The immune system and lung cancer

-OR-

If the immune system is so great, why didn't it work in the first place?

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Disclosures

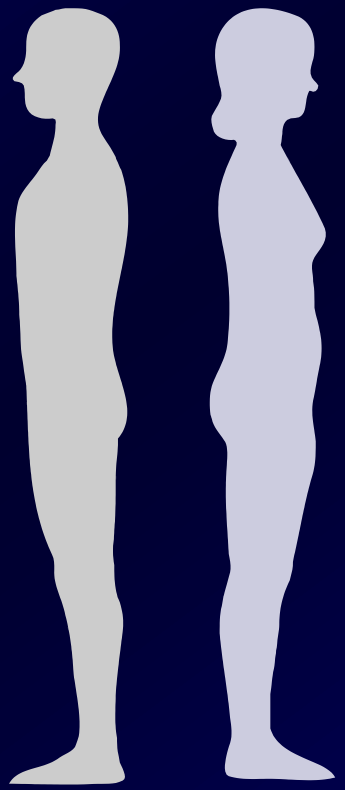
- Bayer Health Care
- Biodesix
- Biothera
- Boehringer Ingelheim
- Bristol Myers-Squibb (BMS)
- Clovis Oncology
- Eisai Inc.
- Genentech/Roche
- GlaxoSmithKline (GSK)
- MedImmune
- Merck
- Novartis
- Peregrine Pharmaceuticals, Inc.
- Pfizer
- Synta Pharmaceuticals Corp.

This includes receipt of grants/research support, receipt of honoraria or consulting fees, and participation in company sponsored speaker's bureaus.

Top Ten Leading Causes of Cancer-related Deaths*

Leading Sites by Sex, United States, 2014 Estimates

Lung and bronchus	28%
Prostate	10%
Colon and rectum	8%
Pancreas	7%
Liver and intrahepatic bile duct	5%
Leukemia	5%
Esophagus	4%
Urinary bladder	4%
Non-Hodgkin lymphoma	3%
Kidney and renal pelvis	3%

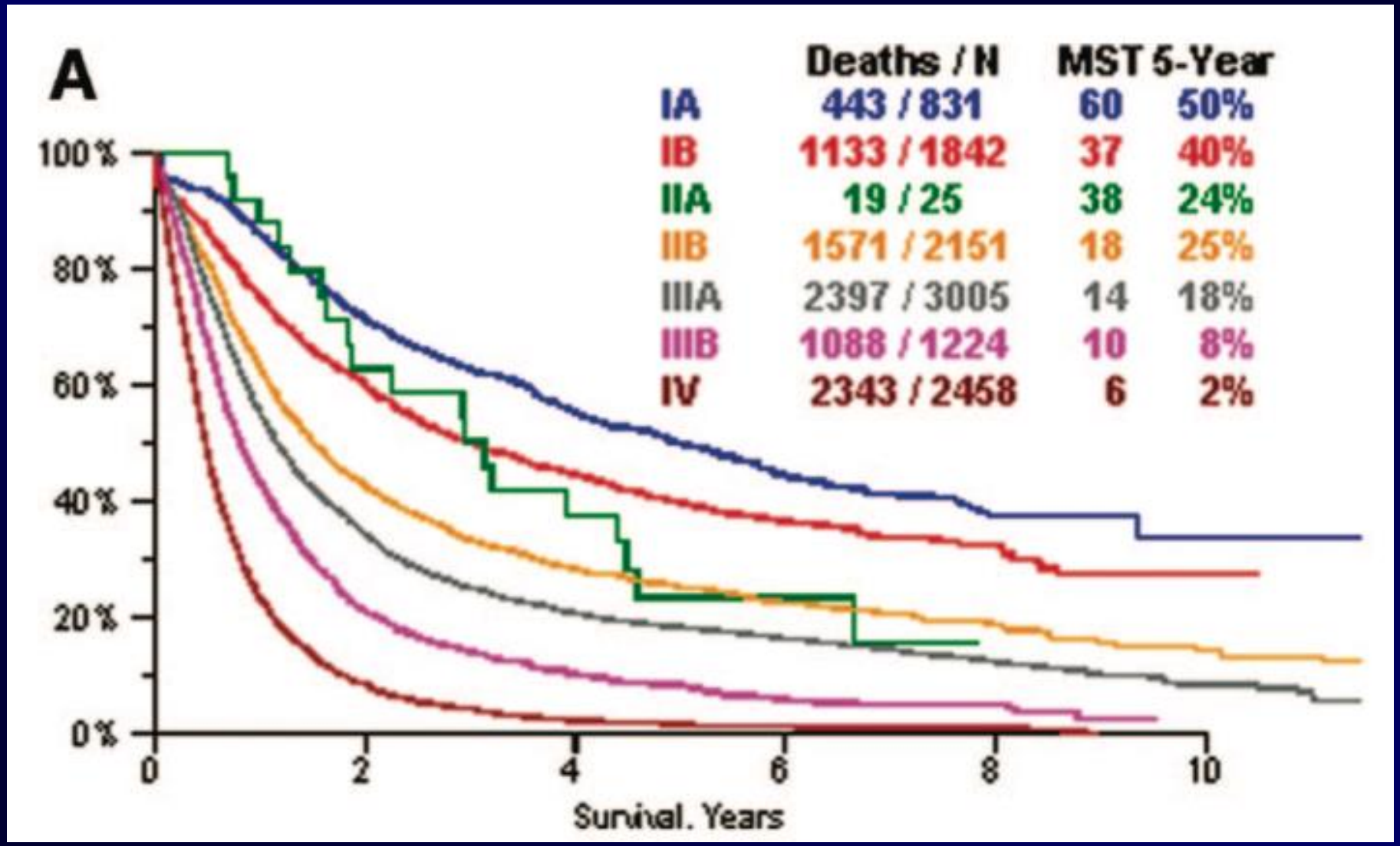


26%	Lung and bronchus
15%	Breast
9%	Colon and rectum
7%	Pancreas
5%	Ovary
4%	Leukemia
3%	Non-Hodgkins lymphoma
3%	Uterine corpus
2%	Brain and other nervous system
2%	Liver and intrahepatic bile duct

*Excludes basal and squamous cell skin cancer and carcinoma in situ, except urinary bladder.

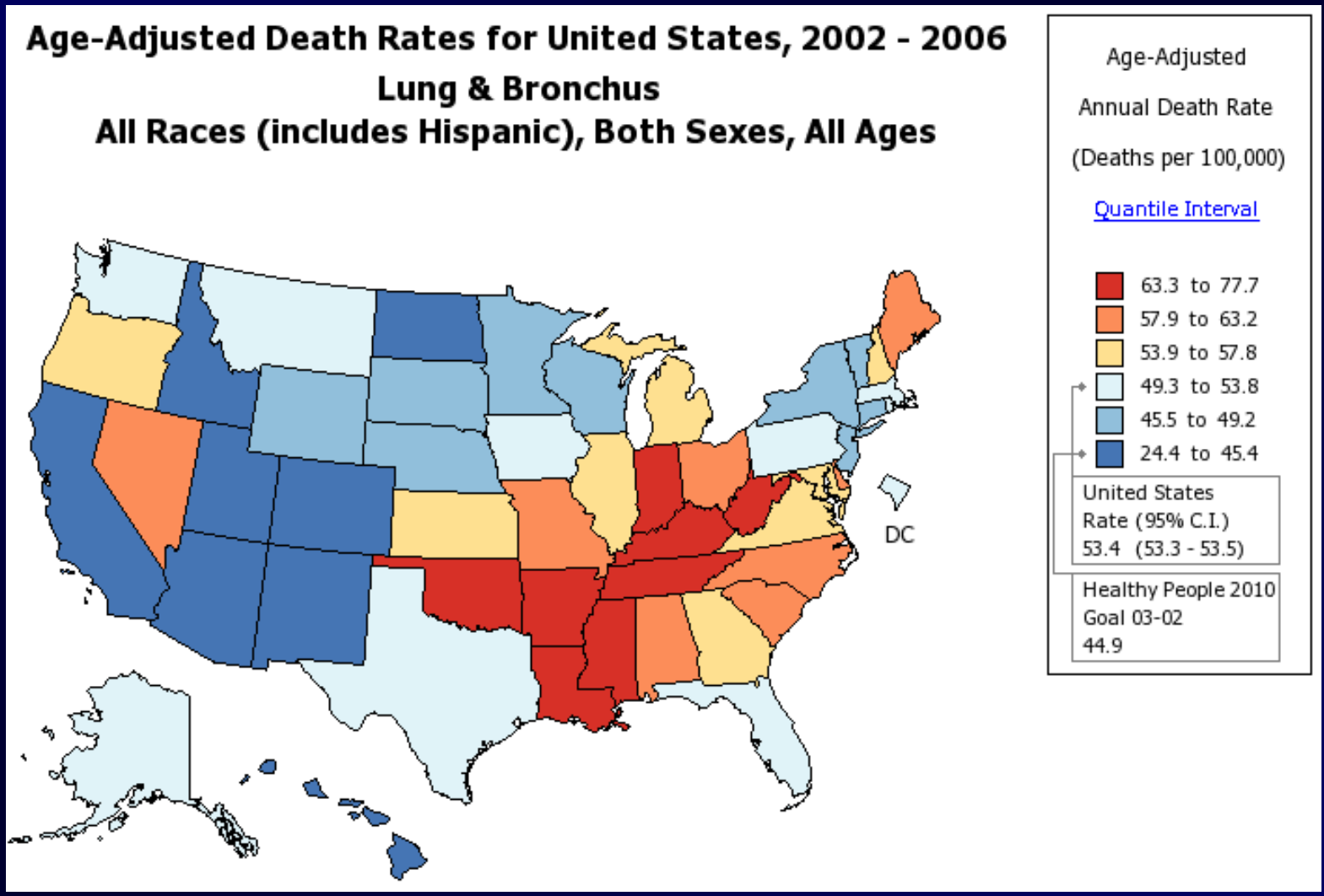
American Cancer Society. *Cancer Facts & Figures*. 2014.

Stage-specific survival, NSCLC



Goldstraw, JTO 2009

Lung Cancer in the region

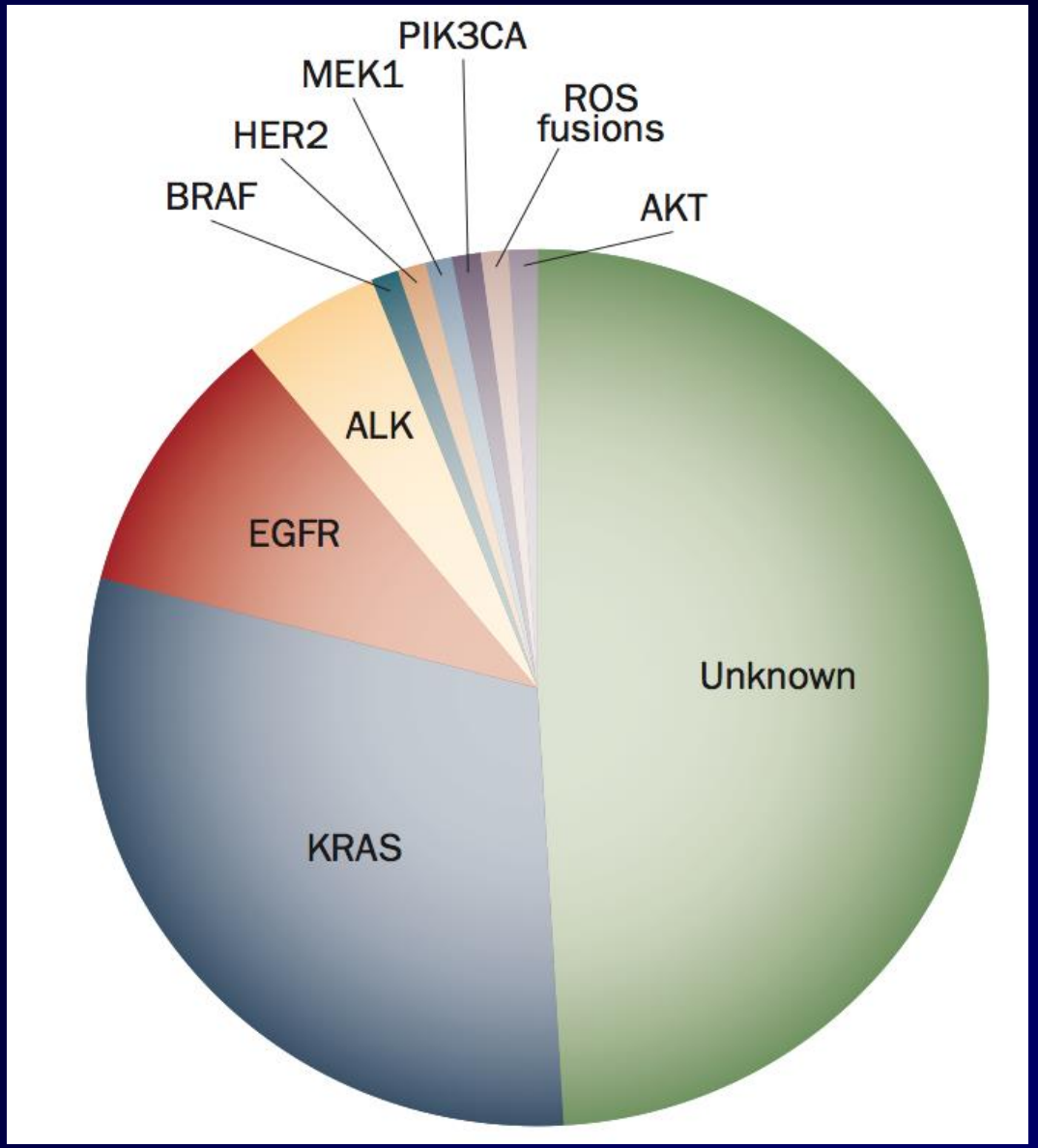


Cancer 5-year survivals

Organ site	1974 5-yr survival	2012 5-yr survival	Improvement
Lung Ca	13%	17%	4%
Colon Ca	50%	64%	14%
Breast Ca	75%	90%	15%
Prostate ca	67%	>99%	32%

Incidence of Oncogene Mutations- Adenocarcinoma

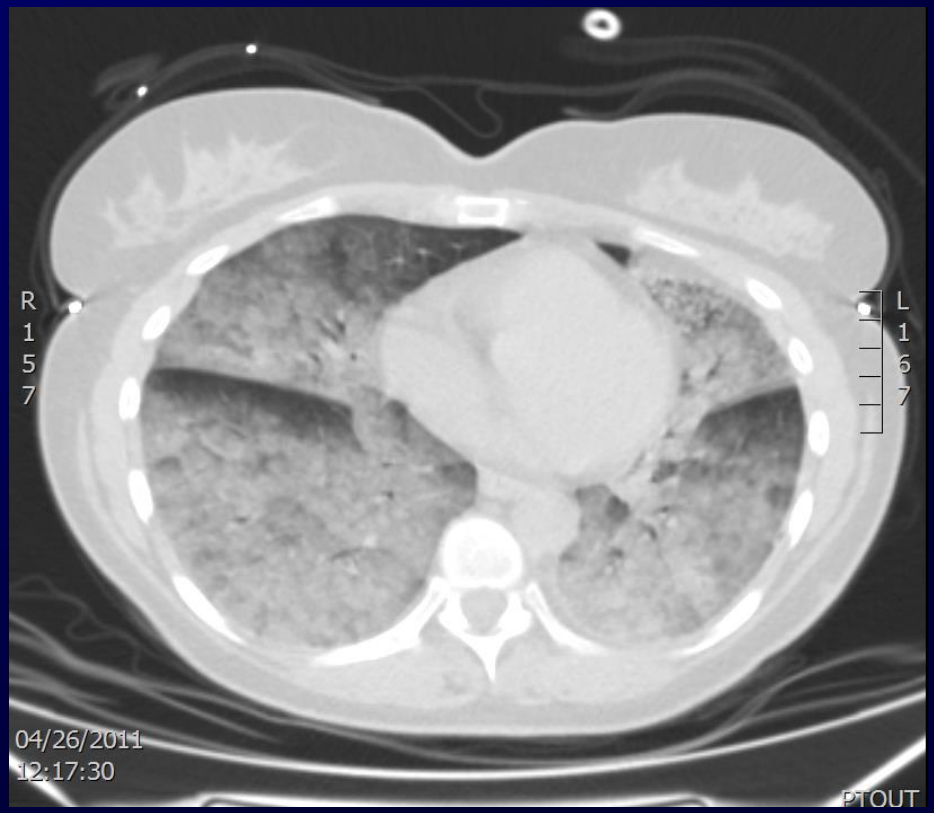
ALK and EGFR targeting are now first-line therapies of choice. Others on the way



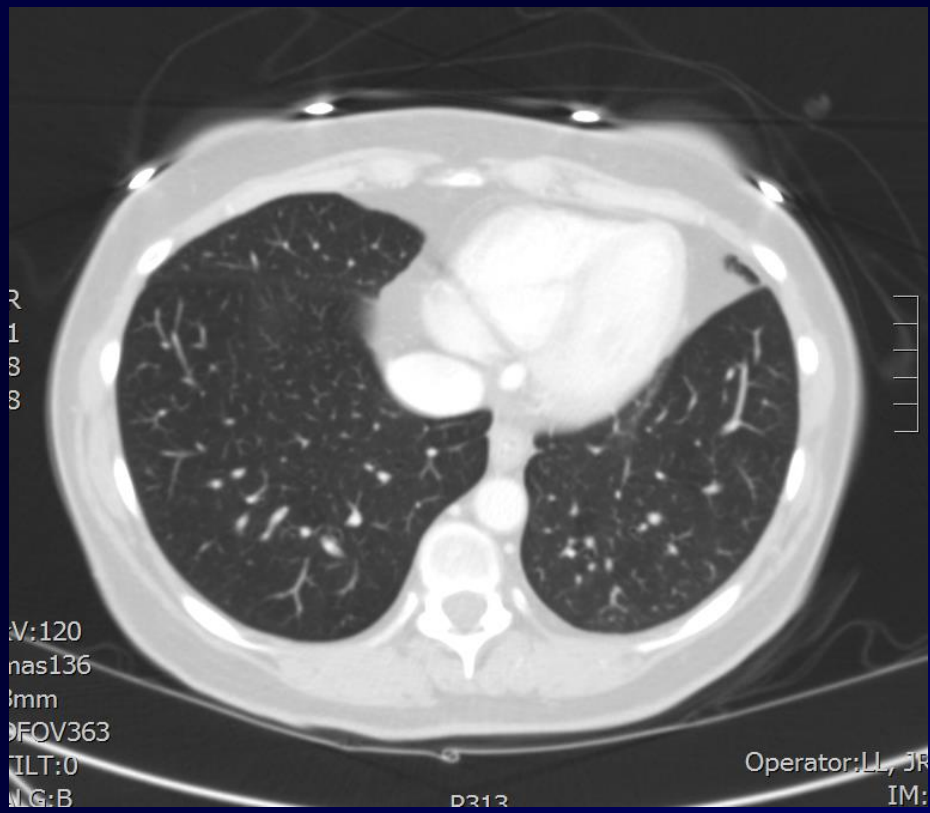
Lovly & Carbone (2011). *Nature Reviews Clinical oncology*, *8*(2), 68–70.

A human model of mouse cancer

Response to crizotinib (Xalkori) in ALK+ NSCLC



4/26/2011



9/27/2011

BUT, responses tend to be short-lived, ca. one year

Immunotherapy

- The immune system has evolved over millions of years to detect and eliminate “non-self”.
- Potentially exquisitely specific and sensitive, able to detect single amino acid changes, even in intracellular proteins.
- Adaptable to novel challenges not previously seen (hundreds of novel protein sequences in lung cancers e.g. mutant oncoproteins)
- Highly regulated to avoid self-toxicity
  - Exactly these regulatory mechanisms are usurped by clinically evident tumors to escape immune elimination
- More promise than reality until now.

Clinically evident tumors must have evaded immune recognition/killing

- **Avoided immune surveillance**
  - **clearance of readily recognized tumor cell clones**
- **Structural alterations of tumor antigen presentation to avoid immune recognition**
  - **In ~5-10% of human tumors: Deletion/mutation of MHC class I, β-2 microglobulin, TAP1**
- **Functional alterations to avoid immune recognition**
  - **For 90-95% of human tumors, we see:**
    - **Failure to induce a response**
    - **Failure of responding T cells to effectively kill tumor targets**
    - **Both soluble and cell surface immune-regulatory factors**
  - **These defects can theoretically be overcome**

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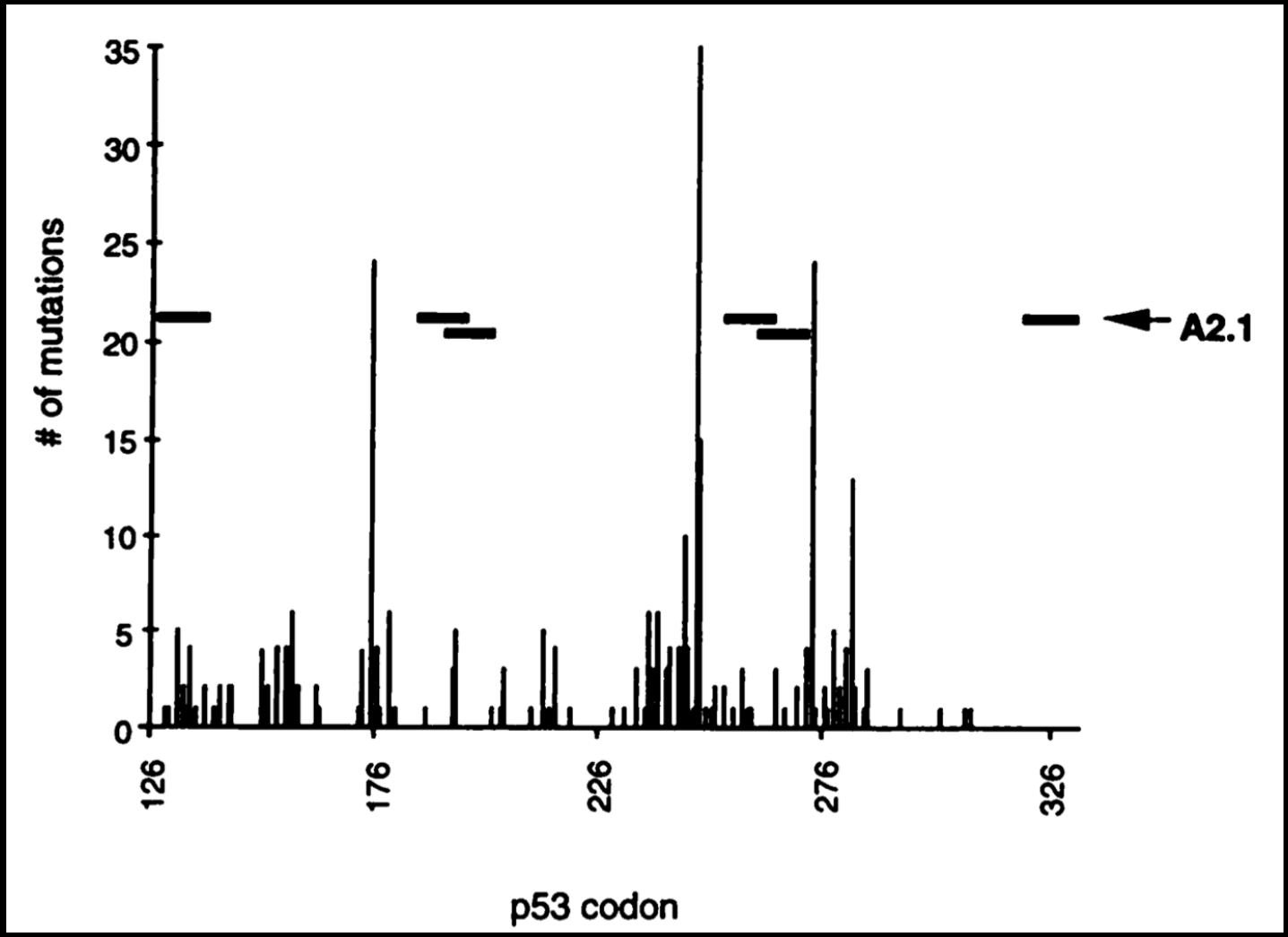
Evidence for selection against mutant epitopes on class I MHC



<u>None</u> of the HLA-A2.1 + patients had a tumor with a p53 mutation in peptides predicted to be efficiently presented on A2.1

Wiedenfeld and Carbone, 1994

No mutations match motif

Table 1 *Comparison of the frequency of HLA A*0201 alleles in tumors bearing missense p53 mutations that either lie within or outside the consensus peptide motif [X(ILM)XXXXXX(VLIA)]*

	Fraction with A*0201 allele	P^a
Mutation in motif	0/6 (0)[b]	0.02
Mutation not in motif	10/28 (36)[b]	NS
General population	46[b]	

[a] Calculated using the binomial test. NS, not significant.
[b] Percentage.

- **Mutations might be selected for those that can't be optimally presented on HLA**
- **Suggests that immune surveillance occurs and that these types of antigens can be effective targets**

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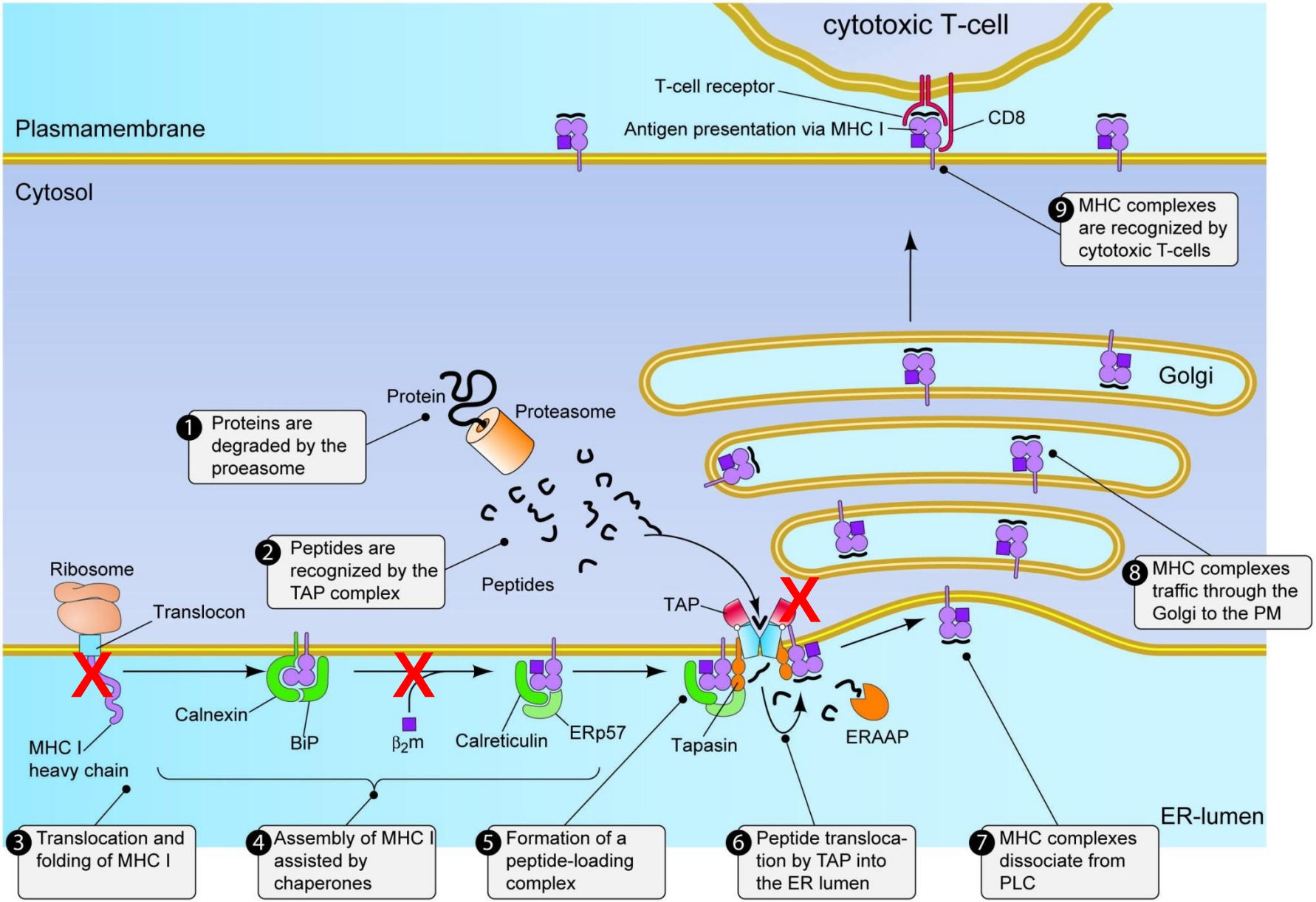
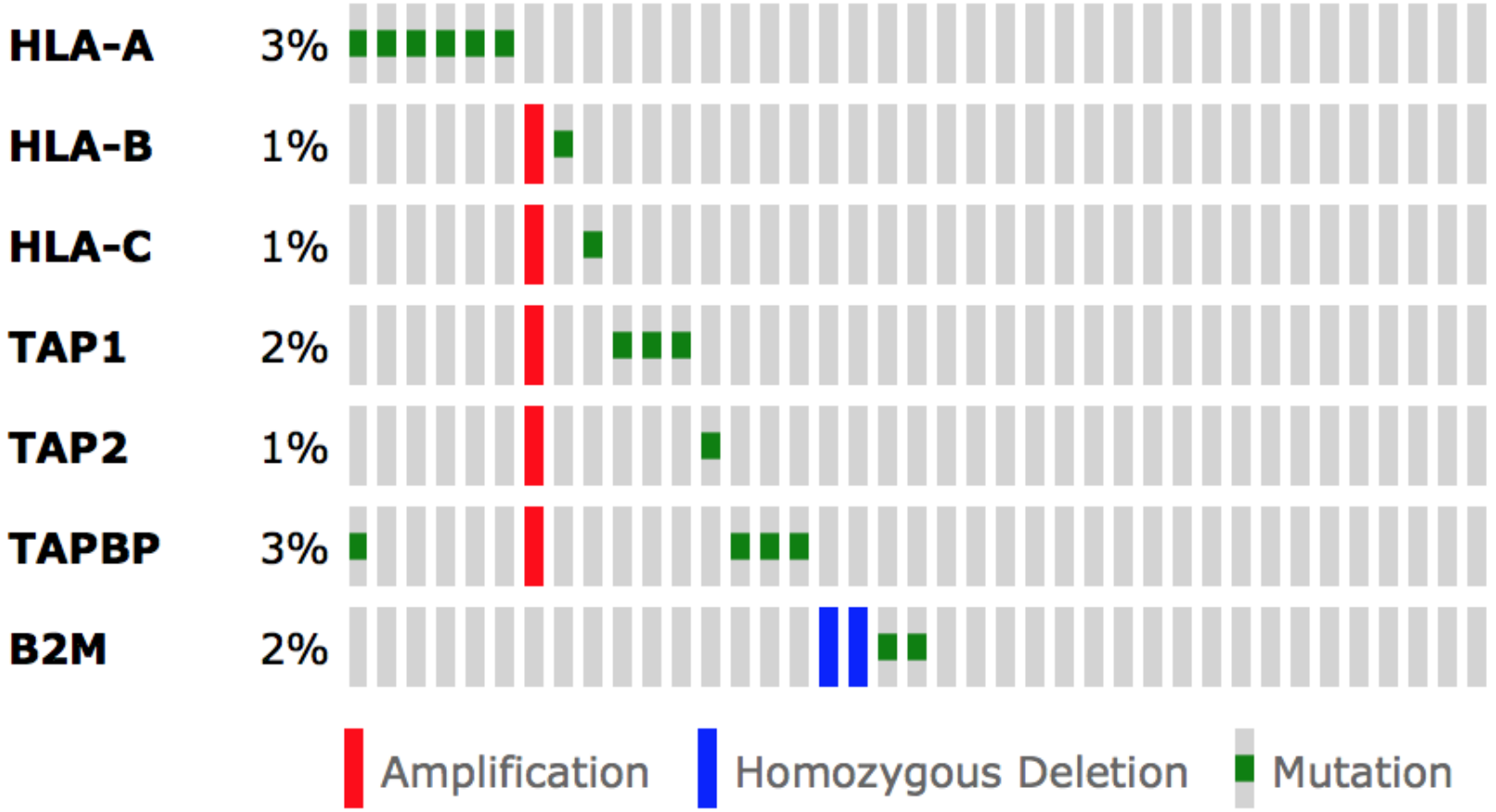
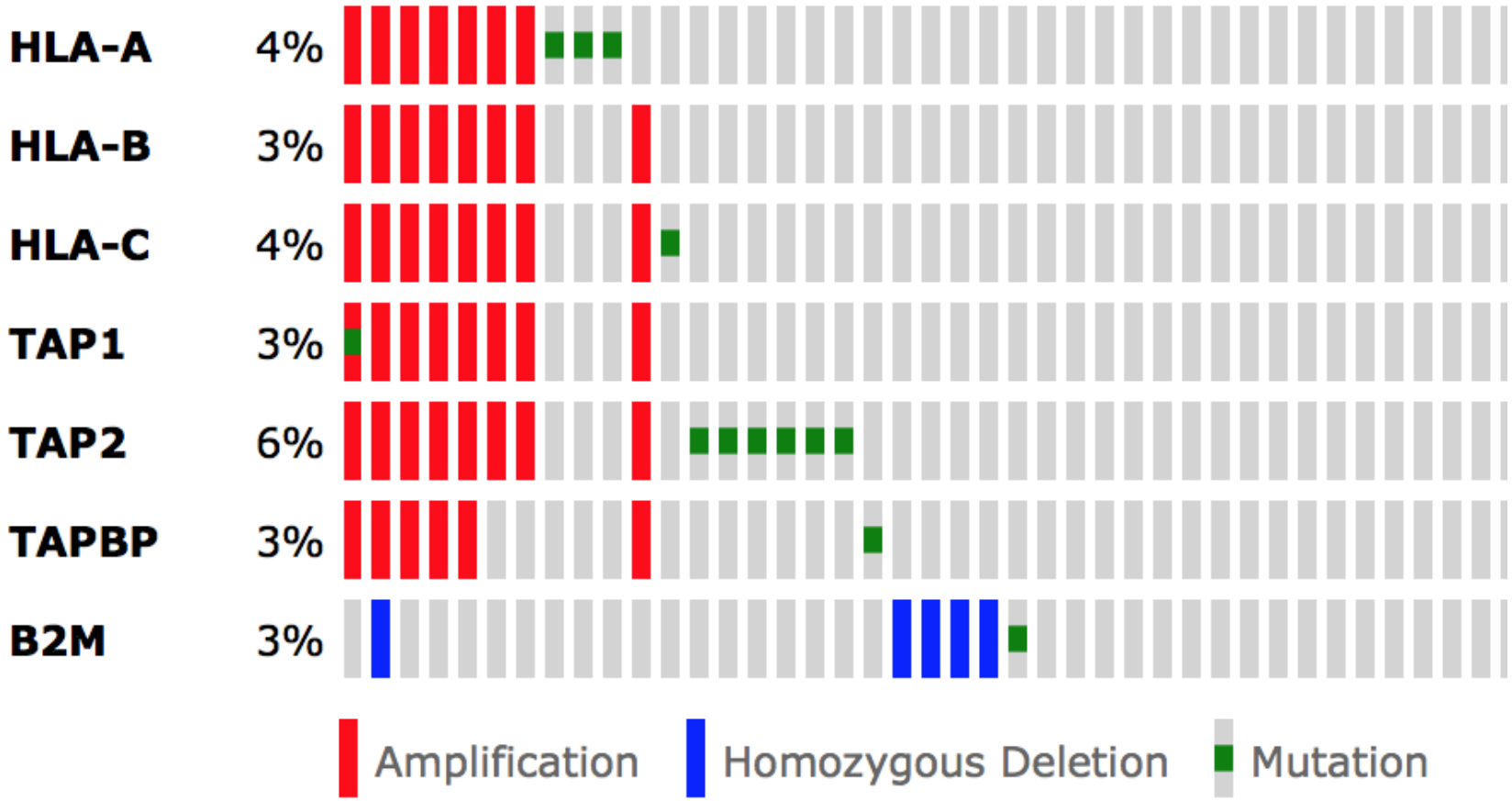
Tumor loss of Class I MHC presentation



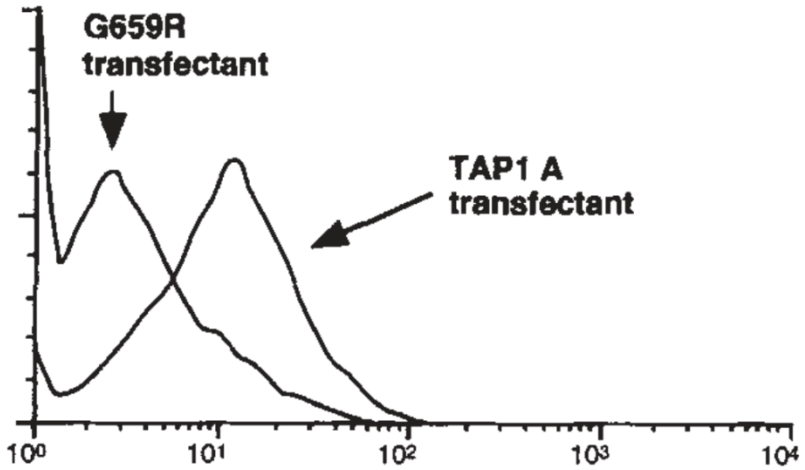
Mutations in antigen presentation - SCC

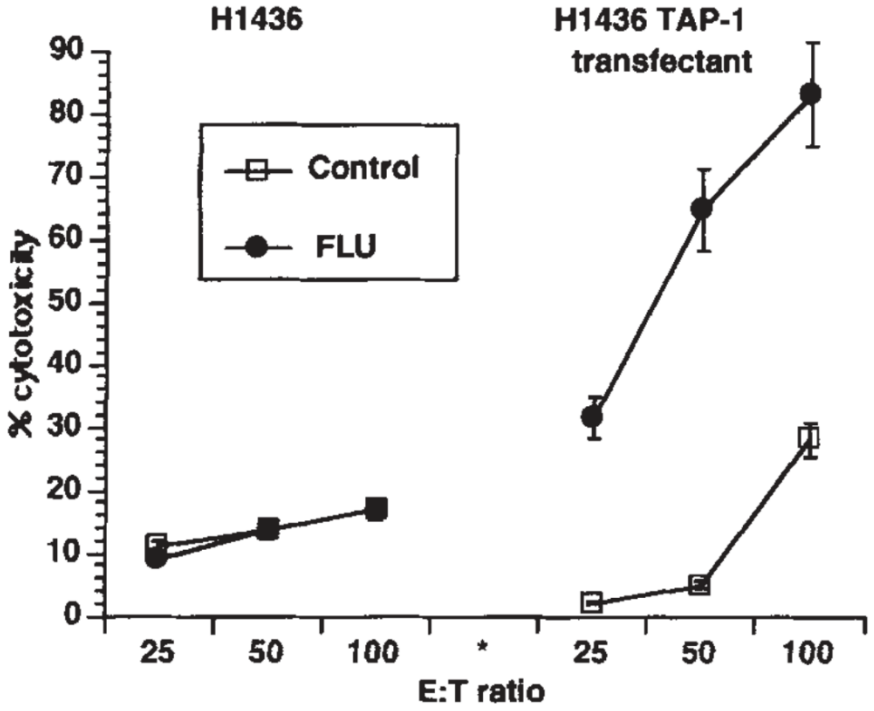


Mutations in antigen presentation - Adeno



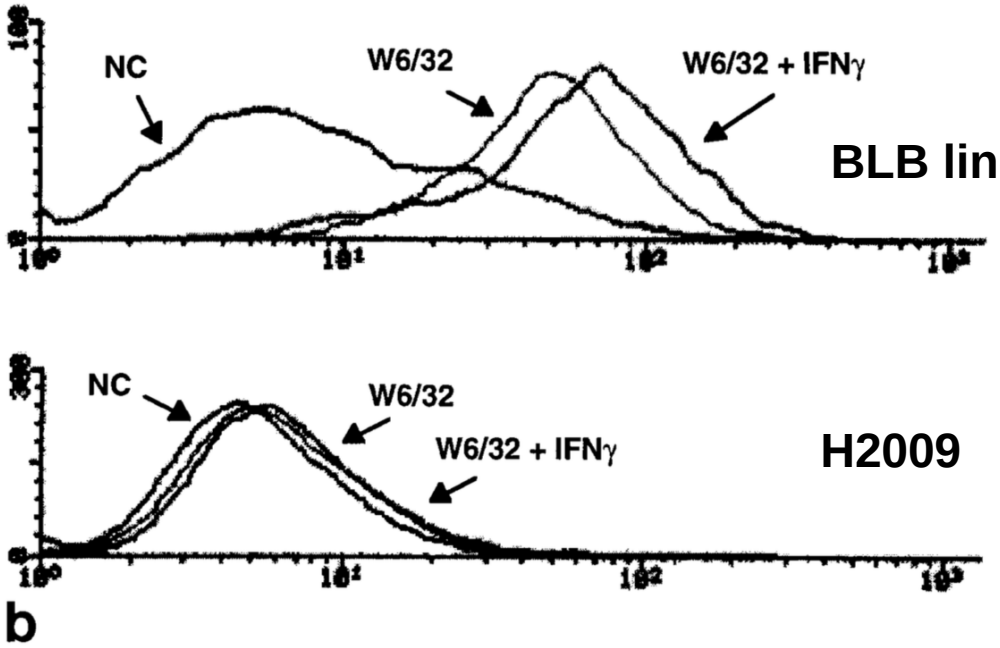
Mutant TAP1 in SCLC

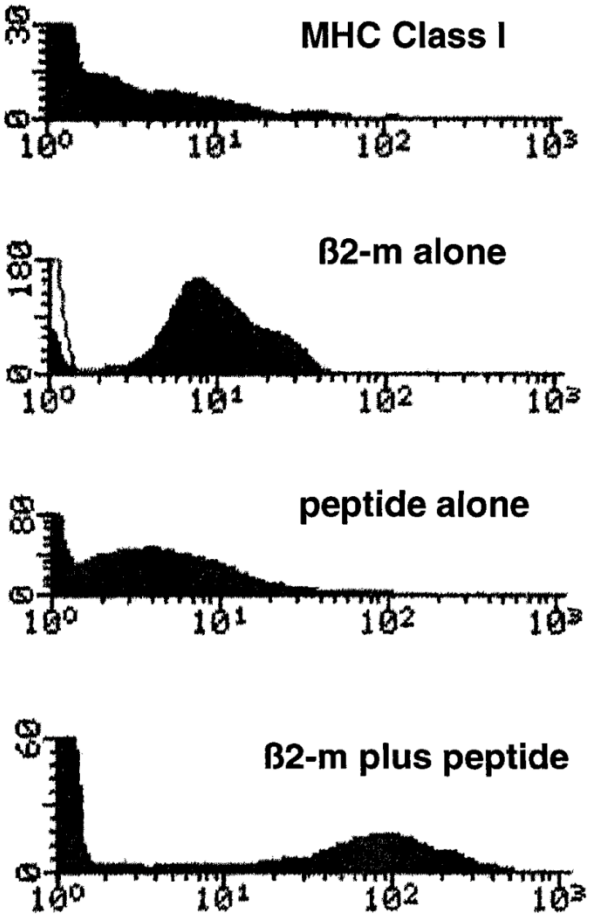




Chen and Carbone, Nat Genet. 1996 Jun;13(2):210-3

Mutant B2M in NSCLC



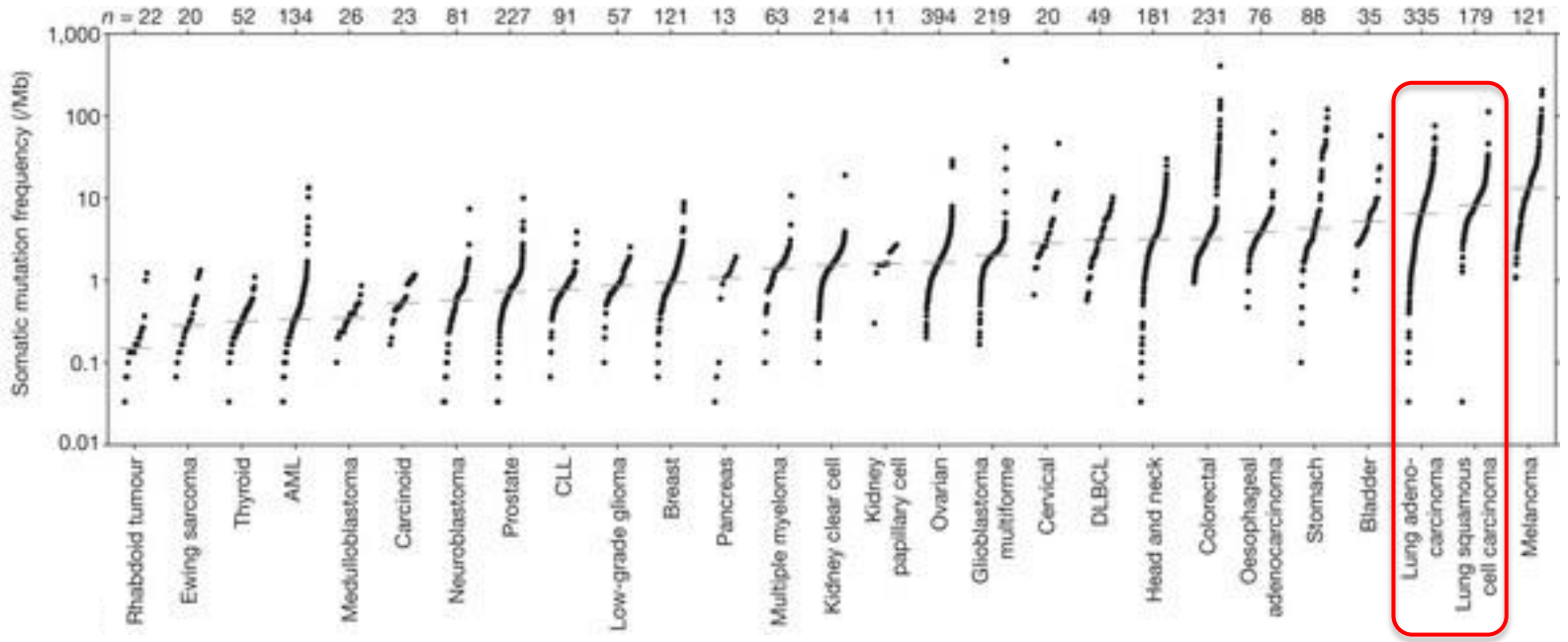


Chen and Carbone, IJC 67, pp 756-763, 1996

Lung Cancers are highly mutagenized -

Can tumor-specific peptides be recognized by the immune system?

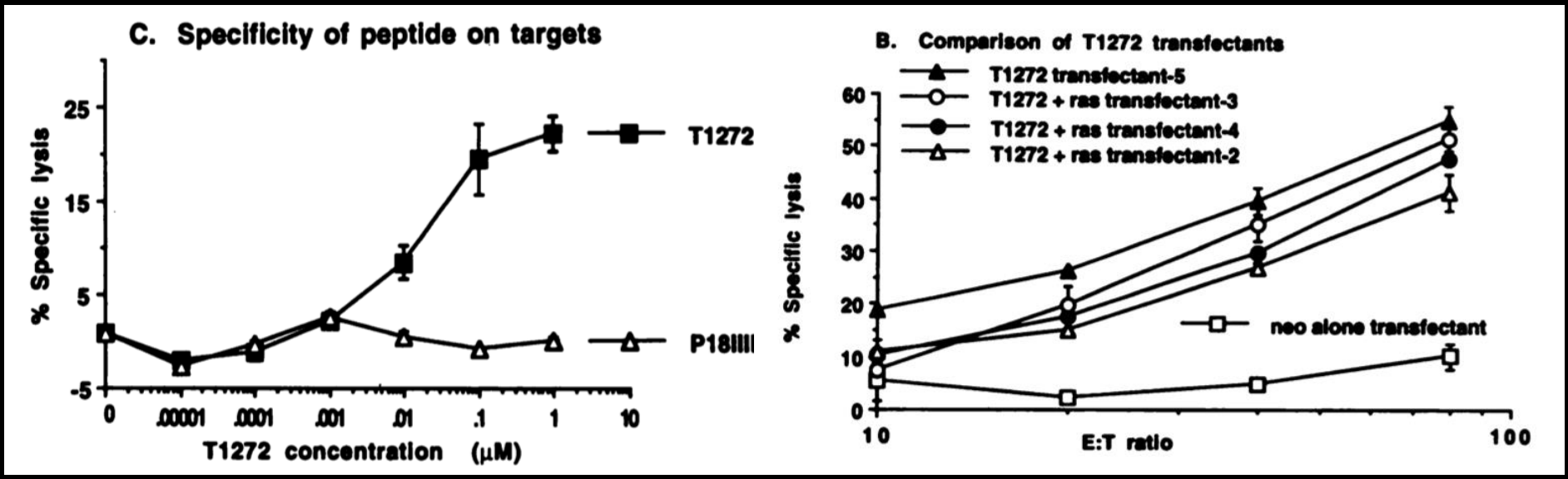
Genetic alterations in cancer cells result in neoantigens that are recognised by the immune system



Lawrence Nature 2013

Mutation rate in lung cancer is higher compared with other cancers

P53-specific killing in murine models



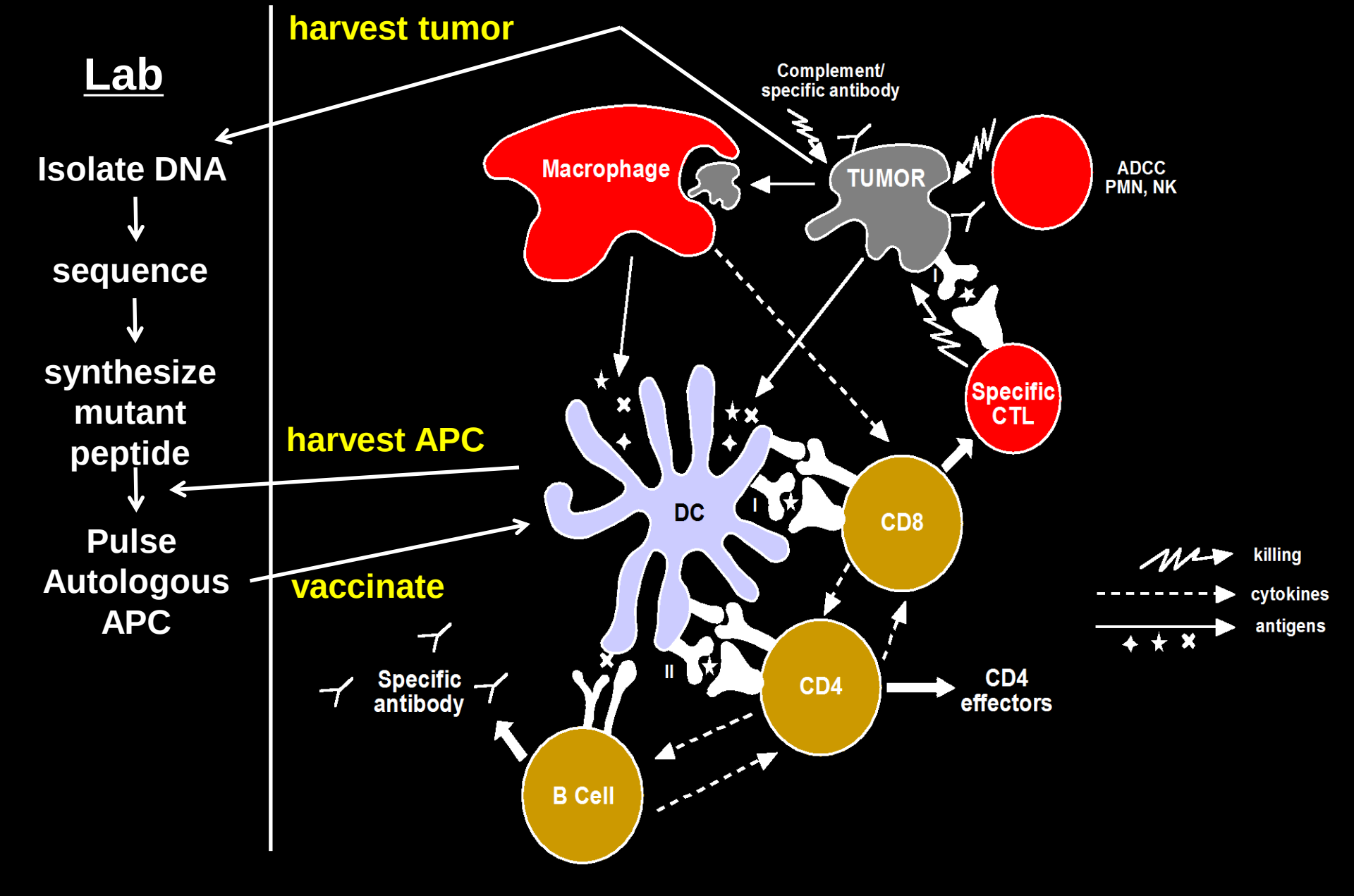
Yanuck, Carbone, Berzofsky, Cancer Research, 1993

Immunization With Mutant *p53- and K-ras–Derived Peptides in Cancer Patients: Immune Response and Clinical Outcome*

David P. Carbone, I. Frank Ciernik, Michael J. Kelley, M. Charles Smith, Sorena Nadaf, Denise Kavanaugh, V. Ellen Maher, Michael Stipanov, David Contois, Bruce E. Johnson, C. David Pendleton, Burkhardt Seifert, Charley Carter, Elizabeth J. Read, Jay Greenblatt, Lois E. Top, Morris I. Kelsey, John D. Minna, and Jay A. Berzofsky

Carbone et al, JCO 2005

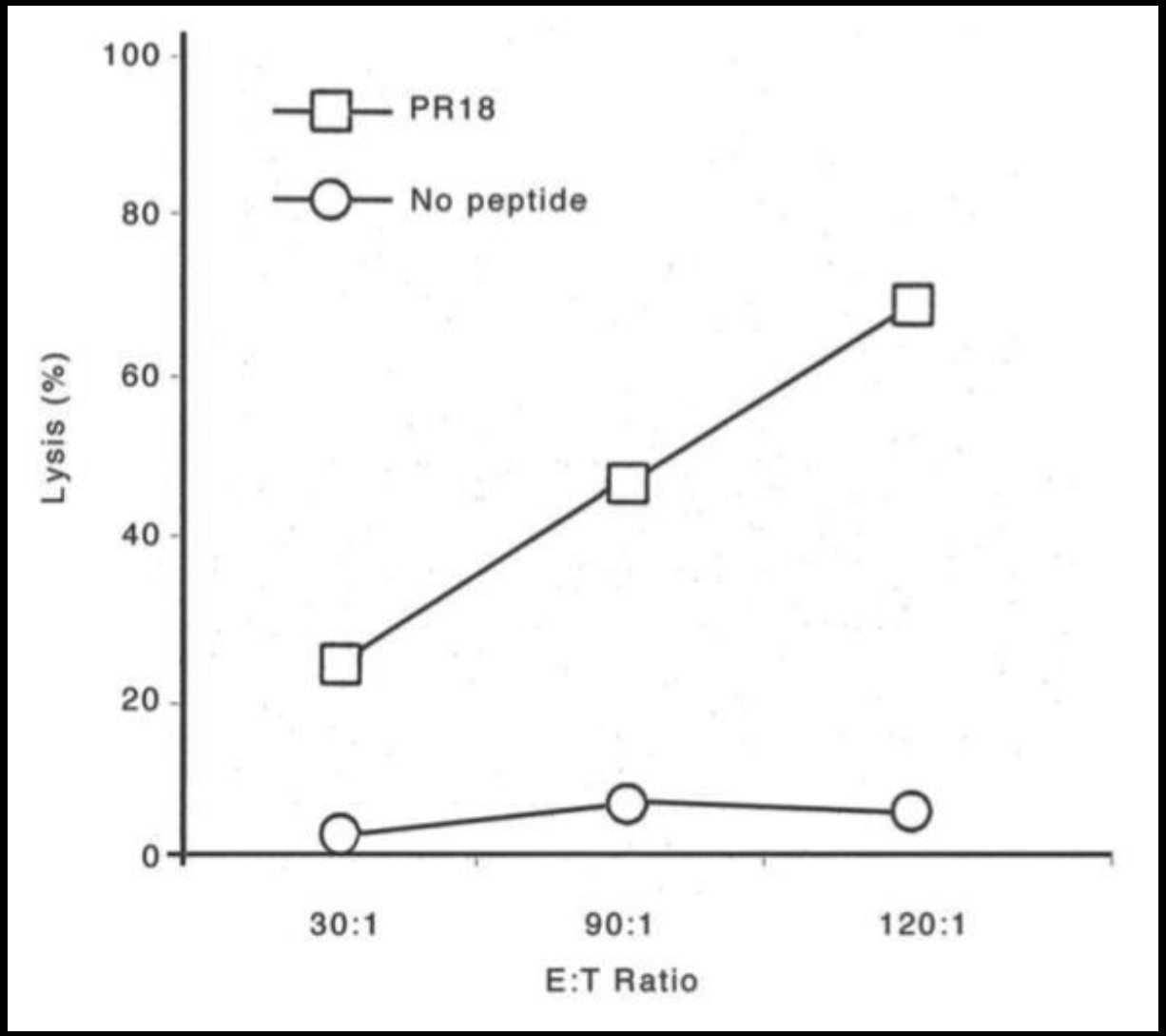
Custom mutant peptide-pulsed DC vaccine



Results

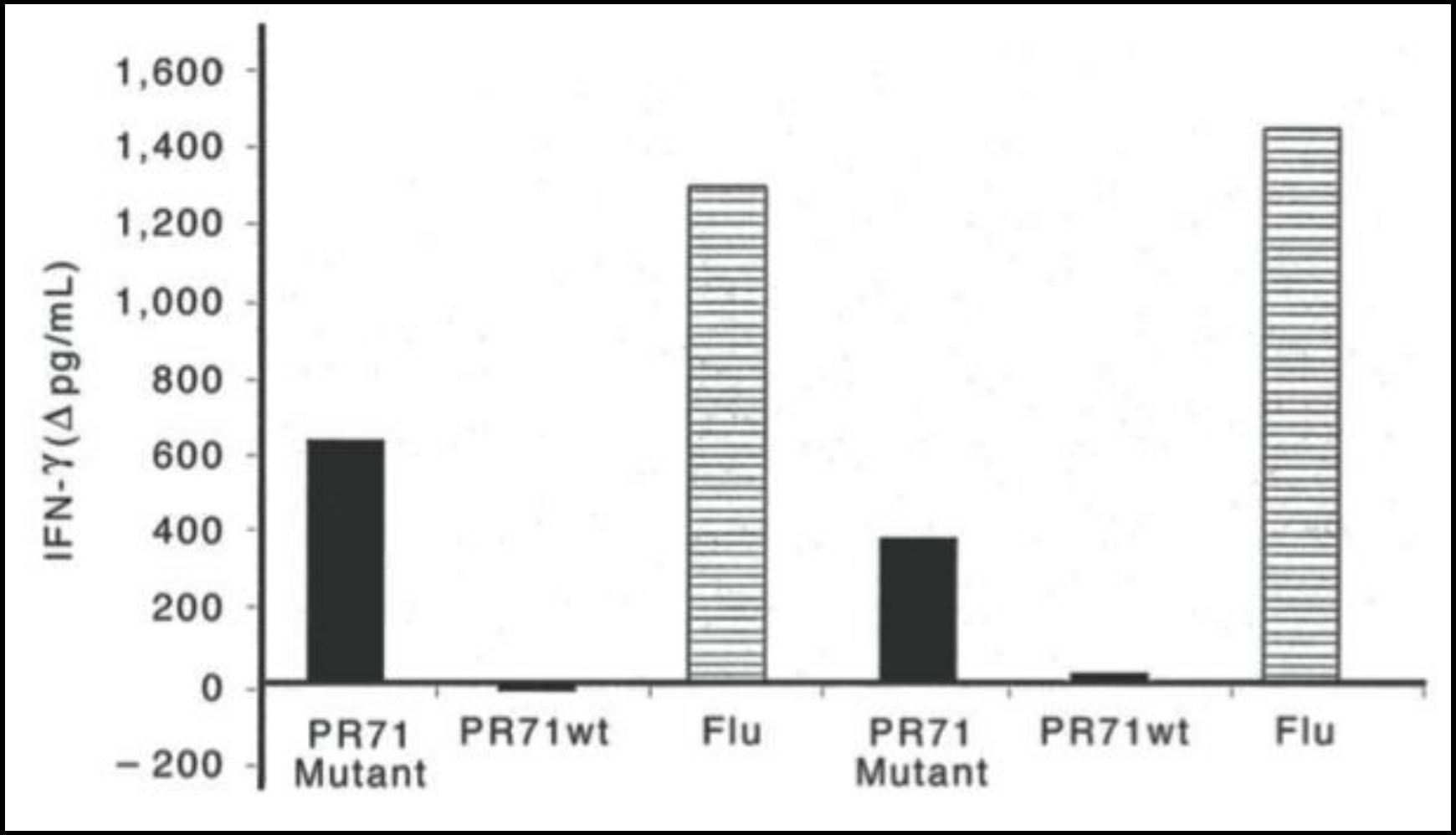
- Some patients had pre-existing mutant-oncogene-specific T-cells
- 26% positive post-vaccine specific immune response
- Median survival 115 days (26 to 685+)
- No objective responses in evaluable patients
- 5 responders had stable disease, 4 to 40 months
- One patient with resected lung metastatic disease and + KRAS responses NED after >5 years
- One KRAS mutant patient recurred with KRAS wild-type disease

Specific CTL to Kras 12 cys



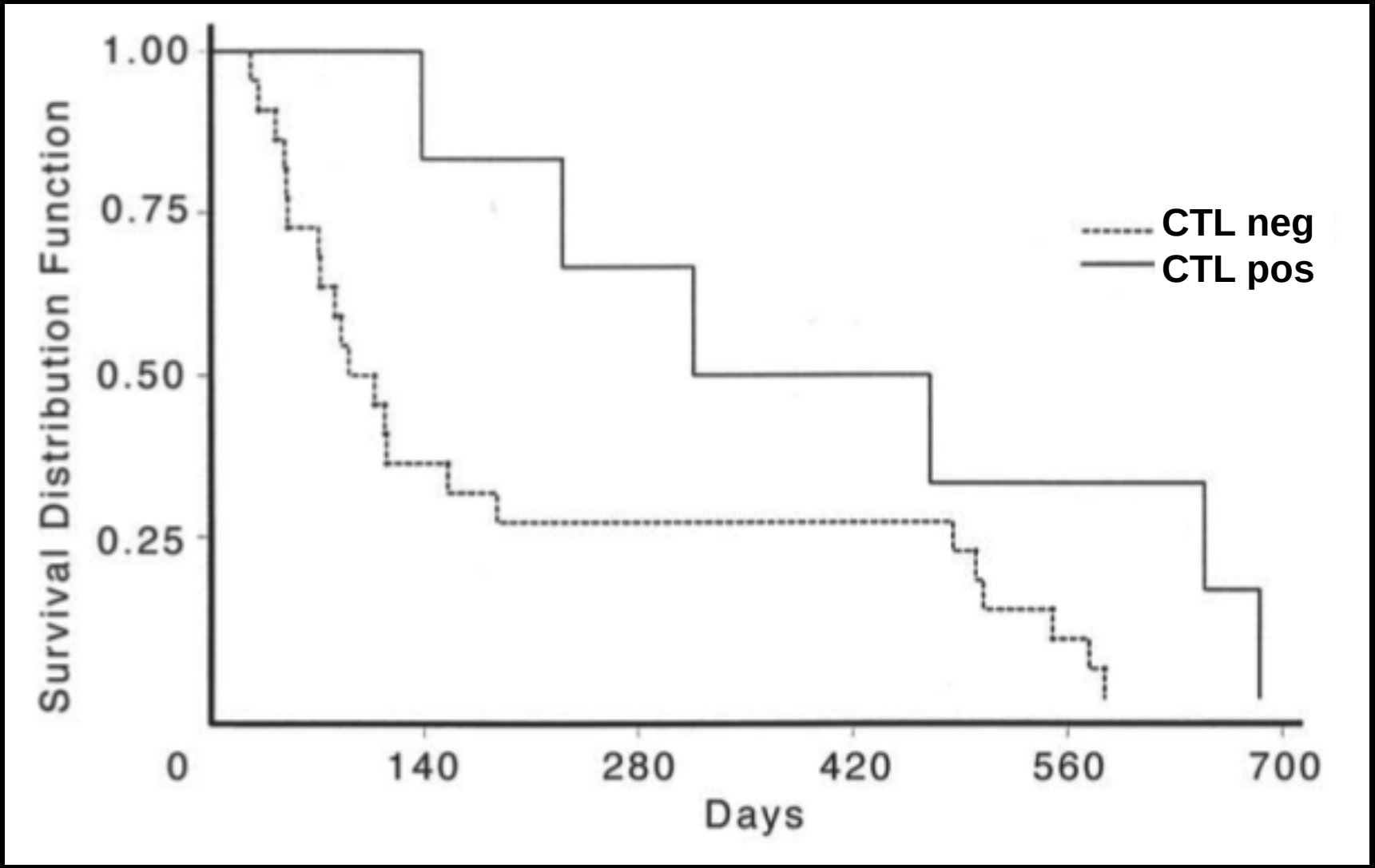
Carbone et al, JCO 2005

CTL to mutant, not wt p53



Carbone et al, JCO 2005

Survival



Carbone et al, JCO 2005

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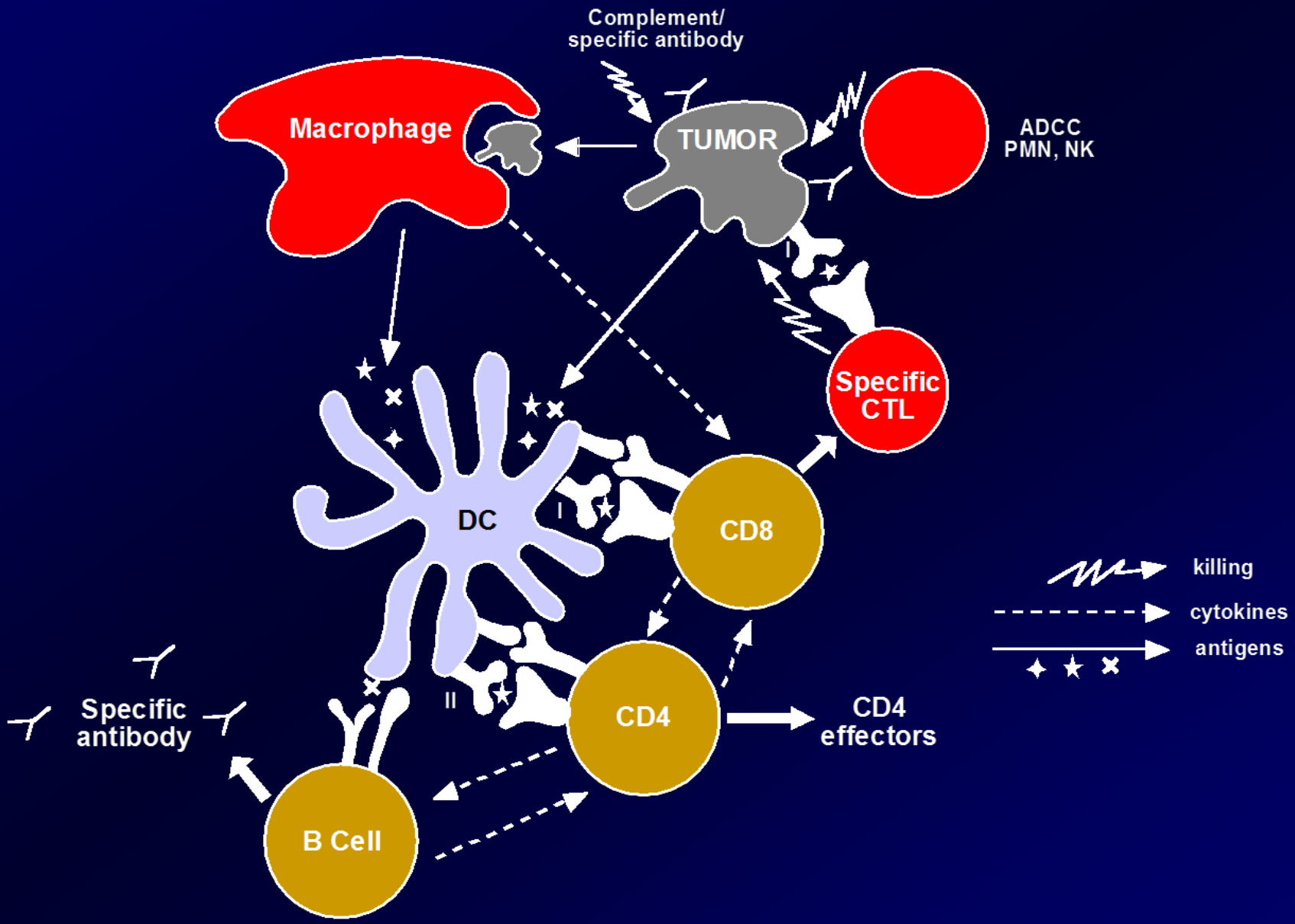
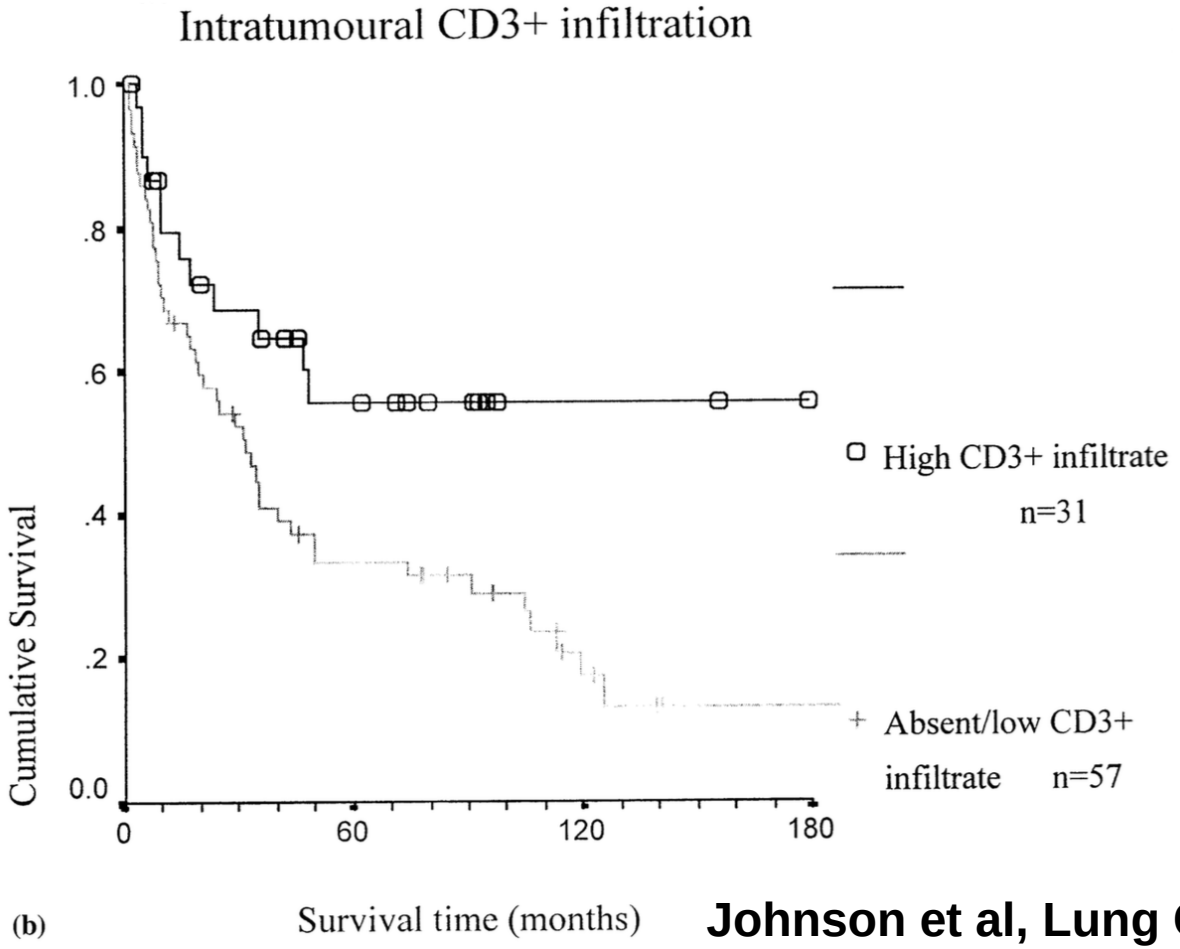
What allows tumors to grow, even when they contain hundreds of highly expressed neoantigens??

Induction of Immunity and Tumor Killing Are Complex and Involve Many Cell-Cell Interactions



T-cell infiltrate and prognosis in lung cancer



Johnson et al, Lung Cancer 27 (2000) 27–35

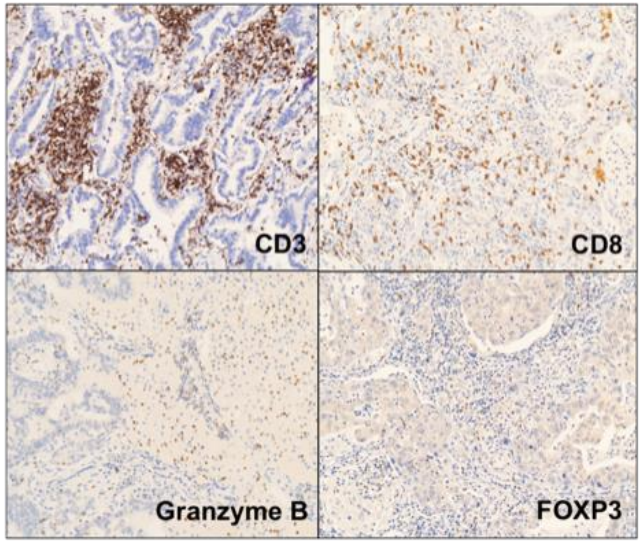
Tumor infiltrating immune cells and secreted factors regulate anti-tumor responses in opposing ways

Tumor progression

Tumor regression

Tumor promoting role

- Regulatory T cells
- Tolerogenic dendritic cells
- N2 neutrophils
- Myeloid derived suppressor cells
- M2 macrophages
- TGF-b
- IL-10
- IDO



From Soo, ASCO 2014

magnification x20

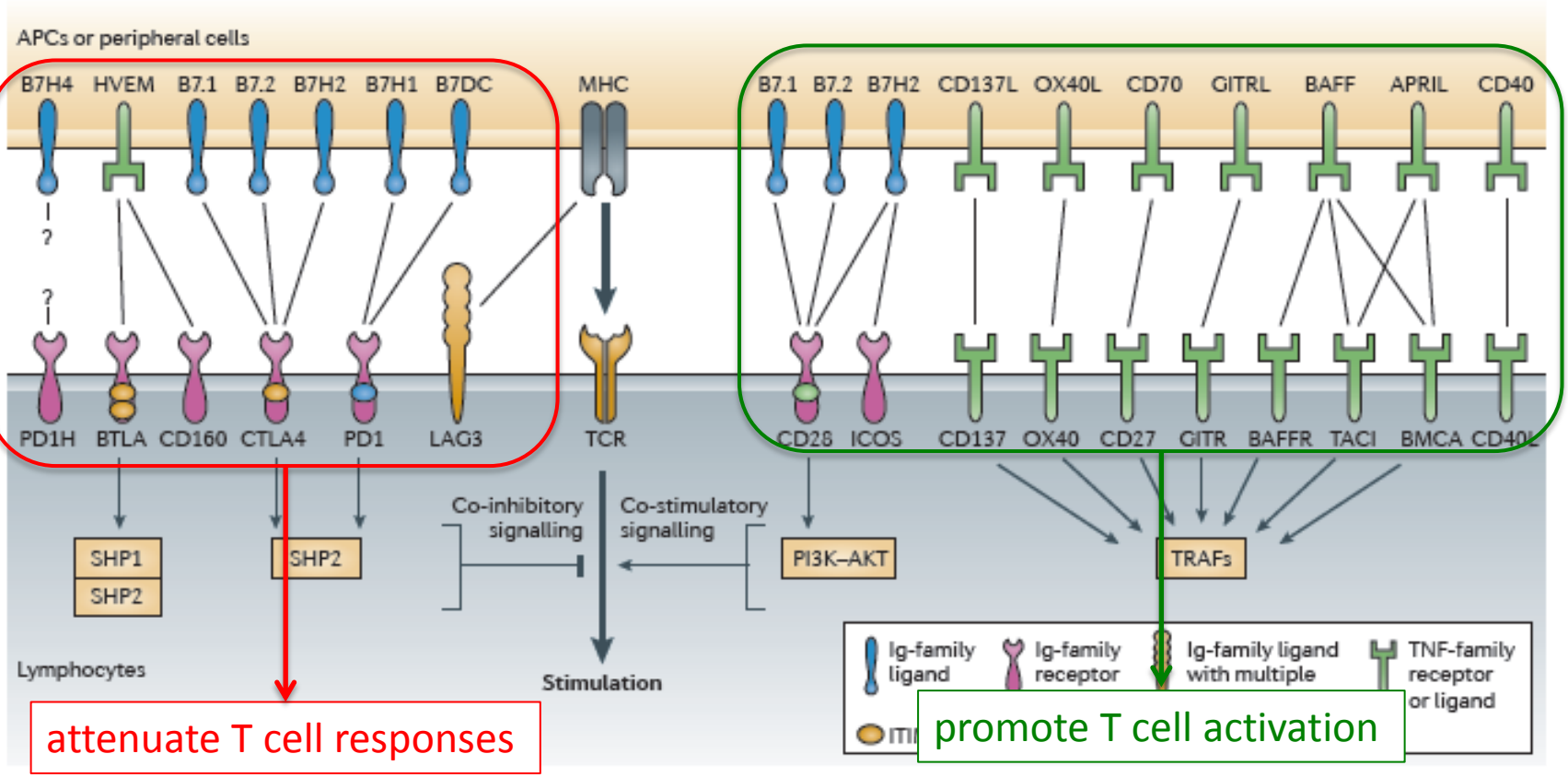
Tumor inhibiting role

- CD4 T helper cells
- CD8 Cytotoxic T cells
- N1 neutrophils
- Natural killer cells
- Mature dendritic cells
- M1 macrophages
- B cells
- IFN-a, -b, -g
- TRAIL
- IL-12
- Perforin

Interaction within the tumor microenvironment modulate anti-tumor immunity, angiogenesis, metastasis, cancer cell proliferation and survival

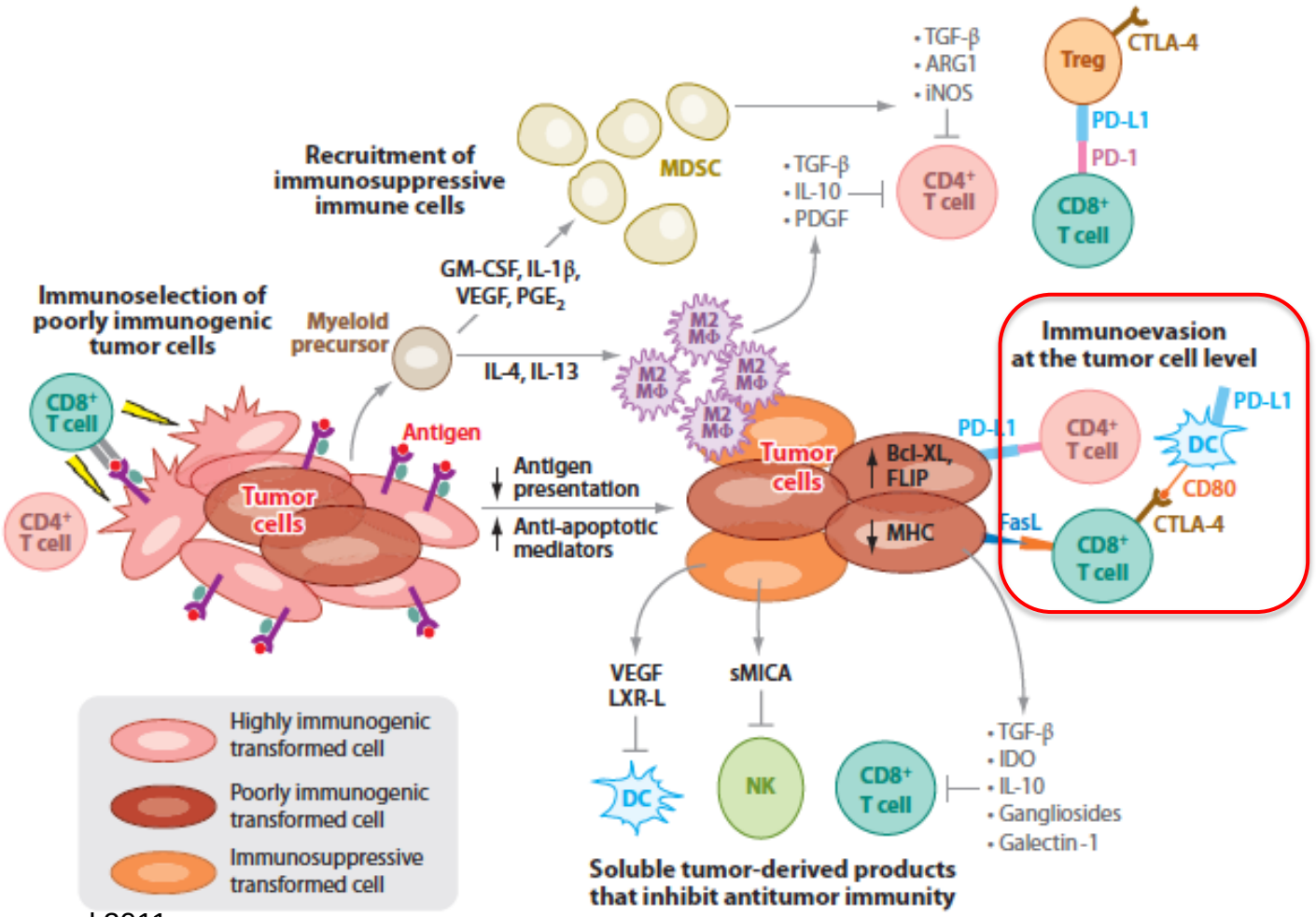
O'Callaghan JTO 2010, Schreiber Science 2011, Vesely Annu Rev Immunol 2011

T cell response is regulated by co-stimulatory and co-inhibitory (checkpoint) factors



Yao Nat Rev Drug Dis 2013

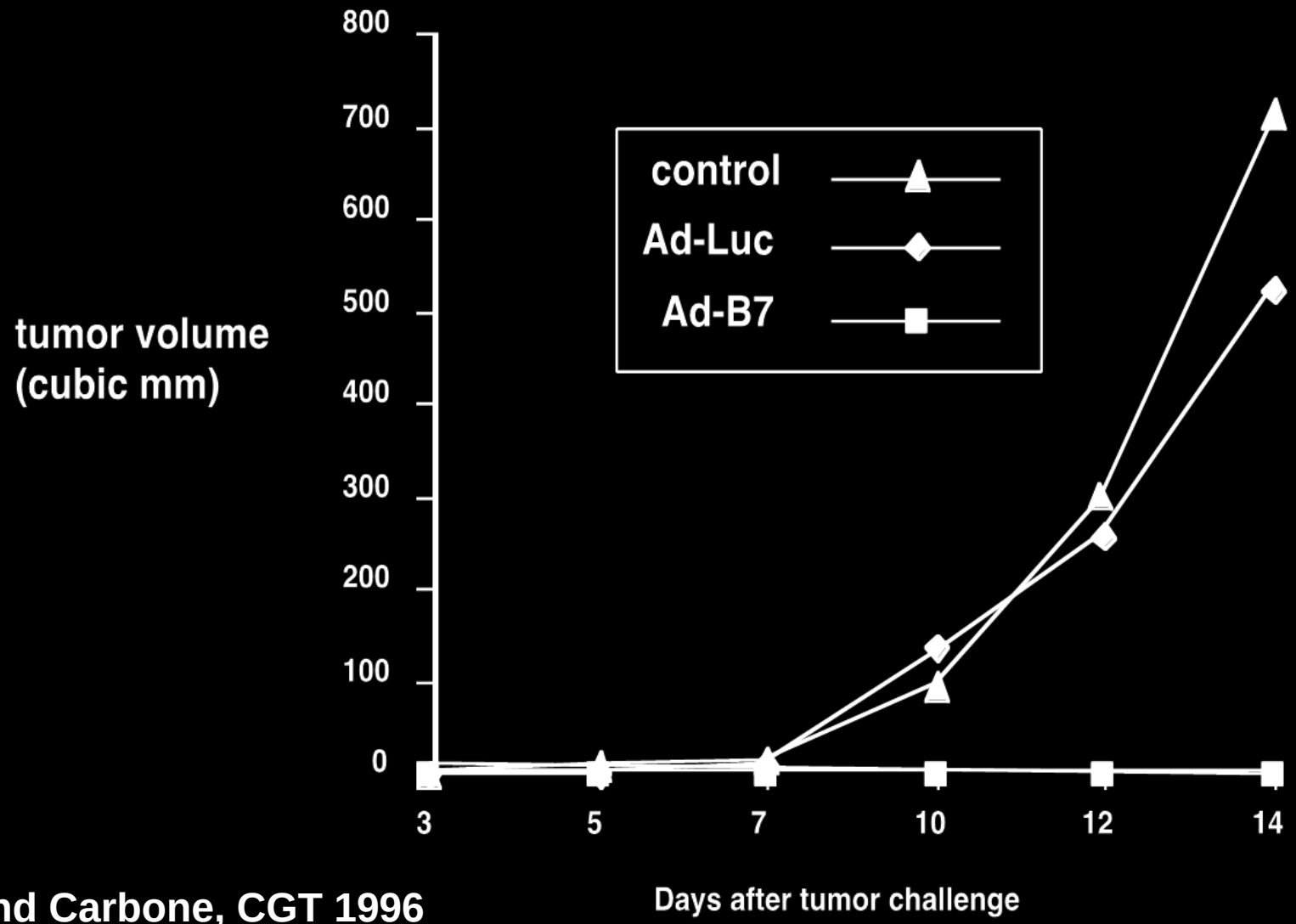
Immune escape mechanisms of tumor



CTLA-4
PD-1

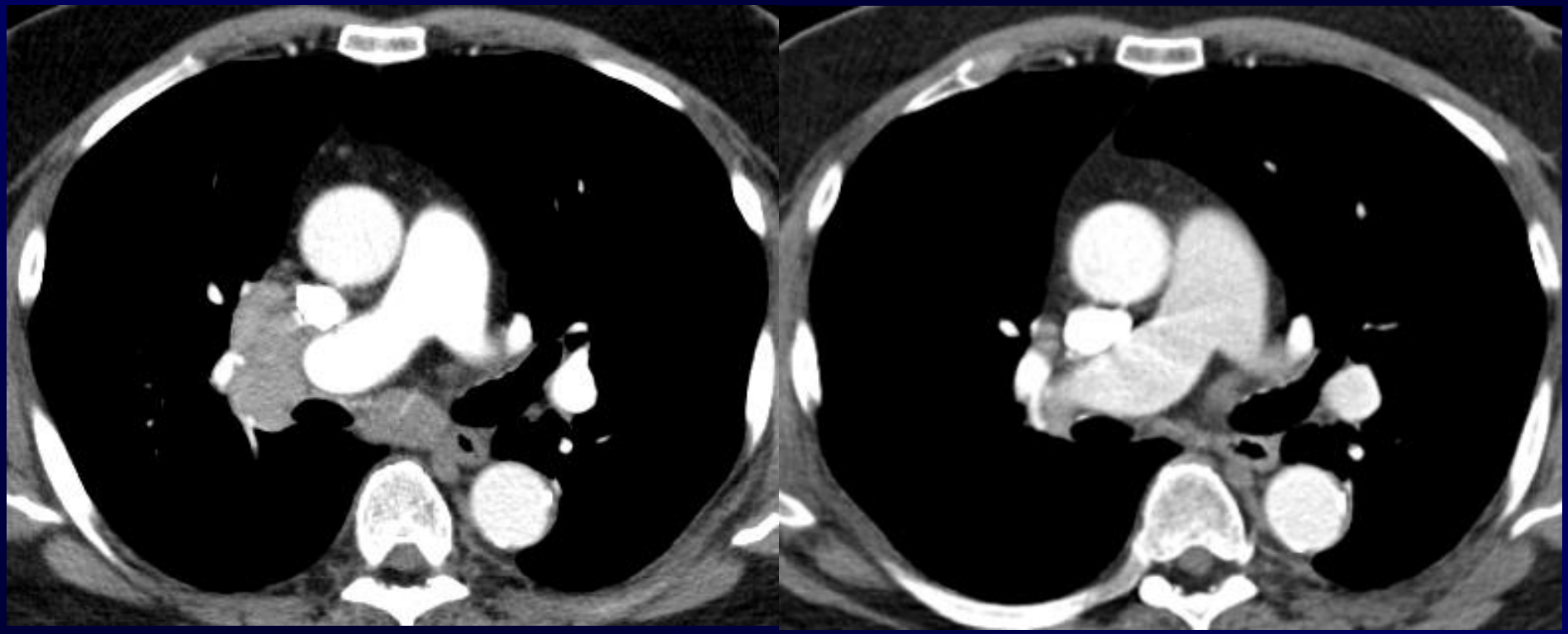
Vesely Annu Rev Immunol 2011

Immune Response Can Fail to Develop Even When Everything's There



Lee and Carbone, CGT 1996

Response to first line anti-PD1



Clinical Development of Inhibitors of PD-1 Immune Checkpoint

Target	Antibody	Molecule	Company	Development stage
PD-1	BMS-936558/ MDX-1106/ONO-4538	Fully human IgG4 mAb	Bristol-Myers Squibb	Phase III multiple tumors
	CT-011	Humanized IgG1 mAb	CureTech	Phase II multiple tumors
	MK-3475	Humanized IgG4 mAb	Merck/MSD	Phase III
PD-L1	BMS-936559/ MDX-1105	Fully human IgG4 mAb	Bristol-Myers Squibb	Phase II
	MedI-4736		MedImmune	Phase I
	MPDL-3280A		Genentech	Phase II/III

NSCLC PD-L1 expression is variable

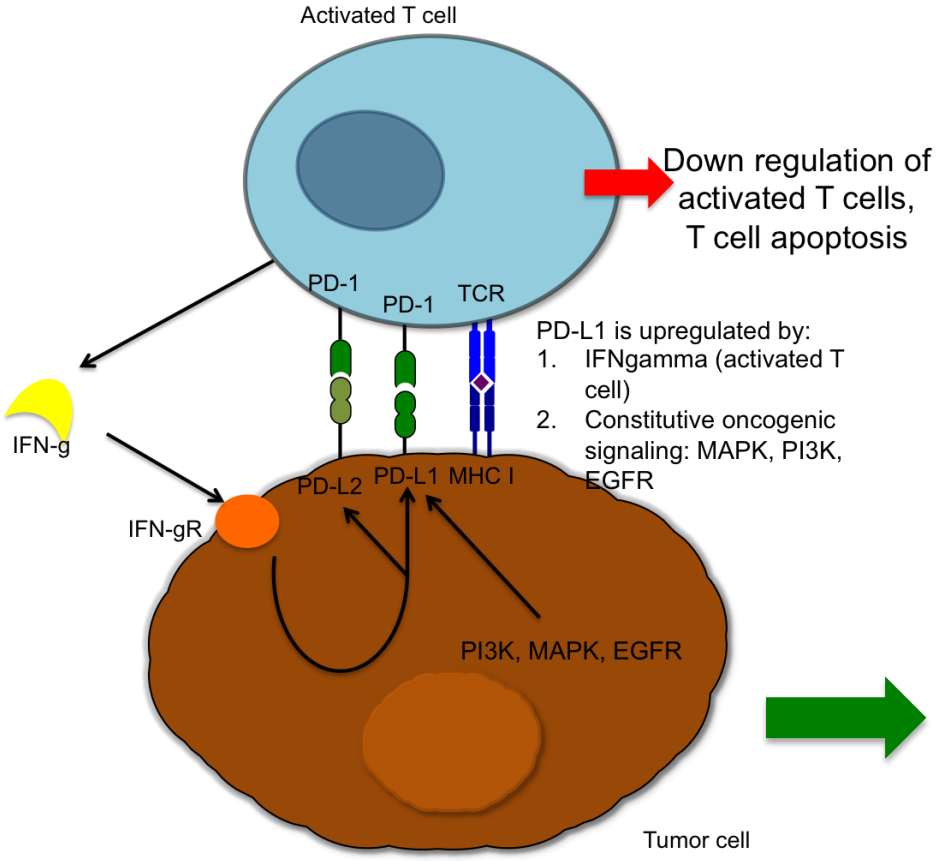
Author	N	Histologic subtype	Pathologic stage	Detection method/Ab clone	Cellular localization	% PD-L1 + ve	Clinpathological association	Association with immune cells/TILs	Prognosis
PD-L1									
Yang [45]	163	ADC	I	IHC anti-PD-L1/Proteintech Group Chicago, IL	Membrane	39,9	Vascular invasion, higher grade differentiation	No association with TILs	RFS: improved, OS: neutral
Velchetti [46]	204 (US)	Mixed	I–IV	QIF/5H1	Membrane	36,1	SCC	Increased inflammatory infiltrate	OS: improved 60 v 27months
	340 (Greece)	Mixed	I–IV	QIF/5H1	Membrane	24,8	Lower stage	Increased inflammatory infiltrate	OS: improved NR v 31months
	173 (US)	Mixed	I–IV	mRNA	Not applicable	50,8	None	Increased inflammatory infiltrate	OS: improved
	314 (Greece)	Mixed	I–IV	mRNA	Not applicable	53,2	None	Increased inflammatory infiltrate	OS: improved
Chen [47]	208	Mixed	I–IV	IHC anti-PD-L1	Cytoplasm, membrane	65,3	Non-smokers, less LN metastasis	Increased macrophages	Not reported
Velchetti [52]	445	Mixed	I–IV	QIF/5H1	Not reported	27,4	Not reported	Not reported	Not reported
	13	Sarcomatoid	I–IV	QIF/5H1	Not reported	69,2	Not reported	Not reported	Not reported
Chen [48]	120	Mixed	I–III	IHC anti-PD-L1/236A/E7	Cytoplasm, membrane	57,5	Not reported	Not reported	OS: reduced
Boland [49]	214	SCC	I–IV	IHC anti PD-L1/5H1	Membrane	19,6	Not reported	No association with TILs	OS: neutral
Mu [50]	109	Mixed	I–III	IHC anti-PD-L1/not reported	Cytoplasm, membrane	53,2	ADC	Increased dendritic cells	OS: reduced PD-L1 + <3 year survival 46%, >3Y survival 12%
Konishi [51]	52	Mixed	I–IV	IHC anti-PD-L1/M1H1	Cytoplasm, membrane	27,2	None	Reduced TILs	OS: neutral 5 year survival 59% v 48%

Ab: antibody; ADC: adenocarcinoma; IHC: immunohistochemistry; LN: lymph node; NR: not reached; OS: overall survival; PD-L1: programmed death-1 ligand; QIF: quantitative fluorescence; RFS: relapsed free survival; SCC: squamous cell carcinoma; TILs: tumor infiltrating lymphocytes.

Sundar Lung Cancer 2014

PD-L1+: 20-70%

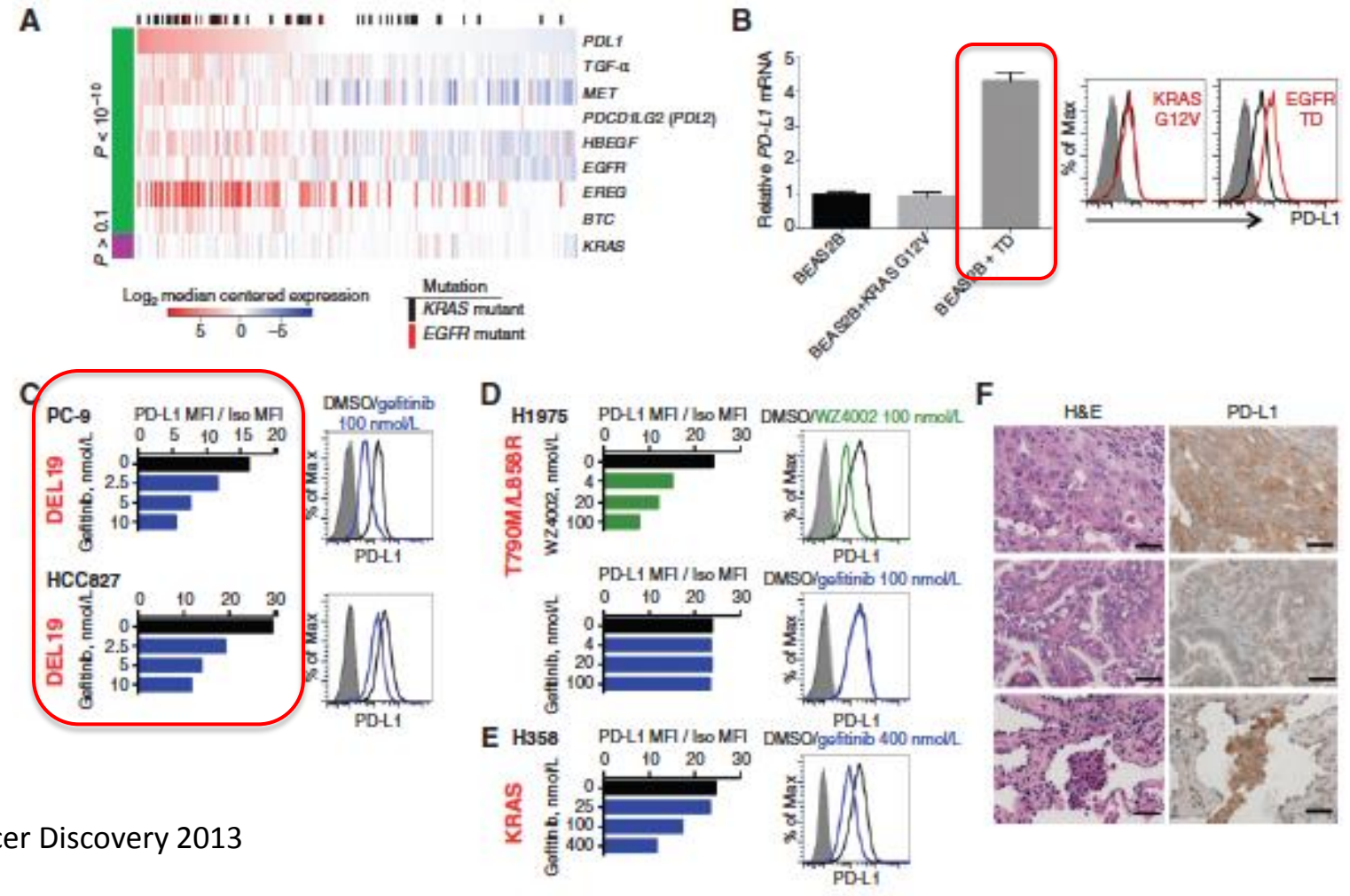
PD-L1 is upregulated by IFN-γ & tumor oncogenic signaling



Impaired T effector function (impaired proliferation, reduced expression of of IL-2, TNFa, IFN-g, perforin)
Persistent expression of inhibitory molecules

Engagement of PD-L1 via PD-1 (reverse signaling):
Induces resistance to cytotoxic T cells

EGFR pathway activation in human bronchial epithelial cells induces PD-L1 expression



Akbay Cancer Discovery 2013

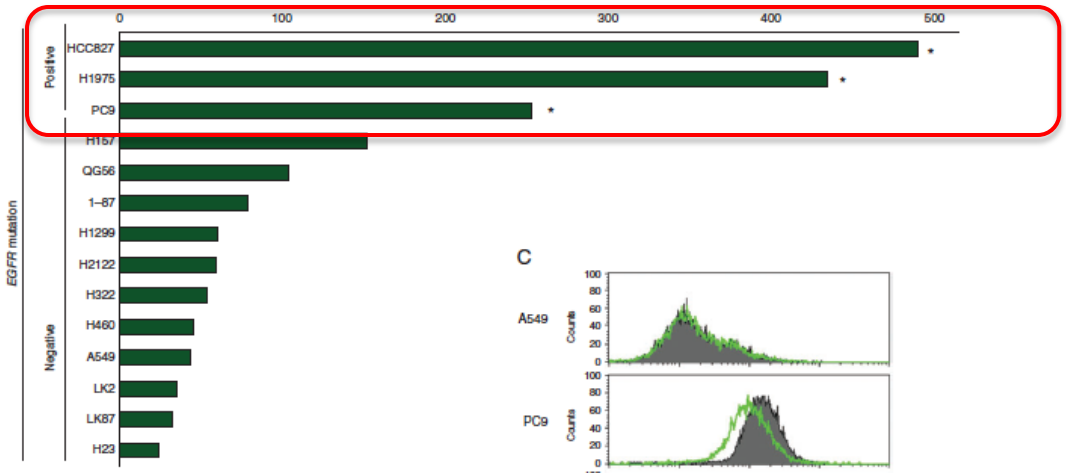
NSCLC harboring *EGFR* mutations is associated with PD-L1 expression

Table 2. Multivariate analysis of the relation between PD-L1 expression in tumor specimens and other patient characteristics

Characteristic		Coefficient (95% CI)	*P* value
p Stage	I versus II/III	2.0 (−18.4 to 22.4)	0.846
Age (years)	≤66 versus >66	5.3 (−13.8 to 24.4)	0.584
Sex	Female versus male	14.5 (−29.0 to 58.1)	0.511
Smoking status	Smoker versus never smoker	−18.6 (−64.6 to 27.4)	0.426
Histology	Adenocarcinoma versus SCC	25.1 (0.5 to 49.8)	0.046
EGFR status	Mutant versus wild type	25.4 (2.9 to 47.9)	0.027

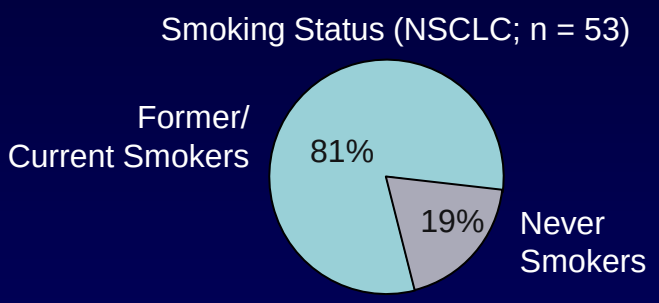
SCC, squamous cell carcinoma.

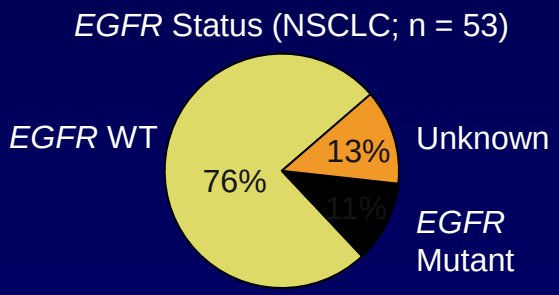
Flow cytometry: mean fluorescence intensity (MFI) for PD-L1 was significantly higher in EGFR mutation-positive cell lines

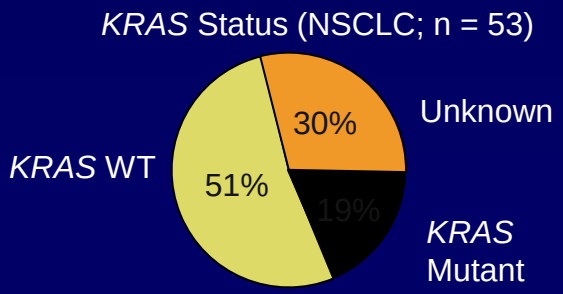


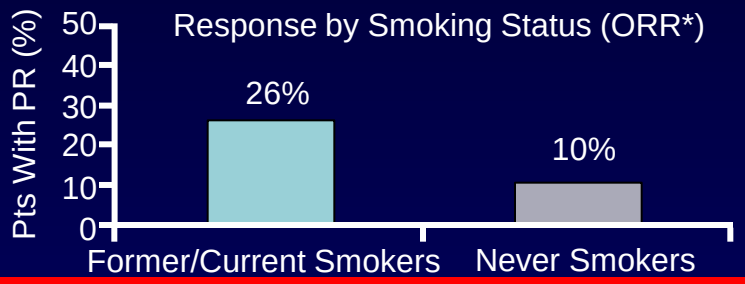
Azuma Ann Oncol 2014

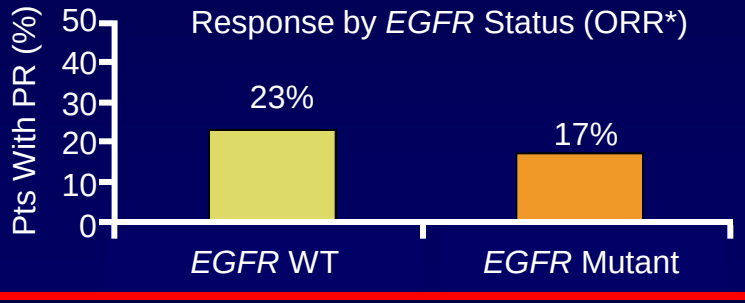
MPDL3280A Phase Ia: Response by Smoking and Mutational Status

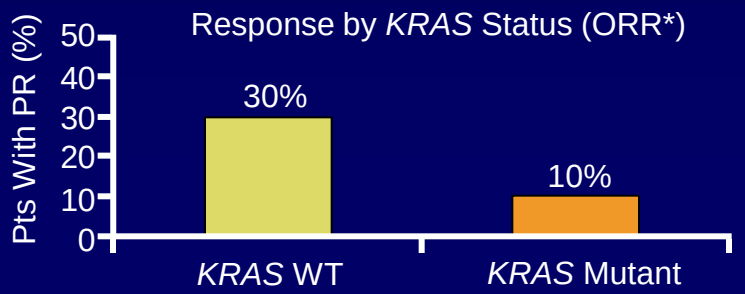








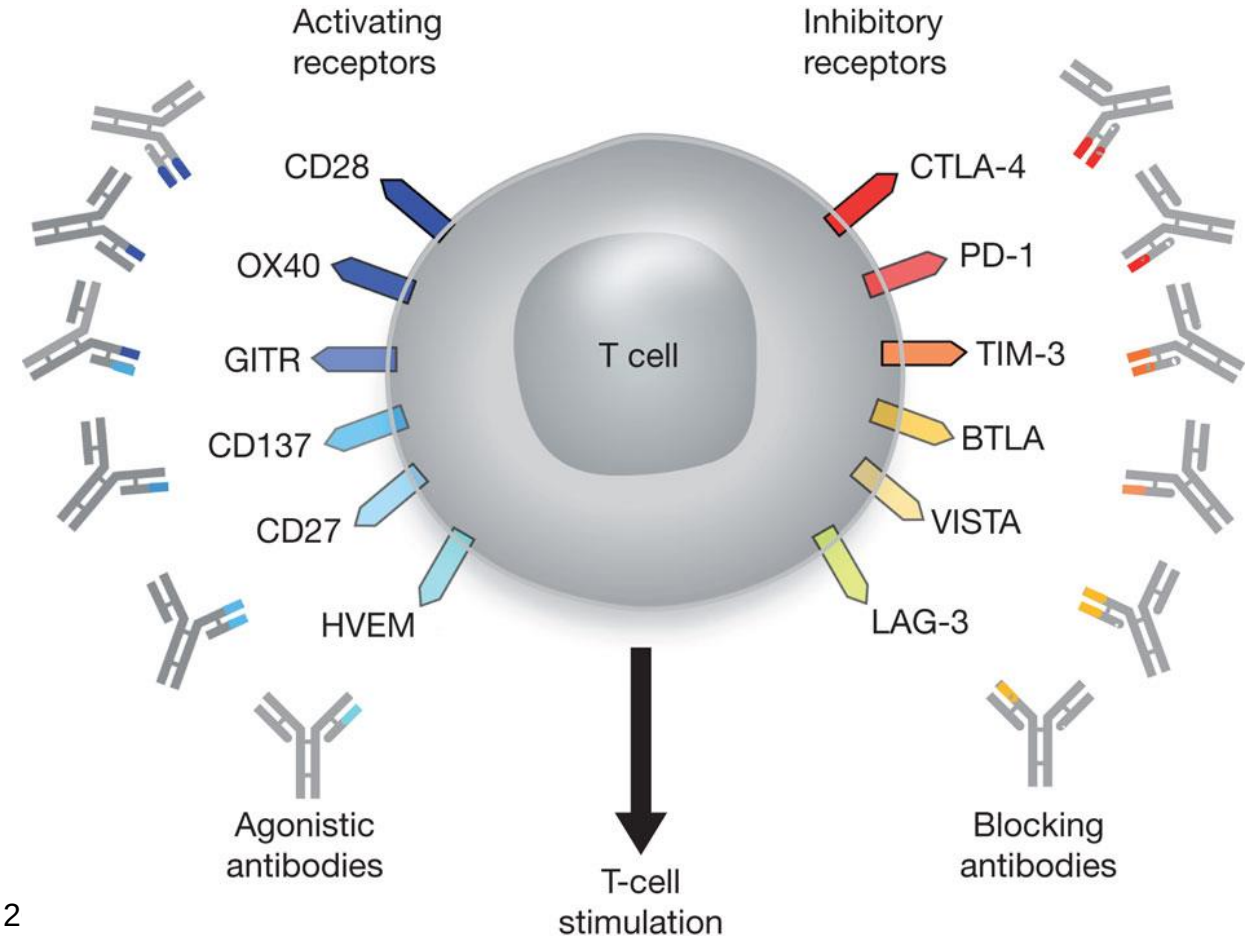




*ORR includes investigator-assessed u/c PR by RECIST 1.1. Patients first dosed at 1-20 mg/kg by October 1, 2012. Data cutoff April 30, 2013.

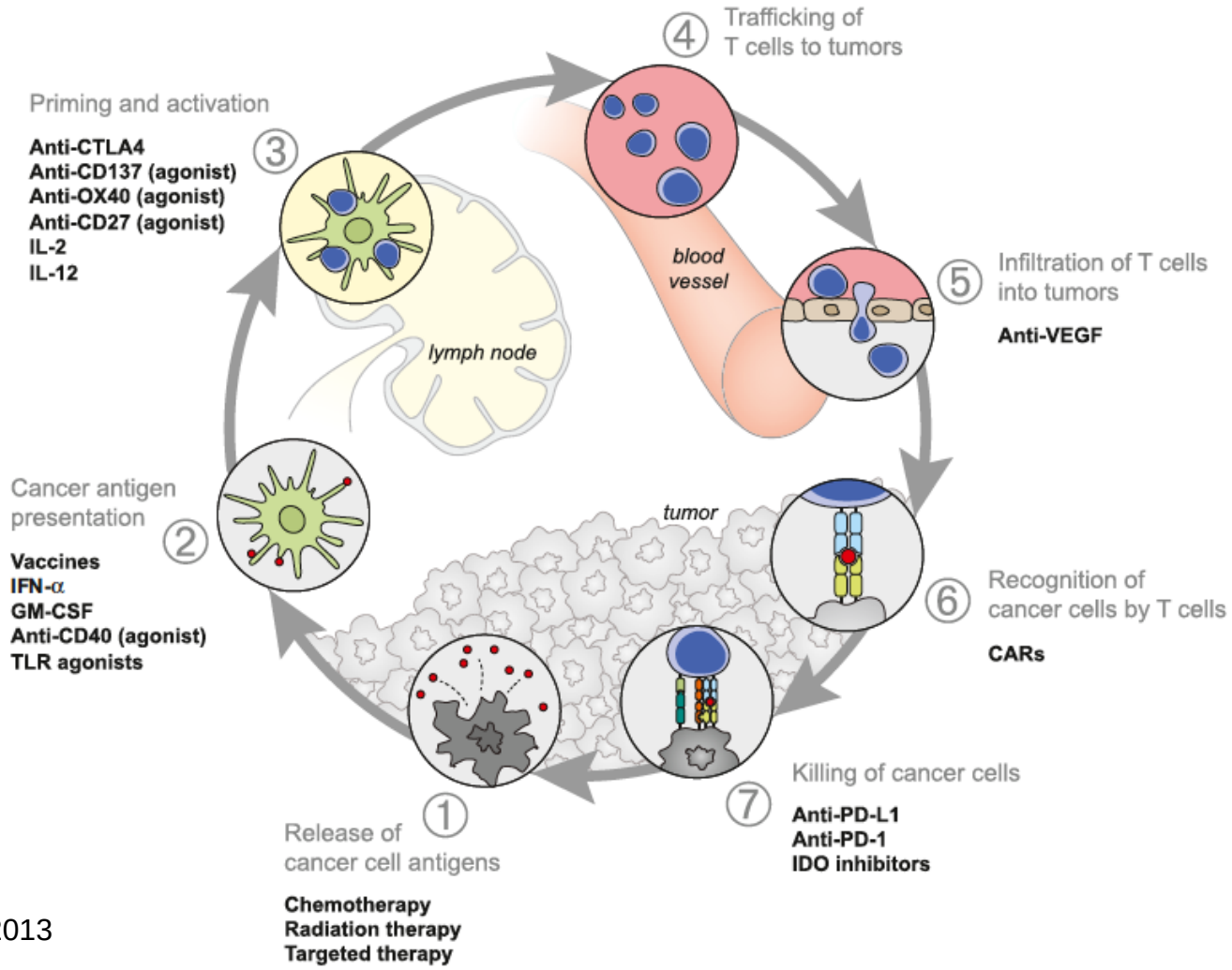
Horn L, et al. WCLC 2013. Abstract MO18.

Emerging co-inhibitory & co-stimulatory immune targets



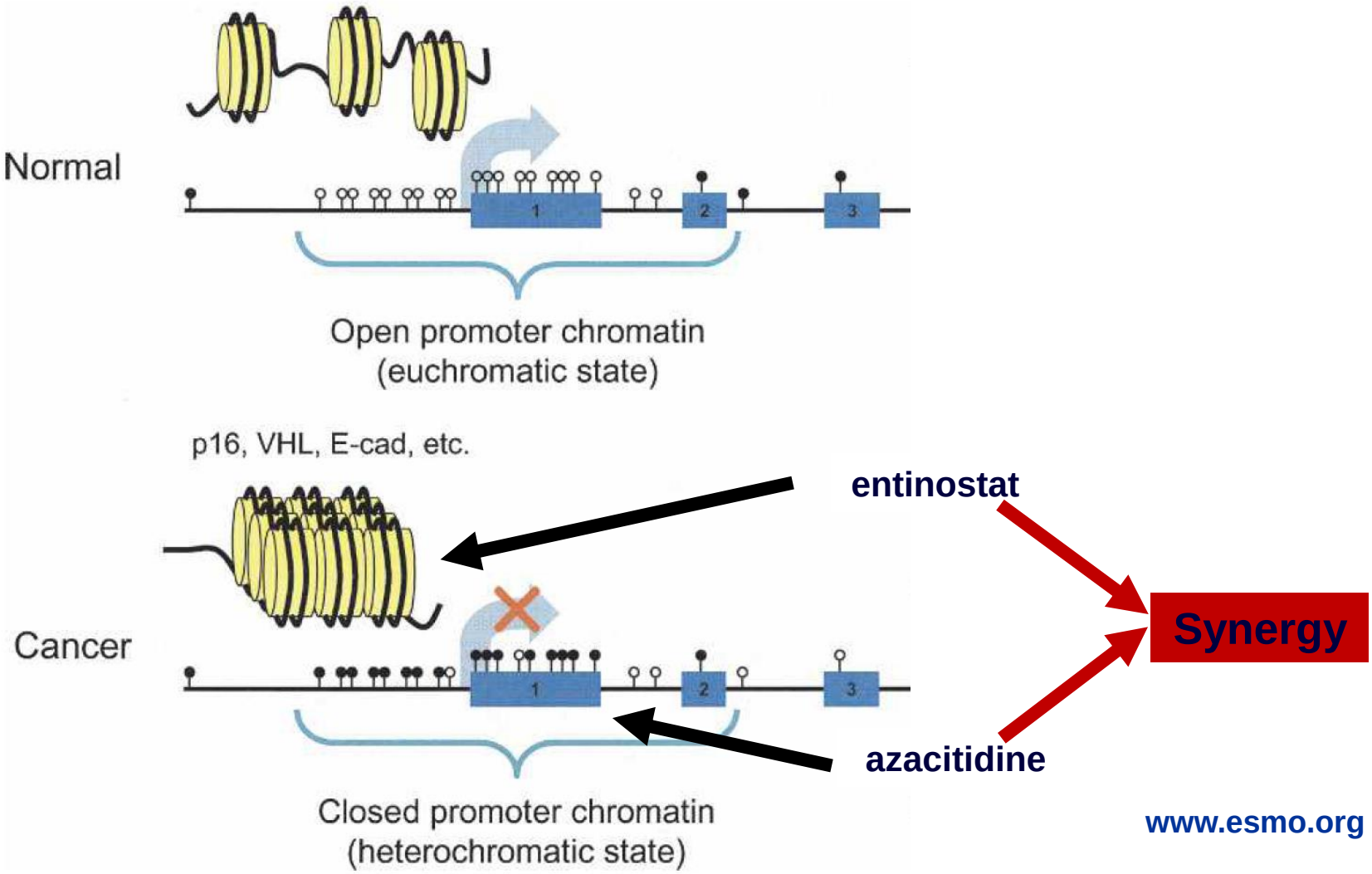
Mellman Nature 2

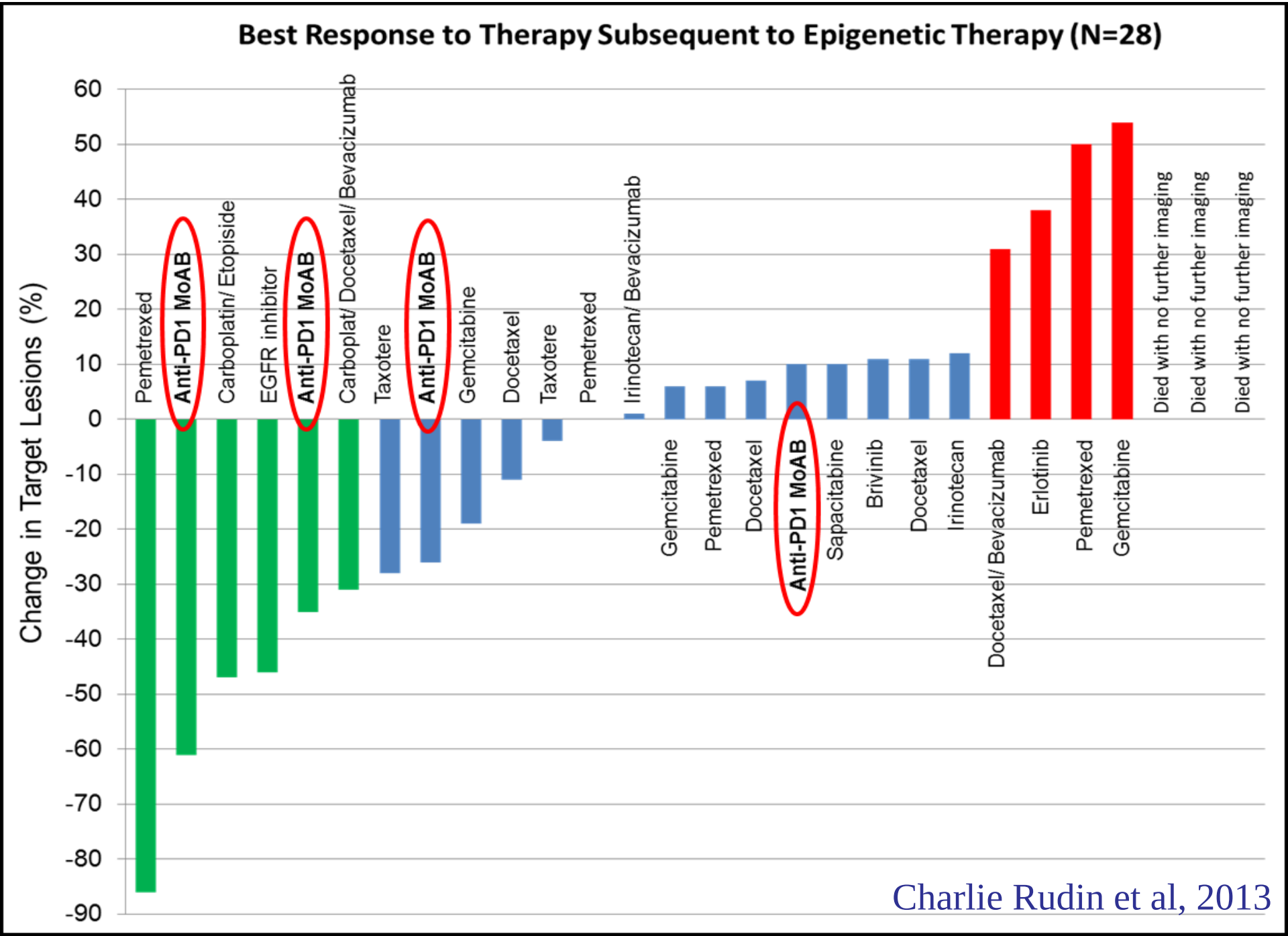
Combination therapies



Chen Immunity 2013

Combination epigenetic therapy

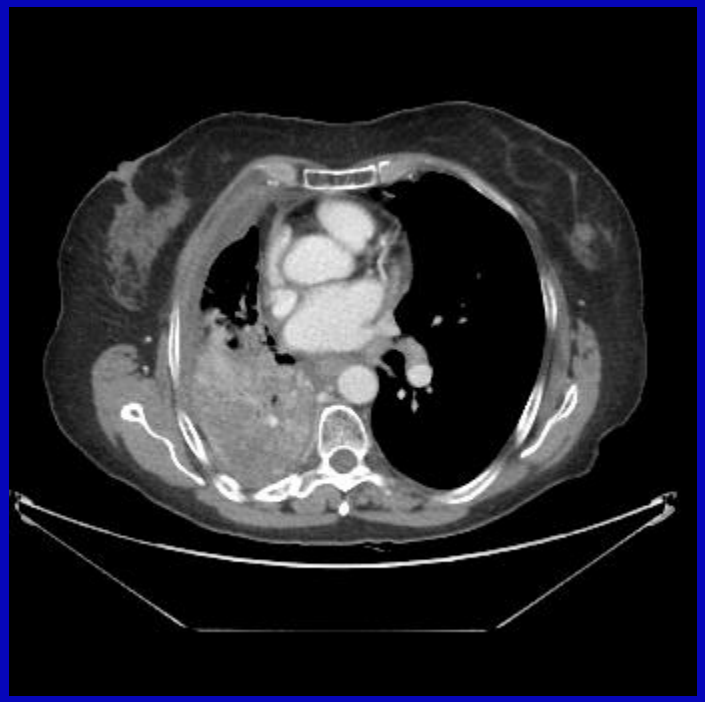




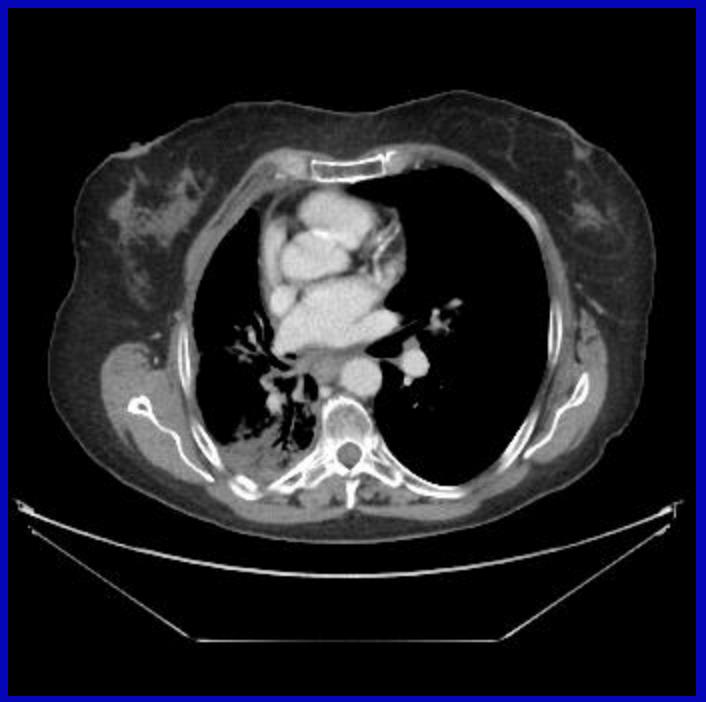
Charlie Rudin et al, 2013

Epigenetic Therapy Followed by Anti-PD-L1: An example of response

10/2011



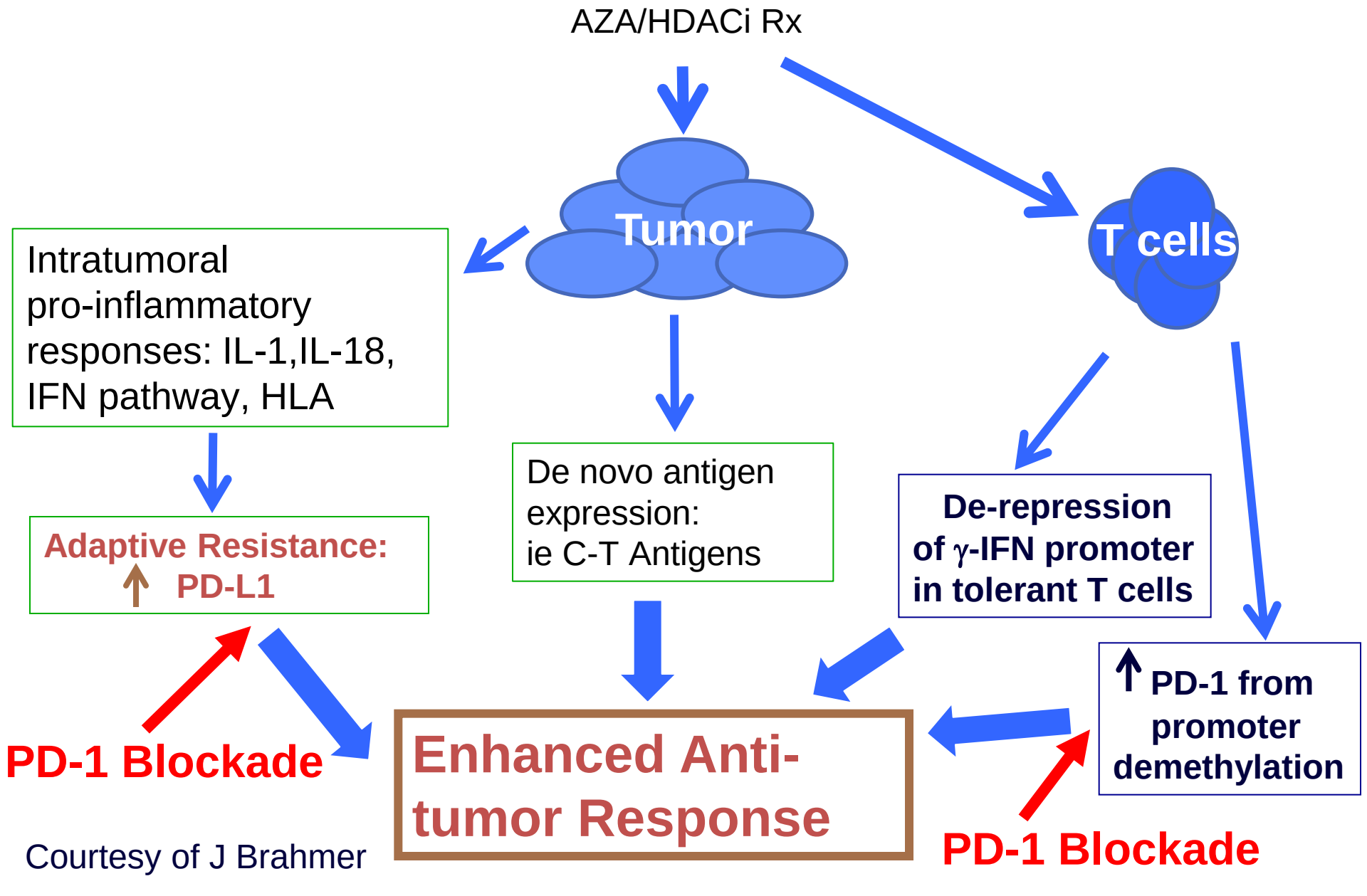
12/2011



Pt 010-6084 – History 64 y/o Diagnosed with IIIB adenoca Rx with XRT+ Tax/carbo, pemetrexed + carbo, Entinostat + 5aza x 6 cycles

M. Brock, C. Rudin, J. Brahmer, S. Baylin

Synergy between Epigenetic Modulation and PD-1 pathway blockade - Unleashing the Perfect Storm against Tumors

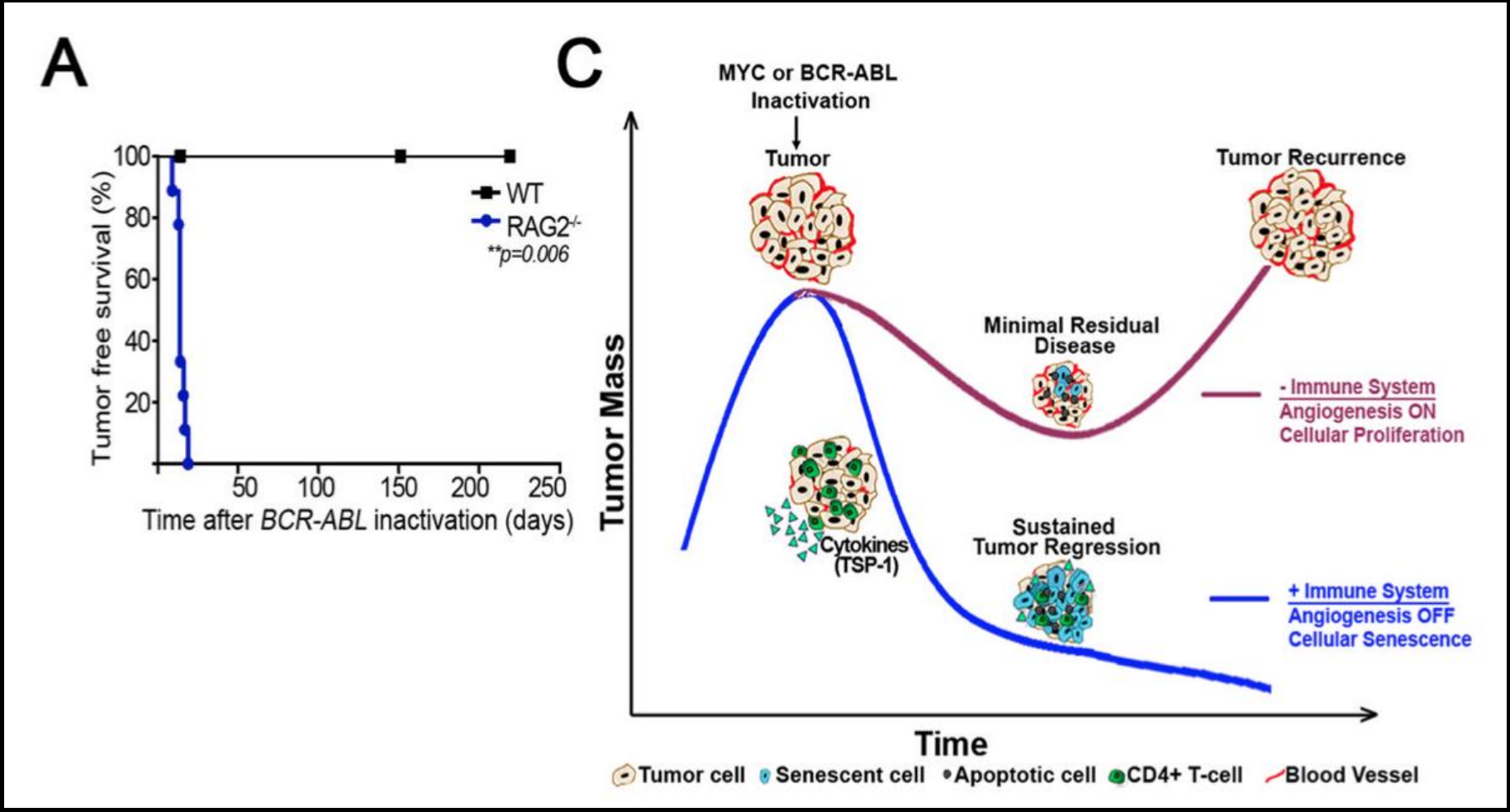


Courtesy of J Brahmer

What about driver-mutated tumors that respond dramatically to blocking the activated driver?

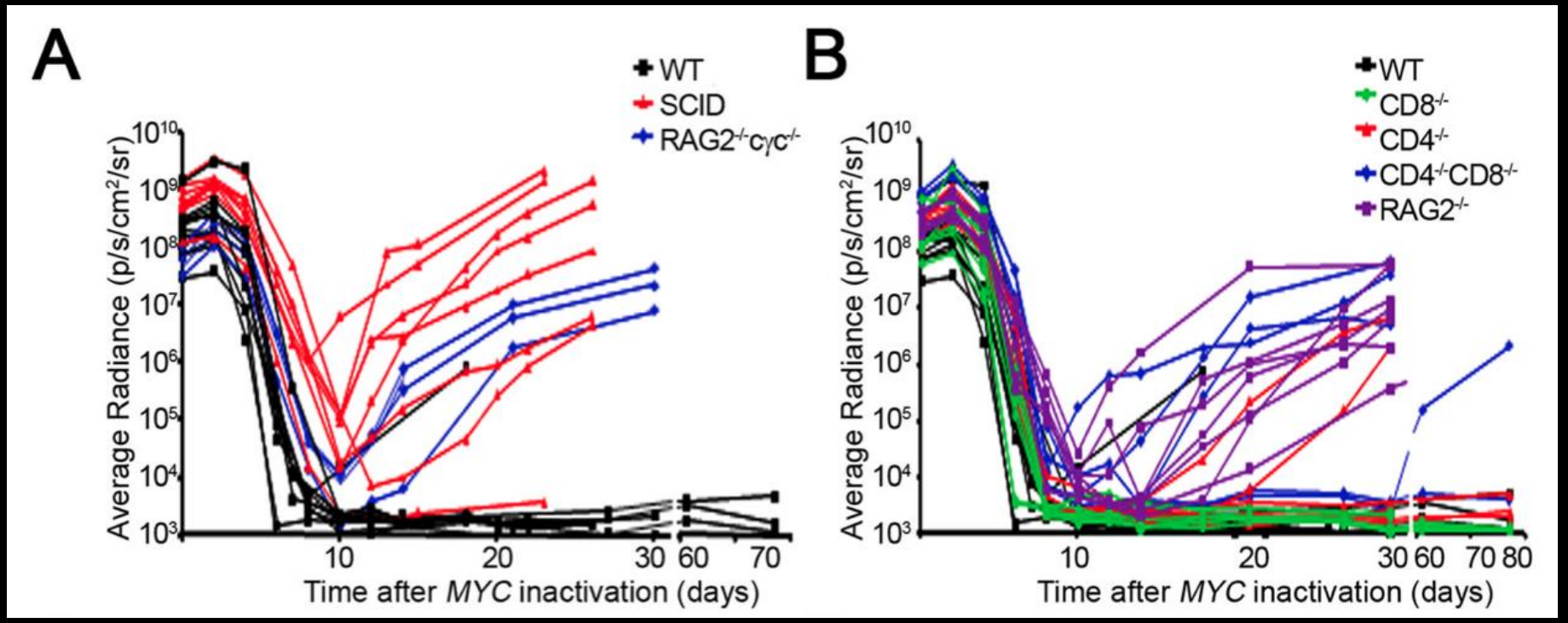
Is the immune system relevant in tumors with “driver oncogenes”?

The immune system and "driver oncogenes"



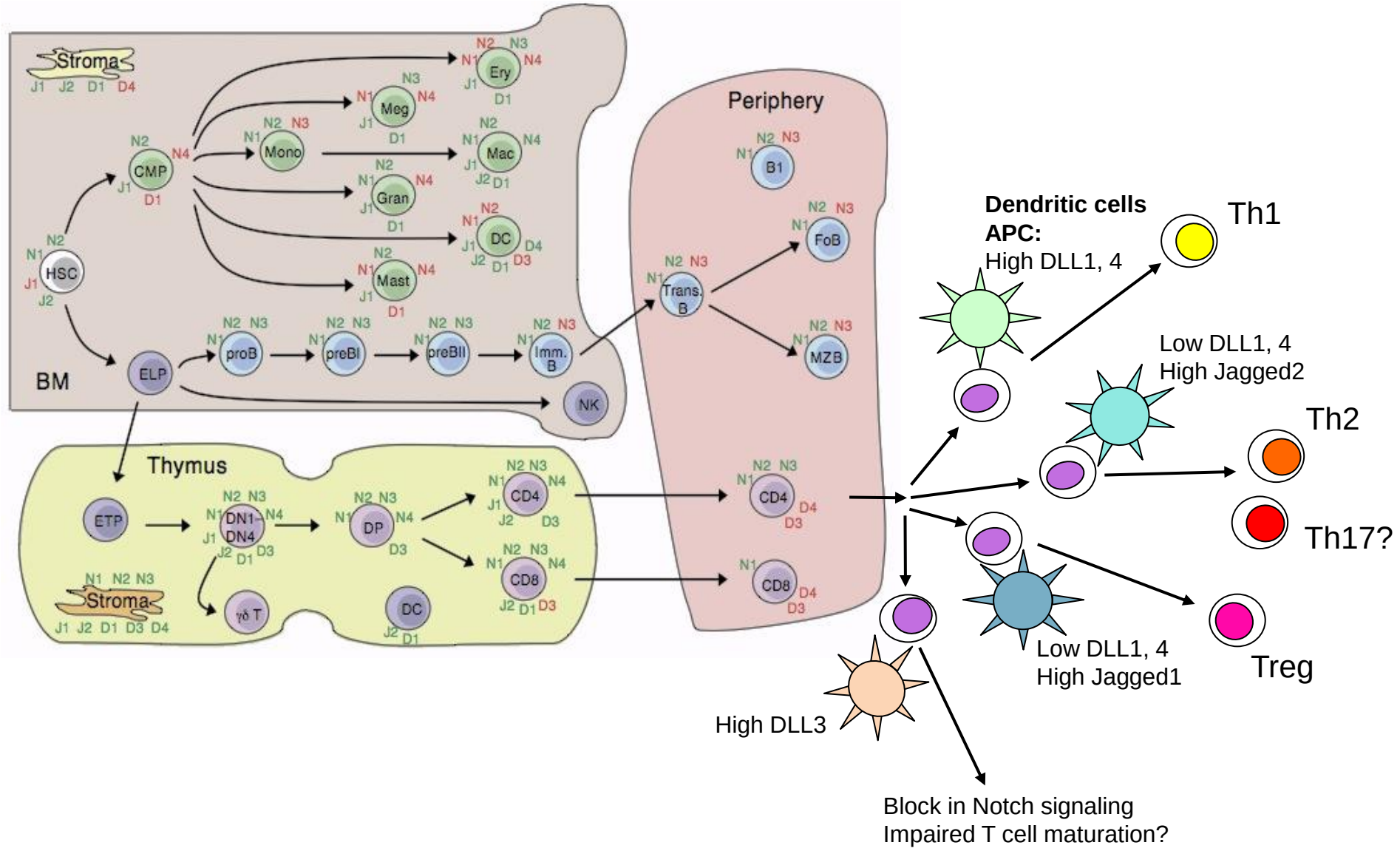
Rakhra and Felsher, Cancer Cell, 2010

Immune system and MYC

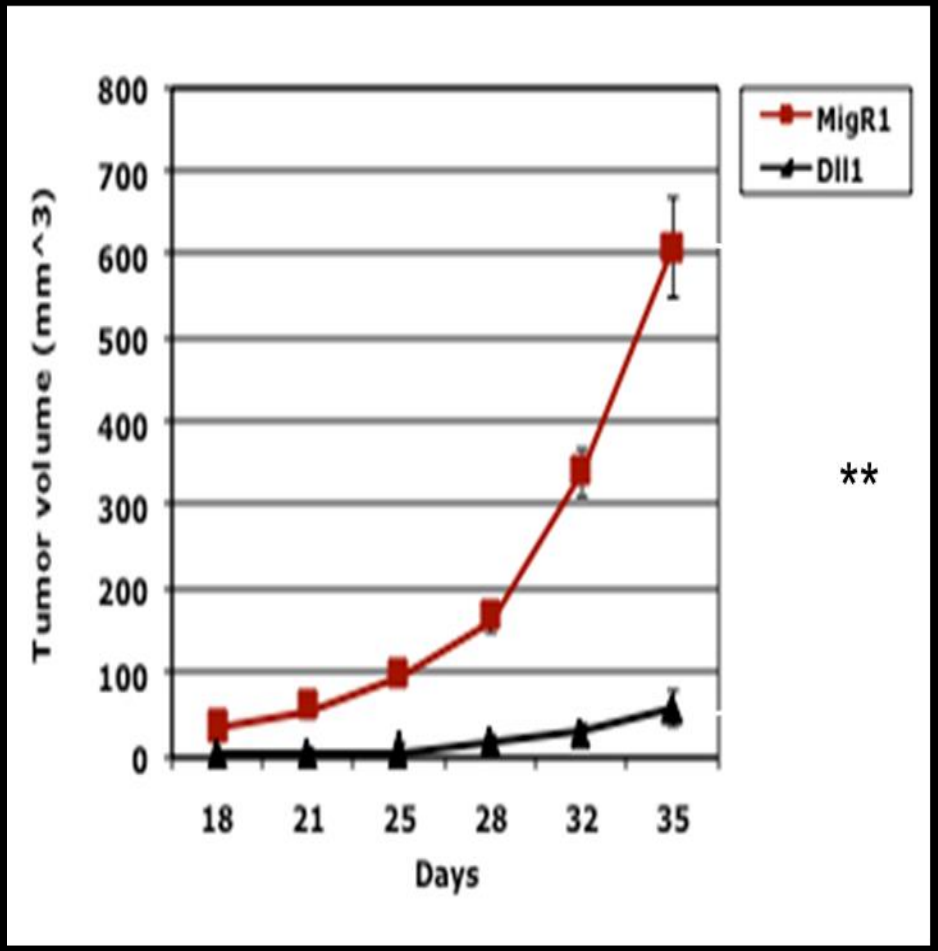


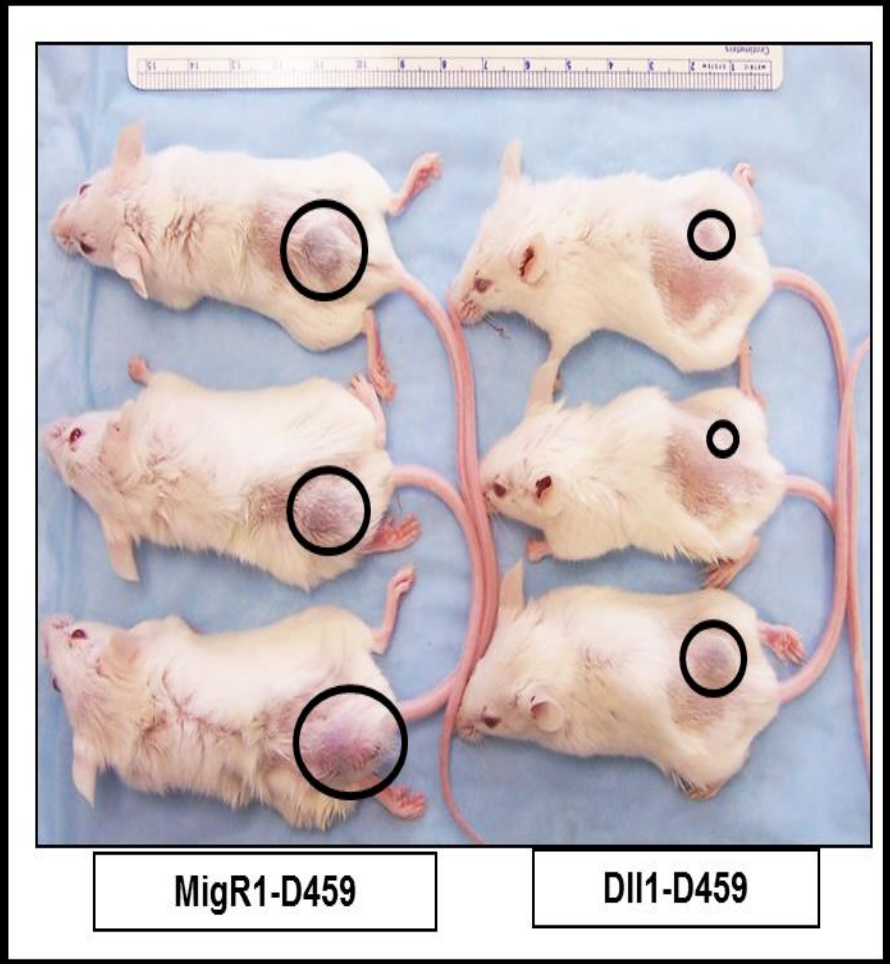
Rakhra and Felsher, Cancer Cell, 2010

Notch is an important regulator of hematopoiesis, development and differentiation of T-lymphocytes



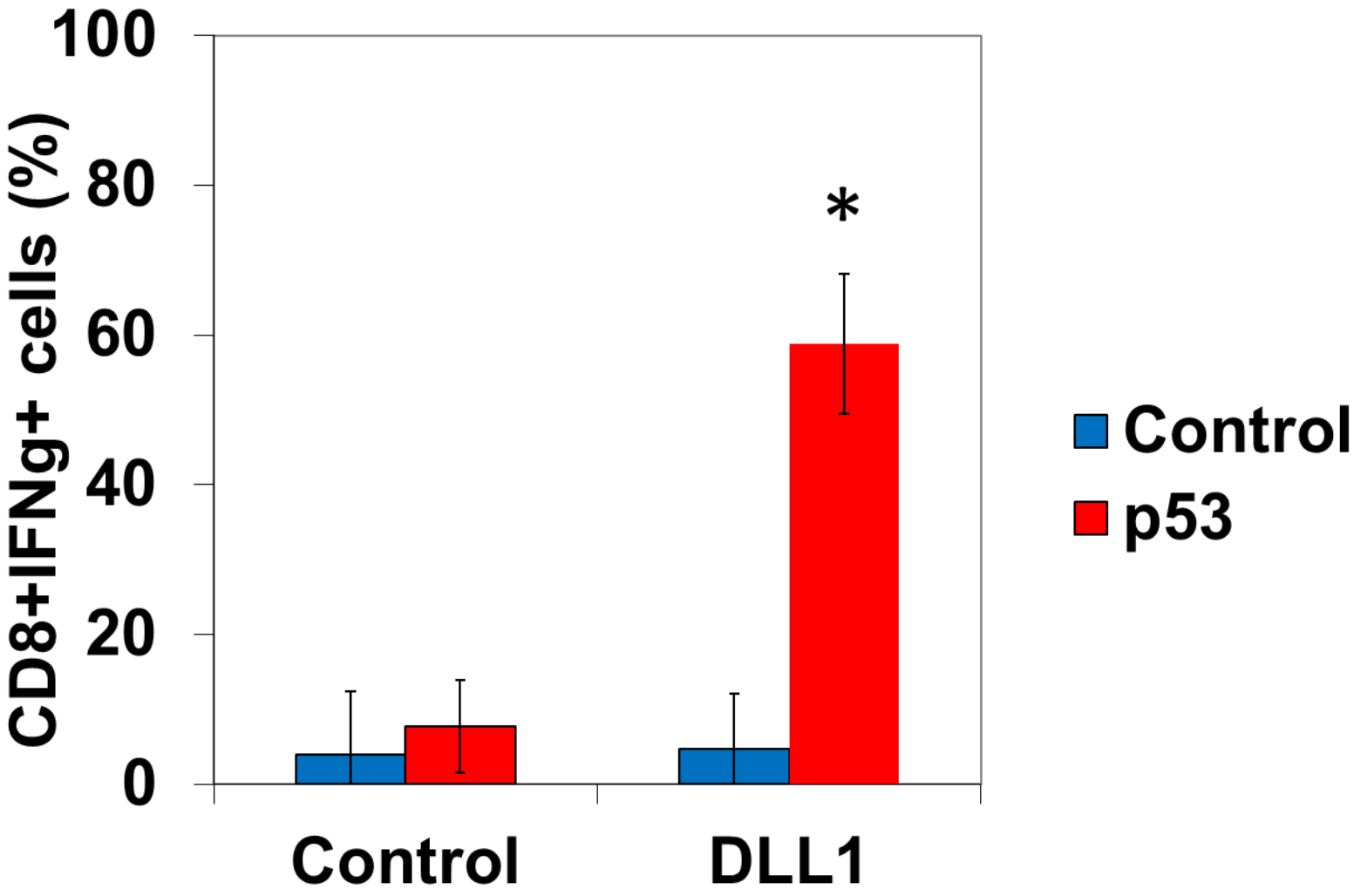
Restoration of DLL-1 in Bone Marrow Inhibits Tumor Growth



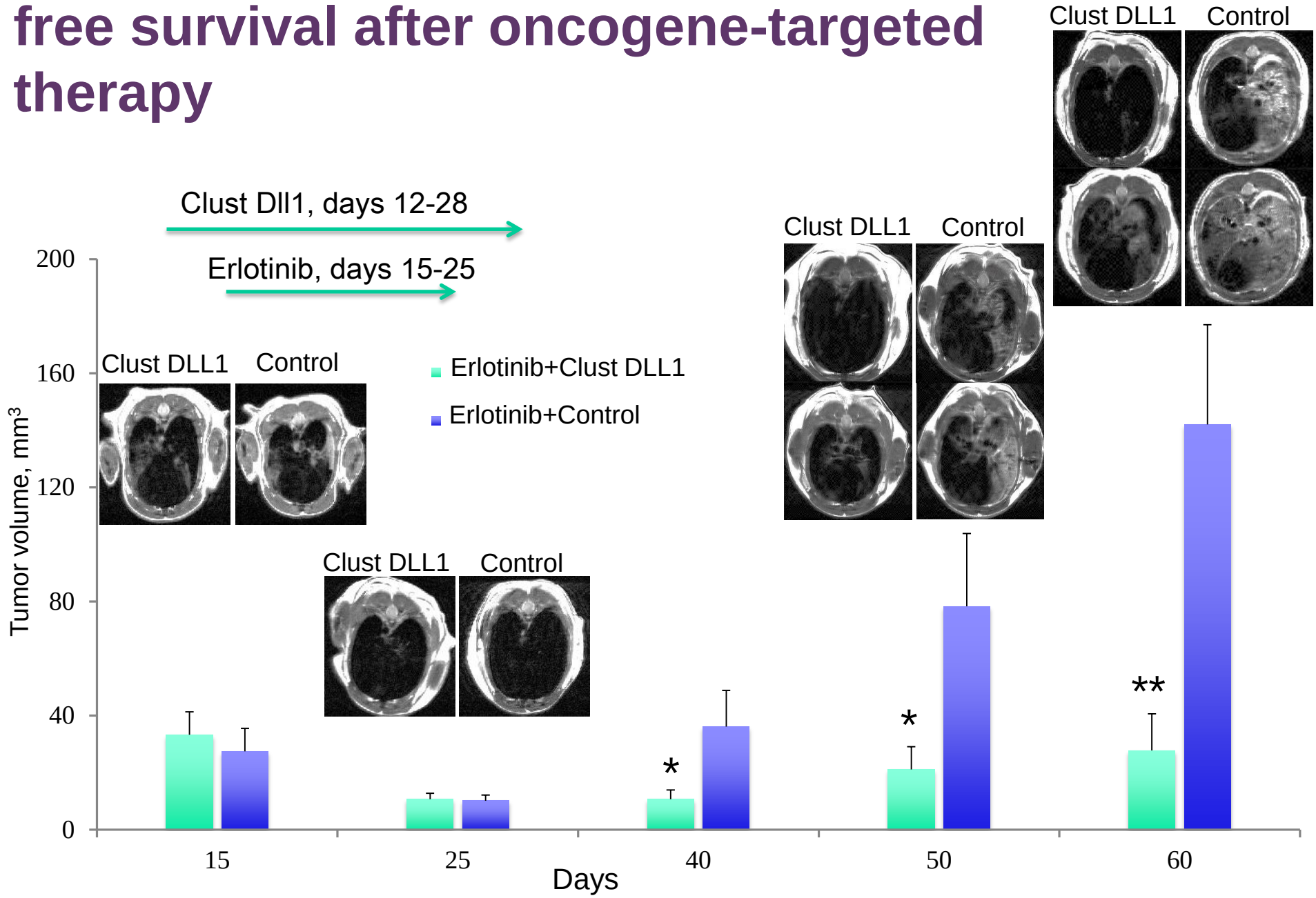


Huang, Dikov, Carbone, CR 2011

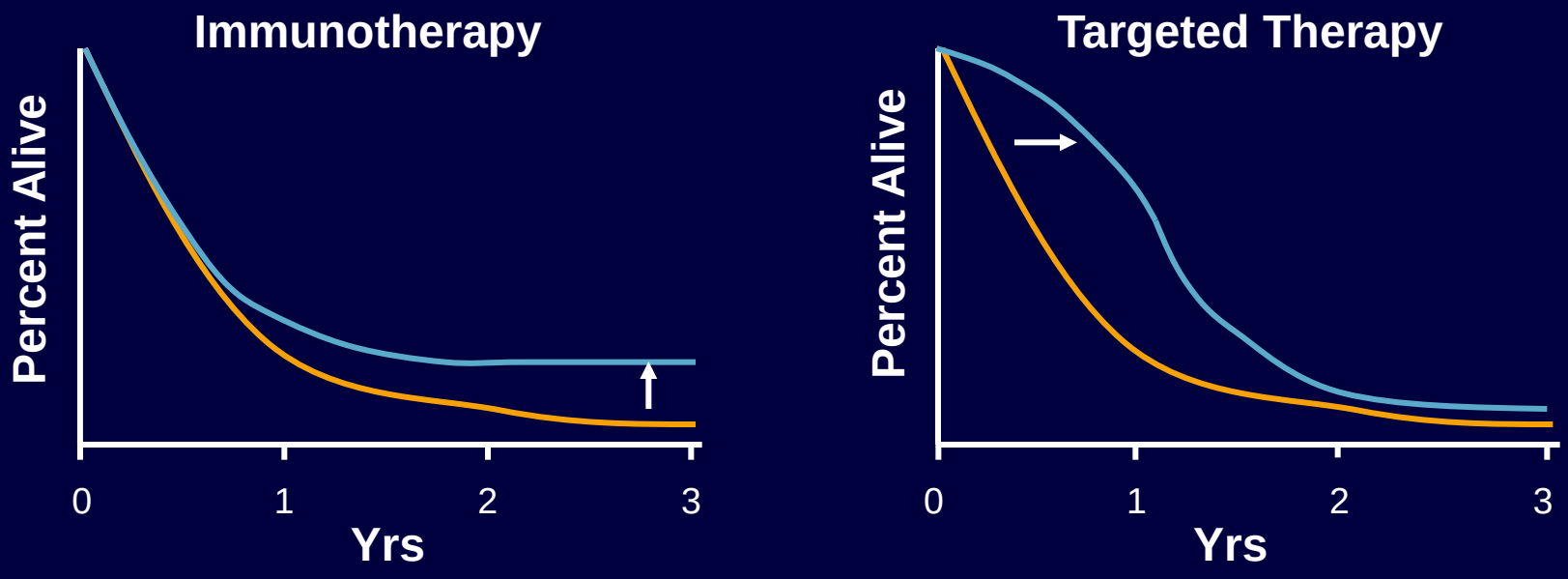
Induction of Mutant p53-Specific Immune Response by Clustered DLL1



Clustered DLL1 improves progression-free survival after oncogene-targeted therapy

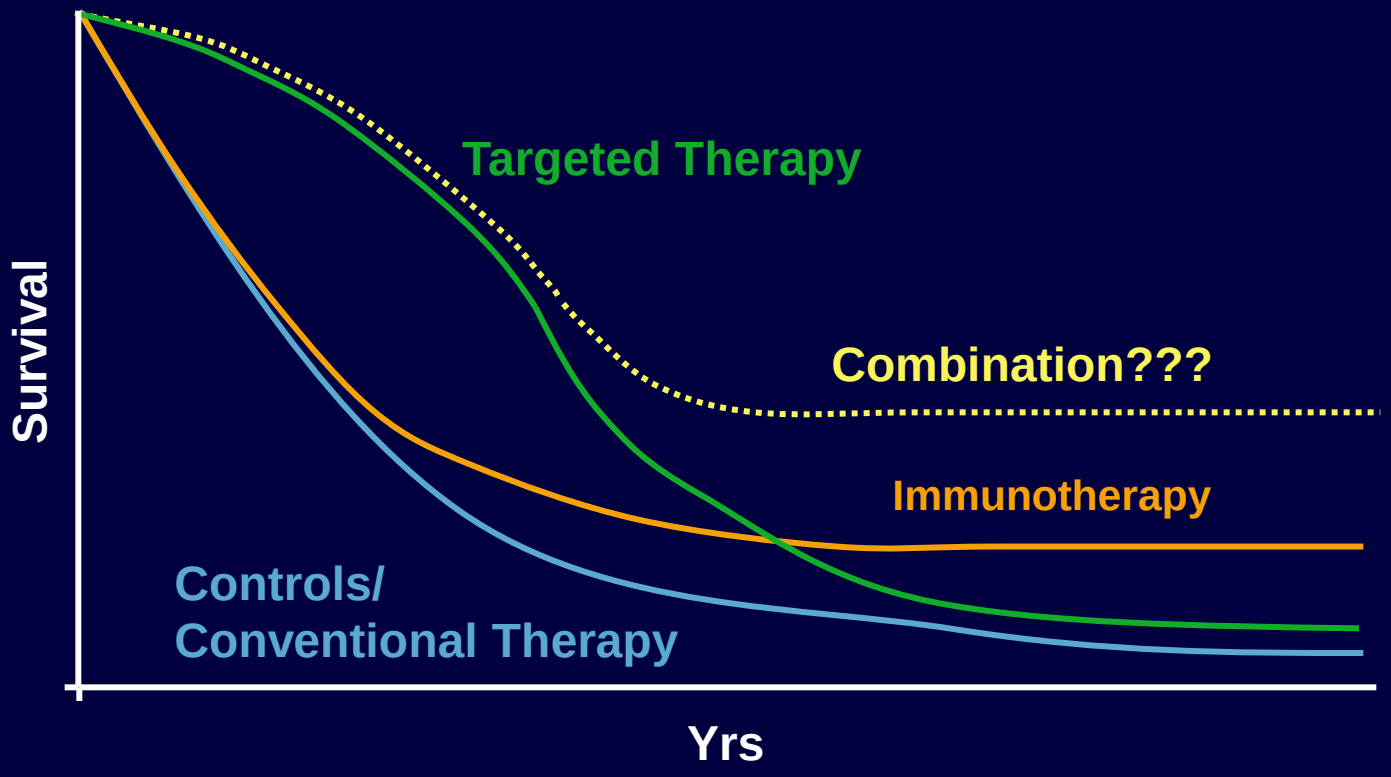


Response Patterns for Immunotherapy Compared With Targeted Therapy



Ribas A, et al. Clin Cancer Res. 2012;18:336-341.

Combining Immunotherapy and Conventional Therapies



Adapted from Ribas A, et al. Clin Cancer Res. 2012;18:336-341.

Conclusions

- **Lung cancer is an immuno-responsive disease!!**
- **T cell mediated immune response is modulated by co-stimulatory and co-inhibitory signals.**
- **Co-inhibitory molecules or immune checkpoint molecules prevent overstimulation of immune responses.**
- **PD-L1 is expressed on tumor cells and negatively regulate immune responses to cancer**
- **Co-signaling stimulatory & inhibitory pathways are important therapeutic targets**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