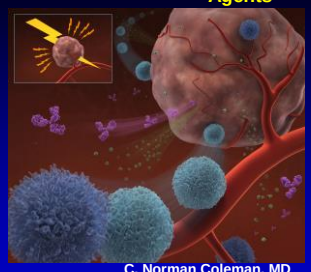
National Cancer Institute

Clinical Trials Using Radiation and Immunomodulatory Agents



Mansoor M. Ahmed Ph.D.
Radiation Research Program
NCI, NIH
Actual Expert

C. Norman Coleman, MD
Radiation Research Program
DCTD, NCI, NIH
The "control" group

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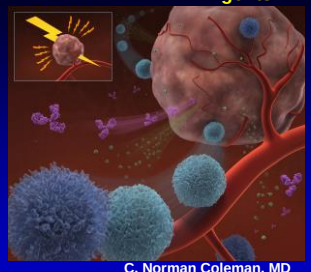

16,000' perspective

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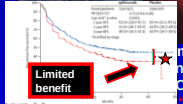
Clinical Trials Using Radiation and Immunomodulatory Agents

It's complex!



Limited benefit

16,000' perspective

Mansoor M. Ahmed Ph.D.
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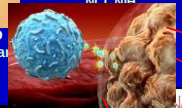

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Outline – concepts to present (from the "casual observer" ~5,000 m)

Osoruri pass, Peruvian Andes 2016

1. The immune system is extraordinarily complex and activation and de-activation are tightly regulated.
2. In cancer immunotherapy there are many targets: tumor cells, innate immune response cells, adaptive immune response cells, stroma & microenvironmental factors (metabolites), normal epithelial cells, endothelial cells and surrounding normal tissue (including lymph nodes).
3. Processes occur over time- not an "all at once" event, although primary event to stimulate response may be key.
4. So, logically "one size doesn't fit all" to harness the immune response. (Maybe we'll be lucky and there is one "best" radiation dose, schedule and target volume ( ).
5. Experiments need good biomarkers and critical thinking so that "null" or disappointing results teach us something.

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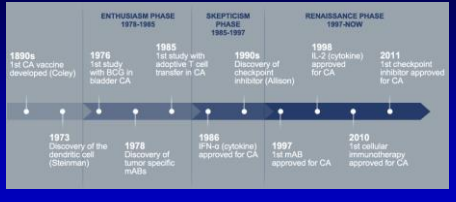
The points I will try to make

1. With the excitement and potential of immunotherapy there are many publications (10,000's) and clinical trials: clinicaltrials.gov listed 112 (not including many industry trials). So many targets- molecular, cellular, interactions.
2. The multi-step process involves cells and signals throughout the body. What volumes should be irradiated, not-irradiated or unknown needs to be defined… and also when to Rx.
3. The clinical results involve mostly melanoma, renal cell cancer, non-small cell lung cancer and some lymphomas. Response criteria need to be adapted for the "pseudoprogression".
4. Many patients do not yet benefit.
5. Radiation goes where one aims it and tissues know they've been hit.
6. Good opportunities for models and analytical minds.

Cancer Immunotherapy Development



2014/2015

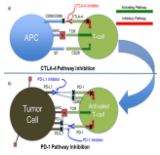
Approval of 2 anti-PD-1 antibodies for advanced melanoma and lung cancer

http://www.fightcancerwithimmunotherapy.com

General classes of immunotherapy

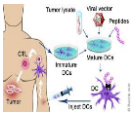
Checkpoint Inhibitors

Left unchecked, immune responses can be so powerful that they will destroy healthy tissue. Thus, specialized immune cells called T cells must pass several biological checkpoints before achieving full strength. Cancer cells often act on these checkpoints in a way that prevents the immune system from attacking the tumor. *New drugs—called checkpoint inhibitors—disable the cancer cells' immunedampening signals, allowing the immune system to do its job.*



Dendritic Cell Vaccine

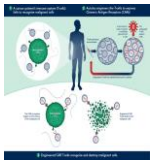
Dendritic cells normally patrol the body looking for bits of proteins called antigens that look unfamiliar. They present the off ending antigens to other immune defenders, known as CD4+ and CD8+ T cells. The T cells then attack any other cells that bear the targeted antigen. *By choosing antigens found on cancer cells but not on healthy ones and mixing the antigens with a patient's own dendritic cells outside the body, researchers create a kind of vaccine* that will seek out and destroy those same cancer cells for years to come.



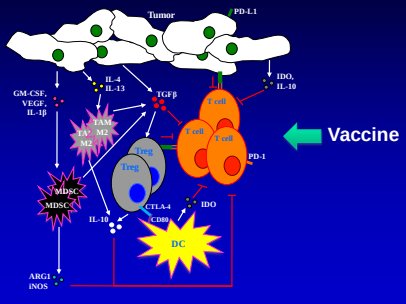
Adapted from Sci Am, April 2016, p48

CAR-T Cells

Chimeric antigen receptor (CAR) T cells combine attributes of two types of immune defenders: T cells and B cells. Molecules called receptors found on a CAR-T cell look like a hybrid of receptors on B cells and T cells. The CAR protein allows this unusual cell to both latch onto select antigens and destroy any cells that bear the target antigen. This mishmash eliminates intermediate steps typically taken by B and T cells, making CAR-T cells virtually unstoppable.

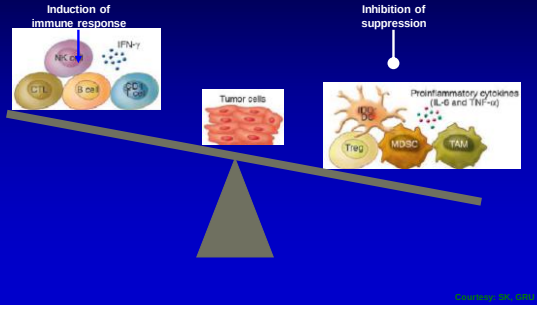


Tumor-Immune Interaction

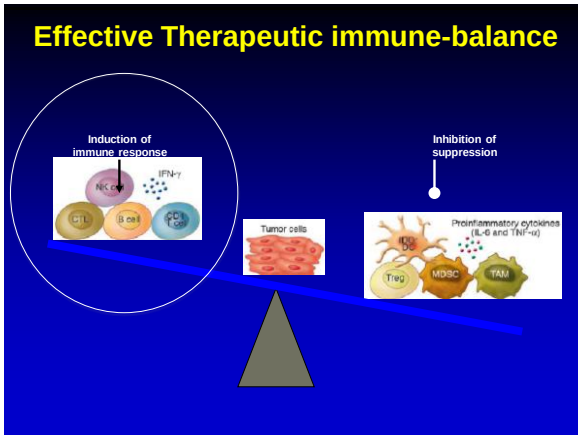


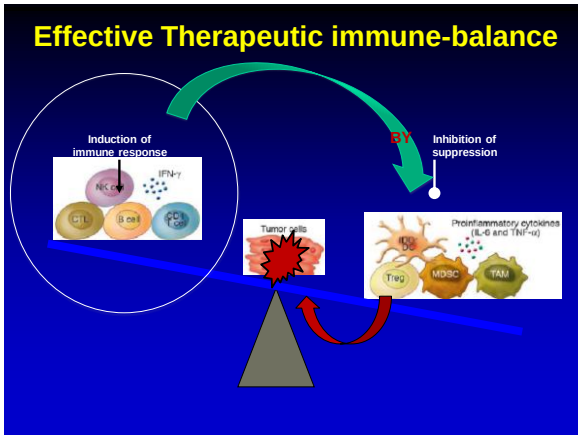
Courtesy: SK, GRU

Effective Therapeutic immune-balance



Courtesy: SK, GRU


Effective Therapeutic immune-balance
Induction of immune response
IFN-γ
NK cell
CTL
B cell
CD4 Cell
Tumor cells
Inhibition of suppression
Proinflammatory cytokines (IL-6 and TNF-α)
IDO
DC
Treg
MDSC
TAM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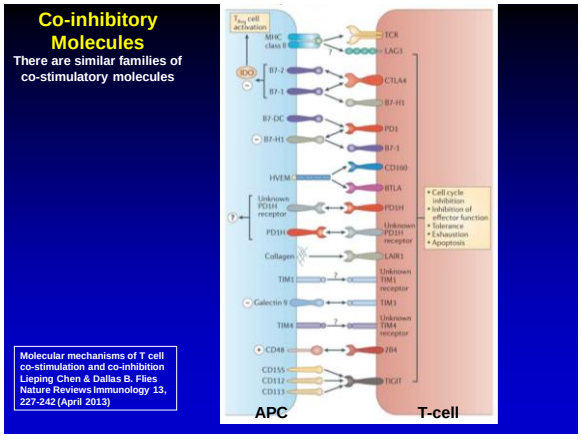
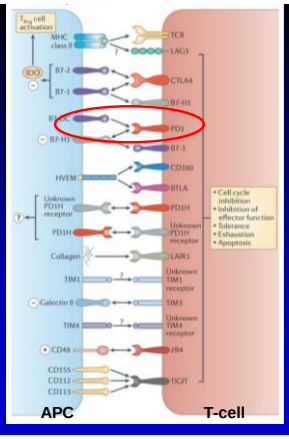
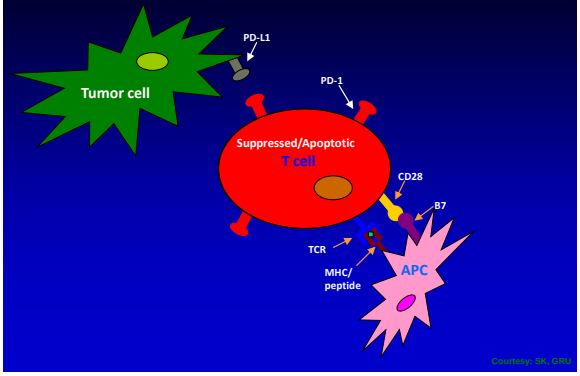

Co-inhibitory Molecules
There are similar families of co-stimulatory molecules
Molecular mechanisms of T cell co-stimulation and co-inhibition Lieping Chen & Dallas B. Flies Nature Reviews Immunology 13, 227-242 (April 2013)
APC
T-cell
TCR
LAG3
CTLA4
B7-H1
PD1
B7-1
CD160
BTLA
PD1H
TIM3
2B4
TIGIT
B7-2
B7-DC
HVEM
Collagen
Galectin 9
TIM4
CD48
CD155
CD112
CD113
IDO
MHC class II
Cell cycle inhibition
Inhibition of effector function
Tolerance
Exhaustion
Apoptosis

Co-inhibitory Molecules

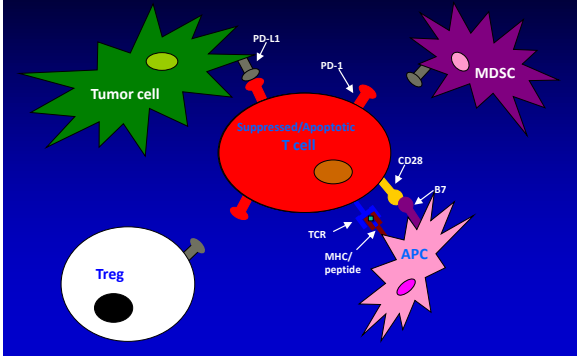


Molecular mechanisms of T cell co-stimulation and co-inhibition Lieping Chen & Dallas B. Flies Nature Reviews Immunology 13, 227-242 (April 2013)

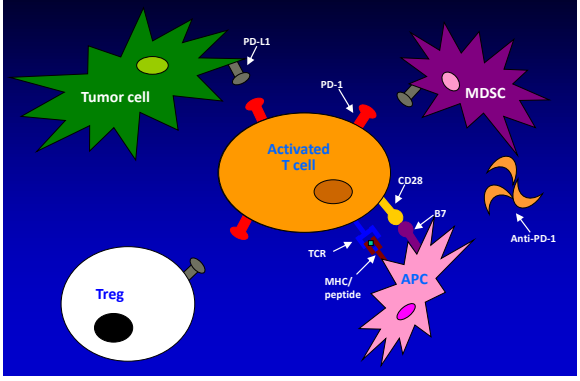
PD-1/PD-L1 Engagement Suppresses Effector T-cells



PD-1/PD-L1 Engagement Suppresses Effector T cells



PD-1/PD-L1 Engagement Suppresses Effector T cells



Combinational Immunotherapy

- Vaccines
- Immune Modulators
  - Immune Agonists
    - Stimulatory cytokines (IL-2, IL-12, IL-15, TLR etc..)
    - Co-stimulatory molecules (OX-40, GITR, 4-1BB)
  - Immune inhibitors
    - Check point inhibitors (CTLA4, PD1/PDL1, LAG3, TIM3, iDO)
    - Inhibitory cytokines/factors (IL-10, TGFβ)
- Standard Therapy
  - Chemotherapy
  - Radiation Therapy
- Small Molecules
- Chimeric Antigen Receptors

Table 1. Final Rankings of Agents with High Potential for Use in Treating Cancer

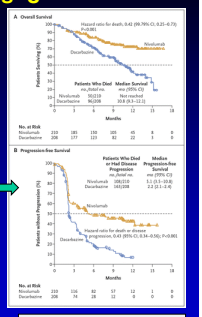
Rank*	Agent	Agent Category
1	IL-15	T-Cell Growth Factor
2	Anti-Programmed Death-1 (PD1)and/or anti–B7-H1 (PD1 Ligand)	**T-Cell Checkpoint Blockade Inhibitor
3	IL-12	Vaccine Adjuvant
4	Anti-CD40 and/or CD40L	Antigen Presenting Cell Stimulator
5	IL-7	T-Cell Growth Factor
6	CpG	Vaccine Adjuvant
7	1-Methyl Tryptophan	Enzyme Inhibitor
8	Anti-CD137 (anti–4-1BB)	T-Cell Stimulator
9	Anti–TGF-beta	Signaling Inhibitor
10	Anti–IL-10 Receptor or Anti–IL-10	Suppression Inhibitor
11	Flt3L	Dendritic Cell Growth Factor/ Vaccine Adjuvant
12	Anti-Glucocorticoid-Induced TNF Receptor (GITR)	T-cell Stimulator
13	CCL21 Adenovirus	T-Cell Attracting Chemokine
14	Monophosphoryl Lipid A (MPL)	Vaccine Adjuvant
15	Poly I:C and/or Poly ICLC	Vaccine Adjuvant
16	Anti-OX40	T-Cell Stimulator
17	Anti–B7-H4	T-Cell Checkpoint Blockade Inhibitor
18	Resiquimod and/or 852A	Vaccine Adjuvant
19	LIGHT and/or LIGHT vector	T-Cell Stimulator
20	Anti–Lymphocyte Activation Gene-3 (LAG-3)	T-Cell Checkpoint Blockade Inhibitor

NCI Immunotherapy Agent Workshop Proceedings

Checkpoint inhibitors- 3

Drug	Trade name	Dosage	Trials – drug label
Ipilimumab (Yevoy)	Anti CTLA-4 antibody	3 mg/kg (mets), or 10 mg/kg (adjuvant) Over 90 min every 3 weeks	Melanoma
Nivolumab (Opdivo)	Anti PD-1 antibody	3mg/kg IV over 60 min every 2 weeks	Melanoma NSCLC Renal Hodgkins
Pembrolizumab (Keytruda)	Anti PD-1 antibody	2mg/kg IV over 30 min every 3 weeks	Melanoma NSCLC

Reason for enthusiasm is obvious!



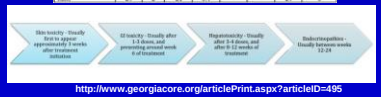
Nivolumab in Previously Untreated Melanoma without *BRAF* Mutation
Robert C, NEJM 372:320, 2015

Table 1 Incidence of Immune-Related Adverse Events Associated With Ipilimumab and Pembrolizumab

	Ipilimumab (n = 1,498)[8] (%)		Pembrolizumab (n = 411)[39] (%)	
Toxicity	**All Grades**	**Grade 3/4**	**All Grades**	**Grade 3/4**
GI (eg, enterocolitis)	33	9.1	1	< 1
Pneumonitis	< 1	< 1	2.9	< 1
Hepatitis	1.6	1.1	< 1	< 1
Dermatologic	45	2.6	11-30	0
Hypophysitis	2.7	2.1	< 1	< 1
Thyroiditis	1.8	< 1	9.5	< 1
Nephritis	< 1	< 1	< 1	< 1

http://www.cancernetwork.com/supplements/identification-and-management-toxicities-immune-checkpoint-blocking-drugs/page/0/3

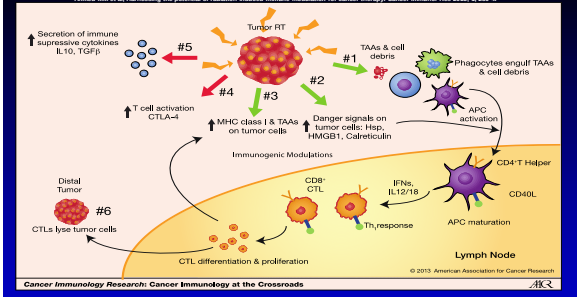
Adverse Reaction	Yervoy (ipilimumab)		Opdivo (nivolumab)				Keytruda (pembrolizumab)	
			Melanoma		NSCLC			
	All grades	Grad e ≥3	All grades	Grade ≥3	All grades	Grade ≥3	All grades	Grade ≥3
Arthralgia	-	-	-	-	-	-	20	0
Constipation	-	-	-	-	24	0	21	0
Cough	-	-	-	-	32	1.7	30	1
Decreased appetite	-	-	-	-	35	2.6	26	0
Diarrhea	32	5	-	-	-	-	20	0
Dyspnea	-	-	-	-	38	9	-	-
Fatigue	41	7	-	-	50	7	47	7
Musculoskeletal pain	-	-	-	-	36	6	-	-
Nausea	-	-	-	-	29	1.7	30	0
Pruritus	31	0	-	-	-	-	30	0
Rash	29	2	21	0.4	-	-	29	0



http://www.georgiacore.org/articlePrint.aspx?articleID=495

A Schematic view of RT-induced immune modulations

Ahmed MM et al, Harnessing the potential of radiation-induced immune modulation for cancer therapy. Cancer Immunol Res 2013; 1, 280–4.



#1: Tumor-associated antigens (TAAs) are released by irradiated dying cancer cells. TAAs and cell debris are engulfed in the tumor microenvironment by phagocytes such as macrophages, neutrophils, and dendritic cells for antigen processing and presentation.
#2: RT-induced cell death releases danger signals including heat-shock protein (Hsp), HMGB1, and calreticulin (eat-me signal for phagocytes).
#3: RT induces increased expression of tumor antigens and MHC class I molecules on tumor cells.
#4: RT-induced T cell activation increases expression of negative stimulatory molecules such as CTLA-4.
#5: Certain radiation doses may increase tumor production/secretion of immunosuppressive cytokines such as IL-10 and TGF-b.
#6: Activated APCs migrate to the draining lymph node, further mature upon encountering T helper cells, release interferons (IFNs) and IL-12/18 to stimulate Th_1 responses that support the differentiation and proliferation of antigen-specific CTLs. Activated antigen-specific CTLs traffic systematically from the draining lymph node to infiltrate and lyse primary and distal tumors.

OPINION

Immunotherapy and stereotactic ablative radiotherapy (ISABR): a curative approach?

Michael B. Bernstein, Sunil Krishnan, James W. Hodge and Joe Y. Chang

Nature Reviews, Clinical oncology August 2016, p 516

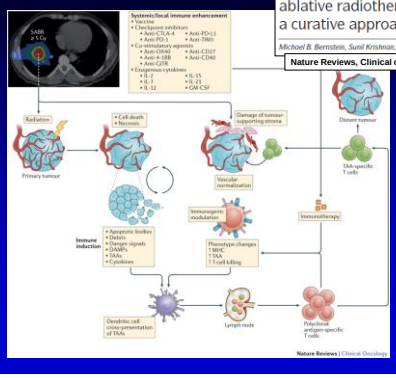


Table 1 | **Examples of published studies of SABR and immunotherapy combinations**

Study details	SABR dose (Gy)/ fractions	SABR target*	Immunotherapy agent	Sequence of treatments	Location of response
Postow et al. (2012)[6]	28.5/3	Paraspinal	Ipilimumab	Immunotherapy, then SABR, then immunotherapy	IF and OF
Hiniker et al. (2012)[7]	54/3	Liver	Ipilimumab	Immunotherapy, then SABR, then immunotherapy	IF and OF
Golden et al. (2013)[8]	30/5	Liver	Ipilimumab	Concurrent	IF and OF
Silk et al. (2013)[9]	14–24/1–5	Brain	Ipilimumab	Immunotherapy then SABR; or SABR then immunotherapy	IF
Stamell et al. (2013)[10]	NR	Brain	Ipilimumab	Concurrent	IF and OF
Karbach et al. (2014)[11]	45/1	Brain	Autologous tumour-lysate-loaded dendritic cells	SABR then immunotherapy	IF and OF
Kiess et al. (2015)[12]	15–24/1	Brain	Ipilimumab	SABR then immunotherapy; or concurrent treatment; or immunotherapy then SABR	IF
Kwon et al. (2014)[13]	8/1	Bone	Ipilimumab	SABR then immunotherapy	IF
Seung et al. (2012)[14]	20/1	Any	IL-2	SABR then immunotherapy	IF and OF

IF, in field; NR, not reported; OF, out of field; SABR, stereotactic ablative radiotherapy. *All targets are metastases and not primary tumours.

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Table 2 | **Selected ongoing clinical trials investigating the efficacy of ISABR**

Institution and study details	SABR dose (Gy)/fraction	SABR Target	Immunotherapy agent	Sequence of treatments	Phase
Johns Hopkins University, NCT01950195 (REF. 45)	NS	Brain, spine	Ipilimumab	Immunotherapy, then SABR, then immunotherapy	I
University of Pennsylvania, NCT01497808 (RADVAX)[46]	NS	NS	Ipilimumab	SABR then immunotherapy	I/II
MD Anderson Cancer Center, NCT02239900 (REF. 47)	• 50/4 • 60/10	Liver, lung, adrenal	Ipilimumab	Concurrent; or immunotherpy then SABR	I/II
Chiles Research Institute, NCT01862900 (REF. 68)	• 15/1 • 20/1	Lung, liver	Anti-OX40	Concurrent	I/II
Stanford University, NCT01769222 (REF. 69)	20/2	Any	Ipilimumab	Concurrent	I/II
New York University, NCT01401062 (REF. 70)	22.5/3	Any	Fresolimumab	Concurrent	I/II
NIH/NCI, NCT02239946 (REF. 71)	• 8/1 • 24/3	Liver	PD-1 inhibitor	SABR then immunotherapy	I
Thomas Jefferson University, NCT01703507 (REF. 72)	• 24/1 • 21/3 • 18/3 • 15/3	Brain	Ipilimumab	Concurrent	I
MD Anderson Cancer Center, NCT02444741 (REF. 73)	50/4	Lung, liver	PD-1 inhibitor	Concurrent	I/II

ISABR, Immunotherapy and stereotactic ablative radiotherapy; NCI, National Cancer Institute; NS, not specified; PD-1, programmed cell death protein 1; SABR, stereotactic ablative radiotherapy.

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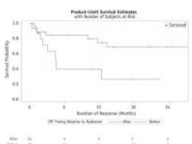
Table 3 Proposed ISABR studies

Table 3 | **Proposed ISABR studies**

Patient population	SABR dose (Gy)/number of fractions	SABR target	Type of immunotherapy	Sequence	Readout
Stage I NSCLC	• 50–60/3–5 • 60–70/8–10	Primary tumour	• Vaccine-MAGE* • Anti-PD-L1	• Immunotherapy followed by SABR • Concurrent ISABR	PET/CT scan, PD-L1, TIL, T_{REG}, CD8/CD4, exome miRNA, cytokine production, CEA-specific T cells
Early stage hepatocellular carcinoma	• 40–60/3–5 • 50–70/8–10	Primary tumour	Anti-PD-L1	Concurrent ISABR	MRI scan, PD-L1, TIL, T_{REG}, CD8/CD4, exome miRNA, cytokine production
Stage IV CRC	• 50–60/3–5 • 60–70/8–10	Dominant liver or lung metastasis	• Vaccine-CEA • Anti-PD-1	• Immunotherapy followed by SABR • Concurrent ISABR	CT scan, MRI of the abdomen, cytokine production, CEA-specific T cells, TILs in treated and off-target metastases, inflammatory cytokine production, PD-L1, T_{REG}, CD8/CD4, exome miRNA
Stage IV NSCLC with spinal/brain metastases	12–25/1	Spinal metastases	• Anti-PD-L1 • Anti-PD-1 + ipilumumab	• Immunotherapy then SABR • Concurrent ISABR	PET/CT, brain MRI, tumour-specific T cells, PD-L1 expression levels, inflammatory cytokine production, TIL, T_{REG}, CD8/CD4, exome miRNA
	15–24/1	Brain metastases			
Stage IV NSCLC	Organ-dependent dose regimens	Oligometastasis	• Anti PD-L1 • Anti PD-1 + ipilumumab	• SABR then immunotherapy • Concurrent ISABR	PET/CT scan to monitor regression at distant metastatic sites, PD-L1 expression levels, TILs in treated primary and untreated metastases, T_{REG}, CD8/4, exome miRNA

CEA, carcinoembryonic antigen; CRC, colorectal cancer; ISABR; Immunotherapy combined and stereotactic ablative radiotherapy; MAGE, melanoma antigen E; NSCLC, non-small-cell lung cancer; PD-1, programmed cell death protein 1; PD-L1, programmed cell death 1 ligand 1; SABR, stereotactic ablative radiotherapy; TIL, tumour-infiltrating lymphocyte; T_{REG}, regulatory T cells; RNA, ribonucleic acid. *MAGE-tumour-associated antigen is expressed in 50% of patients with NSCLC[74], with no immunohistochemical staining of this antigen observed in normal lung tissue samples[75].

Bernstein, M. B. *et al*. (2016) Immunotherapy and stereotactic ablative radiotherapy (ISABR): a curative approach? *Nat. Rev. Clin. Oncol.* doi:10.1038/nrclinonc.2016.30



Ipilimumab Timing Relative to Radiation	Total	Censored	Median Duration of Response (95% CI)	6-Month Response Rate (95% CI)	12-Month Response Rate (95% CI)	Log-Rank P Value
After	16	7 (44%)	4.5 Months (1.8, NE)	0.448 (0.195, 0.674)	0.448 (0.195, 0.674)	0.01
Before	28	21 (75%)	NE (13.4, NE)	0.843 (0.634, 0.938)	0.747 (0.518, 0.878)	
Total	44	28 (64%)				

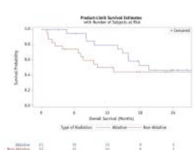
Fig. 1Patients treated with ipilimumab (IPI) before radiation therapy had higher 6- and 12-month response rates, as well as increased response duration, compared with those who received ipilimumab after radiation therapy. Abbreviations: CI = confidence interva...

Rosie QinRosie Qin, Adam OlsonRosie QinRosie Qin, Adam Olson, Bhavana SinghRosie QinRosie Qin, Adam OlsonR...

Safety and Efficacy of Radiation Therapy in Advanced Melanoma Patients Treated With Ipilimumab

International Journal of Radiation Oncology*Biology*Physics, 2016

http://dx.doi.org/10.1016/j.ijrobp.2016.04.017



Type of Radiation	Total	Censored	Median OS (95% CI)	6-Month Survival Rate (95% CI)	12-Month Survival Rate (95% CI)	Log-Rank P Value
Ablative	21	10 (48%)	19.6 Months (14.8, 38.1)	0.951 (0.703, 0.993)	0.787 (0.540, 0.918)	0.43
Non-Ablative	23	11 (48%)	10.2 Months (6.7, NE)	0.727 (0.491, 0.867)	0.485 (0.266, 0.674)	
Total	44	21 (48%)				

NE=Non-Estimable.

Fig. 2Patients receiving ablative radiation therapy had numerically higher 6- and 12-month overall survival (OS) rates versus patients treated with non-ablative radiation therapy. Abbreviation: CI = confidence interval.

Rosie QinRosie Qin, Adam OlsonRosie QinRosie Qin, Adam Olson, Bhavana SinghRosie QinRosie Qin, Adam OlsonR...

Safety and Efficacy of Radiation Therapy in Advanced Melanoma Patients Treated With Ipilimumab

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Radiation can

- Impact both innate and adaptive immunity
- Provide a source of robust tumor antigens
- Induce cytokines that can help to alter the profile and function of immune infiltrates
- Remodels the stromal and angiogenic compartments of the tumor microenvironment

More importantly

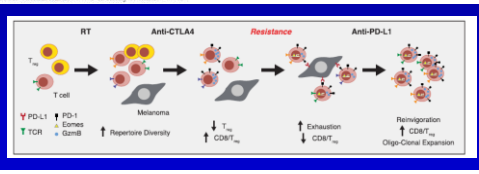
Surviving tumor cells after radiation therapy are more sensitive to immune-mediated killing

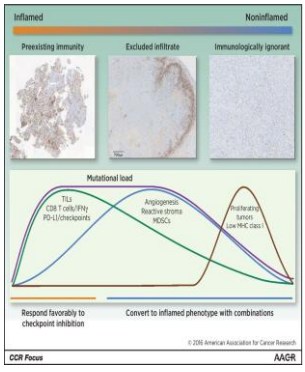
LETTER

doi:10.1038/nature14292

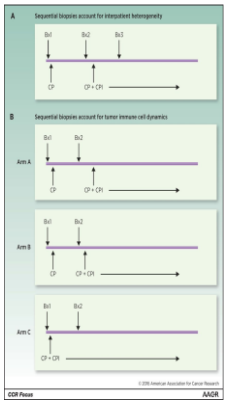
Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer

Christina Twyman-Saint Victor[1,2]*, Andrew J. Rech[2]*, Amit Maity[3,4], Ramesh Rengan[3,4]†, Kristen E. Pauken[5,6], Erietta Stelekati[5,6], Joseph L. Benci[2,3], Bihui Xu[2,3], Hannah Dada[2,3], Pamela M. Odorizzi[5,6], Ramin S. Herati[5,6], Kathleen D. Mansfield[5,6], Dana Patsch[3], Ravi K. Amaravadi[2,4], Lynn M. Schuchter[2,4], Hemant Ishwaran[7], Rosemarie Mick[4,8], Daniel A. Pryma[8,9], Xiaowei Xu[4,10], Michael D. Feldman[8,10], Tara C. Gangadhar[2,4], Stephen M. Hahn[2,4]†, E. John Wherry[5,6]§, Robert H. Vonderheide[2,3,4]§ & Andy J. Minn[2,3,4]§





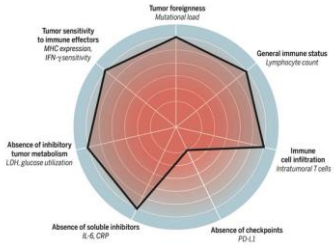
The Where, the When, and the How of Immune Monitoring for Cancer Immunotherapies in the Era of Checkpoint Inhibition Priti S. Hegde1Vaios Karanikas2 and Stefan Evers Clin Cancer Res; 22(8); 1865–74.



The Where, the When, and the How of Immune Monitoring for Cancer Immunotherapies in the Era of Checkpoint Inhibition Priti S. Hegde1Vaios Karanikas2 and Stefan Evers
Clin Cancer Res; 22(8); 1865–74.

The "cancer immunogram"

Christian U. Blank1, John B. Haanen, Antoni Ribas, Ton N. Schumacher
Netherlands Cancer Institute and UCLA
Science 352: 658, (May 2) 2016



The cancer immunogram.
The radar plot depicts the seven parameters that characterize aspects of cancer-immune interactions for which biomarkers have been identified or are plausible. *Potential biomarkers for the different parameters are shown in italics. Desirable states are located in blue; progressively undesirable states are shown in the red gradient. The black line connecting the data values for each parameter represents a plot for a single hypothetical patient.* In the case shown, it may be argued that single-agent PD-1 blockade, rather than combined PD-1 and CTLA-4 blockade, could be a first treatment of choice. For details on this case and other hypothetical patient cases, see (2).

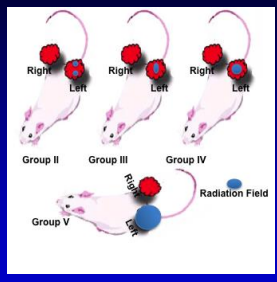
- **Few trials testing RT parameters in combination with checkpoint blockade**
  - **One trials testing low-dose ultrafractionated radiation (<1 Gy per fraction) with PD-L1.**
    - **Low-dose radiation was an effective inducer of tumor infiltrating T-cells in mouse models (Klug et al. Cancer Cell 2013)**
    - **Low-dose RT allows exploration of synergistic effects on local control**
  - **Only one trial explicitly evaluating RT dose (40 patients, 5 histology, multiple timings), not using combined checkpoint blockade**

MOUSE MODEL AND SCHEMES

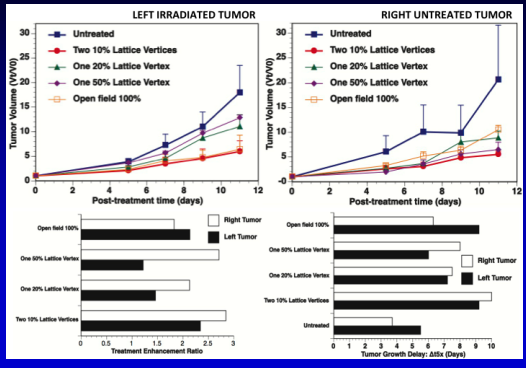
The Lewis lung carcinoma 1 (LLC1), a mouse cancer cell was used to develop syngenic tumors in C57BL/6 mice.

Tumors were initiated by *sub-cutaneous* injection of 2 x 10^6 LLC1 cells into the flanks of both left and right hind legs of each mouse.

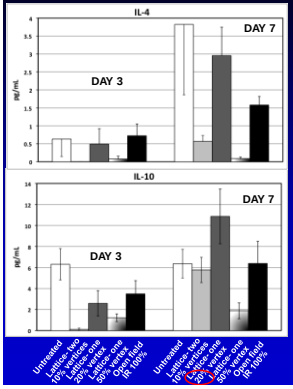
Tumors were allowed to grow to an area of 5 mm x 5 mm before irradiation and sorted within 10% differences in the intra and inter-tumor volumes.



Single fraction, high-dose LRT significantly delayed growth of both local and distant tumors



Immune responses in LLC xenograft tumor model: Lattice high-dose radiation induces increased secretion of inflammatory cytokines



There was a significant decrease in both IL-4 and IL-10 in serum obtained from one 50% lattice vertex at both time points. Treatment with two 10% lattice vertices significantly reduced IL-4 and IL-10 secretion in serum although IL-10 levels returned to normal on day 7.

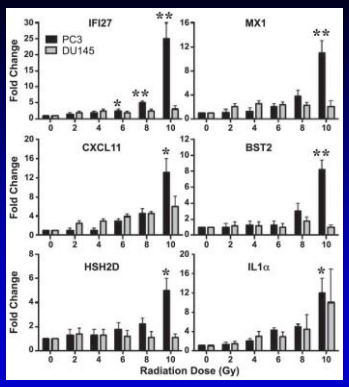


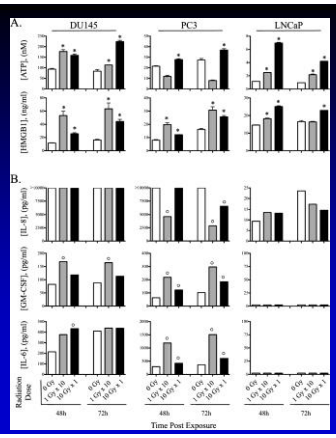
FIG. 1. Inflection point kinetics of immune genes in multifractionated treated PC3 and DU145 cells as assessed by real-time RT-PCR. PC3 and DU145 cells were exposed to 1–10 Gy of radiation delivered as 1 Gy multifractionated treatment. Fold change in IFI27, MX1, CXCL11, BST2, HSH2D and IL1A expression 24 h after cells received 1–10 Gy of radiation was determined by RT-PCR. Data shown are fold change (AV ± SEM) of 3 biologically distinct experiments. *$P < 0.05$, **$P < 0.01$.

List of Immune Genes Modulated Greater than Twofold in PC3 Cells 24 h after Single-Dose or Multifractionated Treatment

Networks and Associated Functional Categories Identified by IPA for PC3, DU 145 and LNCaP Cells 24 h after Exposure to 10 Gy of Radiation Administered as a Single-Dose or Multifractionated Regimen (1 Gy × 10)

Cell type	Treatment (24 h)	Score	Functions (number of genes in the category implicated)
PC3	Single dose	18	Connective tissue disorders, inflammatory disease, skeletal and muscular disorders (6)
	Multifractionated	57	Infectious disease, dermatological diseases and conditions, antimicrobial response (21)
		17	Hematological system development and function, tissue morphology, inflammatory response (8)
		10	Cell-to-cell signaling and interaction, metabolic disease, infectious disease (5)
DU145	Single dose	21	Cellular development, hematological system development and function, hematopoiesis (7)
	Multifractionated	12	Infectious disease, antimicrobial response, inflammatory response (3)
LNCaP	Single dose	23	Cellular development, cellular growth and proliferation, hematological system development and function (8)
	Multifractionated	27	Cellular development, hematological system development and function, hematopoiesis (10)
		15	Infectious disease, carbohydrate metabolism, lipid metabolism (6)

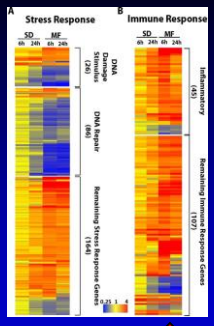
Notes. IPA score refers to statistical significance, with all immune response genes with at least a twofold change and $P < 0.05$, which are mapped to the functional networks available in the Ingenuity Pathway Knowledge Base. Focus molecules indicate the number of genes that could be mapped to molecules out of a possible 35 molecules in each network.



Fractionation can induce an adaptive response- Immune response genes upregulated in tumors and endothelial cells:

Dose, Fx and volume matter

May even vary dose and schedule ?!



Differential expression of stress and immune response pathway transcripts and miRNAs in normal human endothelial cells subjected to fractionated or single-dose radiation.

Palayoor ST. Mol Cancer Res. 12(7):1002-15; 2014

Challenges

Immune-modulation of tumor microenvironment and tumor cells by radiation

•Quality of radiation (high versus low-LET), dose, size, fractionation (low-dose versus high-dose fractionation) and dose-rate (high-dose rate versus low-dose rate), and schedule (hypofractionation versus multifractionation

•Irradiation of complete tumor volume or partial volume adequate for effective modulation of tumor immune microenvironment
- Gross tumor volume (GTV) or GTV plus LN
- Evoking it by irradiating normal tissue

•HLA class I loss or low TCR diversity or checkpoint expression or TILs.

•Balance between radio-induction of immune suppressive cytokines and radio-induction of immune activating cytokines

Challenges

Effective combinations of radiation and immunotherapy

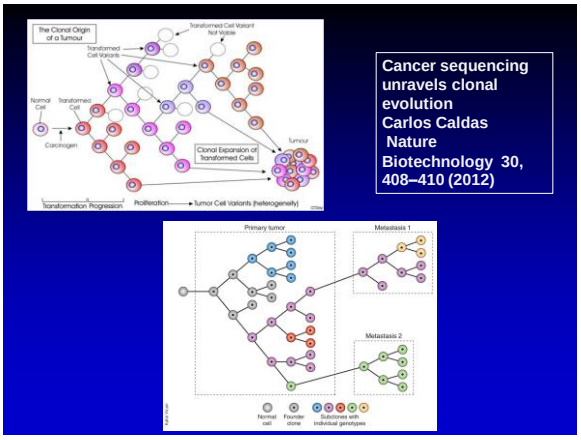
•Relevant pre-clinical models (NSG-PDX, GEMMs and canine) or from clinical trials (reverse translational)

•Radiation effect on normal tissues and its impact on the efficacy of radiation + checkpoint blockade therapy
- Efficacy area versus safety area
- Use of traditional endpoints for safety and efficacy

•Significant challenges in the selection of opportune biomarkers of immunogenicity when radiation is combined with immunotherapy

•Remember tumor cell heterogeneity- many clones to start with and there is ongoing evolution.

•Combinations with molecular targeted agents will add complexity of pathway activation/suppression, tumor adaptation and impact on normal tissues!



Cancer sequencing unravels clonal evolution
Carlos Caldas
Nature Biotechnology 30, 408–410 (2012)

ETCTN
Experimental Therapeutics Clinical Trials Network
Team Driven. Cancer Therapy Focused.

A Phase II Trial of Durvalumab and Tremelimumab Alone or in Combination with High or Low-dose Radiation in Metastatic Colorectal and NSCLC

PD-L1 Project Team

Jonathan Schoenfeld, Arta Monjazeb, Mansoor Ahmed, Stephen Hodi

NIH NATIONAL CANCER INSTITUTE

Questions asked by this trial

- This clinical trial is designed to answer novel and critical questions which are unlikely to be answered by existing trials
  - Does RT synergize with dual check-point blockade (Saint-Victor et al Nature 2015)
  - What is the influence of RT dose (Chandra et al Oncoimmunology 2015)
  - Can RT overcome resistance to T-cell exclusion mediated resistance to dual checkpoint blockade (Spranger et al Nature 2015)
  - Does RT increase intratumoral TCR diversity and how does this correlate with response to RT + dual checkpoint blockade (Saint-Victor et al Nature 2015)
  - Does RT increase tumor antigenic load and how does this correlate with response to RT + dual checkpoint blockade (Rizvi et al Science 2015)

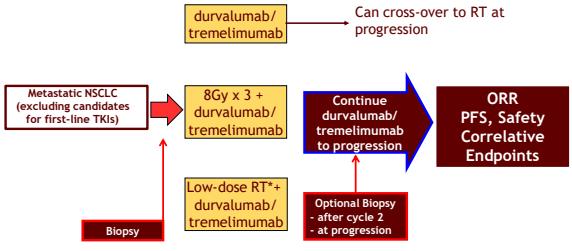
Study Hypotheses

Overall Hypothesis:

Due to complimentary immunologic effects (increasing the magnitude and diversity of the T-cell response in the tumor microenvironment), radiation will synergize with dual checkpoint blockade.

- Two cohorts:
  - **Metastatic NSCLC (~15-20% response to anti-PD-1 therapy)**
    - Hypothesis: The addition of low or high dose radiation to combined blockade of PD-L1 (durvalumab) and CTLA-4 (tremelimumab) will increase response rate
  - **Metastatic colorectal cancer, microsatellite stable (few to no responders to anti-PD-1 therapy)**
    - Hypothesis: Low or high dose radiation combined with PD-L1/CTLA-4 blockade will lead to a measurable response rate

Trial Schema – NSCLC Cohort



*0.5 Gy BID x2 days repeated q4weeks with durvalumab / tremelimumab

Mathematical modeling of cancer immunotherapy and its synergy with radiotherapy
R. Serre1, S. Benzekry2, L. Padovani3, C. Meille4, N. André1, 5, 6, J. Ciccolini1, F. Barlesi1, 7, X. Muracciole•3, D. Barbolosi*1
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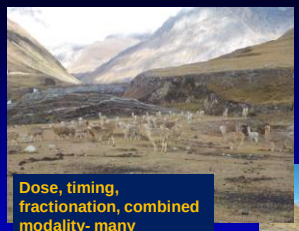
[7] Multidisciplinary Oncology & Therapeutic Innovations Unit, AP-HM, Marseille, France

Mathematical model of radiation and immunology
The model is described by 5 discrete-time equations:
Tumor dynamics (equation 1)
Antigen dynamics (equation 2)
Lymphocytes (or immune effectors) dynamics (equation 3)
Primary immune response (equation 4)
Secondary (or memory) immune response (equation 5)

In press Cancer Research

Immunotherapy and radiation oncology



Dose, timing, fractionation, combined modality- many parameters to understand so *avoid the* (cute for sure) *herd mentality*….

…or important potential clinical benefit for ourpatients may lead to *inappropriate* "ruin" of what our "focused biology" can accomplish!

Immunotherapy and radiation oncology

Entering a new era for which great opportunities exist, based on critical science (much yet to be learned)