VI ZOOM Journal Club 2016
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Immunoterapia

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Policlinico

Immunotherapy

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Cancer and immune system

PD-1/PD-L1 pathways

RT and immunotherapy

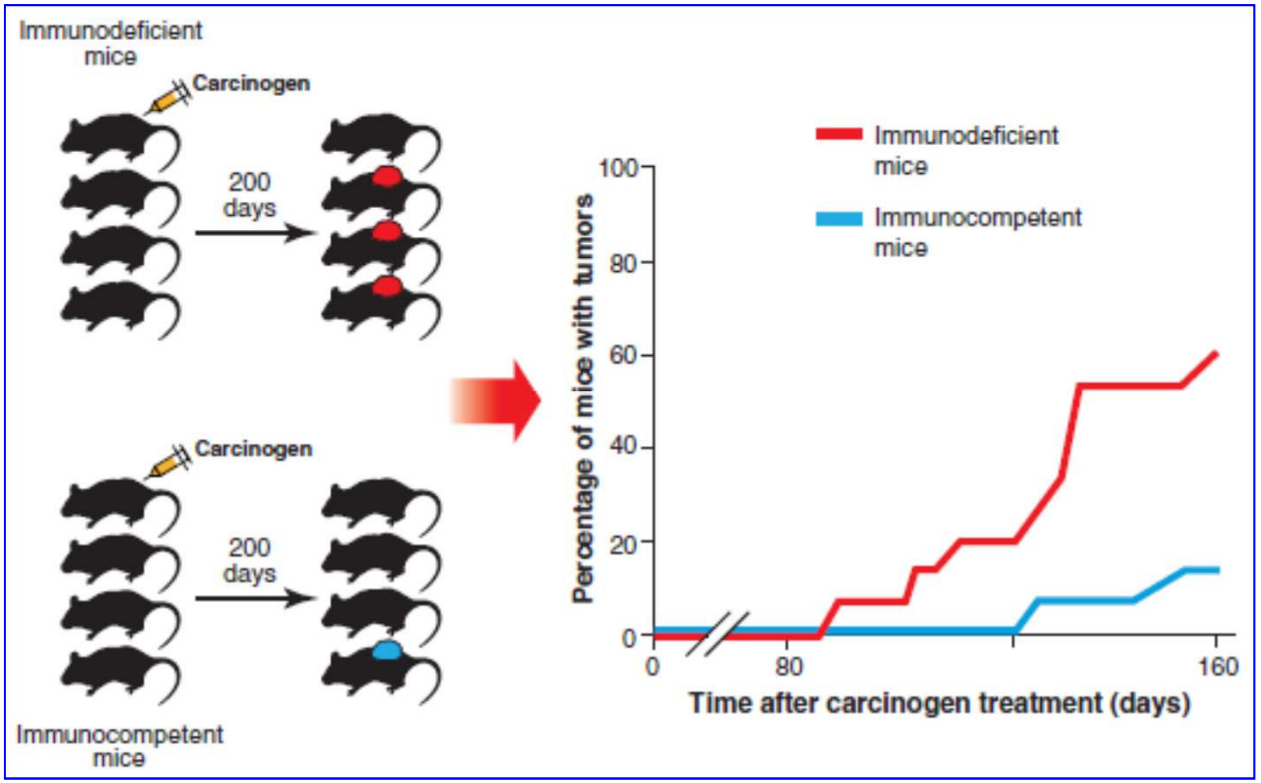
Clinical trials in breast cancer

Cancer and immune system

JNCI Journal of the National Cancer Institute

Effect of Host Immune Capability on Radiocurability and Subsequent Transplantability of a Murine Fibrosarcoma2

Degree of immunocompetence of the host influence the response to cancer therapy

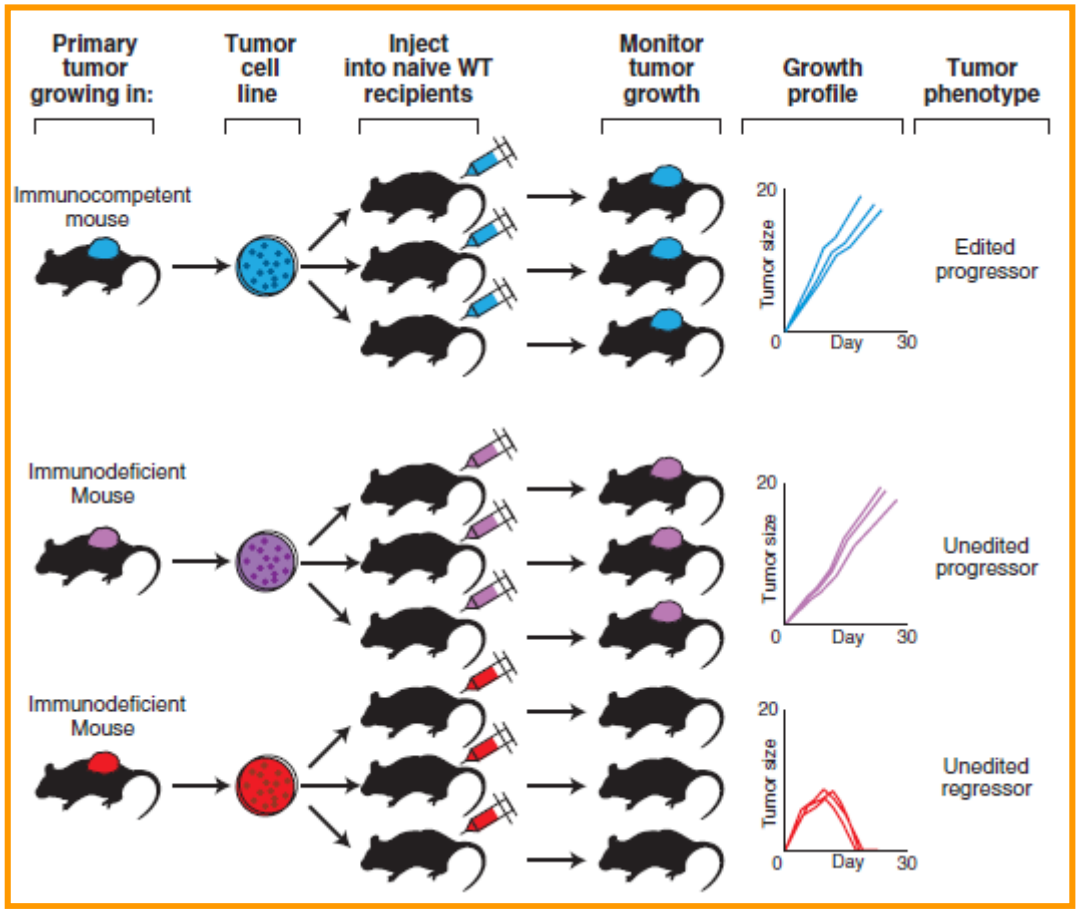


Shankaran V. et al., *Nature* **2001;** 410:1107

Stone H.B. et al., *JNatlCancerInst* **1979;** 63:1229-35

Cancer and immune system

Cancer Immunoediting



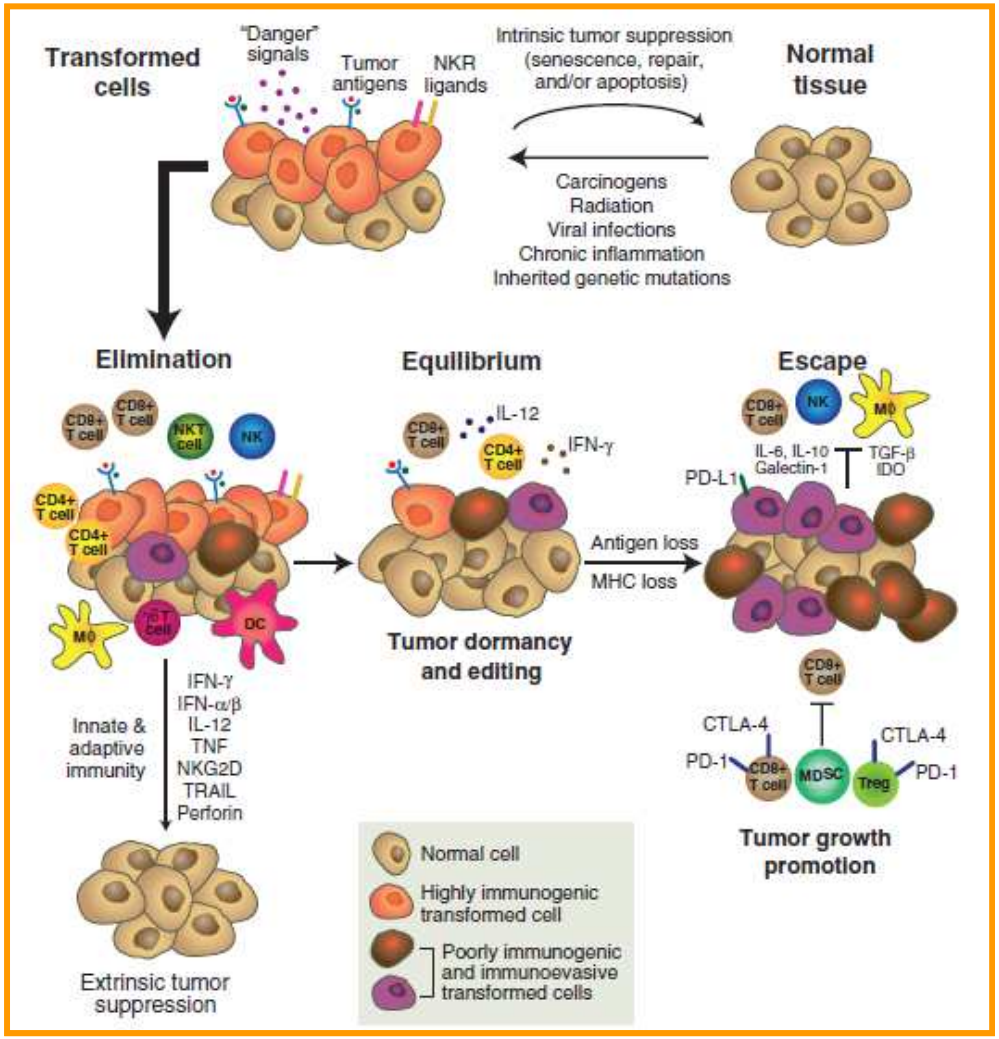
Immune system controls not only tumor quantity but also tumor quality (**immunogenicity**)

Tumors formed in mice that lacked an intact immune system are more immunogenic (***unedited***) than similar tumors derived from immunocompetent mice (***edited***)

Shankaran V. et al., *Nature* **2001;** 410:1107

Cancer and immune system

Cancer Immunoediting



Elimination: *innate* and *adaptive* immunity work together to destroy developing tumors before they become clinically apparent

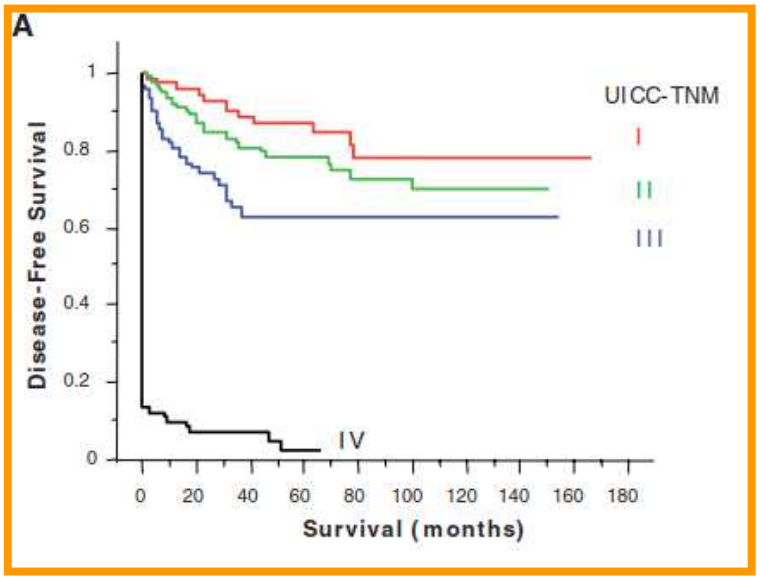
Equilibrium: *adaptive immune system* prevents tumor cell outgrowth and also sculpts the immunogenicity of the tumor cells; maintains residual tumor cells in a functional state of dormancy

Escape: tumor cells that have acquired the ability to circumvent immune recognition and/or destruction emerge as progressively growing, visible tumors

Schreiber RD et al., *Science* **2011;** 331:1565

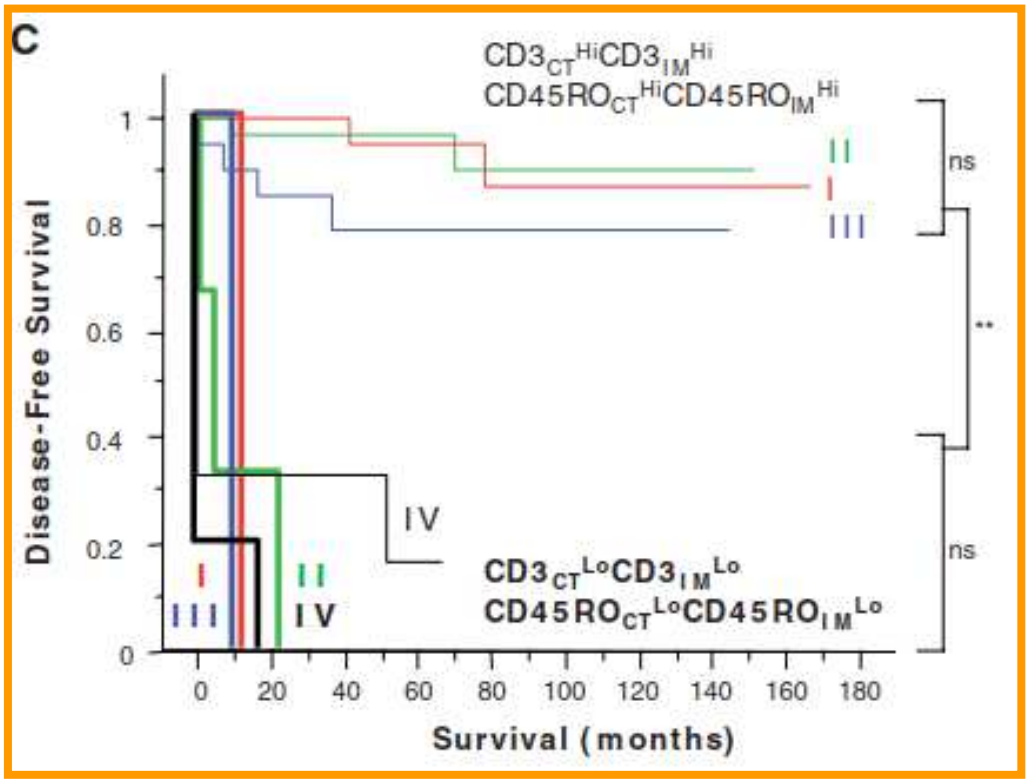
Cancer and immune system

Immune cells predict clinical outcome



Immunological data: type, density, and location of immune cells within the tumor samples

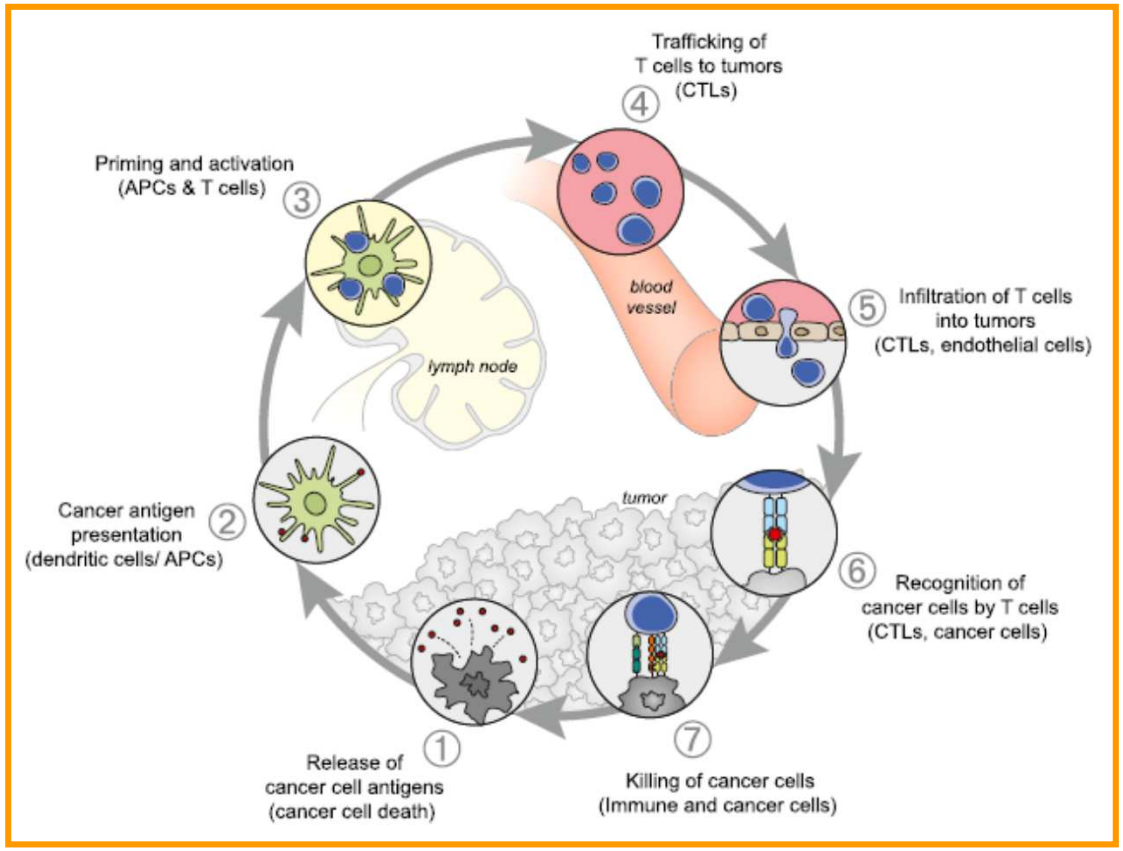
Adaptive immune response influences the behavior of human tumors



Cancer and immune system

Cancer-Immunity Cycle

For an anticancer immune response to lead to effective killing of cancer cells, a series of stepwise events must be initiated and allowed to proceed and expand iteratively

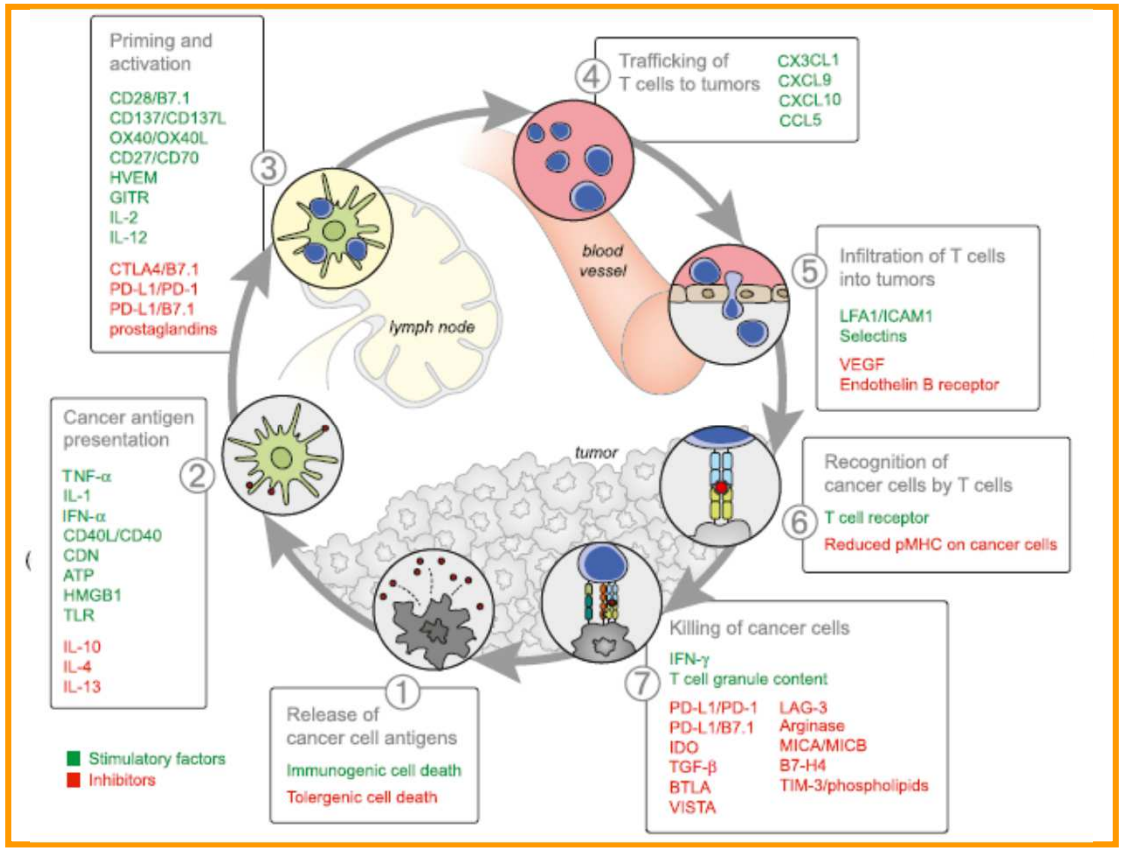


Chen D.S. et al., *Immunity* **2013;** 39:1-10

Cancer and immune system

Cancer-Immunity Cycle

Each step of the Cancer-Immunity Cycle requires the coordination of numerous factors, both stimulatory and inhibitory



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Cancer and immune system

PD-1/PD-L1 pathways

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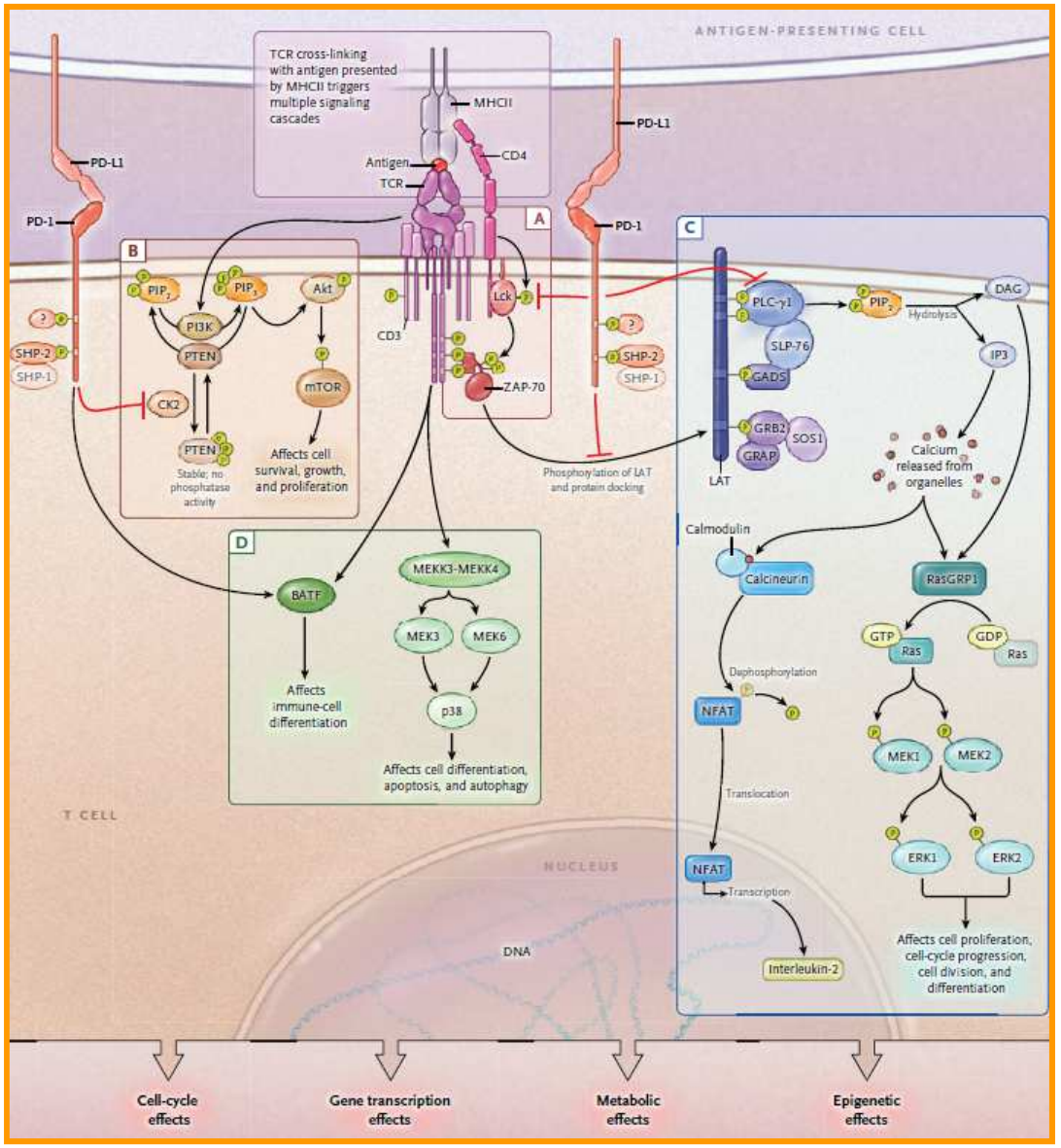
PD-1/PD-L1 pathways

PD-1 Signaling Pathways in T Cells

TCR crosslink with antigen presented by MHC I-II molecules → tyrosines of the cytoplasmic tail of *PD-1 phosphorylated*
Activation of the *PI3K–Akt–mTOR* pathway and activation of the Ras–*MEK–ERK* pathway are *inhibited*.
Other signaling events, such as the activation of the p38 pathway, remain unaffected or enhanced

The imbalanced activation of signaling pathways *alters cell-cycle progression, gene transcription, metabolism, and epigenetic programs in **T cells**.*



Boussiotis V. A. *The New England journal of medicine* **2016;** 375: 1767-78

PD-1/PD-L1 pathways

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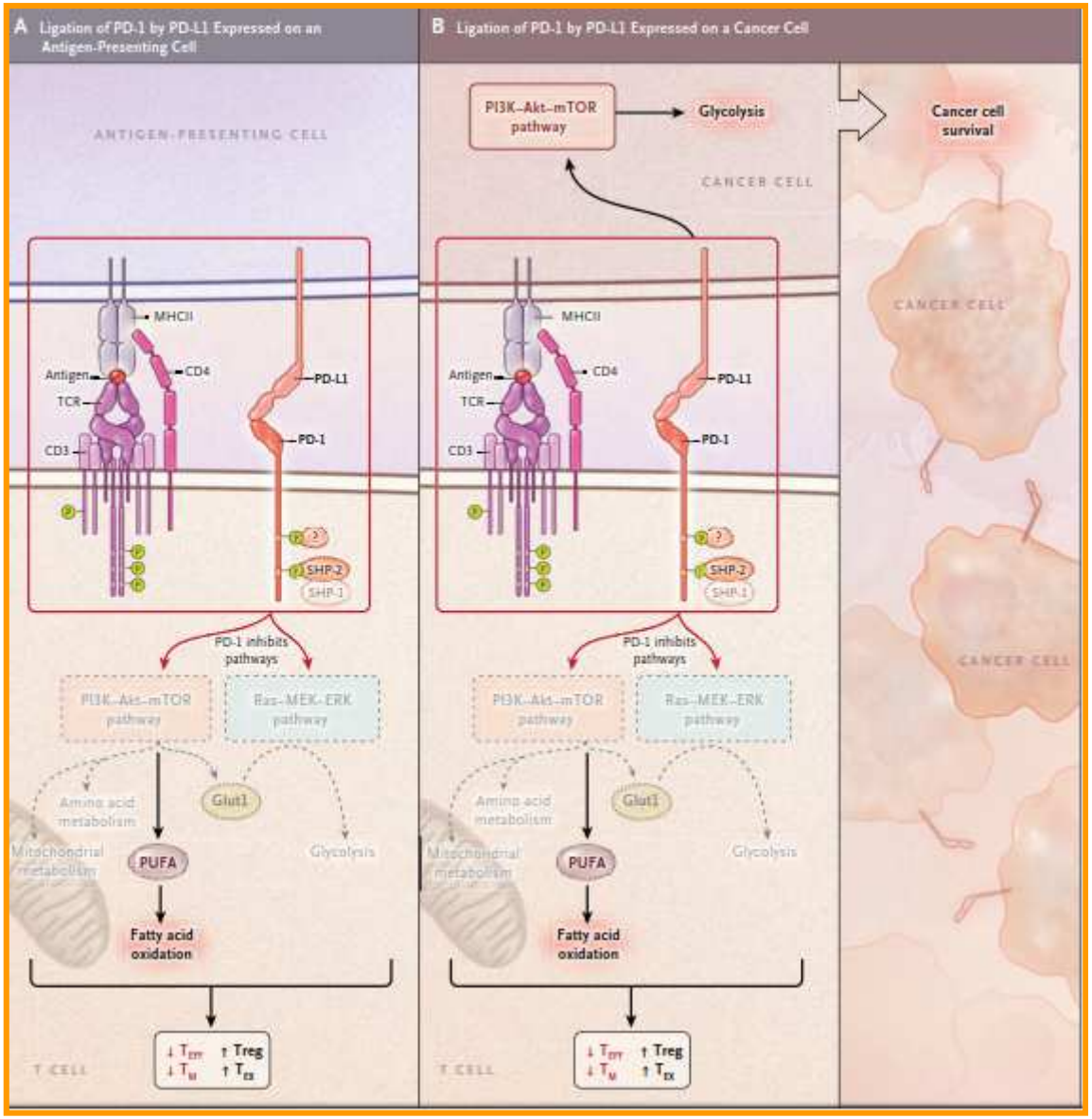

Alteration of metabolism by the PD-1 Checkpoint Pathway

PD-1-PD-L1 alters T-cell metabolic: inhibit glycolysis, AA and mitochon metabolism, promoting the accumulation of PUFA and activation of fatty acid oxidation

T_{eff} and T_m → Treg and Tex

In cancer cells, expression of PD-L1 might result in increased levels of PI3K–Akt–mTOR activation and an elevated rate of tumor-intrinsic glycolysis as a consequence of *improved survival*

Boussiotis V. A. *The New England journal of medicine* **2016;** 375: 1767-78

PD-1/PD-L1 pathways

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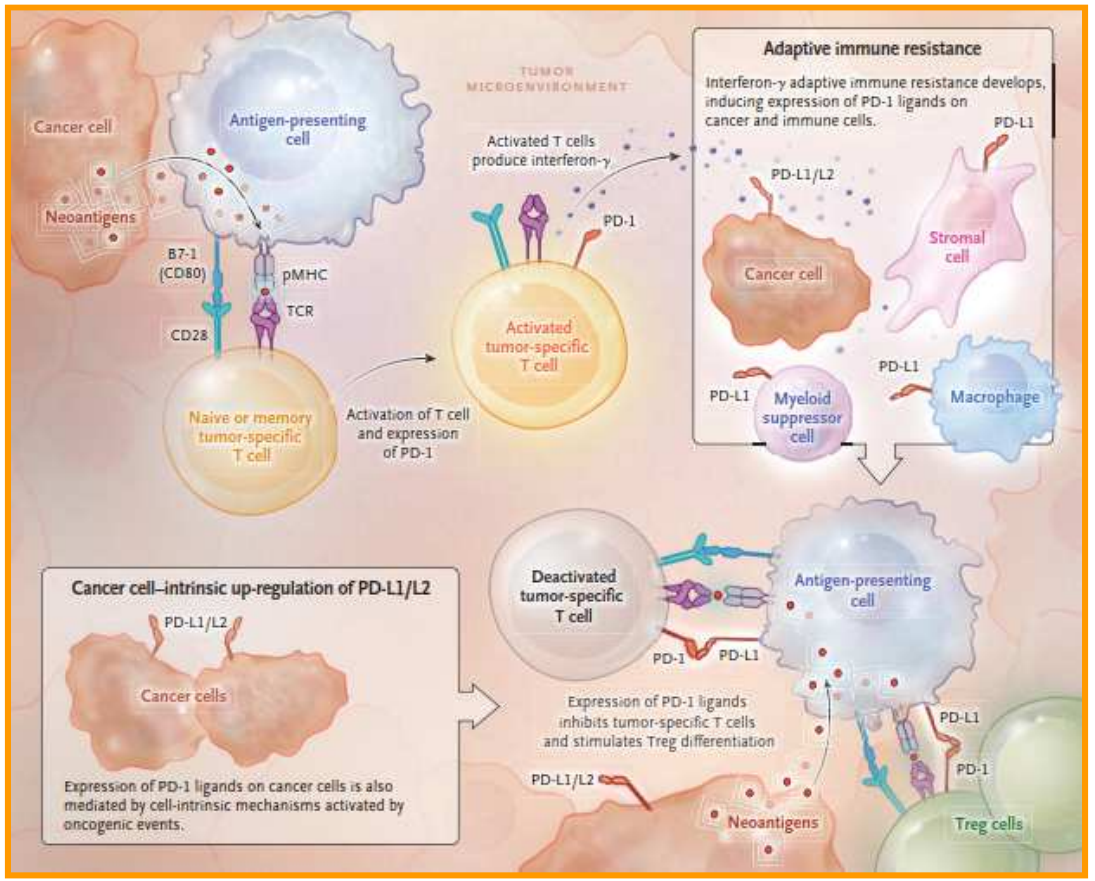
Biologic Aspects of PD-1 – PD-L 1/L 2 for Cancer Therapy

Expression of **PD-L1** on tumor through various mechanisms:

Cell-intrinsic mechanisms that are activated by oncogenic events (activation of EGFR, MAPK, or PI3K–Akt pathways)

By inflammatory cytokines (interferon-γ)

Targeting of the PD-1 checkpoint pathway results in an ***expansion*** of oligoclonal populations of ***tumor-infiltrating CD8+ T cells***



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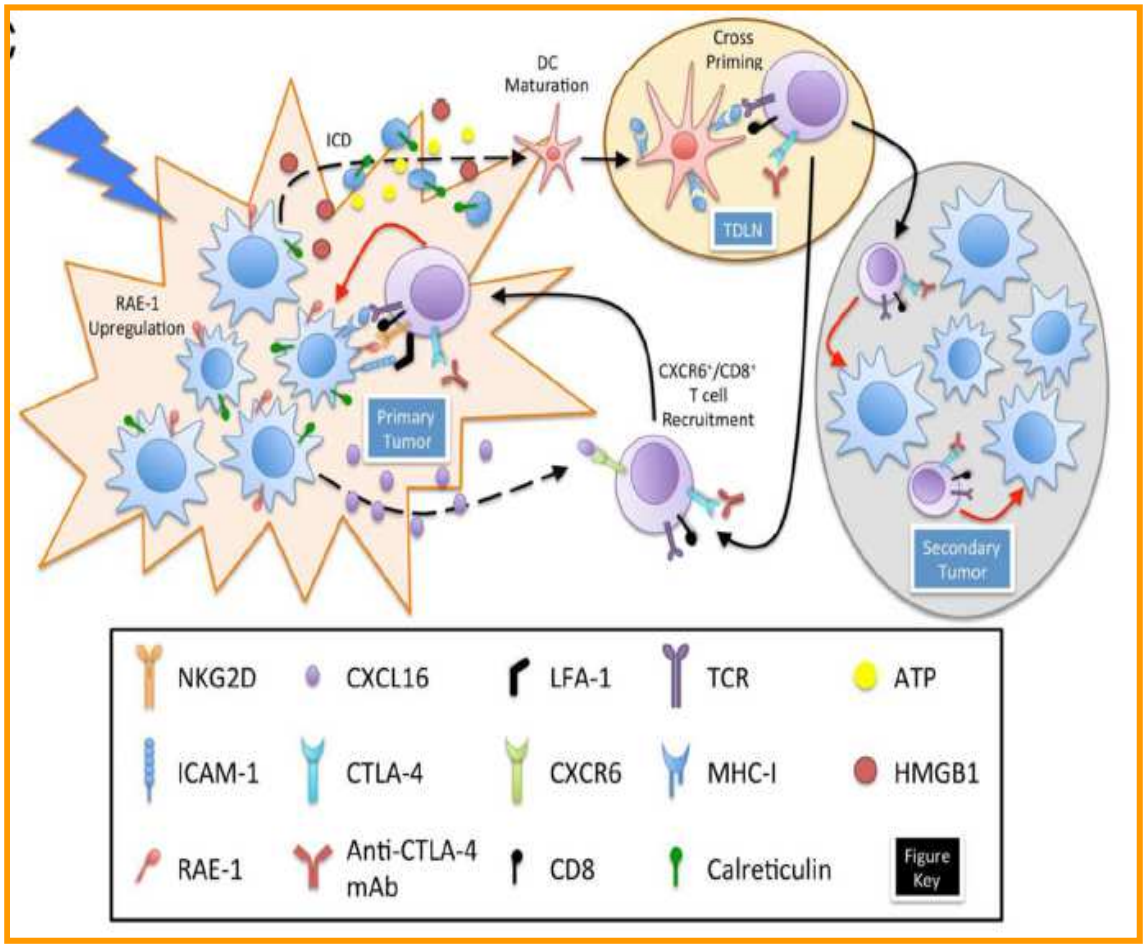
RT and immunotherapy

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



Immunogenic cell death

Cell surface translocation of **calreticulina** (DC "eat-me" signal)

HMGB1: immune system's *nuclear weapon*
(nuclear protein, acts as a cytokine and danger-associated molecular pattern protein that mediates responses to infection, injury and inflammation)

ATP release: involves the autophagic machinery, activation of the DC inflammasome → IL-1

Golden EB et al., *OncoImmunology* **2014;** 3:e28133 Golden EB et al., *Semin Radiat Oncol* **2015;** 25:11-7

RT and immunotherapy

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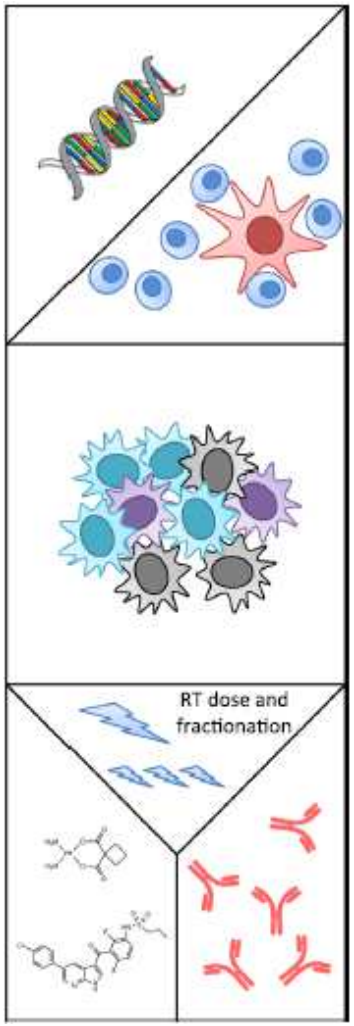
Immunogenic cell death



Host characteristics

SNP: TLR4 (reduce the binding of HMGB1 to TLR4. inhibiting HMGB1-dependent DC cross- presentation); P2XR7 (ATP acts on P2XR7 of DC's and triggers inflammasome→ IL-1-β. → activation CD8+), Intestinal Microbiota

Tumor characteristics

Immunogenic and non immunogenic tumor types (may be converted into endogenous vaccines with the addition of immune checkpoint inhibitors or immune adjuvants with radiotherapy)

Treatment characteristics

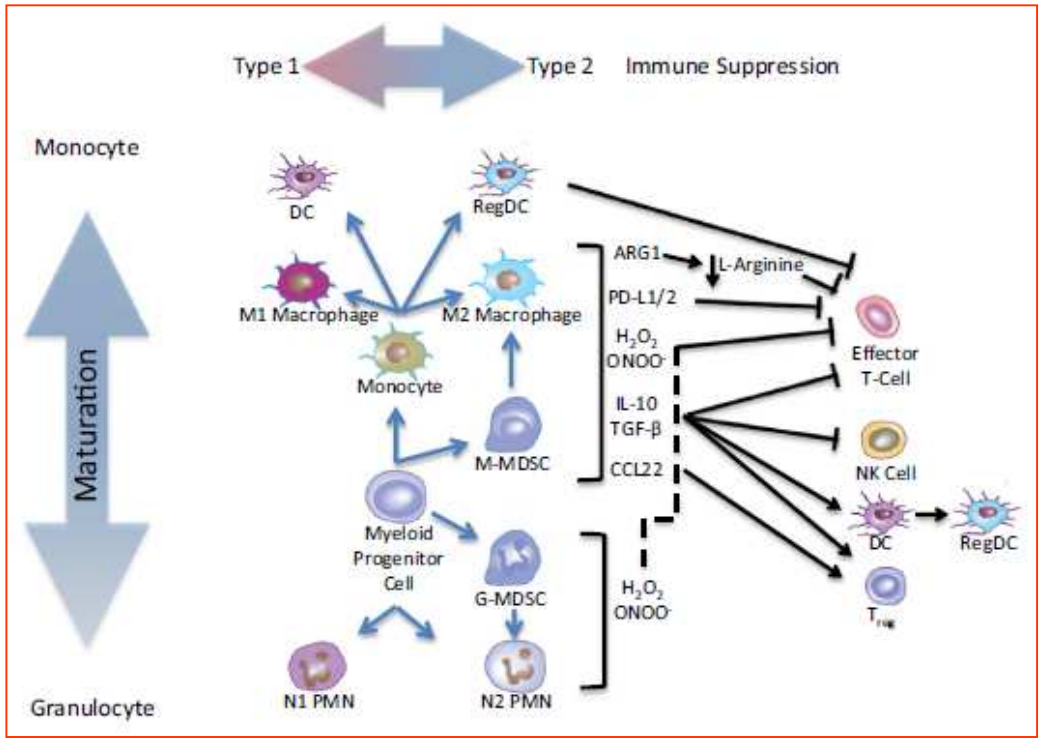
Dose and fractionation (fractionated regimens; LDR reprogram macrophages toward a iNOS+/M1 phenotype); concomitant CHT (but some CHT agents may counteract the immunogenicity of radiotherapy)

Golden EB et al., *Semin Radiat Oncol* **2015;** 25:11-7

RT and immunotherapy

Myeloid-Derived Cells in Tumors

Influenced by their *environment*, myeloid cells can acquire a phenotype ranging from proinflammatory (type1) to immunosuppressive (type2)



Type2 suppression mechanisms

Arginase production: depletes L-arginine and inhibitsT-cell function

Reactive oxygen species: modify receptors for antigens and chemokines on T-cells, impairing their function

Cytokines production: IL-10 and TGF-β impair effector T-cells and NK and convert DCs into regulatory DCs

Chemokines: CCL22 selectively attract Tregs, andTGF-β directly stimulates Tregs

Vatner RE et al., *Semin Radiat Oncol* **2015;** 25:18-27

RT and immunotherapy

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RT and Myeloid-Derived Cells

Recruitment

Increase serum CSF-1 and HIF-1→ M2 TAMs and MDSCs → **tumor growth** and **immune evasion**

Repolarization

- RT (>2 Gy/fr) can repolarize TAMs toward an *M2 phenotype*
- Lower doses of RT (0,5-2Gy) polarize TAMs toward an *M1 phenotype* (production of iNOS → lymphocyte recruitment to tumors →improved tumor control)

Re-presentation

Re-presentation of tumor antigens by myeloid cells

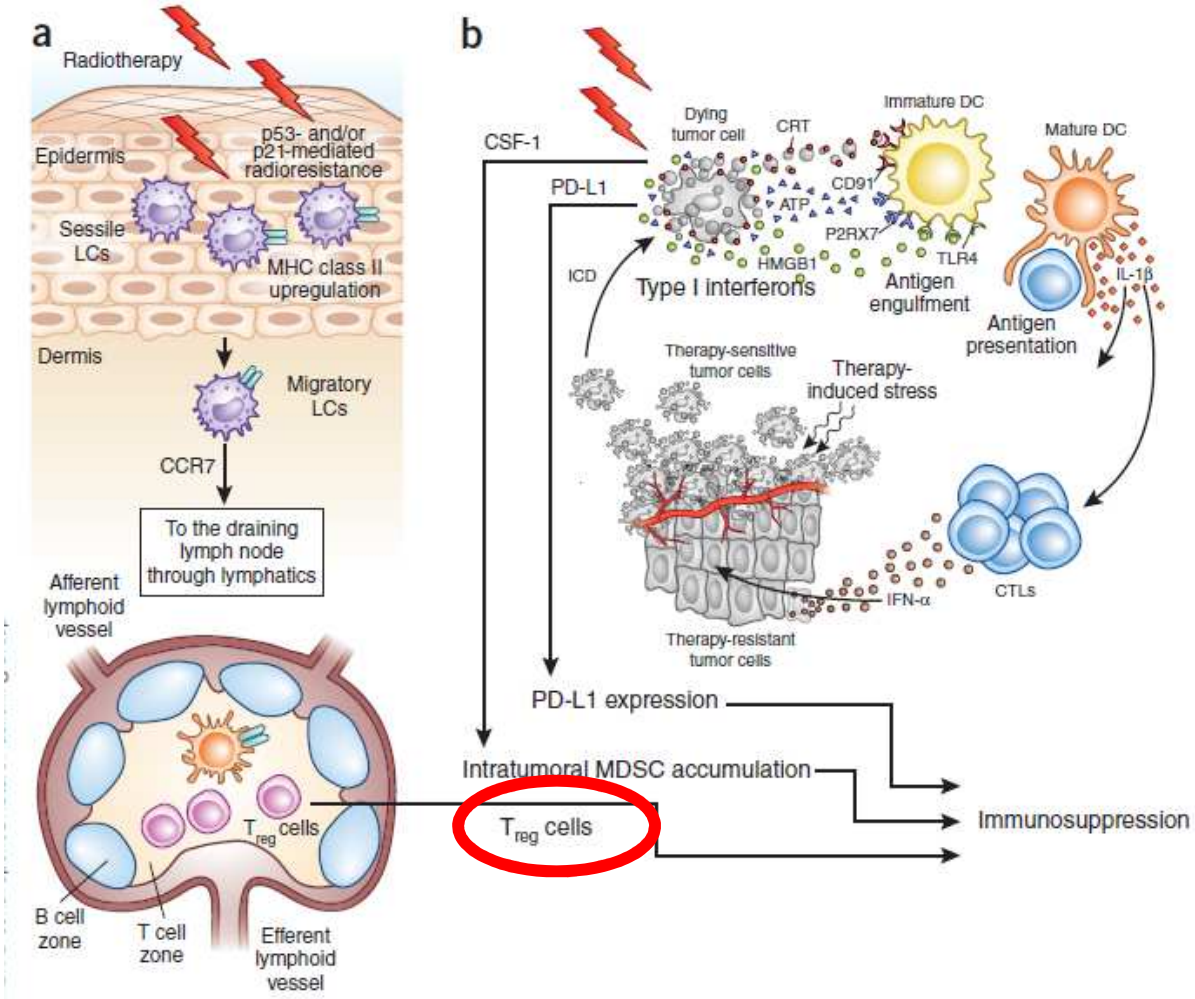
Myeloid-derived cells in the tumor often counteract development of an effective immune response

Schaue D et al., *Front Oncol* **2012;** 2:90

Chiang CS et al., *Front Oncol* **2012;** 2:89

RT and immunotherapy

Subversion of anticancer immunosurveillance



Irradiation induces ***Langerhans cells*** to migrate from the skin to lymph nodes, where they **stimulate regulatory Tcells**

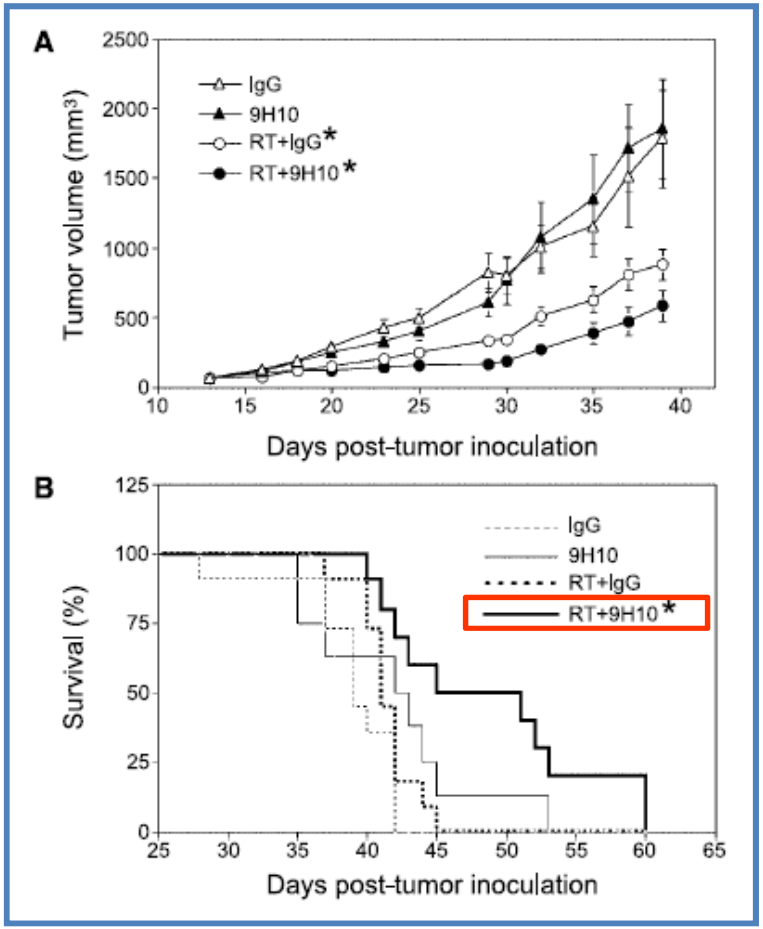
Price JG et al., *Nature immunology* **2015; 16**:1060

Zitvogel L et al., *Nature immunology* **2015; 16**:1005

RT and immunotherapy

Radiotherapy with CTLA- 4 blockade

Blockade CTLA-4 induce antitumor immunity limited to relatively immunogenic tumors



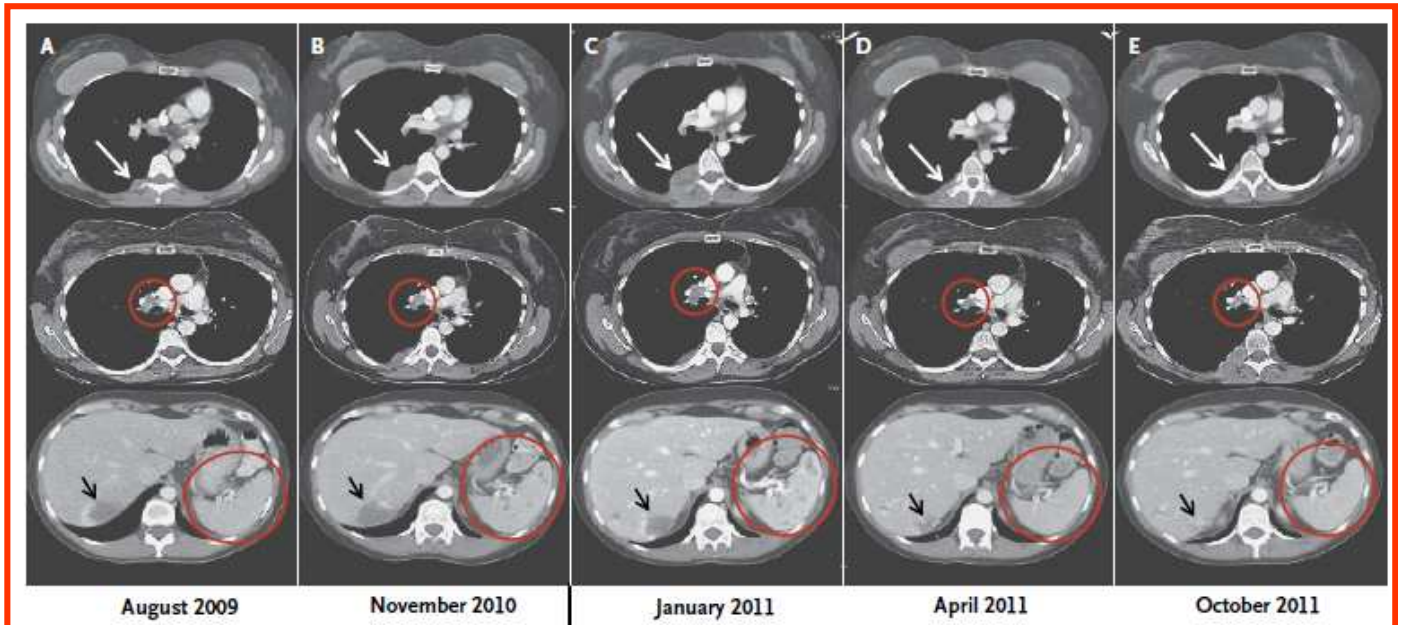
- In poorly immunogenic tumor, 9H10 (Ab against CTLA-4) alone did not have any effect on primary tumor growth or survival
- RT was able to delay the growth of the irradiated tumor, but ***in the absence of 9H10*** SVV was similar to that of control mice
- Mice treated with ***RT + 9H10*** had a statistically significant survival advantage (correlated with inhibition of lung metastases formation and required CD8+ but not CD4+ T cells)

The combination of **RT + CTLA- 4 blockade** is a promising immunotherapeutic strategy against poorly immunogenic metastatic cancers

Demaria S et al., *Clinical Cancer Research* **2005;** 11: 728

RT and immunotherapy

Radiotherapy with CTLA- 4 blockade



RT → **in situ tumor vaccine**

Abscopal responses can be seen in tumor types that do not respond to anti–CTLA-4 treatment

Postow MA et al., *N Engl J Med* **2012;** 366:925-31

RT and immunotherapy

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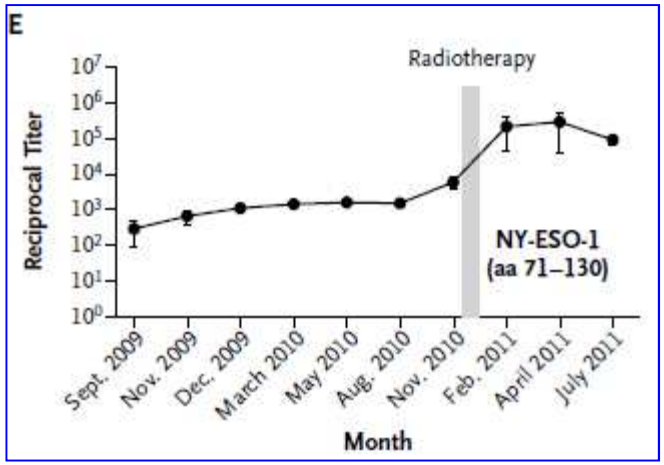
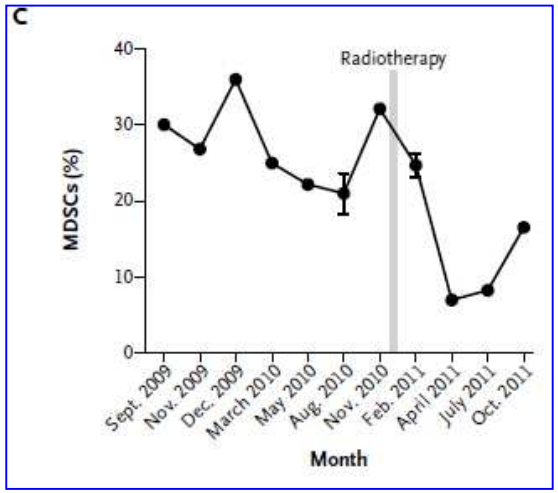
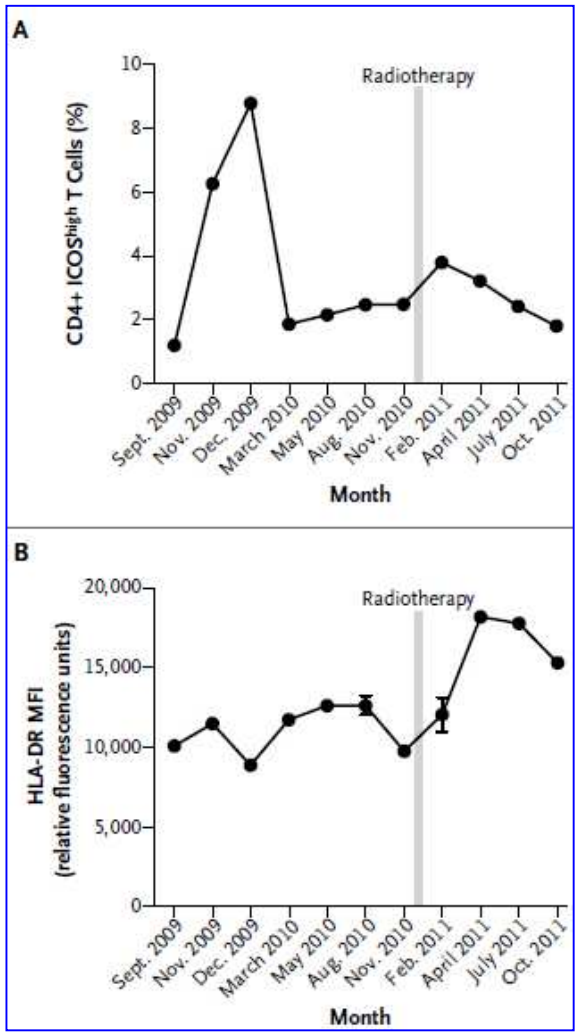
Radiotherapy with CTLA- 4 blockade







Ab-anti NY-ESO-1 protein diminished

CD4+ ICOS^{high} increased during ipi induction but decreased before RT; after RT, there was a second increase in the levels

Increase HLA-DR expression on monocytes after RT

Decline in levels of myeloid-derived suppressor cells

RT sensitizer of tumors to immune checkpoint inhibitors

Postow MA et al., *N Engl J Med* **2012;** 366:925-31

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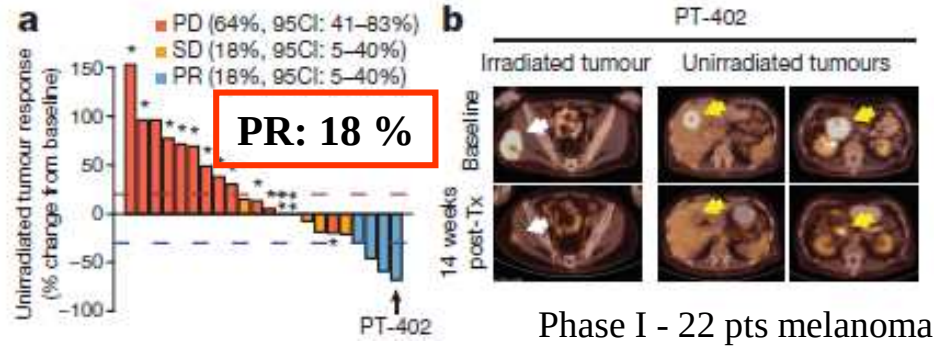
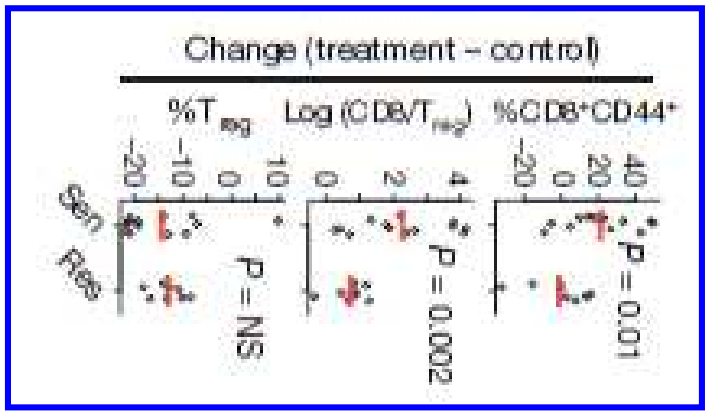
RT and immunotherapy

Radiotherapy with dual checkpoint blockade

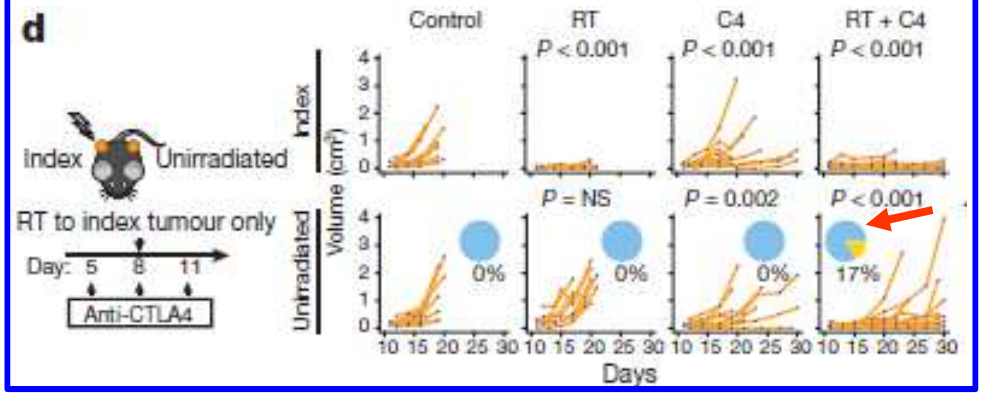
Patients with melanoma (RT + C4)



Phase I - 22 pts melanoma



B16-F10 melanoma mouse model



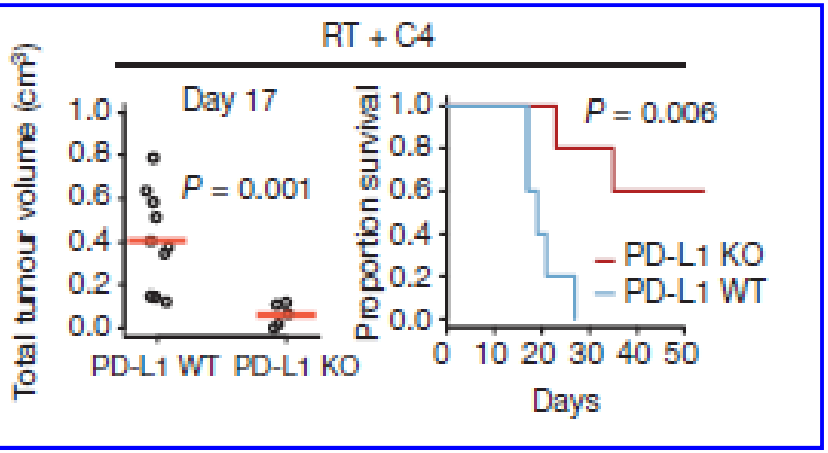
In resistant tumours, CD8/T_{reg} ratio failed to increase after RT + anti-CTLA4

$CD8^+$ $CD44^+$ T cells did not significantly expand

Twyman-Saint Victor C et al., *Nature* **2015;** 520:373-77

RT and immunotherapy

Radiotherapy with dual checkpoint blockade

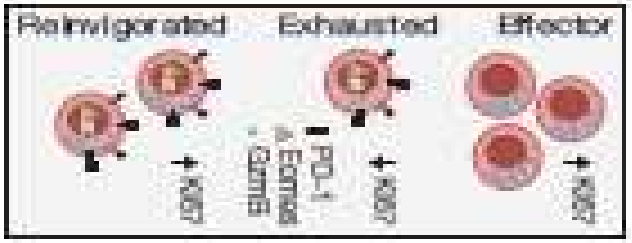


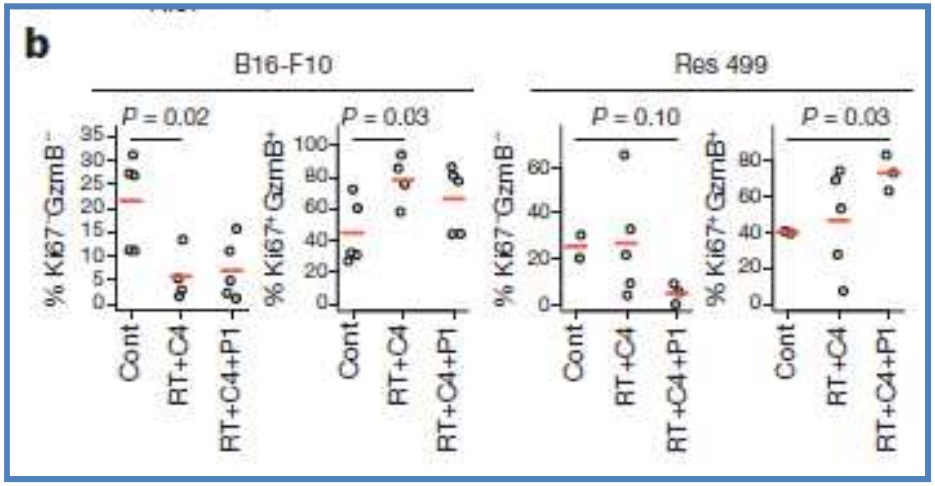
In B16-F10 tumors, RT + anti-CTLA4 markedly increased Ki67$^+$ GzmB$^+$

In contrast, in resistant tumors, Ki67$^+$ GzmB$^+$ only marginally increased after radiation + anti-CTLA4;

anti-PD-L1 increased Ki67$^+$ GzmB$^+$.

Genetic elimination of PD-L1 on Res 499 cells restored response to radiation + anti-CTLA4, increasing survival from 0% to 60%





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RT and immunotherapy

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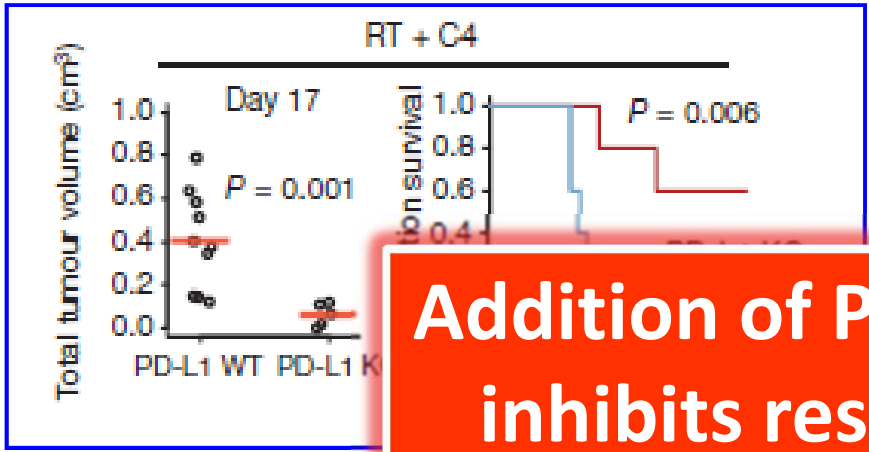
Radiotherapy with dual checkpoint blockade



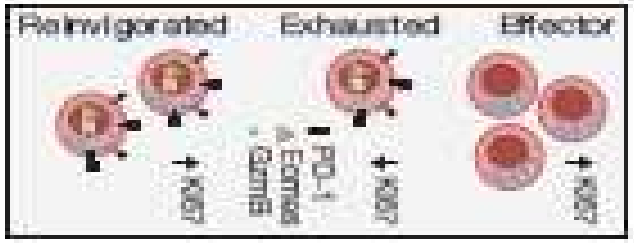
In B16-F10 tumors, RT + anti-CTLA4 markedly increased Ki67$^+$ GzmB$^+$

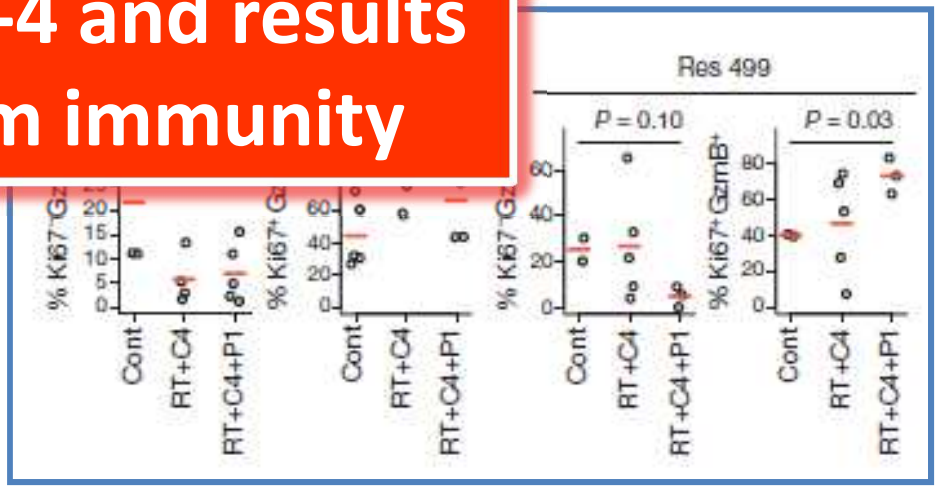
In contrast, in resistant tumors, Ki67$^+$ y increased after 4;

Ki67$^+$ GzmB$^+$.

Addition of PD-L1 blockade inhibits resistance after RT+antiCTLA-4 and results in long-term immunity

Genetic elimina
Res 499 cells r
radiation + anti-
survival from 0% to 60%





Twyman-Saint Victor C et al., *Nature* **2015;** 520:373-77

RT and immunotherapy

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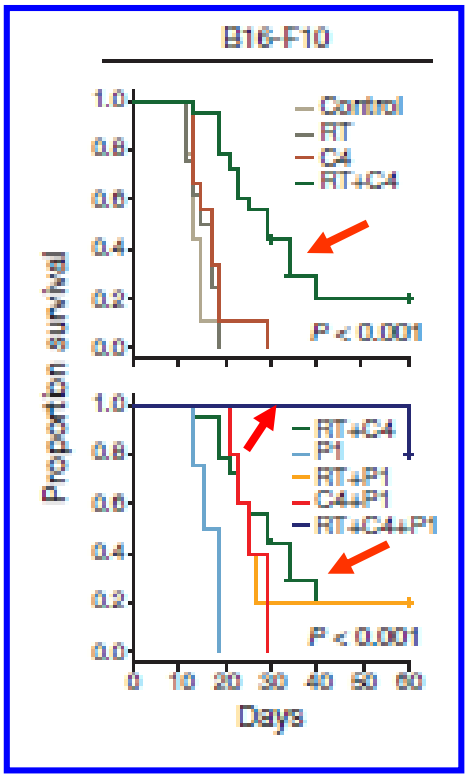
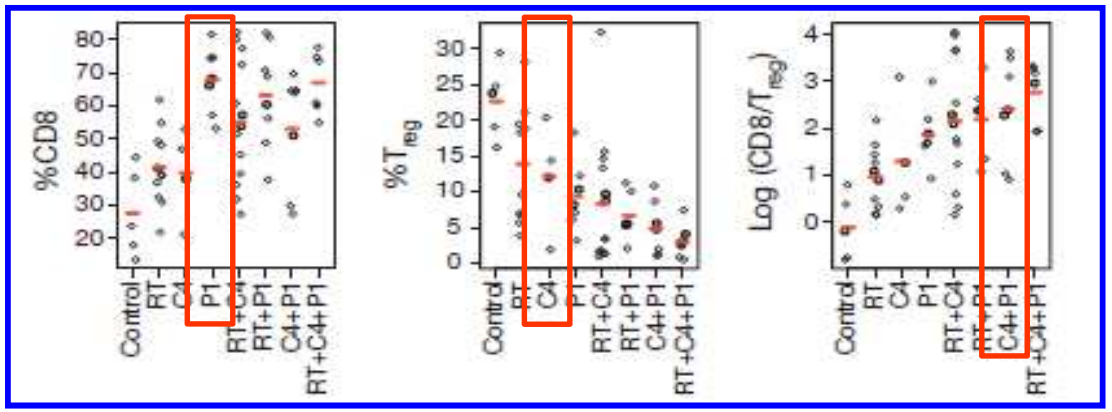
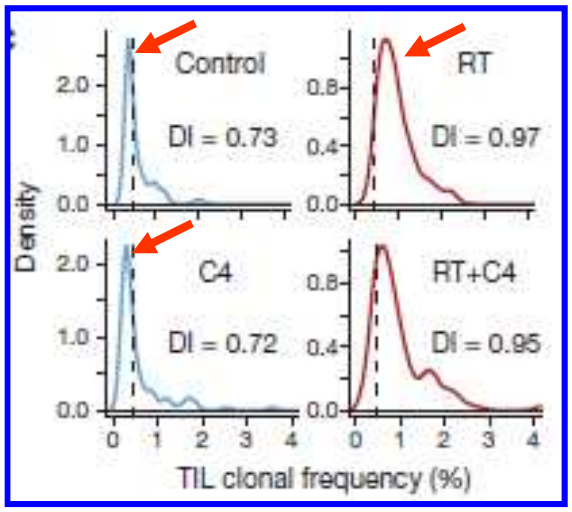
Radiotherapy with dual checkpoint blockade

RT is needed to achieve high complete response rates







anti-CTLA4 → decrease in Treg

anti-PD-L1 → increase CD8 TIL

Radiotherapy diversifies the TCR repertoire of CD8+ TILs from unirradiated tumours

Twyman-Saint Victor C et al., *Nature* **2015;** 520:373-77

RT and immunotherapy

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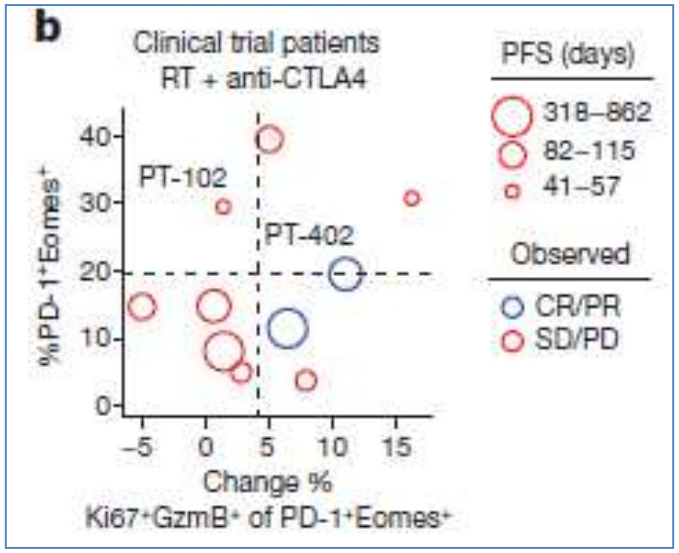
Radiotherapy with dual checkpoint blockade

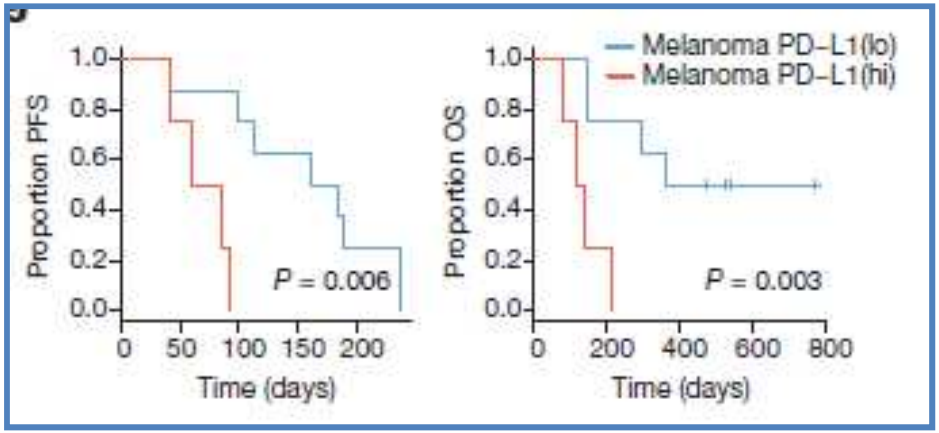


For **patients with PR**: Ki67+GzmB+ increased in PD-1+Eomes+ CD8 T cells after treatment while the proportion of PD-1+ Eomes+ Tcells remained at or below the mean

Patients with a high percentage of PD-1+ Eomes+ Tcells post-treatment did **not have partial responses** and had a short progression-free survival

PD-L1 high status was associated with persistent exhaustion.

None of the patients with PD-L1hi on melanoma cells had a complete response/partial response and all rapidly progressed and died



Twyman-Saint Victor C et al., *Nature* **2015;** 520:373-77

RT and immunotherapy

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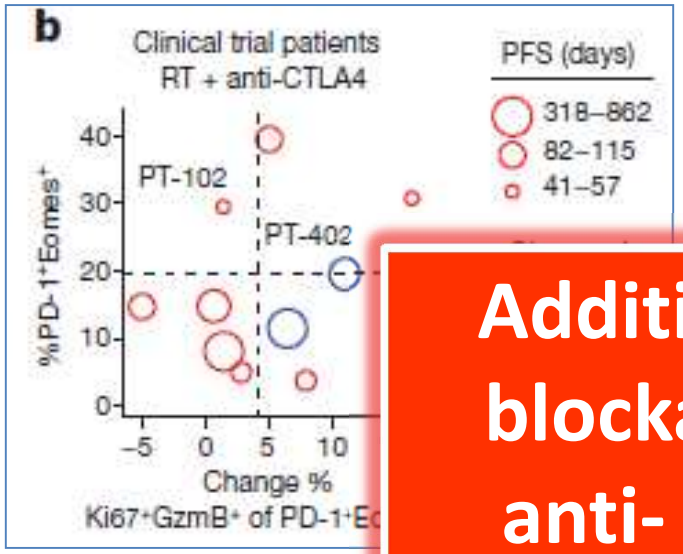
Radiotherapy with dual checkpoint blockade



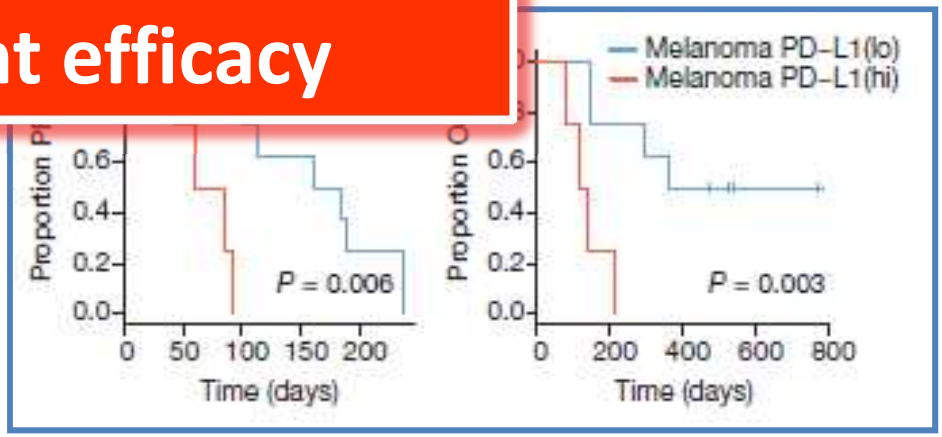
For **patients with PR**: Ki67+GzmB+ increased in PD-1+Eomes+ CD8 T cells after treatment while the proportion of PD-1+ Eomes+ Tcells remained at or

PD-1+ Eomes+
partial responses
rvival

Addition of PD-L1/PD-1 blockade to radiation + anti- CTLA4 may show significant efficacy

PD-L1 high status v
persistent exhaustion.

None of the patients with PD-L1hi on melanoma cells had a complete response/partial response and all rapidly progressed and died



Twyman-Saint Victor C et al., *Nature* **2015;** 520:373-77

RT and immunotherapy

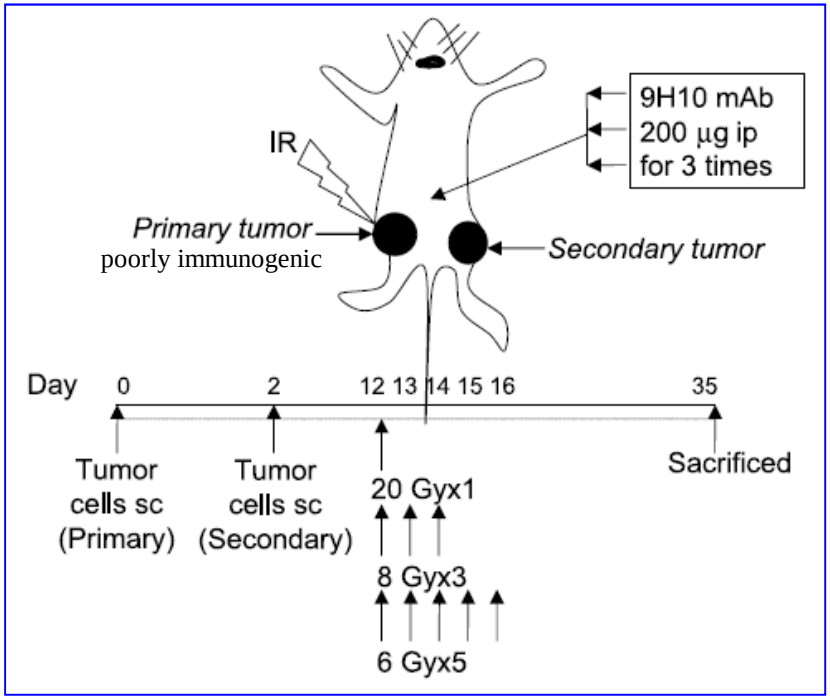
Fractionated dose in vivo

- Few studies have addressed the effects of dose fractionation
- The contribution of the different mechanisms of MHC-I up-regulation by radiation described *in vitro* remains to be demonstrated *in vivo* (signaling by host cells may dominate *in vivo*, cross-talk between irradiated tumor cells and the local immuno- logical microenvironment)

Two poorly immunogenic tumor models not expressing model antigens (mammary and colorectal carcinoma)

Three RT regimens

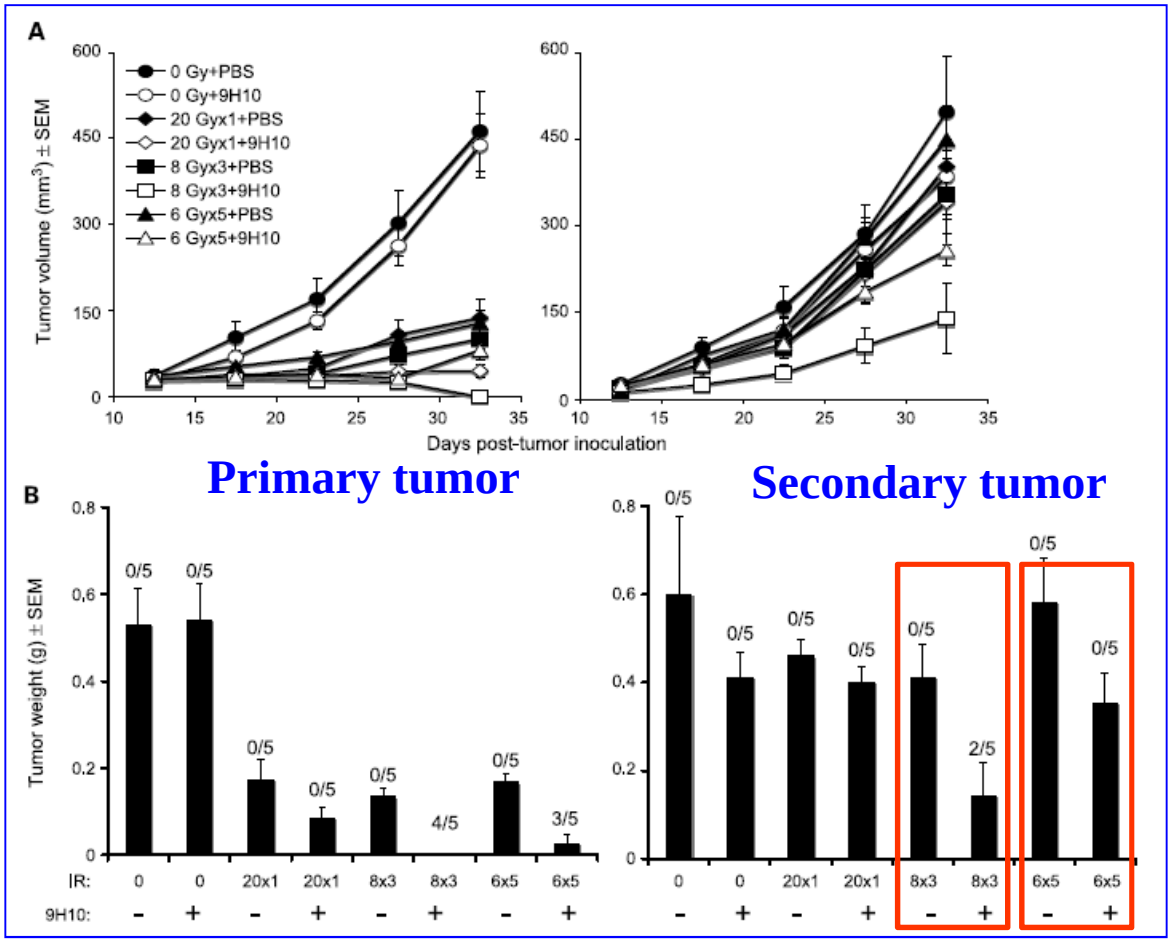
Anti-CTLA-4



Dewan MZ et al., *Clin Cancer Res* **2009;** 15:5379–88

RT and immunotherapy

Fractionated dose in vivo



Combination of 9H10 and RT regimens achieved enhanced tumor response at the primary site ($P < 0.0001$)

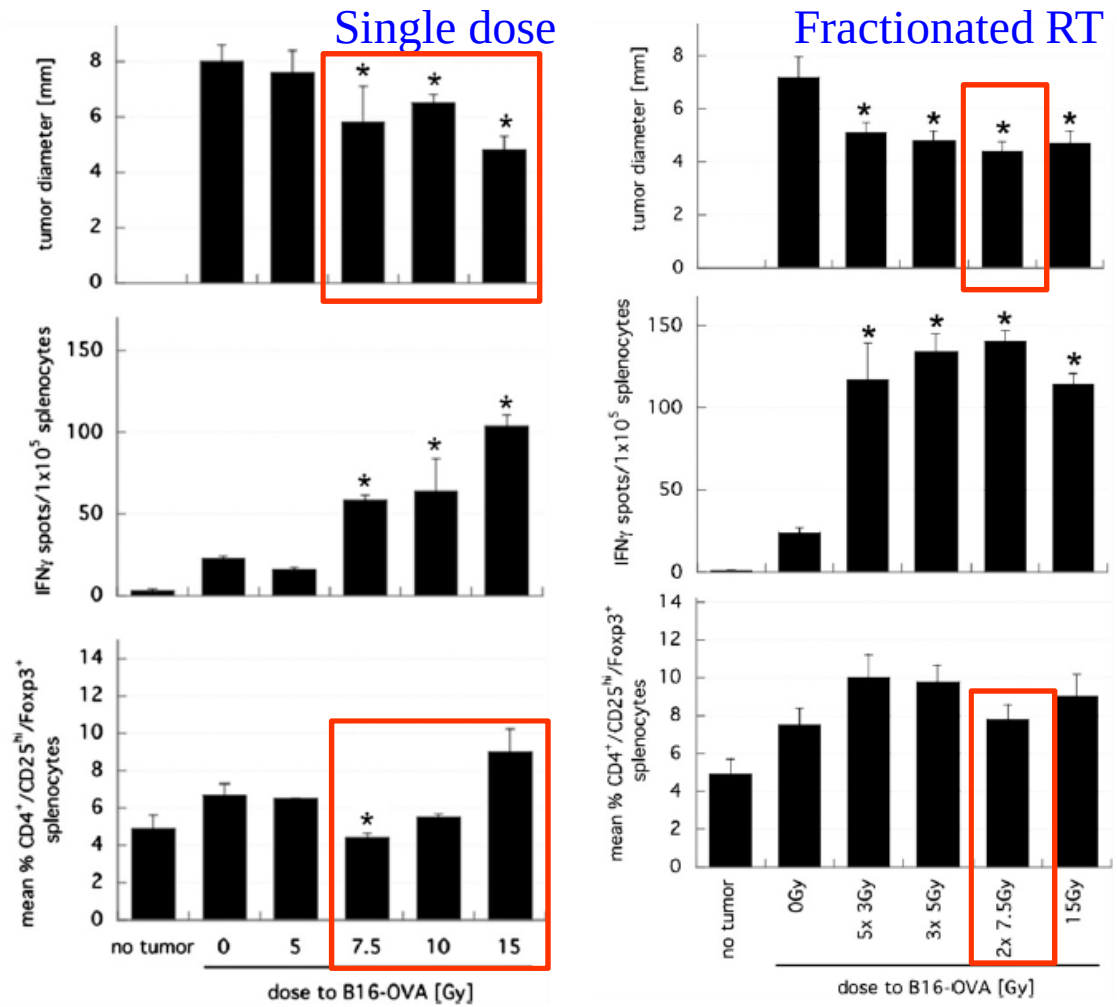
Abscopal effect occurred **only** in mice treated with combination of 9H10 and **fractionated RT** ($P < 0.01$)

The frequency of CD8+ T cells showing tumor-specific IFN-γ production was proportional to the inhibition of the secondary tumor

Dewan MZ et al., *Clin Cancer Res* **2009;** 15:5379–88

RT and immunotherapy

Fractionated dose in vivo



Radiation can be an immune adjuvant, but the ***response varies with the size of dose per fraction***.

The ultimate challenge is to optimally integrate cancer immunotherapy into radiation therapy

Schaue D et al., *IJROBP* **2012;** 83:1306–10

Immunotherapy

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Cancer and immune system

PD-1/PD-L1 pathways

RT and immunotherapy

Clinical trials in breast cancer

Ongoing Trials

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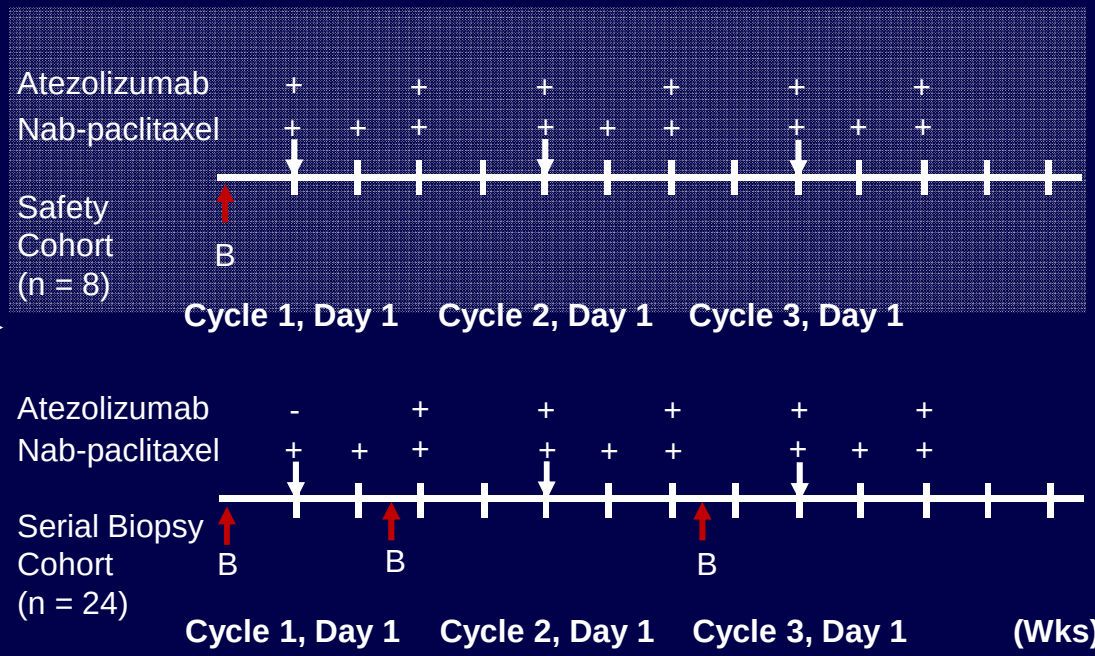
ClinicalTrials.gov
A service of the U.S. National Institutes of Health

1	Recruiting	**Study of Pembrolizumab (MK-3475) Monotherapy for Metastatic Triple-Negative Breast Cancer (MK-3475-086/KEYNOTE-086)** **Condition:** Breast Cancer **Intervention:** Biological: Pembrolizumab
2	Recruiting	**Study of Single Agent Pembrolizumab (MK-3475) Versus Single Agent Chemotherapy for Metastatic Triple Negative Breast Cancer (MK-3475-119/KEYNOTE-119)** **Condition:** Metastatic Triple Negative Breast Cancer **Interventions:** Biological: pembrolizumab; Drug: capecitabine; Drug: eribulin; Drug: gemcitabine; Drug: vinorelbine
3	Recruiting	**Study of Pembrolizumab (MK-3475) Plus Chemotherapy vs. Placebo Plus Chemotherapy for Previously Untreated Locally Recurrent Inoperable or Metastatic Triple Negative Breast Cancer (MK-3475-355/KEYNOTE-355)** **Condition:** Triple Negative Breast Cancer (TNBC) **Interventions:** Biological: Pembrolizumab; Drug: Nab-paclitaxel; Drug: Paclitaxel; Drug: Gemcitabine; Drug: Carboplatin; Drug: Normale Saline Solution
4	Recruiting	**Safety and Efficacy Study of Pembrolizumab (MK-3475) in Combination With Chemotherapy as Neoadjuvant Treatment for Participants With Triple Negative Breast Cancer (TNBC) (MK-3475-173/KEYNOTE 173)** **Condition:** Triple Negative Breast Neoplasms **Interventions:** Biological: Pembrolizumab; Drug: Nab-paclitaxel; Drug: Anthracycline (doxorubicin); Drug: Cyclophosphamide; Drug: Carboplatin; Drug: Paclitaxel
5	Recruiting	**Abrogation of Chronic Monoclonal Antibody Treatment-induced T-cell Exhaustion With DURVALUMAB in Advanced HER-2 Negative Breast Cancer** **Conditions:** Metastatic Breast Cancer; Bevacizumab-alone Maintenance Treatment Progression **Interventions:** Drug: Durvalumab; Drug: Bevacizumab
6	Recruiting	**A Study of FAZ053 Single Agent and in Combination With PDR001 in Patients With Advanced Malignancies.** **Conditions:** Advanced Solid Tumors; Non-Small Cell Lung Carcinoma (NSCLC); Triple Negative Breast Cancer (TNBC); Endometrial Cancer; Anaplastic Thyroid Cancer **Interventions:** Drug: FAZ053; Drug: PDR001
7	Recruiting	**Study of the Effects of Pembrolizumab in Patients With Advanced Solid Tumors** **Conditions:** Squamous Cell Cancer of Head and Neck; Triple Negative Breast Cancer; Epithelial Ovarian Cancer; Malignant Melanoma; Advanced Solid Tumors **Intervention:** Biological: Pembrolizumab
8	Not yet recruiting	**Durvalumab and Endocrine Therapy in ER+/Her2- Breast Cancer After CD8+ Infiltration Effective Immune-Attractant Exposure**

Atezolizumab + Nab-Paclitaxel in Metastatic TNBC: Phase Ib Study Design

- GP28328: a multicenter, multicohort phase Ib study; arm F includes pts with TNBC (metastatic or unresectable, locally advanced)[1,2]

Pts with TNBC ≤ 2 previous systemic cytotoxics, ECOG PS 0/1, no CNS cancer or untreated/active CNS metastases, available tumor sample →



Atezolizumab (800 mg) + nab-paclitaxel (125 mg/m^2), as long as clinical benefit received; nab-paclitaxel for at least 4 cycles, unless disease progression or unacceptable toxicity; if discontinued, atezolizumab as monotherapy

- Primary endpoint: safety and tolerability
- Secondary endpoints: response per RECIST v1.1 (ORR, DoR, PFS) and immune-modified response criteria; pharmacokinetics; biomarker analyses

1. Adams S, et al. ASCO 2016. Abstract 1009.
2. ClinicalTrials.gov. NCT01633970.

Slide credit: clinicaloptions.com

Atezolizumab + Nab-Paclitaxel in Metastatic TNBC: Pt Population

Characteristic	Pts (N = 32*)
Median age, yrs (range)	56 (32-84)
ECOG PS, n (%)	
▪ 0	6 (19)
▪ 1	26 (81)
Metastatic sites, n (%)	
▪ Visceral	15 (47)
▪ Nodal only	2 (6)
▪ Other	15 (47)
Median number of previous systemic therapies, n (range)	5 (1-10)
Number of previous systemic therapies (including [neo]adjuvant therapy), n (%)†	
▪ 1-2	2 (6)
▪ 3-4	13 (41)
▪ ≥ 5	17 (53)
Previous taxane use, n (%)	28 (88)

*Safety evaluable population: ≥ 1 dose atezolizumab.
†Individual agents counted separately.

Adams S, et al. ASCO 2016. Abstract 1009.

Atezolizumab + Nab-Paclitaxel in mTNBC: Safety and Tolerability (Primary Endpoint)

- Median safety follow-up: 6.1 mos (range: 1.7-17.1)
- Median duration of exposure: 5.4 mos (range: 0-17) for atezolizumab; 4.2 mos (range: 0-12) for nab-paclitaxel
- No reported deaths were related to study treatment

Treatment-Related AE (Grade 3/4 AEs Occurring in ≥ 1% of Pts), %	Pts (N = 32)	
	All Grades	Grade ≥ 3
All	100	69
Neutropenia/decreased neutrophil count	66	46
Thrombocytopenia and decreased platelet count	16	9
Diarrhea	41	6
Anemia	22	6
Decreased white blood cell count	9	6

Adams S, et al. ASCO 2016. Abstract 1009.

Slide credit: clinicaloptions.com

Atezolizumab + Nab-Paclitaxel in mTNBC: Safety and Tolerability (Primary Endpoint)

Atezolizumab-Related AE (Any Grade AE in ≥ 10% of Pts), %	Pts (N = 32)	
	All Grades	Grade ≥ 3
Fatigue	34	--
Neutropenia/decreased neutrophil count	28	9
Pyrexia	25	--
Diarrhea	19	3
Peripheral neuropathy/peripheral sensory neuropathy	19	--
Nausea	16	--
Alopecia	13	--
Headache	13	--
Pruritus	13	--

- Additional atezolizumab-related grade 3/4 AEs: syncope, type 1 diabetes mellitus, anemia, thrombocytopenia/platelet count decreased (n = 3), febrile neutropenia, AST increased, white blood cells decreased, and pneumonia mycoplasmal (n = 1 except where indicated)

Adams S, et al. ASCO 2016. Abstract 1009.

Slide credit: clinicaloptions.com

Atezolizumab + Nab-Paclitaxel in mTNBC: Efficacy (Secondary Endpoints)

Best Overall Response (RECIST v1.1)	First Line (n = 13)	Second Line (n = 9)	Third Line+ (n = 10)	All (N = 32)
Confirmed ORR, % (95% CI)	46 (19-75)	22 (3-60)	40 (12-74)	38 (21-56)
CR, %	8	0	0	3
PR, %	38	22	40	34
SD, %	38	67	30	44
PD, %	15	0	30	16
Missing or not estimable, %	0	11	0	3
Median DoR, mos (range)	NE (2.9 to 11.5+)	NE (9.1 to 13.1+)	NE (1.9+ to 5.6+)	

- Among 12 responders, 6 (50%) remain on atezolizumab; 1 for > 17 mos
- Median DoR not reached; PFS and OS data not yet mature
- Responses observed in pts regardless of PD-L1 expression level; trend toward increase in baseline TILs for responding pts

Adams S, et al. ASCO 2016. Abstract 1009.

Slide credit: clinicaloptions.com

Atezolizumab + Nab-Paclitaxel in Metastatic TNBC: Conclusions

- Atezolizumab + nab-paclitaxel well tolerated and active in metastatic TNBC[1]
  - Safety profile similar to that of single agents
  - Durable responses achieved across all lines of therapy
  - Clinical response seen regardless of PD-L1 expression
- Ongoing phase III randomized trial evaluating this combination in previously untreated metastatic TNBC[2]

1. Adams S, et al. ASCO 2016. Abstract 1009.
2. ClinicalTrials.gov. NCT02425891.

Conclusions

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RT and Immunotherapy

- Immune system controls not only tumor quantity but also tumor quality (*immunoediting*)
- Tumor Rejection by the immune system: "5th R" of radiobiology → RT to convert tumor into *an in situ vaccine* (*immunogenic cell death*)
- RT *can also counteract* development of an effective immune response
- RT + CTLA- 4 or PD-1/PD-L1 blockade is a promising immunotherapeutic strategy against poorly immunogenic cancers
- The response varies with the size of dose per fraction (fractionated > single dose)

It is the very beginning of a novel field.

More research is warranted to define the many mechanisms underlying the crosstalk with the immune system and to establish how best to harness ionizing radiation in this new role

Conclusions

Grazie per l'attenzione

«…Everyone else would climb a peak by looking for a path somewhere on the mountain …someone would climb another mountain altogether and from that distant peak would shine a searchlight back on the first peak…»