Disclosures

- Consulting/honoraria from:
  - Genentech/Roche
  - Pfizer
  - Novartis
  - BioDesix
  - Merck
  - EMD Serono
  - GSK
  - Boehringer Ingelheim
  - Amgen
  - Briston-Myers Squibb

The biology of the immune system in lung cancer

-OR-

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

David P. Carbone, MD PhD

Director, James Thoracic Center

The Ohio State University Wexner Medical Center

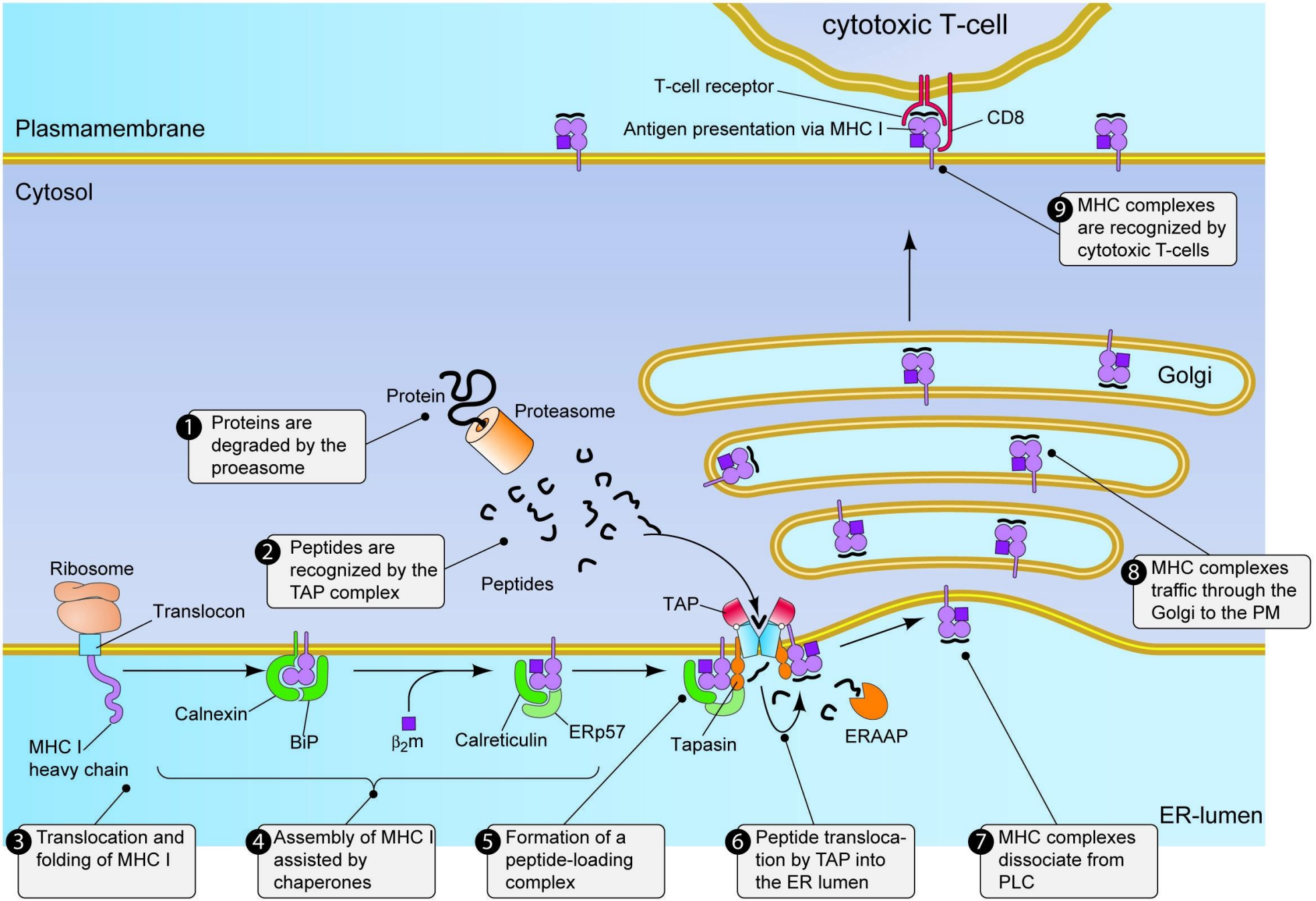
Immunotherapy Advantages

- The immune system has evolved over millions of years (or a few thousand if you are a southern republican in the USA) to detect and eliminate “non-self”.
- Potentially exquisitely specific and sensitive, able to detect single amino acid changes, even in intracellular proteins.
- Highly regulated to avoid self-toxicity
- Adaptable to novel challenges not previously seen (hundreds of novel protein sequences in lung cancers e.g. mutant oncoproteins)

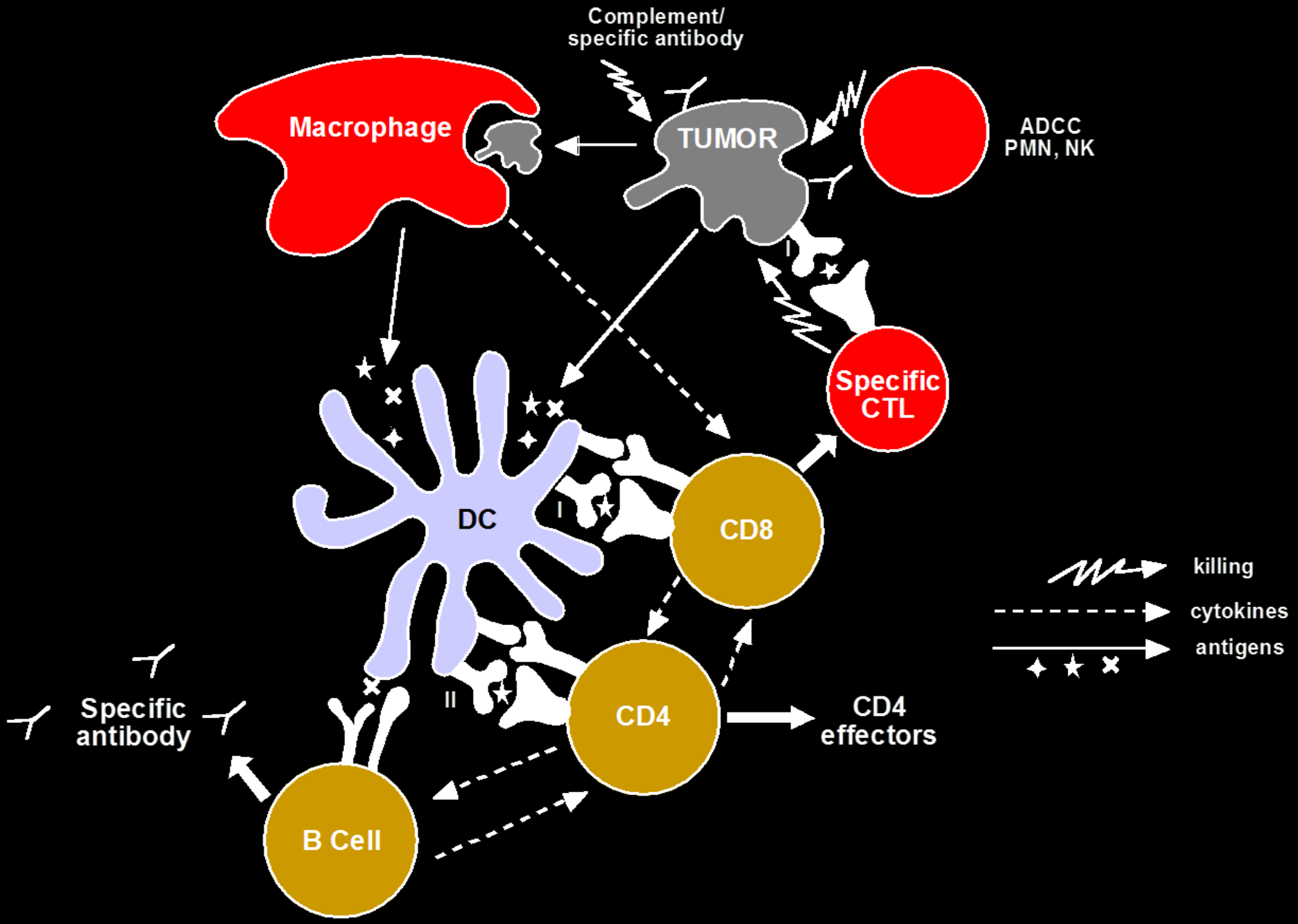
Effector arms for tumor clearance

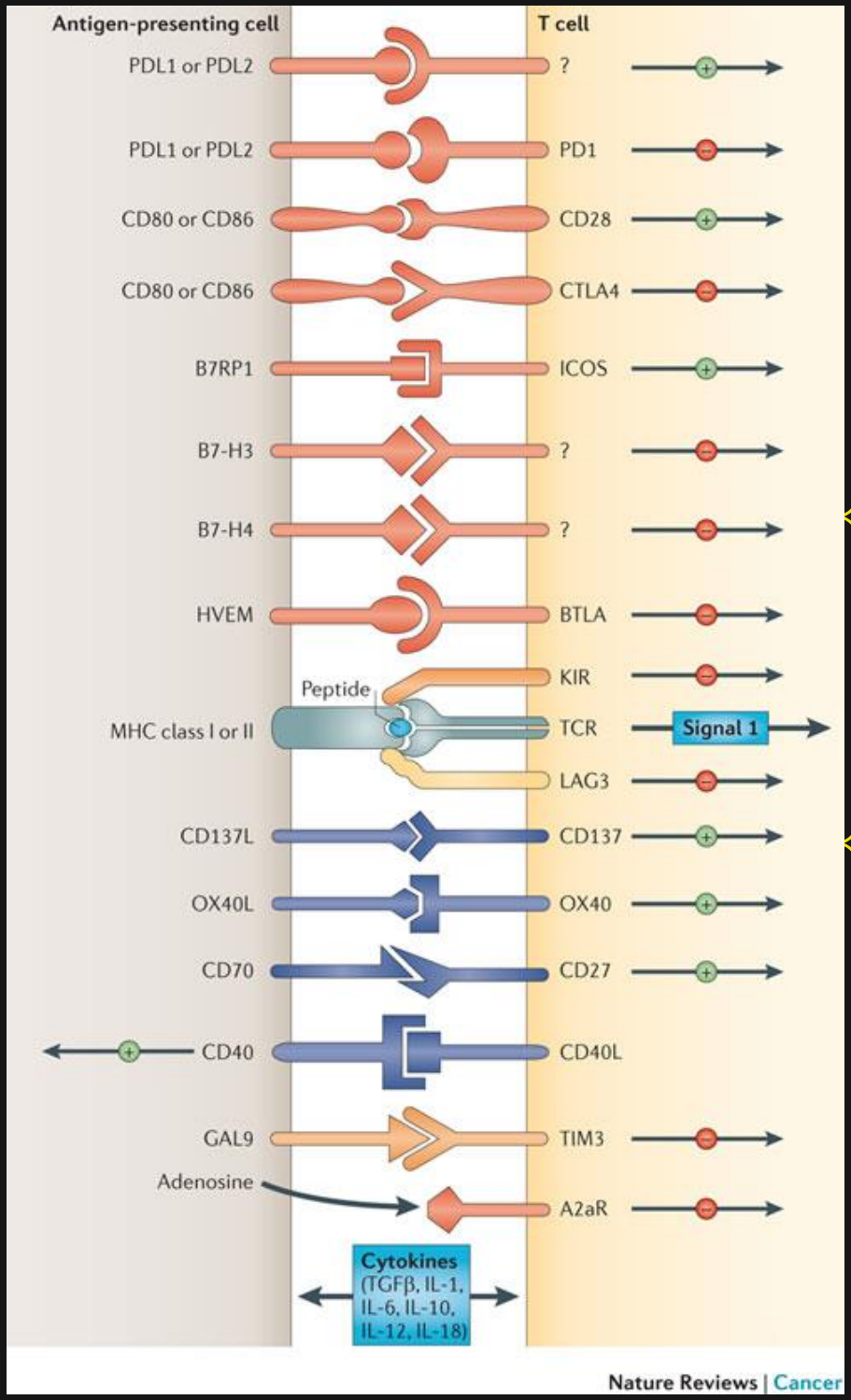
- “Natural Killers”- detect non-self by lack of self-markers, e.g. MHC class I
- Granulocytes/Macrophages – directly clear non-self
- Antibody Dependent Cellular Cytotoxicity
  - Dependent on cell surface antigens bound by certain subclasses of antibodies that activate complement
- Cellular immunity
  - Evolved to clear intracellular pathogens by detecting neoantigens presented on class I MHC
  - Capable of specifically recognizing somatically mutated oncoproteins.

Class I MHC presentation of intracellular antigens



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





Regulation of T Cell Responses Via Multiple Co-Stimulatory and Inhibitory Interactions

- T cell response to antigen is mediated by peptide-MHC recognized by TCR (first signal – specificity)
- B7 family of membrane-bound ligands bind both co-stimulatory and inhibitory receptors (second co-stimulatory signal)

Pardoll DM Nature Rev Cancer 12, 252, 2012

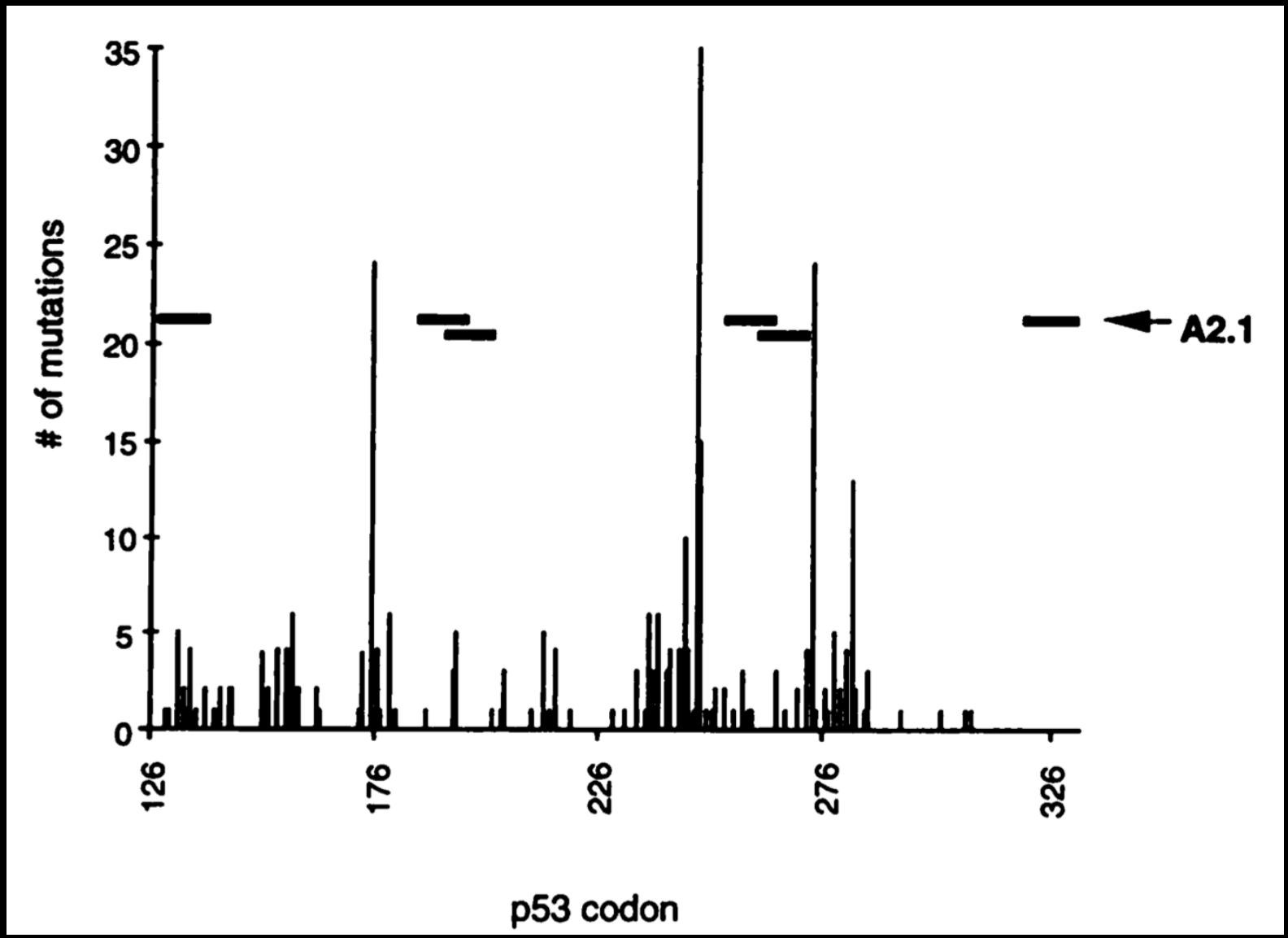
Using the immune system for therapy

- Passive humoral
  - Blocking antibodies
    - Cetuximab, bevacizumab
  - Antibody drug conjugates
  - Bispecific antibodies
- Passive cellular
  - Chimeric antigen receptor (CAR) T cells
- Vaccines
  - Defined antigen, whole tumor cells (auto/allo)
- Immunomodulatory
  - Cytokines (e.g. IL2, bevacizumab)
  - Ipilimumab, PD1

Lung Cancers are highly mutagenized -

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

Evidence for selection against mutant epitopes on class I MHC



None 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

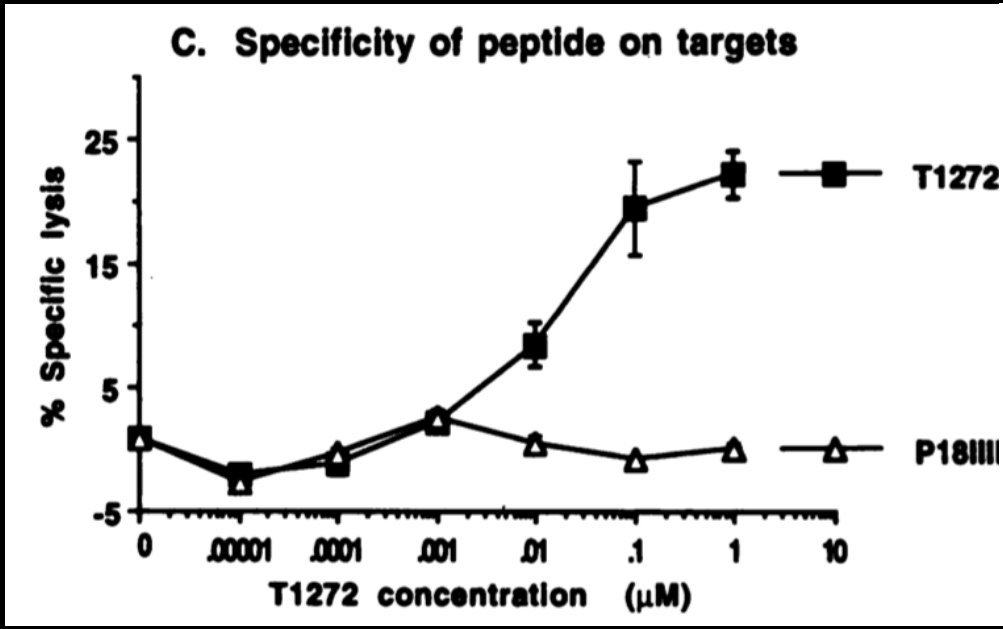
Table 1 *Comparison of the frequency of HLA A*0201 alleles in tumors bearing missense p53 mutations that either lie within or outside the consensus peptide motif [X(ILM)XXXXXX(VLIA)]*

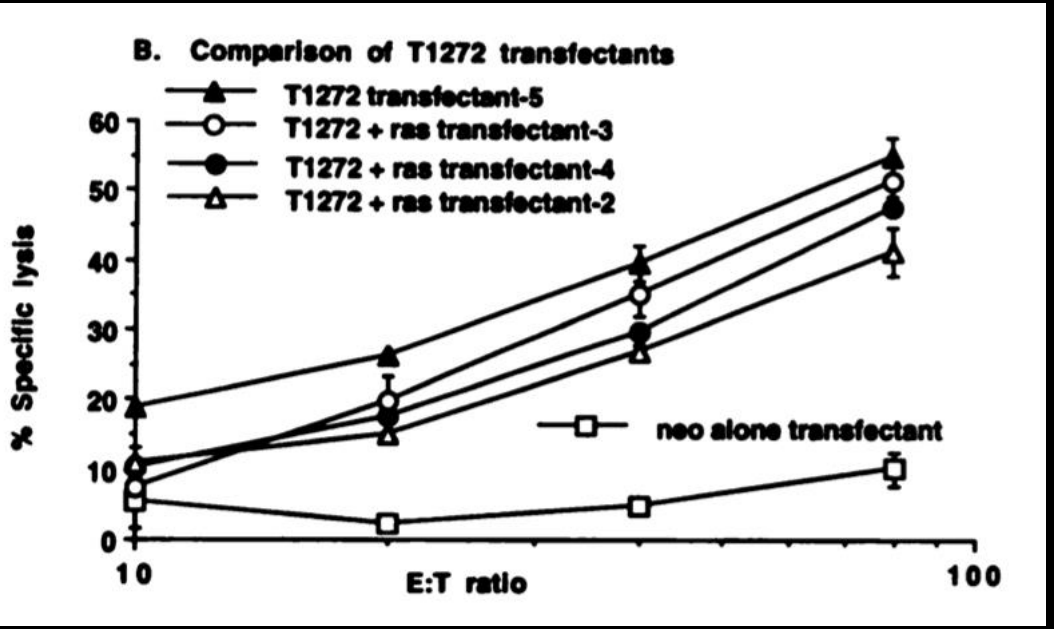
	Fraction with A*0201 allele	P^a
Mutation in motif	0/6 (0)[b]	0.02
Mutation not in motif	10/28 (36)[b]	NS
General population	46[b]	

[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**

P53-specific killing in murine models





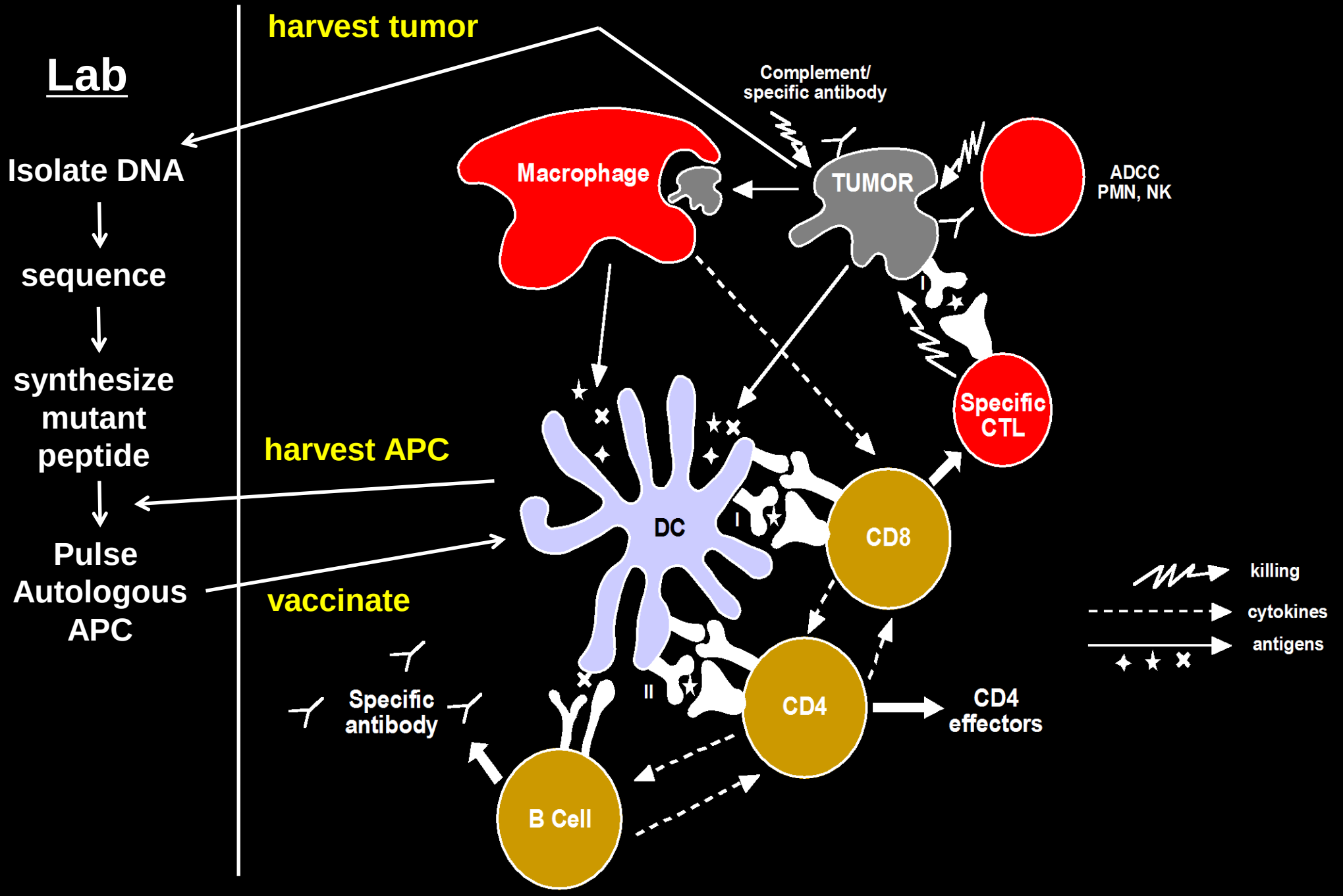
Yanuck, Carbone, Berzofsky, Cancer Research, 1993

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

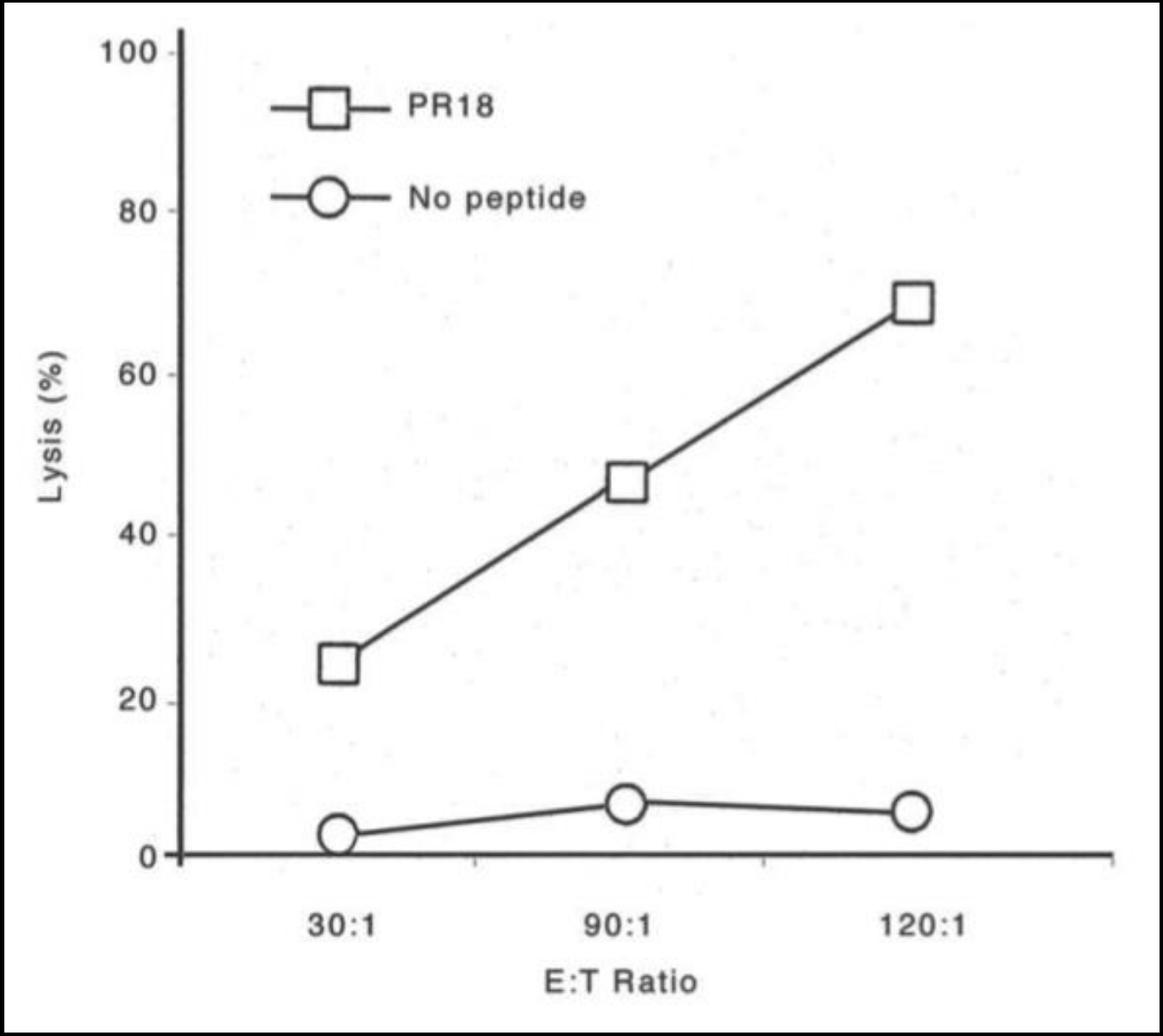
Custom mutant peptide-pulsed DC vaccine



Results

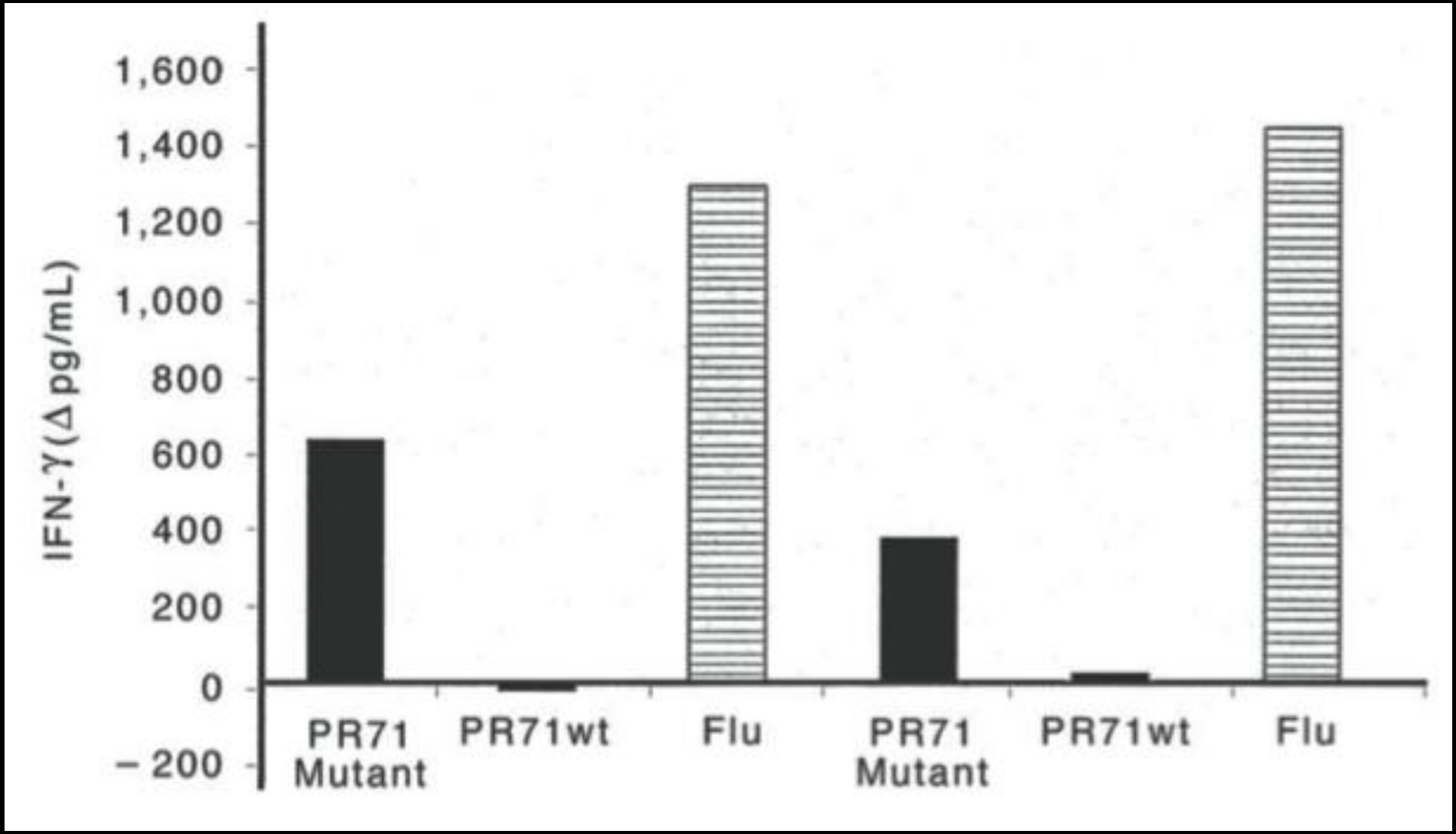
- 26% positive post-vaccine specific immune response
- Median survival 115 days (26 to 685+)
- No objective responses in evaluable patients
- 5 had stable disease, 4 to 40 months
- One colon cancer patient with resected lung metastatic disease and + kras responses NED after >5 years
- One KRAS mutant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

Specific CTL to Kras 12 cys



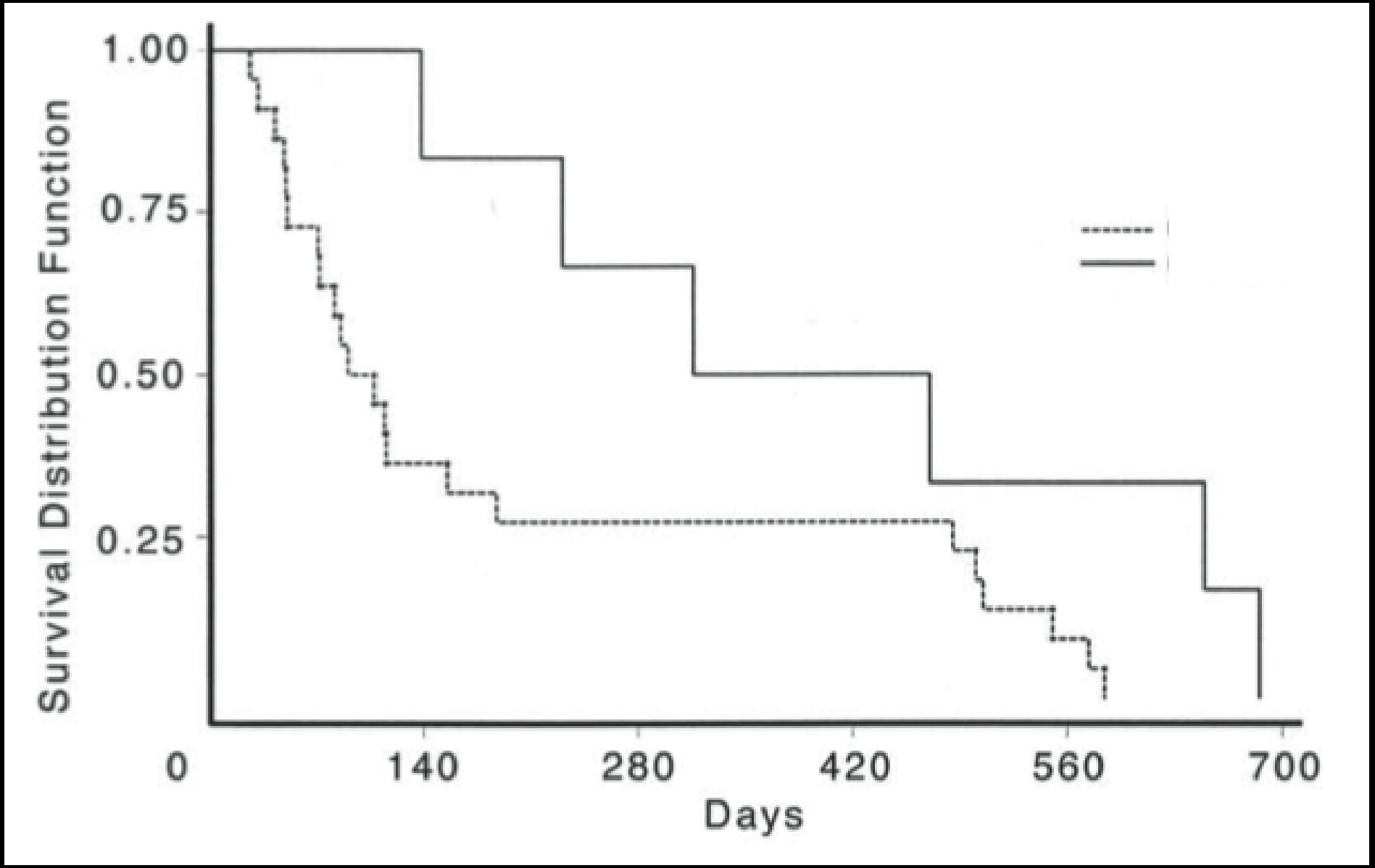
Carbone et al, JCO 2005

CTL to mutant, not wt p53



Carbone et al, JCO 2005

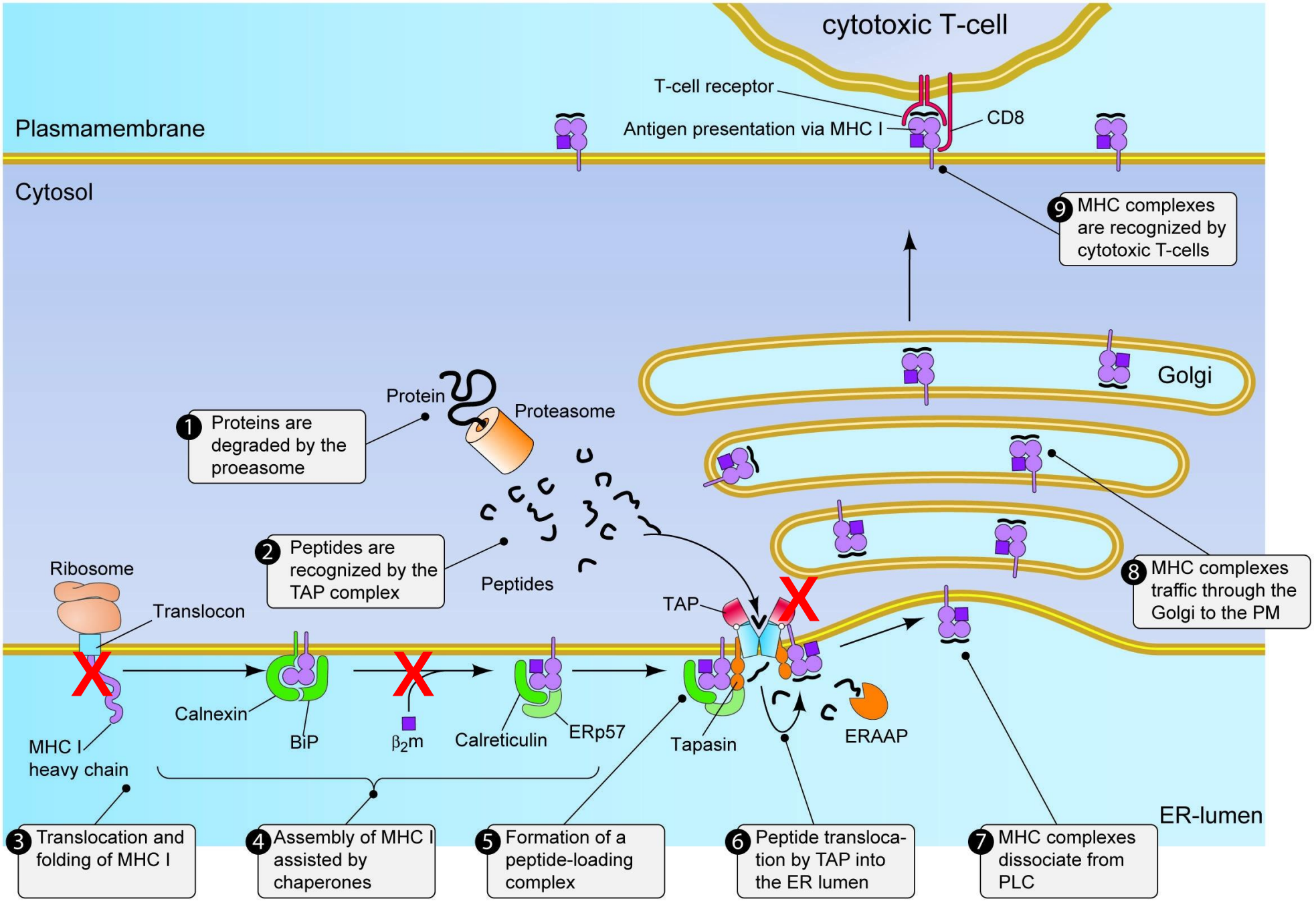
Survival



Carbone et al, JCO 2005

Lung cancers are highly mutagenized - are there structural defects in immune pathways that render tumors non-responsive?

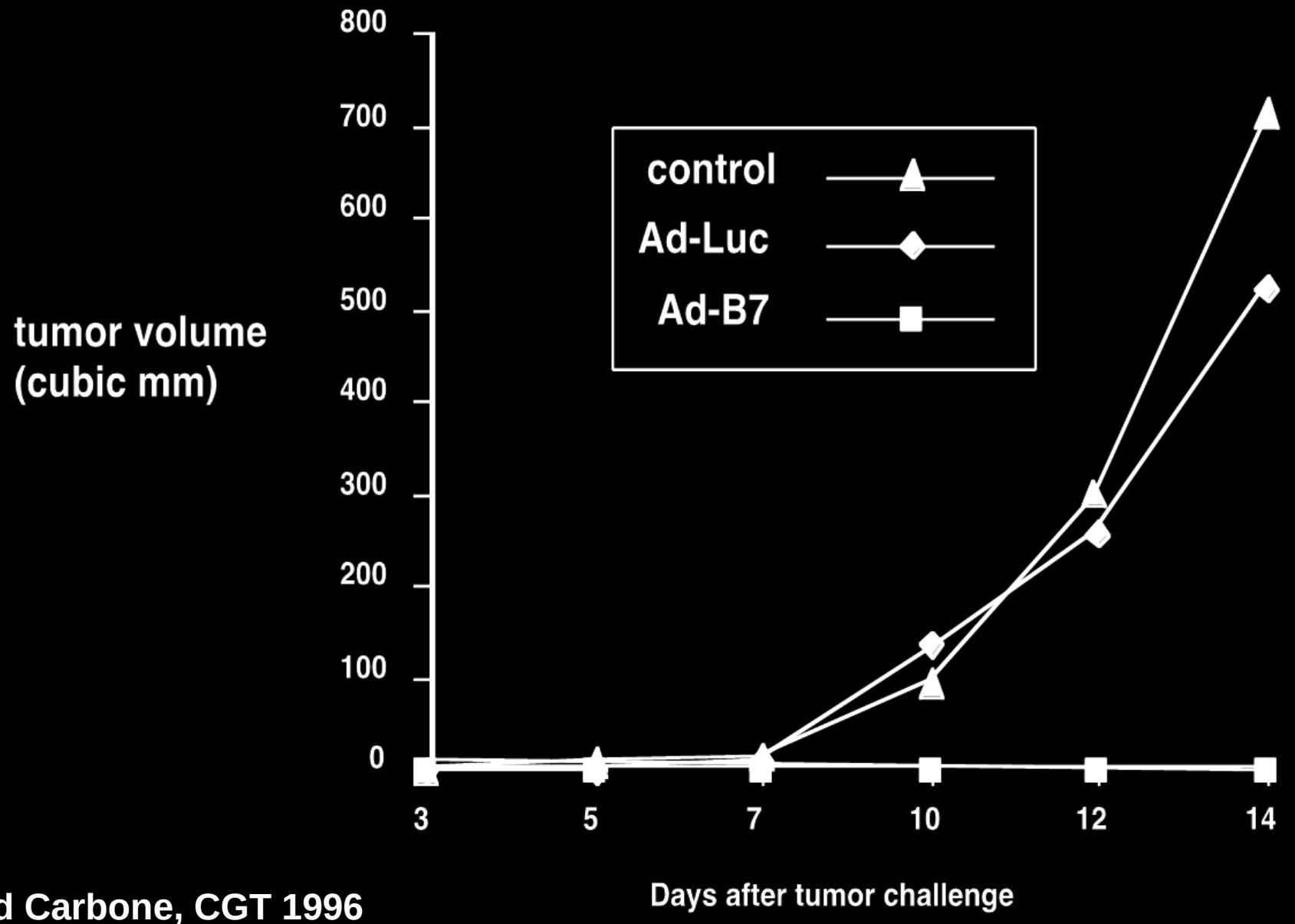
Tumor loss of Class I MHC presentation



Clinically evident tumors must have evaded immune recognition/killing

- Immune surveillance
  - clearance of readily recognized tumor cell clones
- Structural alterations of tumor antigen presentation
  - In 5-10% of human tumors:
    Deletion/mutation of MHC class I, β-2 microglobulin, and TAP1
- Functional
  - 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 functional defects can theoretically be overcome**

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

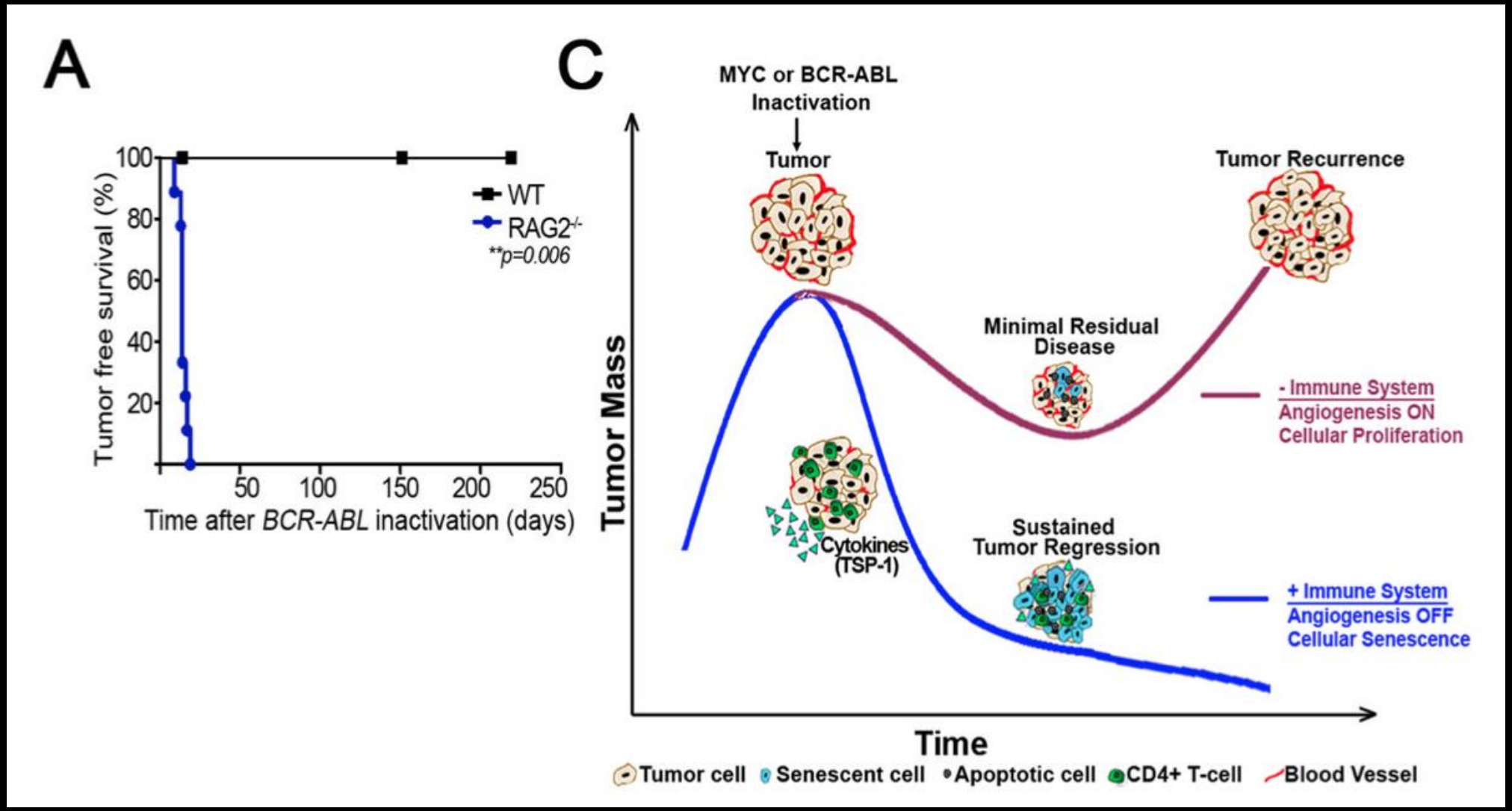


Lee and Carbone, CGT 1996

Driver-mutated tumors respond dramatically to blocking the activated driver

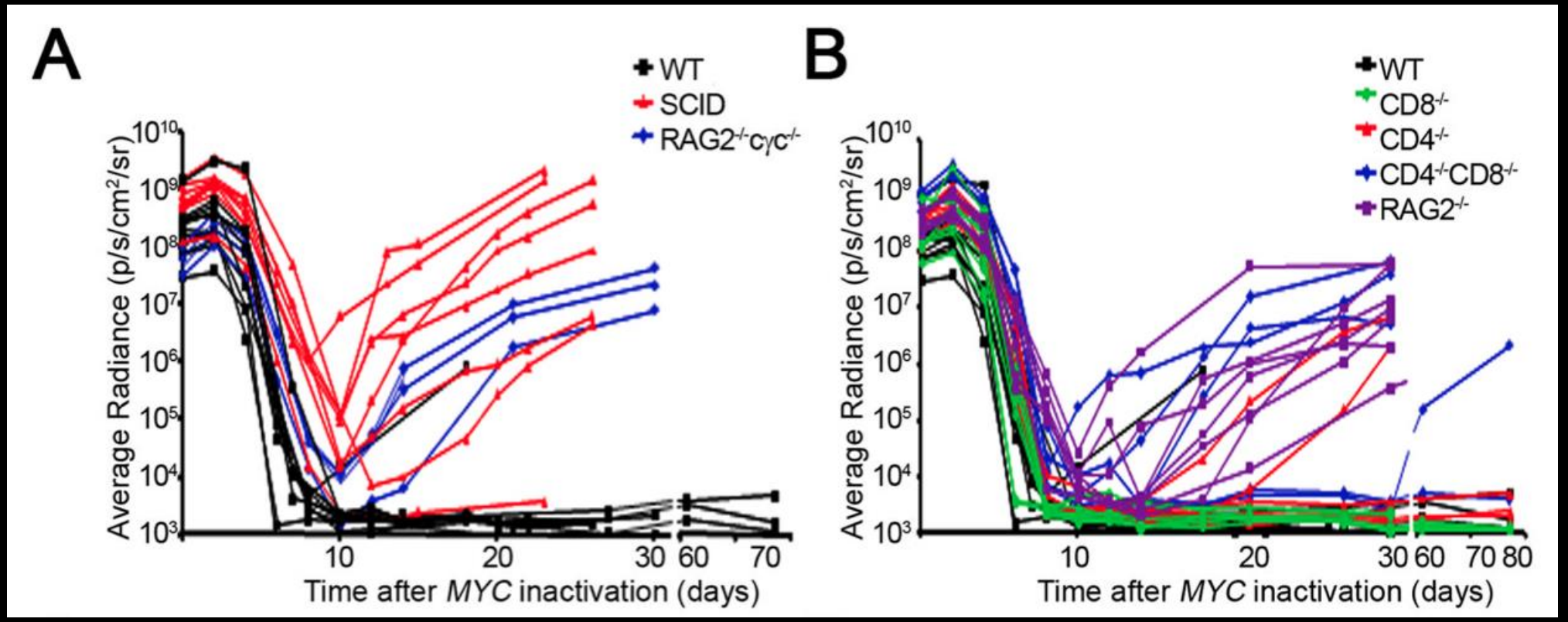
Is the immune system relevant in tumors with “driver oncogenes”?

The immune system and "driver oncogenes"



Rakhra and Felsher, Cancer Cell, 2010

Immune system and MYC

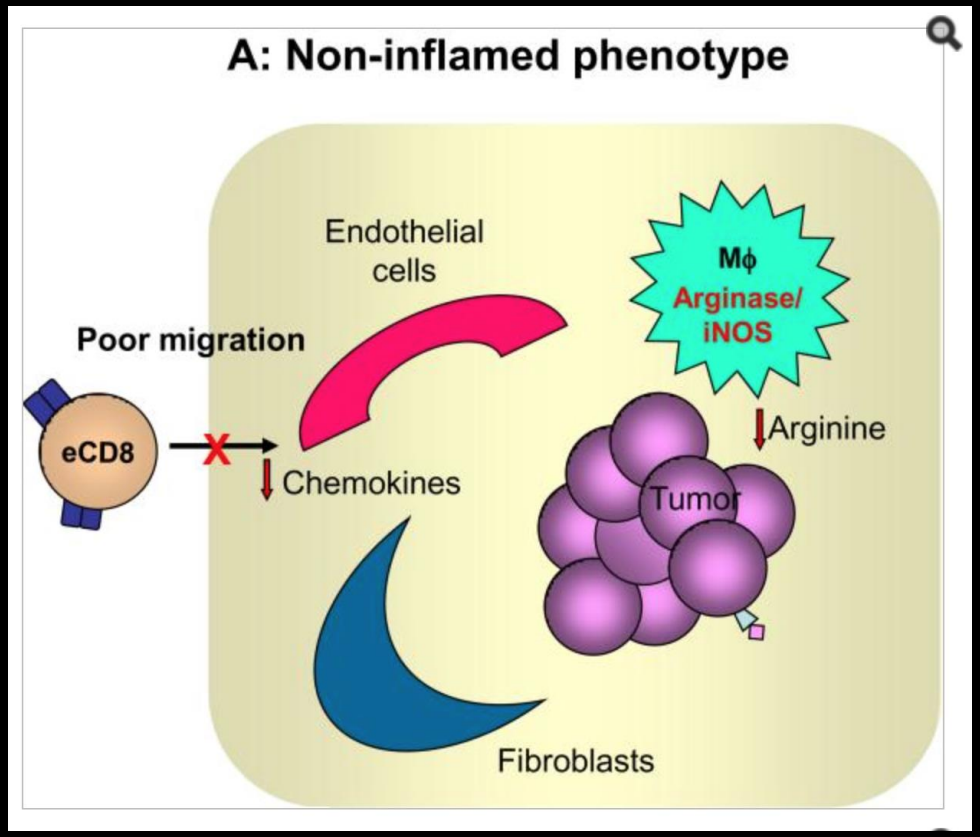


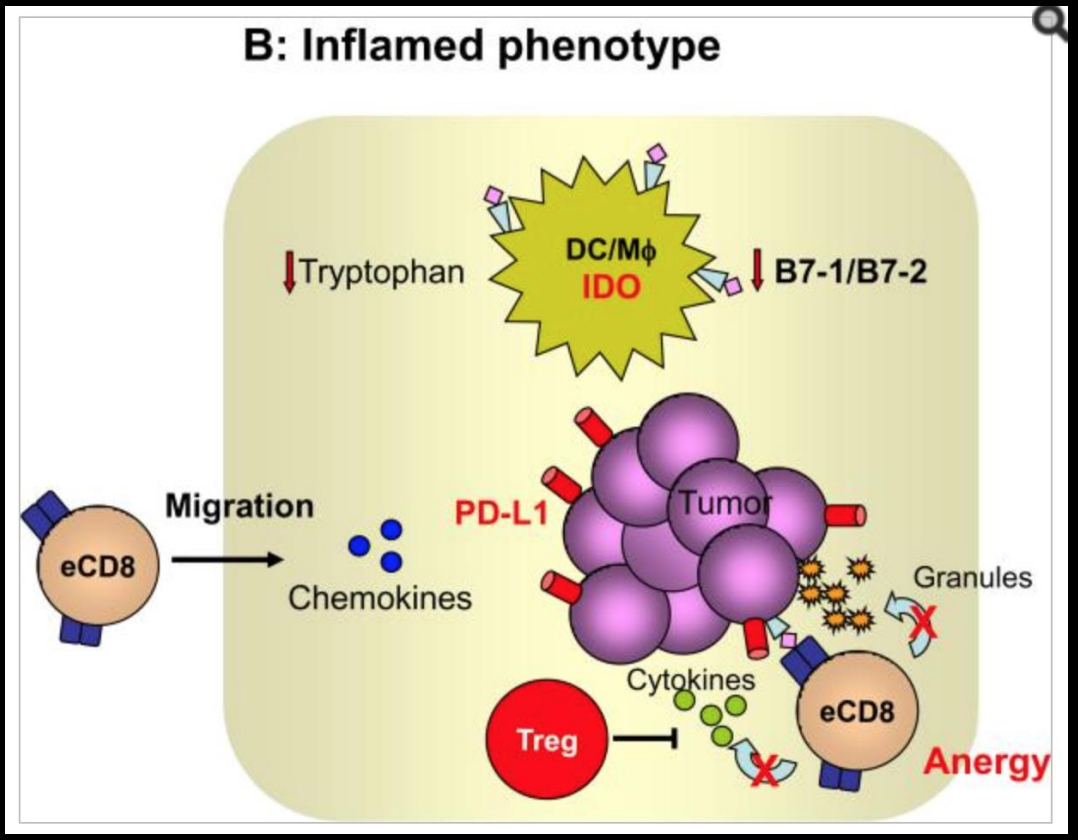
Rakhra and Felsher, Cancer Cell, 2010

Functional defects?

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

“Inflamed Tumor Phenotype”





Distinct subsets of tumors may escape from immune response in different ways

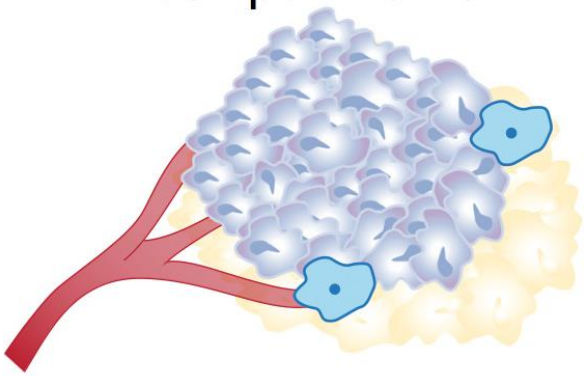
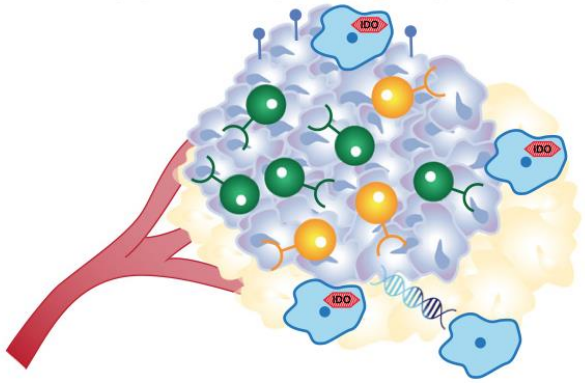
Gajewshi, Curr Opin Immunol. 2011 April; 23(2): 286–292.

Inflamed tumor phenotype barriers

- **T-cell Trafficking**
  - **CCL2, CCL3, CCL4, CCL5, CXCL9, and CXCL10**
- **Negative regulatory mechanisms in the tumor environment**
  - **IDO, PDL1, Tregs**
- **Innate immune sensing of tumor cells**
  - **Dependent on IFN-gamma**
- **“Inflamed Tumor Signature” currently being tested as a biomarker for benefit in GSK melanoma and lung cancer MAGE-1 vaccine trials**

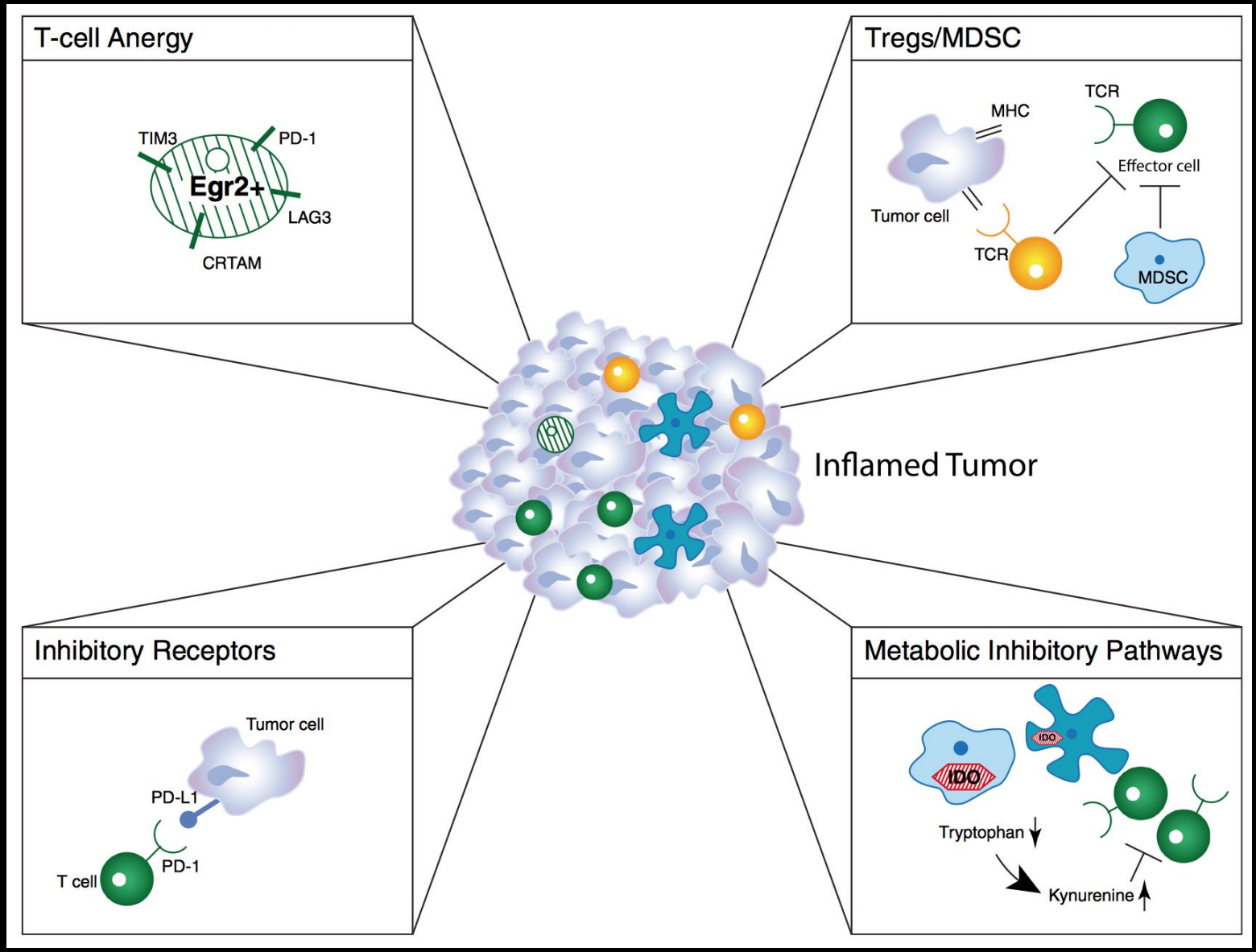
Gajewshi, Curr Opin Immunol. 2011 April; 23(2): 286–292.

Differences between tumors with “inflamed” and “non-inflamed” immunophenotypes and potential therapeutic interventions.

T cell-poor tumor	T cell-inflamed tumor
	
Reasons for immune evasion	
Lack of innate immune activation Lack of chemokine Dense stroma Immunosuppressive oncogene expression	Expression of inhibitory factors T-cell anergy Presence of regulatory immune cells
Therapeutic interventions	
Innate immune activation Stroma disruption Manipulation of oncogene signaling pathway	α-PD-1/PD-L1 Treg depletion IDO inhibition Homeostatic cytokines

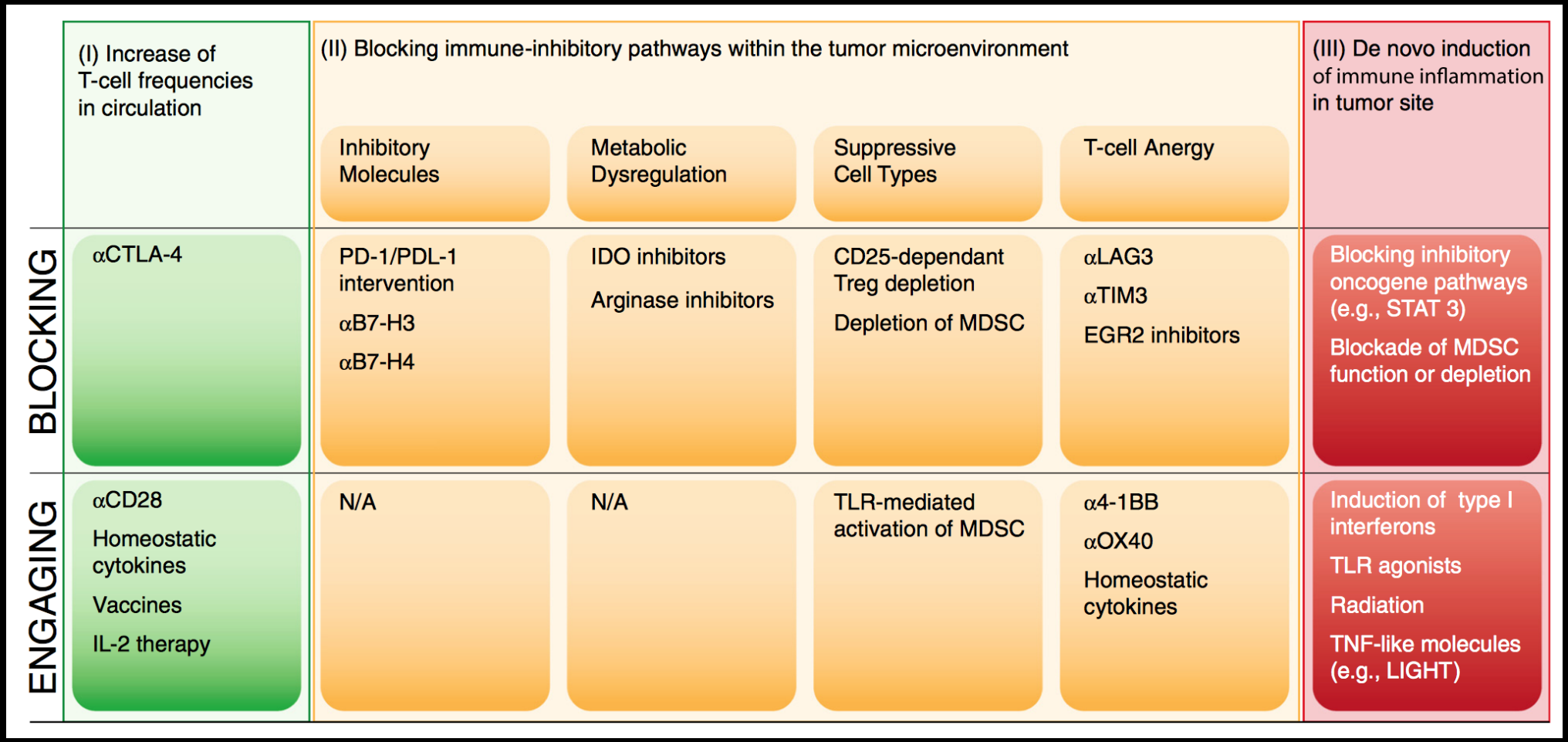
Spranger, Journal for ImmunoTherapy of Cancer 2013, 1:16

Dominant inhibitory mechanisms in the tumor microenvironment that suppress anti-tumor immunity



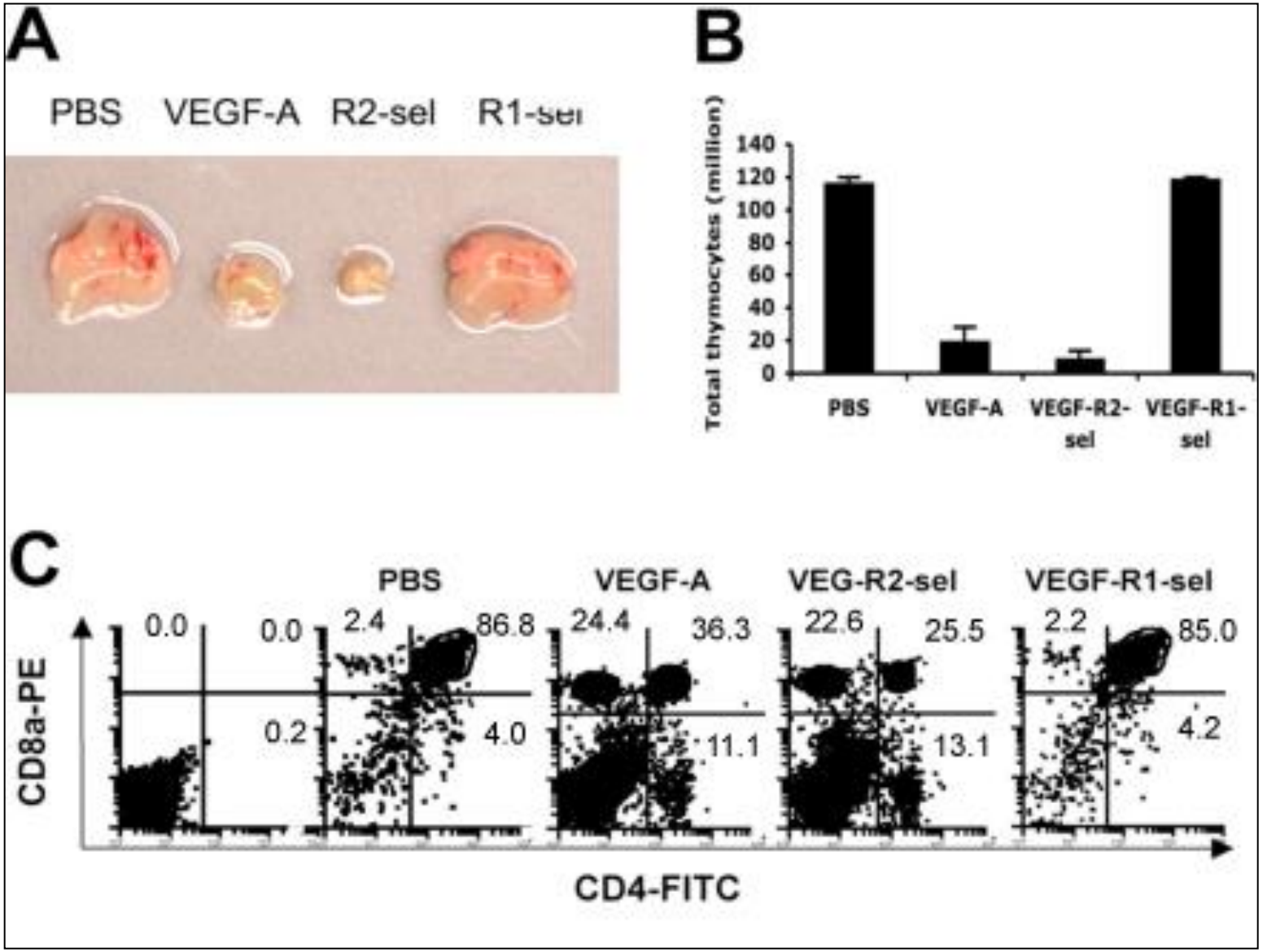
Spranger, Journal for ImmunoTherapy of Cancer 2013, 1:16

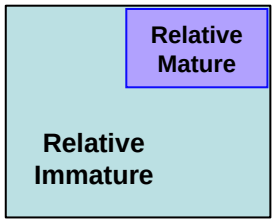
Categories of potential interventions



Spranger, Journal for ImmunoTherapy of Cancer 2013, 1:16

VEGF inhibits thymic T-cell development via VEGFR-2

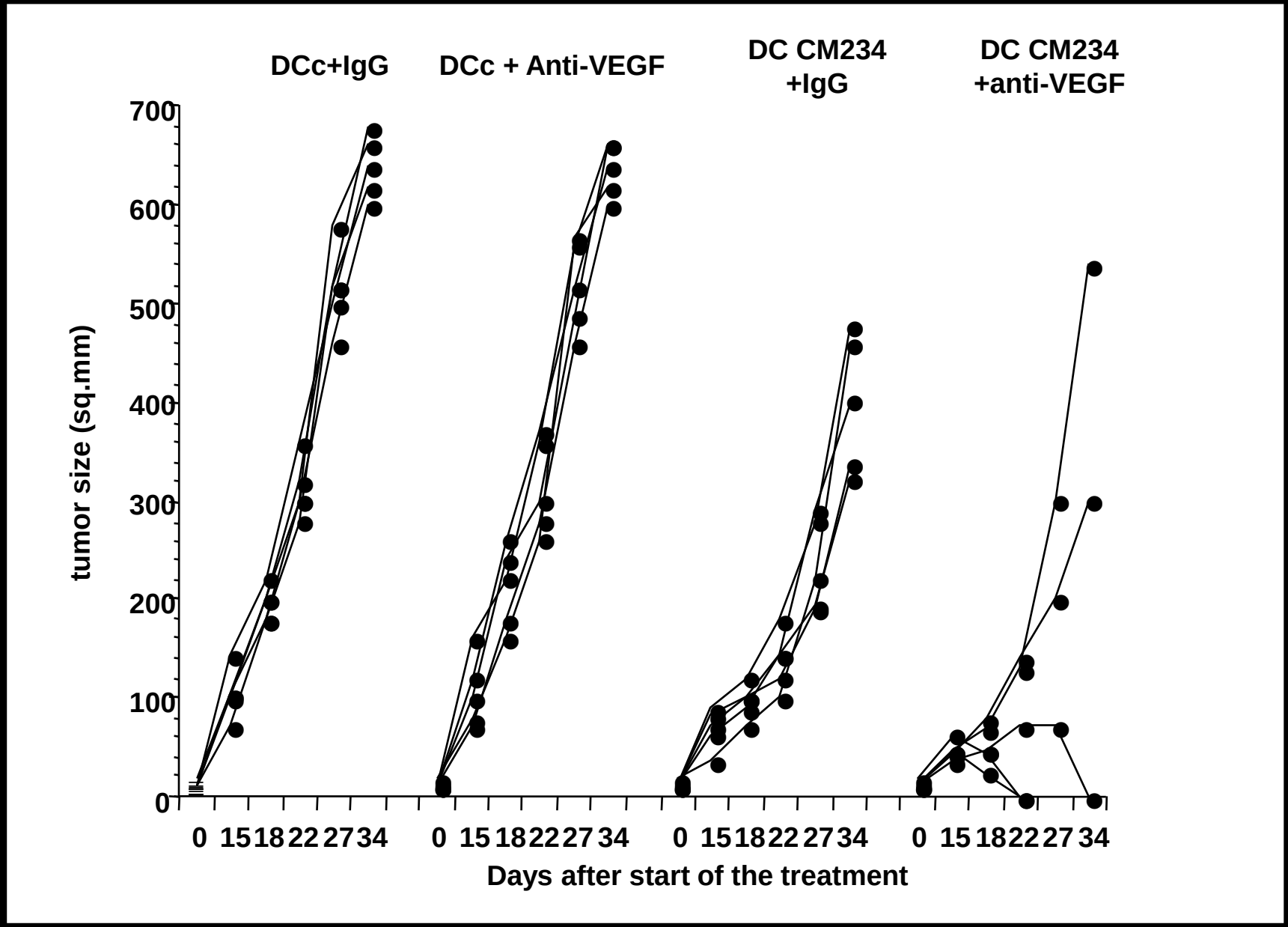




R2-sel: VEGF mutant specifically binds to VEGFR-2
R1-sel: VEGF mutant specifically binds to VEGFR-1

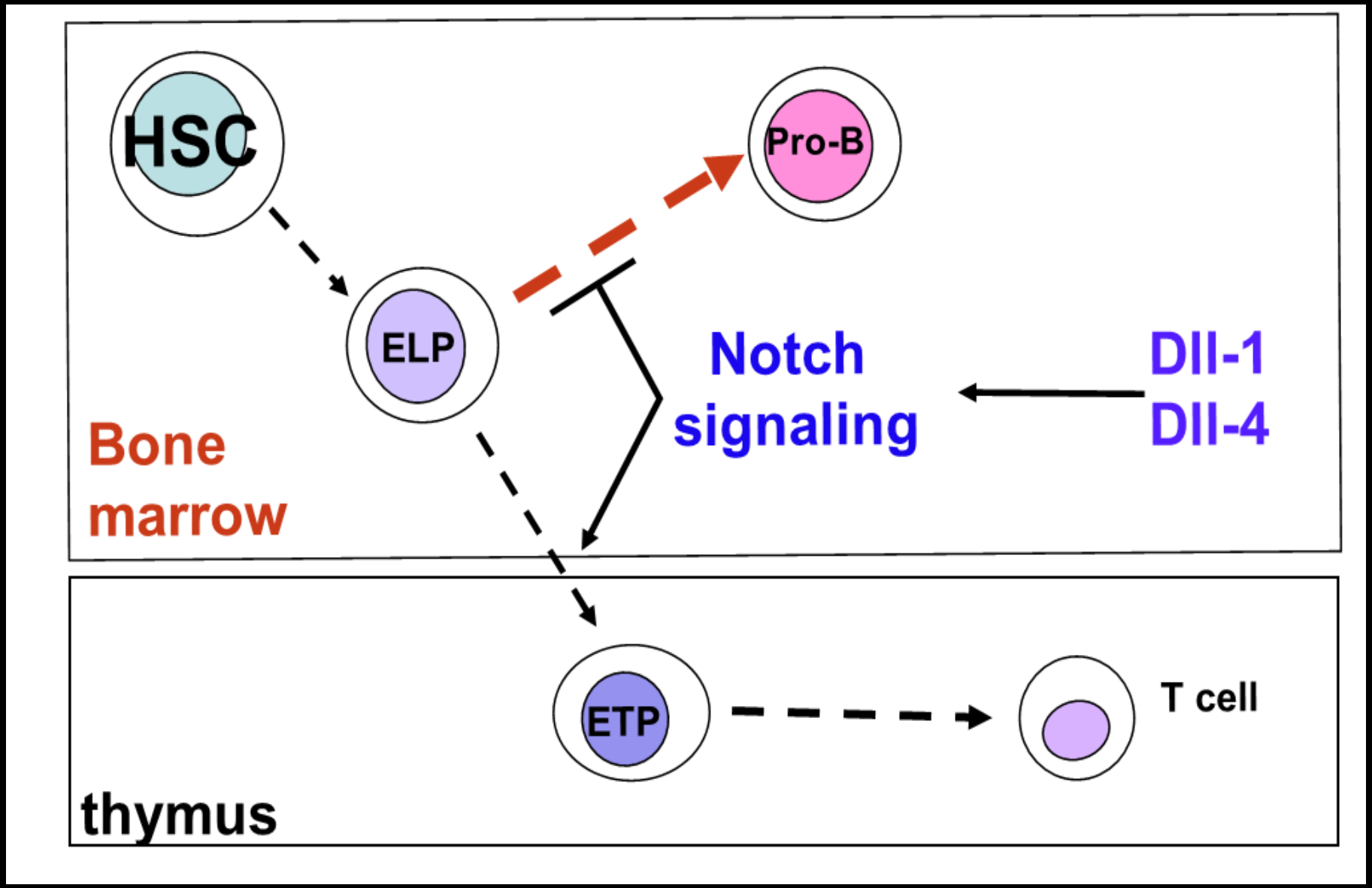
Huang, Y. and Carbone. Blood 2007;110:624-631

Anti-VEGF improves the efficacy of p53-directed immunotherapy



Gabrilovich and Carbone, CCR 1999

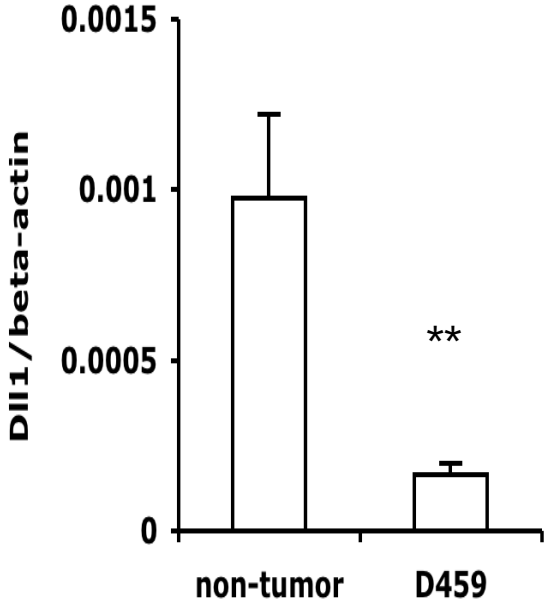
Notch Signaling Regulates Lymphopoiesis

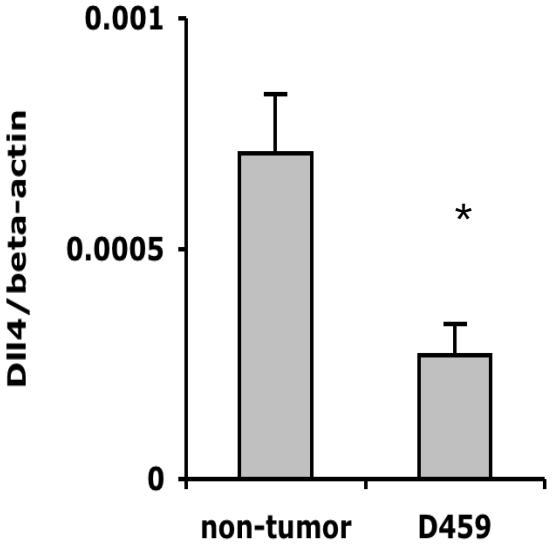


HSC: hematopoietic stem cell
ELP: early lymphoid progenitor
ETP: early T cell progenitor

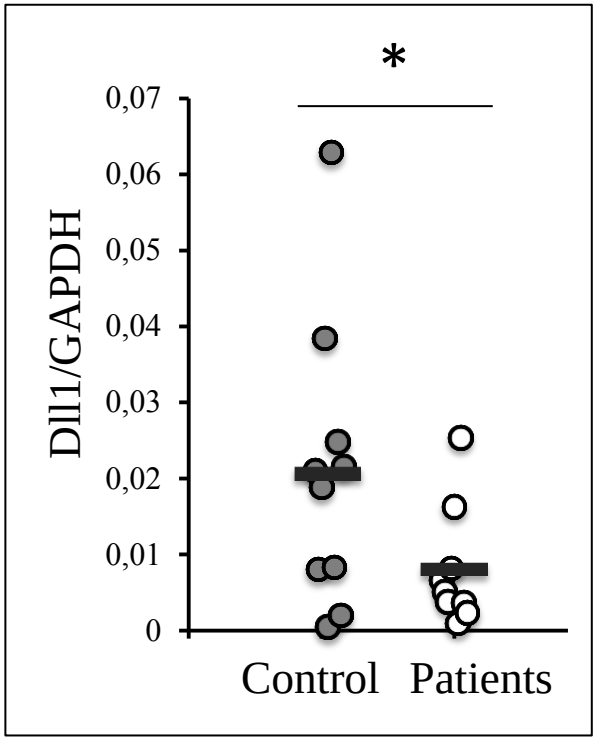
Notch Ligand Downregulation in Both mouse and Human Bone Marrow

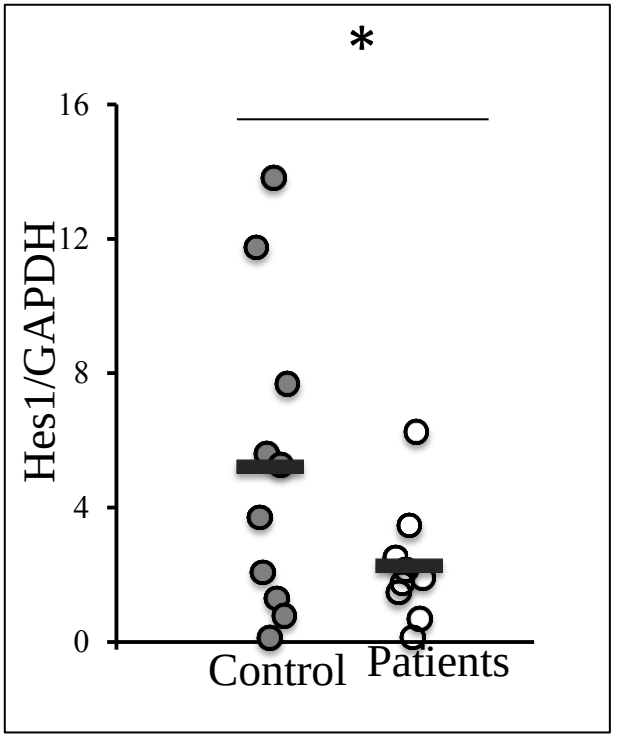
Mice



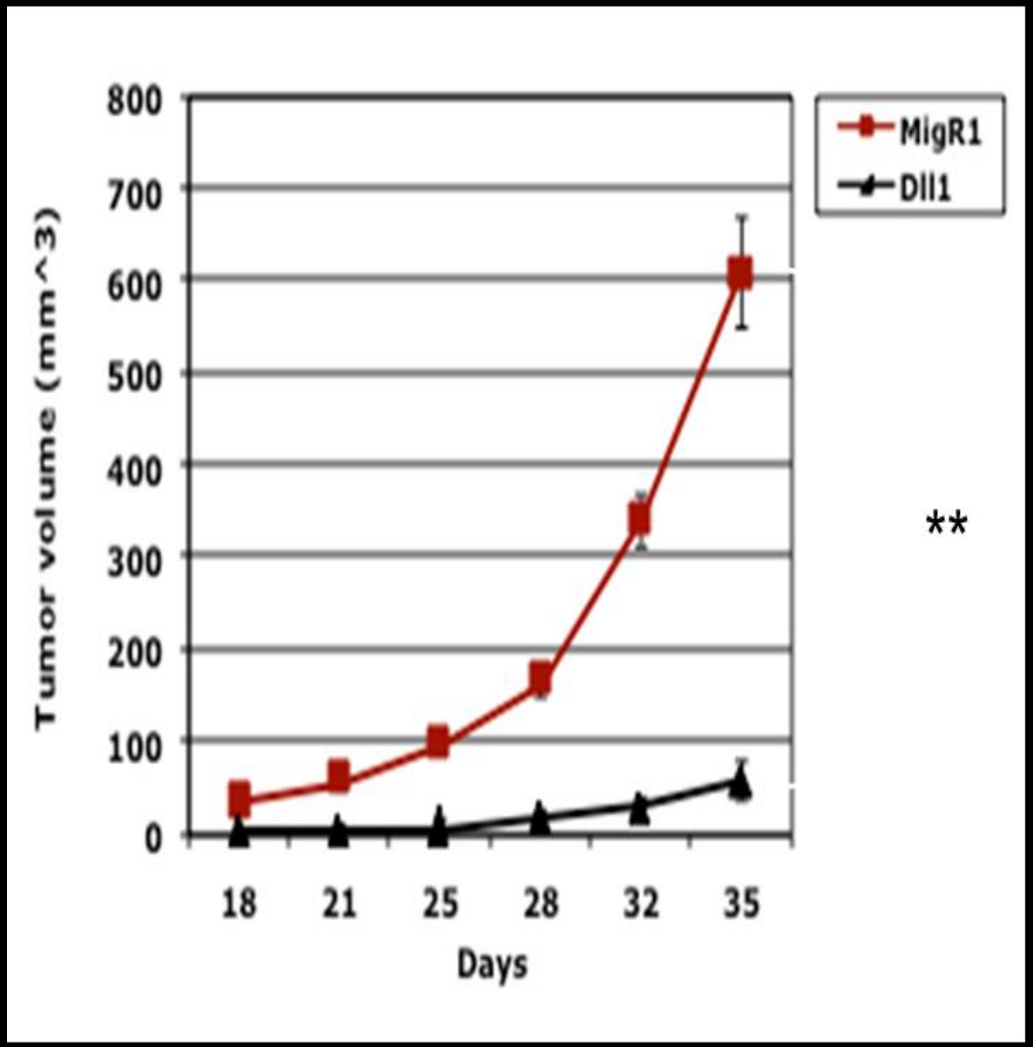


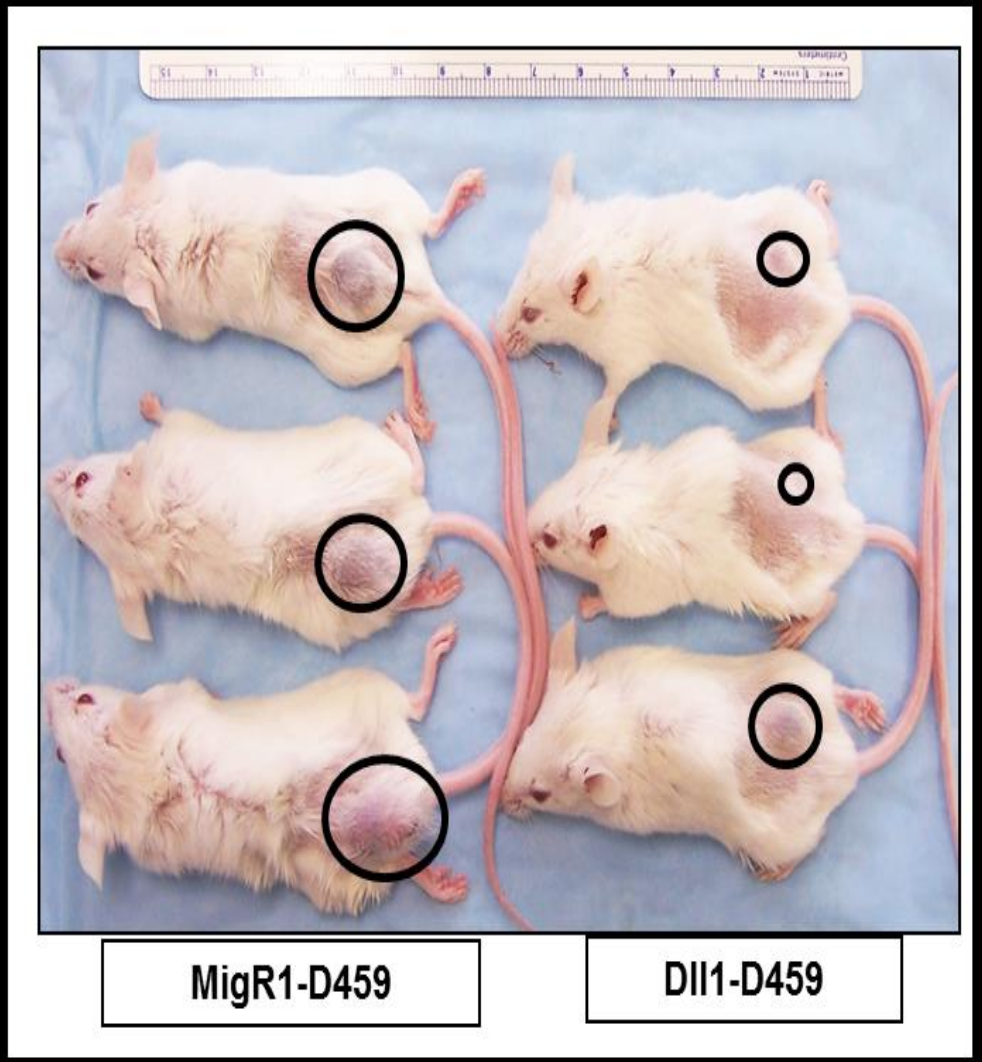
Humans





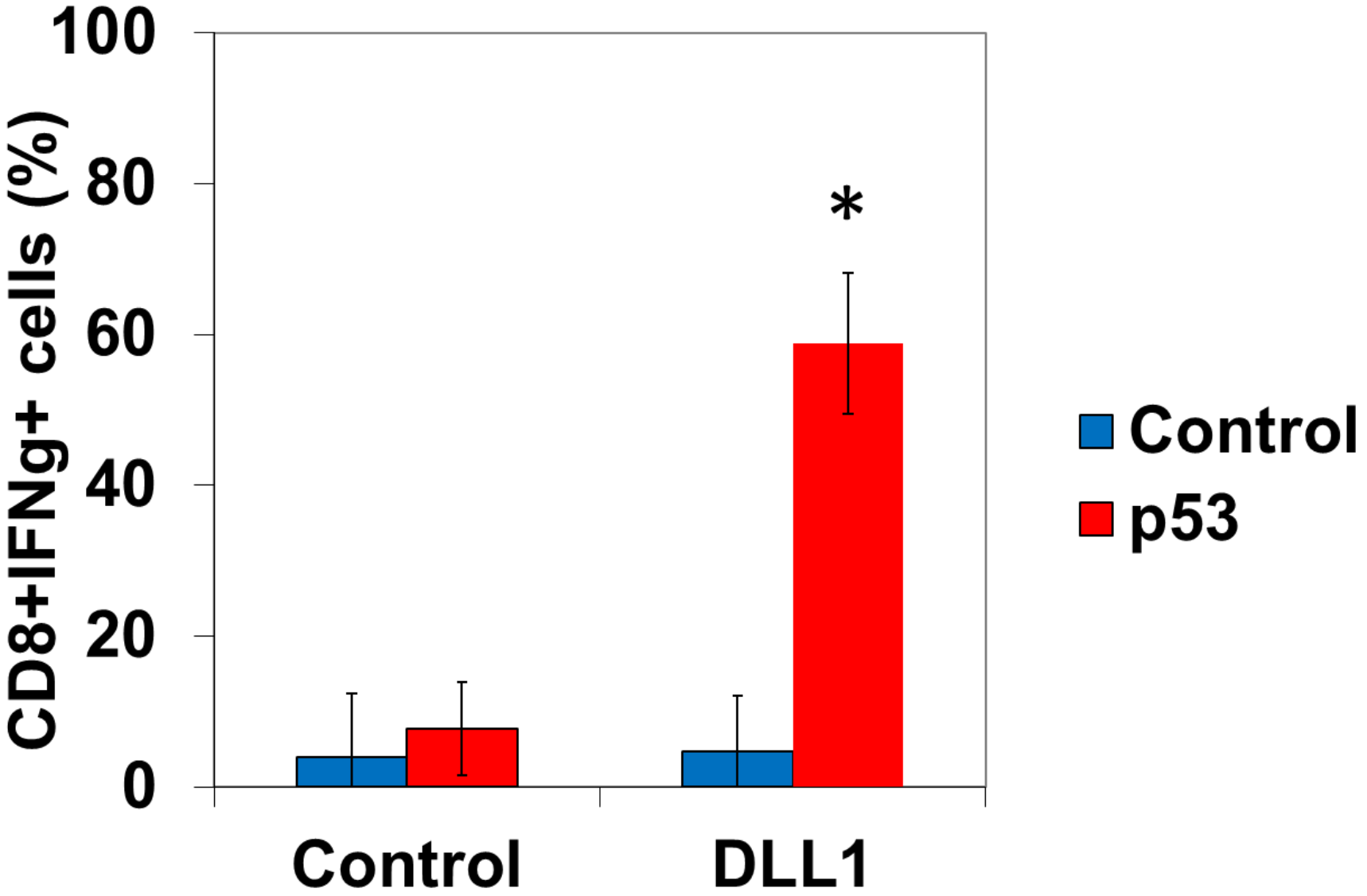
Restoration of DLL-1 in Bone Marrow Inhibits Tumor Growth



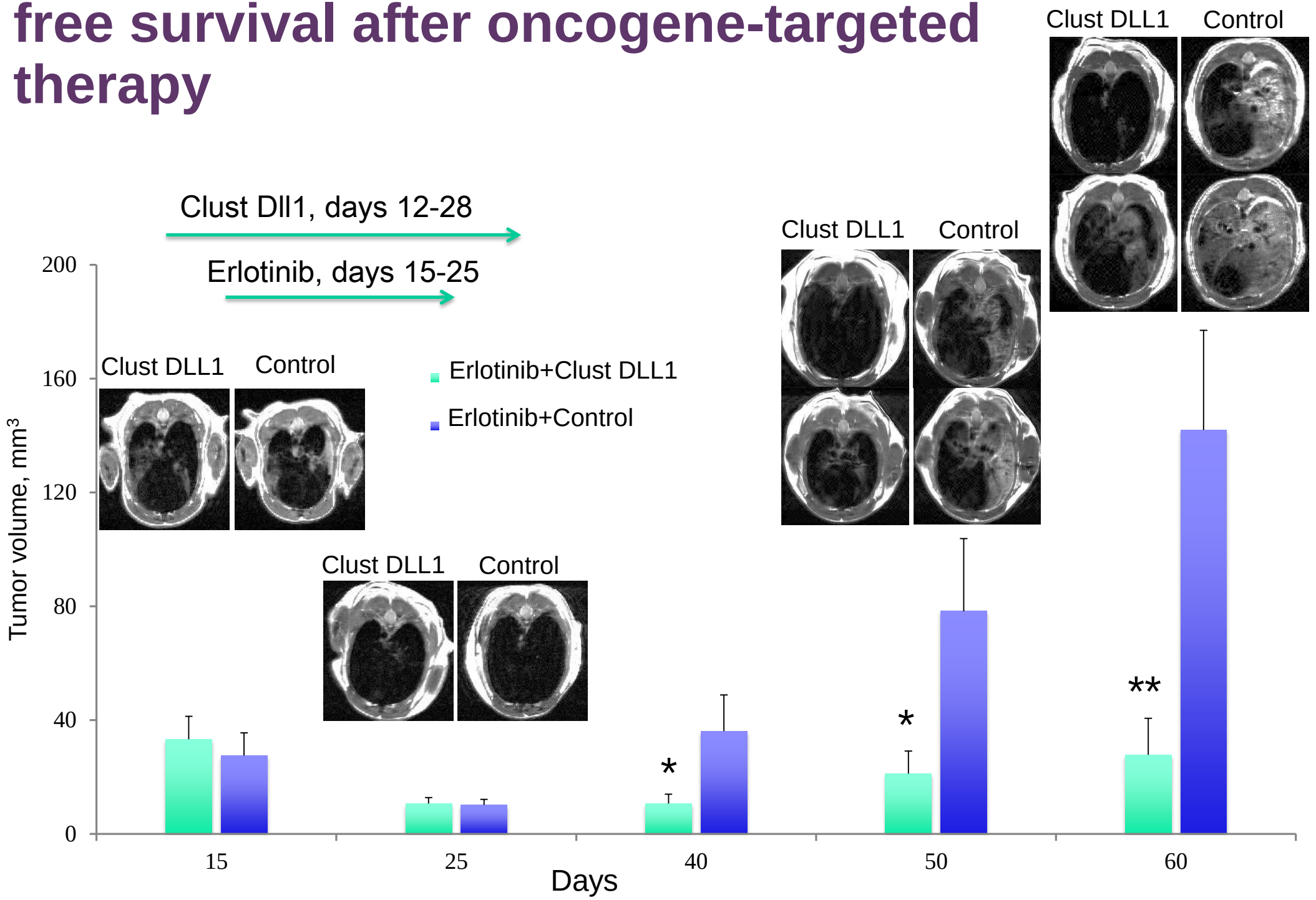


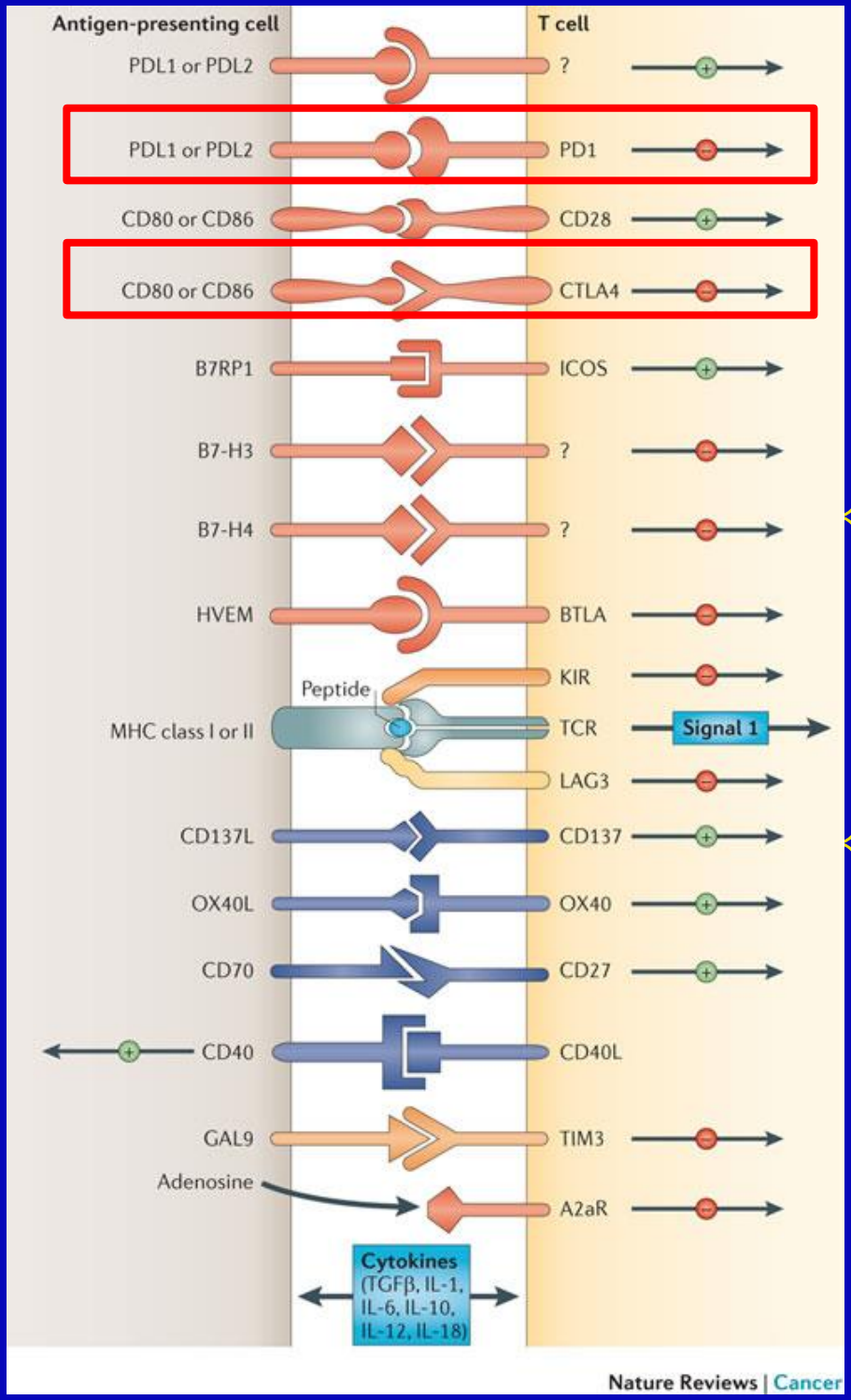
Huang, Dikov, Carbone, CR 2011

Induction of Mutant p53-Specific Immune Response by Clustered DLL1



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





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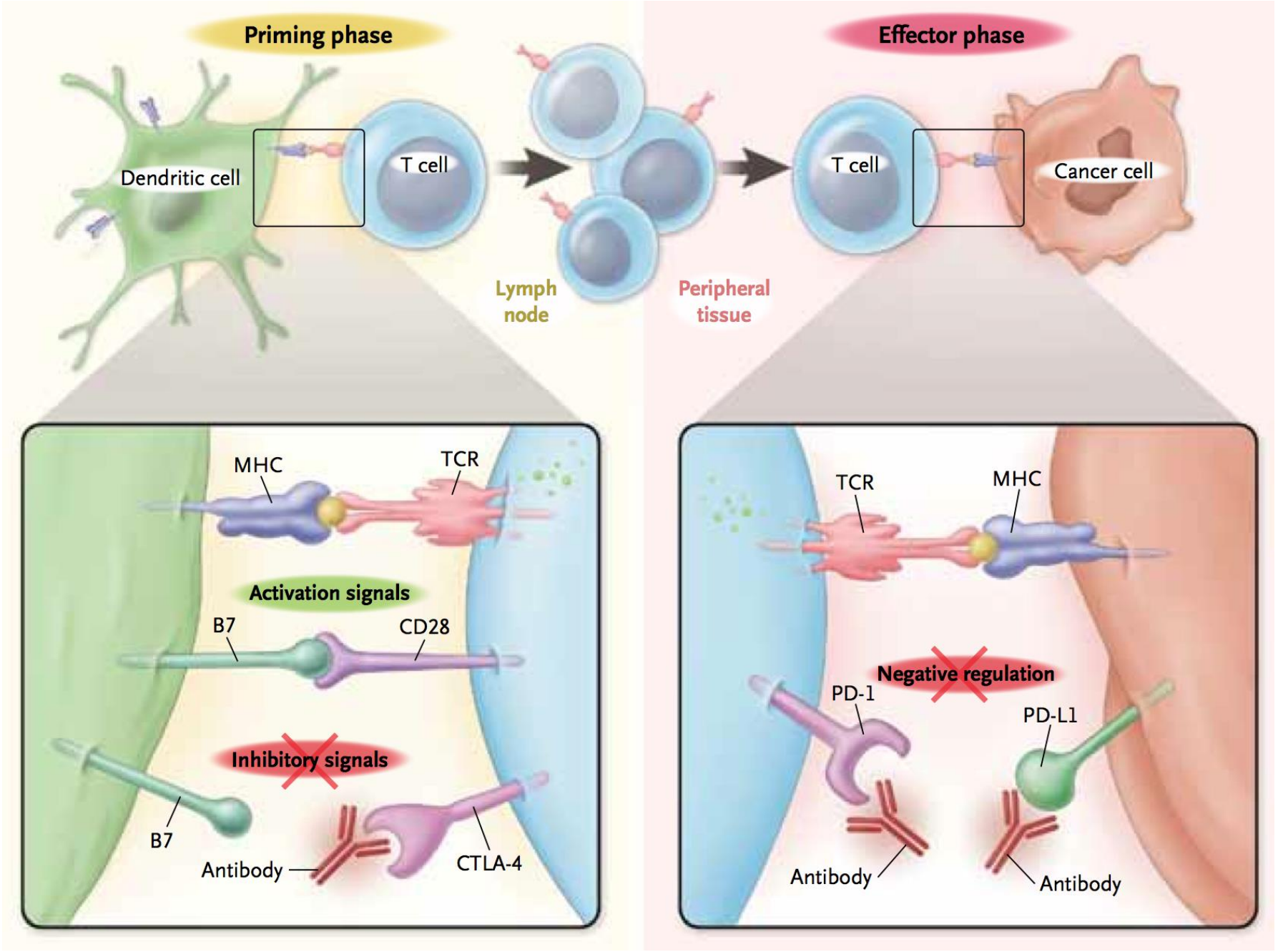
Pardoll DM Nature Rev Cancer 12, 252, 2012

CTLA-4 vs. PD-1



Ribas, NEJM 2012

Conclusions

- Clinically evident tumors have clearly avoided an effective immune response
- Most of this avoidance is functional, and not structural – potentially surmountable
- Understanding the mechanisms of this avoidance has led to several proposed immunomodulatory therapeutic approaches with early promise in the clinic