Immune Checkpoints

John Haanen

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Overview

- CTLA4
- PD1/PD-L1
- Other checkpoints

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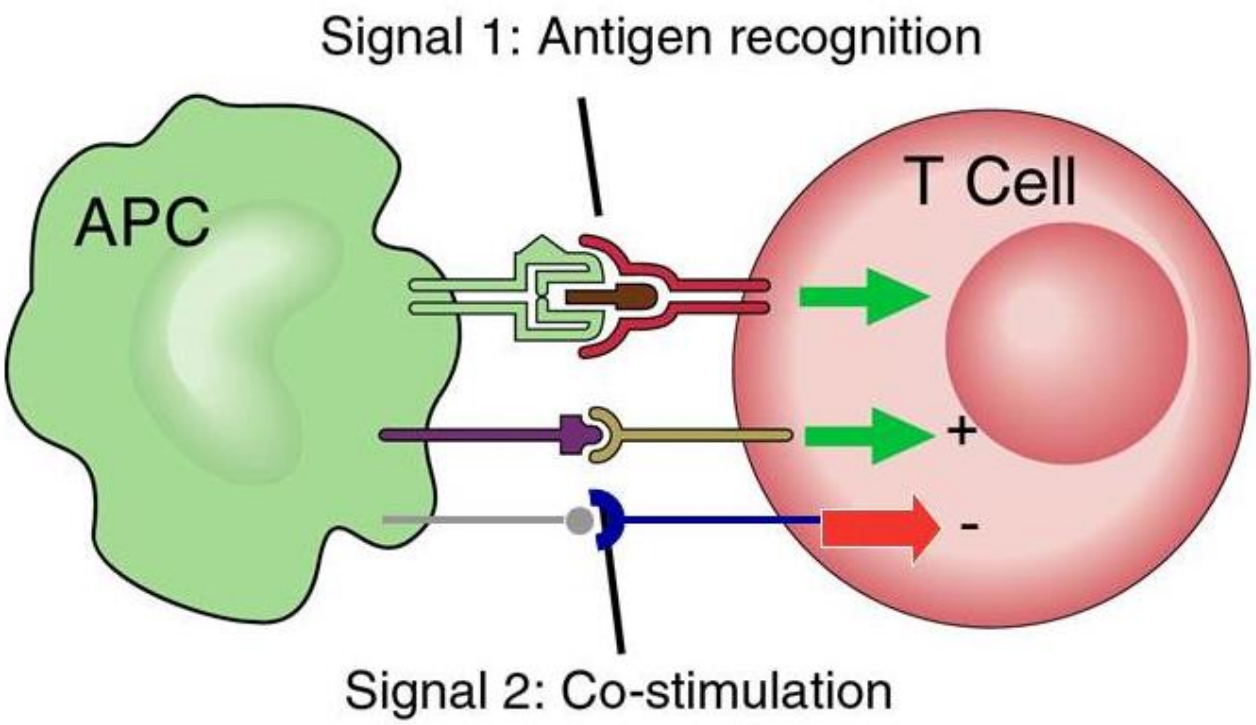
T cell signaling



There are positive and negative second signals

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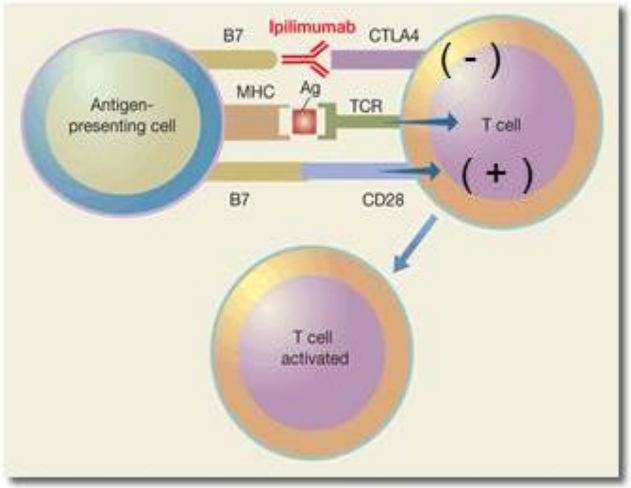
Cancer Immunotherapy: CTLA4

- CTLA-4 suppresses T cell activation and inhibits T cell function
- CTLA-4 regulates T cell tolerance
  -- CTLA-4 KO mice develop lethal lympho-proliferative syndrome
- CTLA-4 (Ipilimumab) first drug in this class approved for tumor immunotherapy



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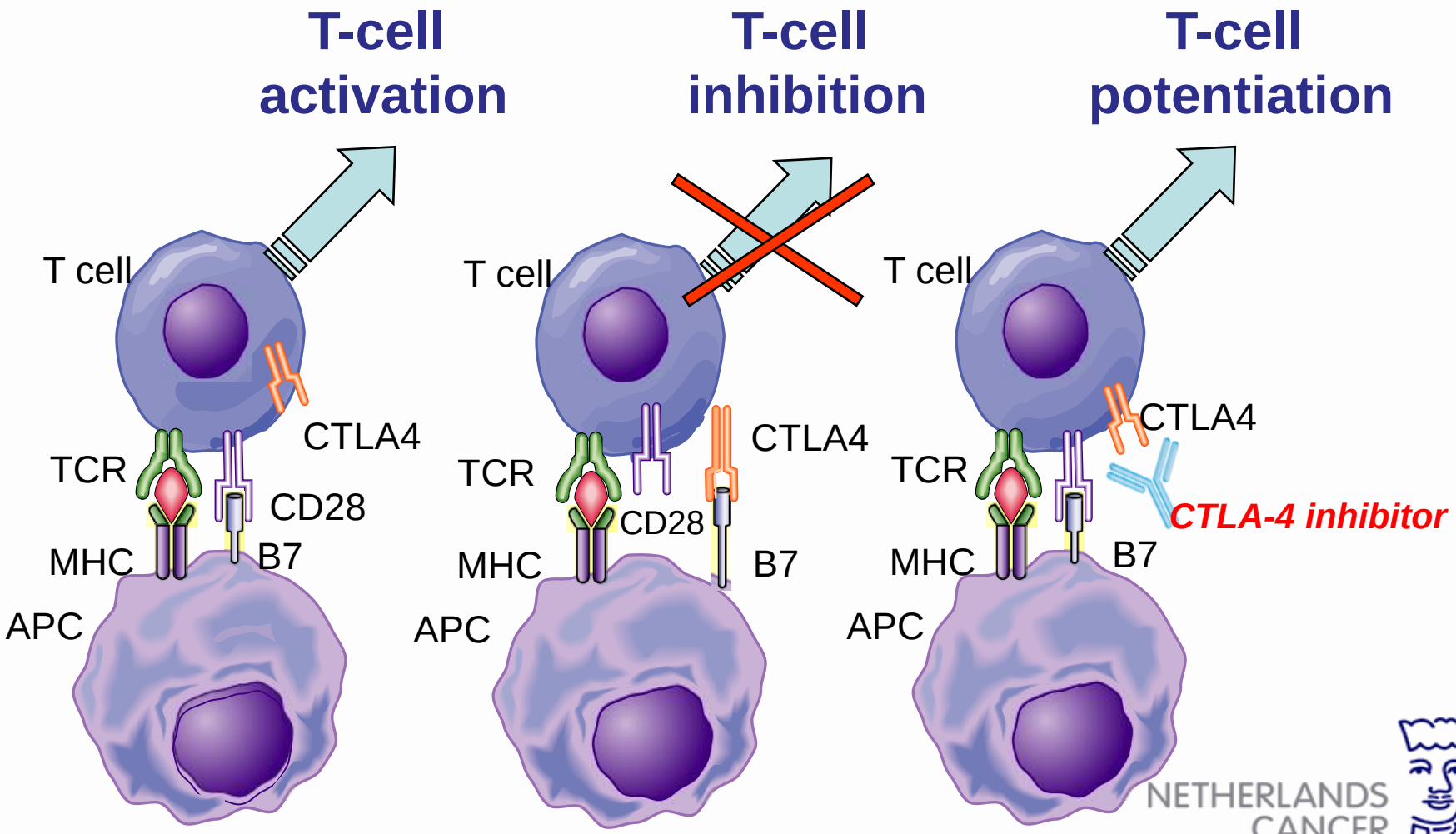
CTLA-4 inhibition



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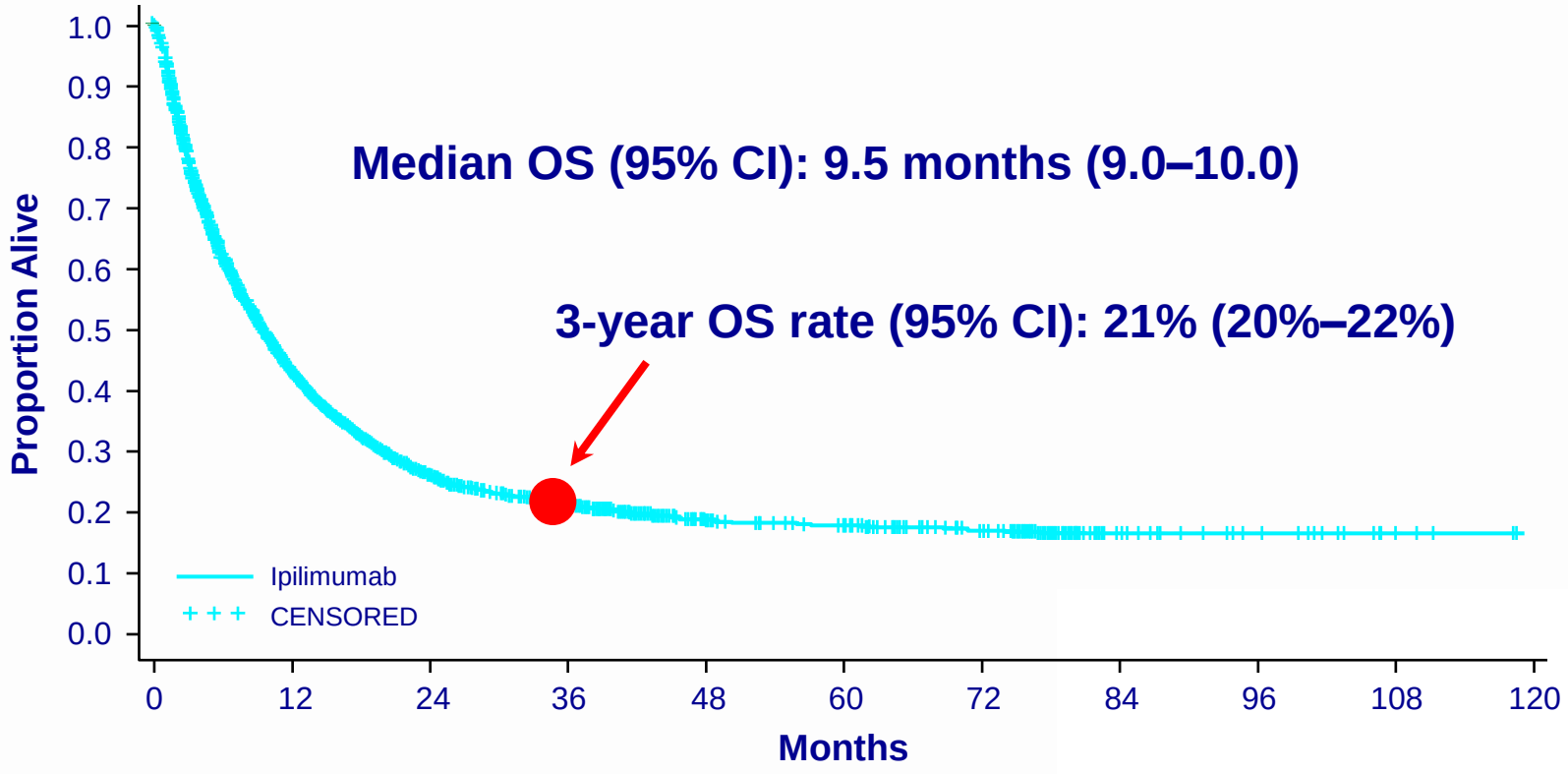
Adapted from Lebbé et al. ESMO 2008

CTLA-4 blockade (ipilimumab) can induce long-term survival

(pooled overall survival analysis including Expanded Access Program data from 4846 patients)

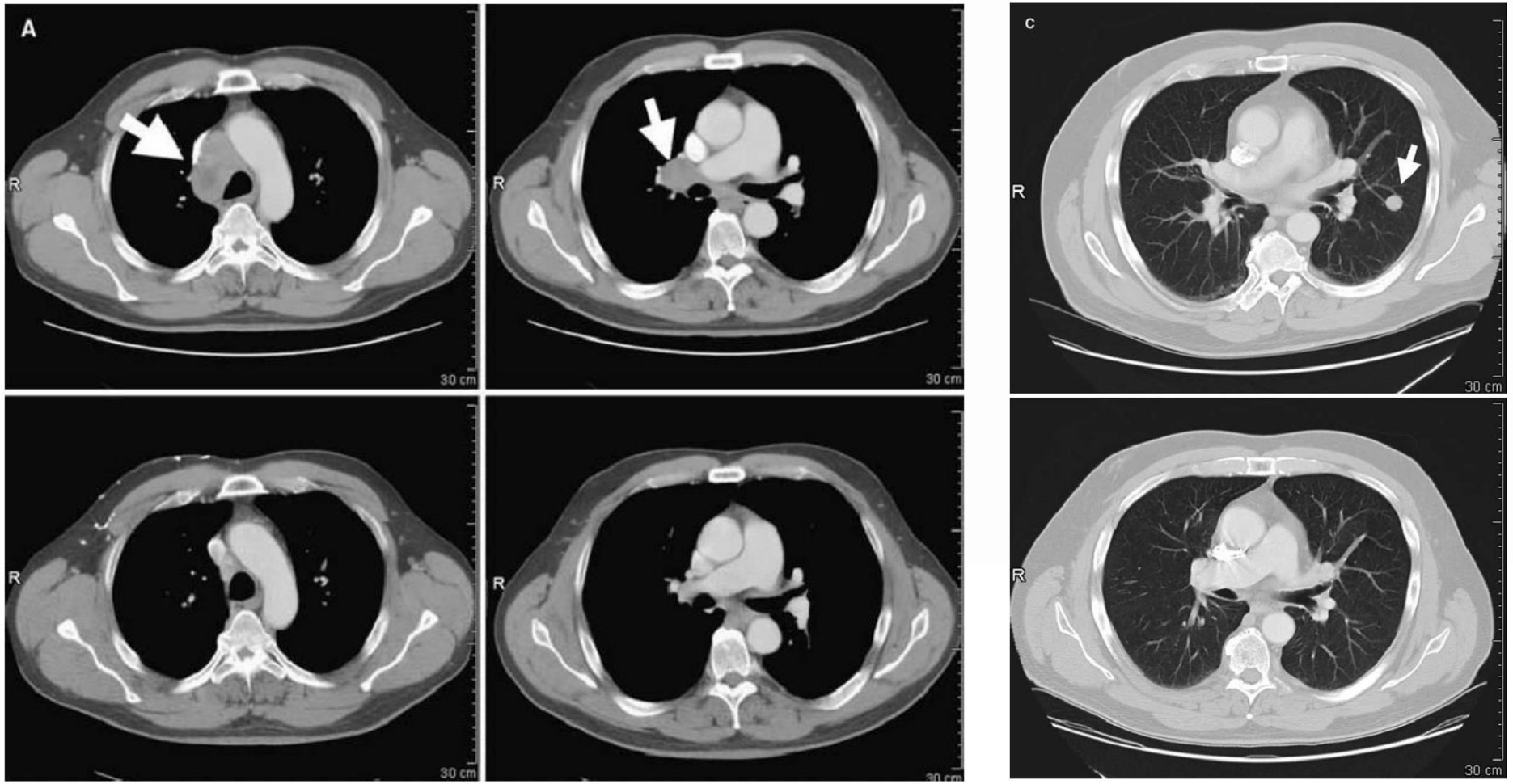


Patients at Risk

Ipilimumab	4846	1786	612	392	200	170	120	26	15	5	0

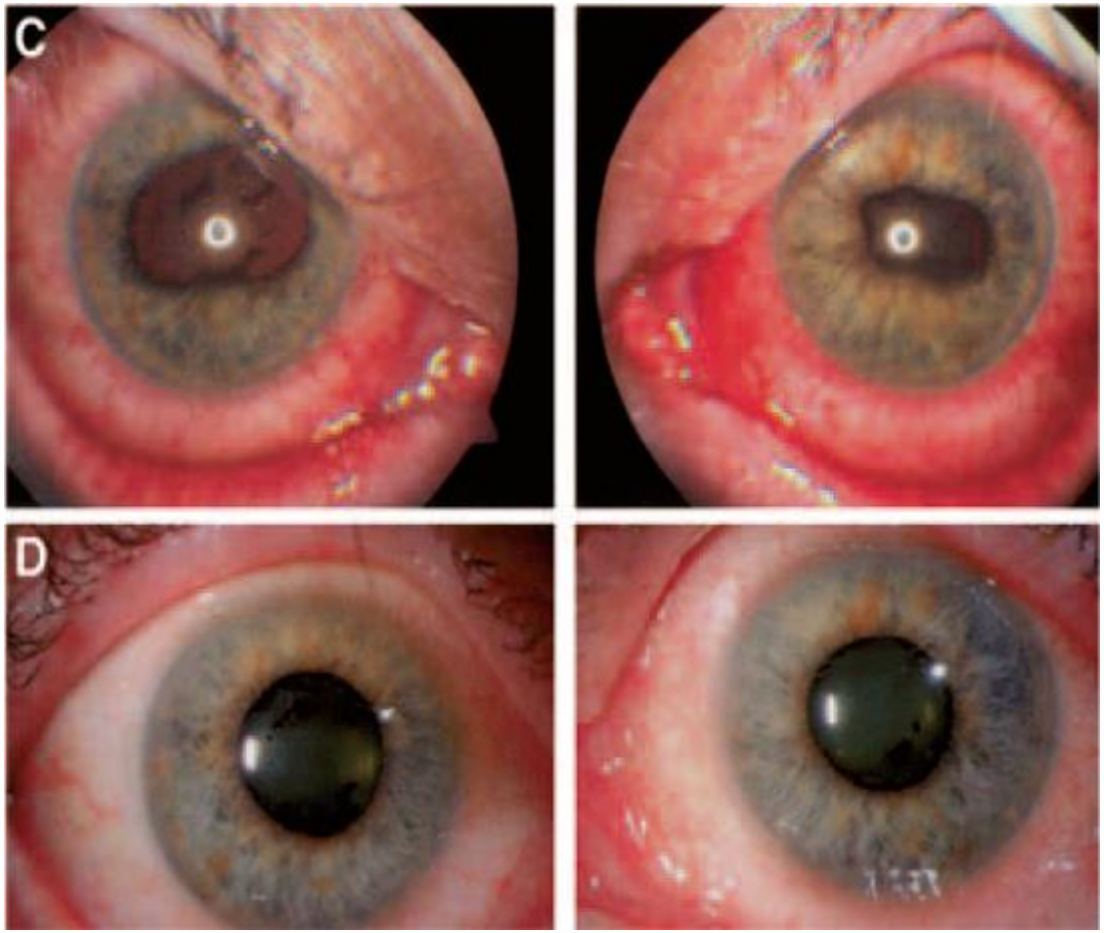
Schadendorf D, et al. ECCO/ESMO 2013: oral presentation 24LBA

Treatment with anti-CTLA-4 mAb



Maker et al., Ann Surg Oncol 2005

Auto-immune uveitis after anti-CTLA-4 treatment



After treatment

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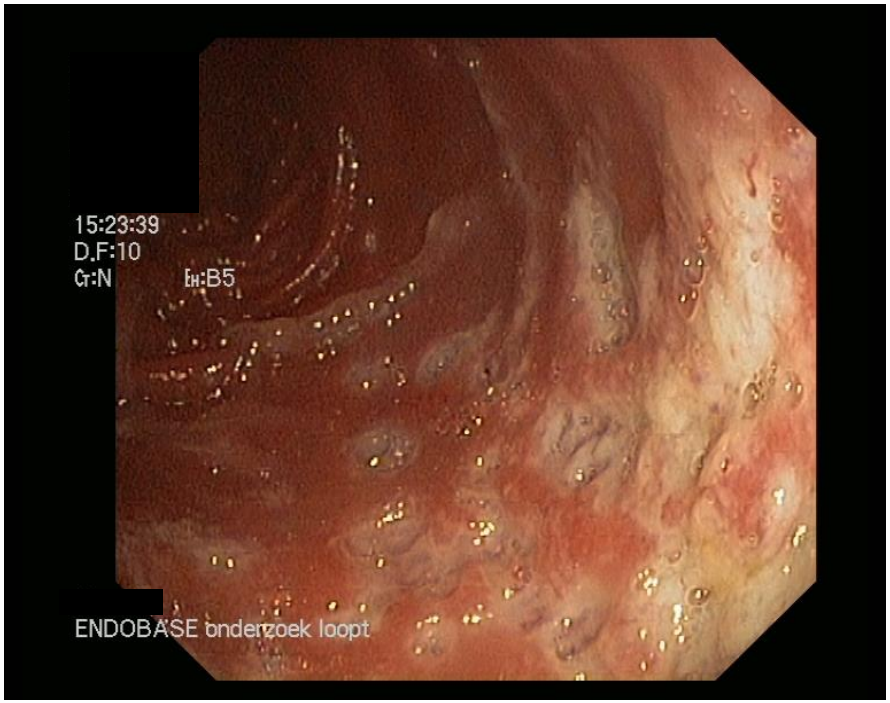
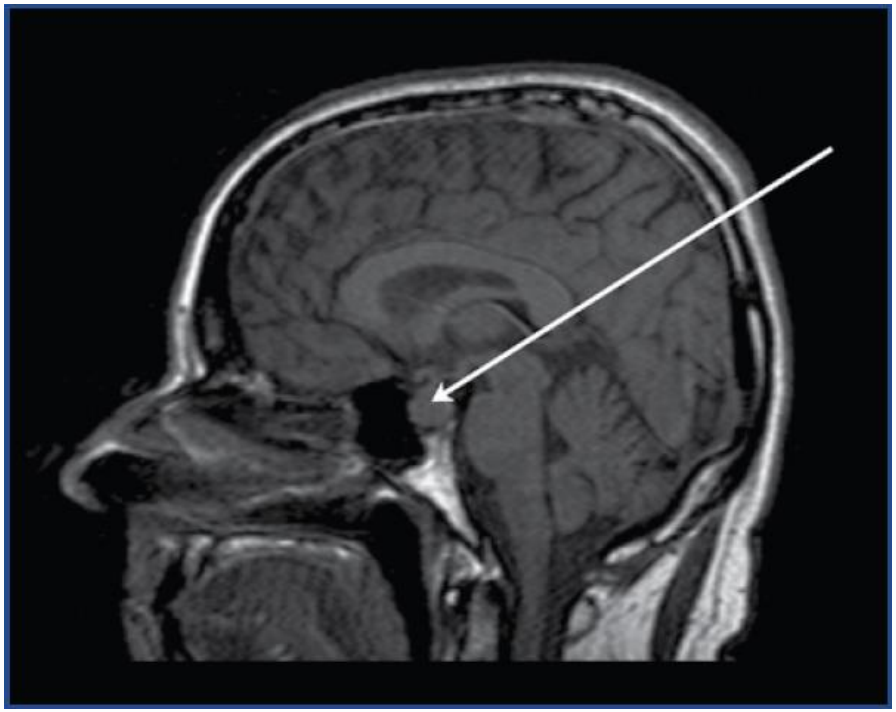
Immune related adverse events upon anti-CTLA-4 mAb treatment

colitis



hypophysitis



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How does CTLA4 blockade lead to anti-tumor immune responses?

Two not mutually exclusive mechanisms have been proposed:

1. Priming of tumor-reactive T cells
   - Against shared tumor associated antigens
   - Against mutated (neo) antigens
2. Depletion of regulatory FoxP3+ T cells from the tumor microenvironment

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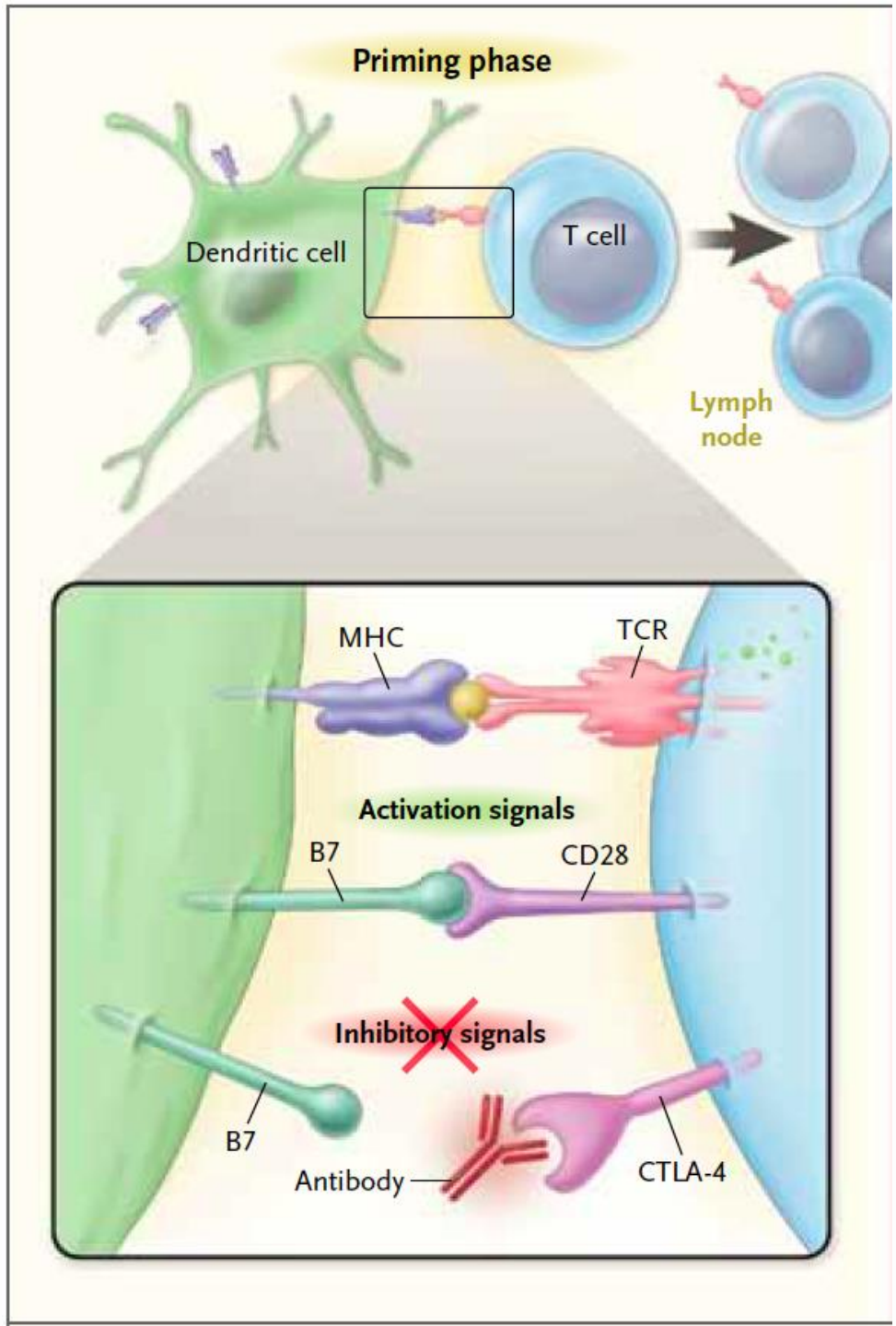
CTLA4 plays a role during T cell priming



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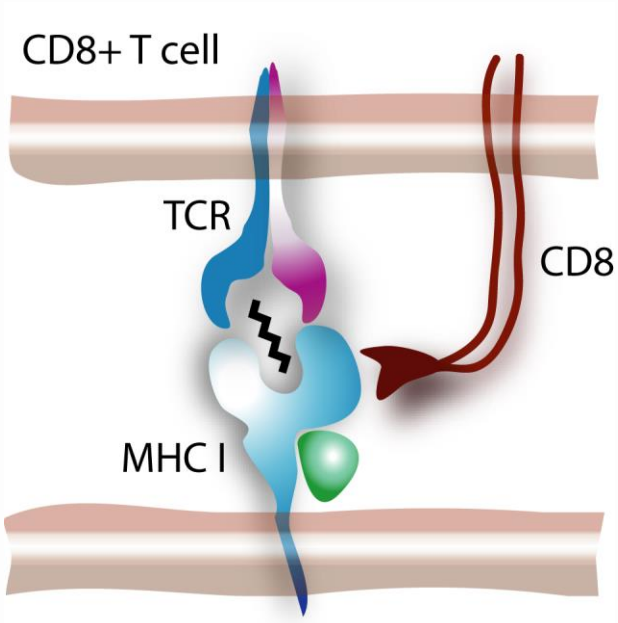
Ribas. N Engl J Med 2012

What could tumor-specific cytotoxic T cells detect on human cancer?



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   Shared between patients

2. 'Neo-antigens', epitopes that arise as a consequence of tumor-specific mutations
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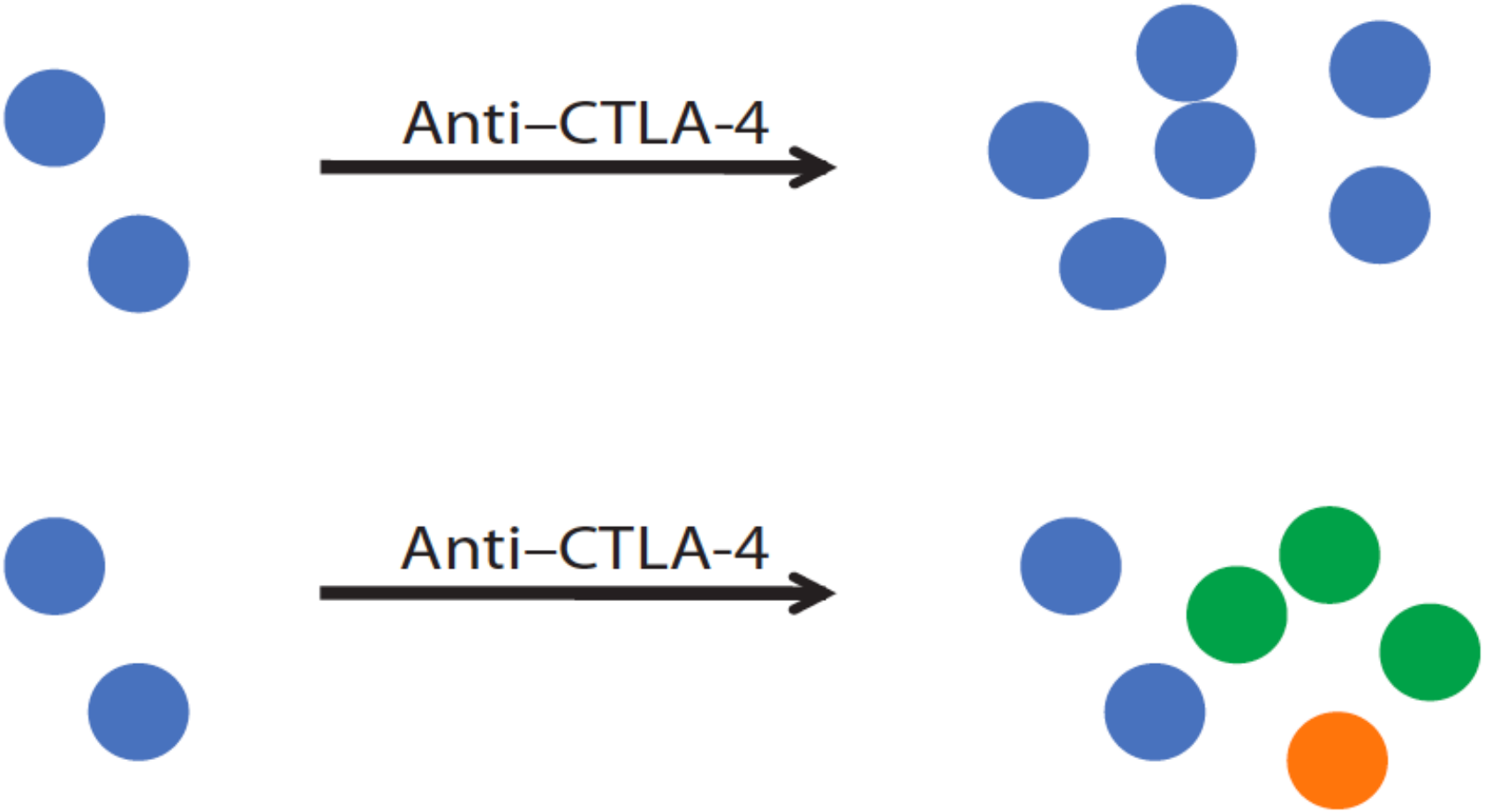

Kvistborg et al., Science Transl Med 2014

Analysis of PBMC from 40 ipilimumab treated melanoma patients for 75 tumor associated antigens

Epitope Sequence	Antigen
ACDPHSGHFV	CDK4
ALKDVEERV	MAGE-C2
ALSVMGVYV	MAGE-A9
ALVDAGVPM	CML28 (EXOSC5)
ALYVDSLFFL	PRAME
AMAPIKVRL	PRDX5 (OMT3-12)
AMLGTHTMEV	gp100 / Pmel17
SAWISKPPGV	SOX10
CLLWSFQTSA	Tyrosinase
CQWGRLWQL	BING-4 (WDR46)
ELAGIGILTV	Melan-A / MART-1
FLWGPRALV	MAGE-A3
FVWLHYYSV	TRP-2
GLIQLVEGV	TRAG-3
GLMDVQIPT	MAGE-A8
GLYDGMEHL	MAGE-A10
ILLRDAGLV	TRAG-3
ILTVILGVL	Melan-A / MART-1
IMDQVPFSV	gp100/Pmel17
KASEKIFYV	SSX-2
KLATAQFKI	CDCA1/NUF3
KMVELVHFL	MAGE-A2
KTWGQYWQV	gp100
SLLMWITQA	NY-ESO1
KVAELVHFL	MAGE-A3
MLGTHTMEV	gp100
AWISKPPGV	SOX10
FLWGPRAYA	DAM-6, -10 (MAGE-B1, -B2)
GVYDGREHTV	MAGE-A4
KVLEFLAKL	MAGE-C2
LATEKSRWS	B-RAF
LKLSGVVRL	RAGE-1
LLDGTATLRL	gp100
LVFGIELMEV	MAGE-A3
LVHFLLLKY	MAGE-A2
LVQENYLEY	MAGE-A2
MLAVISCAV	HERV-K-MEL

Epitope Sequence	Antigen
SVYDFFVWL	TRP-2
TLDSQVMSL	TRP-2
TLNDECWPA	Meloe-1
VIWEVLNAV	MAGE-C2
VLDGLDVLL	PRAME
VLGEAWRDQV	TRAG-3
VLHWDPETV	NY-MEL-1
VLPDVFIRCV	GnT-V
VLYRYGSFSV	gp100
VYDFFVWLHY	TRP-2
YLEPGPVTA	gp100
YLEYRQVPV	MAGE-A6
YLQLVFGIEV	MAGE-A2
YMDGTMSQV	Tyrosinase
GILGFVFTL	Influenza A
GLCTLVAML	EBV BMF1
NLVPMVATV	CMV pp65
MLLAVLYCL	Tyrosinase
MLMAQEALAFL	LAGE-1
RLMKQDFSV	gp100
RLPPKPPLA	Meloe-2
RLPRIFCSC	gp100
RLQGISPKI	SSX-2
SLADTNSLAV	gp100
SLLMWITQC	LAGE-1
VLPDVFIRC	GnT-V
YMMPVNSEV	CDCA1/NUF2
TLDEKVAELV	MAGE-C2
PLPPARNGGL	RAGE-1
QLSLLMWIT	NY-ESO1
KVLEYVIKV	MAGE-A1
LLFGLALIEV	MAGE-C2
SLDDYNHLV	TRP-2
SLGWLFLLL	TAG-1
KVAELVRFL	MAGE-A8
SLLMWITQCFL	NY-ESO1
SLLQHLIGL	PRAME
SLYSFPEPEA	PRAME

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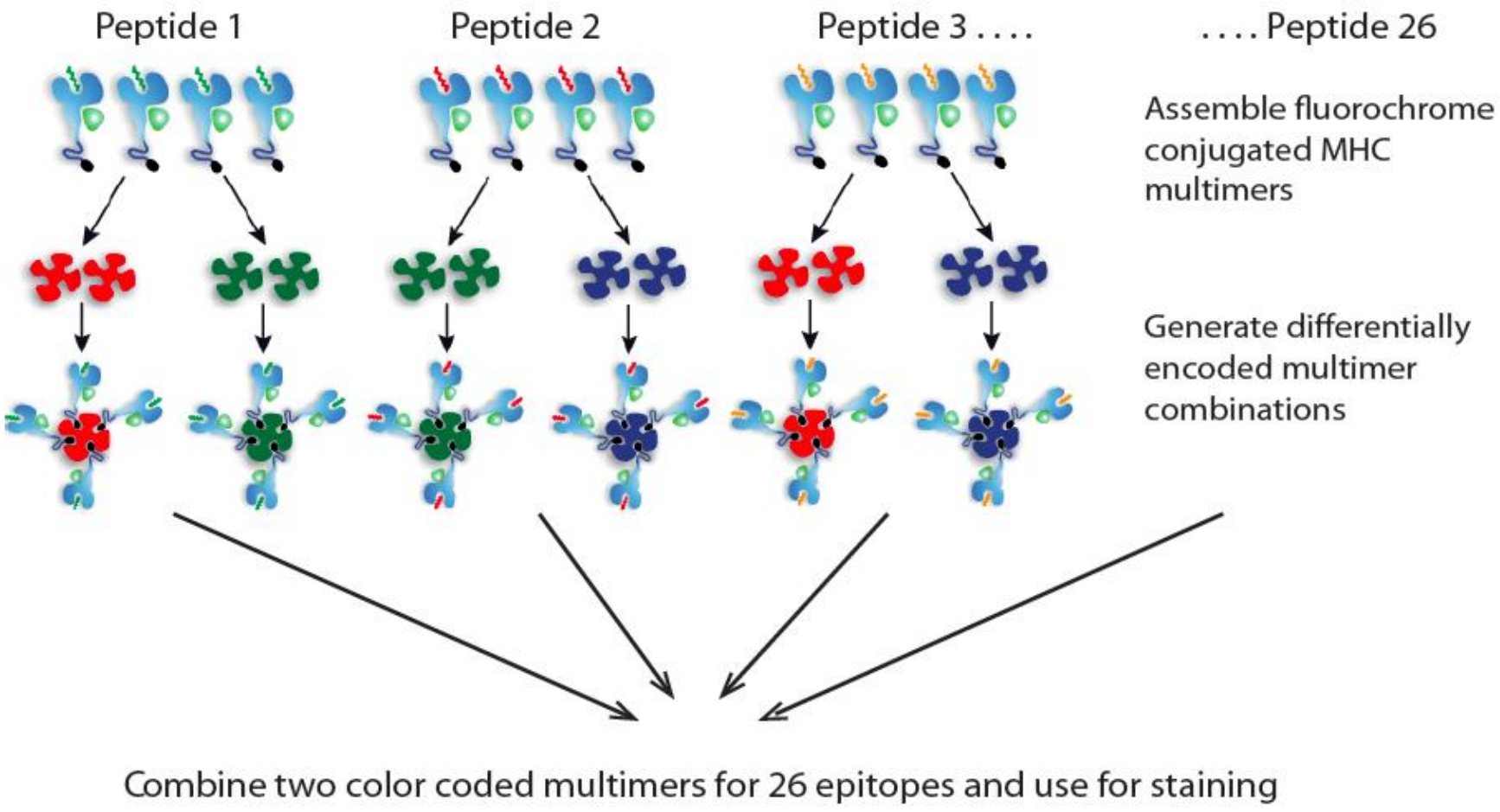
Kvistborg et al., Science Transl Med 2014

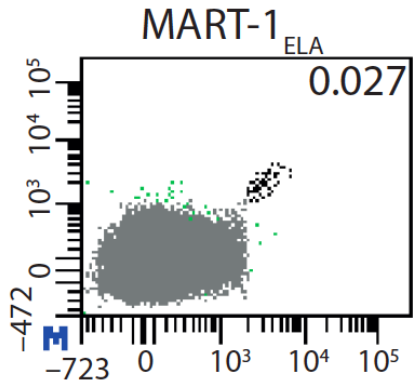
Analysis performed by flow cytometry

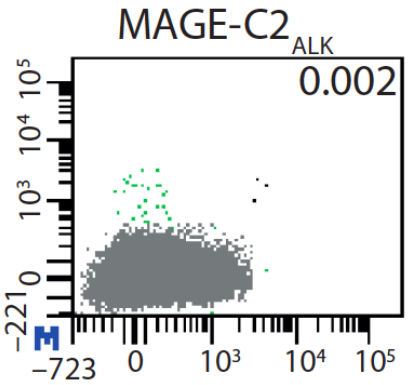


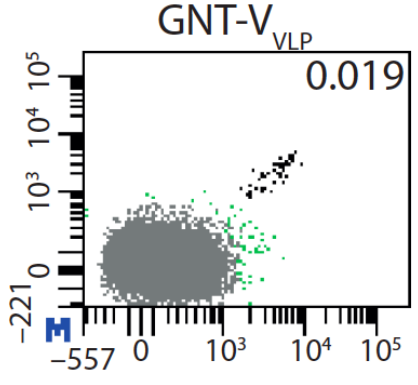
Toebes et al., Nat Med 2006; Anderson et al. Nat Protoc 2012

Flow results…

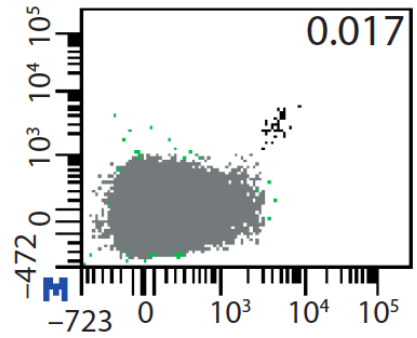
Pretherapy

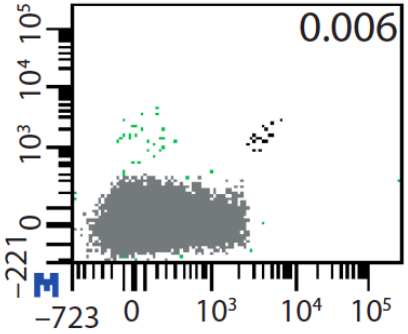


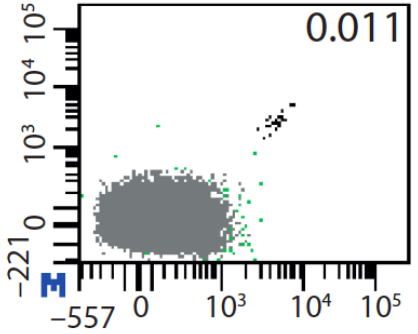




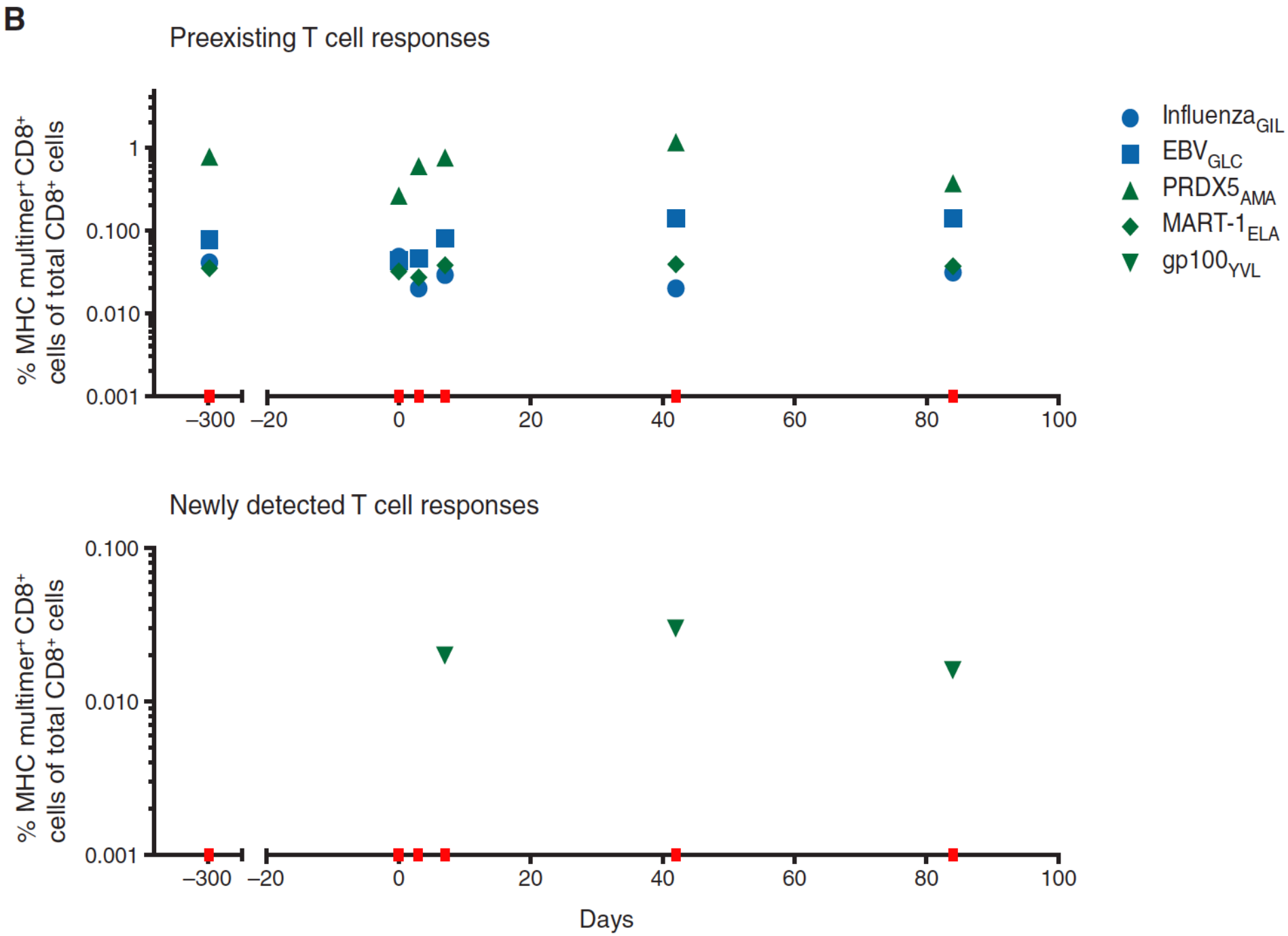
Posttherapy



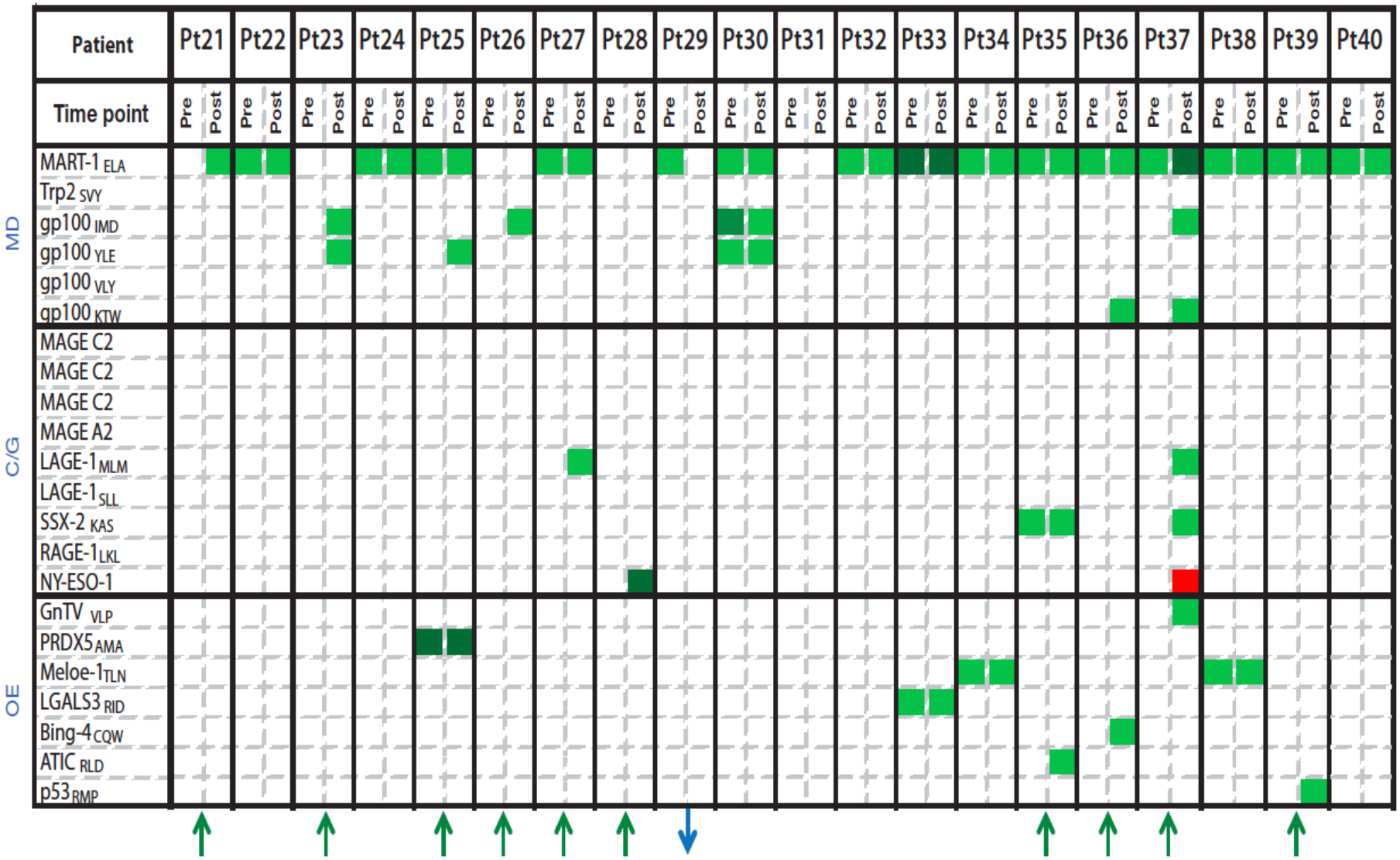




Kvistborg et al., Science Transl Med 2014

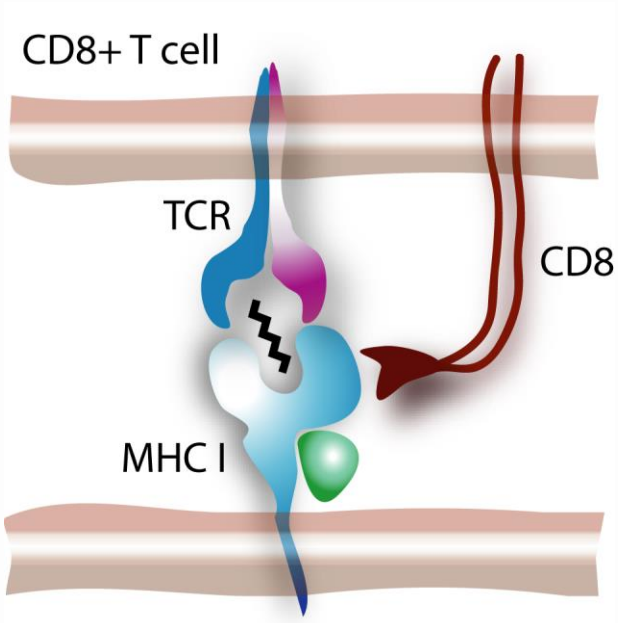

B
Preexisting T cell responses
% MHC multimer+ CD8+ cells of total CD8+ cells
1
0.100
0.010
0.001
–300
–20
0
20
40
60
80
100
Influenza GIL
EBV GLC
PRDX5 AMA
MART-1 ELA
gp100 YVL
Newly detected T cell responses
% MHC multimer+ CD8+ cells of total CD8+ cells
0.100
0.010
0.001
–300
–20
0
20
40
60
80
100
Days

Ipilimumab treatment leads to broadening of the anti cancer IR



Kvistborg et al., Science Transl Med 2014

What could tumor-specific cytotoxic T cells detect on human cancer?



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   Shared between patients

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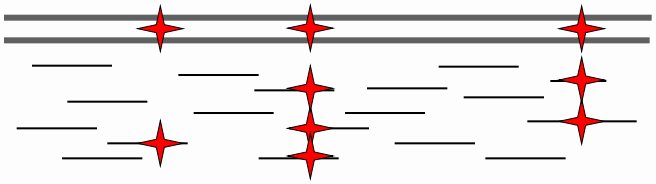
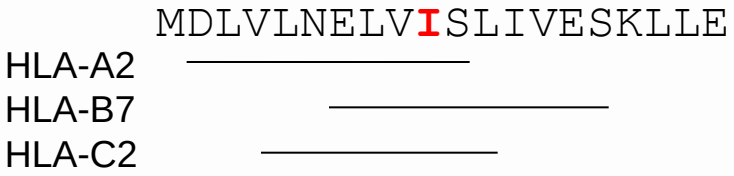
Analyzing the neo-antigen-specific T cell repertoire in human cancer?

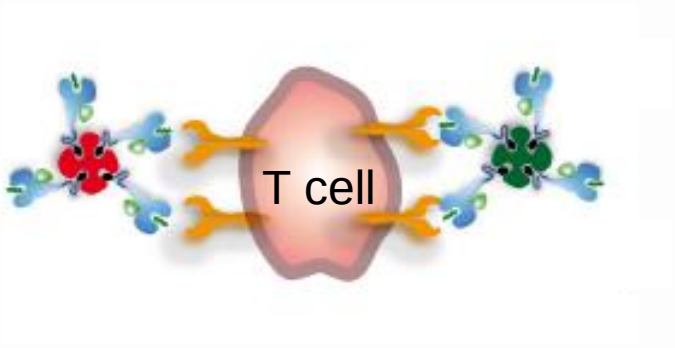


Generate map of tumor-specific mutations (ExomeSeq)

Determine which mutated genes are expressed (RNASeq)



Predict epitopes for each mutation/ each HLA-allele *in silico*



Screen for T cell recognition of mutated epitopes

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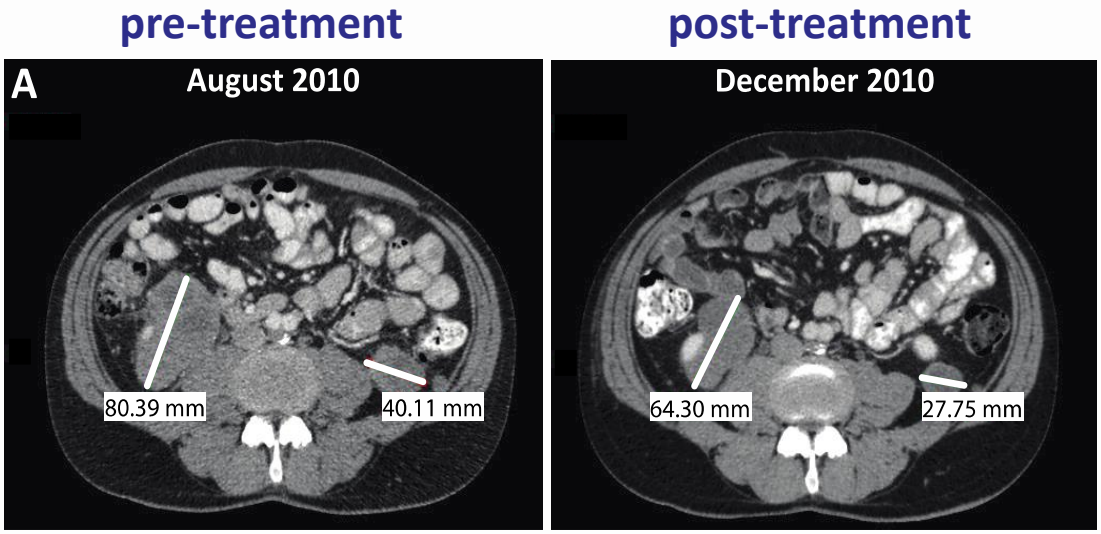
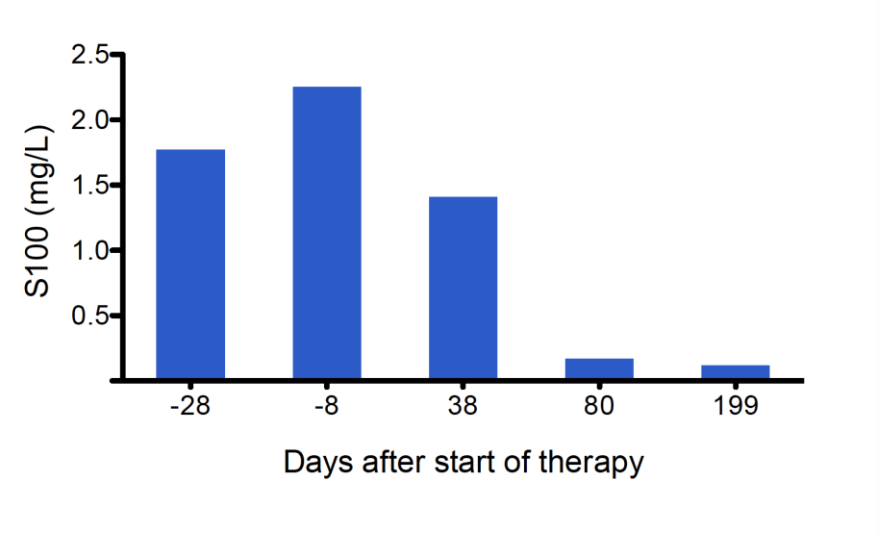
Pt 002: Partial response upon anti-CTLA4 treatment





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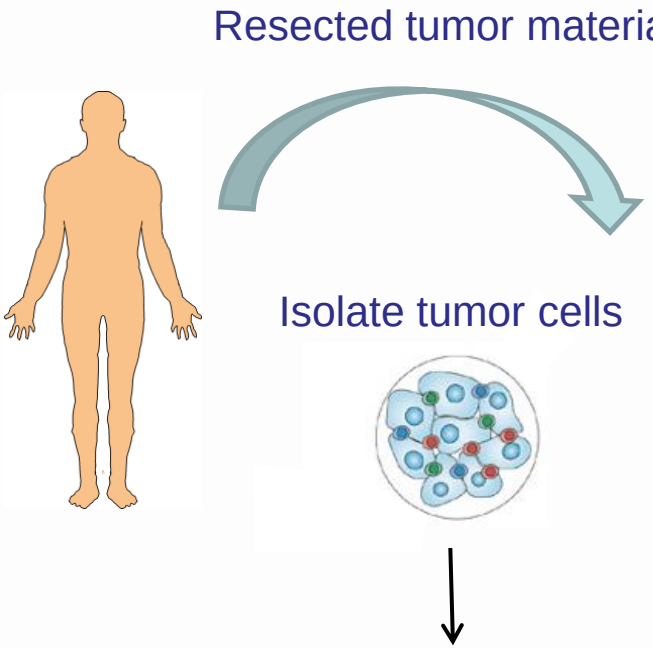
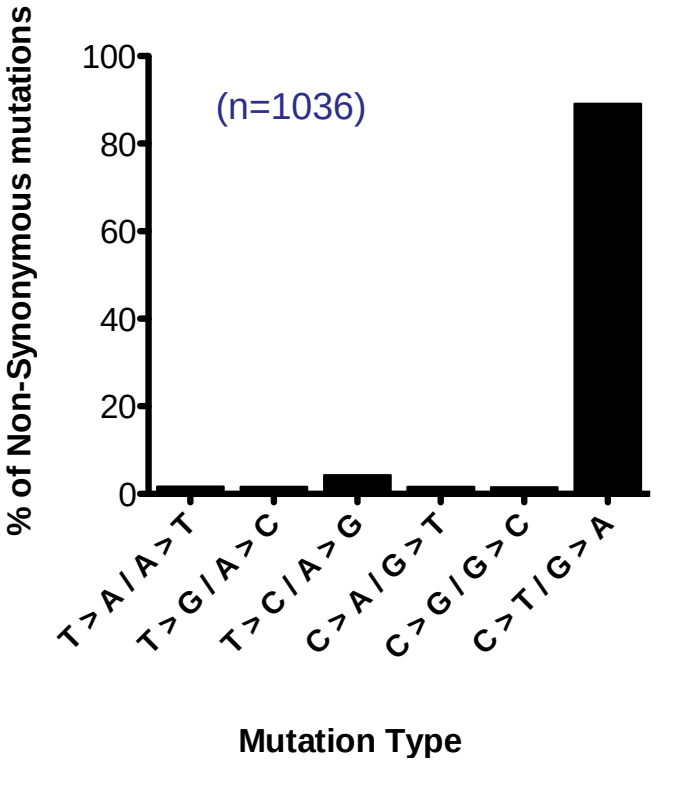
Van Rooij et al., J Clin Oncol 2013

Analyzing the neo-antigen-specific T cell repertoire in human cancer?





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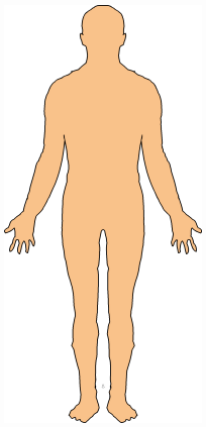
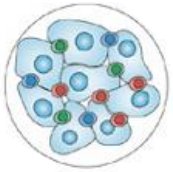
Analyzing the neo-antigen-specific T cell repertoire in human cancer?

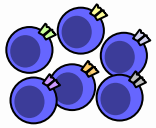
Resected tumor material



Isolate tumor cells



Isolate tumor-infiltrating T cells



Screen with MHC multimer technology

Identify tumor-specific mutations

Predict potential epitopes

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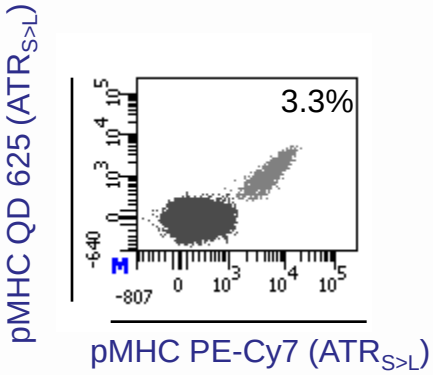
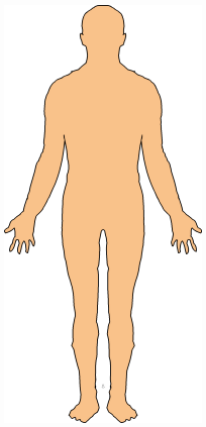
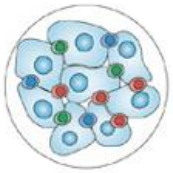
Strong T cell response against an $ATR_{S>L}$ neo-epitope within the tumor



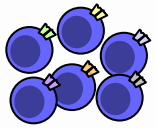
Resected tumor material



Isolate tumor cells



Isolate tumor-infiltrating T cells



Screen with MHC multimer technology

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Predict potential epitopes

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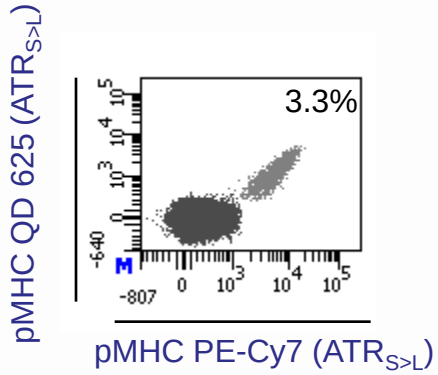
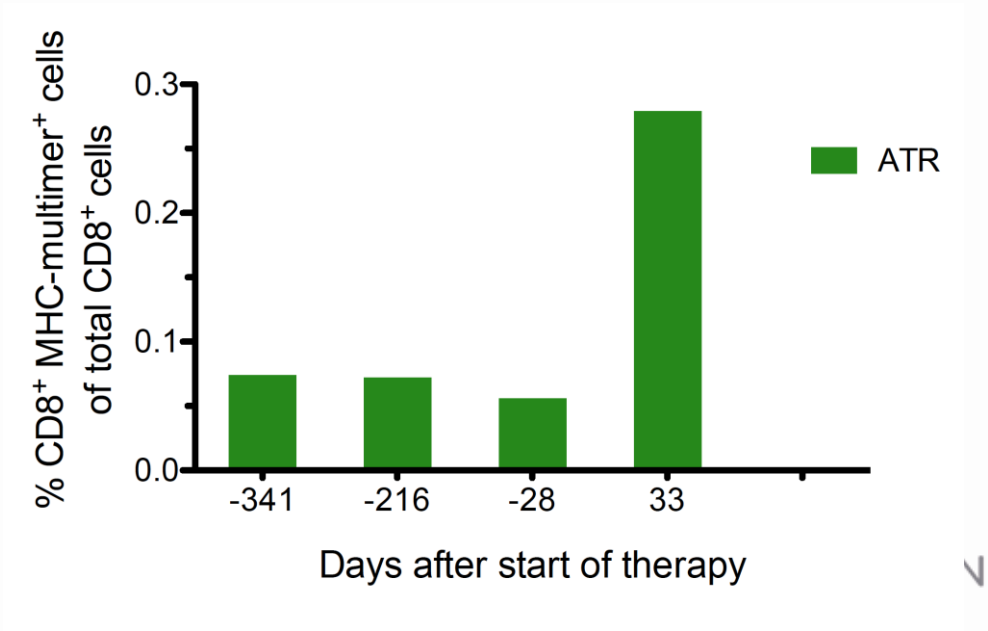
Increased magnitude of neo-antigen-specific T cell response under anti-CTLA4





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van Rooij, van Buuren *J Clin Oncol* 2013

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Fc-dependent depletion of tumor-infiltrating regulatory T cells co-defines the efficacy of anti–CTLA-4 therapy against melanoma

by Tyler R. Simpson, Fubin Li, Welby Montalvo-Ortiz, Manuel A. Sepulveda, Katharina Bergerhoff, Frederick Arce, Claire Roddie, Jake Y. Henry, Hideo Yagita, Jedd D. Wolchok, Karl S. Peggs, Jeffrey V. Ravetch, James P. Allison, and Sergio A. Quezada

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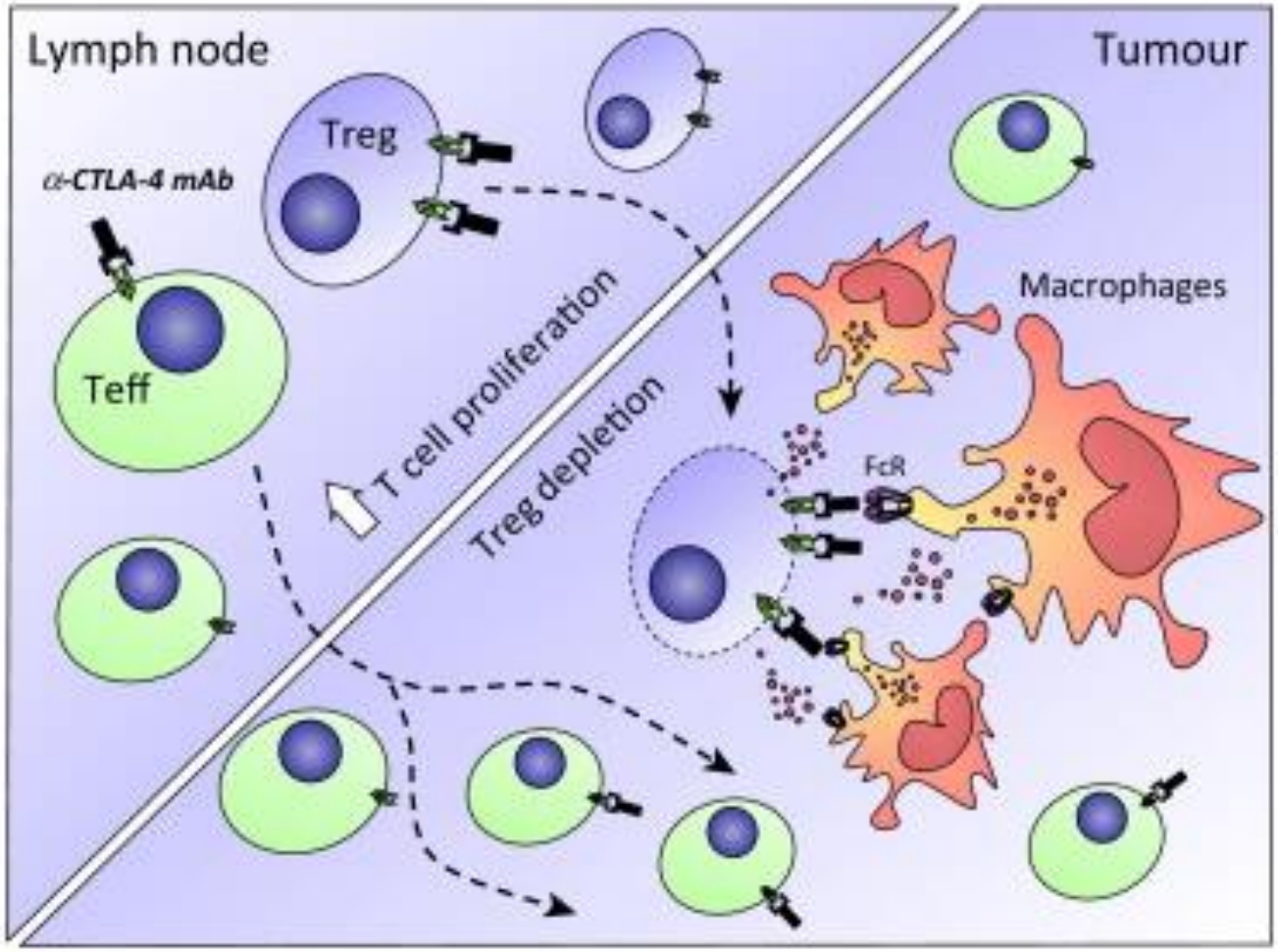
Simpson et al., J Exp Med 2013

Anti-CTLA4 plays a role in T cell priming and Treg depletion



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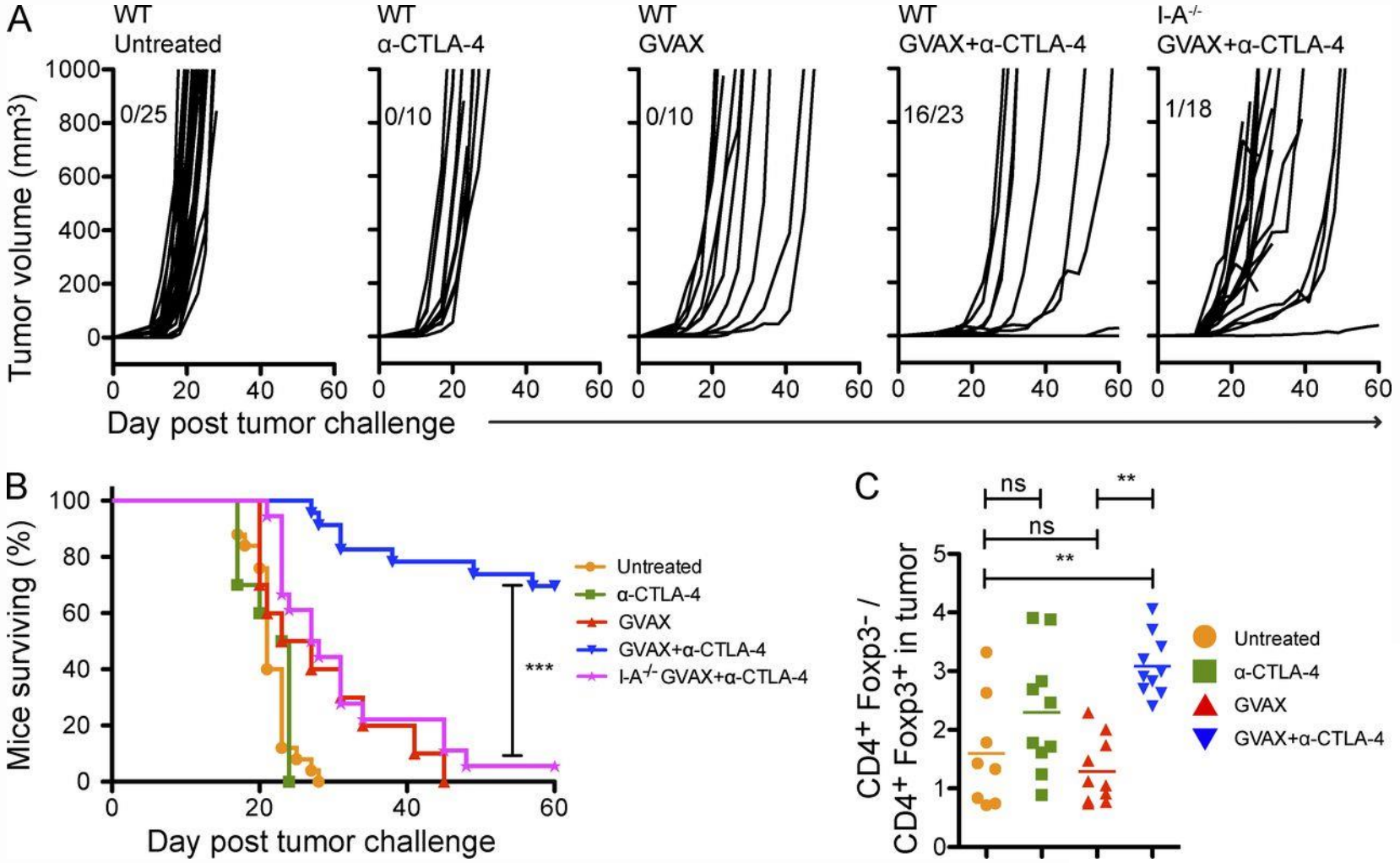
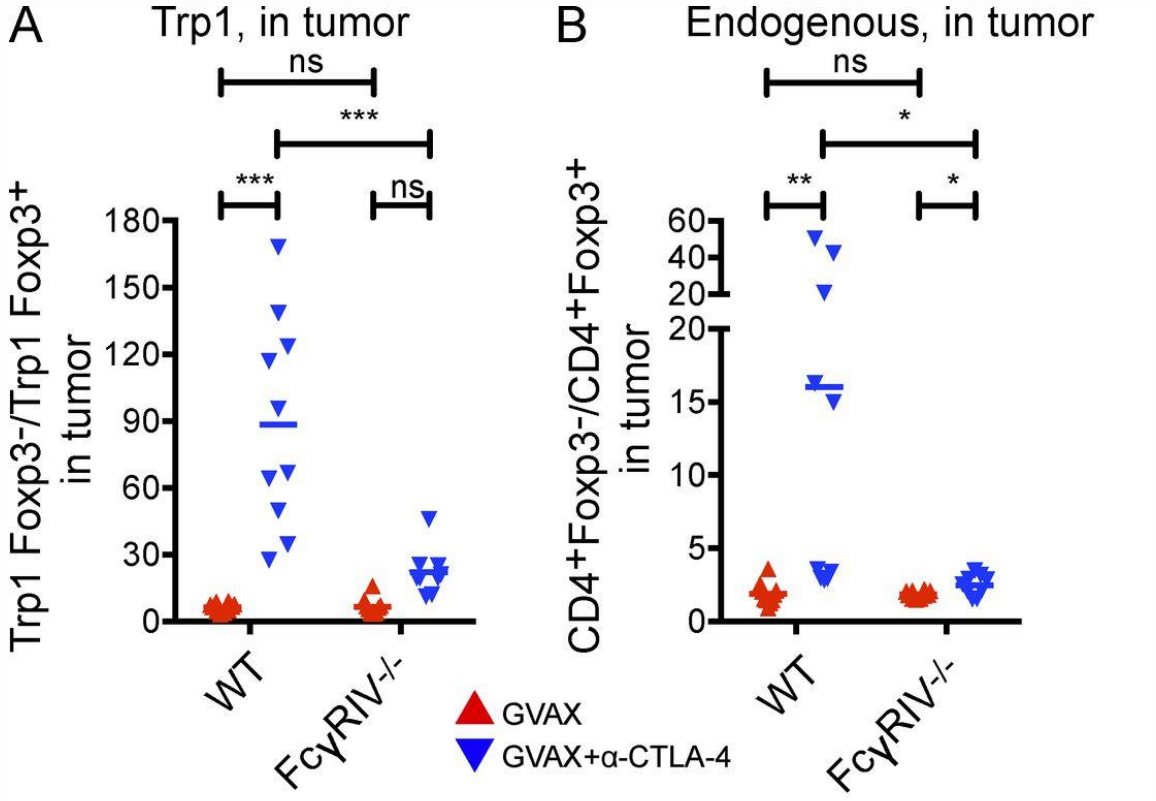
Furness et al., Trends in Immunol 2014

GVAX+α–CTLA-4 combination therapy protects against tumor outgrowth through a CD4+ T cell–dependent mechanism.



Simpson T R et al. J Exp Med 2013;210:1695-1710

α–CTLA-4 therapy does not increase the intratumoral T eff/T reg cell ratio in FcγRIV−/− mice



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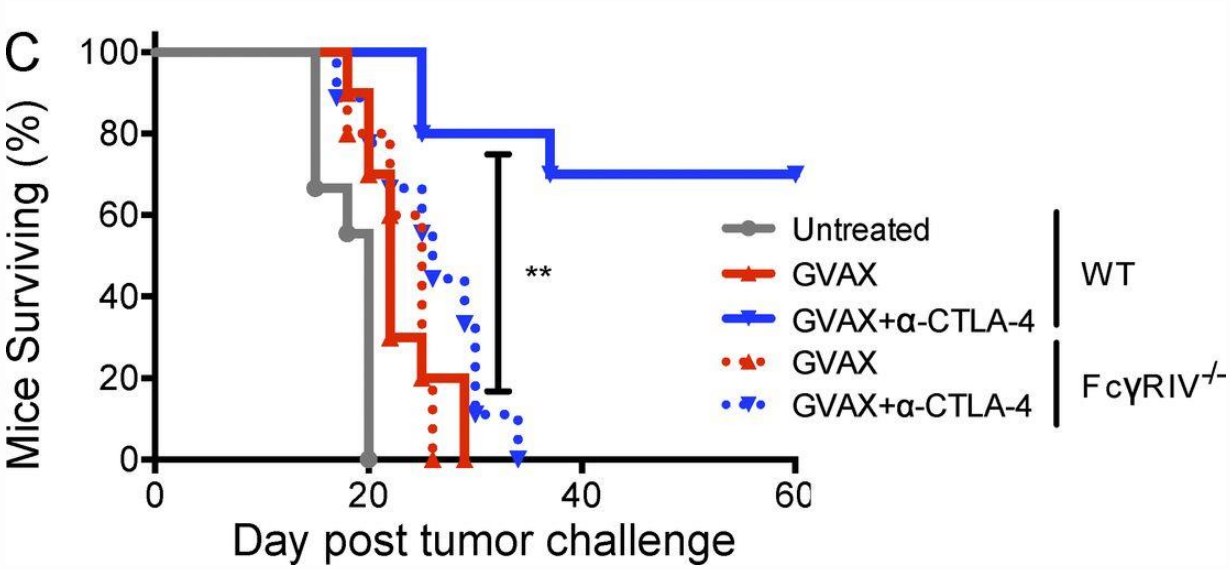
Simpson T R et al. J Exp Med 2013;210:1695-1710

α–CTLA-4 therapy in FcγRIV−/− mice fails to elicit tumor protection



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Simpson T R et al. J Exp Med 2013;210:1695-1710

Does Treg depletion also occur in humans with ipilimumab?

Table 3. Immunomodulatory antibodies in clinical development.

Target	Antibody	Species	Isotype	Predicted ADCC	Company
CTLA-4	Ipilimumab	Humanised	IgG1	Yes	Bristol-Myers Squibb
	Tremelimumab	Humanised	IgG2	No	AstraZeneca/Pfizer

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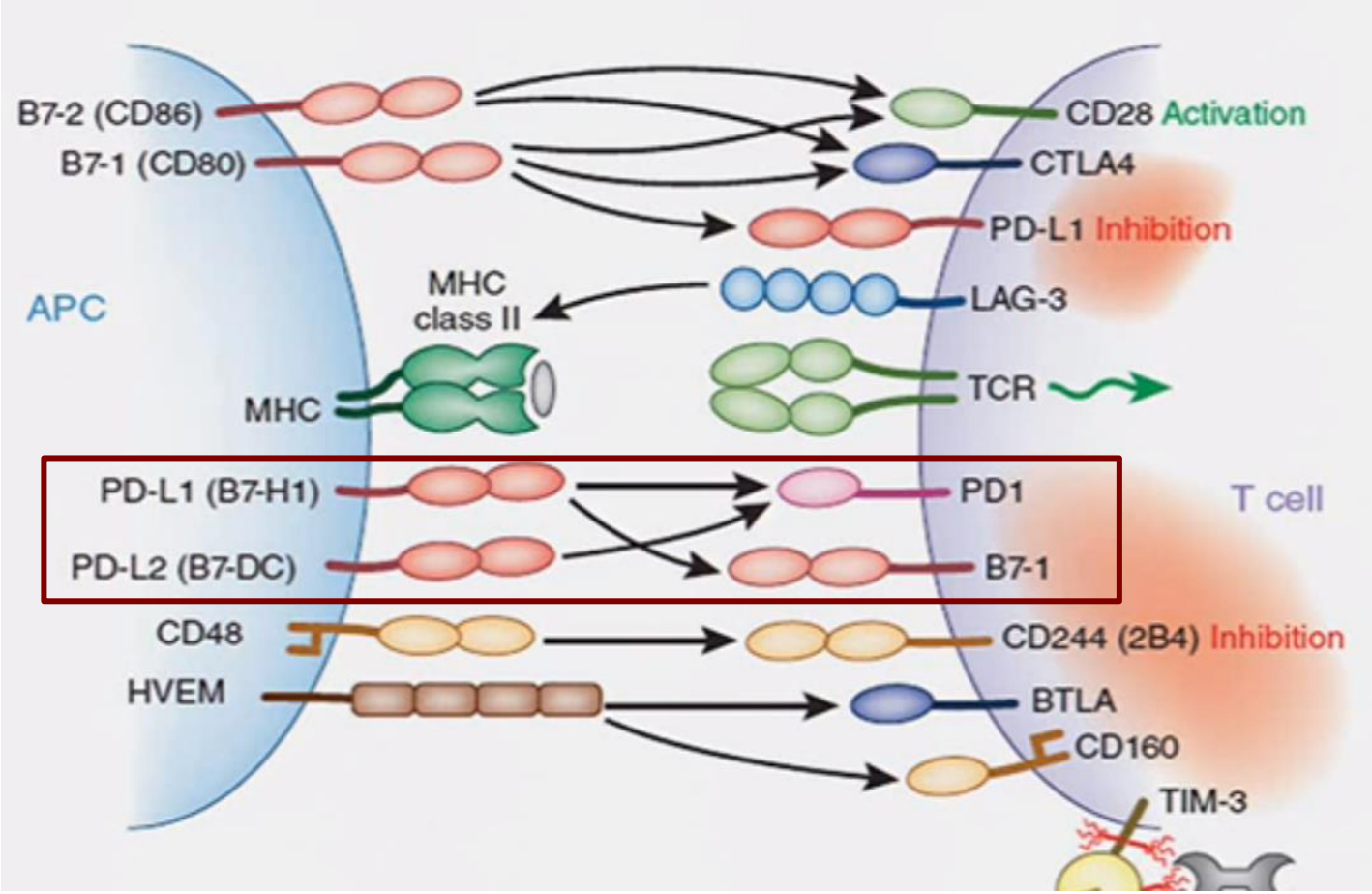
Furness et al., Trends in Immunol 2014

Summary CTLA4 blockade

- CTLA4 is an important regulator of peripheral tolerance
- Blockade of CTLA4 can result in anti-tumor immunity and auto-immunity
- CTLA4 plays a role during induction of the immune response
- Blockade results in broadening of the anti-tumor immune response
- CTLA4 is highly expressed by Tregs
- Anti-CTLA4 antibody treatment may deplete Tregs from the tumor

PD1 and PD-L1 checkpoint



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Freeman & Sharpe. Nat Immunol 2013

Programmed Death-1 receptor (PD1)

- Discovered in 1992 by Honjo and coworkers
  - Upregulated gene in relation to apoptosis
- Member of the Ig superfamily
- Cytoplasmic domains with ITIM and ITSM
  - Recruites phosphatases
  - Inhibits PI3K and AKT activity
- Inducibly expressed by CD4 and CD8 T cells, NKT cells, B cells, monocytes and subtypes of DC
- Expressed by both effector and regulatory T cells
- PD1/PD-L1 interaction involved in tolerance and chronic inflammation
- PD1/PD-L1 contributes to functional T cell exhaustion during chronic infection and cancer

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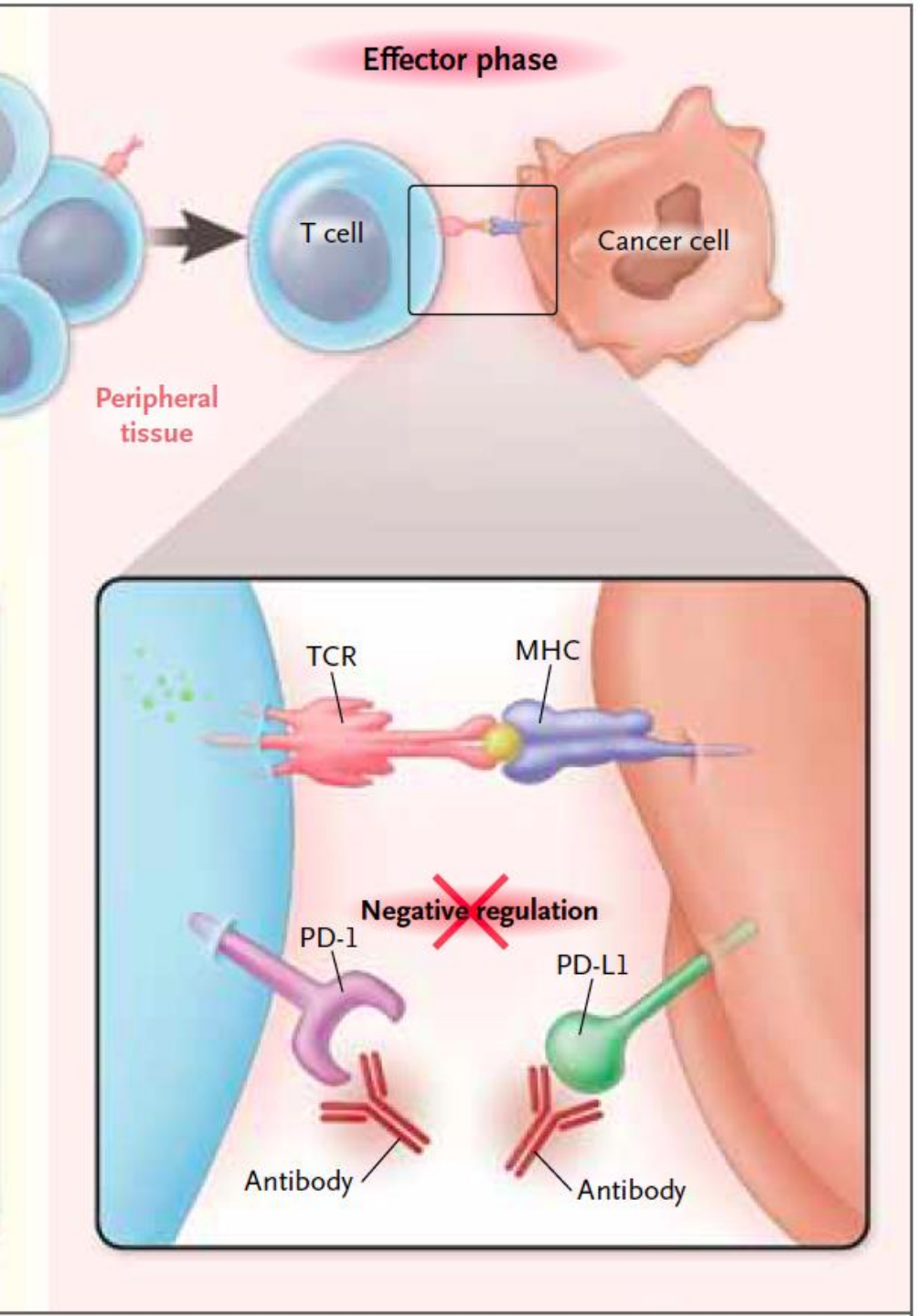
PD1/PD-L1 play a role at the tumor/effector phase



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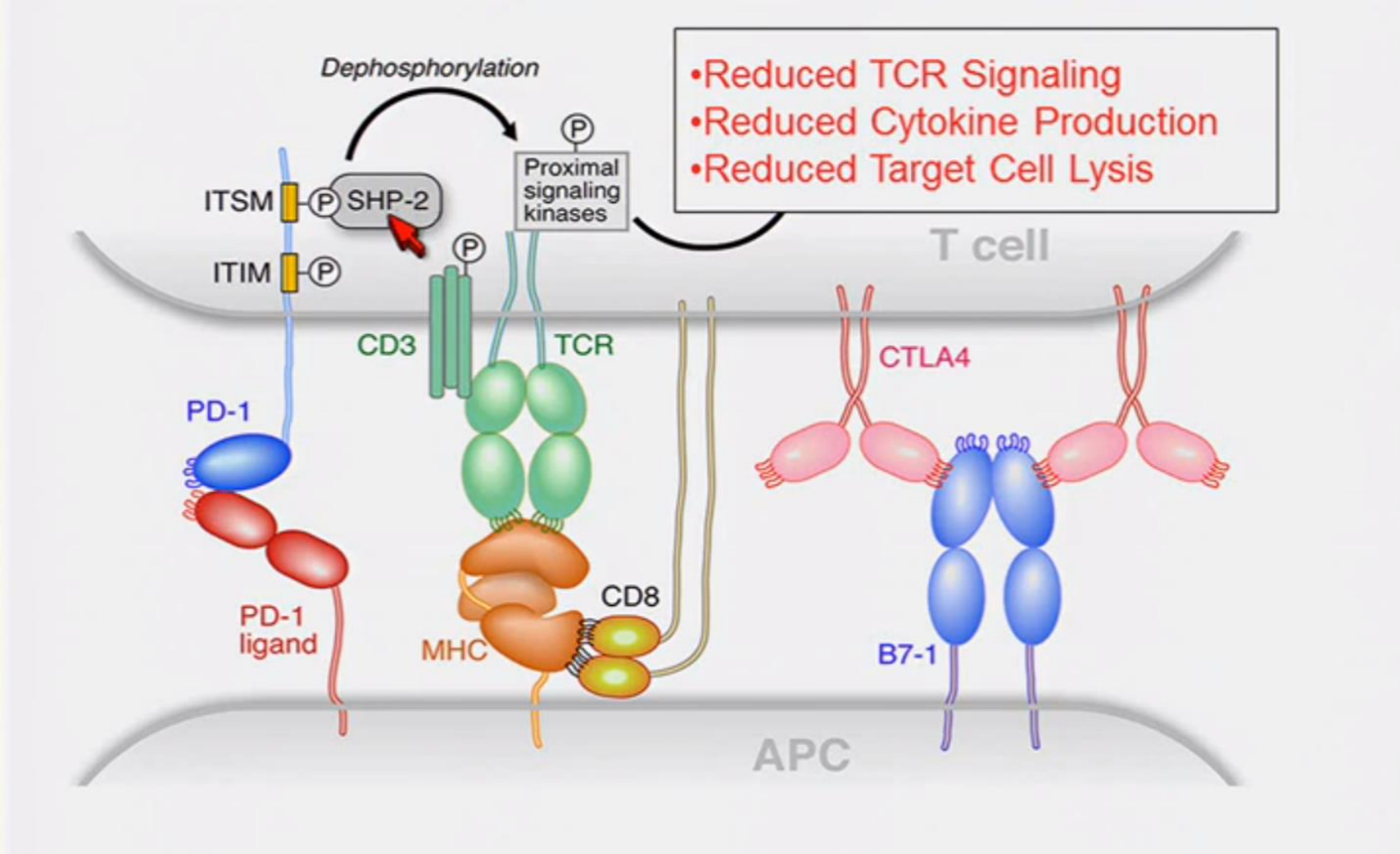
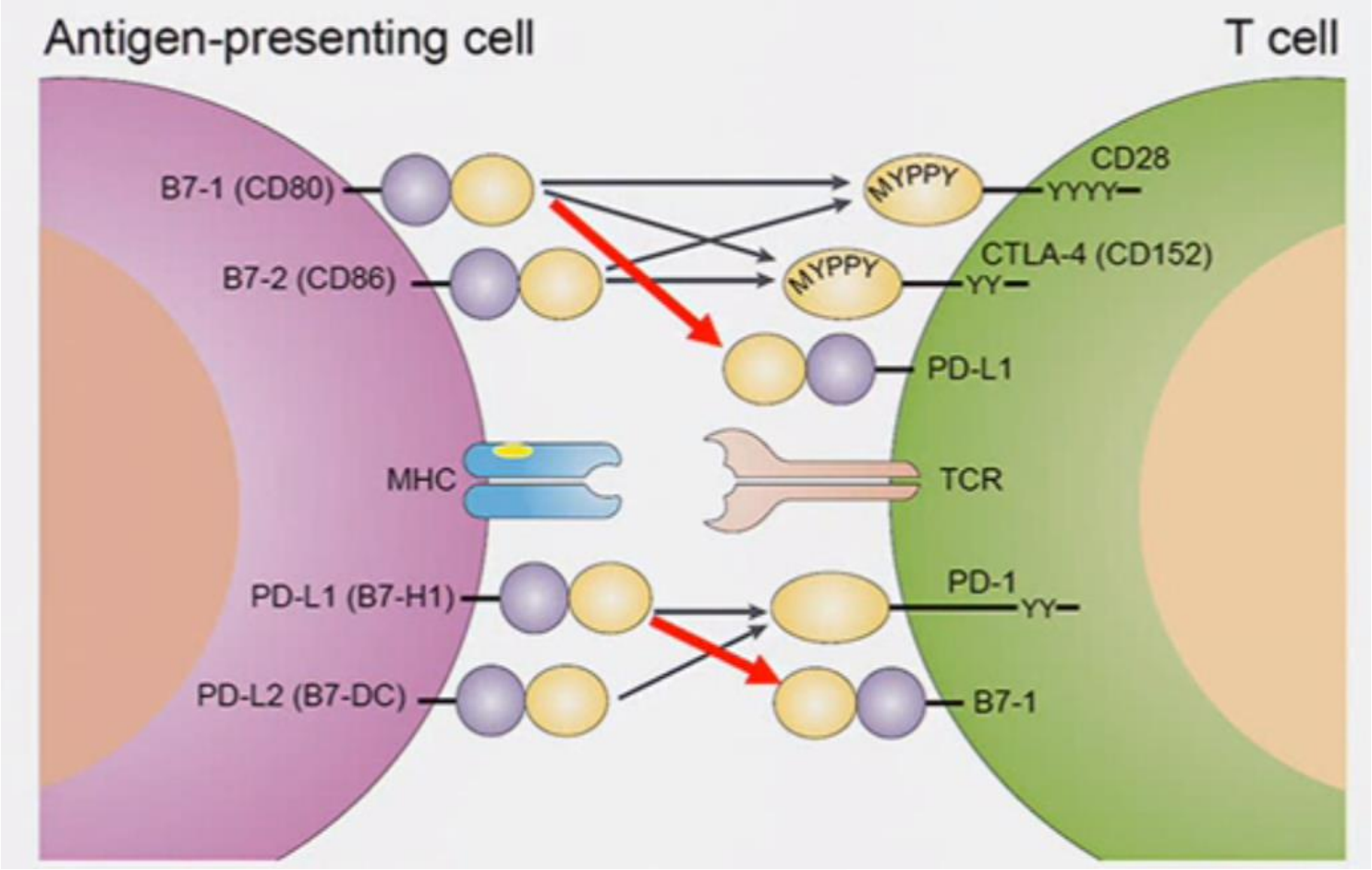
Ribas. N Engl J Med 2012

PD-1 pathway inhibits T cell response directly downstream of the TCR



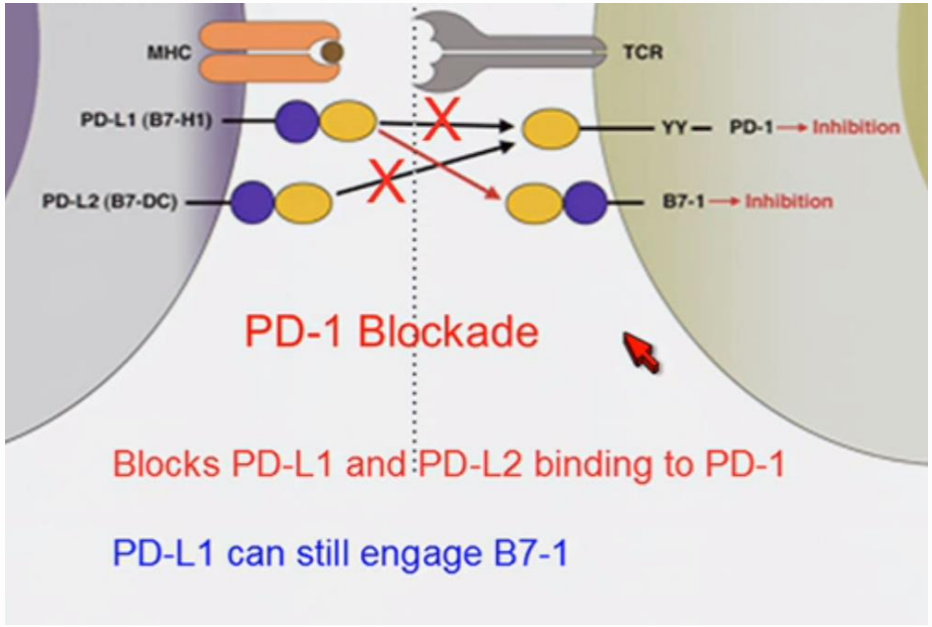
Freeman PNAS 2008

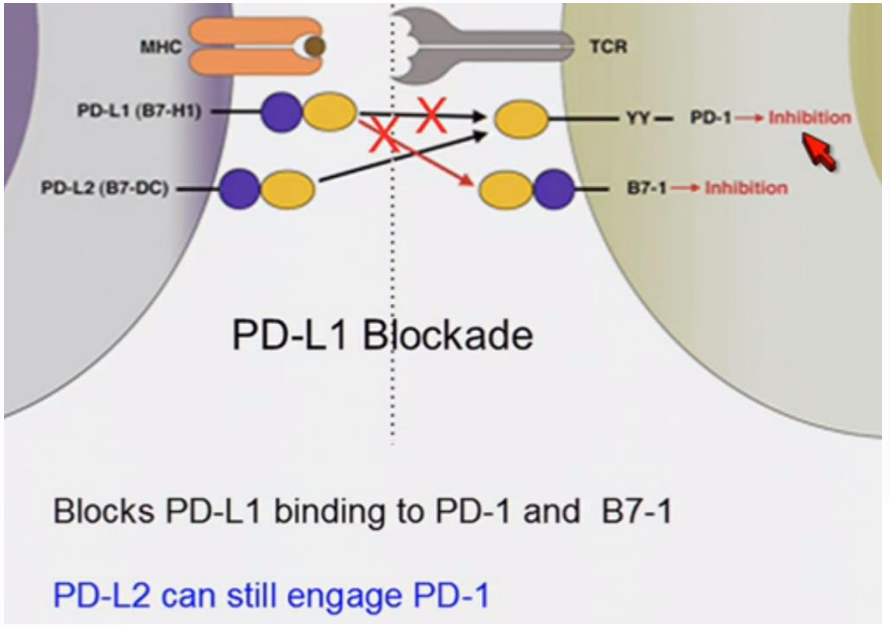
PD-1/PD-L1 interaction with B7-1



Arlene Sharpe, ASCO 2013

Blocking PD-1 versus PD-L1





Arlene Sharpe, ASCO 2013

Expression of PD1 ligands

	PD-L1 (B7-H1)	PD-L2 (B7-DC)
Hematopoietic cells	DC, macrophages, B cells, T cells, BM-derived mast cells	DC, macrophages, B cells, Th2 cells, BM-derived mast cells
Non-hematopoietic cells	Vascular endothelium, epithelia, muscle, liver, pancreatic islets, placenta, eye	Few, airway epithelia
Stimuli	Interferons (α, β, γ)	IL-4 + GM-CSG
Binding partners	PD1, B7.1	PD1
Expression by tumors	Melanoma, RCC, HNSCC, ovary, NSCLC	

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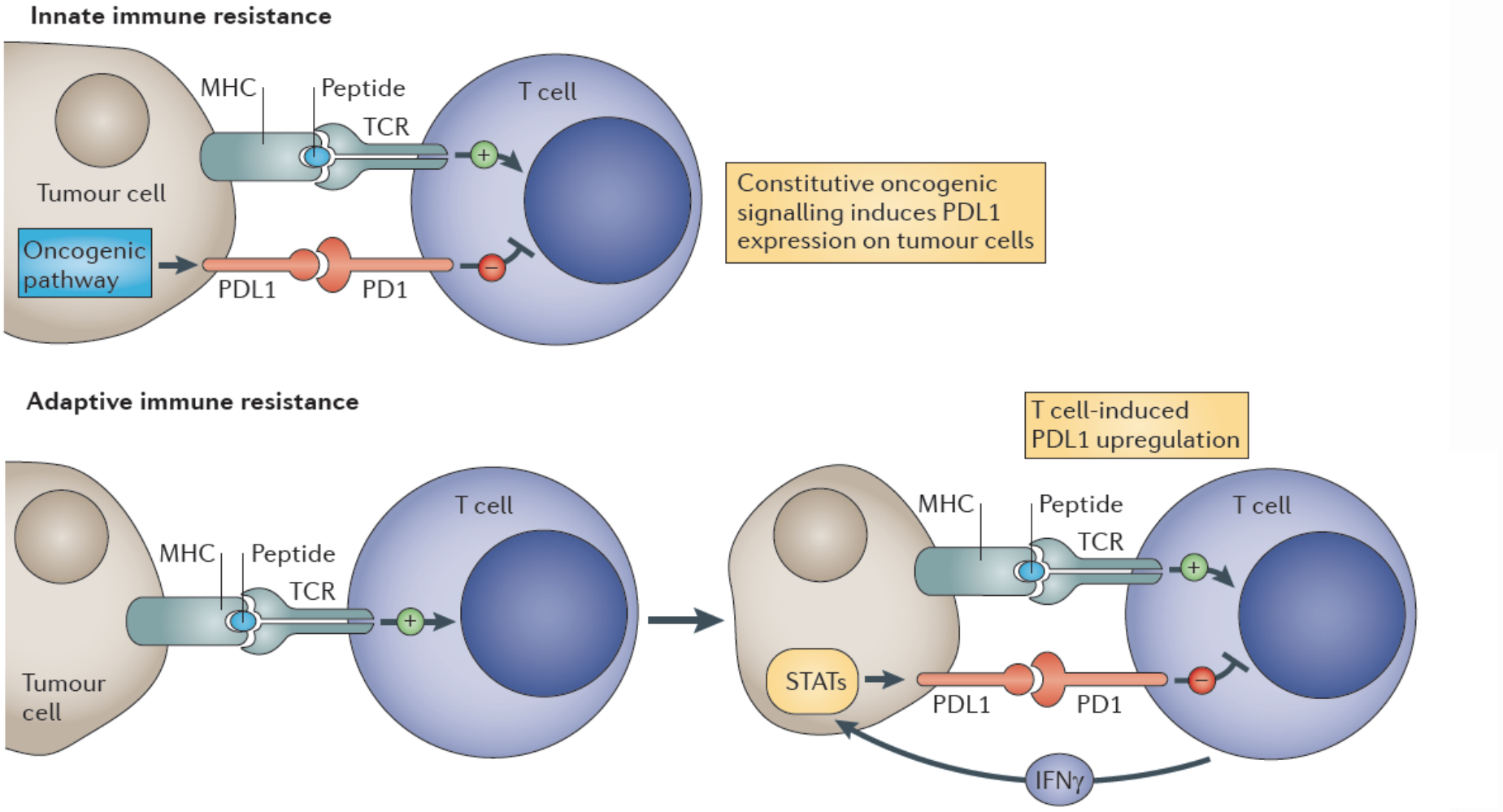
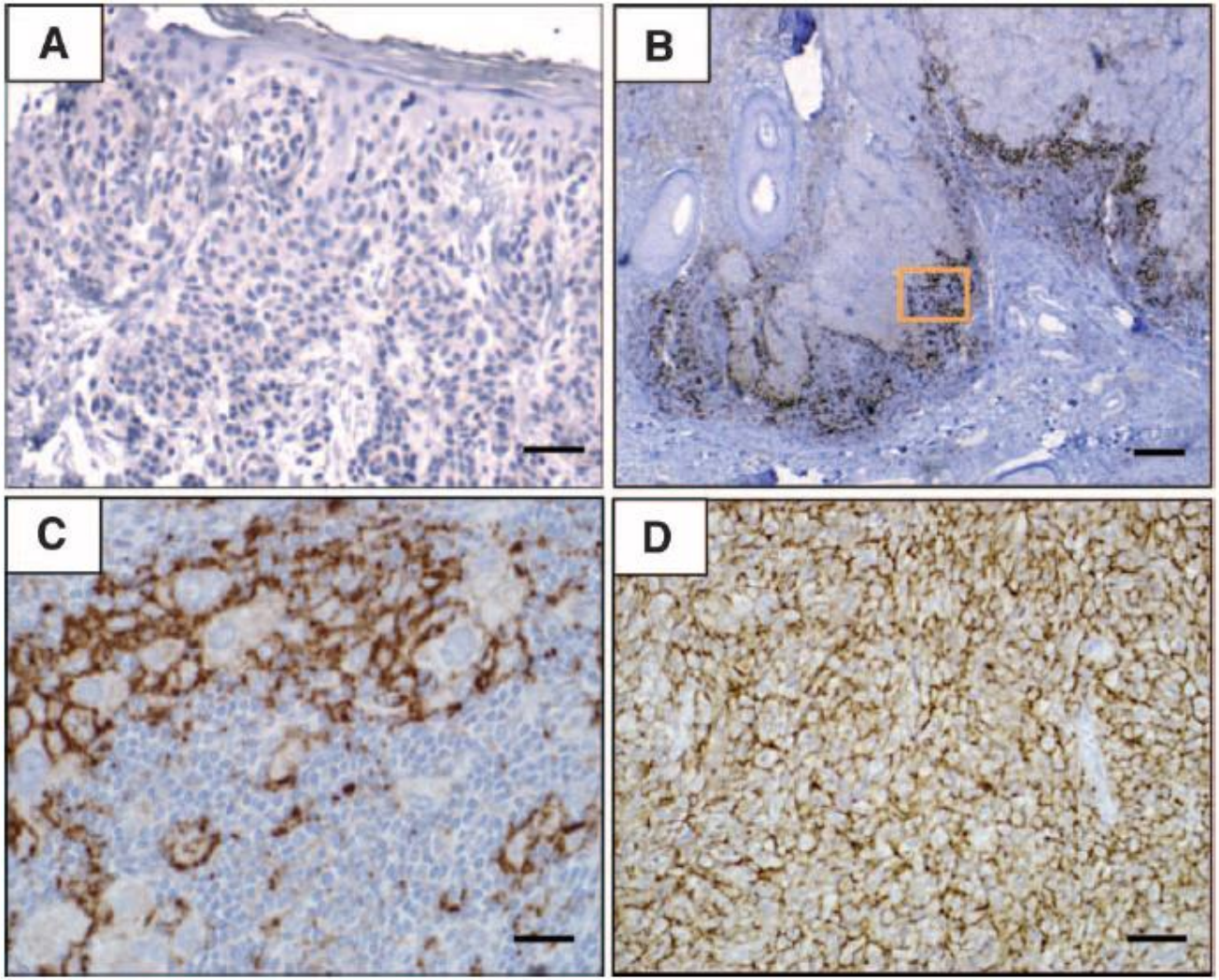
PD-L1 on human tumor cells mediates T cell inhibition



Pardoll DM, Nat Rev Cancer 2012

Expression of PD-L1 by melanoma



A: melanocytic nevus

B: primary melanoma

C: magnification of B

D: subcutaneous metastasis

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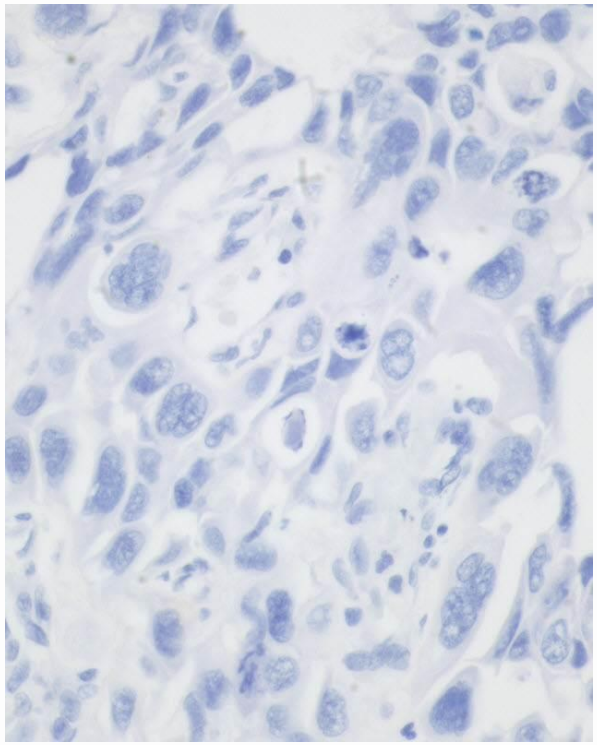
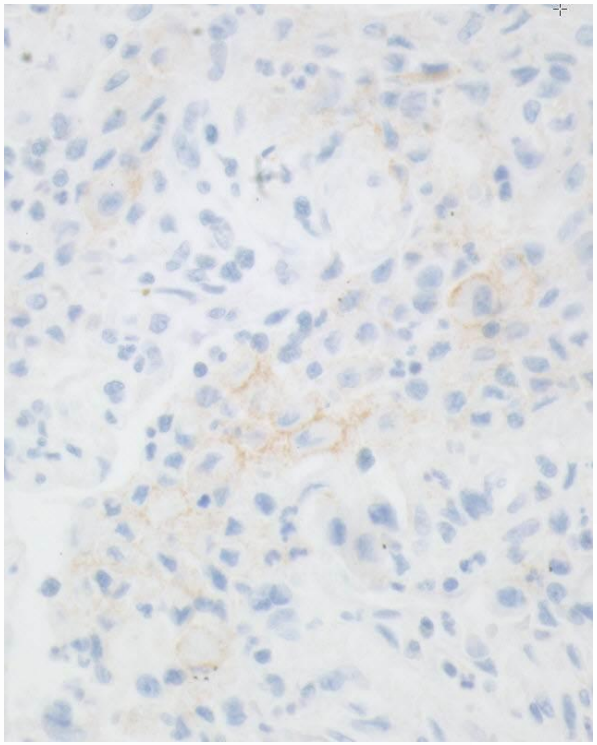
Taube et al. Science Transl Med 2012

PD-L1 NSCLC Sample IHC staining



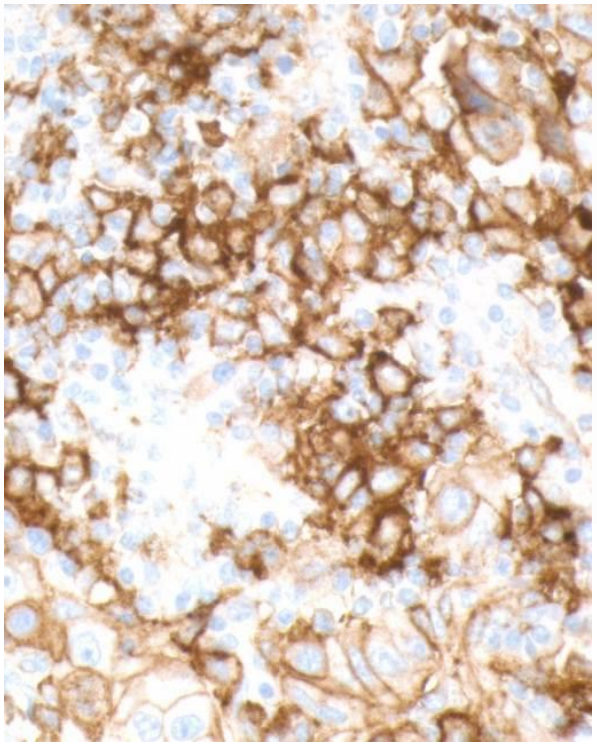
PD-L1 = 0% positive

Negative



PD-L1 = 2% positive

Weak Positive (1%-49%)

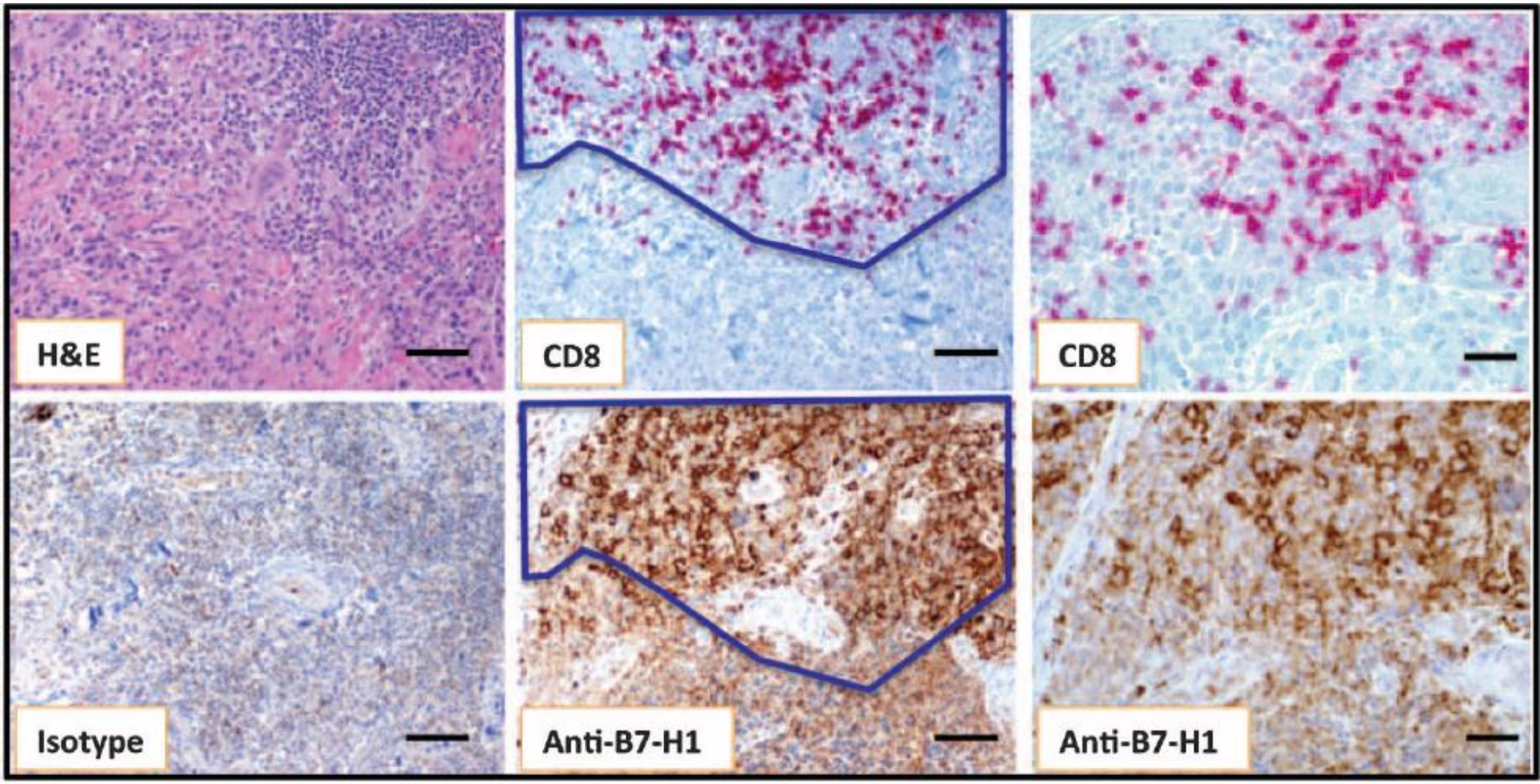


PD-L1 = 100% positive

Strong Positive (50%-100%)

Courtesy of N Rizvi

Expression of PD-L1 co-localizes with TILs



Taube et al. Science Transl Med 2012

PD-1 pathway is a good target for cancer immunotherapy

- PD-1 is highly expressed on tumor-infiltrating T cells and these are functionally exhausted cells

- Blockade of PD-1 or PD-L1 can reinvigorate exhausted TILs, enhancing their expansion, cytokine production, and cytolytic functions

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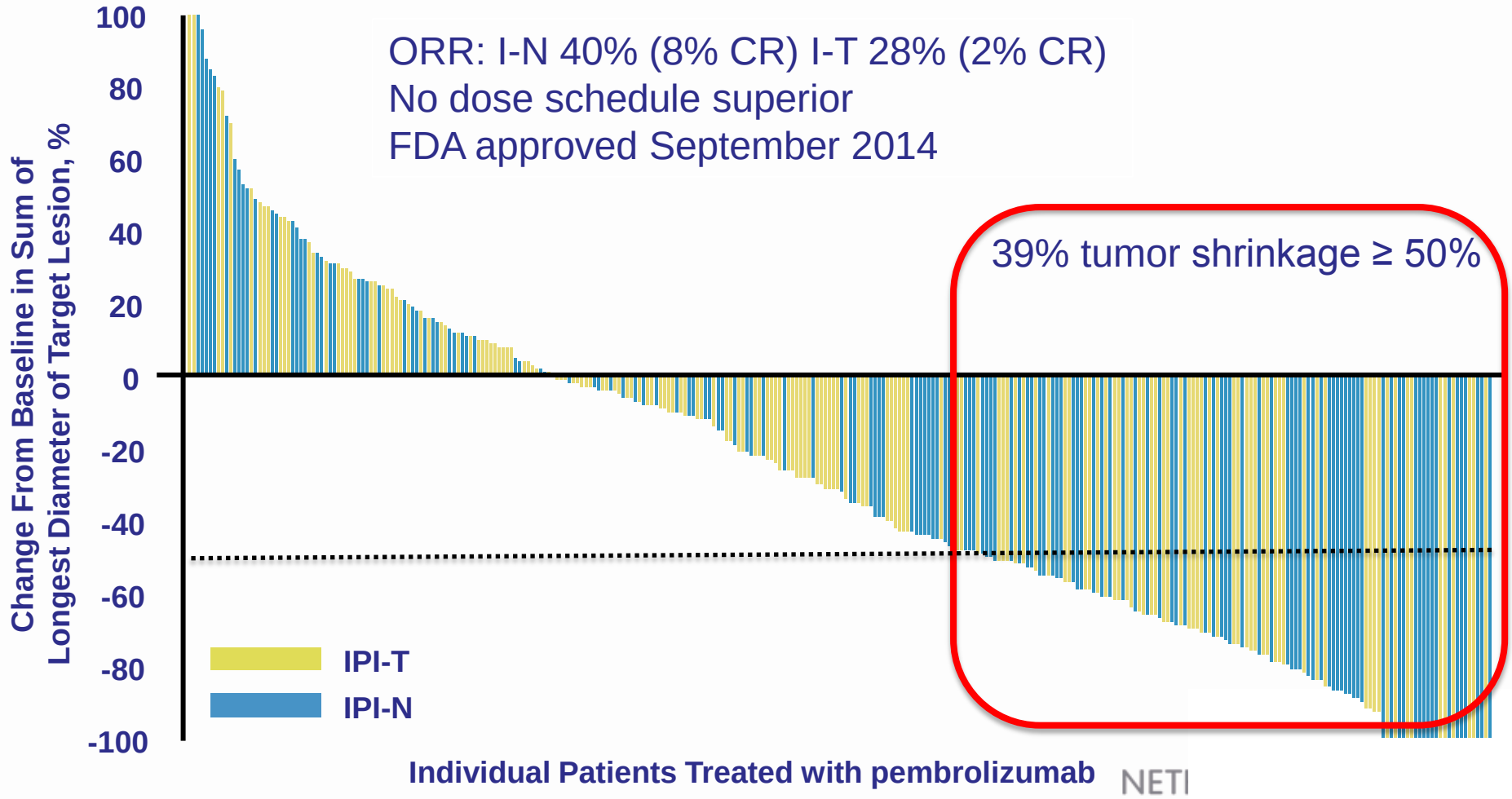
Anti-PD1/anti-PD-L1 mAbs currently in clinical testing

	Target molecule	
Source	PD1	PD-L1 (B7-H1)
Amplimmune Inc./GSK	AMP-224 (PD-L2/IgG1) fusion protein	
BMS	Nivolumab (MDX-1106)(human IgG4)	MDX-1105/BMS-936559 (human IgG4)
CureTech/Teva	Pidilizumab (CT-011) (humanized IgG1)	
MSD	Pembrolizumab (humanized IgG4)	
Roche/Genentech		MPDL3280A (engineered human IgG1)
MedImmune		MEDI-4736 (engineered human IgG1)
MedImmune	AMP-514	

Adapted from Topalian et al., Curr Opin Immunol 2012

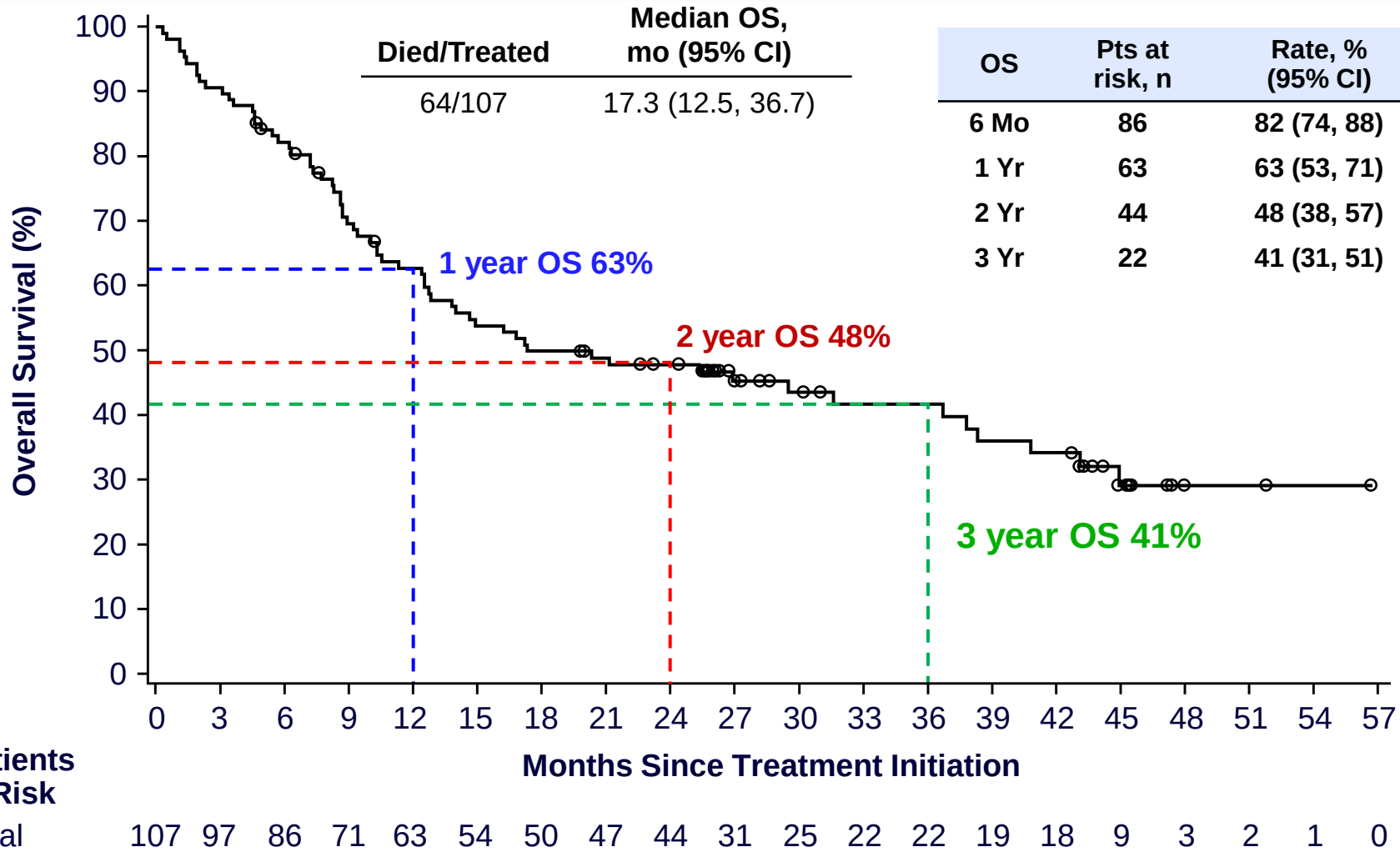
Maximum Percent Change from Baseline in Tumor Size[a] (Central Review, RECIST v1.1)



[a]In patients with measurable disease at baseline by RECIST v1.1 by central review and ≥1 postbaseline assessment (n = 317).
Percentage changes >100% were truncated at 100%.
Analysis cut-off date: October 18, 2013.

Presented by: Antoni Ribas at ASCO 2014

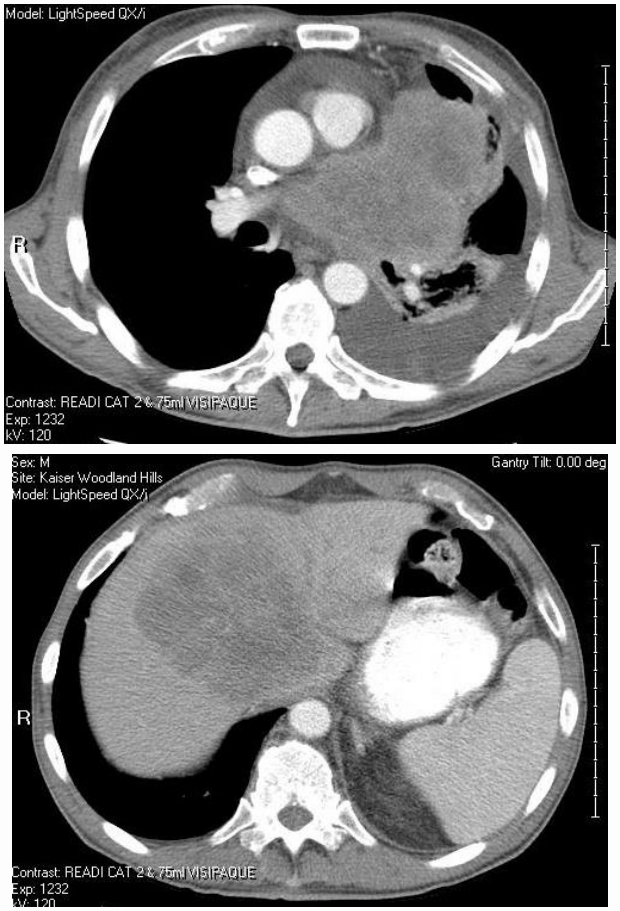
Overall Survival for Patients with Melanoma Treated with Nivolumab



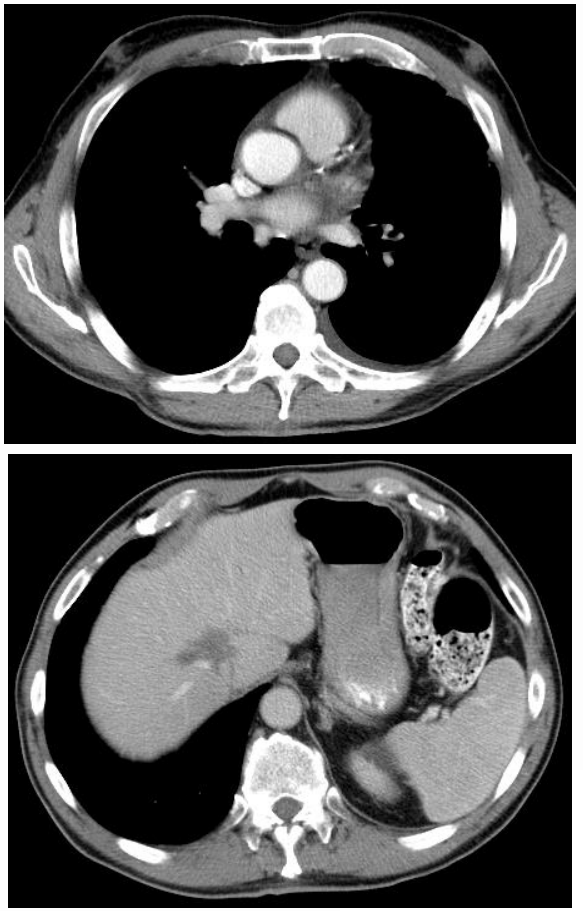
Presented by: F. Stephen Hodi at ASCO 2014

Pembrolizumab

Baseline: April 13, 2012



April 9, 2013



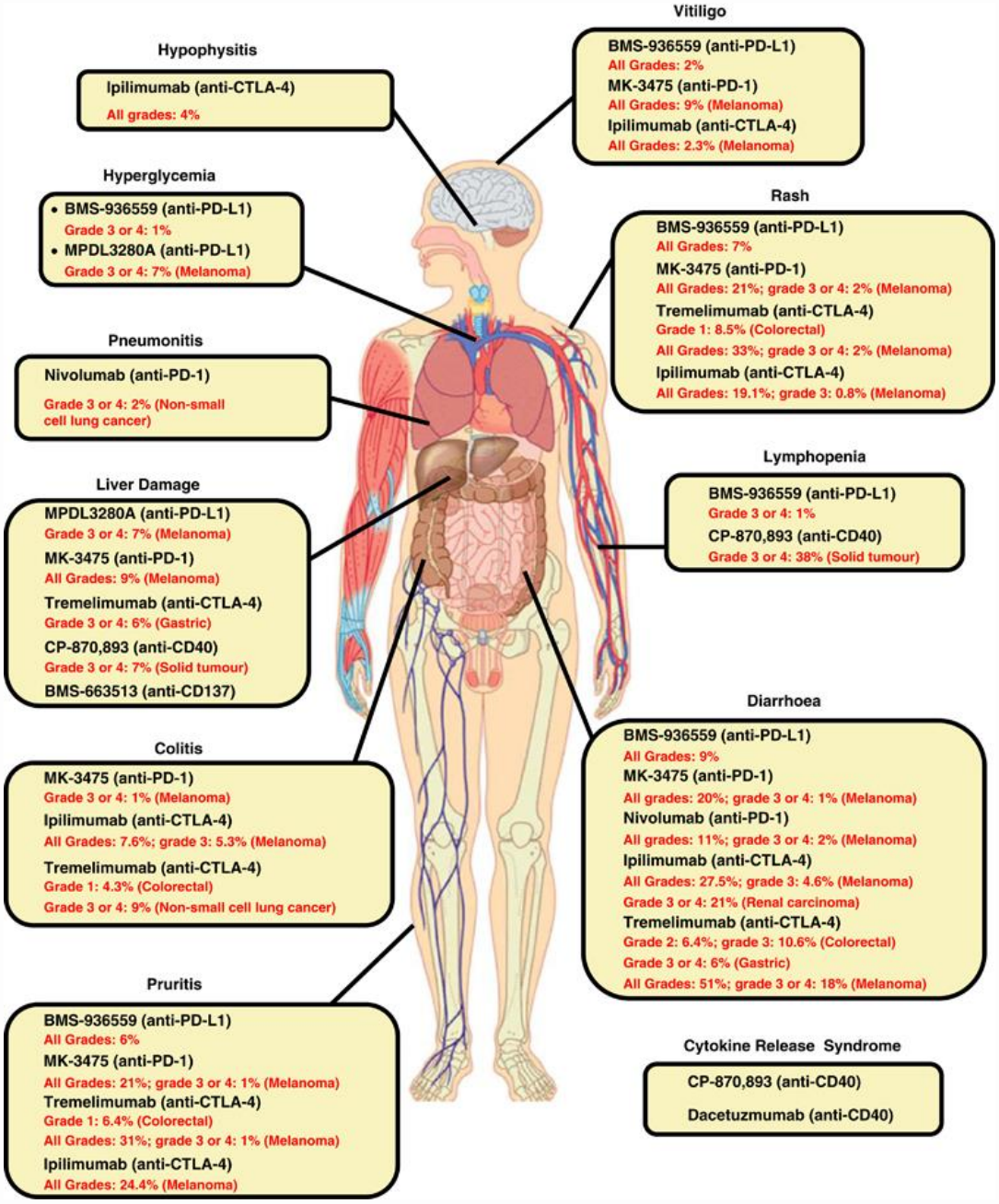
72-year-old male with symptomatic progression after bio-chemotherapy, HD IL-2, and ipilimumab

Images courtesy of A. Ribas, UCLA

Conclusion PD1/PD-L1 blockade

- PD1/PD-L1 are involved in peripheral tolerance
- PD1 is expressed by activated and “exhausted” T cells
- Tumors may express PD-L1 as a result of immune interrogation by T cells or as a result of an oncogenic event
- Blockade of PD1/PD-L1 interaction at the tumor site results in tumor destruction and clinical response
- Tumors expressing PD-L1 have a higher chance of responding to PD1 blockade compared to PD-L1 negative tumors
- PD1/PD-L1 blockade is an active treatment for many tumor types (melanoma, RCC, NSCLC, bladder cancer)

Immune related adverse events



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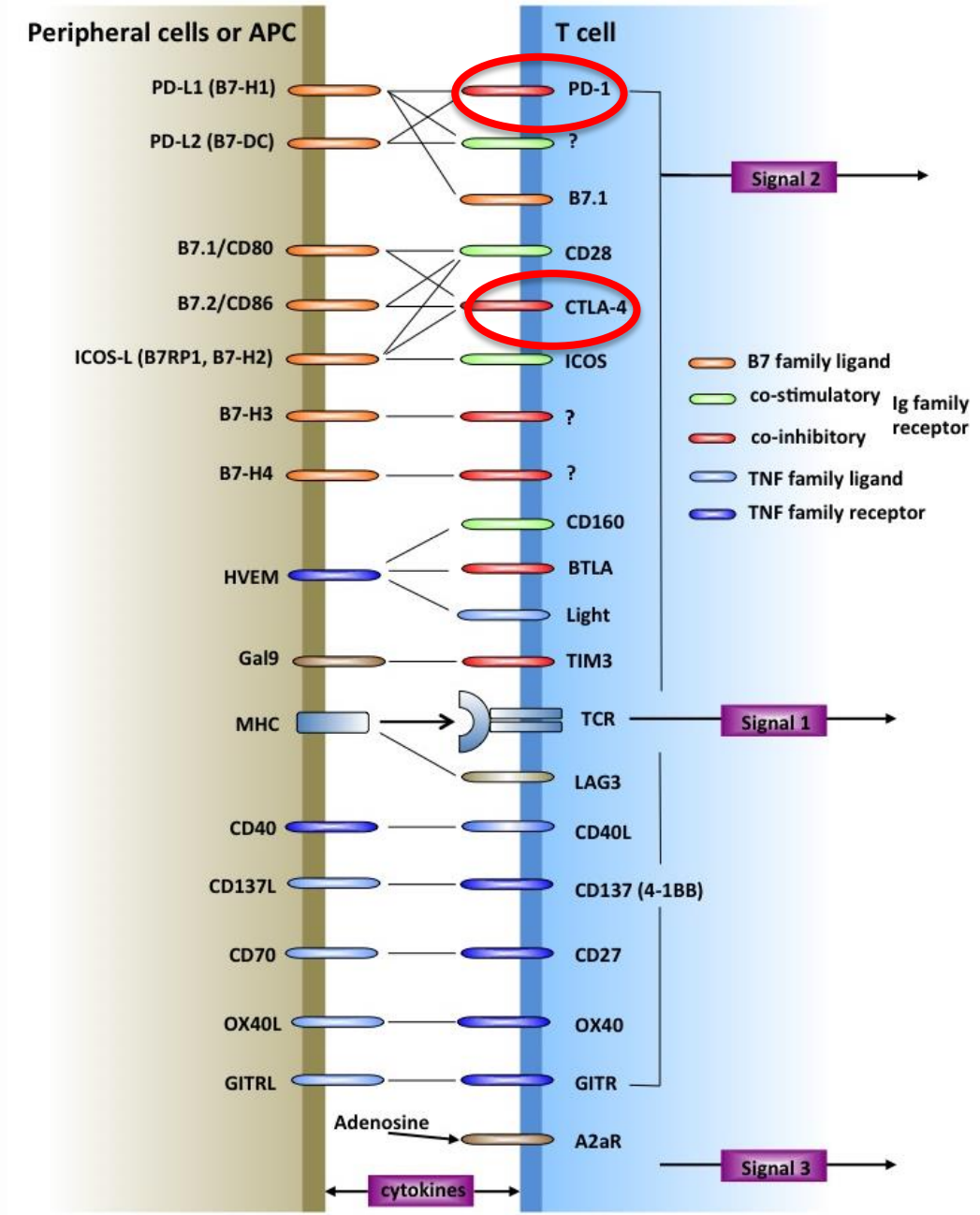
Liu et al, Clinical & Translational Immunology (2014)

Checkpoint molecules



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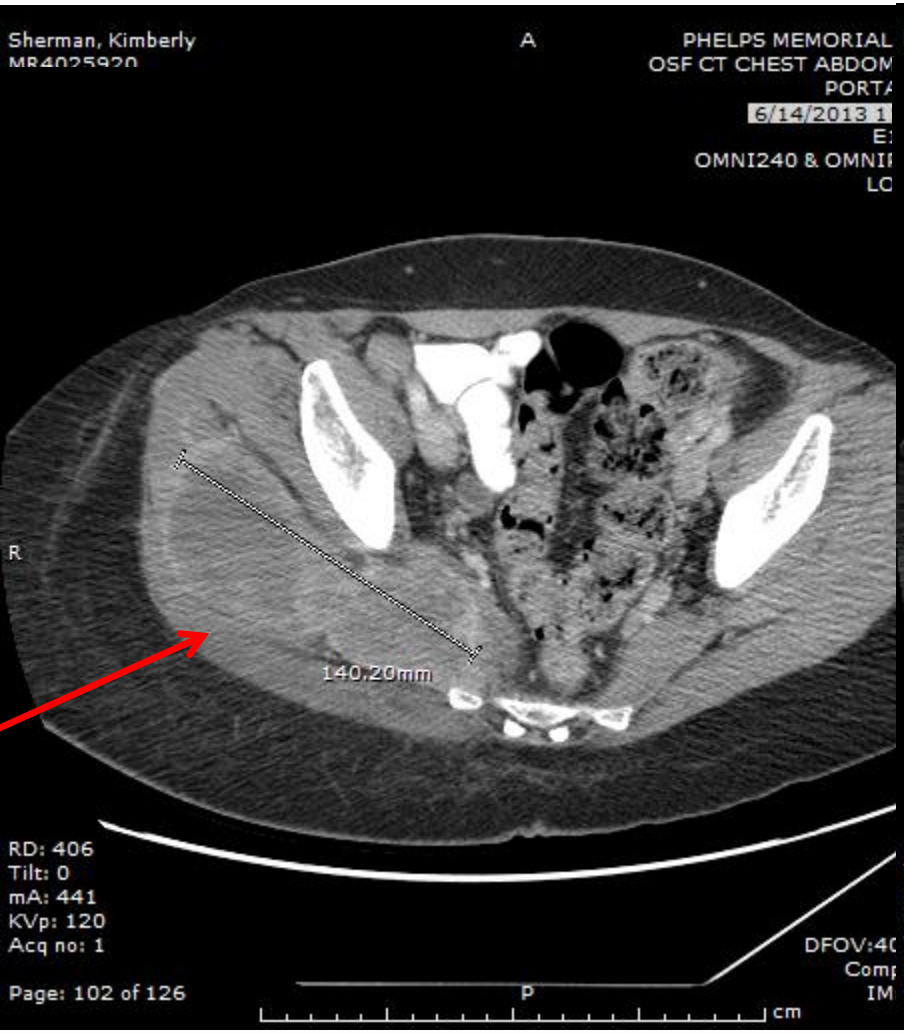
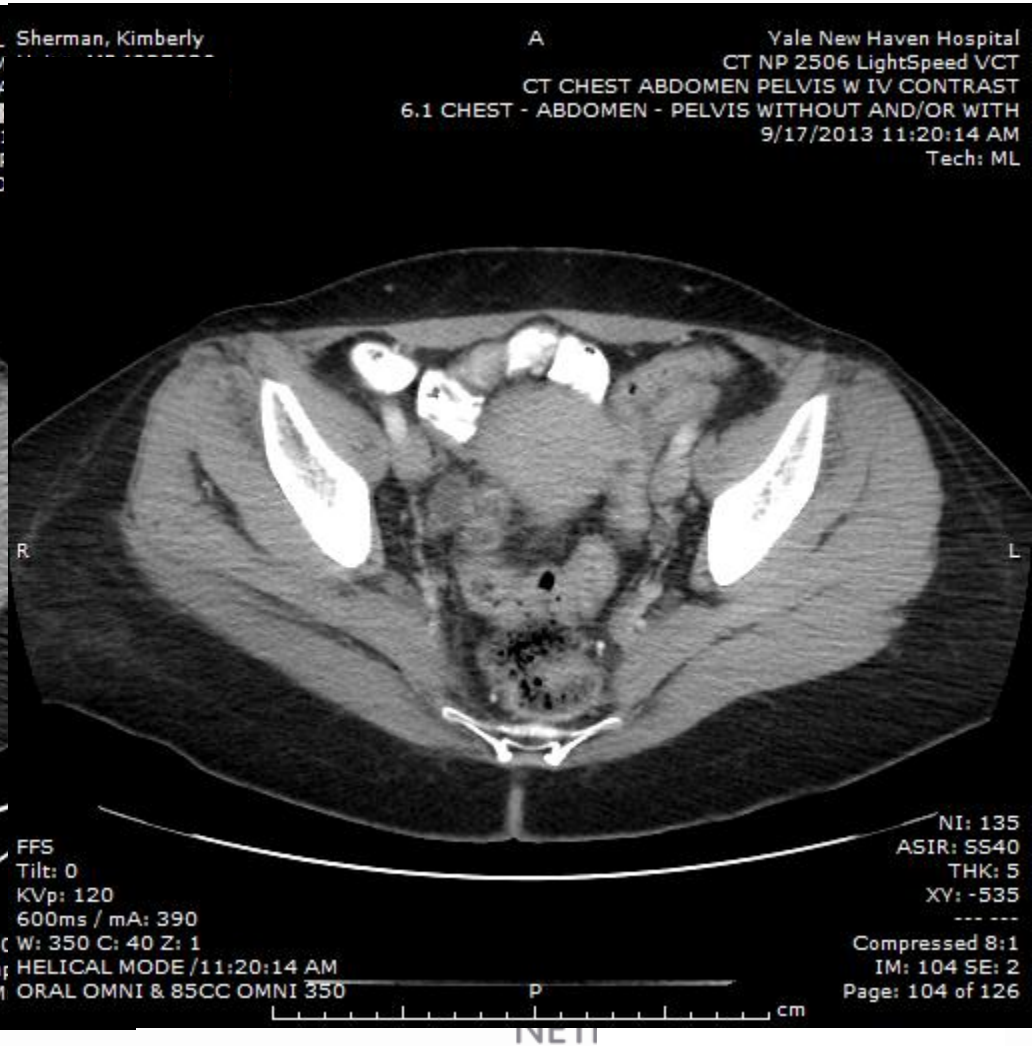
Blank Curr Opin Oncol 2013

ipilimumab + nivolumab, response at 12 weeks

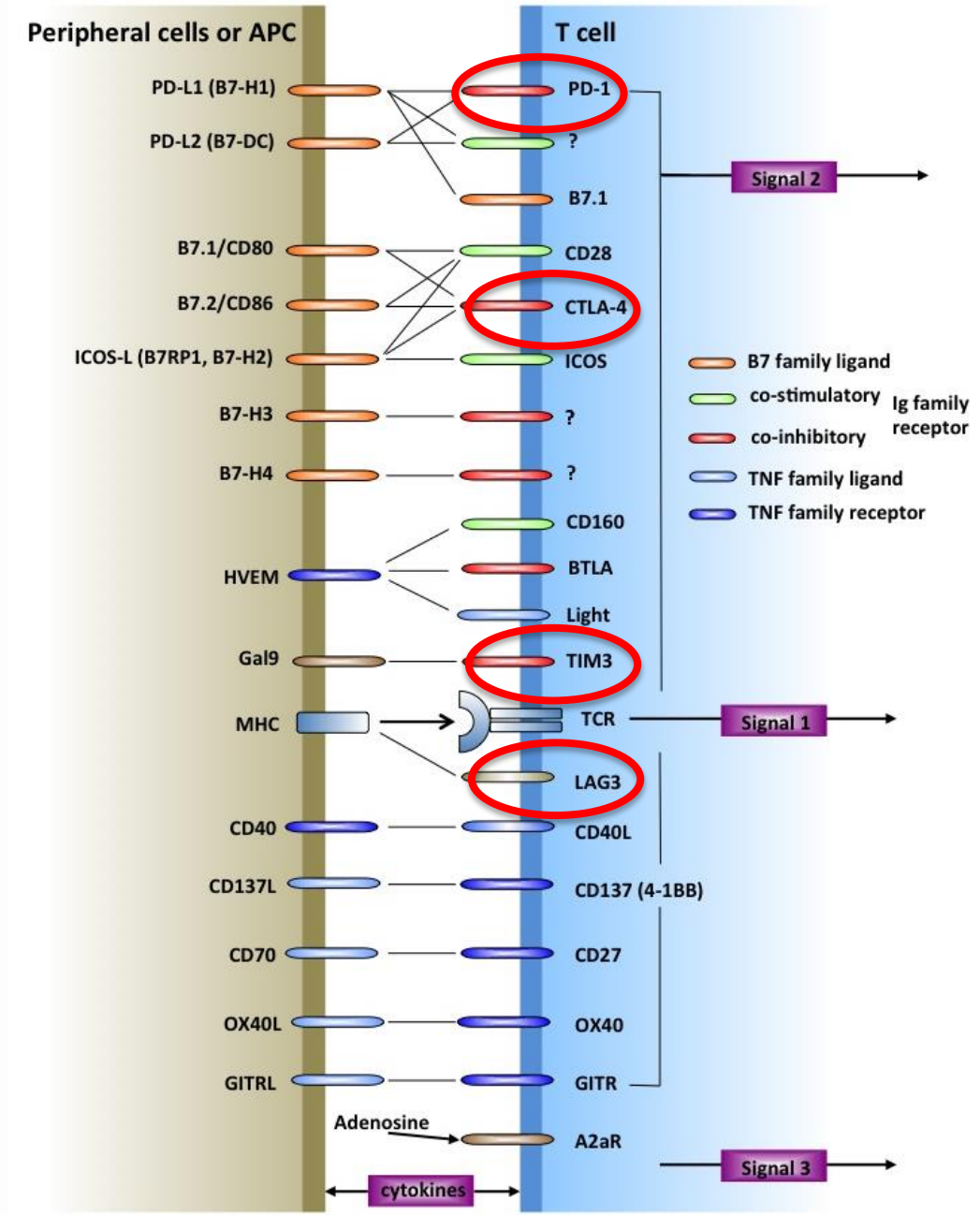




Prior therapy with HD-IL2, multiple resections, Vemurafenib, and RT; LDH > 2000 at baseline; LDH nearly normal within 3 weeks

Courtesy of R. Kefford

Checkpoint molecules



Blank Curr Opin Oncol 2013

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Immune Checkpoints

- Blocking of immune checkpoint has revolutionized immunotherapy of cancer
- Many cancer types show reactivity for immune checkpoint inhibition
- No true biomarkers have been found to highly predict response to treatment
- Immune checkpoint inhibitors induce a new class of adverse events
- Immune checkpoint inhibitors can be combined with other treatment

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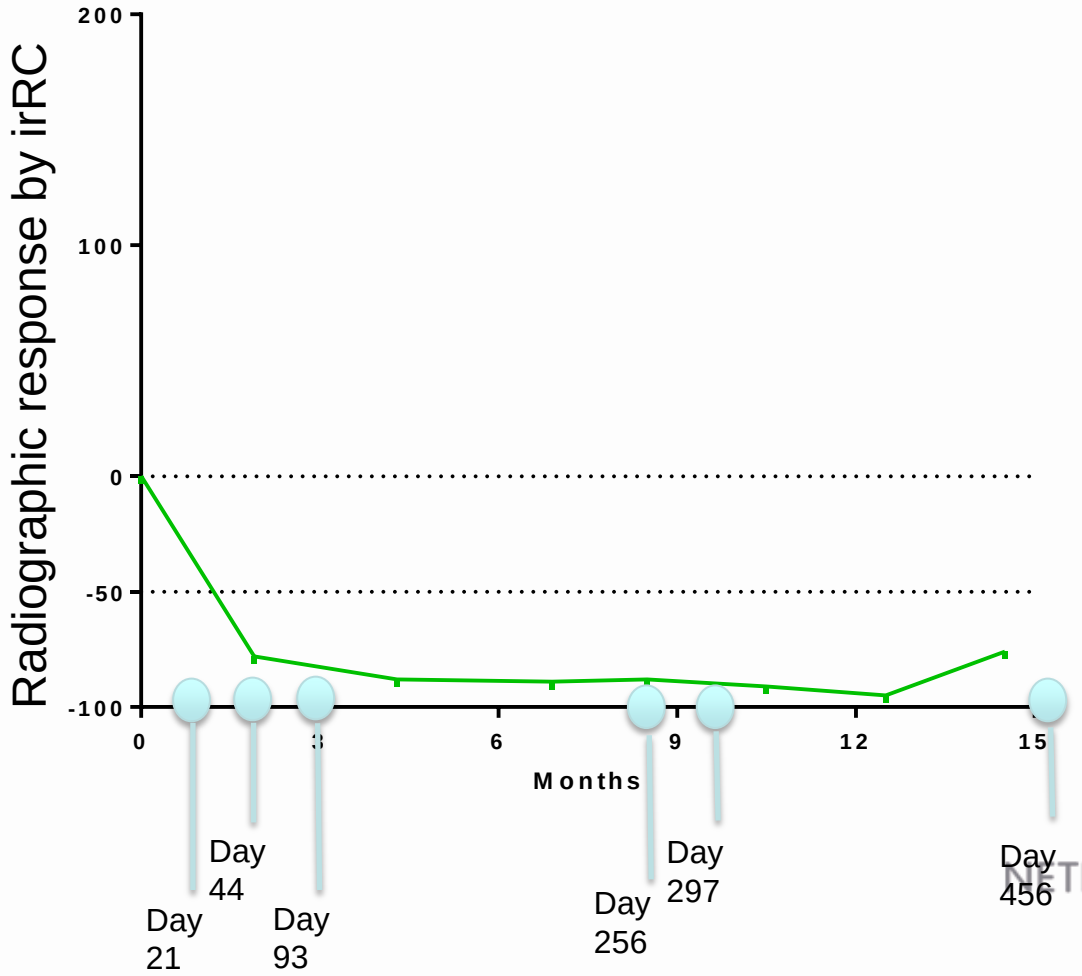
NSCLC: clinical responder to PD-1 blockade (MSKCC)



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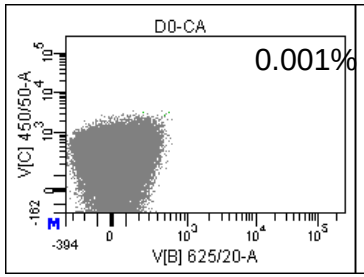
Induction of neo-antigen specific T cell reactivity upon PD-1 blockade

Day 0



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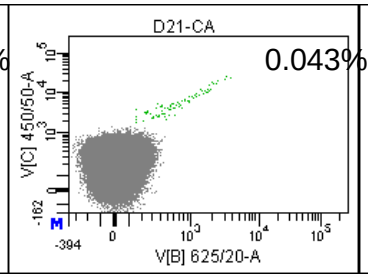

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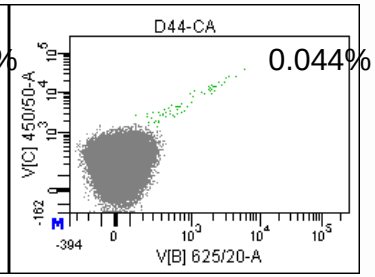
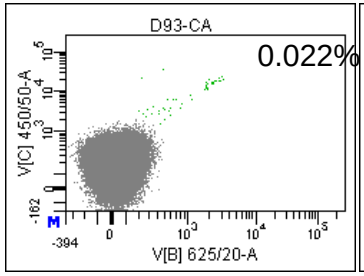


Day 93



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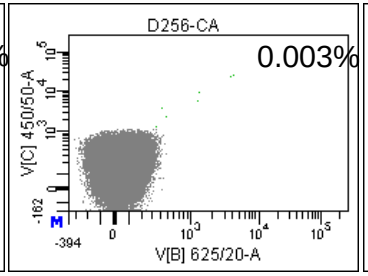

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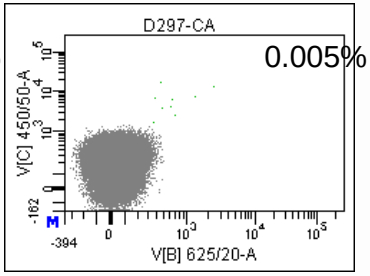

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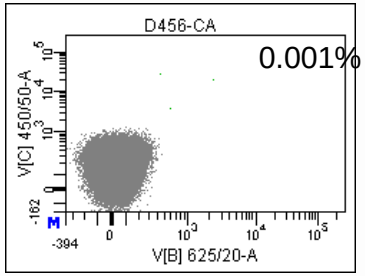

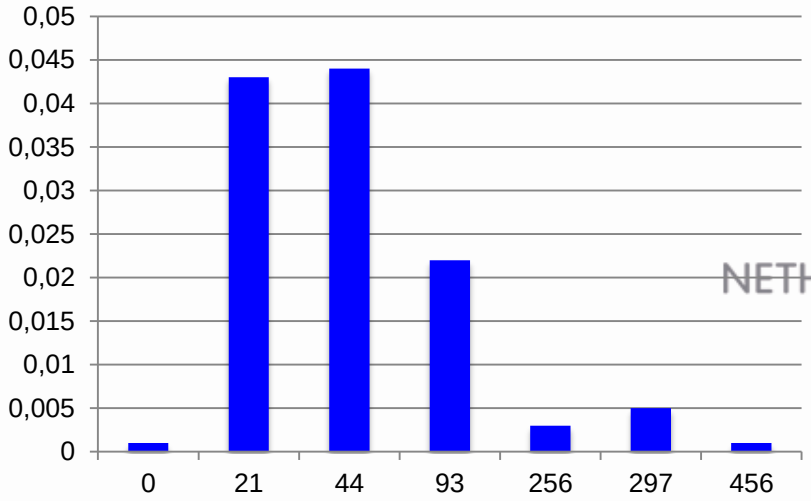

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