Combination Immune Therapy

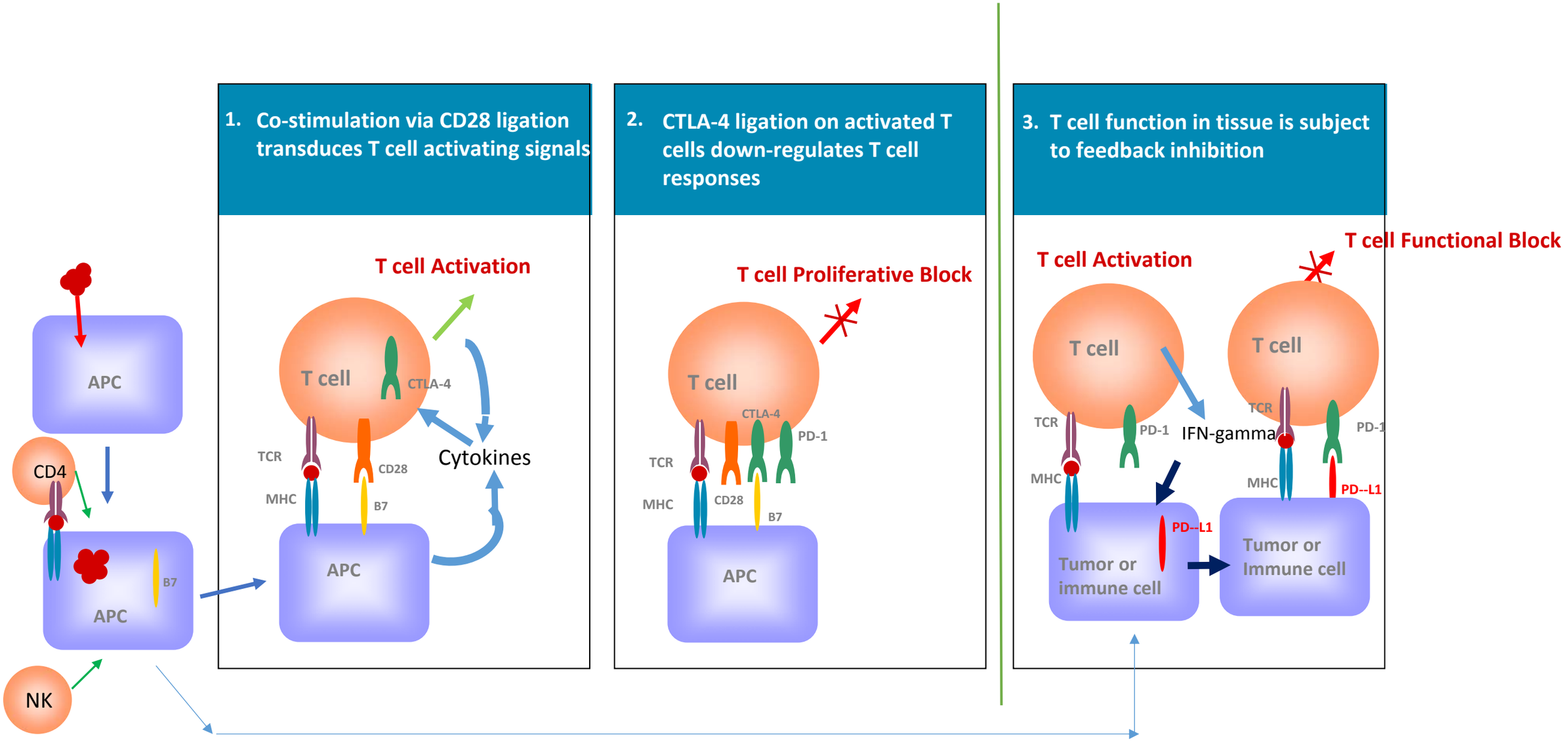
Translational Medicine Plenary

SWOG

April 17, 2017

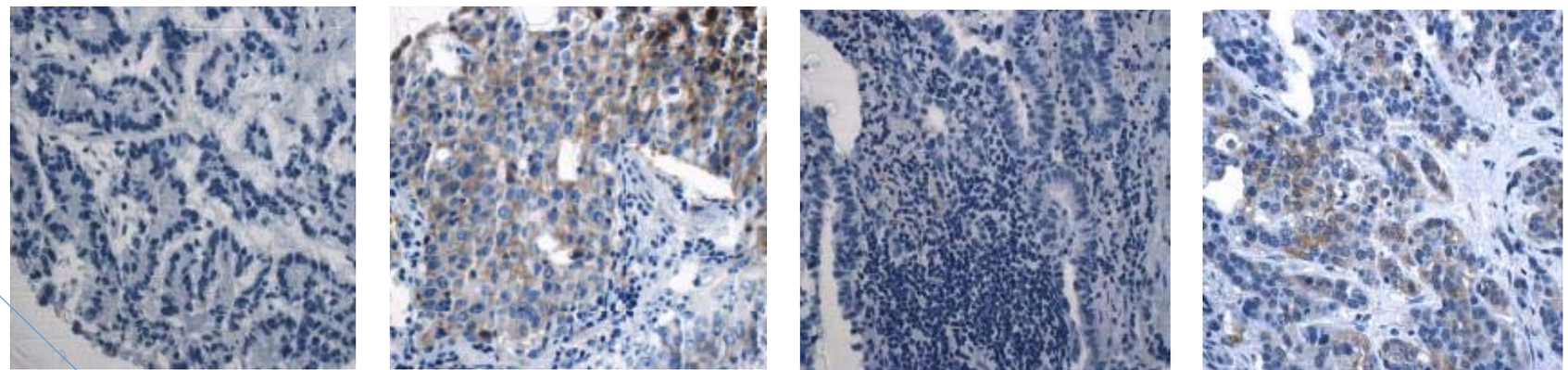
San Francisco

T-cell Activation, Proliferation, and Function is Controlled by Multiple Agonist and Antagonist Signals



Presence of PD-L1 or TILs[1]

NSCLC



Schalper and Rimm, Yale University

Melanoma

45% 41% 13% 1%

Taube et al

Table 2. Correlation of B7-H1 expression by melanocytes with the presence of immune cell infiltration.

Histology	Total	Number of cases/total cases (%)				*P**
		B7-H1⁺†		B7-H1⁻		
		TIL⁺‡	TIL⁻	TIL⁺	TIL⁻	
Benign nevi	40	14/14 (100)	0/14 (0)	4/26 (15)	22/26 (85)	<0.0001
Primary melanomas (in situ or invasive)	54	19/19 (100)	0/19 (0)	15/35 (43)	20/35 (57)	<0.0001
Metastases	56	23/24 (96)	1/24 (4)	7/32 (22)	25/32 (78)	<0.0001
All	150	56/57 (98)	1/57 (2)	26/93 (28)	67/93 (72)	<0.0001

*Fisher's exact test, two-sided, was conducted on the 2 × 2 matrix defined by B7-H1 (±) expression and TIL (±) for each lesion type. †More than 5% melanocytes with membranous expression on IHC. ‡Including mild, moderate, and severe lymphocyte infiltrates and their associated histiocytes/macrophages.

Tumor-specific T cells are contained in the PD-1+ TIL population and are functional after in vitro culture

The Journal of Clinical Investigation http://www.jci.org Volume 124 Number 5 May 2014

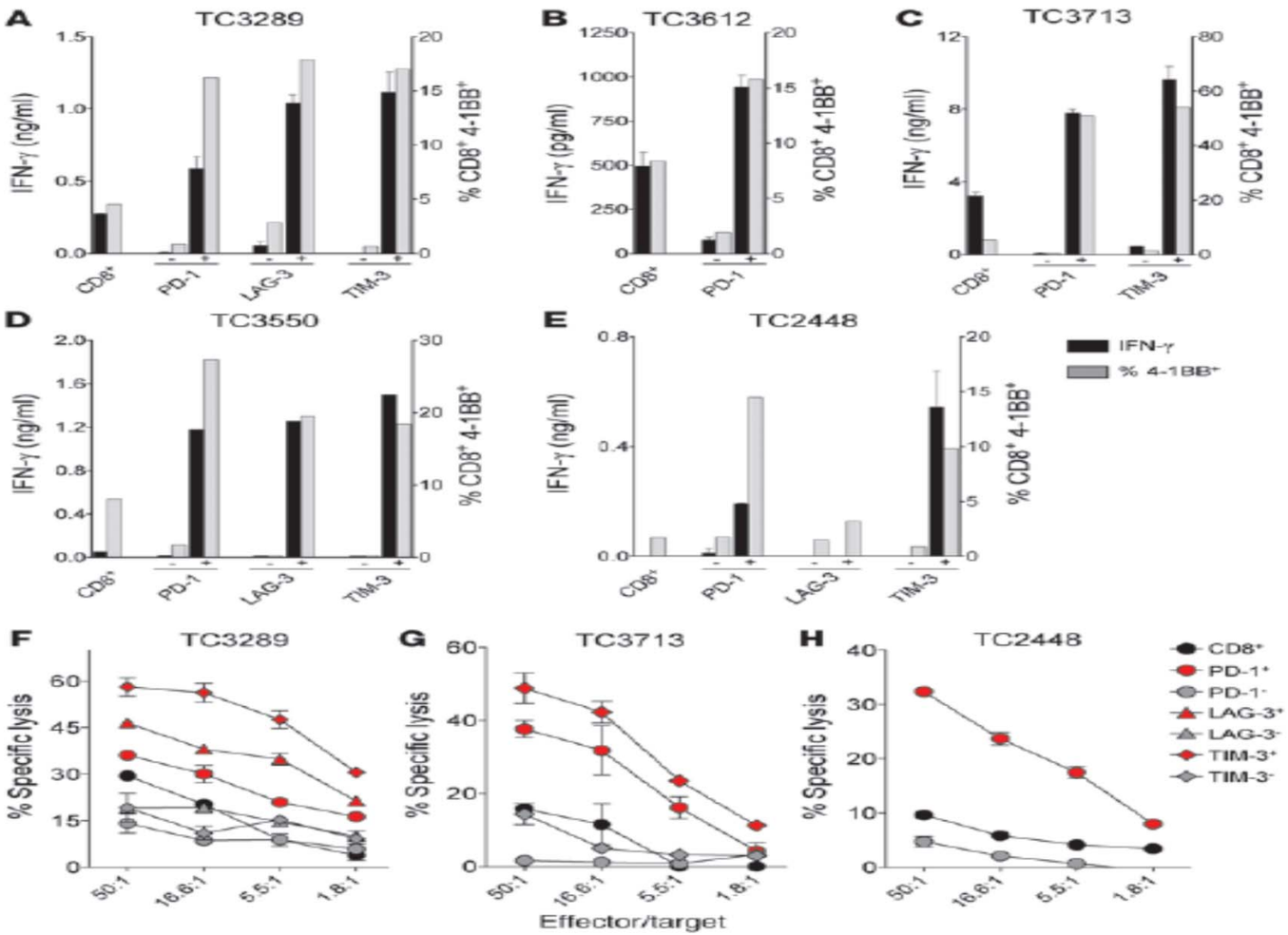


Figure 3
Recognition and lysis of autologous tumor by CD8+ TILs sorted based on PD-1, LAG-3, and TIM-3 expression. Bulk $CD3^+CD8^+$ TILs were sorted to high purity from FrTu3289, FrTu3612, FrTu3713, FrTu3550, and FrTu2448 based on positive or negative expression of PD-1, LAG-3 and/or TIM-3, and expanded in vitro for 15 days. (**A**–**E**) Response of fresh tumor–derived TILs to their respective autologous tumor cell lines, TC3289 (**A**), TC3612 (**B**), TC3713 (**C**), TC3550 (**D**) and TC2448 (**E**). Reactivity was assessed by measuring IFN-γ release (duplicates, mean ± SD) and frequency of 4-1BB upregulation. (**F**–**H**) Cytolytic activity of fresh tumor–derived TILs in response to their respective autologous tumor cell lines, TC3289 (**F**), TC3713 (**G**), and TC2448 (**H**). Percentage of specific lysis at different effector/target ratios is shown as mean ± SD.

Spectrum of PD-1/PD-L1 Antagonist Activity

Active

- **Melanoma**
- **Renal cancer (clear cell and non-clear cell)**
- **NSCLC – adenocarcinoma and squamous cell**
- Small cell lung cancer
- **Head and neck cancer**
- Gastric and gastroesophageal junction
- **MMR-repair deficient tumors (colon, cholangiocarcinoma)**
- Bladder
- Triple negative breast cancer
- Ovarian
- Hepatocellular carcinoma
- Thymoma
- Mesothelioma
- Cervical
- **Hodgkin lymphoma**
- Diffuse large cell lymphoma
- Follicular lymphoma
- T-cell lymphoma (cutaneous T-cell lymphomas, peripheral T-cell lymphoma)
- **Merkel cell**

Minimal to no activity

- Prostate cancer
- MMR+ (MSS) colon cancer
- Myeloma
- Pancreatic cancer

Major PD-1/PD-L1 antagonists

- Nivolumab (anti-PD-1)
- Pembrolizumab (anti-PD-1)
- Atezolizumab (MPDL3280, anti-PD-L1)
- Durvalumab (anti-PD-L1)
- Avelumab (anti-PD-L1)

Objective Response to anti-PD-1 by PD-L1 Expression Level (MERCK assay)

Figure 2. IHC score by percentage of PD-L1 expression as determined using the 22C3 antibody.

IHC score	0	1	2	3	4	5
Proportion of positive cells, %[1]	0	<1	≥1 - <10	≥10 - <33	≥33 - <66	≥66 - 100
PD-L1 expression status	Negative		Positive			

IHC = immunohistochemical; PD-L1 = programmed death ligand 1.
[1]Proportion of tumor and inflammatory cells positive for PD-L1 22C3 membrane staining.

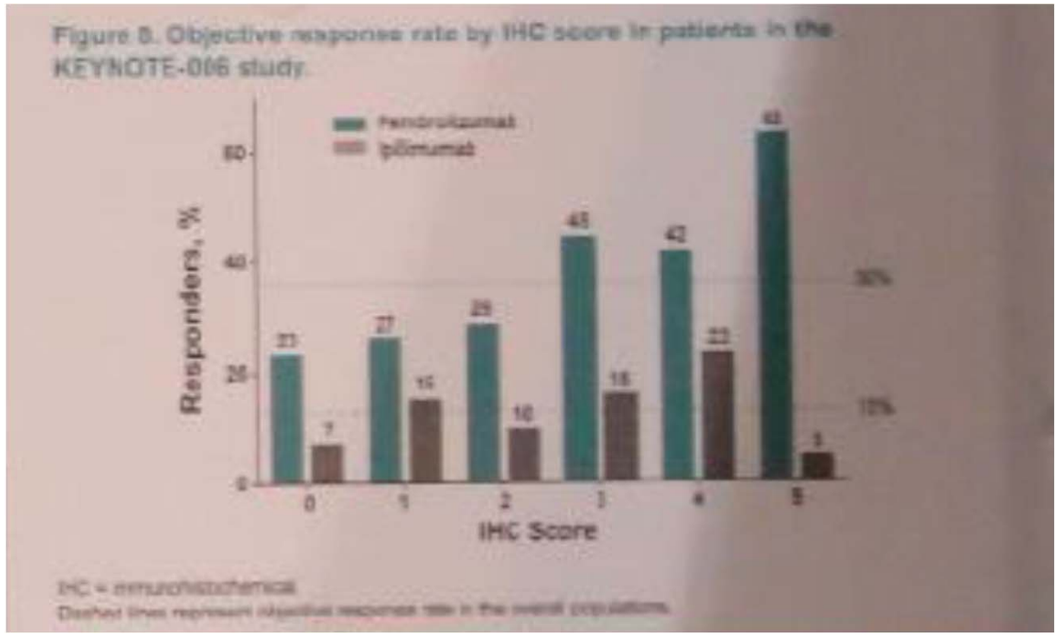
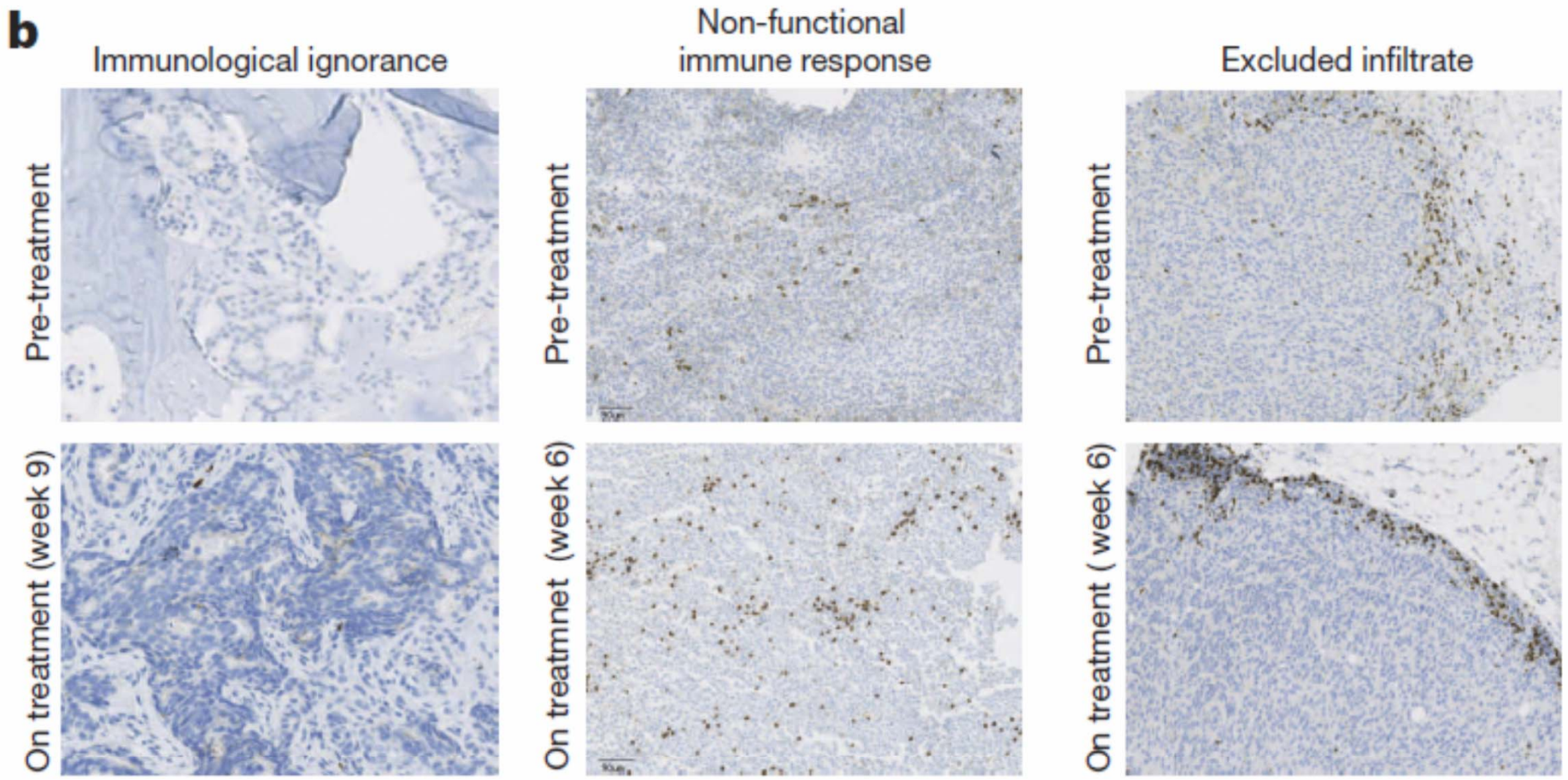


Figure 8. Objective response rate by IHC score in patients in the KEYNOTE-006 study.

IHC = immunohistochemical.
Dashed lines represent objective response rate in the overall population.

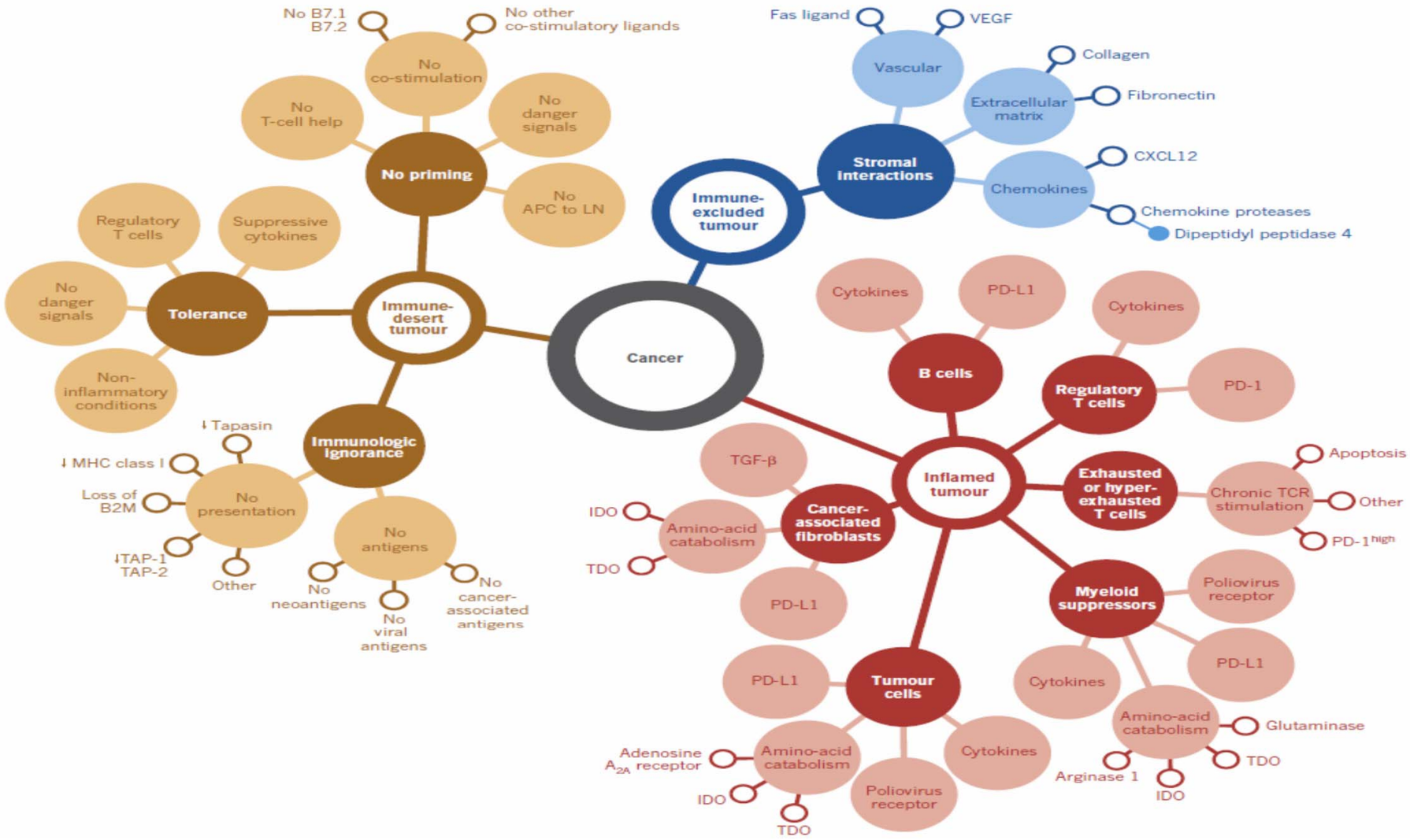
Dolled-Filhart M[1]; Toland G[2]; Stanforth D[2]; Roach C[2]; Jansson M[2]; Ebbinghaus S[1]; Emancipator K[1]
[1]Merck & Co., Inc., Kenilworth, NJ, USA; [2]Dako North America, Inc., Carpinteria, CA, USA

ASCO 2015



Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients

Roy S. Herbst[1], Jean-Charles Soria[2], Marcin Kowanetz[3], Gregg D. Fine[3], Omid Hamid[4], Michael S. Gordon[5], Jeffery A. Sosman[6], David F. McDermott[7], John D. Powderly[8], Scott N. Gettinger[1], Holbrook E. K. Kohrt[9], Leora Horn[10], Donald P. Lawrence[11], Sandra Rost[3], Maya Leabman[3], Yuanyuan Xiao[3], Ahmad Mokatrin[3], Hartmut Koeppen[3], Priti S. Hegde[3], Ira Mellman[3], Daniel S. Chen[3] & F. Stephen Hodi[12]



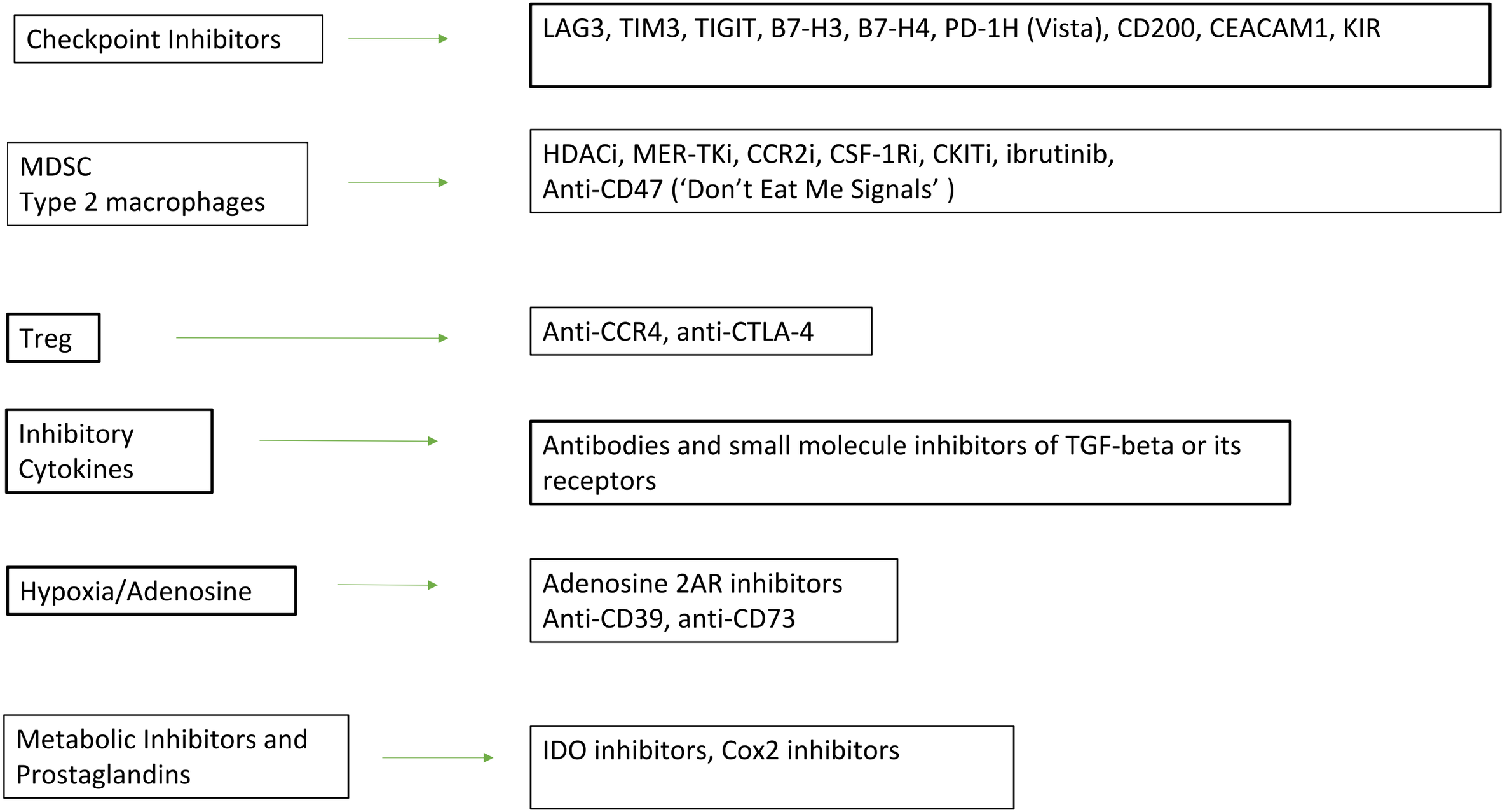
Elements of cancer immunity and the cancer–immune set point

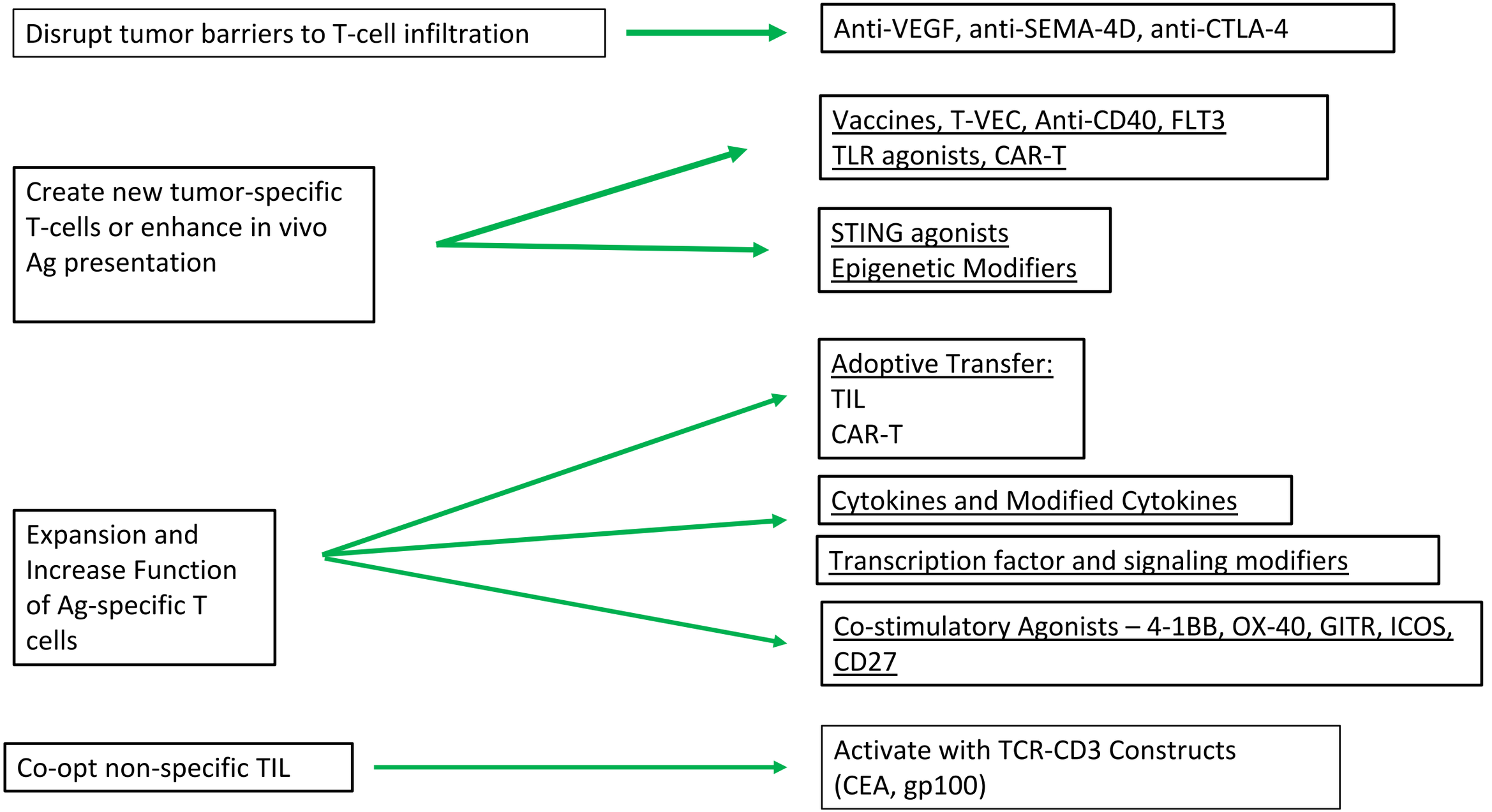
Daniel S. Chen[1] & Ira Mellman[1]

Antigen Presenting Cell or Tumor	T-lymphocyte	Function (excluding Treg)
Peptide-MHC	T cell receptor	Signal 1
CD80/CD86 (B7.1, B7.2)	CD28/CTLA-4	Stimulatory/inhibitory
CEACAM-1	CEACAM-1	inhibitory
CD70	CD27	stimulatory
LIGHT	HVEM	stimulatory
HVEM	BTLA, CD160	inhibitory
PD-L1 (B7-H1)	**PD-1** and CD80	Inhibitory (Th1)
PD-L2 (B7-DC)	PD1 and ?	Inhibitory (Th2) or stimulatory
OX40L	OX40	stimulatory
4-1BBL	CD137	stimulatory
CD40	CD40L	Stimulatory to DC/APC
B7-H3	?	Inhibitory or stimulatory
B7-H4	?	inhibitory
PD-1H (Vista)	?	inhibitory
GAL9	TIM-3	inhibitory
MHC class II	LAG-3	inhibitory
B7RP1	ICOS	stimulatory
MHC class I	KIR	Inhibitory or stimulatory
GITRL	GITR	stimulatory
CD48	2B4 (CD244)	inhibitory
HLA-G, HLA-E	ILT2, ILT4; NKG2a	inhibitory
MICA/B, ULBP-1, -2, -3, and -4+-	NKG2D	Inhibitory or stimulatory
CD200	CD200R	inhibitory
CD155	TIGIT/CD226	Inhibitory/stimulatory

Other Inhibitory Factors

IDO
Arginase
Treg
MDSC
Macrophages
TGF-beta
IL-10?
VEGF
Adenosine

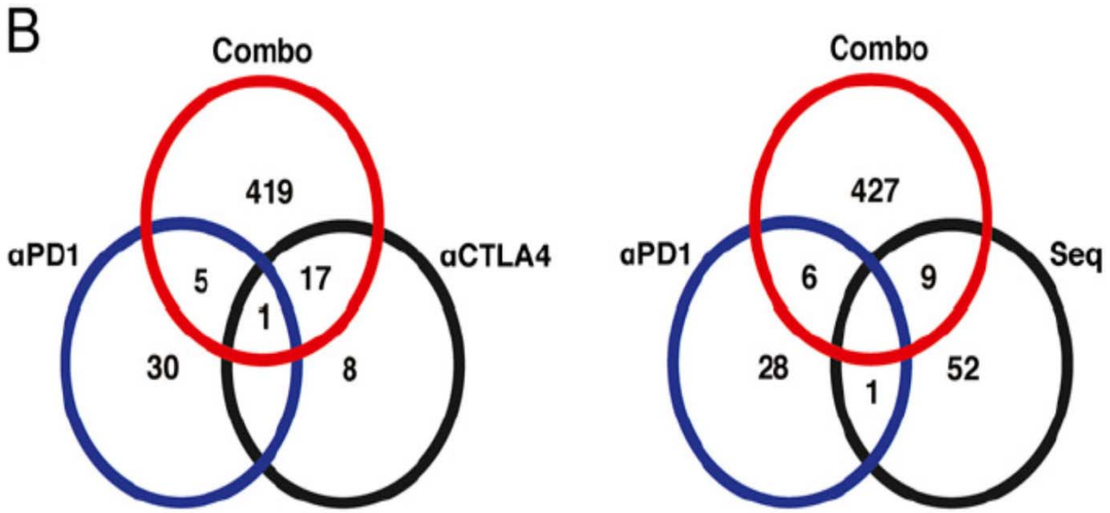

Checkpoint Inhibitors
LAG3, TIM3, TIGIT, B7-H3, B7-H4, PD-1H (Vista), CD200, CEACAM1, KIR
MDSC
Type 2 macrophages
HDACi, MER-TKi, CCR2i, CSF-1Ri, CKITi, ibrutinib,
Anti-CD47 (‘Don’t Eat Me Signals’)
Treg
Anti-CCR4, anti-CTLA-4
Inhibitory
Cytokines
Antibodies and small molecule inhibitors of TGF-beta or its receptors
Hypoxia/Adenosine
Adenosine 2AR inhibitors
Anti-CD39, anti-CD73
Metabolic Inhibitors and
Prostaglandins
IDO inhibitors, Cox2 inhibitors


Disrupt tumor barriers to T-cell infiltration
Anti-VEGF, anti-SEMA-4D, anti-CTLA-4
Create new tumor-specific T-cells or enhance in vivo Ag presentation
Vaccines, T-VEC, Anti-CD40, FLT3
TLR agonists, CAR-T
STING agonists
Epigenetic Modifiers
Expansion and Increase Function of Ag-specific T cells
Adoptive Transfer:
TIL
CAR-T
Cytokines and Modified Cytokines
Transcription factor and signaling modifiers
Co-stimulatory Agonists – 4-1BB, OX-40, GITR, ICOS, CD27
Co-opt non-specific TIL
Activate with TCR-CD3 Constructs
(CEA, gp100)

Combination Therapy with Anti–CTLA-4 and Anti–PD-1 Leads to Distinct Immunologic Changes In Vivo

Rituparna Das, Rakesh Verma, Mario Sznol, Chandra Sekhar Boddupalli, Scott N. Gettinger, Harriet Kluger, Margaret Callahan, Jedd D. Wolchok, Ruth Halaban, Madhav V. Dhodapkar and Kavita M. Dhodapkar

J Immunol 2015; 194:950-959; Prepublished online 24 December 2014;
doi: 10.4049/jimmunol.1401686
http://www.jimmunol.org/content/194/3/950



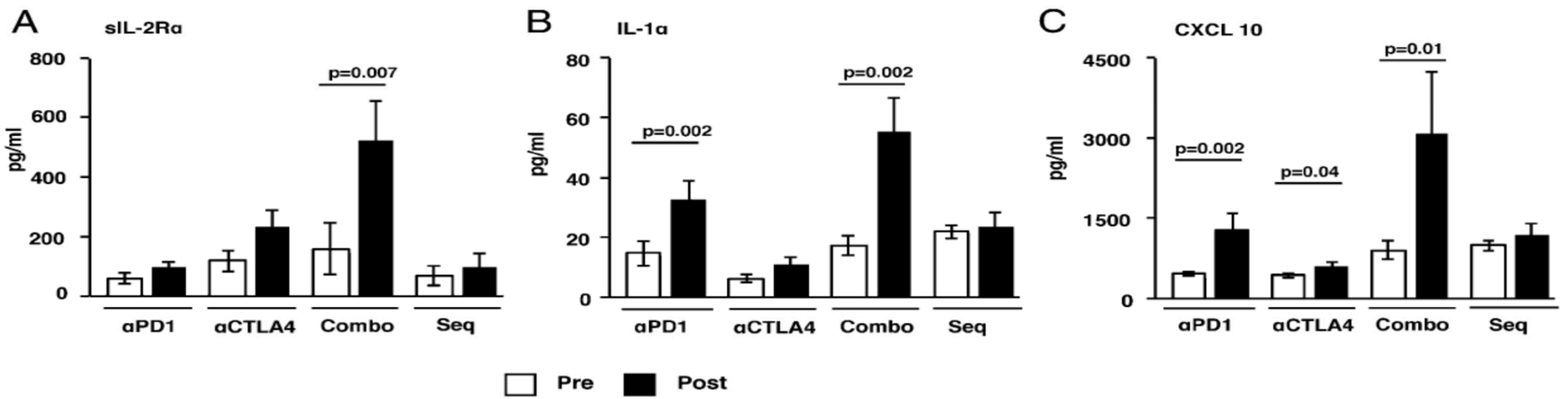
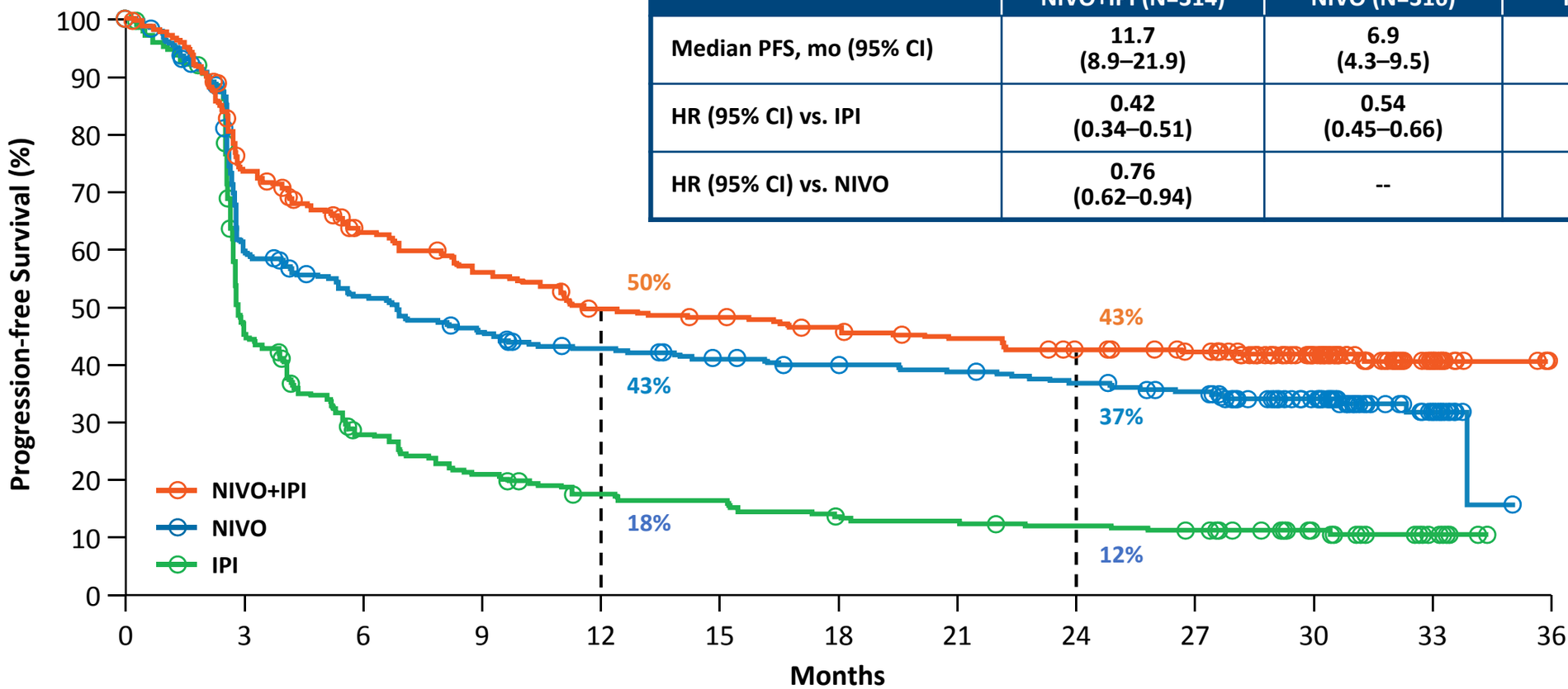


FIGURE 3. Changes in plasma chemokine and cytokines of patients treated with checkpoint blockade inhibitors. Plasma collected before and after therapy with anti–PD-1, anti–CTLA-4, Combo therapy, as well as Seq therapy was analyzed for presence of cytokines and chemokines using 39-plex luminex assay. All samples were tested in duplicate. Figure shows data for levels of cytokines and chemokines (mean and SEM) that were differentially secreted. (**A**) sIL-2Rα levels, (**B**) IL-1α levels, and (**C**) CXCL10/IP10 levels in plasma of patients pretherapy and posttherapy.

CA209-067: Updated Progression-Free Survival, Larkin et al, AACR 2017

	NIVO+IPI (N=314)	NIVO (N=316)	IPI (N=315)
Median PFS, mo (95% CI)	11.7 (8.9–21.9)	6.9 (4.3–9.5)	2.9 (2.8–3.2)
HR (95% CI) vs. IPI	0.42 (0.34–0.51)	0.54 (0.45–0.66)	--
HR (95% CI) vs. NIVO	0.76 (0.62–0.94)	--	--



Patients at risk:

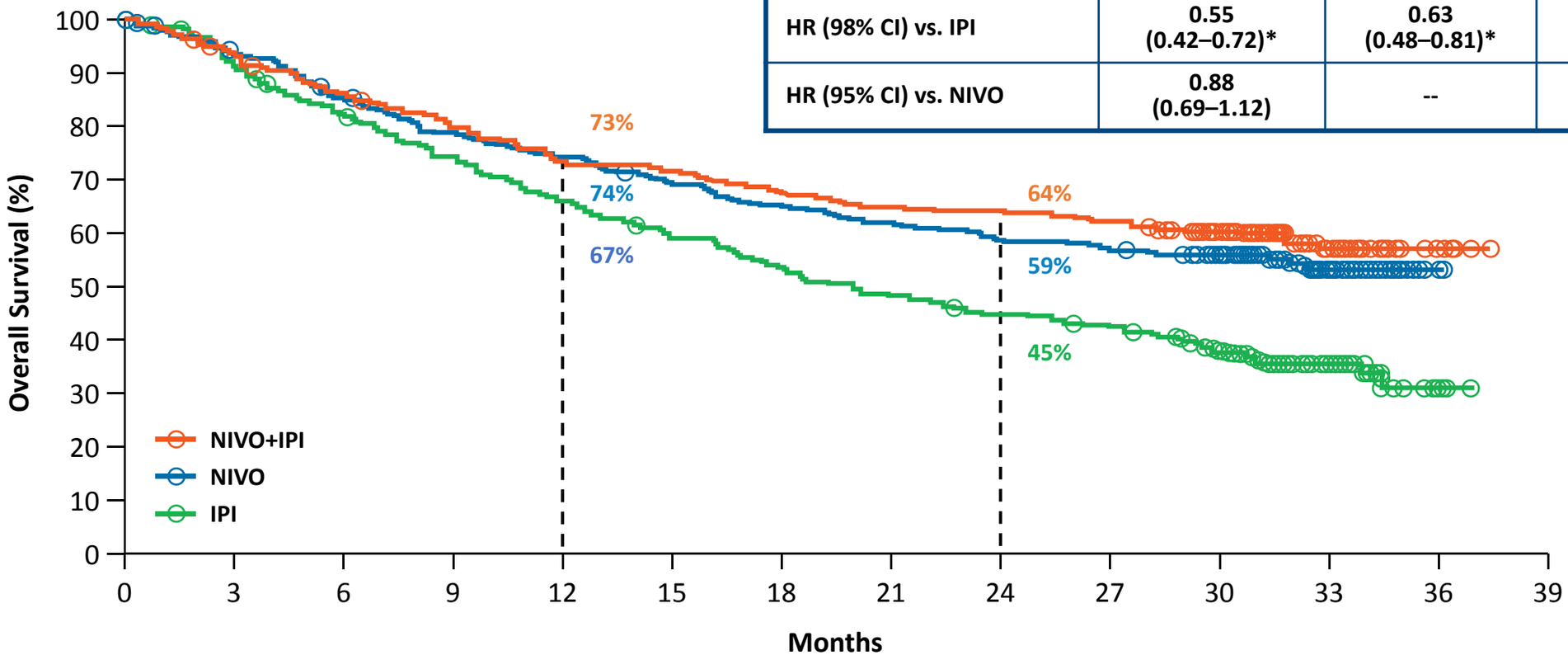
NIVO+ IPI	314	218	176	156	137	132	125	118	110	104	71	16	0
NIVO	316	178	151	132	120	112	107	103	97	88	62	16	0
IPI	315	136	77	58	46	43	35	33	30	27	16	5	0

Database lock: Sept 13, 2016, minimum f/u of 28 months

CA209-067:Overall Survival, Larkin et al, AACR 2017

	NIVO+IPI (N=314)	NIVO (N=316)	IPI (N=315)
Median OS, mo (95% CI)	NR	NR (29.1–NR)	20.0 (17.1–24.6)
HR (98% CI) vs. IPI	0.55 (0.42–0.72)*	0.63 (0.48–0.81)*	--
HR (95% CI) vs. NIVO	0.88 (0.69–1.12)	--	--

*$P<0.0001$



Patients at risk:

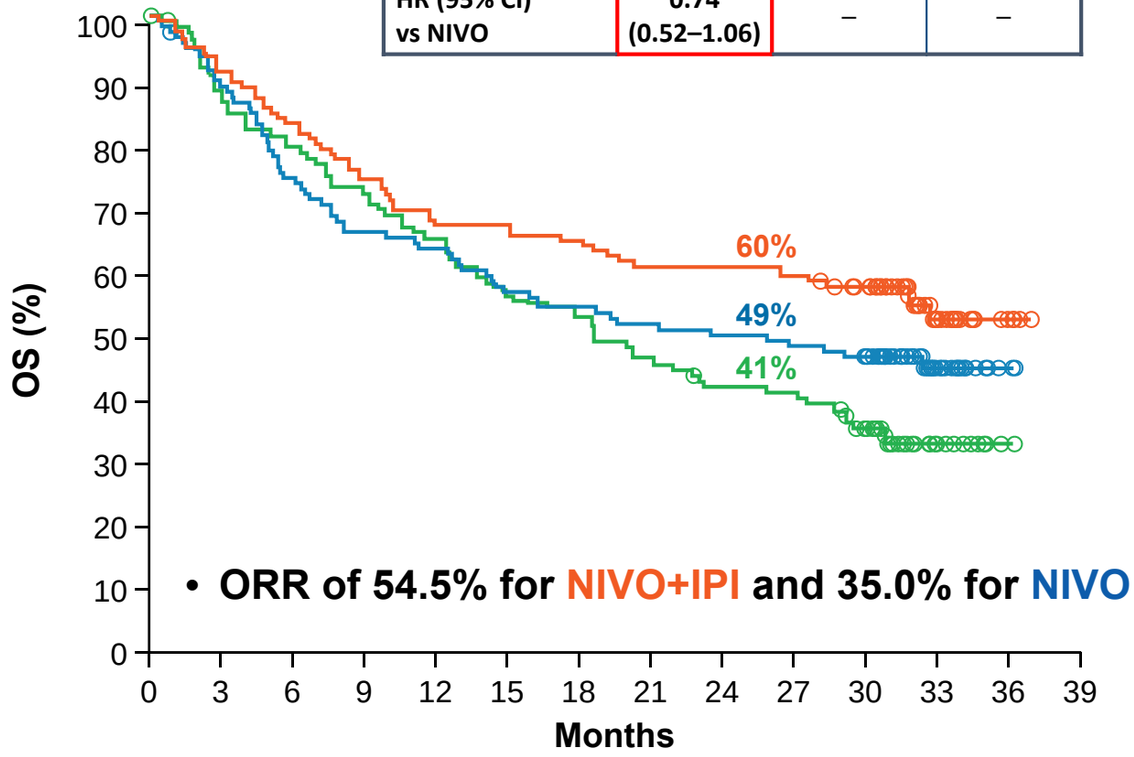
NIVO+IPI	314	292	265	247	226	221	209	200	198	192	170	49	7	0
NIVO	316	292	265	244	230	213	201	191	181	175	157	55	3	0
IPI	315	285	254	228	205	182	164	149	136	129	104	34	4	0

Database lock: Sept 13, 2016, minimum f/u of 28 months

CA209-067:Overall Survival, Larkin et al, AACR 2017

PD-L1 Expression Level <1%

<1% PD-L1	NIVO+IPI	NIVO	IPI
Median OS, mo (95% CI)	NR (26.5–NR)	23.5 (13.0–NR)	18.6 (13.7–23.2)
HR (95% CI) vs NIVO	0.74 (0.52–1.06)	–	–

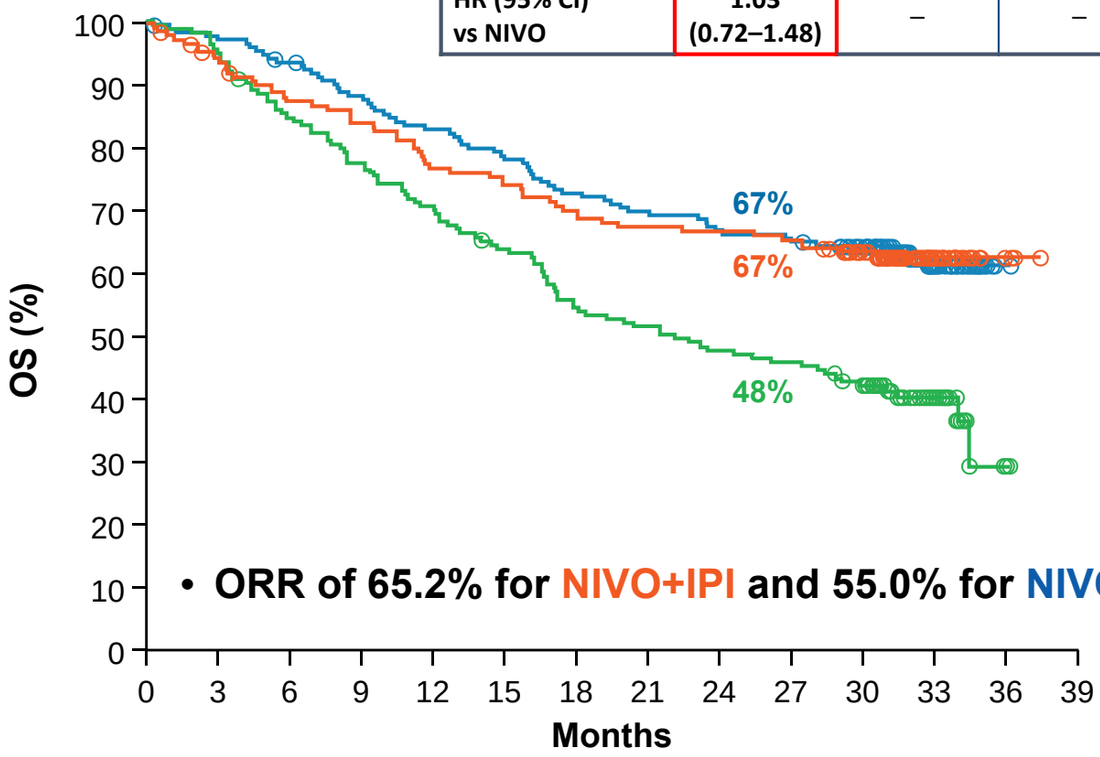


Patients at risk:

NIVO+IPI	123	113	102	91	82	82	79	74	74	72	66	18	4	0
NIVO	117	103	86	76	73	65	62	59	57	55	50	16	2	0
IPI	113	96	87	79	71	61	57	50	44	43	32	10	1	0

PD-L1 Expression Level ≥1%

≥1% PD-L1	NIVO+IPI	NIVO	IPI
Median OS, mo (95% CI)	NR	NR	22.1 (17.1–29.7)
HR (95% CI) vs NIVO	1.03 (0.72–1.48)	–	–



Patients at risk:

NIVO+IPI	155	144	132	127	116	112	105	102	101	99	85	27	3	0
NIVO	171	165	158	148	139	131	122	117	112	109	98	36	1	0
IPI	164	155	138	126	115	102	89	83	77	74	64	21	2	0

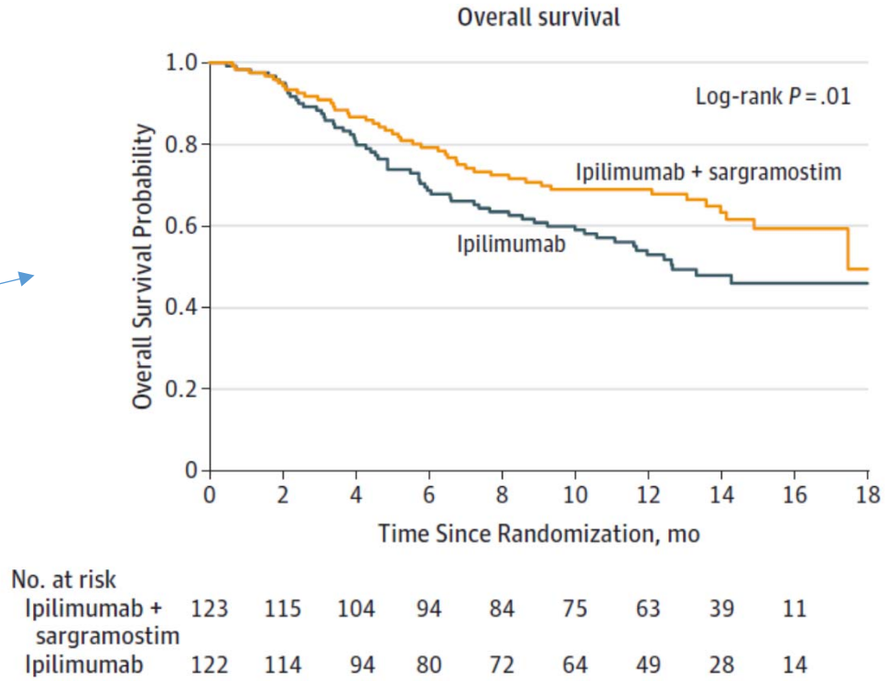
Positive Signals for Combinations (Phase 1-2)

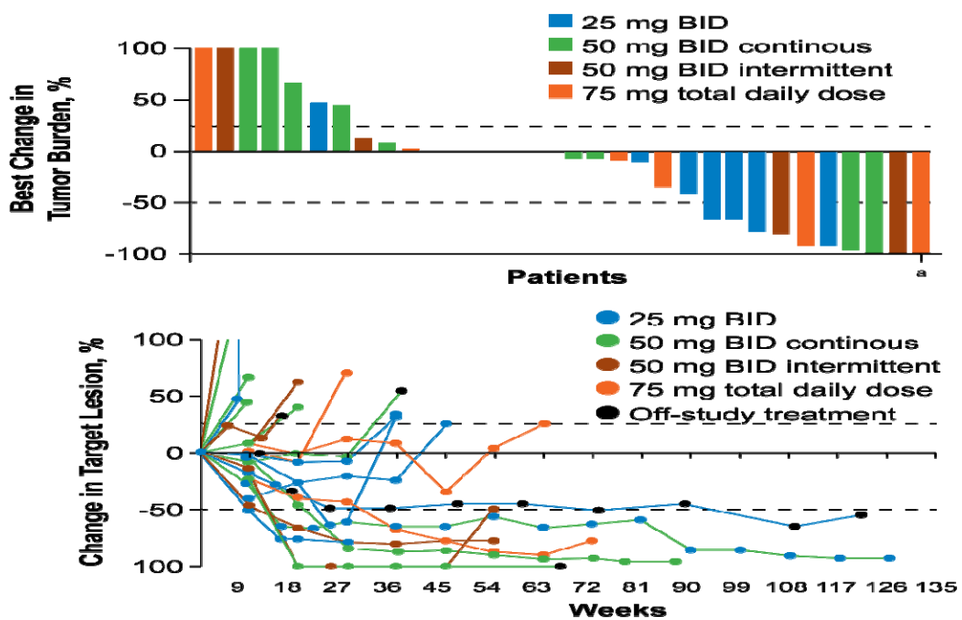
- Anti-CTLA-4 + Anti-PD-1 (melanoma, RCC, NSCLC, SCLC) (*prostate*)
- Anti-CTLA-4 + (GM-CSF, IFNa, IL-2, anti-VEGF, IDO, TVEC) – melanoma
- Pembro + TVEC – melanoma
- Pembro + IDOi - melanoma
- MEKi + atezo (anti-PD-L1) – MSS CRC and melanoma
- Anti-PD-1 + Anti-KIR (SCCHN)
- Anti-PD-1 + anti-CD137 (PD-L1? melanoma)
- Atezo + bevacizumab (anti-VEGF) - RCC
- VEGFRi (sunitinib, pazopanib, axitinib) + anti-PD-1 – RCC
- Anti-PD-1 + chemotherapy – NSCLC

RED = randomized trial

Promising anti-CTLA-4 Combinations

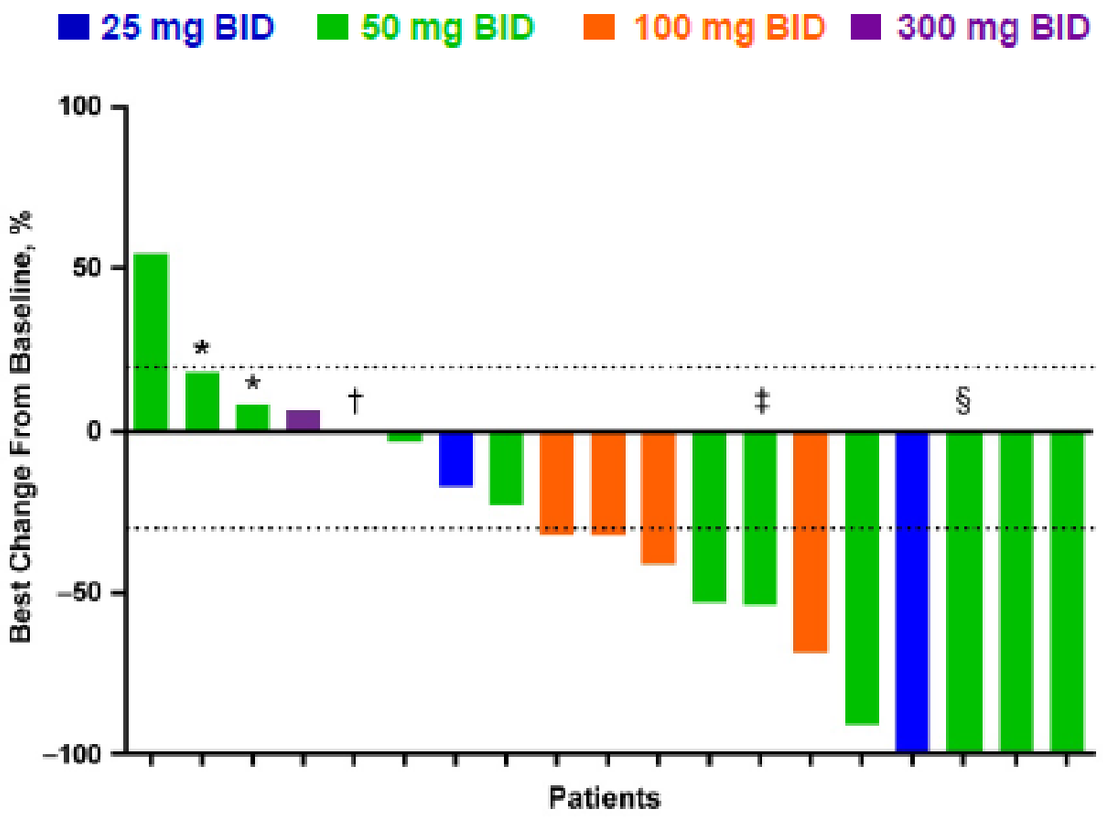
- GM-CSF (randomized trial)
- Bevacizumab (ORR- 19%, phase 2 median TTP - 9 months, OS- 25.1 months→ randomized trial)
- High dose IL-2 (phase 2)- ORR- 25%, OS- 16 months, 6 CR
- Interferon-alfa (phase 2) – ORR- 26%, PFS- 6.4 mths, OS- 21 months
- IDOi (phase 2) – 24% ORR by iRC
- T-VEC (phase 2) -
- RT (local effects)





Epacadostat + Pembrolizumab, Hamid et al, SMR 2015

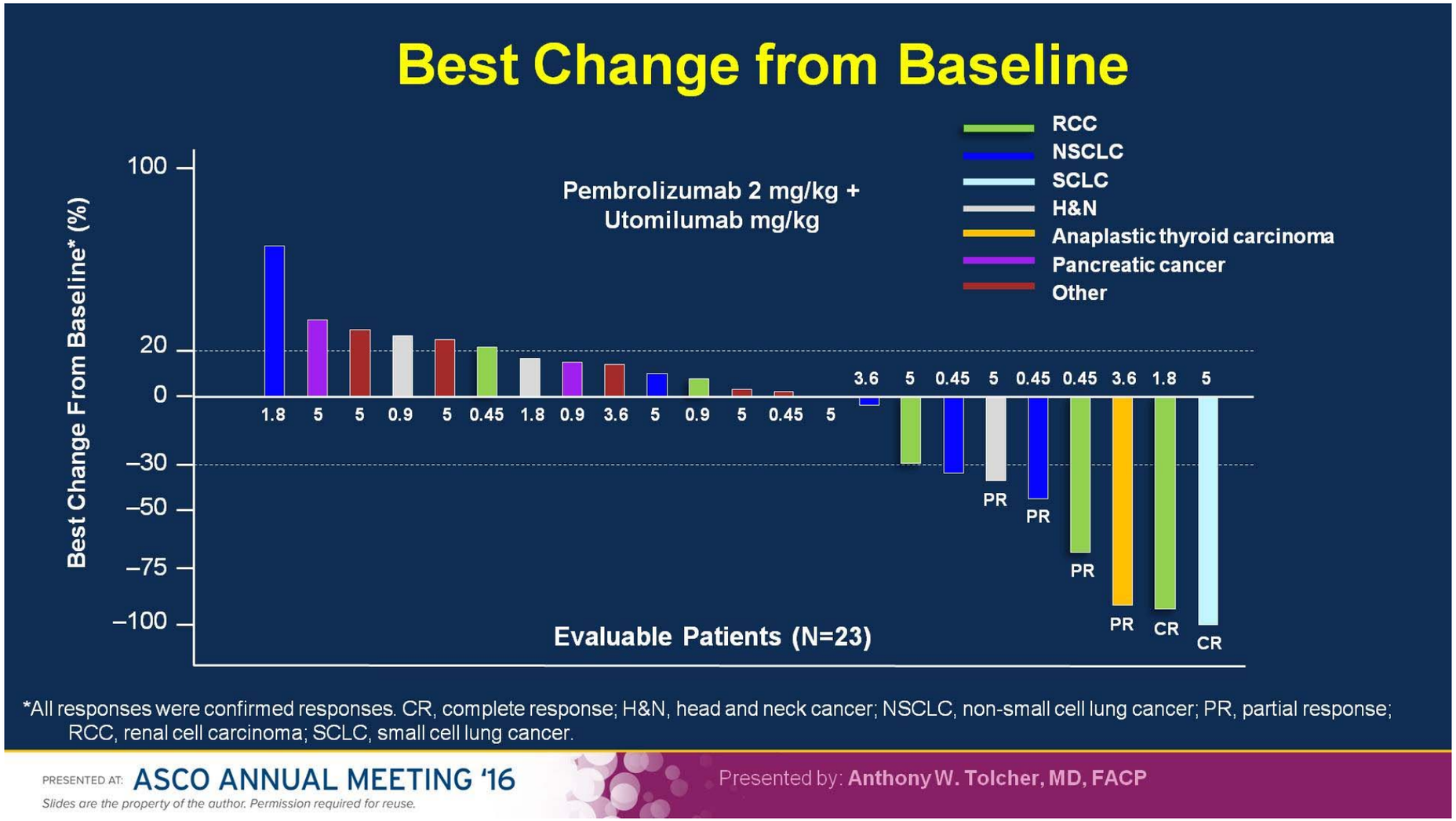
Best Percent Change From Baseline in Target Lesions: Melanoma



ORR = 53% in 19 patients

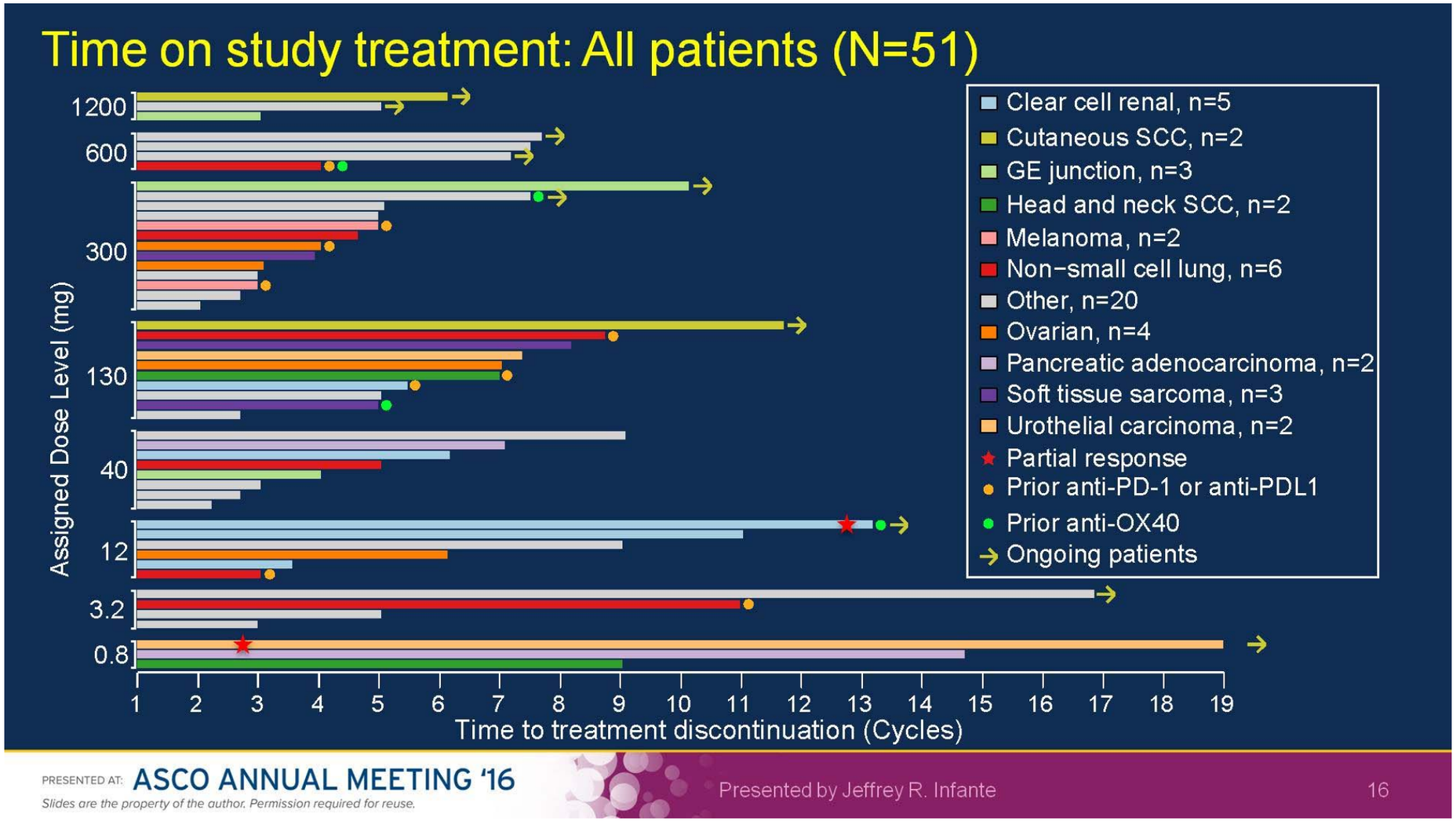
*Overall response is PD (SD for target lesions; PD for nontarget lesions).
†Overall response is PD (target lesions not assessed; PD per new lesions).
‡Overall response is PD (PR for target lesions; PD per new lesions).
§Overall response is PR (CR for target lesions; non-CR/non-PD for nontarget lesions).

Phase 1 anti-CD137 + Pembrolizumab



Presented By Anthony Tolcher at 2016 ASCO Annual Meeting

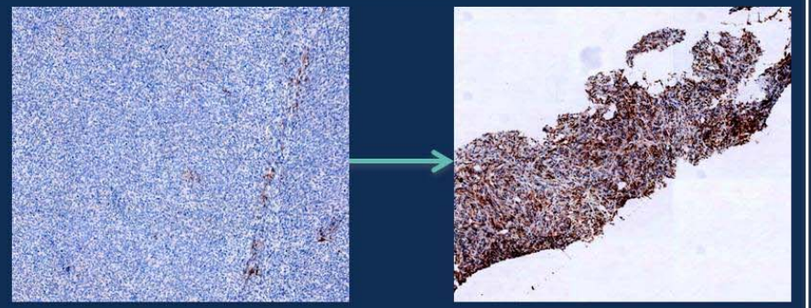
Phase 1 of Anti-OX40 + Atezolizumab



Presented By Jeffrey Infante at 2016 ASCO Annual Meeting

Evidence of PD-L1 induction in patients previously treated with single agent anti-PD-1

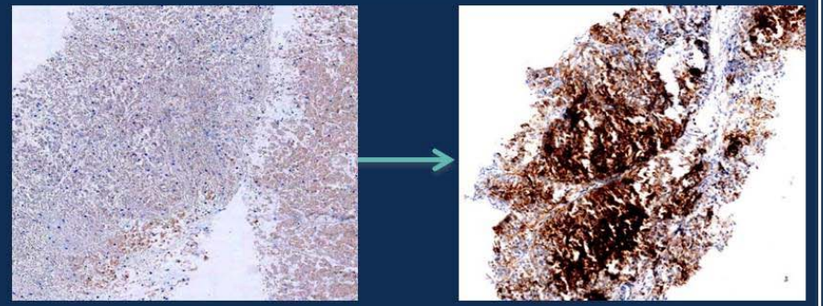
Melanoma #1



Baseline
(~8wk after last dose of pembrolizumab)

After 1 cycle MOXR
(300 mg) + atezo

Melanoma #2



Baseline
(~7wk after last dose of pembrolizumab)

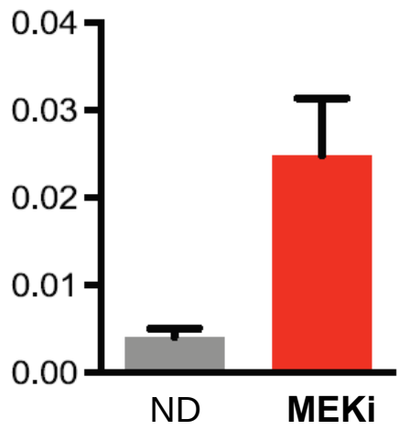
After 1 cycle MOXR
(300 mg) + atezo

Presented By Jeffrey Infante at 2016 ASCO Annual Meeting

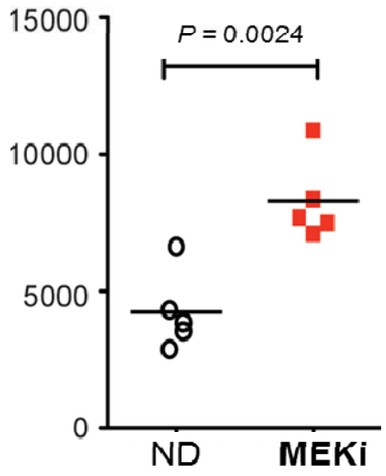
MEK and PD-L1 Inhibition: A Rational Combination

- MEK inhibition has a direct effect on T cells and the tumor microenvironment[1]
  - **Intratumoral T cell accumulation** and **class I MHC up-regulation**, leading to **synergy with PD-L1 inhibition** in CT26 syngeneic mouse model

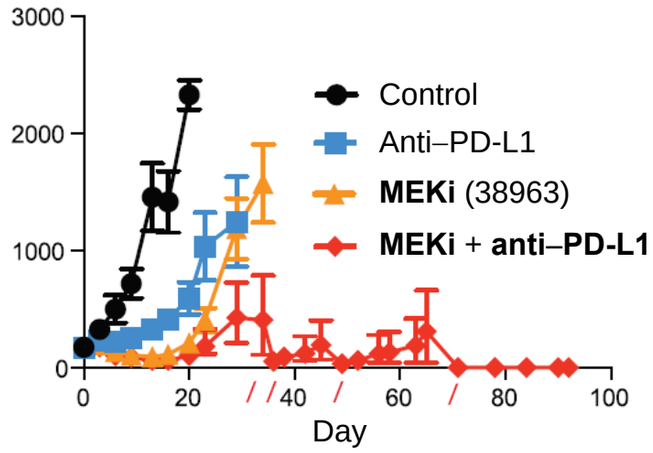
CD8+ T cell per Tumor Cell



Class I MHC



Tumor Volume (mm^3)



1. Ebert P et al. Immunity 2016.
MEKi, MEK inhibitor; ND, no drug (vehicle alone).

Cobimetinib + Atezolizumab

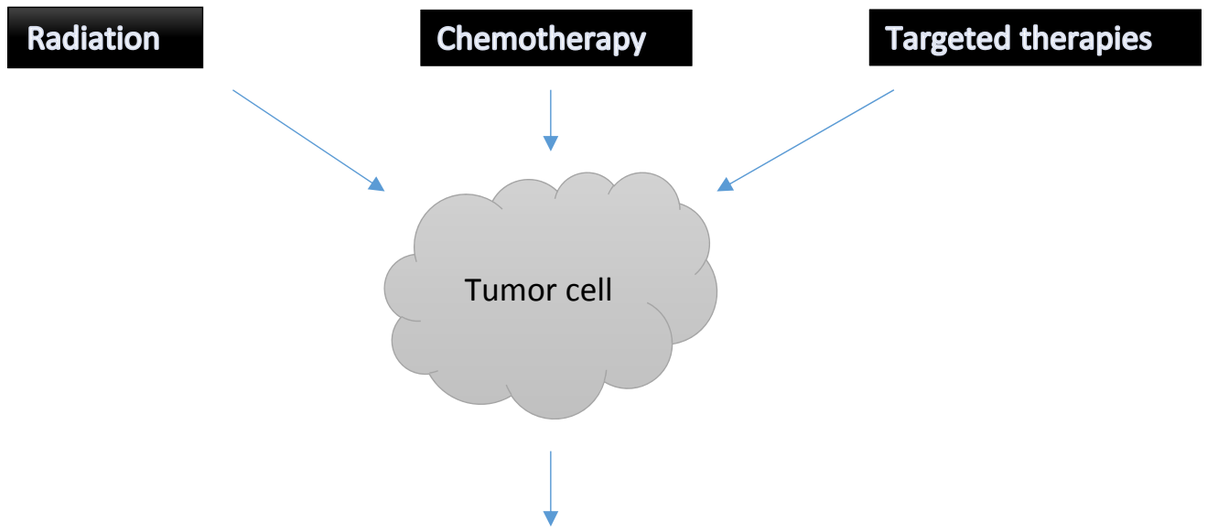
Efficacy: Confirmed Objective Response

Confirmed Response per RECIST v1.1	*KRAS* mutant CRC Cohort (n = 20)	All CRC Patients (N = 23)
ORR (95% CI)	20% (5.7, 43.7)	17% (5.0, 38.8)
PR	20%	17%
SD	20%	22%
PD	50%	52%
NE	10%	9%

- Response did not correlate with PD-L1 status: IC0 (n = 2), IC1 (n = 1) and IC3 (n = 1)

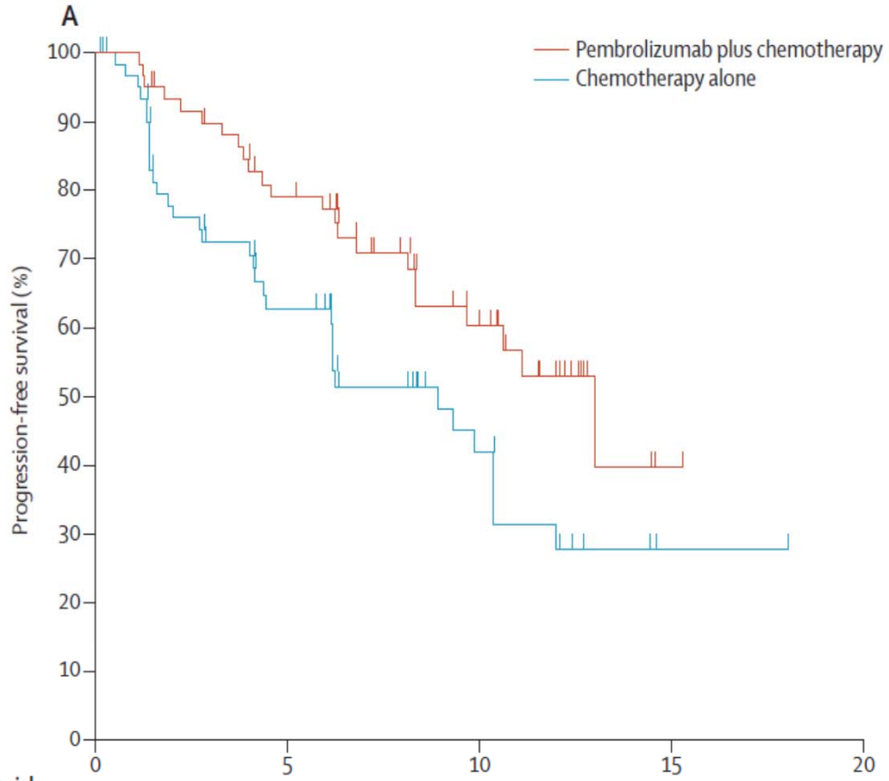
NE, not evaluable; ORR, overall response rate; PD, progressive disease; PR, partial response; SD, stable disease.
Efficacy-evaluable patients. Data cutoff, February 12, 2016.

Presented By Johanna Bendell at 2016 ASCO Annual Meeting

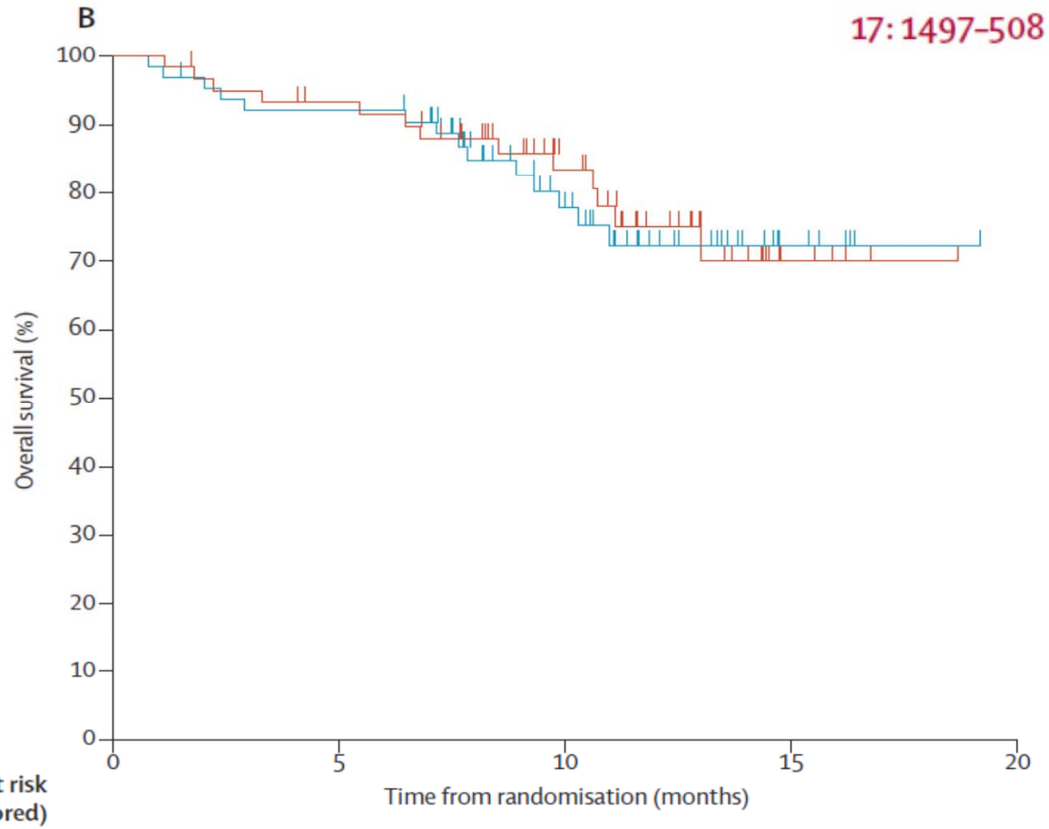


- Reduces Tumor bulk – Improves T-cell: tumor target ratio
- Separate mechanism of kill – 'synergize' with T-cell mechanism of killing
- Reduces T-cell inhibitory substances produced by tumor
- Alters tumor barriers (vasculature/pressure) to T-cell penetration
- Kills tumor cells in a manner that increases their recognition by T-cells and APC (vaccination)
- Alters T-cell signaling/gene expression to produce T-cell attractants

Lancet Oncol 2016;
17: 1497–508



Number at risk (number censored)					
Pembrolizumab plus chemotherapy	60 (0)	43 (5)	20 (20)	1 (36)	0 (37)
Chemotherapy	63 (0)	32 (10)	13 (21)	1 (29)	0 (30)



Number at risk (number censored)					
Pembrolizumab plus chemotherapy	60 (0)	53 (3)	33 (18)	5 (42)	0 (47)
Chemotherapy	63 (0)	57 (1)	31 (20)	6 (43)	0 (49)

Carboplatin and pemetrexed with or without pembrolizumab for advanced, non-squamous non-small-cell lung cancer: a randomised, phase 2 cohort of the open-label KEYNOTE-021 study

*Corey J Langer, Shirish M Gadgeel, Hossein Borghaei, Vassiliki A Papadimitrakopoulou, Amita Patnaik, Steven F Powell, Ryan D Gentzler, Renato G Martins, James P Stevenson, Shadia I Jalal, Amit Panwalkar, James Chih-Hsin Yang, Matthew Gubens, Lecia V Sequist, Mark M Awad, Joseph Fiore, Yang Ge, Harry Raftopoulos, Leena Gandhi, for the KEYNOTE-021 investigators**

Abstract # 410

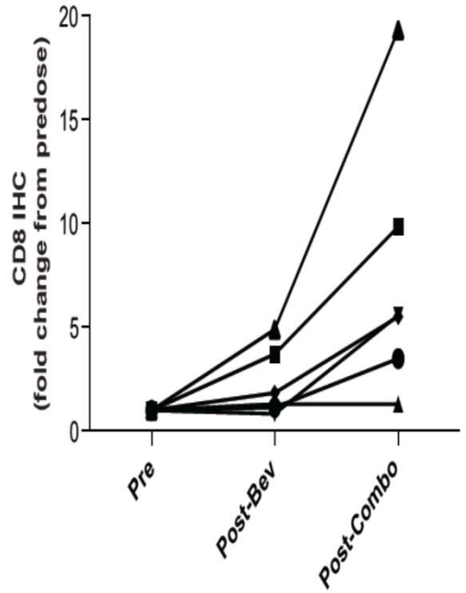
Phase 1b evaluation of MPDL3280A (anti-PDL1) in combination with bevacizumab (bev) in patients (pts) with metastatic renal cell carcinoma (mRCC)

Mario Sznol,[1] David F. McDermott,[2] Suzanne Jones,[3] James W. Mier,[2] Daniel Waterkamp,[4] Bo Liu,[4] Jeffrey Wallin,[4] Roel Funke,[4] Johanna Bendell[3]

[1]Yale Cancer Center, New Haven, CT, USA, [2]Beth Israel Deaconess Medical Center, Boston, MA, USA, [3]Sarah Cannon Research Institute, Nashville, TN, USA, [4]Genentech, Inc, South San Francisco, CA, USA

ASCO GU 2015

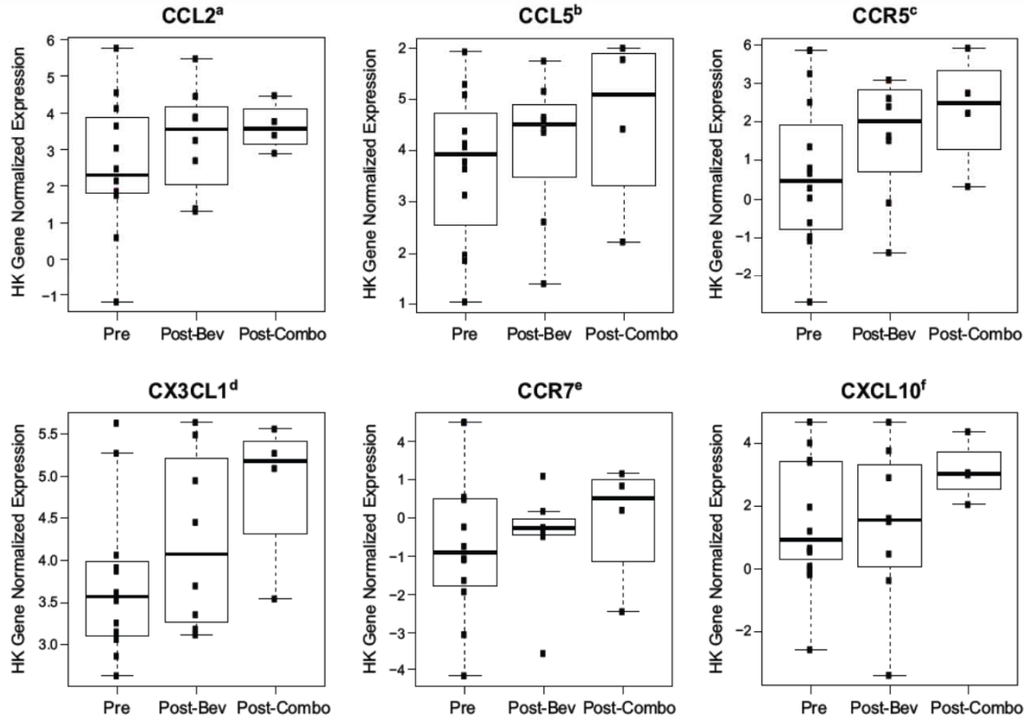
Figure 7. CD8 Staining in the Tumors of Patients With RCC After Treatment With MPDL3280A + Bevacizumab



IHC, immunohistochemistry.

- The increase in CD8+ cells was greatly enhanced in patients after treatment with MPDL3280A + bevacizumab

Figure 8. Chemokine Expression in the Tumors of Patients With RCC After Treatment With MPDL3280A + Bevacizumab



HK, housekeeping gene.

[a] CCL2 is generally produced by tissue injury or infection and serves as a chemoattractant for monocytes, T cells and dendritic cells.

[b] CCL5 is a chemoattractant for T cells, eosinophils and basophils.

[c] CCR5 is the receptor for CCL5.

[d] CX3CL1 is a potent chemoattractant for T cells and monocytes and is primarily expressed in endothelial cells.

[e] CCR7 is a chemoattractant for T cells and stimulates dendritic cell maturation.

[f] CXCL10 is secreted by monocytes, endothelial cells and fibroblasts in response to IFNγ and serves as a chemoattractant for immune cells.

Sunitinib or Pazopanib in Combination with Nivolumab

ASCO 2014

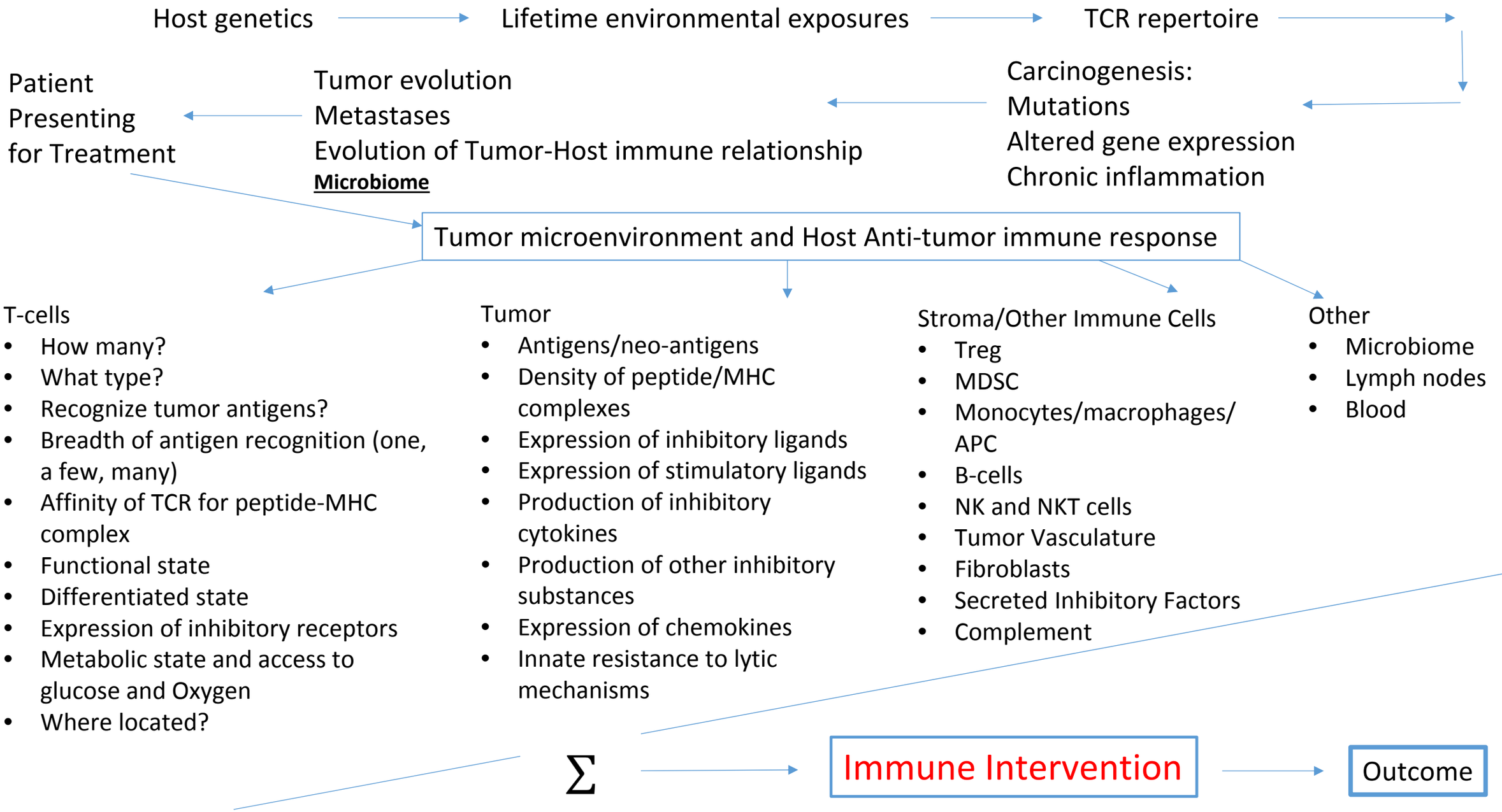
Antitumor activity (per RECIST 1.1)

	S + N (n=33)	P + N (n=20)
Confirmed ORR, n (%) 95% CI	17 (52) 33.5-69.2	9 (45) 23.1-68.5
Median duration of response, weeks (range)	37.1 (18.1-80+)[a]	30.1 (12.1-90.1+)[b]
Ongoing responses, % (n/N)	59 (10/17)	33 (3/9)
Best overall response, n (%) Complete response Partial response Stable disease Progressive disease Unable to determine	 1 (3) 16 (48) 10 (30) 1 (3) 4 (12)	 0 9 (45) 7 (35) 4 (20) 0

[a]Median follow-up 54.7 weeks; [b]Median follow-up 76.5 weeks.
Duration of response defined as time between date of first response and date of disease progression or death (whichever occurs first).
ORR, objective response rate.

11

Presented By Asim Amin at 2014 ASCO Annual Meeting


Host genetics
Lifetime environmental exposures
TCR repertoire
Carcinogenesis:
Mutations
Altered gene expression
Chronic inflammation
Tumor evolution
Metastases
Evolution of Tumor-Host immune relationship
Microbiome
Patient
Presenting
for Treatment
Tumor microenvironment and Host Anti-tumor immune response
T-cells
How many?
What type?
Recognize tumor antigens?
Breadth of antigen recognition (one, a few, many)
Affinity of TCR for peptide-MHC complex
Functional state
Differentiated state
Expression of inhibitory receptors
Metabolic state and access to glucose and Oxygen
Where located?
Tumor
Antigens/neo-antigens
Density of peptide/MHC complexes
Expression of inhibitory ligands
Expression of stimulatory ligands
Production of inhibitory cytokines
Production of other inhibitory substances
Expression of chemokines
Innate resistance to lytic mechanisms
Stroma/Other Immune Cells
Treg
MDSC
Monocytes/macrophages/ APC
B-cells
NK and NKT cells
Tumor Vasculature
Fibroblasts
Secreted Inhibitory Factors
Complement
Other
Microbiome
Lymph nodes
Blood
Σ
Immune Intervention
Outcome

Do Host* Factors Play a Role in in Response and Toxicity of anti-PD-1? Combined Analyses of Nivolumab in Metastatic Melanoma

Table 2. Impact of Treatment-Related Select AEs and IM Use on Response to Nivolumab Therapy

		Any-Grade Treatment-Related Select AEs*				Grade 3 to 4 Treatment-Related Select AEs		Patients Receiving Systemic IM	
	All Patients (N = 576)	Any (n = 255)	None (n = 321)	1-2 (n = 242)	≥ 3 (n = 13)	Yes (n = 18)	No (n = 558)	Yes (n = 114)	No (n = 462)
ORR, No. of patients (%)	181 (31.4)	124 (48.6)	57 (17.8)	113 (46.7)	11 (84.6)	5 (27.8)	176 (31.5)	34 (29.8)	147 (31.8)
95% CI	27.6 to 35.4	42.3 to 54.9	13.7 to 22.4	40.3 to 53.2	54.6 to 98.1	9.7 to 53.5	27.7 to 35.6	21.6 to 39.1	27.6 to 36.3
P		< .001		< .0001†	< .001†	1.00		.736	

Abbreviations: AE, adverse event; IM, immune-modulating agent; ORR, objective response rate.
*Data in these columns are for patients with the indicated numbers of any-grade treatment-related select AEs: any AE, no AEs, 1-2 AEs, and ≥ 3 AEs.
†Versus no treatment-related select AEs.

J Clin Oncol 35:785-792. © 2016 by American Society of Clinical Oncology

*Genetic, Tumor–Induced Systemic Immune Modulation, Other Systemic Immune Modulation

Conclusions

- Nearly infinite combination possibilities
- Not all combinations need to be based on immune checkpoint inhibitor
- Not all combinations with immune checkpoints need to be based on anti-PD-1
- Will be difficult to understand critical signals required for each individual patient (biomarkers)
- Different subsets of cells in tumor may require different signals
- For some targets (CD28, possibly CD40, CD3 agonists) specific delivery to tumor may be necessary
- Scheduling may be important because concurrent administration may have unexpected effects – may need prolonged exposure to ‘priming agent’
- Dose ratios for combinations are unclear – tumor concentrations to optimally block or stimulate unclear
- Toxicities could be very severe but most are manageable based on Ipilimumab/nivolumab experience
- Focus on critical and possibly non-redundant signals – PD-1/CTLA-4 (Treg)/4-1BB + OX-40/VEGF/hypoxia/IL-2/CD40, TLR, MDSC + monocyte-macrophages
- Downstream IFNg signaling in tumor cells may be important
- Modulation of host/systemic factors may be important

Dilemma for Clinical Development

- Complexity of biology and intra- and inter- patient heterogeneity →
  - Multiple mechanisms of resistance
  - May require combination of agents
- Many more ideas to test than available patients
- Cost of clinical trials is exceedingly high
- Must convincingly show benefit (survival, prolonged tumor regression, improvement in symptoms) for regulatory approval
  - Superior value for reimbursement
- But, without selection:
  - single arm trials to detect signal of activity will be unreliable (affecting only a small number of enrolled population)
  - Require large phase 3 randomized studies to provide definitive evidence of effect

Tumor antigen–specific CD8 T cells infiltrating the tumor express high levels of PD-1 and are functionally impaired

(Blood. 2009;114:1537-1544)

Mojgan Ahmadzadeh,[1] Laura A. Johnson,[1] Bianca Heemskerk,[1] John R. Wunderlich,[1] Mark E. Dudley,[1] Donald E. White,[1] and Steven A. Rosenberg[1]

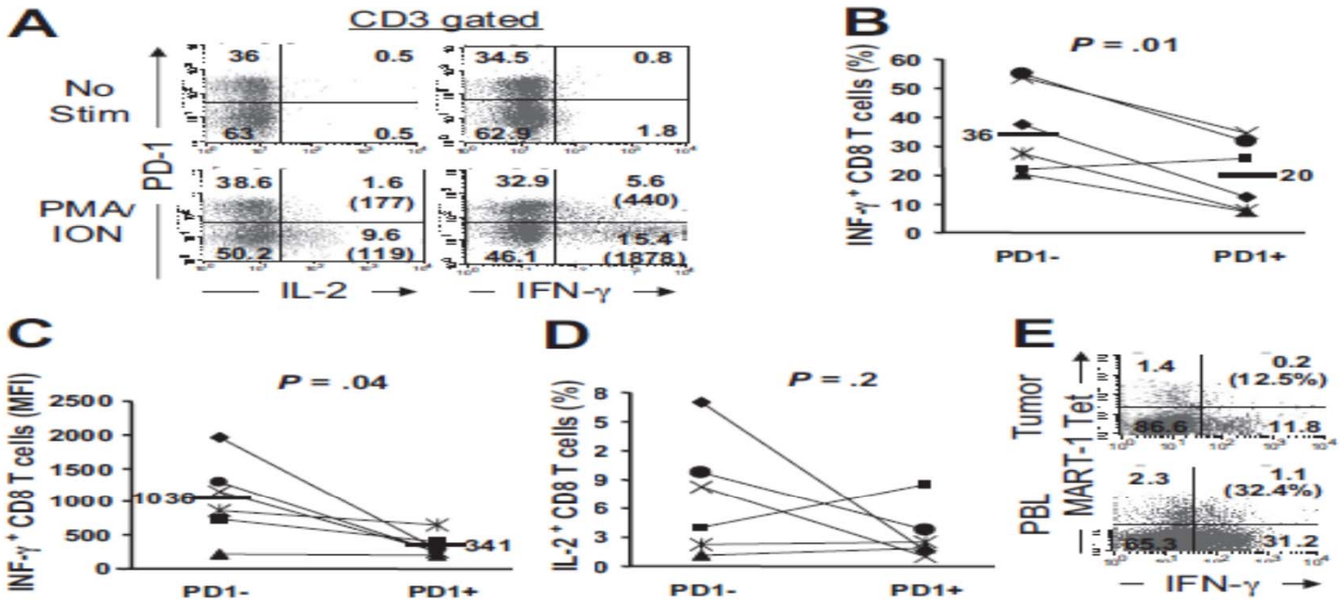
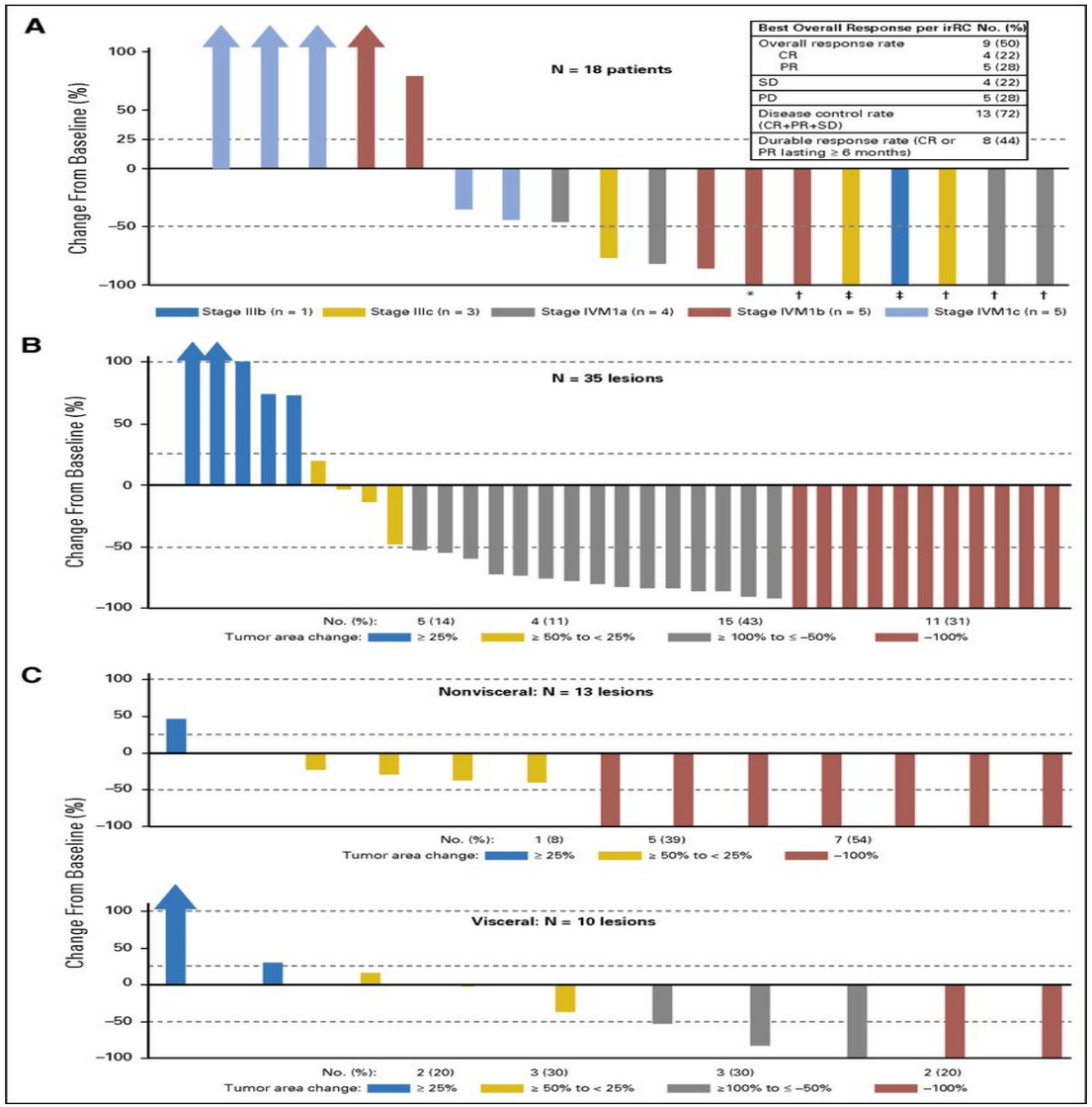


Figure 6. PD-1 expression on tumor-infiltrating T cells correlates with impaired effector function. Tumor digests and peripheral blood sample from patients with metastatic melanoma were thawed and immediately stimulated with PMA/I for 6 to 8 hours in the presence of monensin. Cells were subsequently stained with anti-CD3, anti-CD8, and anti–PD-1 mAb along with anti–IL-2 and anti–IFN-γ mAbs. (A) Dot plots were gated on $CD3^+$ T cells. The numbers represent the percentages of T cells in each quadrant and the value in parentheses represents the MFI for each quadrant. (B) The percentage of $CD3^+CD8^+$ T cells that were IFN-γ^+ is depicted for PD-1^+ and PD-1^- CD8 TILs. (C) The MFI for IFN-γ^+ $CD3^+CD8^+$ T cells are depicted for PD-1^+ and PD-1^- CD8 TILs. (D) The percentage of $CD3^+CD8^+$ T cells that were IL-2^+ is depicted for PD-1^+ and PD-1^- CD8 TILs for 6 patients. *P* values are calculated based on the paired *t* test. (E) IFN-γ production by MART-1 tetramer$^+$ CD8 T cells in tumor digests versus peripheral blood (PBL) from the same patient is shown. The percentage values represent the fraction of MART-1 tetramer$^+$ CD8 T cells that produced IFN-γ.

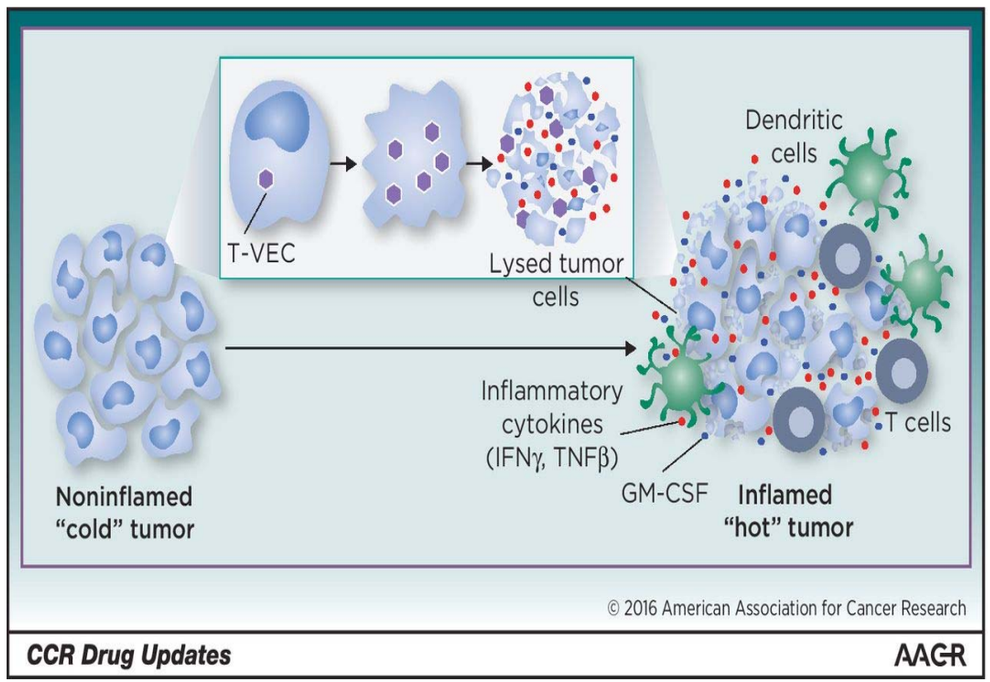
T-VEC Activity (ORR by iRC) in Combination with Immune Checkpoints



JOURNAL OF CLINICAL ONCOLOGY ASCO

Igor Puzanov et al. JCO doi:10.1200/JCO.2016.67.1529

- + Ipilimumab (Puzanov et al) – 9/18 50%
- + Pembrolizumab (Long et al) – 14/21 66%



Melanoma TIL – Expression of Co-inhibitory and Co-stimulatory Receptors (Gros et al)

The Journal of Clinical Investigation http://www.jci.org Volume 124 Number 5 May 2014