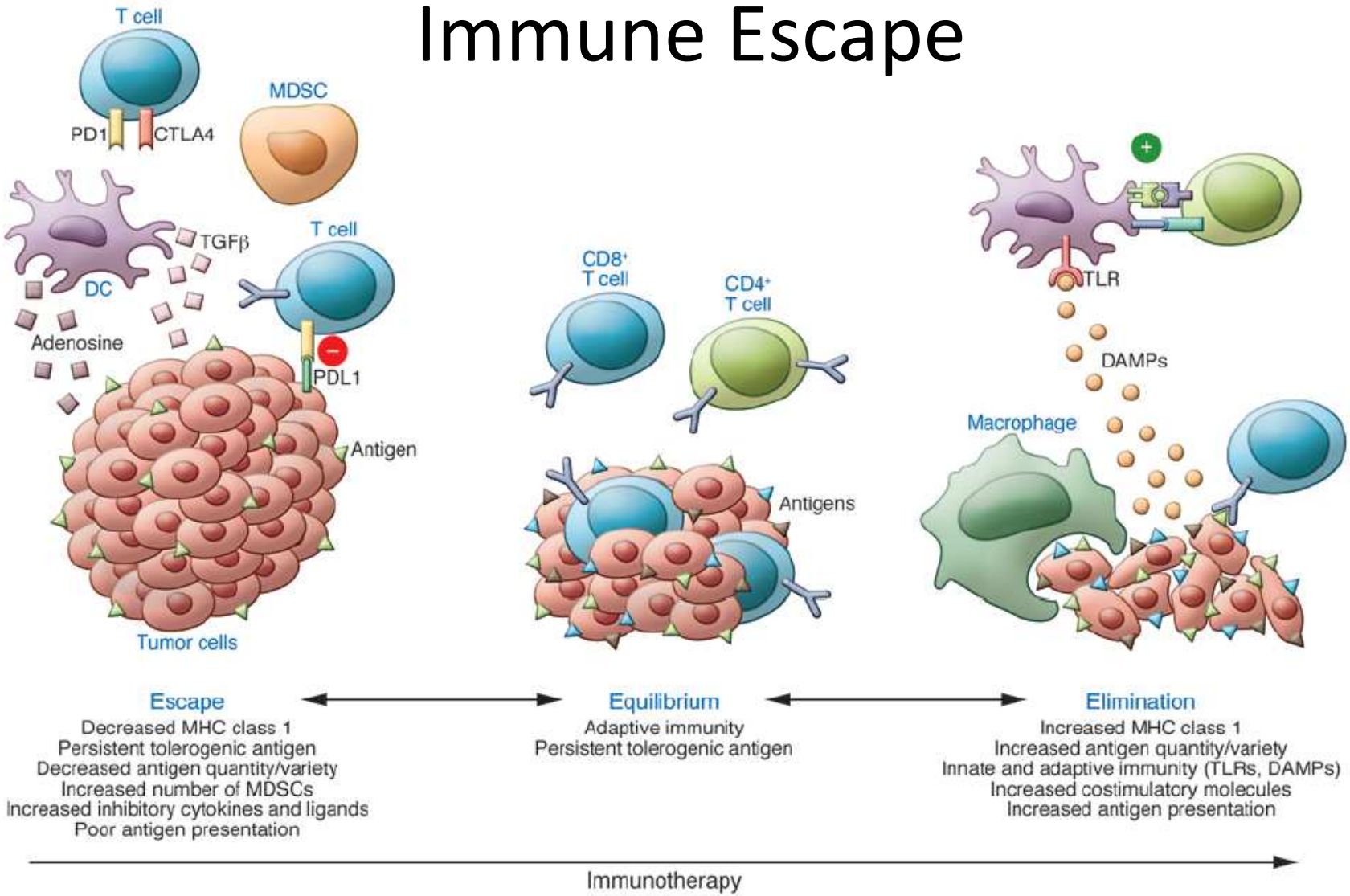
Immunotherapy Combinations with Small Molecules, Surgery and Radiation

David L. Bartlett, MD
University of Pittsburgh

Immunotherapy: Overcoming Immune Escape



The Journal of Clinical Investigation http://www.jci.org Volume 123 Number 7 July 2013

Targeted therapy + Immunotherapy

- Targeted agents can block oncogenic events in cancer cells without impacting lymphocyte function
- Inducing apoptosis may increase sensitivity to recognition and attack by CTLs
- Pathway modulation may activate CTL
- Combine
  - Targeted therapy-high response/short duration
  - Immunotherapy low response but durable
  - Achieve high rate of durable responses

Summary of Targeted agents and their Function

Drug	Class	Target	Pathway
Gefitinib, erlotinib	Tyrosine kinase inhibitor	EGFR	PI3K/Akt – survival MAPK – proliferation
Cetuximab	Monoclonal antibody	EGFR	PI3K – survival MAPK – proliferation
Crizotinib	Tyrosine kinase inhibitor	ALK	MAPK – proliferation
Bevacizumab	Monoclonal antibody	VEGF	Angiogenesis
Sunitinib, sorafenib	Tyrosine kinase inhibitor	VEGF	Angiogenesis
Vorinostat	Small molecule inhibitor	Histone deacetylase	Epigenetic silencing
Cixutumumab	Tyrosine kinase inhibitor	IGF-1R	PI3K/Akt, DNA damage
Figitumumab	Monoclonal antibody	IGF-1R	PI3K/Akt, DNA damage
Celecoxib	Small molecule inhibitor	COX-2	EGFR signaling – PI3K/Akt – MAPK

Abbreviations: EGFR, epidermal growth factor receptor; VEGF, vascular endothelial growth factor; IGF, insulin-like growth factor; PI3K, phosphatidylinositide 3-kinase; MAPK, mitogen-activated protein kinase; COX-2, Cyclooxygenase-2.

Sechler et al. Pharmacogenomics and Personalized Medicine 2013:6

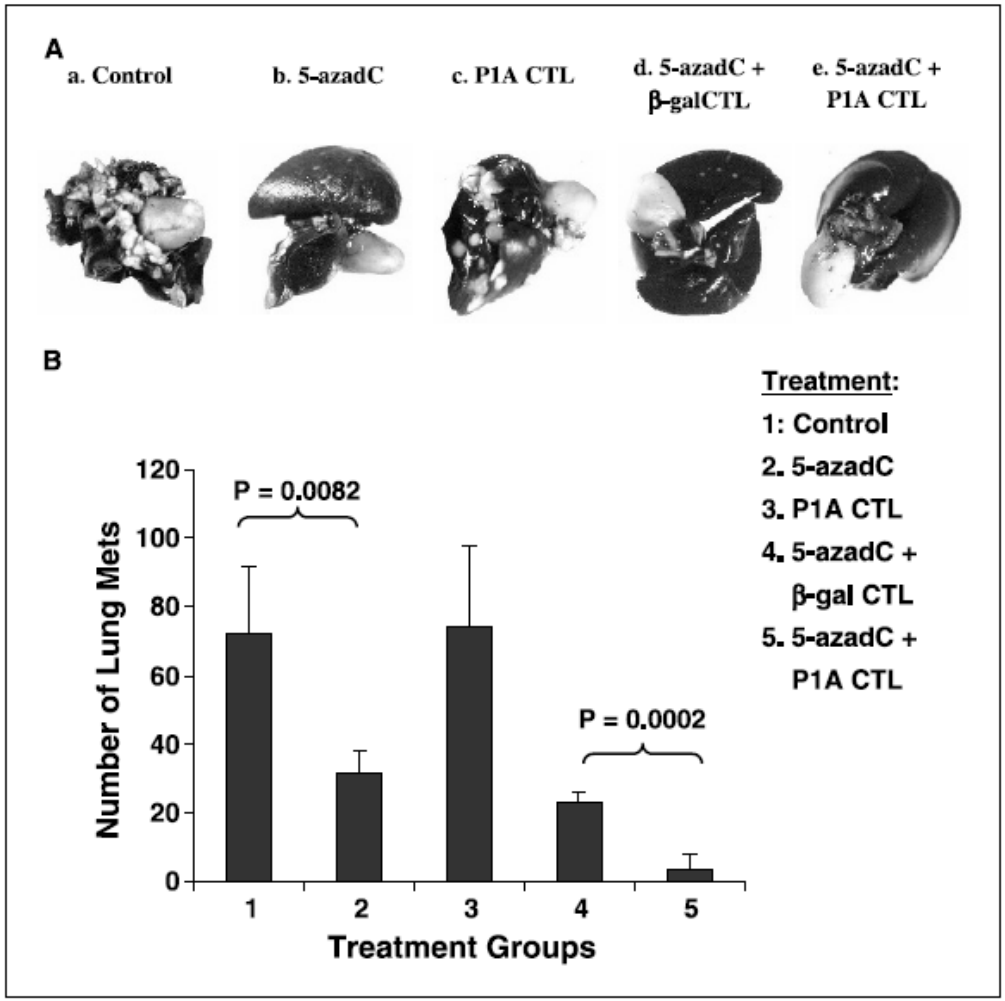
Epigenetic Therapy and Immunotherapy

- Demethylating agents and HDAC inhibitors
  - Pro-apoptotic
    - More sensitive to cytotoxic immune effector cells
  - Increase MHC expression
    - And other molecules involved in ag presentation and processing
  - Improve expression of tumor antigens
  - Improve expression of ligands for NK activating receptors

De novo Induction of a Cancer/Testis Antigen by 5-Aza-2′-Deoxycytidine Augments Adoptive Immunotherapy in a Murine Tumor Model

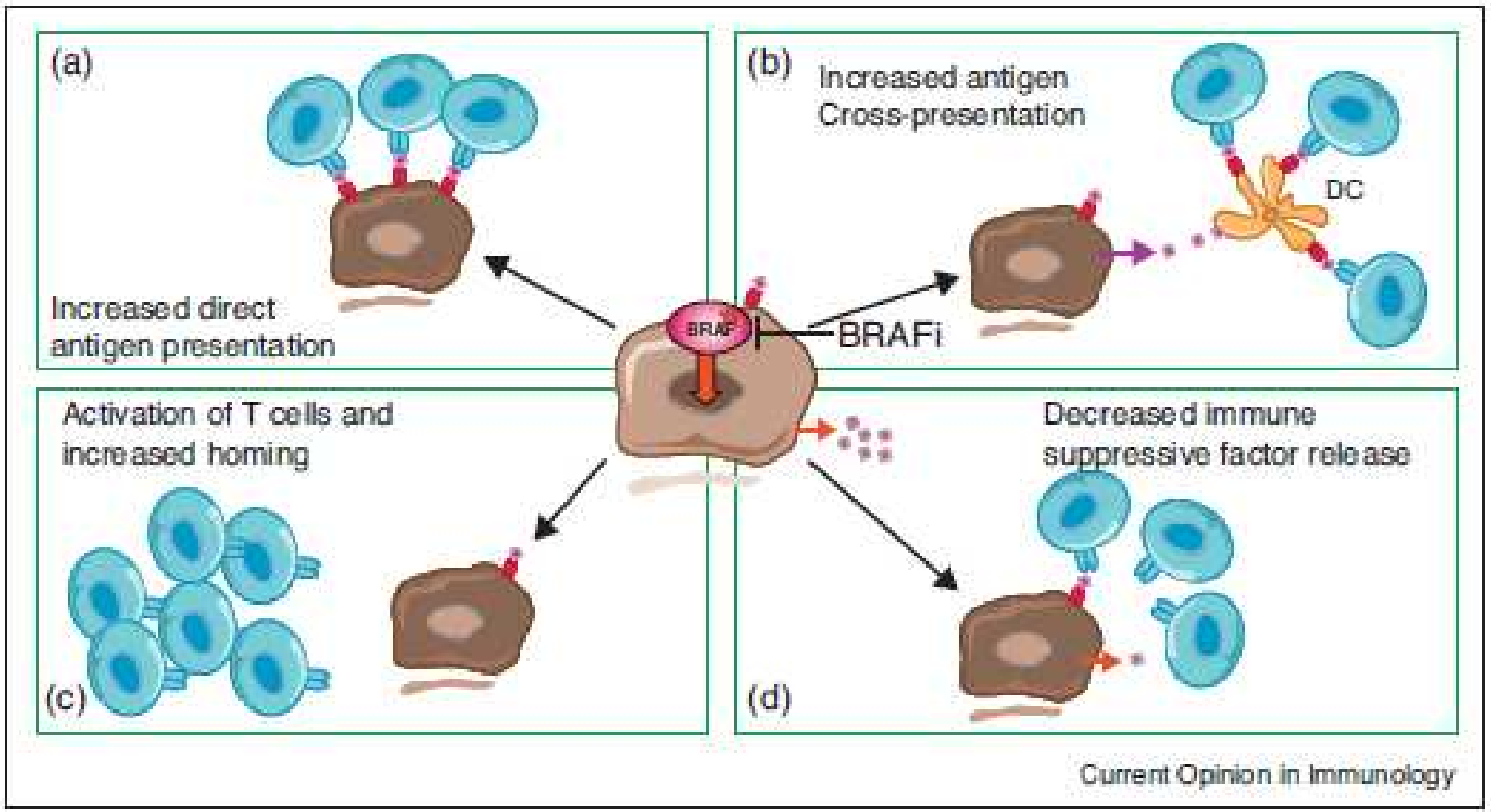
Table 1. *P1A* gene expression as detected by RT-PCR assays

Tumor type	Cancer cells	Relative P1A expression	
		−5azadC	+5azadC
Mastocytoma	P815	+++	+++
Colon carcinoma	CA07/A	−	+++
	CA51	+/−	+++
	CT26	−	+++
	MC38	−	+++
Lung carcinoma	LC12	−	+++
	LLC1	−	+++
	LM2	−	+++
	M109	−	+++
Lymphoma	A20	+/−	+++
	CH-1	−	+++
	EL4	+	+++
	TIML4	+	+++
	YAC-1	−	+++
Sarcoma	MCA 102	−	+++
	MCA 205	−	+++
	WEHI 164	−	+++
Mammary tumor	4T1	−	+++
	C127I	−	+++
Hepatoma	Hepa 1-6	+	+++
Melanoma	B16	−	+++
Neuroblastoma	Neuro-2a	−	+++



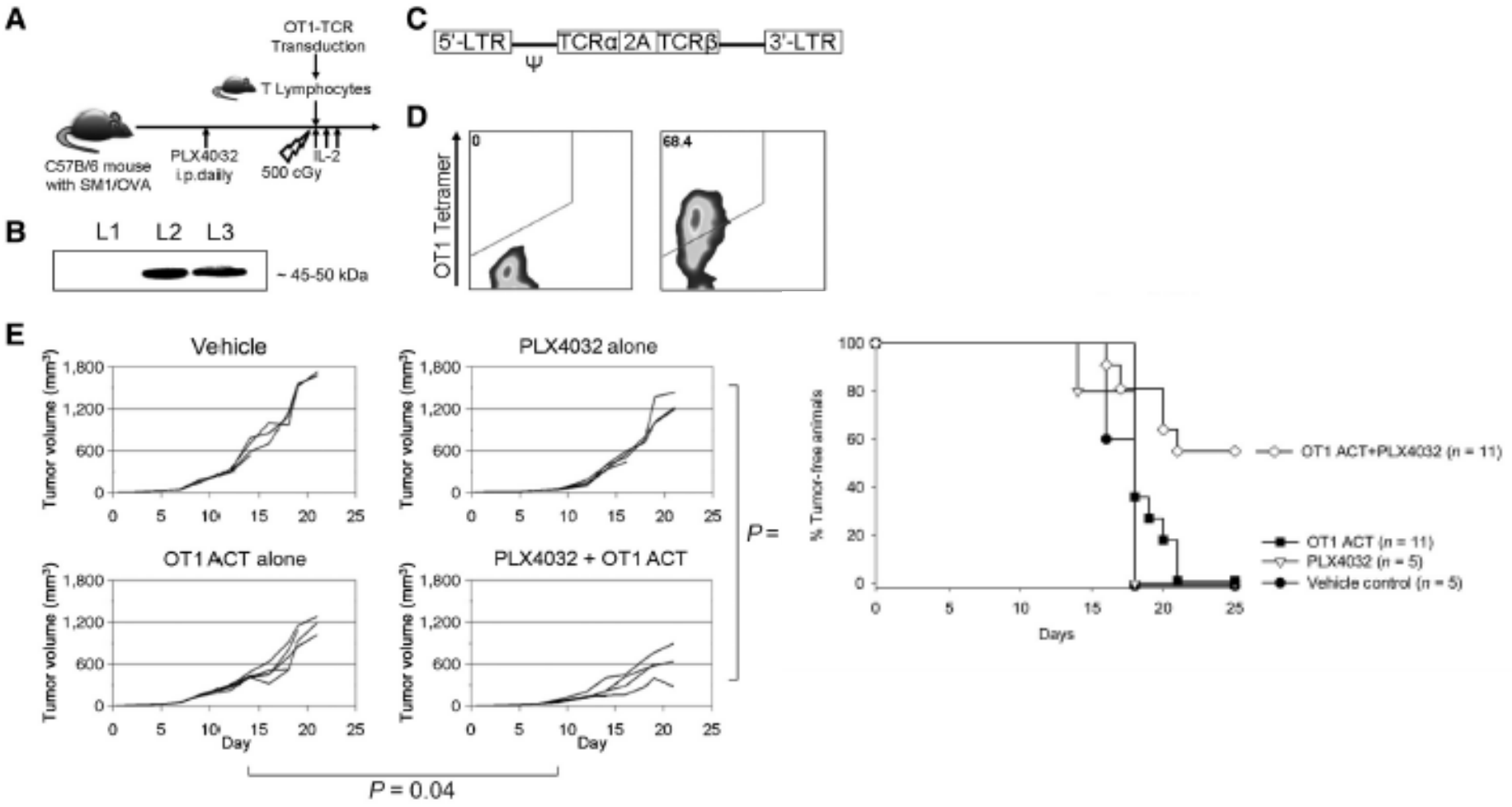
Guo et al. **Cancer Res 2006; 66: (2). January 15, 2006**

MAPK inhibitors
(BRAF inhibitors – vemurafenib, dabrafenib)

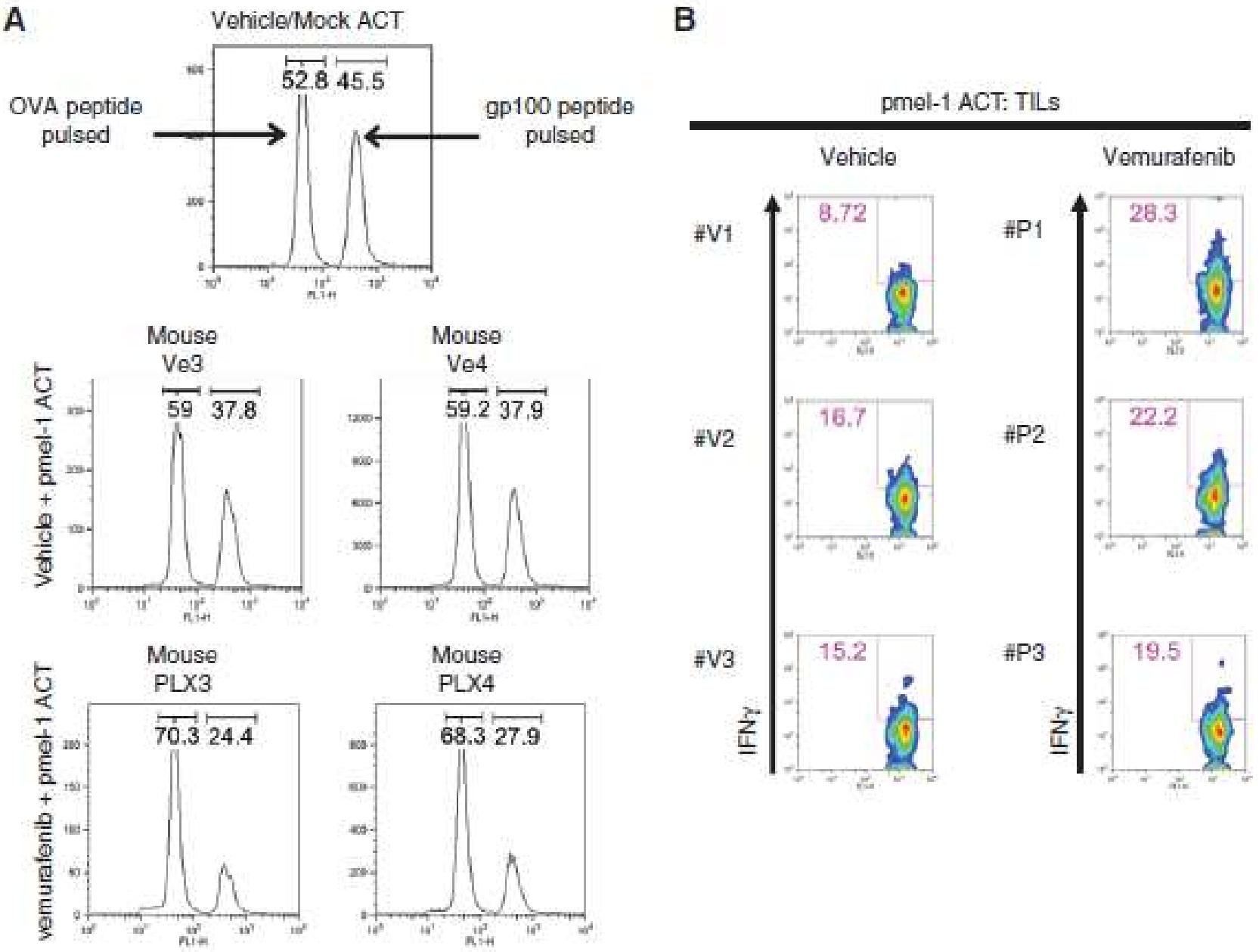


Current Opinion in Immunology 2013, **25**:291–296

BRAF Inhibitor Vemurafenib Improves the Antitumor Activity of Adoptive Cell Immunotherapy – Kuya et al



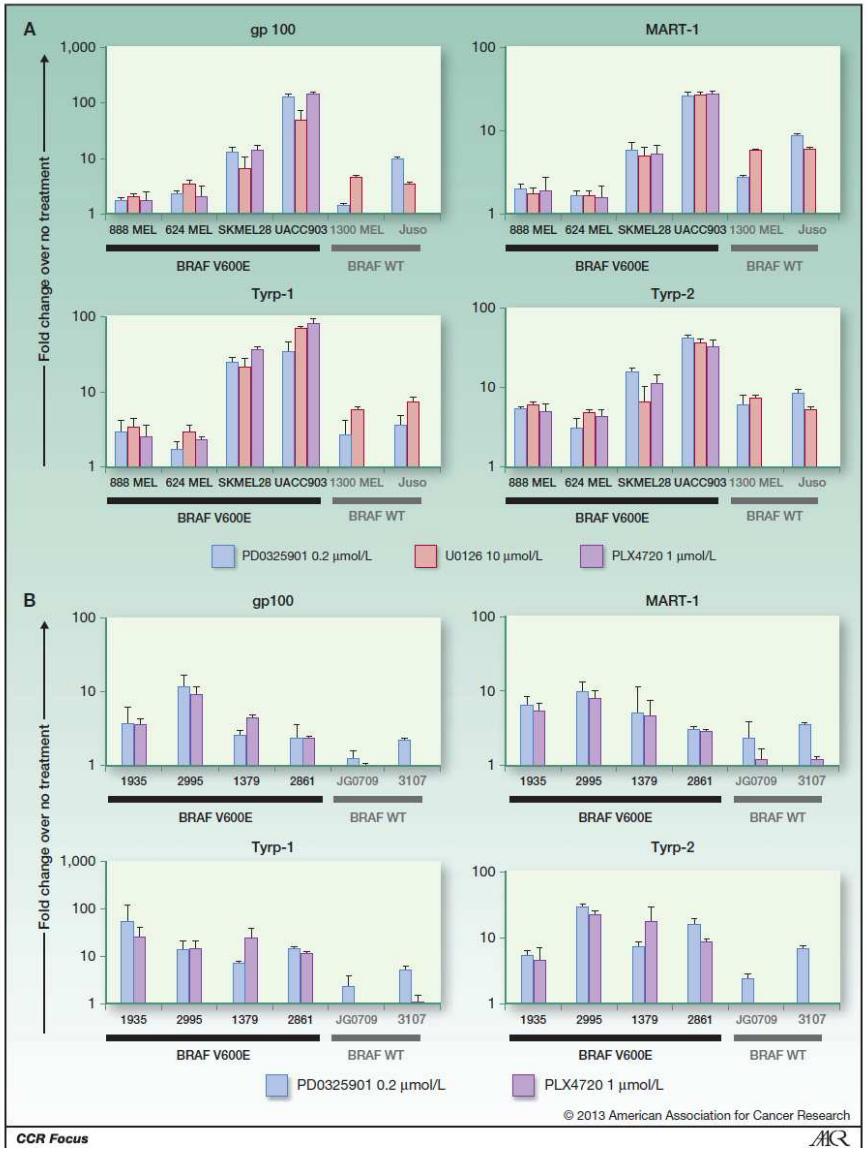
Cancer Res; 72(16) August 15, 2012

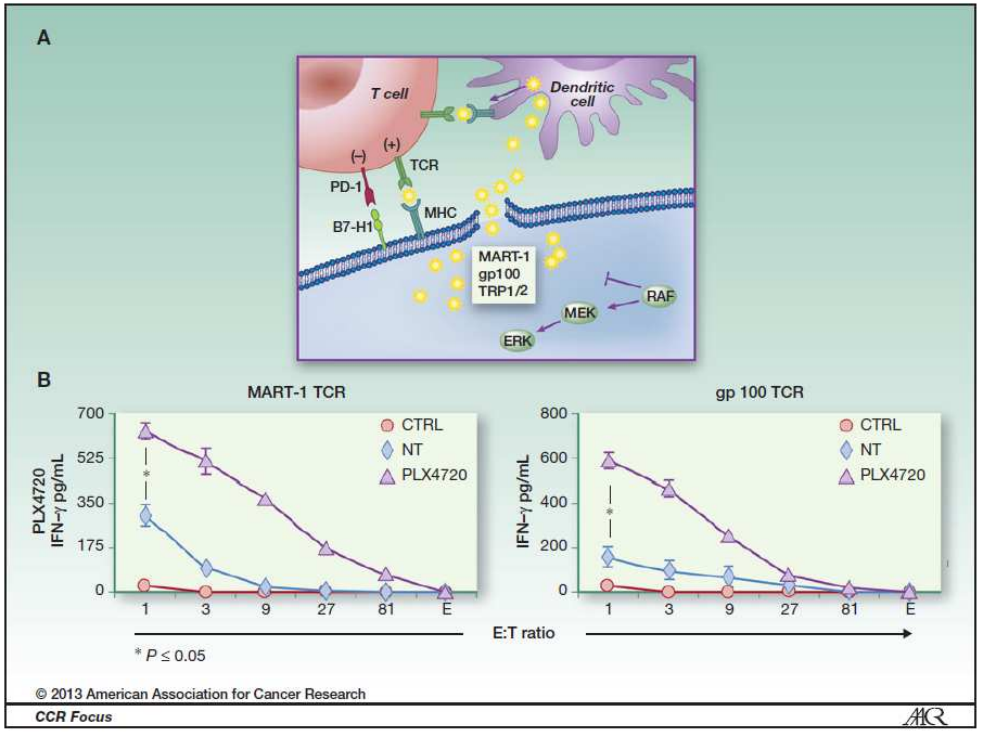


Cancer Res; 72(16) August 15, 2012

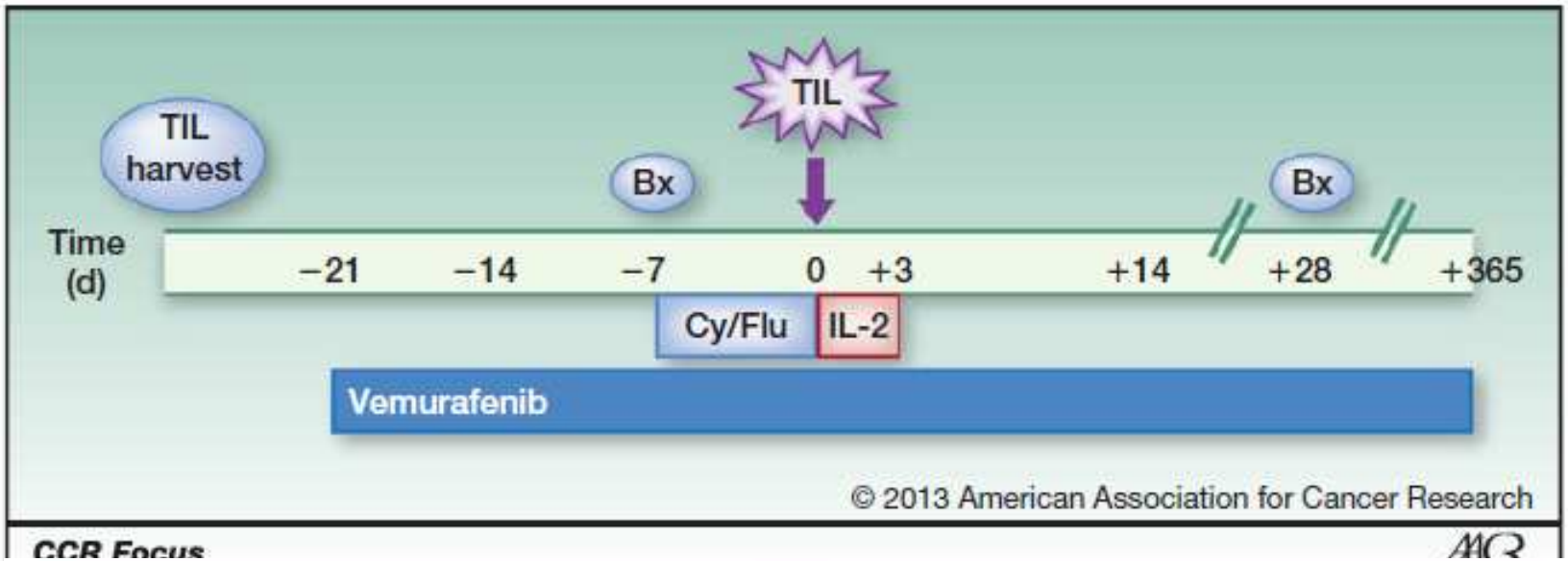
Adoptive T-cell Transfer Therapy and Oncogene-Targeted Therapy for Melanoma: The Search for Synergy

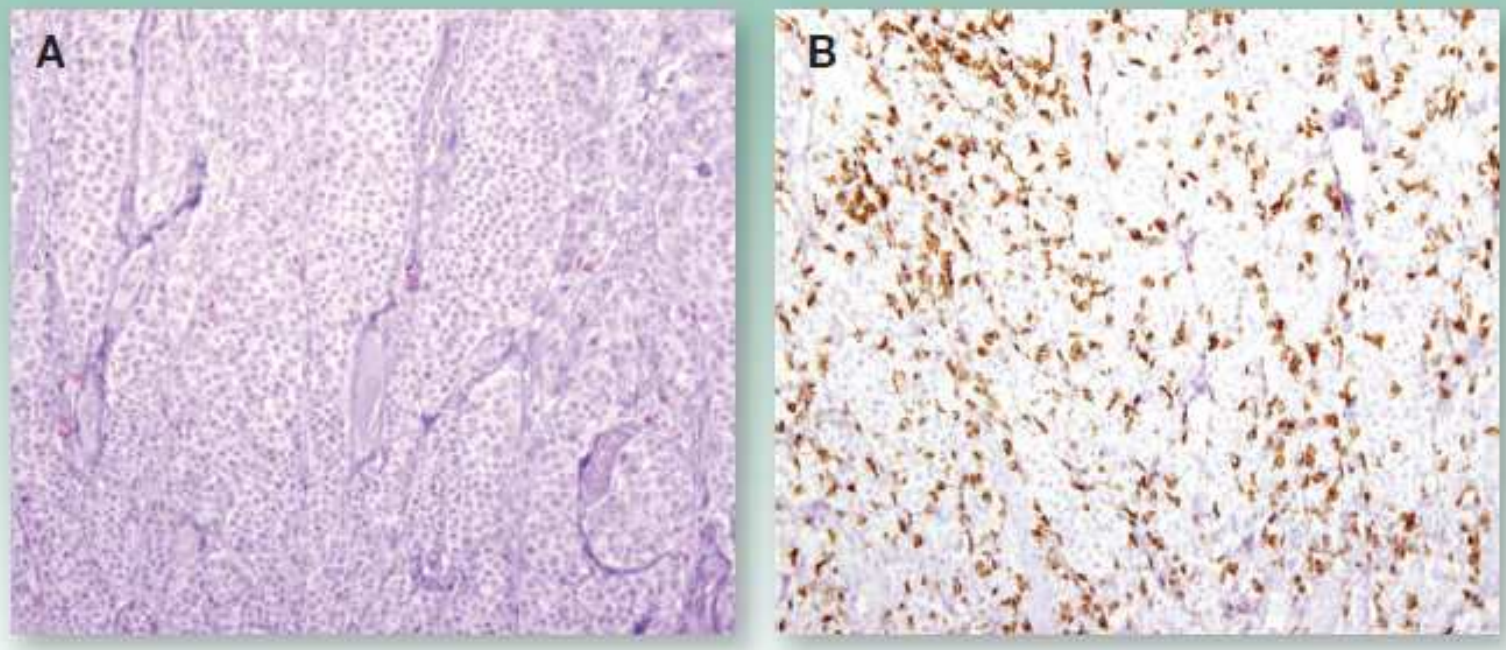
Mei Li M. Kwong[1], Bart Neyns[2], and James C. Yang[1]





Clin Cancer Res; 19(19) October 1, 2013



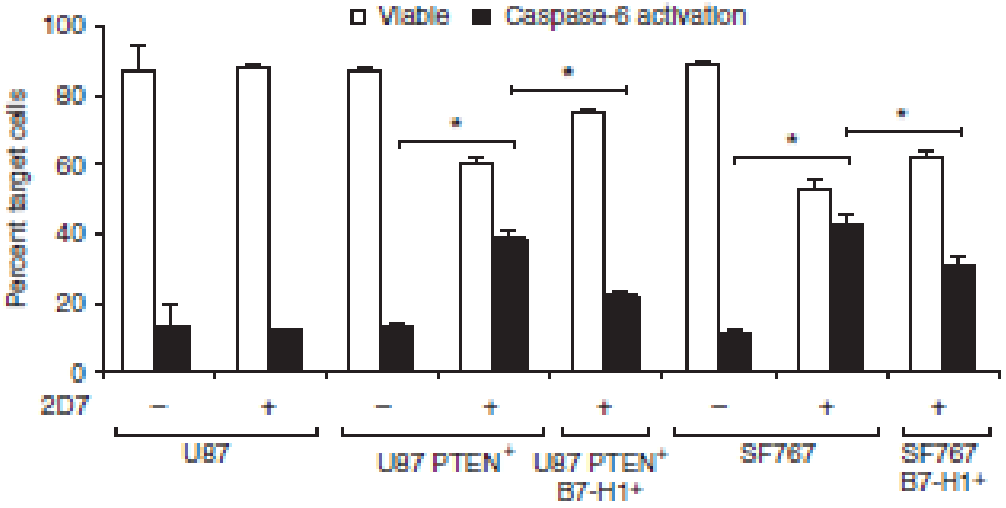


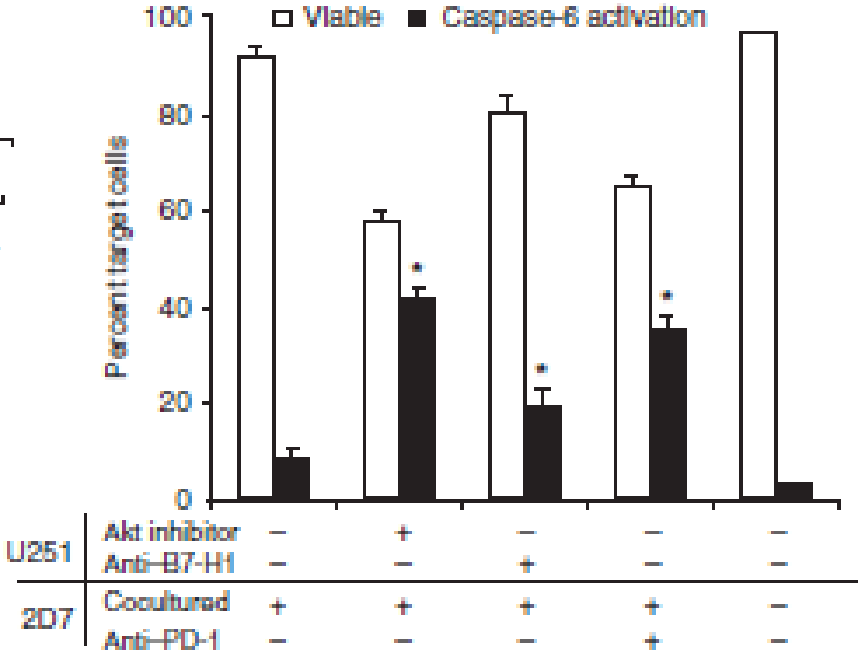
Clin Cancer Res; 19(19) October 1, 2013

PI3K/AKT/mTOR inhibitors

- mTOR inhibitors generate long-lived memory CD8+ T cells
- Loss of PTEN in glioblastoma multiforme is associated with increased PD-L1 and immune evasion via activation of PI3K.
  - Reversed by PI3K inhibitors
- mTOR inhibitors improve DC function

Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma



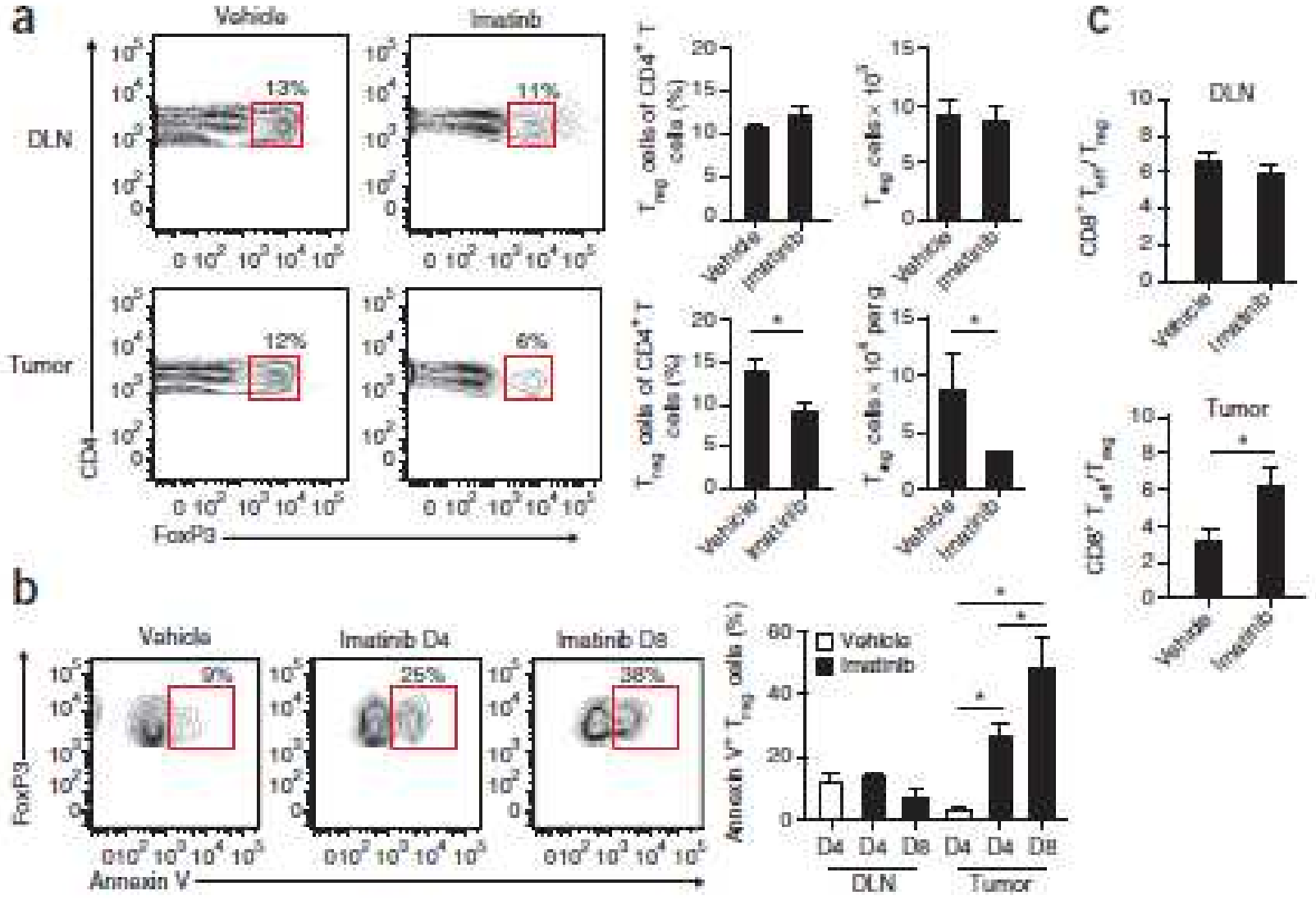


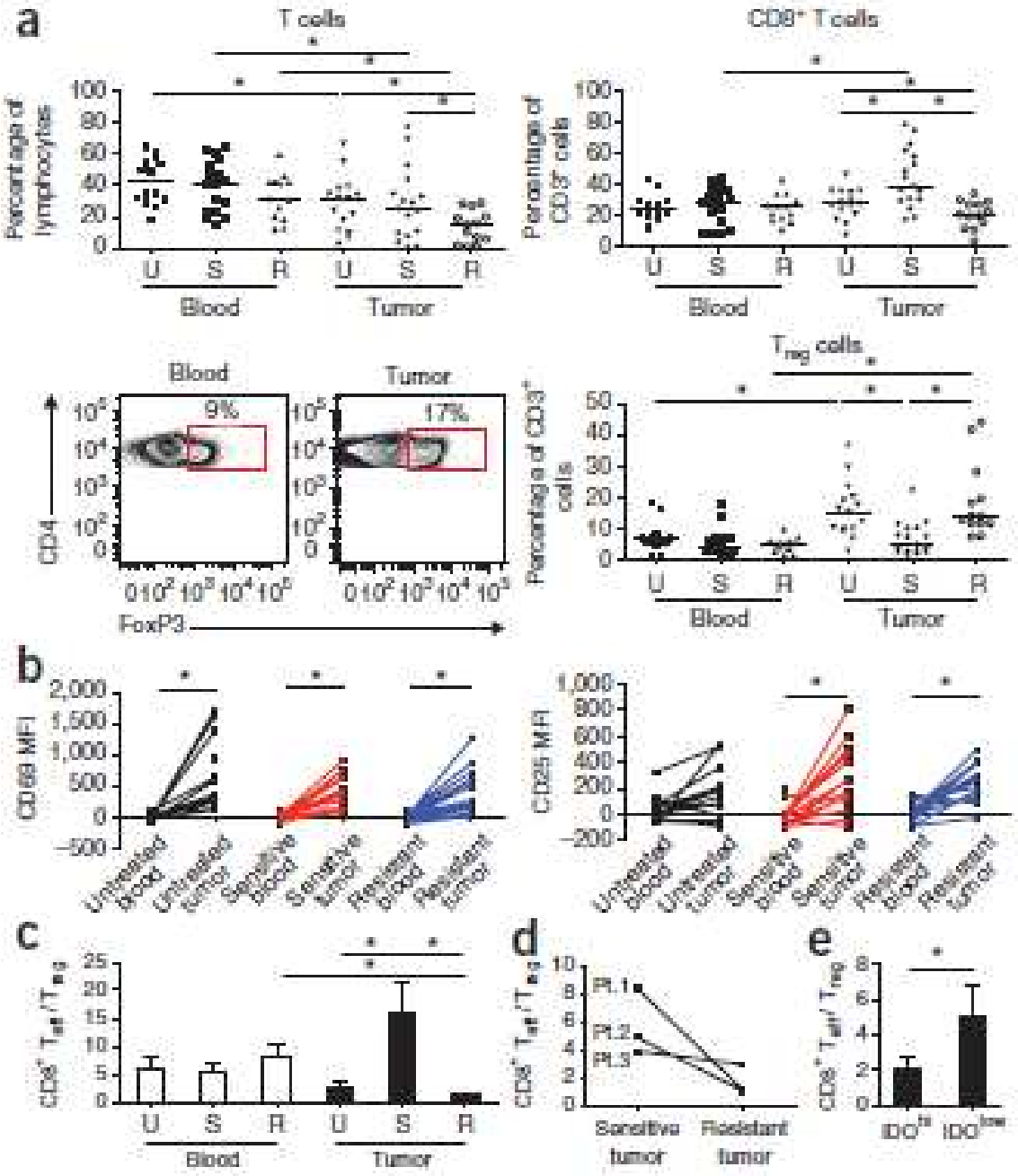
Parsa et al. VOLUME 13 | NUMBER 1 | JANUARY 2007 NATURE MEDICINE

C-Kit Inhibitors

- Activates favorable cross-talk between DC and NK cells
- Imatinib antitumor response is lost with CD8+ T cell depletion and enhanced by CTLA-4 blockade
- Dasatinib (TKI) therapy strongly potentiated by immune stimulation with agonist anti-OX40 antibody therapy

Imatinib potentiates antitumor T cell responses in gastrointestinal stromal tumor through the inhibition of Ido




a
T cells
CD8+ T cells
Percentage of lymphocytes
Percentage of CD3+ cells
U S R U S R
Blood
Tumor
Blood
Tumor
9%
17%
CD4
FoxP3
Treg cells
Percentage of CD3+ cells
b
CD69 MFI
CD25 MFI
Untreated blood
Untreated tumor
Sensitive blood
Sensitive tumor
Resistant blood
Resistant tumor
c
CD8+ Teff/Treg
d
Pt.1
Pt.2
Pt.3
Sensitive tumor
Resistant tumor
e
IDOhi
IDOlow

Hepatotoxicity with Combination of Vemurafenib and Ipilimumab

Ribas et al.

Table 1. Data for Patients with Grade 3 Elevations in Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) Levels While Receiving Combination Therapy with Vemurafenib and Ipilimumab.*

Study Cohort and Patient No.	No. of Doses of Ipilimumab before ALT–AST Elevation	Time to Onset of ALT–AST Elevation after First Dose of Ipilimumab	Treatment	Time to Resolution of ALT–AST Elevation	Toxicity Relapse with Repeated Ipilimumab
First cohort					
4	1	21 days	Glucocorticoids; vemurafenib discontinued for 5 days and then restarted with dose reduction; ipilimumab permanently discontinued	4 days	NA
5	2	36 days	Glucocorticoids; vemurafenib discontinued for 4 days and then restarted with dose reduction; ipilimumab continued (2 doses)	6 days	No
6†	1	21 days	Glucocorticoids; vemurafenib discontinued for 5 days and then restarted with dose reduction; ipilimumab continued (1 dose)	6 days	No
8	1	19 days	Glucocorticoids; vemurafenib discontinued for 4 days and then restarted with dose reduction; ipilimumab continued (1 dose)	12 days	Yes
Second cohort					
10	1	15 days	Glucocorticoids; vemurafenib discontinued for 7 days and then restarted with dose reduction; ipilimumab permanently discontinued	10 days	NA
16‡	1	13 days	Vemurafenib and ipilimumab permanently discontinued	20 days	NA

* The first cohort started with a run-in period of 1 month of single-agent vemurafenib (960 mg orally twice daily), followed by four infusions of ipilimumab (3 mg per kilogram of body weight every 3 weeks) and concurrent twice-daily doses of vemurafenib. The second cohort received a lower dose of vemurafenib (720 mg twice daily) together with the full dose of ipilimumab. NA denotes not available.
† This patient also had a grade 2 increase in the total bilirubin level.
‡ This patient also had a grade 3 increase in the total bilirubin level.

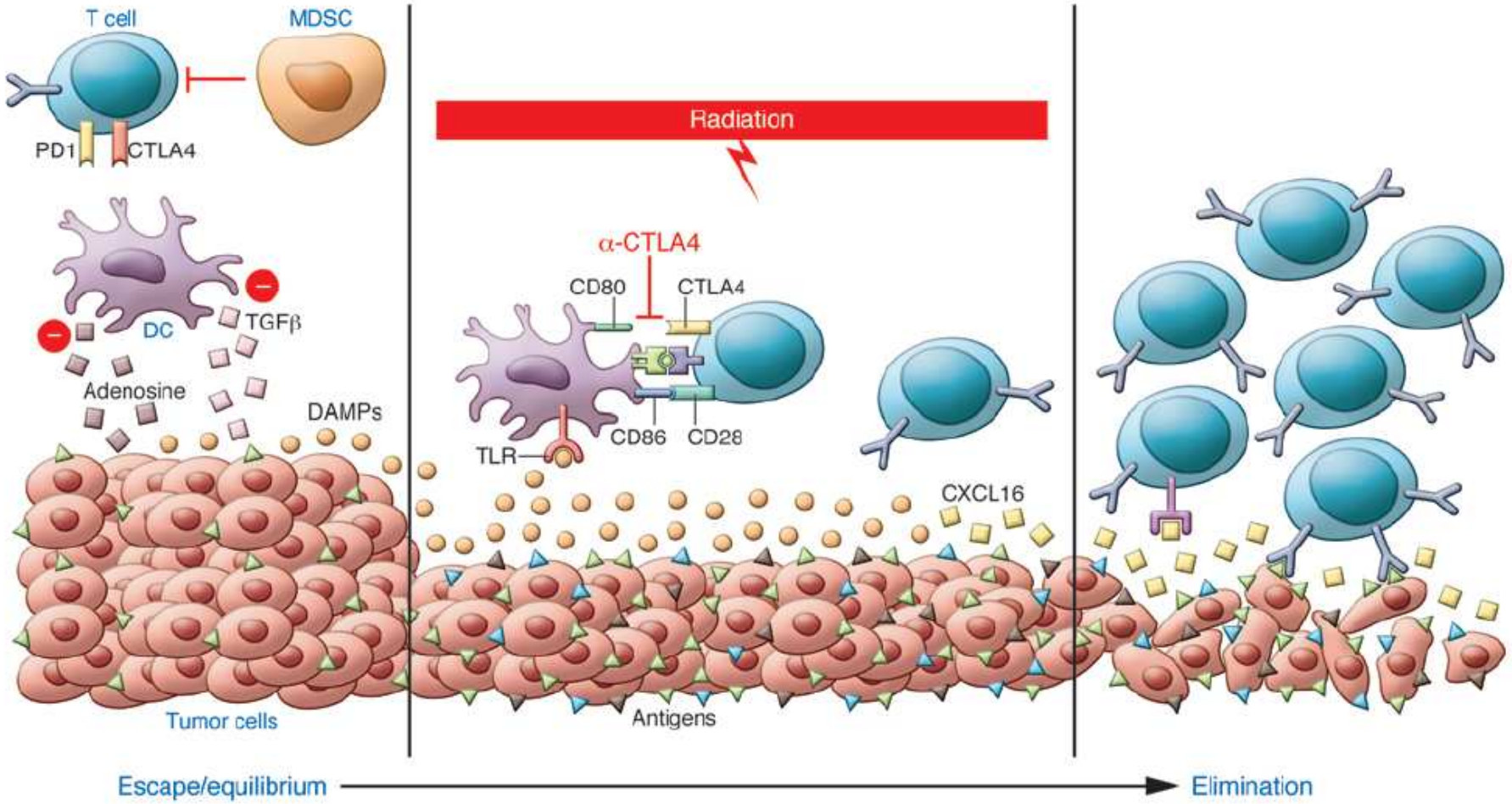
"Our findings reinforce the need for carefully conducted trials of new combination Therapies, even when both agents have regulatory approval and have distinct Mechanisms of action."

Radiation

- Tumor irradiation exposes the complex antigenic tumor environment by generating new peptides and increasing the pool of intracellular peptides for cross-presentation.
- Radiation augments MHC-I expression.
- Radiation recruits hematopoietic and DCs into tumor
- Radiation causes HMGB-1 release, promoting activation and maturation of APCs
- Irradiated tumors upregulate death receptors (e.g., FAS), promoting the cytotoxic effect of T cells at the tumor site

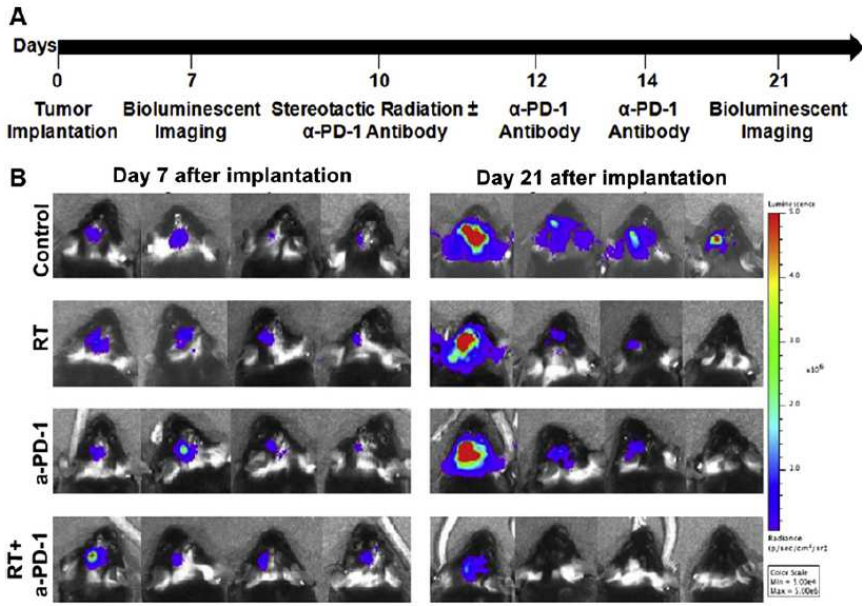
Radiation and immunotherapy: a synergistic combination

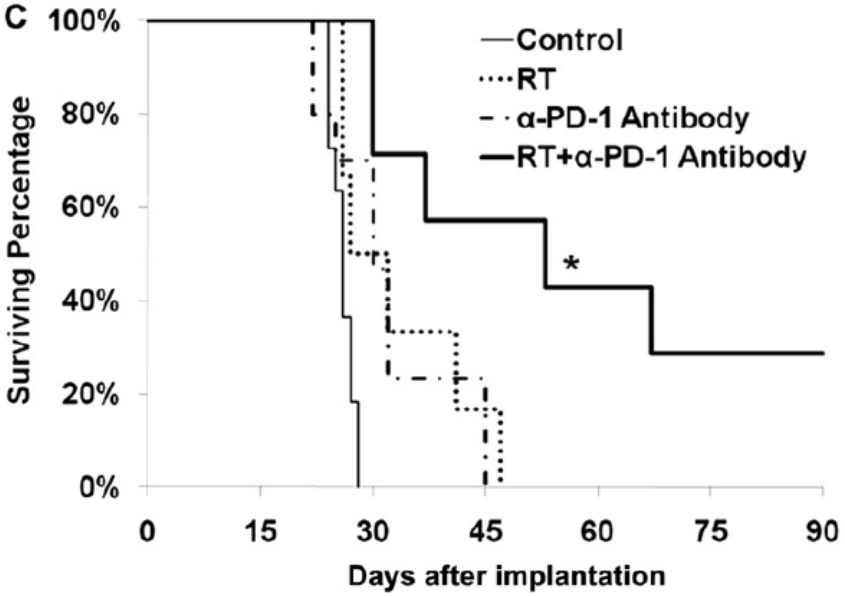
Anusha Kalbasi,[1] Carl H. June,[2,3] Naomi Haas,[3] and Neha Vapiwala[1]



Anti-PD-1 Blockade and Stereotactic Radiation Produce Long-Term Survival in Mice With Intracranial Gliomas

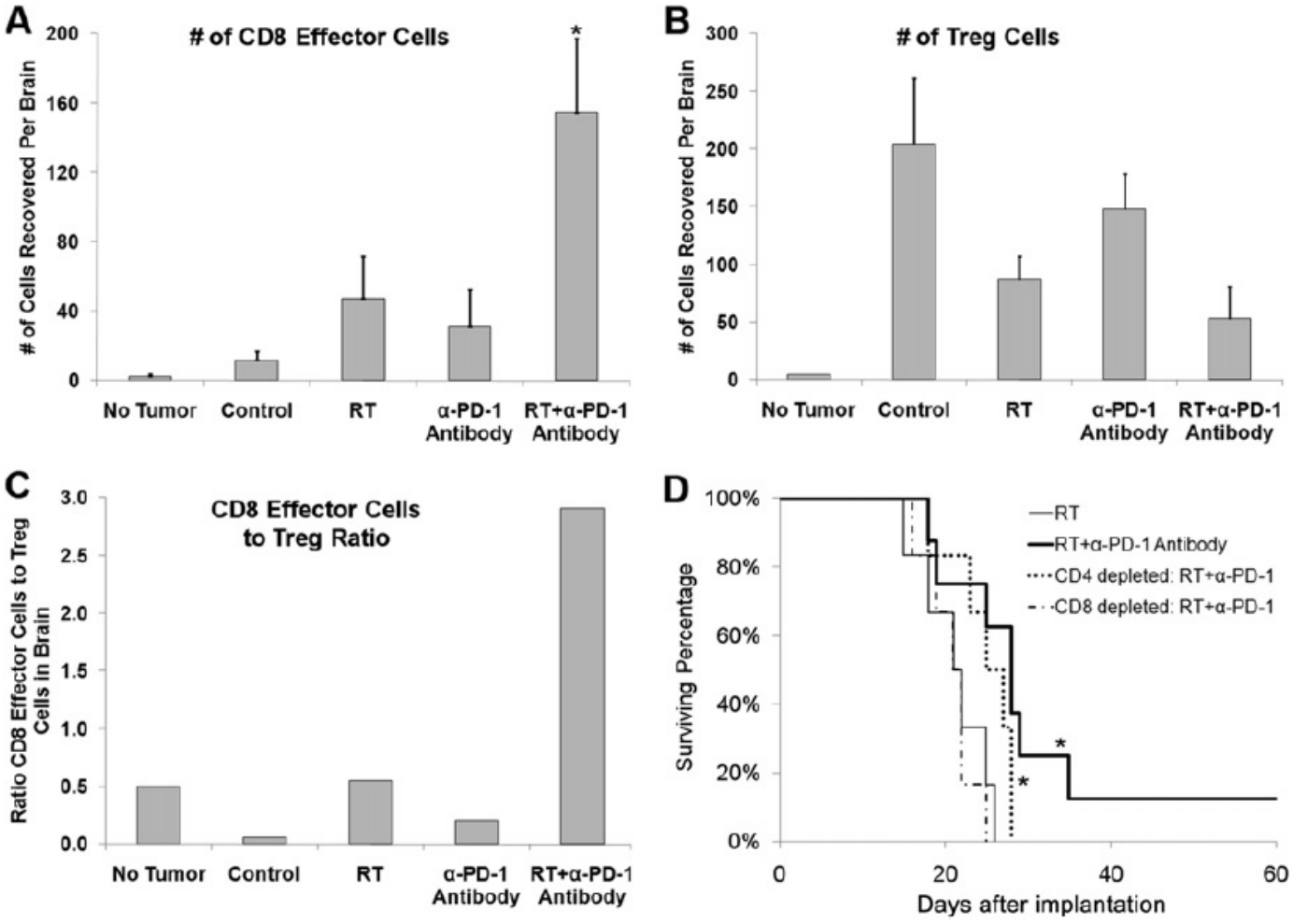
Zeng et al.





Int J Radiation Oncol Biol Phys, Vol. 86, No. 2, pp. 343–349, 2013

Mice Treated with RT+anti-PD-1 antibody show increased cytotoxic T cells and decreased regulatory T cells



Int J Radiation Oncol Biol Phys, Vol. 86, No. 2, pp. 343–349, 2013

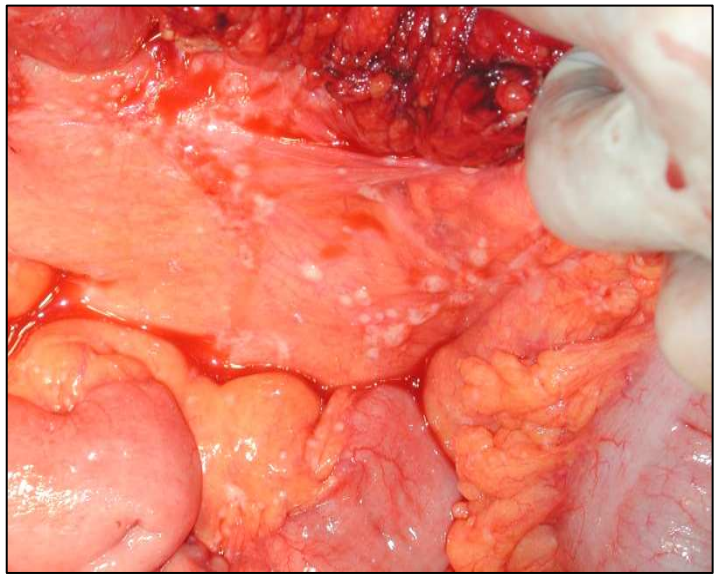
Ongoing Trials Studying Combination RT and Immunotherapy

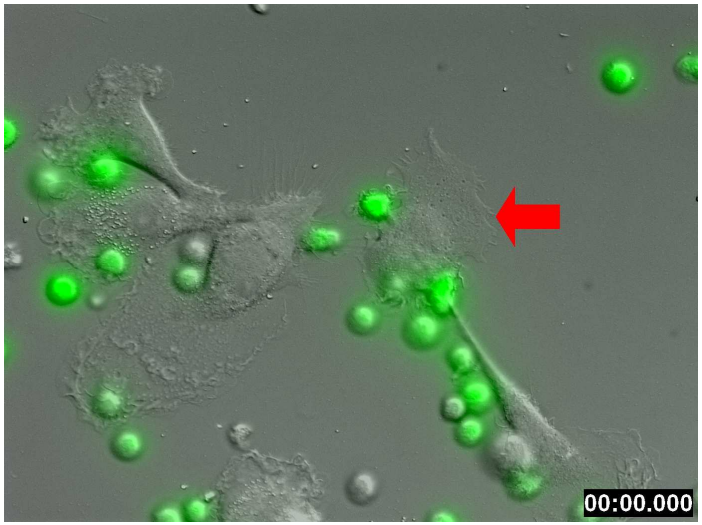
ClinicalTrials.gov identifier	Disease site	Design	Phase	Primary outcome measure	Immunotherapy	RT	Treatment timing
NCT01449279	Melanoma (advanced)	1 arm: ipilimumab prior to palliative RT	1	Safety	Ipilimumab	Palliative	RT <2 days after ipilimumab
NCT01689974	Melanoma (advanced)	2 arms, randomized: ipilimumab 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

Immunotherapy in Surgical Settings: The Principle

Shrinking Tumor → **Remove Gross Residual Tumor** → **Removal of micro-metastases**

Chemotherapy → **Surgery** → **Immunotherapy**

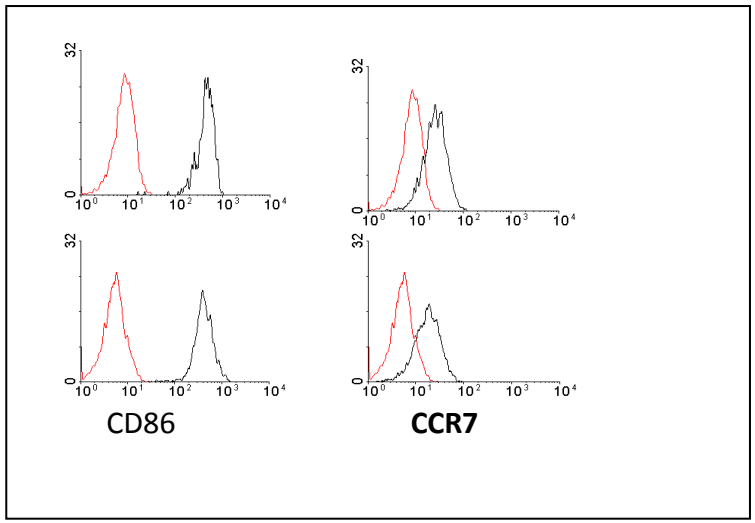


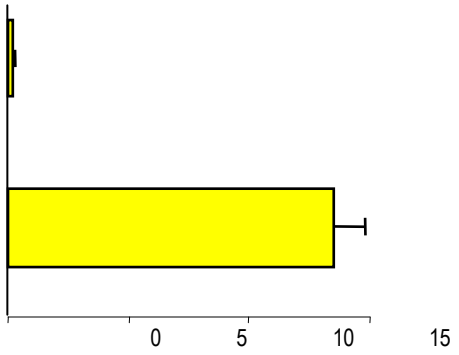


αDC1 "Vaccines": High-IL-12-producing Mature DCs Induced by ***Mediators of Anti-Viral Immunity***

TNFα / IL1β
IL6 / PGE2
("***Standard" DC***)

TNFα / IL1β
pI:C / IFNα / IFNγ
(***αDC1/Polarized DC***)





DC maturation marker **IL-12p70 (ng/ml)**

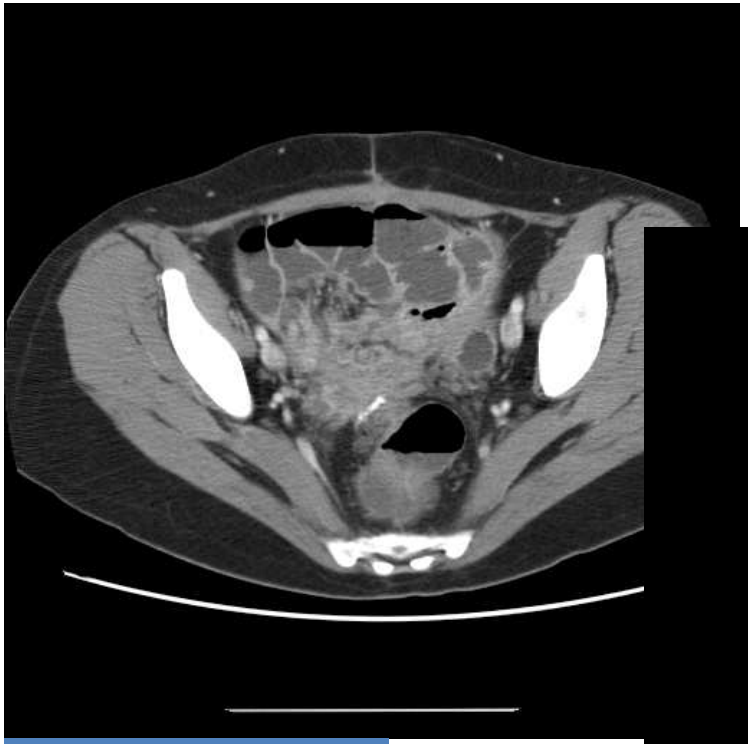
Mailliard, Kalinski et al. Canc. Res. 2004, 64: 5934

- DC-produced IL-12 needed for **induction of tumor-specific CTLs** *(Mailliard, Canc. Res 2004; Butterfield, J. Immunother. 2008; Watchmaker, JI 2010; DeBenedette J. Immunother. 2011)*
- DC-produced IL-12 needed for **activation of NK cells** *(Gustafsson K., Canc. R es.2008)*
- DC-produced IL-12 needed for **Th1 cell induction** *(Kalinski P., JI 1997, Wesa A. J.It 2007)*
- DC-produced IL-12 **predicts prolonged TTP in cancer patients** (*Okada H., JCO 2011)*

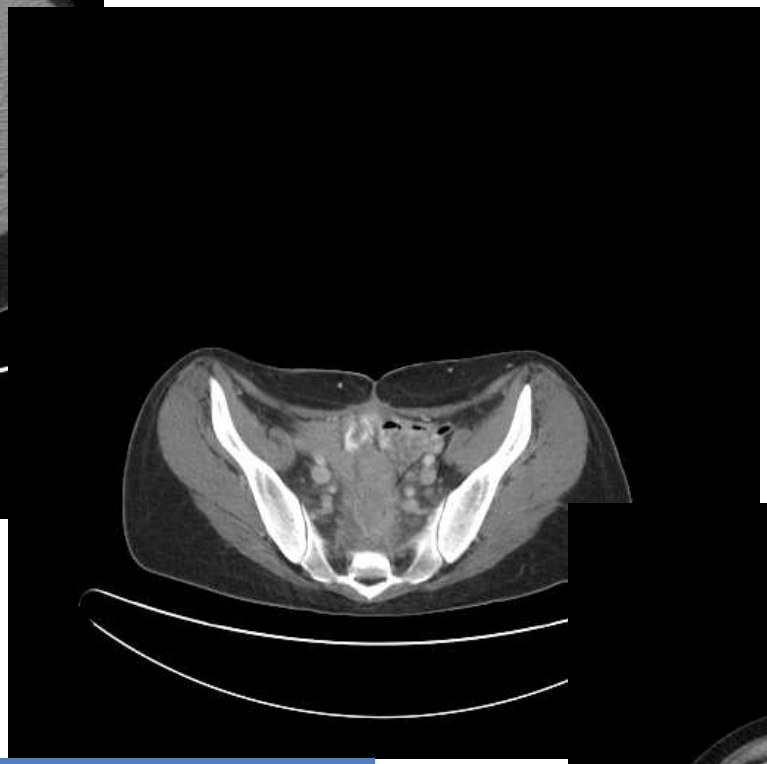
Pilot

- 38 year old female
- PMH: UC (HNPCC)
- FH: Strong FH of colon cancer

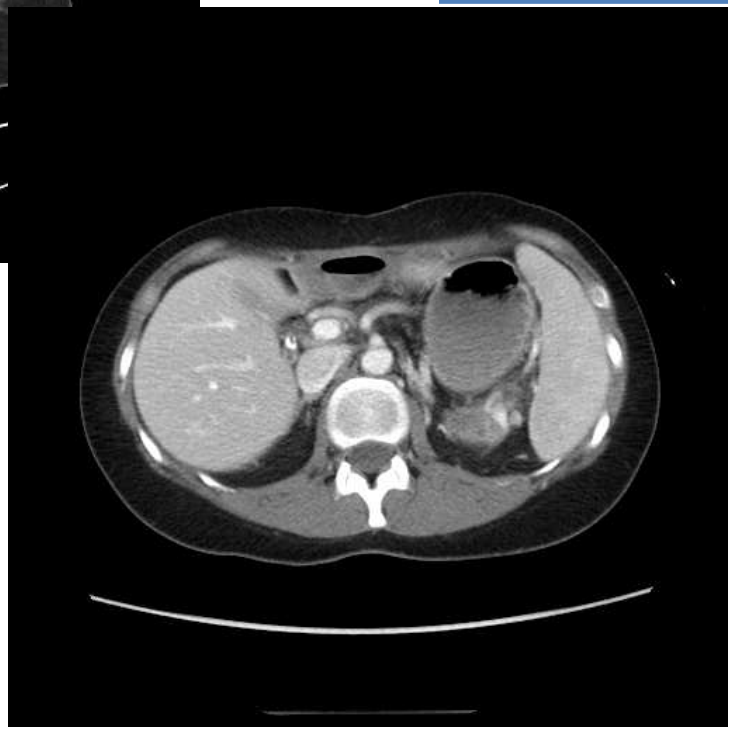
- 2004:
  - Diagnosed with colon cancer
  - TAC + Ileorectal anastamosis + Right oophorectomy
  - Adjuvant chemotherapy



2005
CRS + HIPEC



2006
CRS + HIPEC



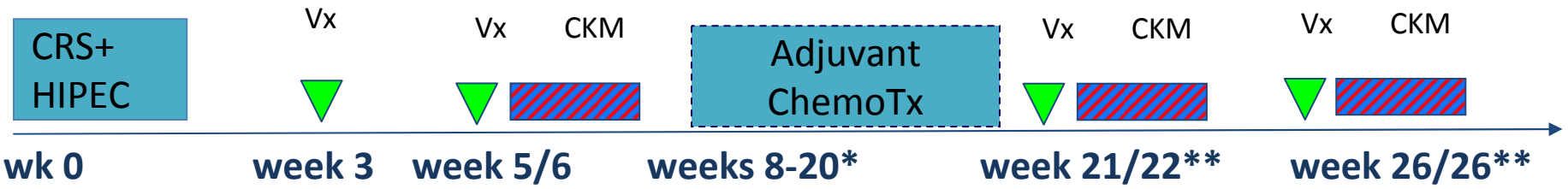
2007
CRS only

Pilot

- Enrolled in αDC1 vaccine trial (UPCI Protocol # 05-063)
  - Cycle 1 (4/2/2008) - αDC1 vaccine ($1.1x10^6$ cells)
  - Cycle 2 (5/1/2008) - αDC1 vaccine ($2x10^6$ cells)
  - Cycle 3 (5/27/2008) - αDC1 vaccine ($1.8x10^6$ cells)

- Follow-up CT scans negative between 9/2008 and 10/2013

UPCI 12-110 (Bartlett): Combination Immunotherapy of *Advanced Peritoneal Cancer (Colon, Appendix, Meso)*



* shorter if clinically indicated

** resumed sooner if indicated (2 weeks after chemo)

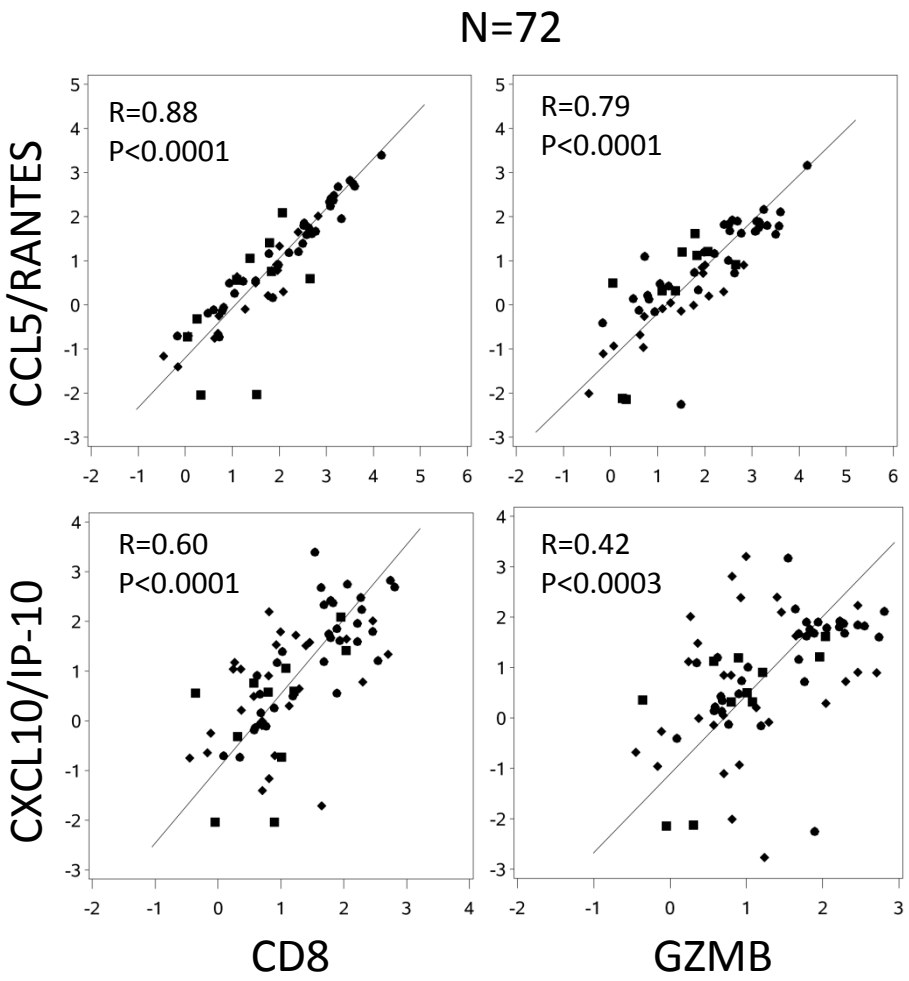
Treatment		
<u>Study cohort</u>	<u>αDC1 vaccine (i.n)</u>	Tumor Conditioning (systemic)
Treatment cohort	6x 10^6 per course (Fridays before CKM)	IFNα +Ampligen +Celecoxib (Mon-Fri after Vx).
Historical Control	(-)	(-)

Endpoints: TTP, 6M PFS, OS, blood immunomonitoring

Surgical Stress and Immunosuppression

- Surgery leads to overproduction of immune suppressive cytokines, chemokines, and COX 2 activation
- These negatively impact tumor growth
- Using immune adjuvants combined with surgery may improve results

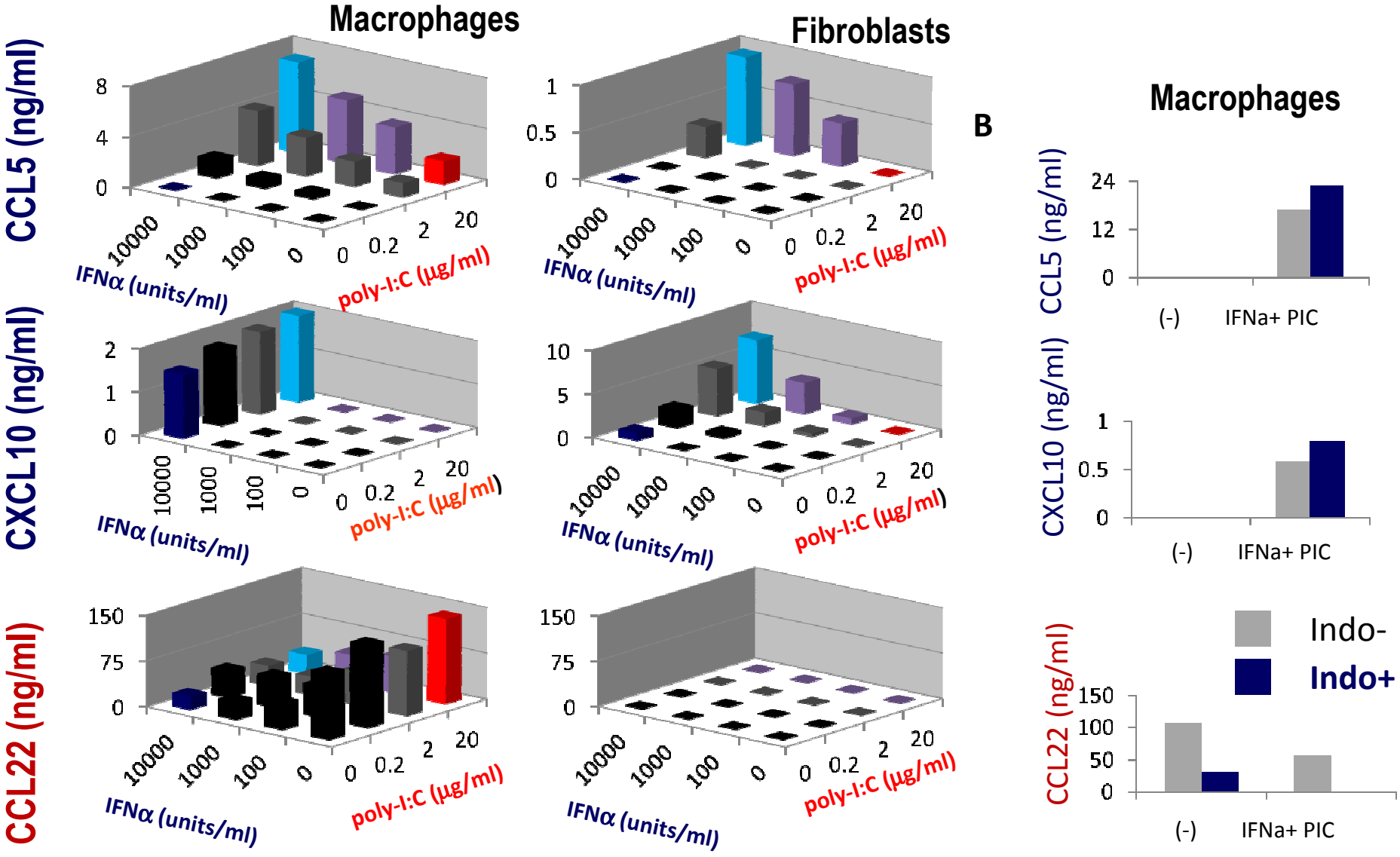
Role of Tumor Environment: Intra-Tumoral CXCL10 & CCL5 Levels Correlate with CTL Infiltration in Metastatic CRC



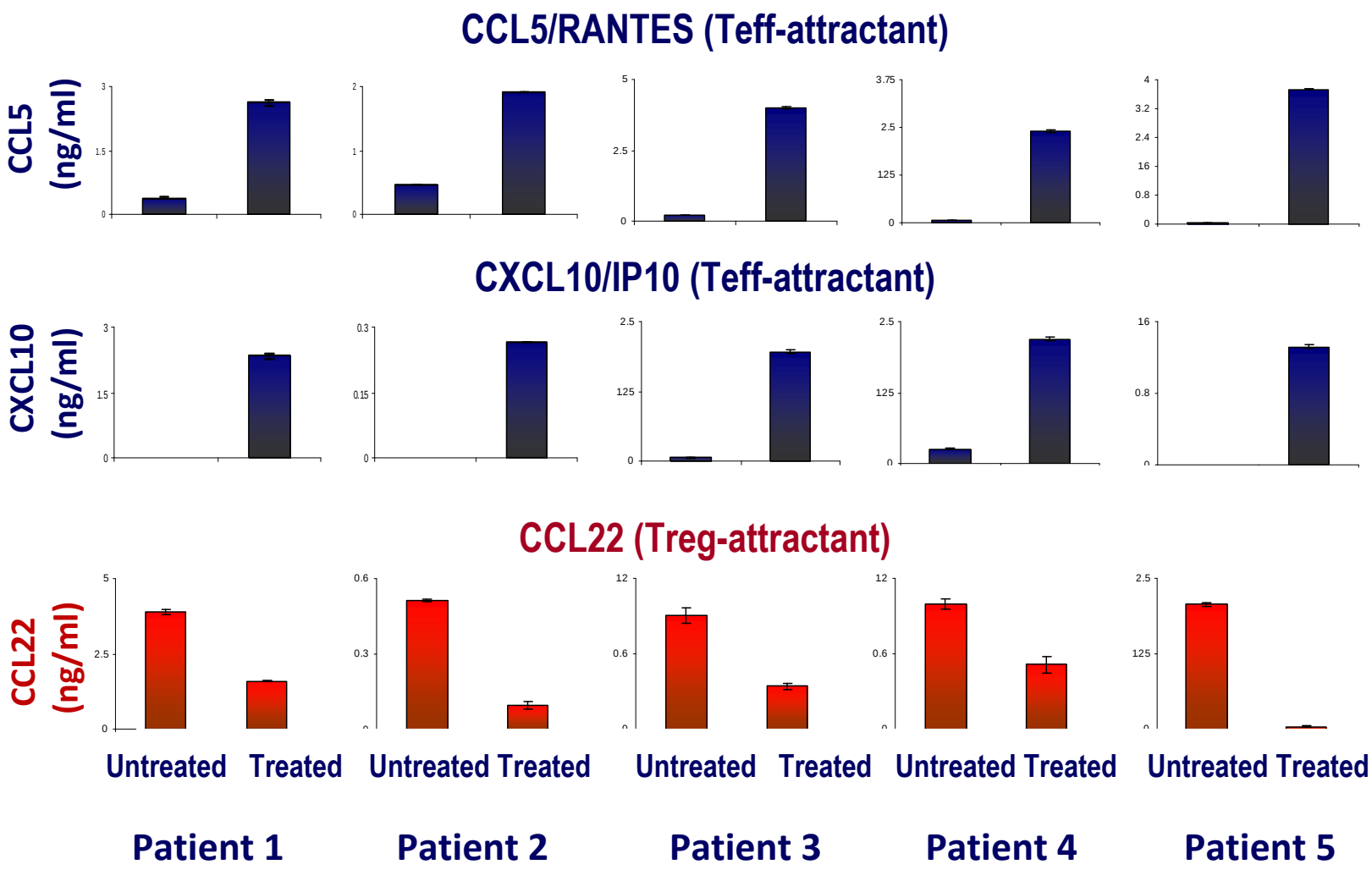
Muthuswamy , Bartlett, Zeh, Zureikat, Kalinski et al. 2012; Canc.Res. 272:3735

1P01 CA132714 (Kalinski-Bartlett-Okada): Directing Tumor-specific T Cells to Tumors

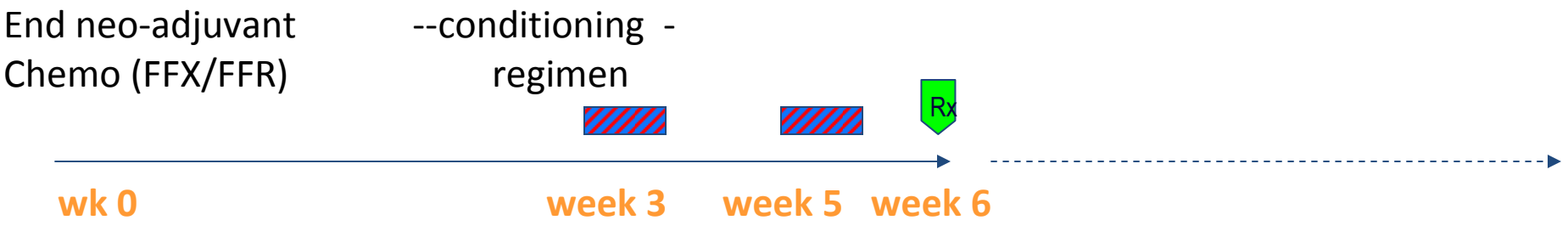
Combination of IFNα, Poly-I:C &COX2 Blockade Induces CTL-attracting CCL5&CXCL10, Blocks T_{reg}-attracting CCL22



Reproducibility of the Combinatorial Modulation of the Teff- & Treg-attracting CKs in Tumor Tissues



UPCI 10-131 (Zureikat): Neoadjuvant Immunotherapy of ***Resectable*** Recurrent CRC (IND 112532; accruing)



Treatment Groups		
<u>Study cohort</u>	<u>αDC1 vaccine</u>	Pharmacologic intervention
Group A	none	None (standard care only)
Group B	none	Ampligen+IFNα+ Celecoxib

Endpoints: **TIL density in resected tumors,** time to recurrence

Conclusions

- Targeted systemic therapy, radiation and surgery can function in concert with immunotherapy to enhance anti-tumor effect without increased toxicity
- A multimodal approach to therapy will be most effective