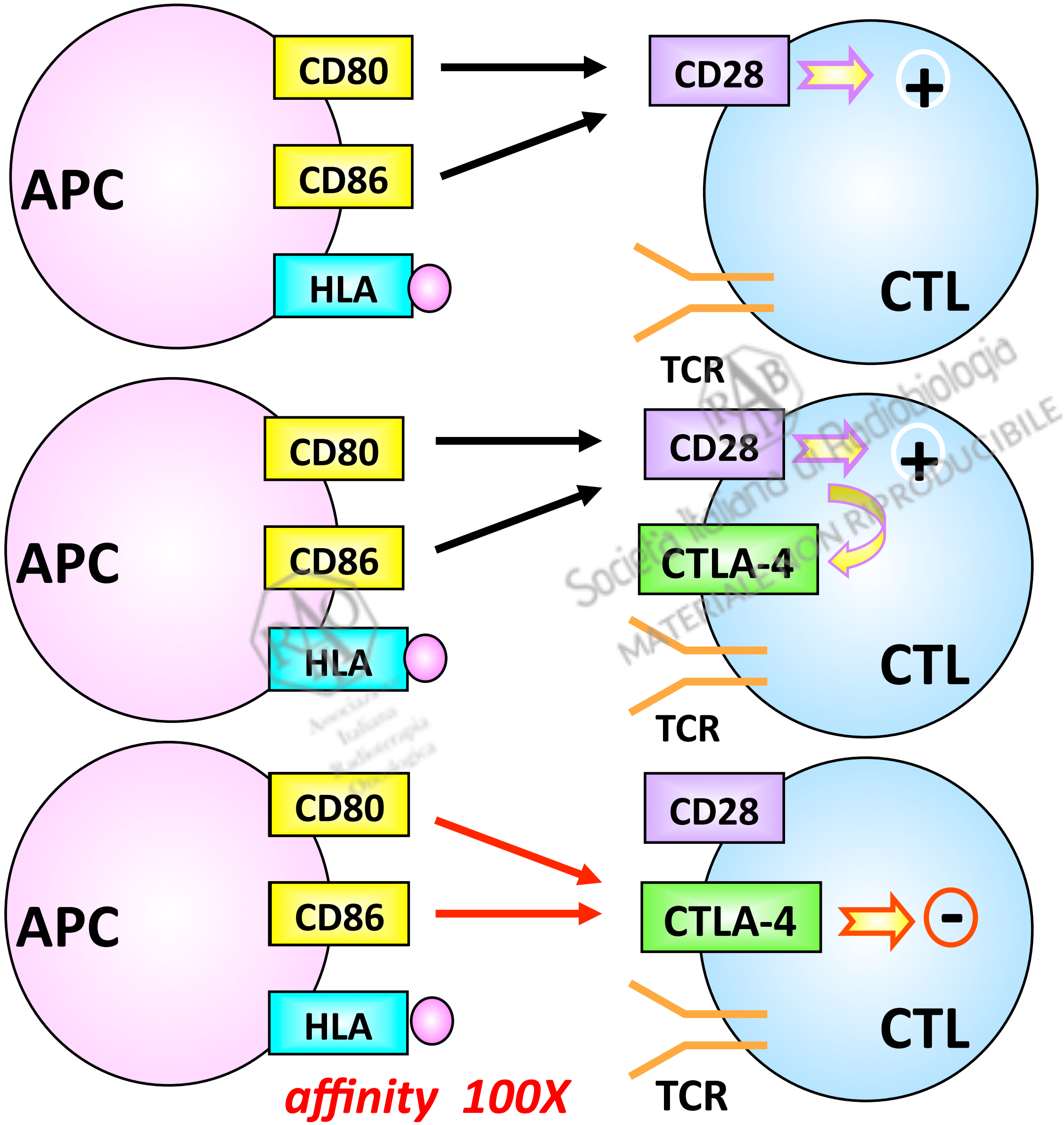


Manipulation of “co-stimulatory – checkpoints” signals



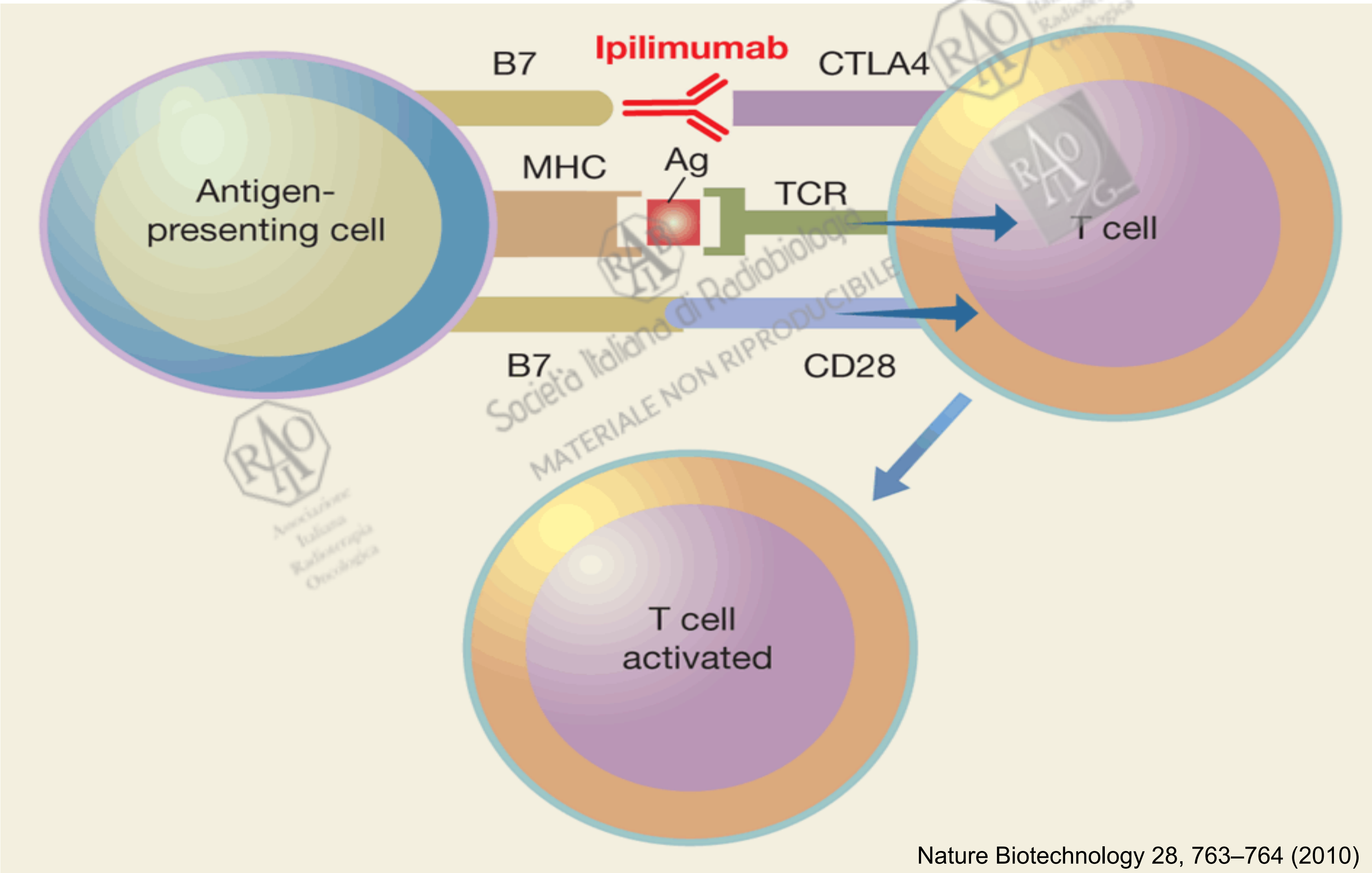
Mellman *et al.* *Nature* **480**, 480-489 (2011) doi:10.1038/nature10673

Manipulation of co-stimulatory signals
The **CTLA-4** pathway



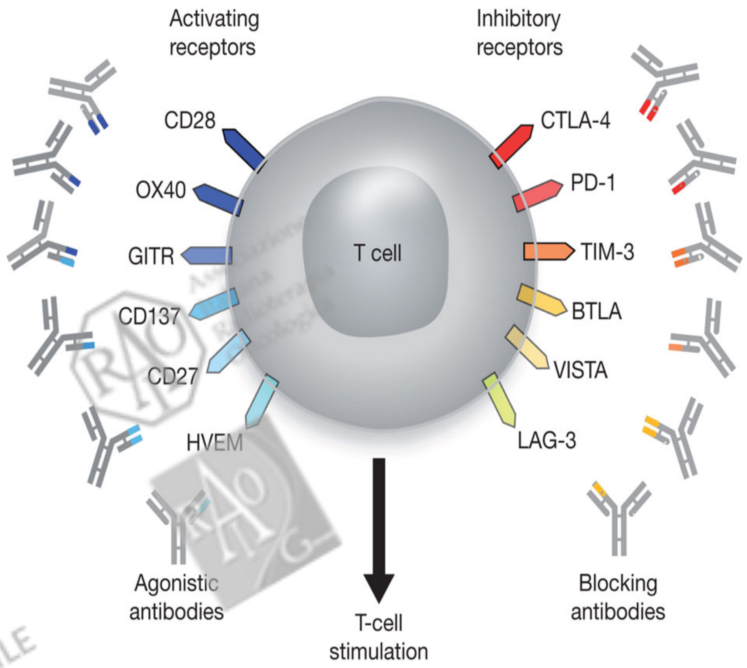
Adapted from : Jean Sibilia, Rhumatologie - CHU de Strasbourg

Ipilimumab (Yervoy®) : fully human monoclonal antibody against CTLA4



Nature Biotechnology 28, 763–764 (2010)

Manipulation of co-stimulatory – checkpoints (future perspectives → **multiple checkpoints blockade**)



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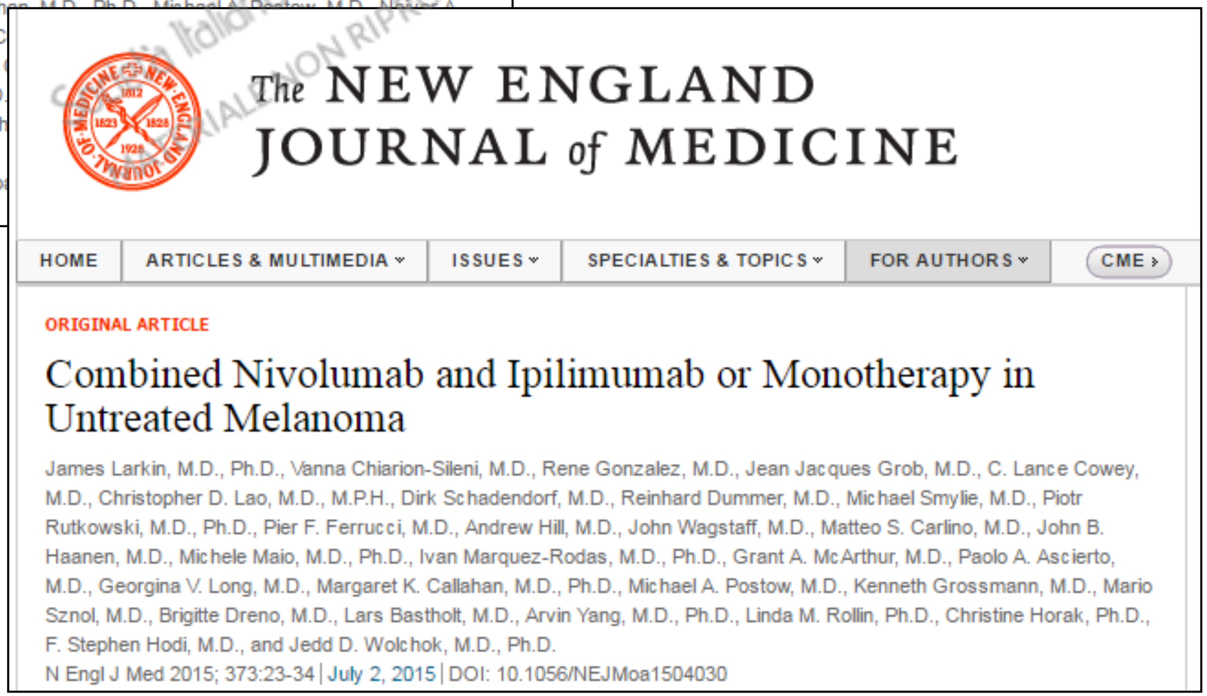
ORIGINAL ARTICLE

Nivolumab plus Ipilimumab in Advanced Melanoma

Jedd D. Wolchok, M.D., Ph.D., Harriet Kluger, M.D., Margaret K. Callahan, M.D., Ph.D., Michael A. Postow, M.D., Naiyer A.
Rizvi, M.D., Alexander M. Lesokhin, M.D., Neil H. Segal, M.D., Ph.D., C
B.S.N., Kathleen Reed, M.S., Matthew M. Burke, M.B.A., M.S.N., Anne
Blessing U. Agunwamba, B.A., Xiaoling Zhang, Ph.D., Israel Lowy, M.D.
M.S., Christine E. Horak, Ph.D., Quan Hong, Ph.D., Alan J. Korman, Ph
Ph.D., and Mario Sznol, M.D.

N Engl J Med 2013; 369:122-133 | July 11, 2013 | DOI: 10.1056/NEJMoa



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ORIGINAL ARTICLE

Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma

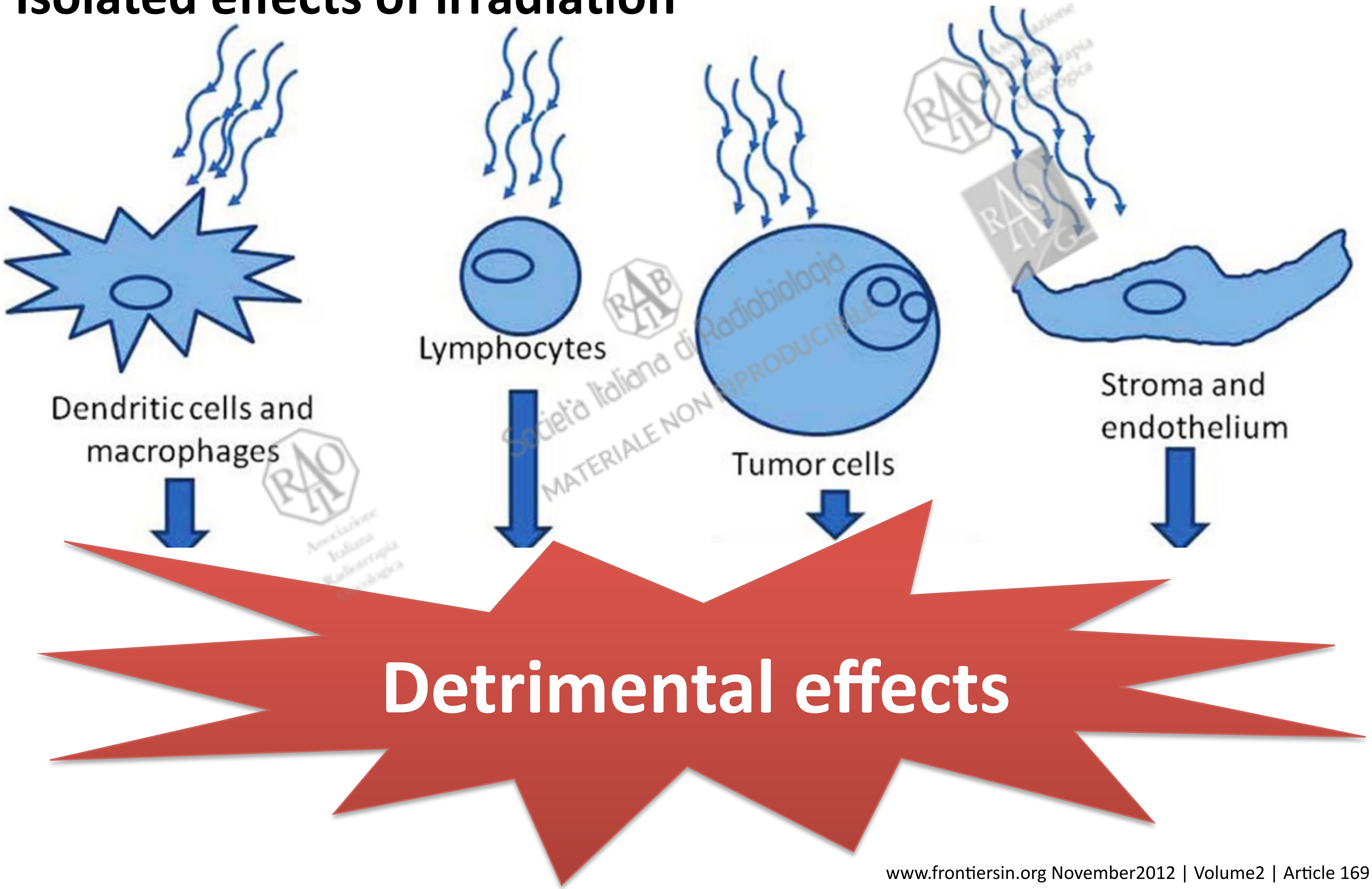
James Larkin, M.D., Ph.D., Vanna Chiarion-Sileni, M.D., Rene Gonzalez, M.D., Jean Jacques Grob, M.D., C. Lance Cowey, M.D., Christopher D. Lao, M.D., M.P.H., Dirk Schadendorf, M.D., Reinhard Dummer, M.D., Michael Smylie, M.D., Piotr Rutkowski, M.D., Ph.D., Pier F. Ferrucci, M.D., Andrew Hill, M.D., John Wagstaff, M.D., Matteo S. Carlino, M.D., John B. Haanen, M.D., Michele Maio, M.D., Ph.D., Ivan Marquez-Rodas, M.D., Ph.D., Grant A. McArthur, M.D., Paolo A. Ascierto, M.D., Georgina V. Long, M.D., Margaret K. Callahan, M.D., Ph.D., Michael A. Postow, M.D., Kenneth Grossmann, M.D., Mario Sznol, M.D., Brigitte Dreno, M.D., Lars Bastholt, M.D., Arvin Yang, M.D., Ph.D., Linda M. Rollin, Ph.D., Christine Horak, Ph.D., F. Stephen Hodi, M.D., and Jedd D. Wolchok, M.D., Ph.D.

N Engl J Med 2015; 373:23-34 | July 2, 2015 | DOI: 10.1056/NEJMoa1504030

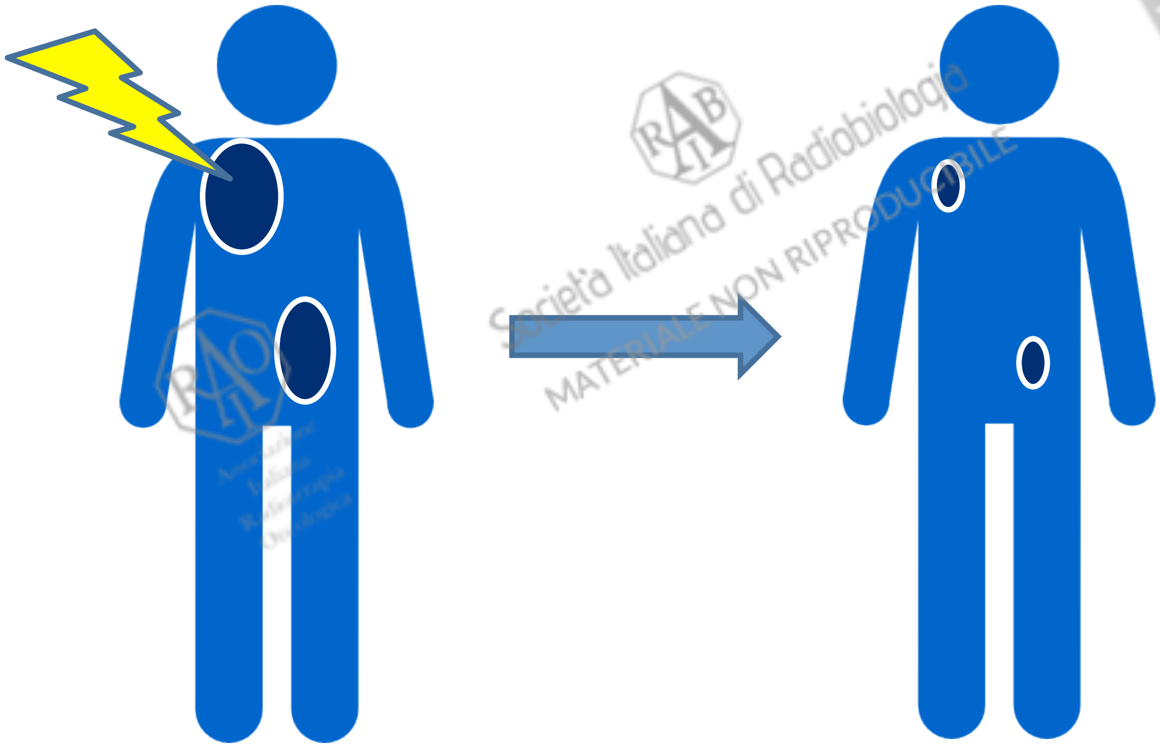
- Basic immunology
- Cancer immunotherapy
- **Immunomodulatory properties of RT**
- RT-immunotherapy combination trials
- Logistical challenges in associating IT and RT

Immunomodulatory properties of RT

Isolated effects of irradiation



Abscopal effect, from the latin *ab* (away from) and the greek *skopos* (target) describe a rare phenomenon in which the effects of RT are seen outside of the treated area.



Mole RH. Whole body irradiation; radiobiology or medicine? *Br J Radiol*. 1953;26(305):234–241.

In 2012, two case reports highlighted the immunoadjuvant effect of RT in melanoma…

Published in final edited form as:
Int J Radiat Oncol Biol Phys. 2013 February 1; 85(2): 293–295. doi:10.1016/j.ijrobp.2012.03.017.

The abscopal effect associated with a systemic anti-melanoma immune response

Emily F. Sta
Lee, MD[f], ar

The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

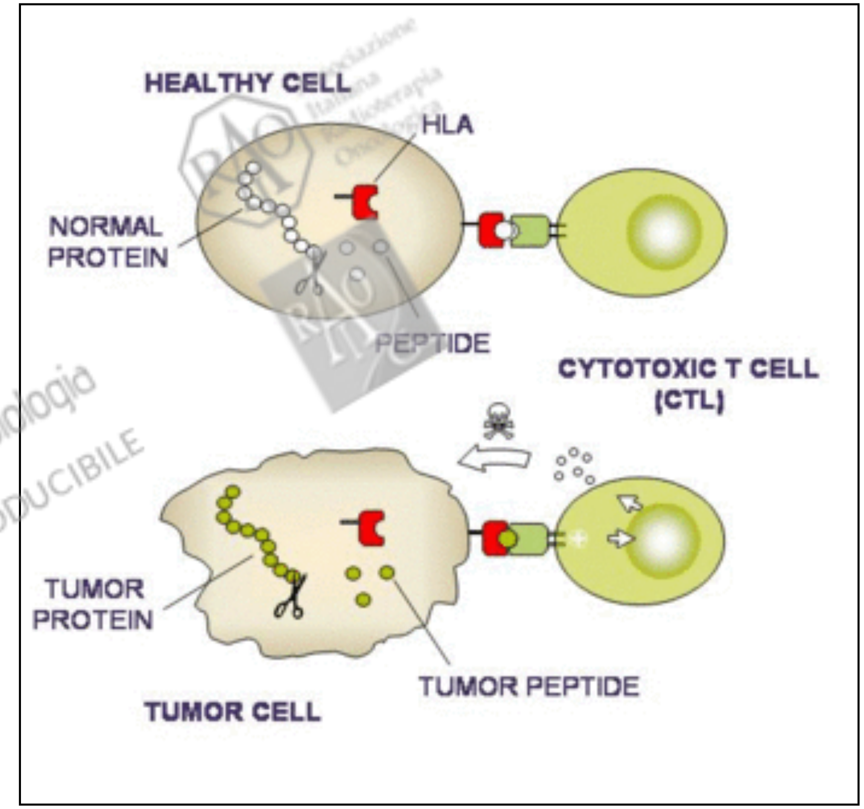
Immunologic Correlates of the Abscopal Effect in a Patient with Melanoma

Michael A. Postow, M.D., Margaret K. Callahan, M.D., Ph.D.,

- preexisting tumor-specific antibody levels rose
- T cell activation markers were enriched
- new antitumor antibodies were identified

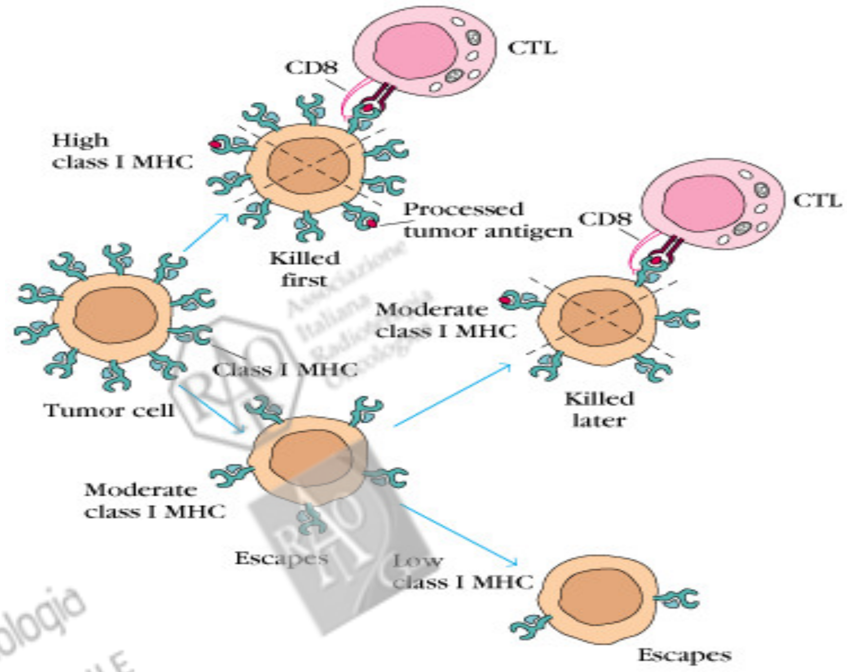
How RT counters immune evasion ?

- Antitumor response to vaccines correlates with the number of antigens to which the immune system mounts a response (variety)
- Tumor can lose the ability to process antigens intracellularly (quantity)



Irradiation is capable to generate new peptides and increase the pool of intracellular peptides presented

Tumors evade antigen presentation (↓ MHC-I molecules = ↓ antigen recognition by CD8+ CTLs)



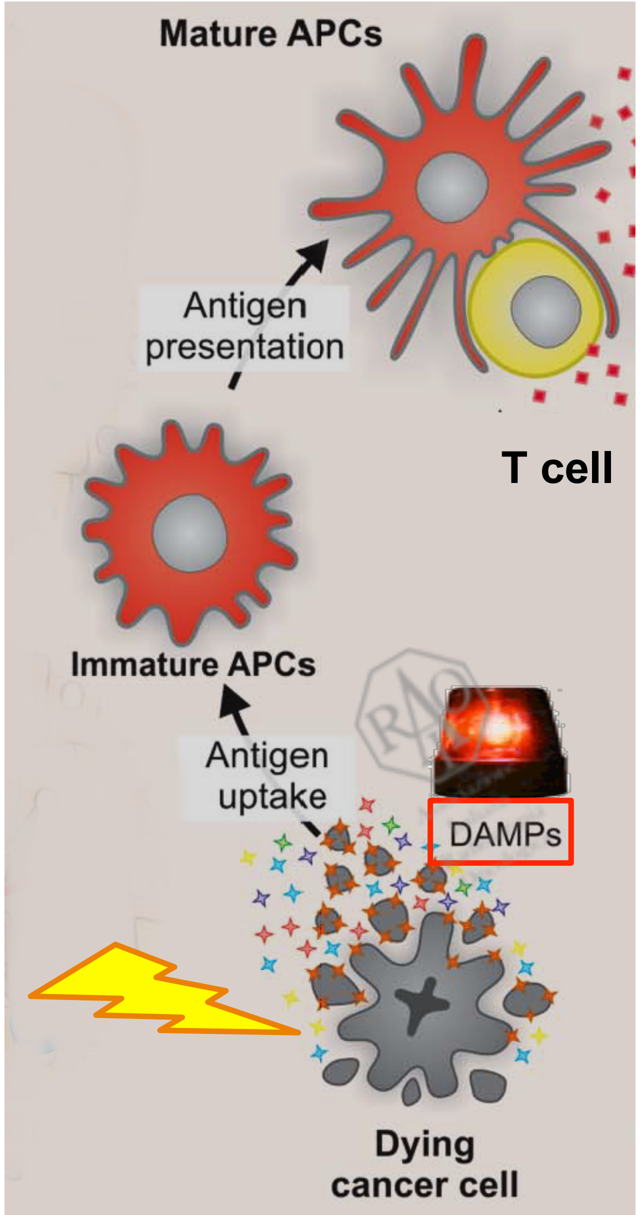
Radiation **augments MHC-I expression**

+ up-regulation on tumor cells of other surface molecules such as :

- **co-stimulatory molecules**
- **adhesion molecules** (e.g. ICAM-1)

Kuby Immunology: 7th Edition (International Edition) by Judy Owen, Jenni Punt, Sharon Stranford

The Immunogenic Cell Death



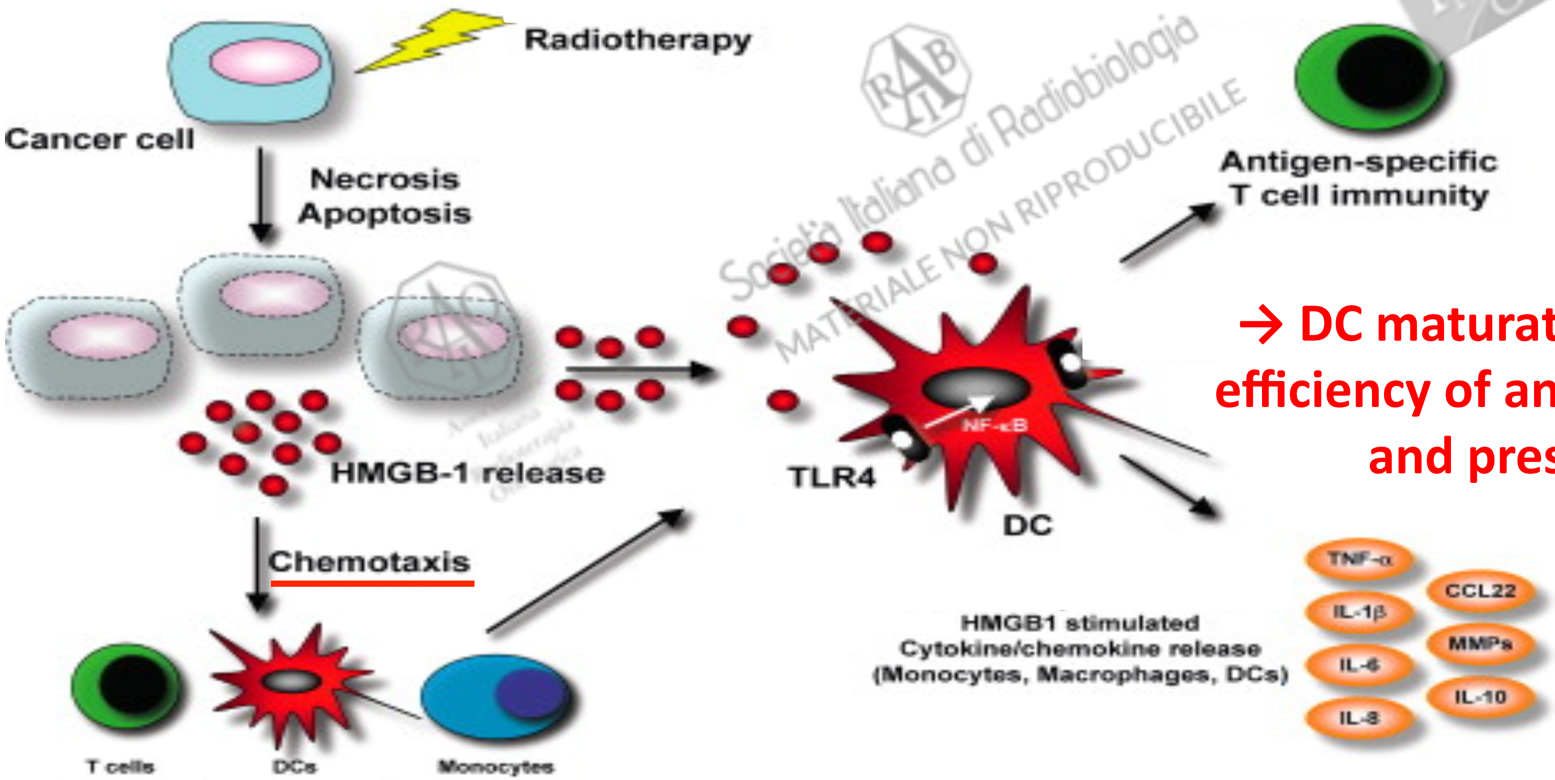
A form of cell death capable to induce an anti-tumor immune response through activation of DCs and consequent priming of cytotoxic T cells.

ICD is characterized by the release "**Damage-associated Molecular Patterns**" (DAMPs) that act as endogenous adjuvants.

Adapted from: Kepp O, et al. Consensus guidelines for the detection of immunogenic cell death. Oncoimmunology. 2014 Dec 13;3(9):e955691.

Immunogenic Cell Death

RT causes dying tumor cells to release "high mobility group box 1" (**HMGB-1**), a "danger signal" that binds TLR4.

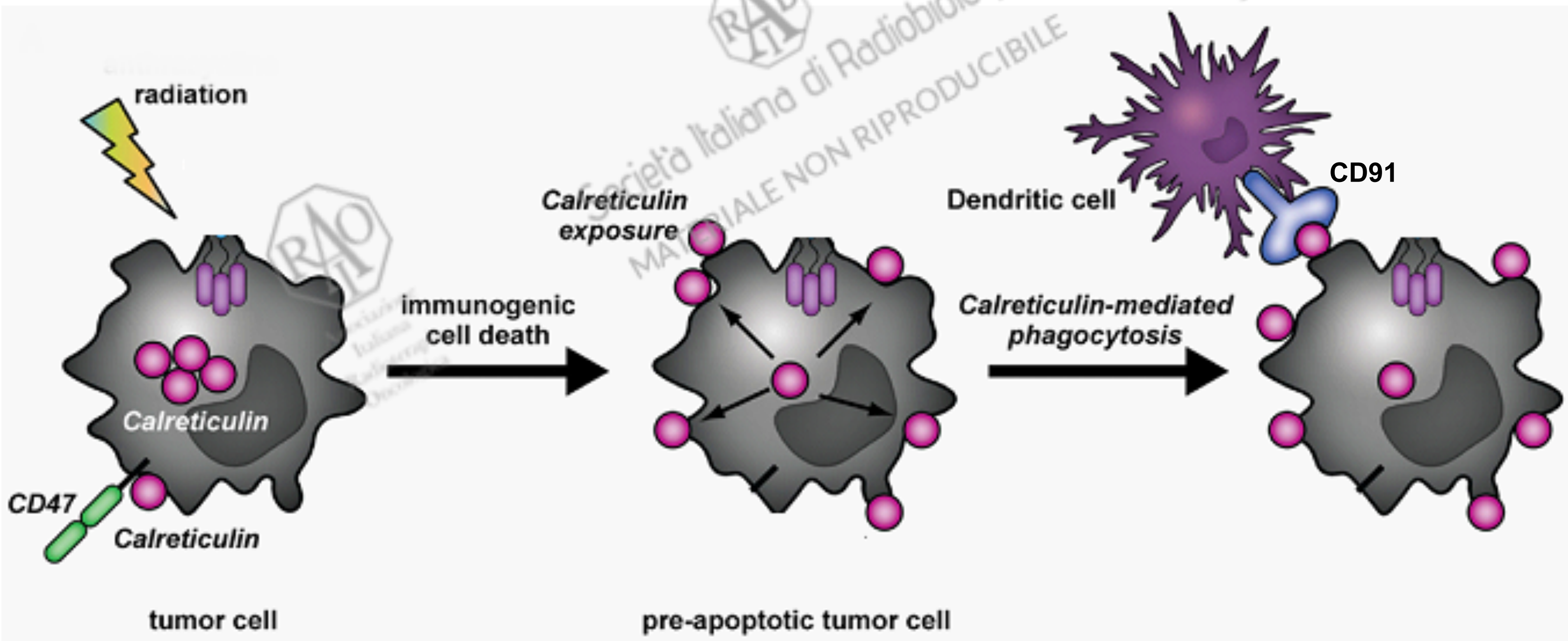


→ **DC maturation (increase in efficiency of antigen processing and presentation)**

Inflammation and Cancer: A Double-Edged Sword Cancer Cell 12, October 2007

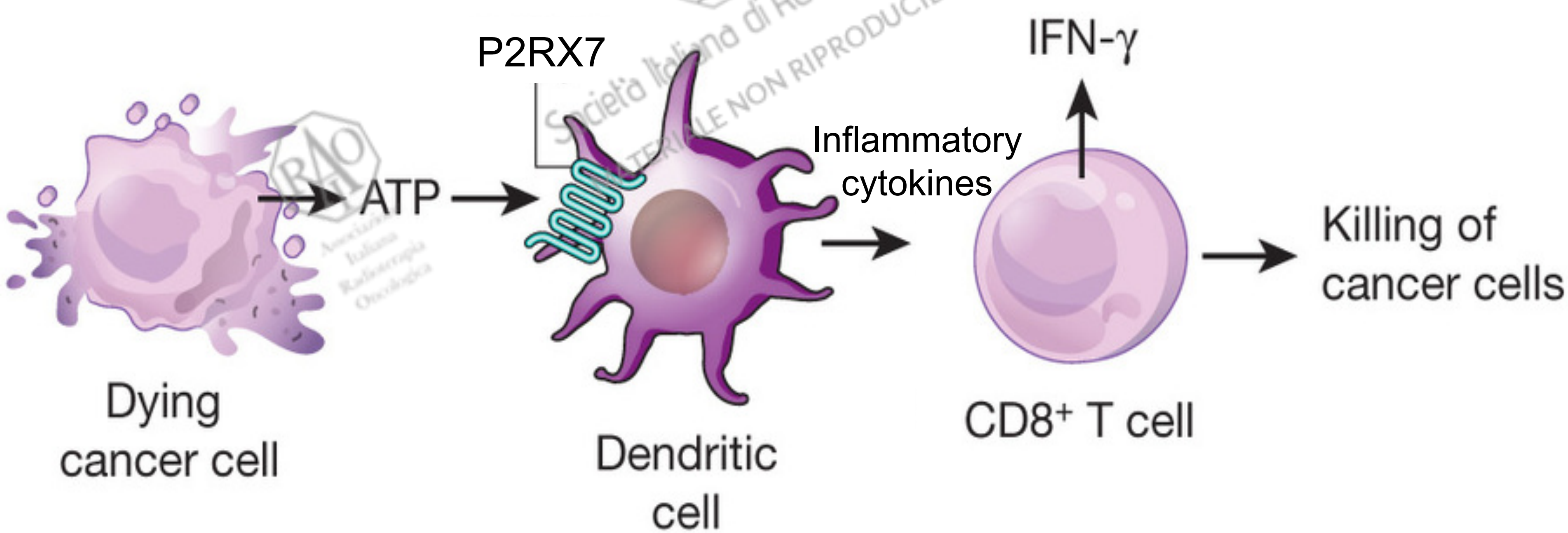
Immunogenic Cell Death

RT causes **calreticulin** translocation to the surface of tumor cells → “**eat me**” signal (+ reduction of CD47 expression - DC “do not-eat-me” signal) = enhancing antigens processing and presentation.



Immunogenic Cell Death

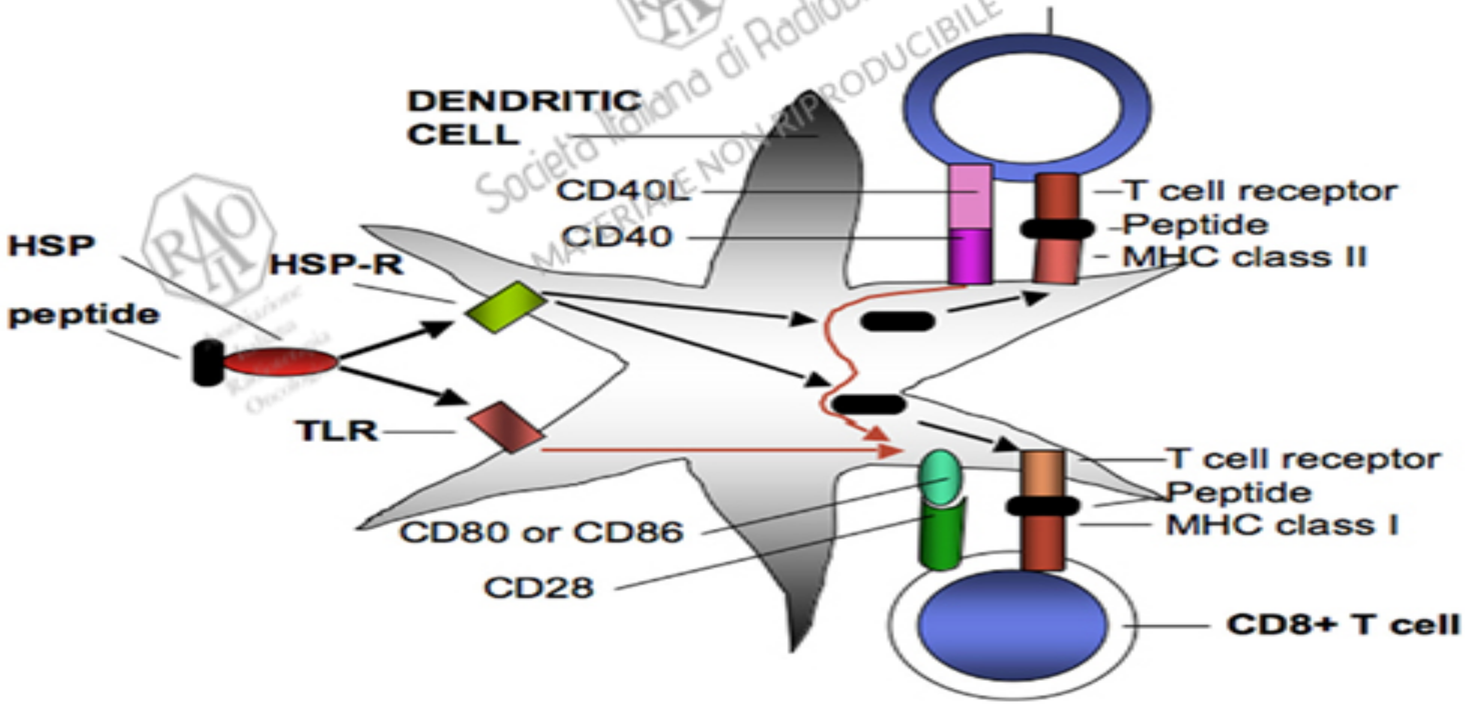
ATP released by dying cells binds to purinergic receptor (P2RX7) on DCs leading to inflammatory **cytokines** production (e.g. IL-1β, TNFα, IL-18).



Adapted from: Idzko M, Ferrari D, Eltzschig HK. Nucleotide signalling during inflammation. Nature. 2014 May 15;509(7500):310-7

Immunogenic Cell Death

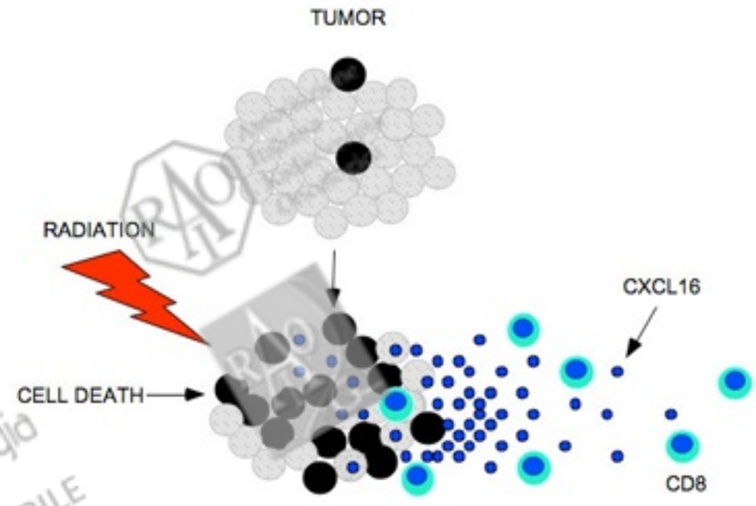
Other important DAMPs translocated to the plasma membrane are **heat-shock proteins** (HSPs) which interact with different APC receptors.



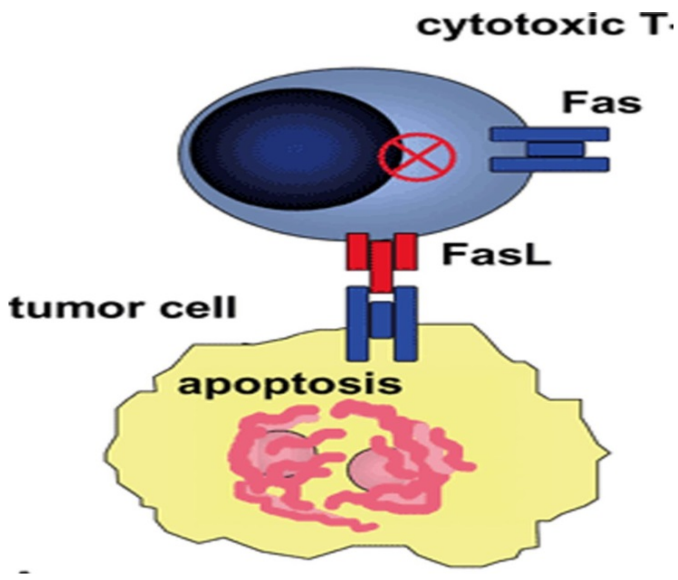
Calderwood SK, Murshid A, Gong J. Heat shock proteins: conditional mediators of inflammation in tumor immunity. Front Immunol. 2012 Apr 9;3:75.

RT: helps the T cell response

Irradiated tumor site release of **chemokines** (such as CXCL16) that recruit cytotoxic T cells



Irradiated tumors upregulate **death receptors** (e.g. FAS), promoting the cytotoxic effect of T cells at the tumor site



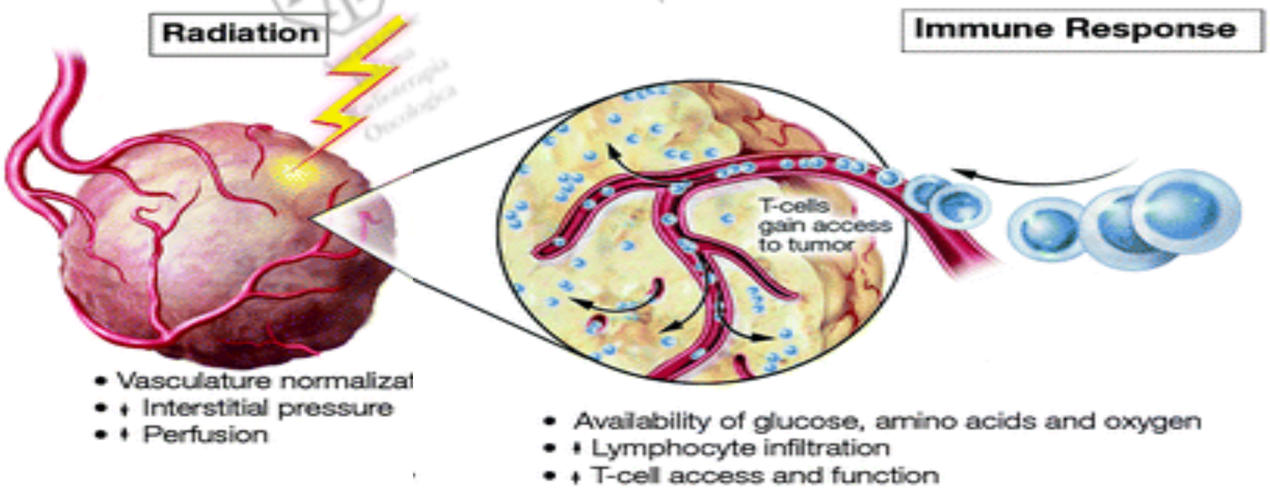
The Journal of Immunology, 2008, 181: 3099-3107

Radiation Therapy vascular effects

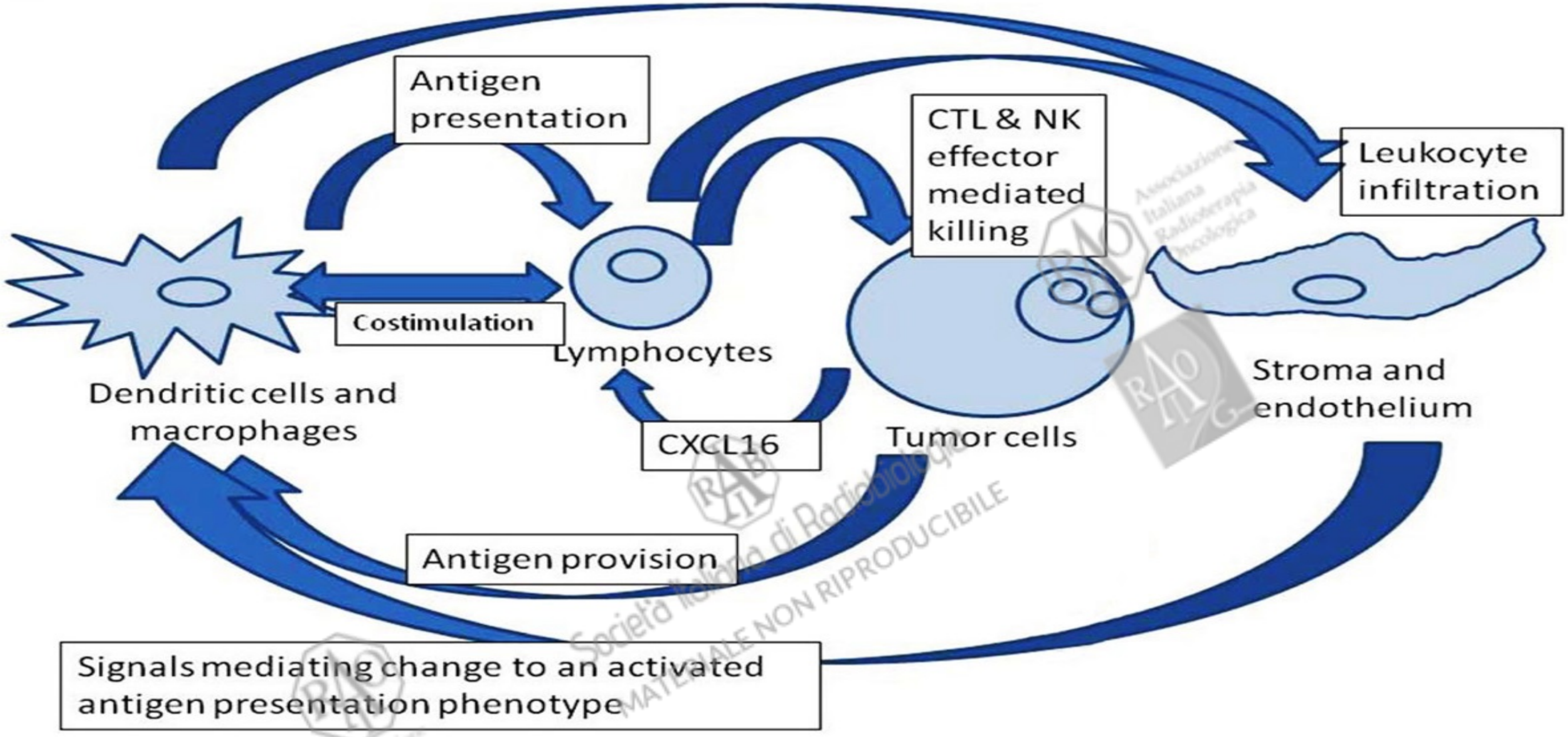
Excessive production of pro-angiogenic factors → abnormal vascular structure → <u>hypoxic microenvironment</u> :

- Hinders immune cells at effectively entering into tumor tissue
- Recruitment of immunosuppressive cells

RT induce normalization of tumor vasculature



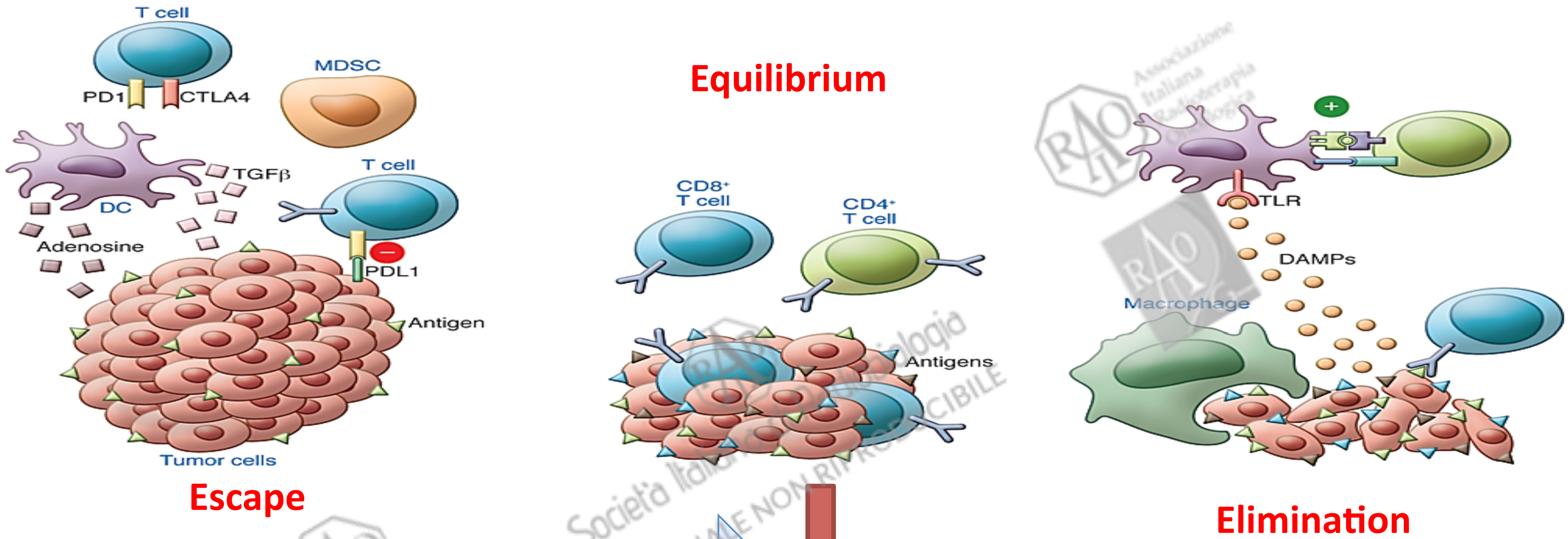
Mol. BioSyst., 2009, 5, 1262-1270
International Journal of Radiation Biology, April 2015; 91(4): 299–305



The combined effects of radiation provide **antigens** along **with adjuvant activating signals** → **"*in situ* anti-tumor vaccine"**

Why are abscopal effects so rare?

Immunosuppression dominates in established tumors



Radiotherapy alone

Immunotherapy alone

Immunotherapy + Radiotherapy ?

→ strong rationale for combining RT with immunotherapy

- Basic immunology
- Cancer immunotherapy
- Immunomodulatory properties of RT
- **RT-immunotherapy combination trials**
- Logistical challenges in associating IT and RT

RT-immunotherapy combination trials (preliminary results)

EFFICACY

- Survival data non available (phase I/II trials)
- Current imaging modalities may be unable to distinguish between inflammation (induced by RT and/or immunotherapy) and persistent disease :
  - "indirect evidences" → peripheral blood examination for cytokine levels, antigen-specific T cell responses, phenotypes of immune cells…
  - "direct evidences" → pathologic evidence of antitumor immunity (essential)

RT-immunotherapy combination trials (preliminary results)

SAFETY

- Patients reported a variety of issues but the majority were relatively minor.
- Toxicities were dependent on the agent given and dosage (e.g. high dose IL-2).

Most common problems were:

- Injection site reactions (pain, swelling and/or local erythema)
- Flu-like symptoms (fever, fatigue, myalgia, chills or arthralgia)

→ The combination of RT and immunotherapy has proven to be safe in a controlled setting.

RT-immunotherapy combination trials (ongoing)

ClinicalTrials.gov identifier	Disease site	Design	Phase	Primary outcome measure	Immunotherapy	RT	Treatment timing
NCT01449279	Melanoma (advanced)	1 arm: ipilimumab prior to palliative RT	1	Safety	Ipilimumab	Palliative	RT <2 days after ipilimumab
NCT01689974	Melanoma (advanced)	2 arms, randomized: ipilimumab prior to RT or ipilimumab alone	2	Tumor response	Ipilimumab	30 Gy in 5 fractions	RT starts 4 days prior to ipilimumab
NCT01557114	Melanoma (advanced)	1 arm: ipilimumab prior to RT	1	Maximum tolerated dose	Ipilimumab	9, 15, 18, 24 Gy in 3 fractions	RT from week 4 to week 10 of ipilimumab
NCT01565837	Melanoma (advanced)	1 arm: ipilimumab prior to SRT	2	Safety, tolerability	Ipilimumab	SRT to 1–5 lesions	RT after first dose of ipilimumab, before week 6
NCT01497808	Melanoma (advanced)	1 arm: SRT prior to ipilimumab	1/2	Dose-limiting toxicity	Ipilimumab	SRT to 1 lesion	RT prior to ipilimumab
NCT00861614	Prostate (castrate resistant)	2 arms, randomized: RT prior to ipilimumab vs. RT alone	3	Overall survival	Ipilimumab	Not specified	RT prior to ipilimumab
NCT01347034	Soft tissue sarcomas	2 arms, nonrandomized: RT alone vs. RT plus dendritic cell therapy, then surgery	2	Immune response	Autologous dendritic cell intratumoral injection	Conventional RT with boost	Dendritic cell injection during RT
NCT01421017	Breast cancer with skin metastases	1 arm: imiquimod to all skin metastases plus RT to select skin metastases	1/2	Tumor response	Topical imiquimod	600 cGy in 5 fractions	Imiquimod starts evening of first RT
NCT00751270	Supratentorial malignant glioma	1 arm: surgical resection with Adv-tk injection, followed by pro-drug (valacyclovir) and RT	1	Safety; immune response	Adv-tk injection into tumor bed	Standard of care	Start RT 3 days after Adv-tk injection, during prodrug therapy
NCT01595321	Pancreatic cancer following resection (stage R0)	1 arm: cyclophosphamide, vaccine, SRT, and FOLFIRINOX	1	Toxicity	Low-dose cyclophosphamide and vaccine	6.6 Gy in 5 fractions	Start RT <12 weeks following operation and 7–14 days after first vaccine dose
NCT01436968	Prostate cancer, localized, intermediate or high risk	2 arms, double-blind, randomized: Adv-tk vs. placebo followed by valacyclovir; EBRT with or without androgen deprivation therapy	3	Disease-free survival	Adv-tk intraprostate injection	Standard EBRT	Adv-tk prior to, immediately prior to, and during EBRT

(NON exhaustive listing)

- Basic immunology
- Cancer immunotherapy
- Immunomodulatory properties of RT
- RT-immunotherapy combination trials
- **Logistical challenges in associating IT and RT**

Logistical challenges → timing of RT with respect to immunotherapy

- RT → adoptive T cell transfer ?

In order to avoid disruption of the T cell response at the tumor site.

- Targeted agents and vaccines → RT ?

Immunotherapy could "prepare" the tumor to the immune-stimulatory effects of RT; however, the cytotoxic effect of RT may disrupt the cellular immune response.

- αOX40 (agonist of an activating molecule) shortly after RT
- αCTLA4 (antagonist of an inhibiting molecule) pre-treatment provides the best environment for enhanced radiation efficacy

→ Ideal timing may differ by immunotherapy and its mechanism of action

→ timing of RT with respect to immunotherapy

Ongoing Ipilimumab + RT clinical trials

Clinical trial	SIte	Start date	Pre-RT	Concurrent/ post-RT	Cancer	RT dosing	Additional therapy
NCT00861614	Bristol-Myers Squibb	May-09		x	Prostate	Not specified	
NCT01557114	Gustave Roussy, Paris	Mar-11		x	Melanoma	5Gyx3, 6Gyx3, 8Gyx3	
NCT01449279	Stanford University	Oct-11		x	Melanoma	Palliative	
NCT01565837	Comprehensive Cancer Centers of Nevada	Aug-12	x		Melanoma	SART	
NCT01711515	NCI	Oct-12		x	Cervical	Fractionated	Cisplatin
NCT01703507	Thomas Jefferson University	Nov-12		x	Melanoma	Whole brain/SRS	
NCT01689974	New York University	Jan-13		x	Melanoma	6Gyx5	
NCT01935921	NCI	Apr-13		x	H&N	Fractionated	Cetuximab
NCT01860430	University of Pittsburgh	Apr-13		x	H&N	Fractionated	Cetuximab
NCT01996202	Duke University	Nov-13			Melanoma	Not specified	
NCT01970527	University of Washington	Mar-14		x	Melanoma	SBRTx3	
NCT02115139	Grupo Español Multidisciplinar de Melanoma	Apr-14	x		Melanoma	Whole brain 3Gyx10	
NCT02107755	Ohio State University	Apr-14	x		Melanoma	SABR	
NCT02097732	University of Michigan	Apr-14	x		Melanoma	SRS	

(non exhaustive listing)

Timing of RT with respect to immunotherapy


Cancer Immunology Research

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Concurrent Radiotherapy and Ipilimumab Immunotherapy for Patients with Melanoma

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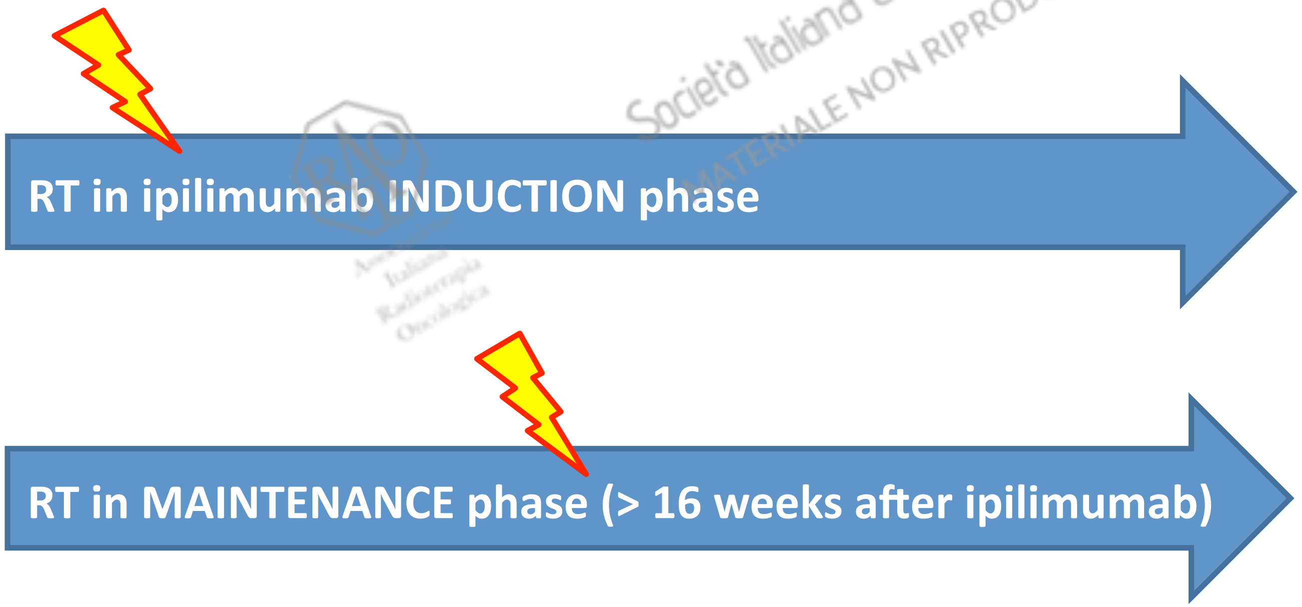


9-month median overall survival

39-month median overall survival

Logistical challenges → fractionation and dosing

Is it better to use:

- conventional *versus* hypo- *versus* hyper-fractionated regimens ?
- total dose ?

Current data are not conclusive...

RT should be «re-invented» as an immunologic tool instead of a cytotoxic treatment

Logistical challenges → choosing a site for RT

Immune response to RT may depend on the site of irradiation → different immune phenomenon at each site.

▪skin, gut and lung ("great activity" of the immune system because of the exposure to external pathogens).

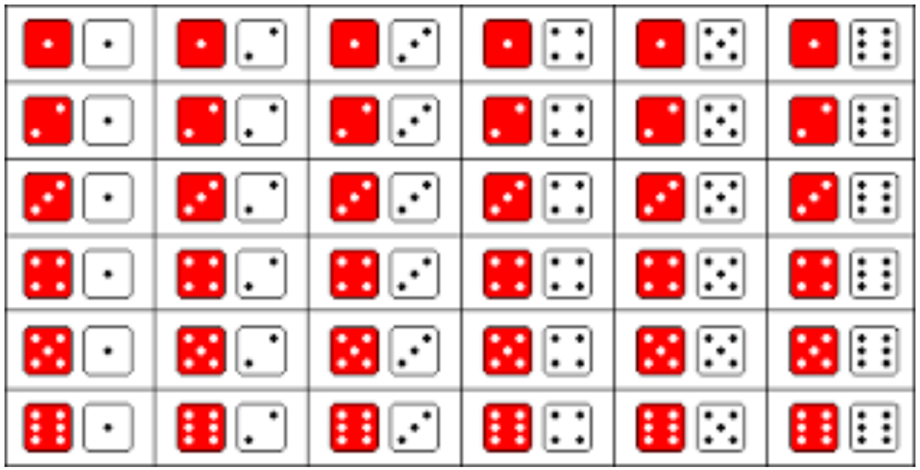
▪liver immunology is different due to its chronic and persistent exposure to toxic metabolites and antigens from the GI tract.

Optimal site to irradiate in metastatic disease ?

Combining RT and immunotherapy: infinite possibilities…

- External beam radiotherapy (EBRT)
- Stereotactic body radiation therapy (SBRT)
- Brachytherapy
- Bone-seeking radionuclides (^{153}Samarium…)
- Radiolabeled antibodies
- Proton therapy

- Unspecific stimulation
- Vaccine-based therapy
- Effector cell transfer
- Immune checkpoint blockade
- Targeted immunotherapeutics



Take-home messages

- RT is able to induce local and systemic immune responses (*in situ* vaccination)
- Radiotherapy may improve efficacy of immunotherapy and *vice versa*
- Strong preclinical data and early clinical observations report safety and efficacy from combined RT + IT treatments
- Logistical aspects (timing, dose/fractionation, site…) of RT/IT combination still need to be fully elucidated

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Thank you for your attention !

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