National Cancer Institute

Strategies and Opportunities for Cancer Therapy with Vaccines Inducing T cells or Antibodies

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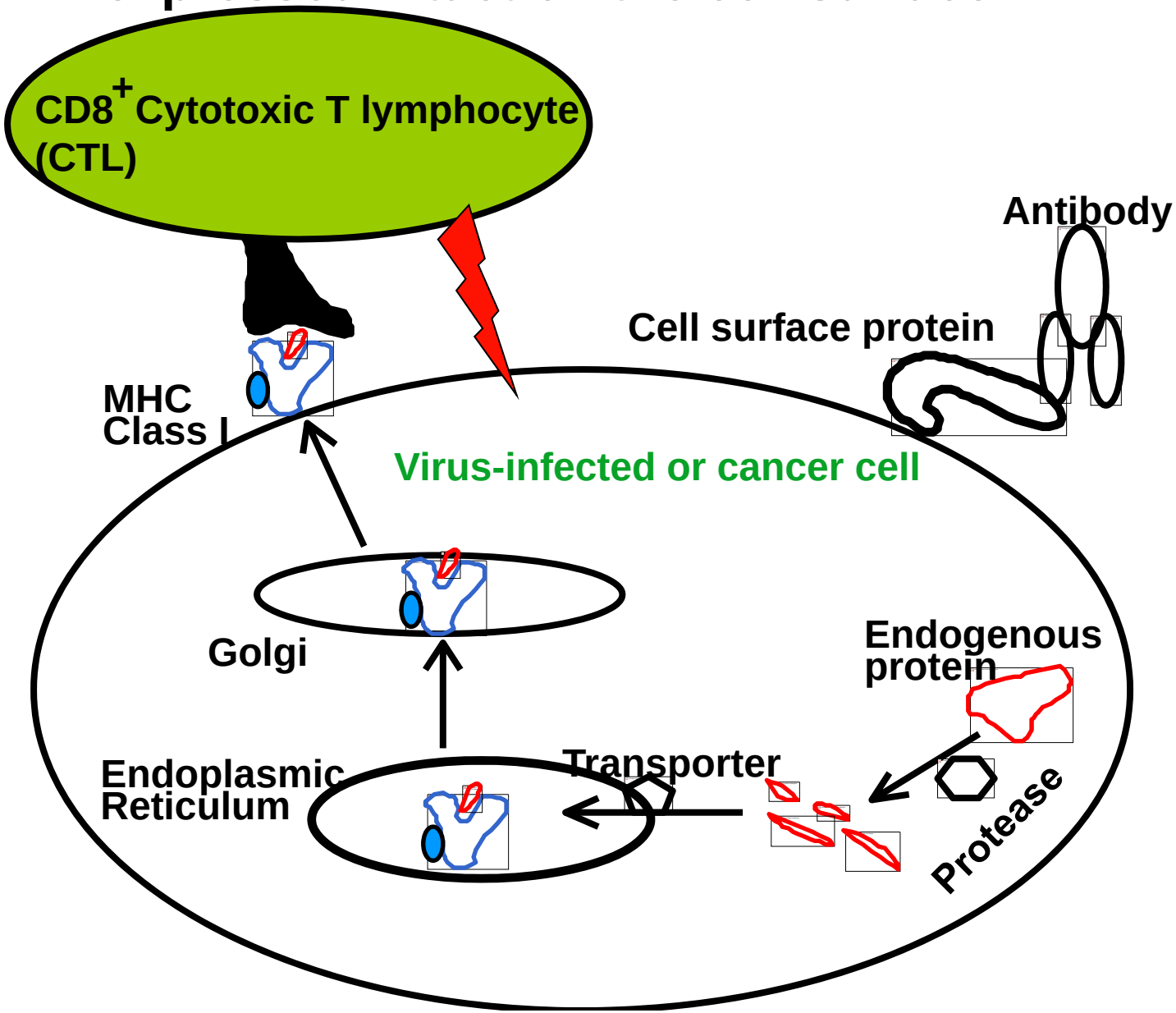
National Institutes of Health

Rationale for Engineered Vaccines

- **Most successful vaccines (except toxoids) have been against viruses causing acute, self-limited infections, for which the most widely used strategy is to mimic the natural infection with an attenuated, inactivated, or subunit vaccine.**
- **However, for cancer or viruses causing chronic infection, such as HIV or hepatitis C virus, the natural disease does not induce sufficient immunity to eradicate the infection.**
- **A vaccine must elicit better immunity than the disease itself.**

CD8$^+$ Cytotoxic T cells can detect endogenous antigenic proteins even if not expressed intact on the cell surface



Types of Tumor Antigens

	Examples
-Overexpressed antigens	Her-2/neu, CEA, TARP
-Altered antigens	
Shared by many tumors	p53, Ras, fusion proteins, MUC1
Unique to a single tumor	Point mutations in various genes
-Tissue-specific antigens	tyrosinase, MART1, gp100
-Novel antigens (in adult)	
Fetal antigens	CEA, oncofetal protein
Viral antigens	HPV E6 or E7, EBV antigens
Clonal antigens	Idiotype

Desirable Characteristics for Tumor Antigens

1. Tumor-selective

2. Essential to tumor cell survival

3. For T-cell antigens
   - Processed
   - Bind MHC
   - Immunogenic

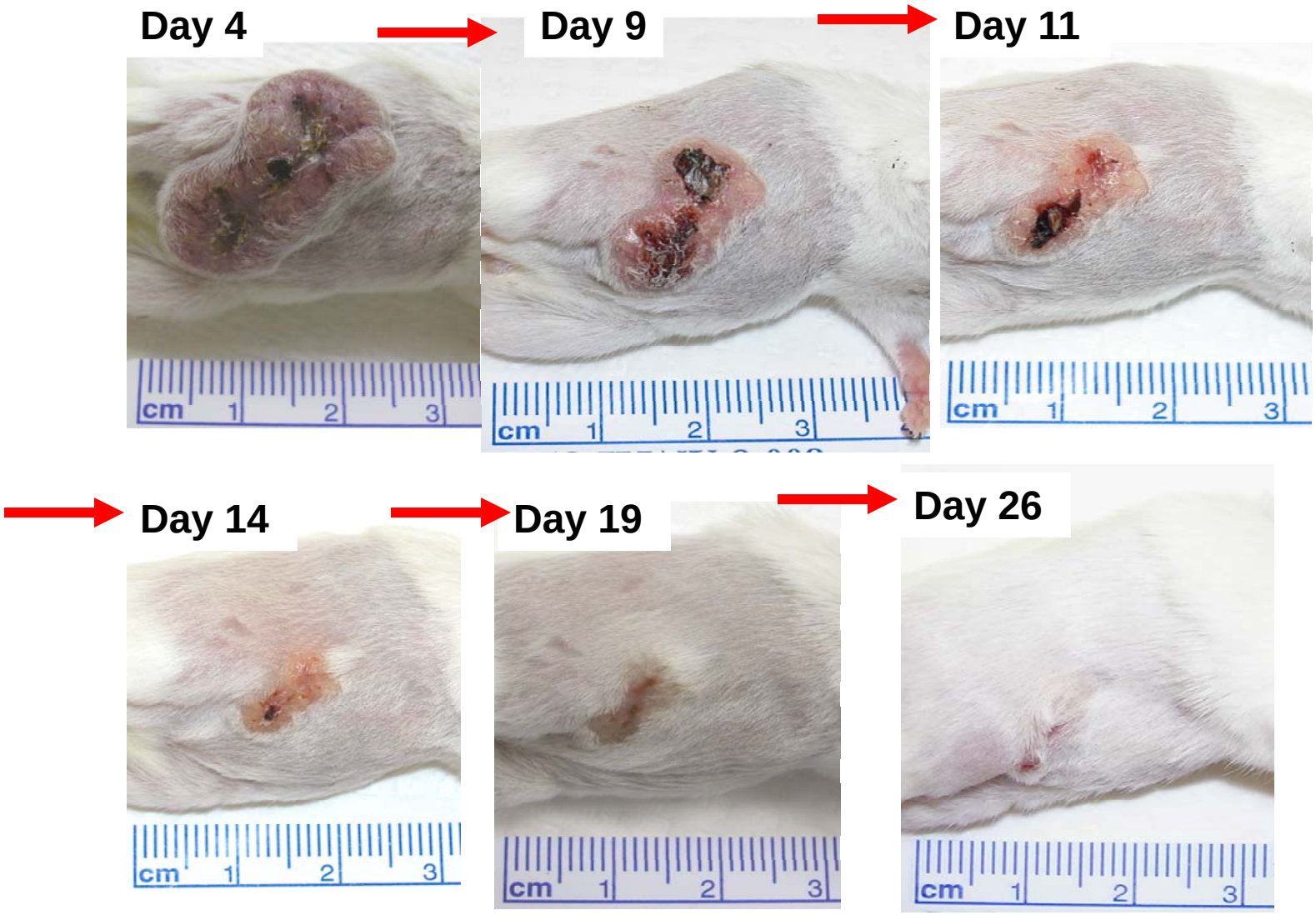
4. For B-Cell antigens
   - Cell Surface Expression
   - Accessibility of Epitopes
   - Immunogenicity
     - In vitro
     - In vivo
     - Pre-existing Antibody response

Potential Mechanisms of Antibody Action against Tumors

- **Antibody-dependent cellular cytotoxicity (ADCC): NK or other cells with Fc receptors bind antibodies and use them to target cells for killing.**
- **Complement-mediated lysis**
- **Inhibition of function of a molecule required for oncogenicity: e.g. HER-2/neu, CD25**

- **Success of antibodies to HER-2/neu (Herceptin) and to CD25 (Zenapax) suggests functional targets may be the most effective.**

Adeno-neuECTM (Her-2) treatment causes regression of established s.c. TUBO mammary carcinomas



Park et al., *Cancer Research* 2008

Adeno-neuECTM (Her-2) vaccine induces regression of established lung tumors from IV injection of TUBO breast cancer cells

Control, sacrificed day 15

Control, sacrificed 30

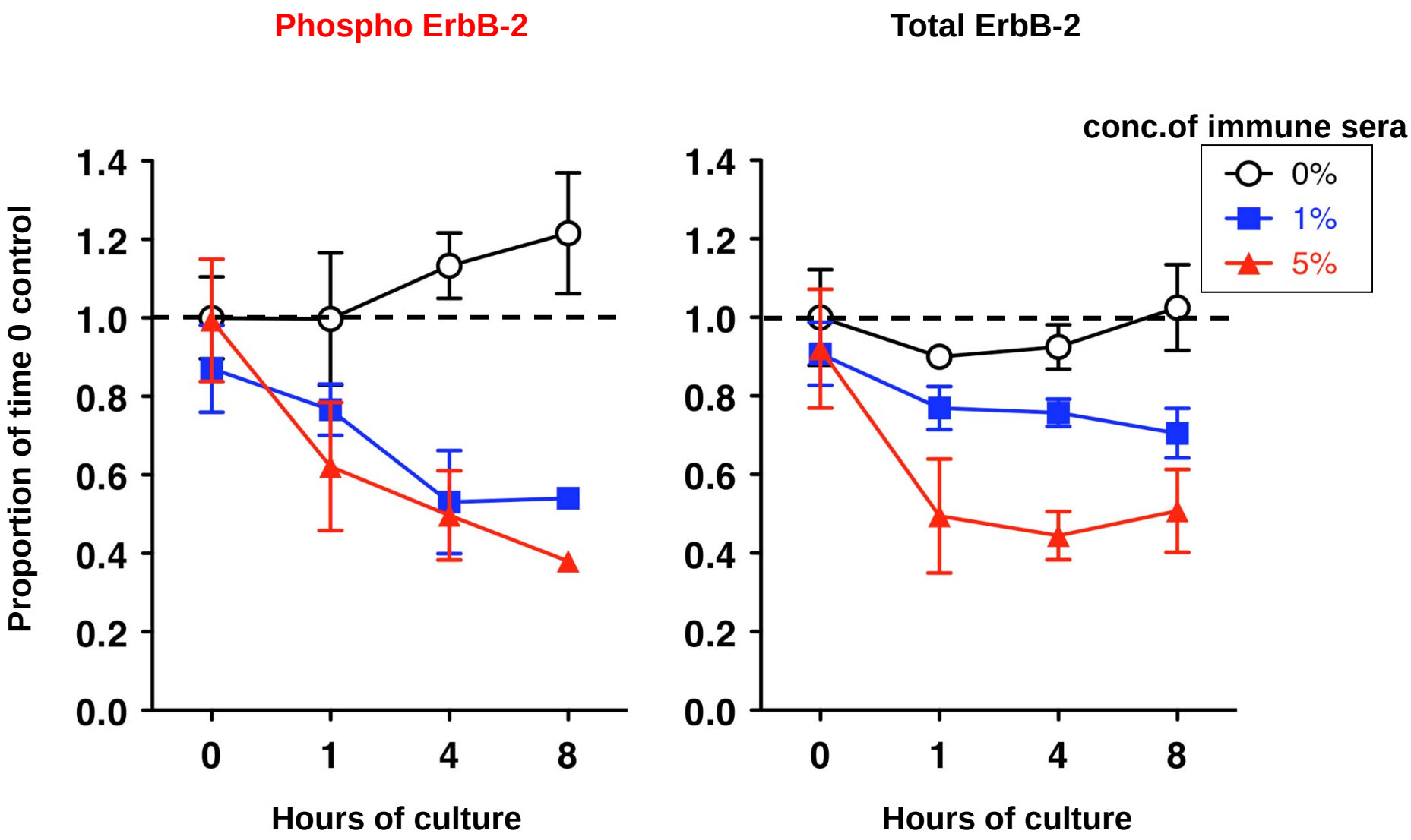
Ad-ECTM day 15,
Sacr day 29

Ad-ECTM day 15,
Sacr day 35

Ad-ECTM day 15,
Sacr day 48

Park et al.,
Cancer Research 2008

Ad-neuECTM serum downmodulates ErbB2 (Her-2) and inhibits its phosphorylation



Park et al., *Cancer Research* 2008

Advantages of Vaccine over Trastuzumab (Herceptin)

- **Antibody induced by vaccine is not dependent on FcRs, but directly inhibits the function of the oncogene product and inhibits tumor growth without other cells. Herceptin requires FcRs.**

- **Polyclonal antibodies elicited may target multiple Her-2 epitopes and be less susceptible to escape mutations than a monoclonal antibody to a single epitope.**

- **Continuous antibody production avoids the need for repeated expensive monoclonal antibody administration (~$100K/yr).**

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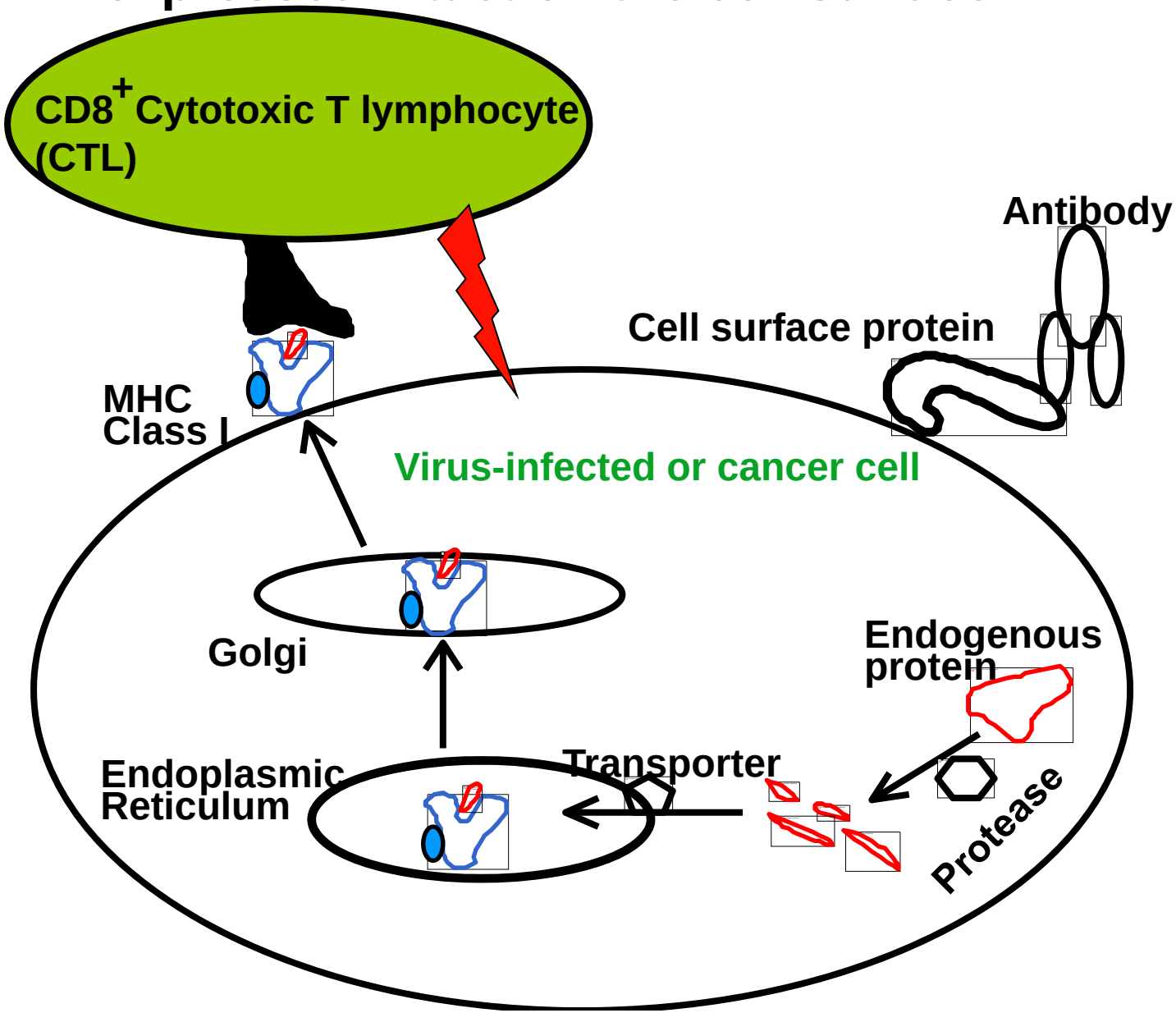
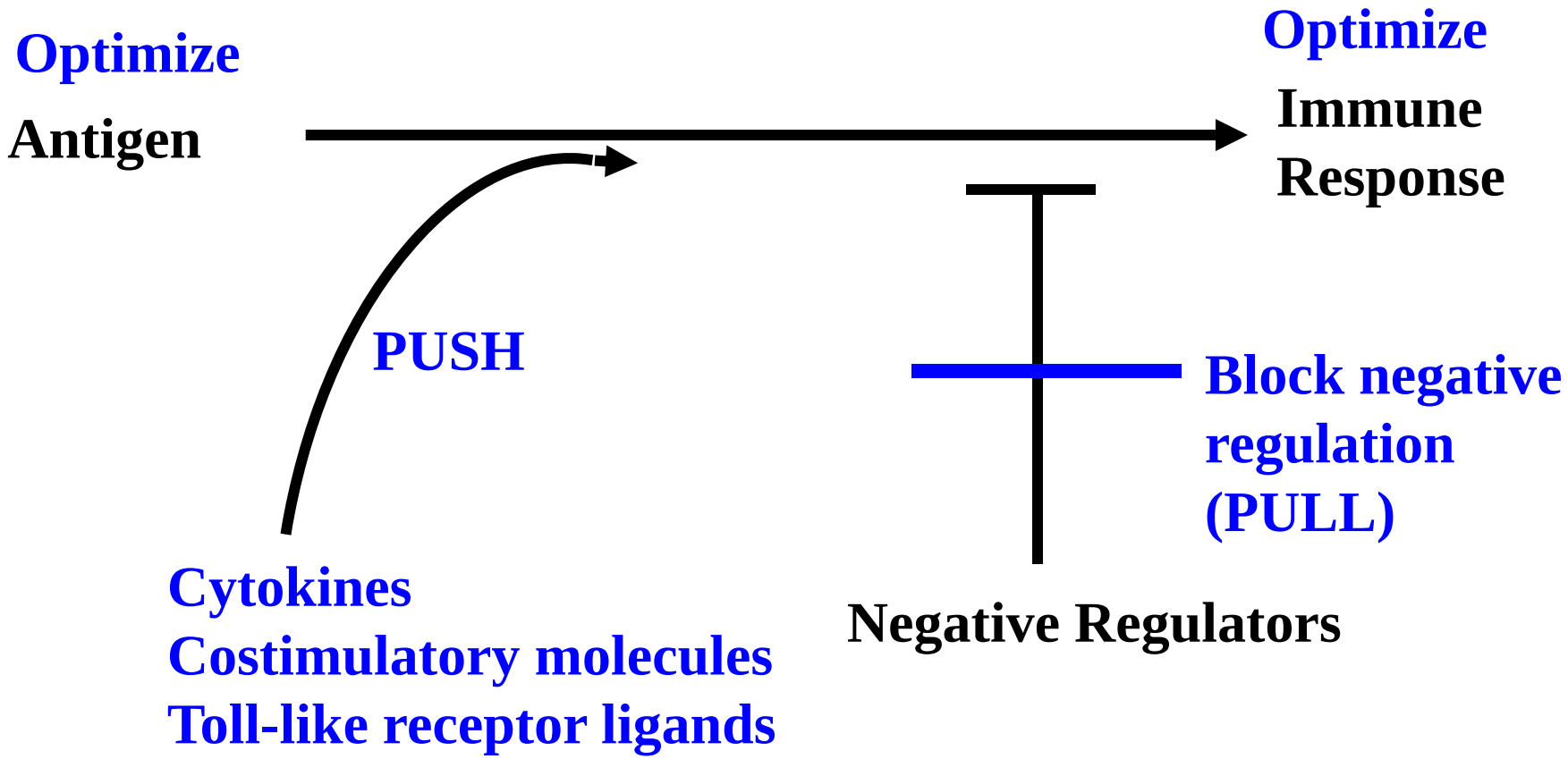


Cancer Vaccine Problems & Strategies

Problems	Strategies to solve
Self antigens to which host is tolerant	**Target subdominant epitopes strengthened by epitope enhancement:** **Modify the amino acid sequence to improve MHC binding.**
Downregulation of MHC or of processing machinery	**Induce higher avidity T cells that can respond to low densities of peptide-MHC**
Poor quality or quantity of immune response **For therapeutic vaccines, inadequate $CD4^+$ T help**	**Use cytokines to improve the quantity and quality and substitute for $CD4^+$ help:**
Suppression of the immune response	**Remove the brakes by blocking negative regulation.**

PUSH-PULL Approach to Optimizing Vaccine-induced Immunity

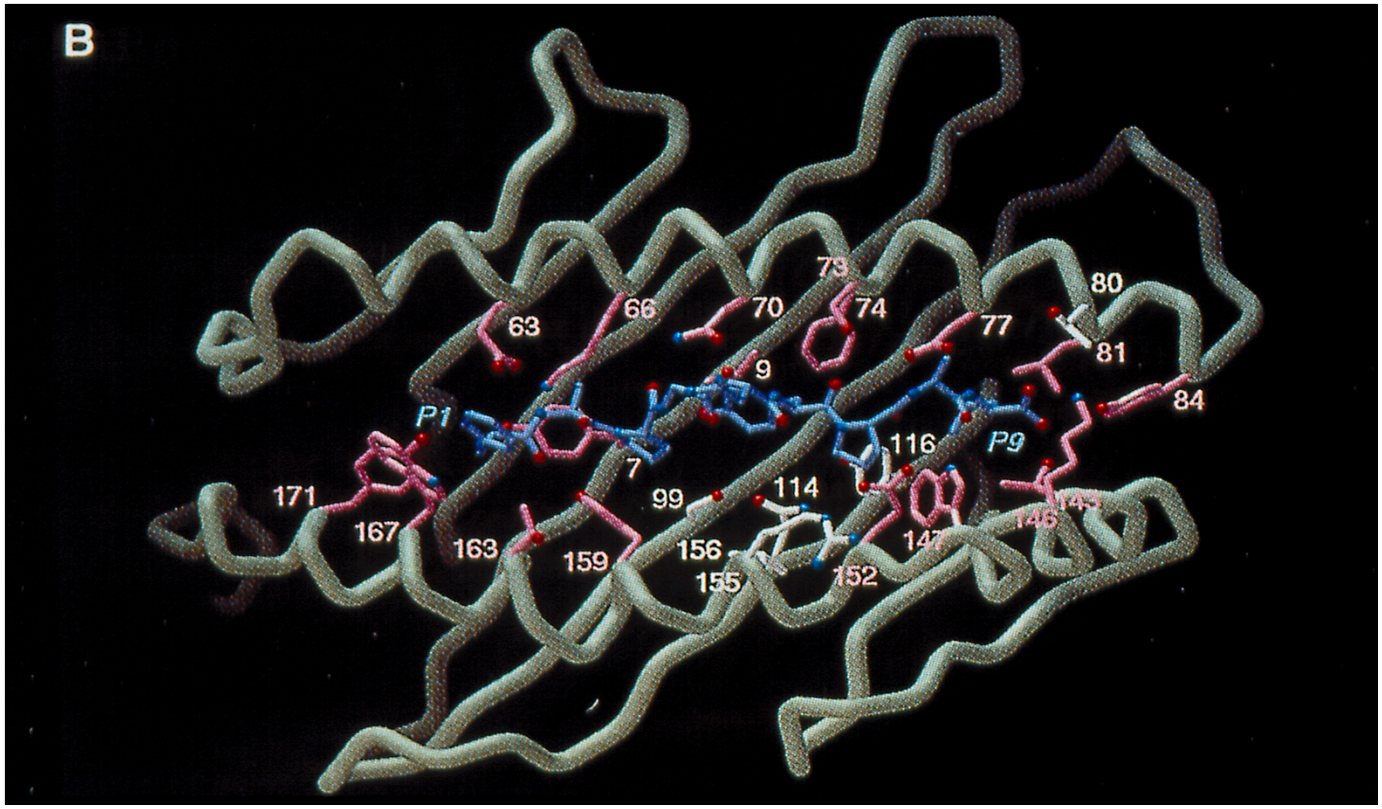


Topics:

- **Improve the antigen: epitope enhancement**
- **Use cytokines to improve the quality and quantity of immune response**
- **Improve CTL quality by increasing avidity with IL-15**
- **Improve CTL quality by using IL-15 to substitute for $CD4^+$ T cell help to induce long-lived memory CTL**
- **Remove the brakes by blocking negative regulation: A new NKT regulatory axis.**

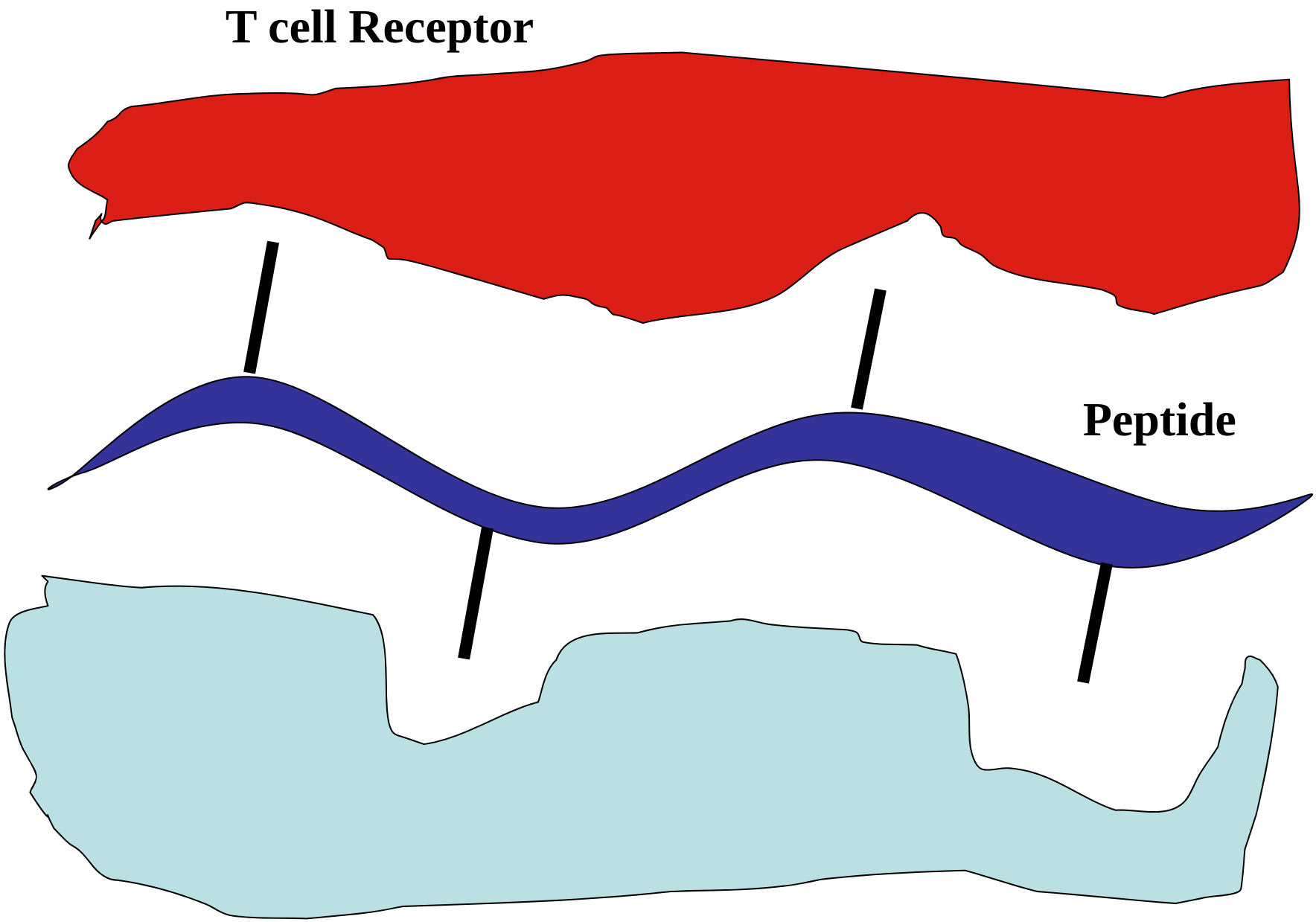
Peptide Fragments of Viral Proteins Bind Specifically in the Groove of Major Histocompatibility Molecules such as HLA-A, B, C

Sendai Virus Peptide Bound to H-2K^{b}

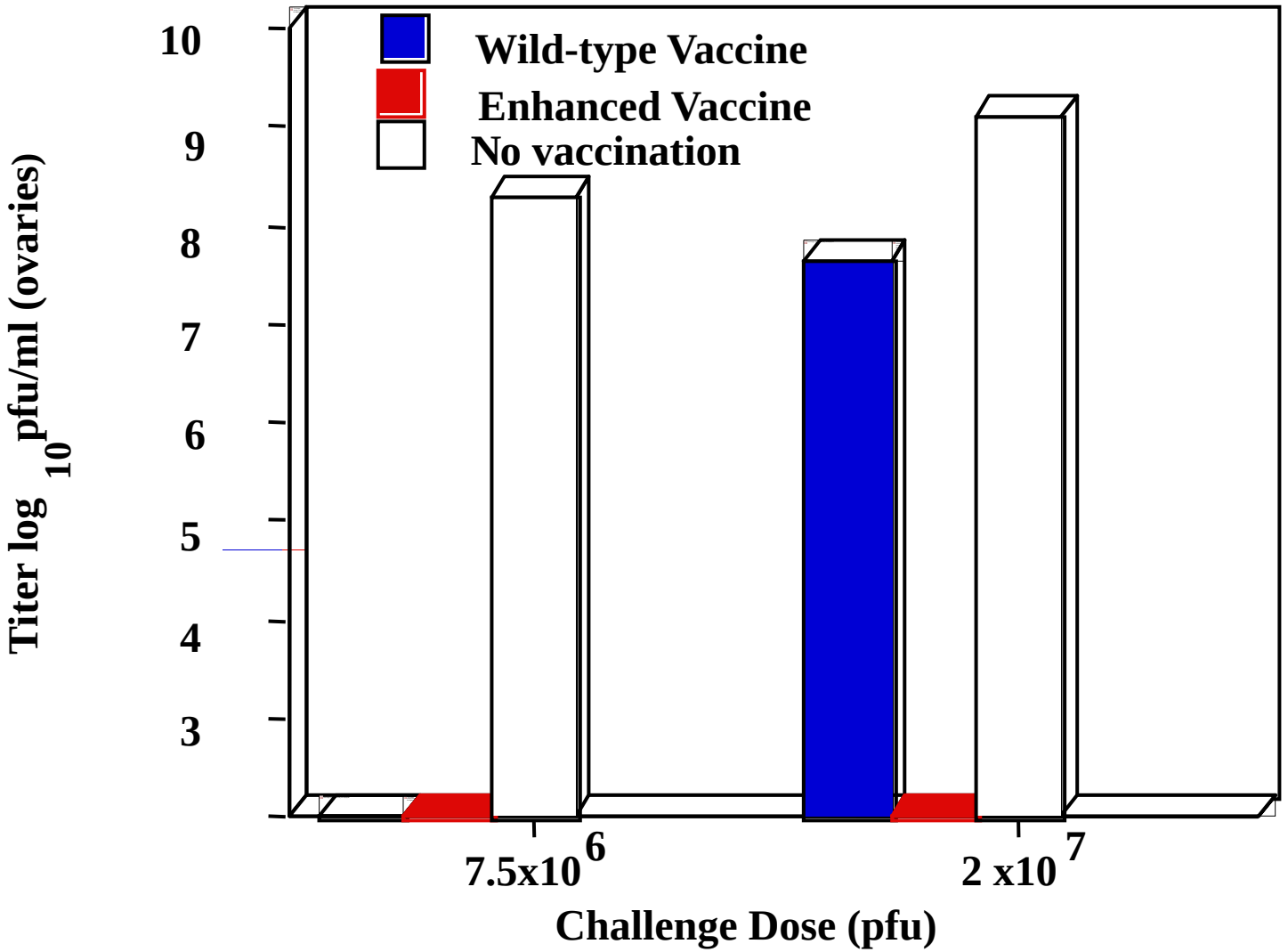


From DH Fremont, M. Matsumura, EA Stura, PA Peterson, & IA Wilson. Science 257: 919-926, 1992

Strategy: Epitope Enhancement by Sequence Modification to Increase Peptide Affinity for the MHC Molecule


T cell Receptor
Peptide
Major Histocompatibility Molecule (HLA)

Enhanced Vaccine Protects Against Higher Viral Challenge



Ahlers et al., *JCI* 108:1677, 2001

TARP: TCRγ Alternative Reading frame Protein

- **Expressed in prostate and breast cancers, but not in other organs**
- **Using different open reading frame from normal TCRγ**
- **Possible role: Oncogenic transformation of the cells**

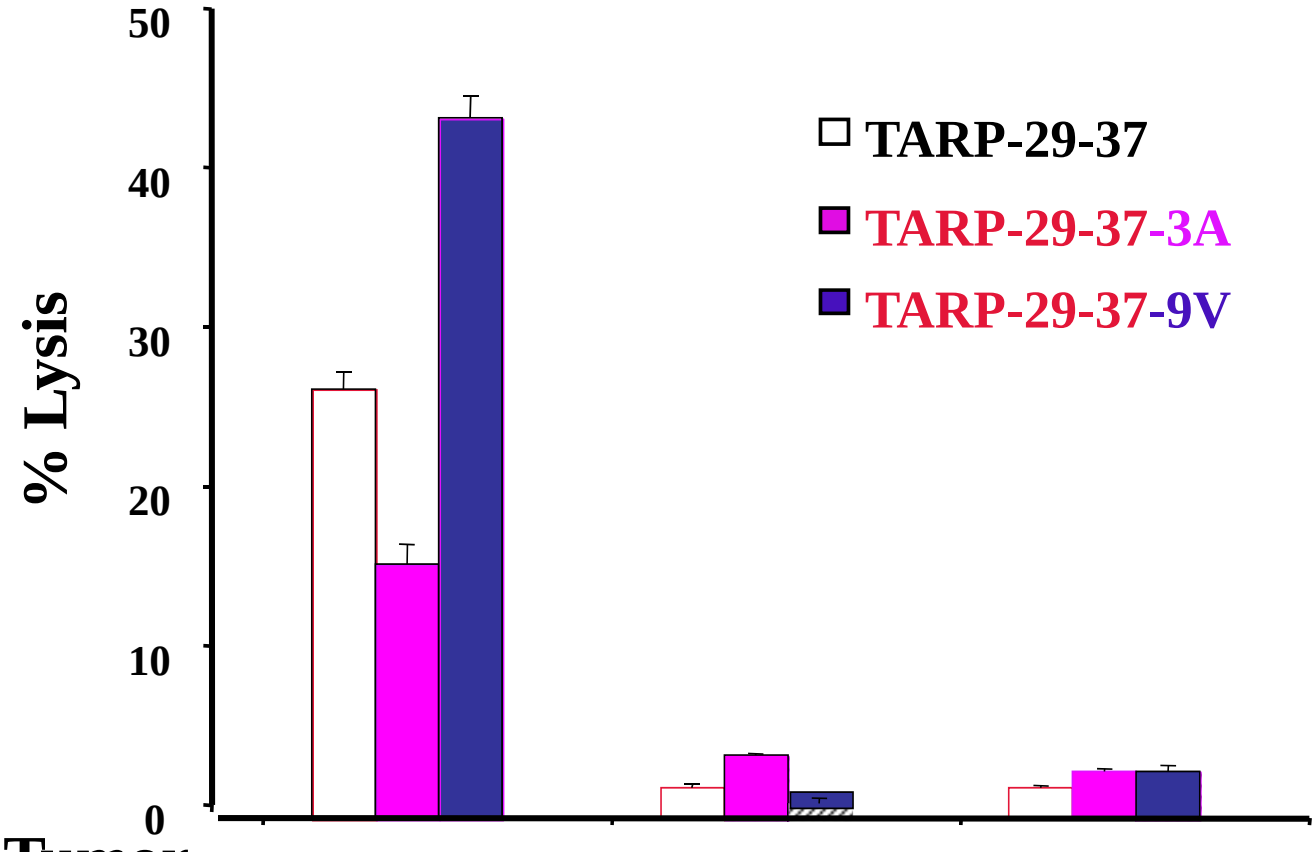
Amino Acid Sequence of TARP

MQMFPPSPLFFFLQLLKQSSRRLEHTF
VFLRNFSLMLLRGIGKKRRATRFWDP
RRGTP (58 residues)

FLRNFSLML = HLA-A2-binding peptide

TARP 29-37

Human CTL raised against an epitope-enhanced TARP peptide can kill human tumor cells expressing TARP and HLA-A2.



Use of Cytokines in Adjuvant to Steer the Immune Response to Vaccines

Cyto-kine	CTL	Prolif-eration	IL-2	IL-4	IFN-γ	Cyto-kine mRNA	Ab Isotype	Neutral-izing Ab
GM-CSF	↑	↑	↑	↑	→		IgG1, 2b	↑
IL-1β	↓	→	→	→	→		IgG1	↑
IL-2	→	↑	→	→	sl. ↑		IgG2a	↑
IL-4	↓	→	→	↑	→		IgG1, 2b	↑
IL-7	→	↑	↑	↑	→		IgG1	→
IL-12	↑	↑/→	→	sl. ↓	↑		IgG1, 2a, 2b	↑
TNFα	↓/→	→	→	↑	→		ND	ND
IFN-γ	→	↑	→	→	↑		IgG2a, 2b	↑

Two peptide vaccine candidates: PCLUS 3-18MN, PCLUS6.1-18-MN
Two mouse genetic backgrounds: BALB/c, B10 congenics

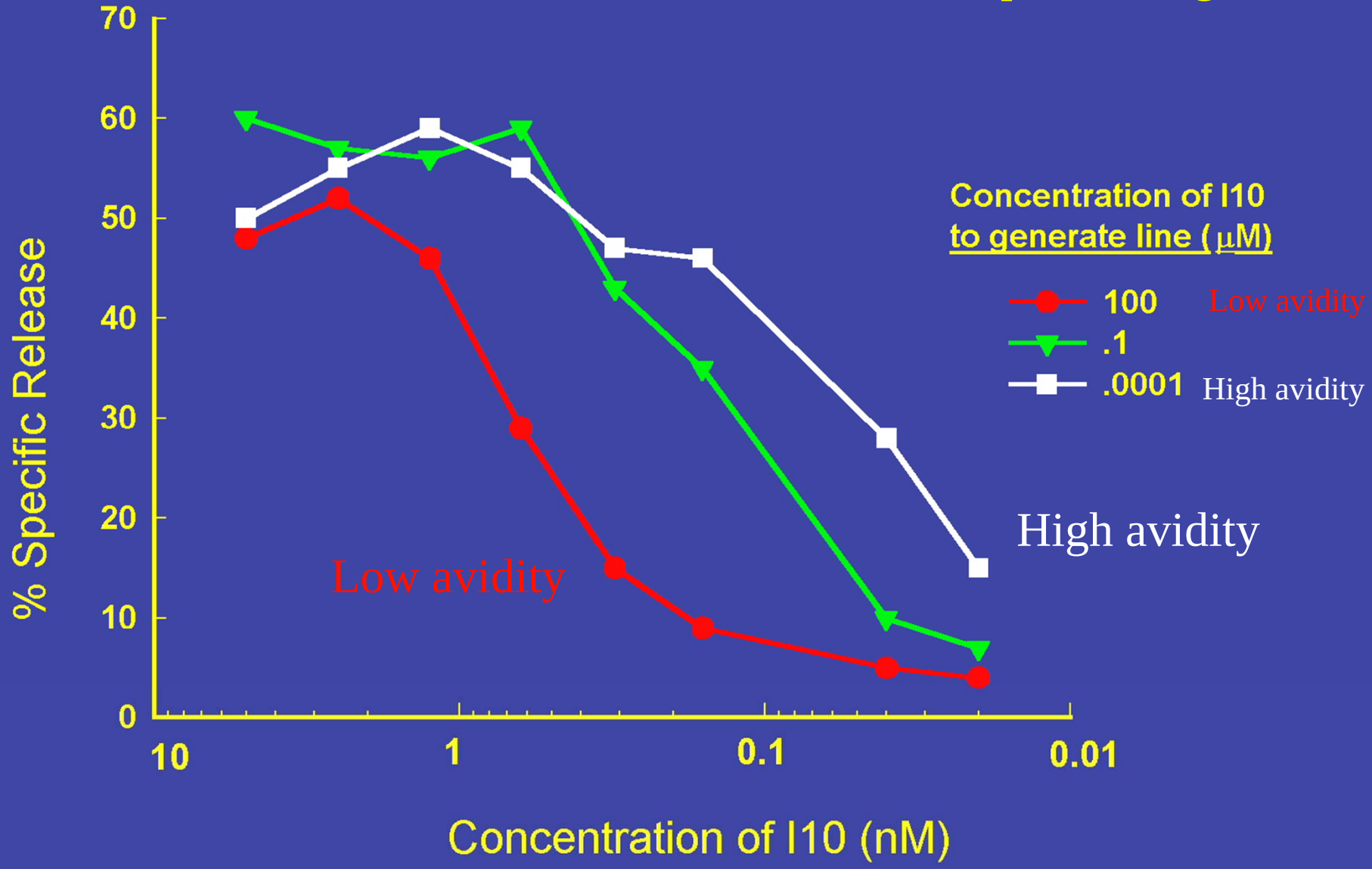
Cytokine Synergies:

IL-12 and GM-CSF synergized for CTL induction

Topics:

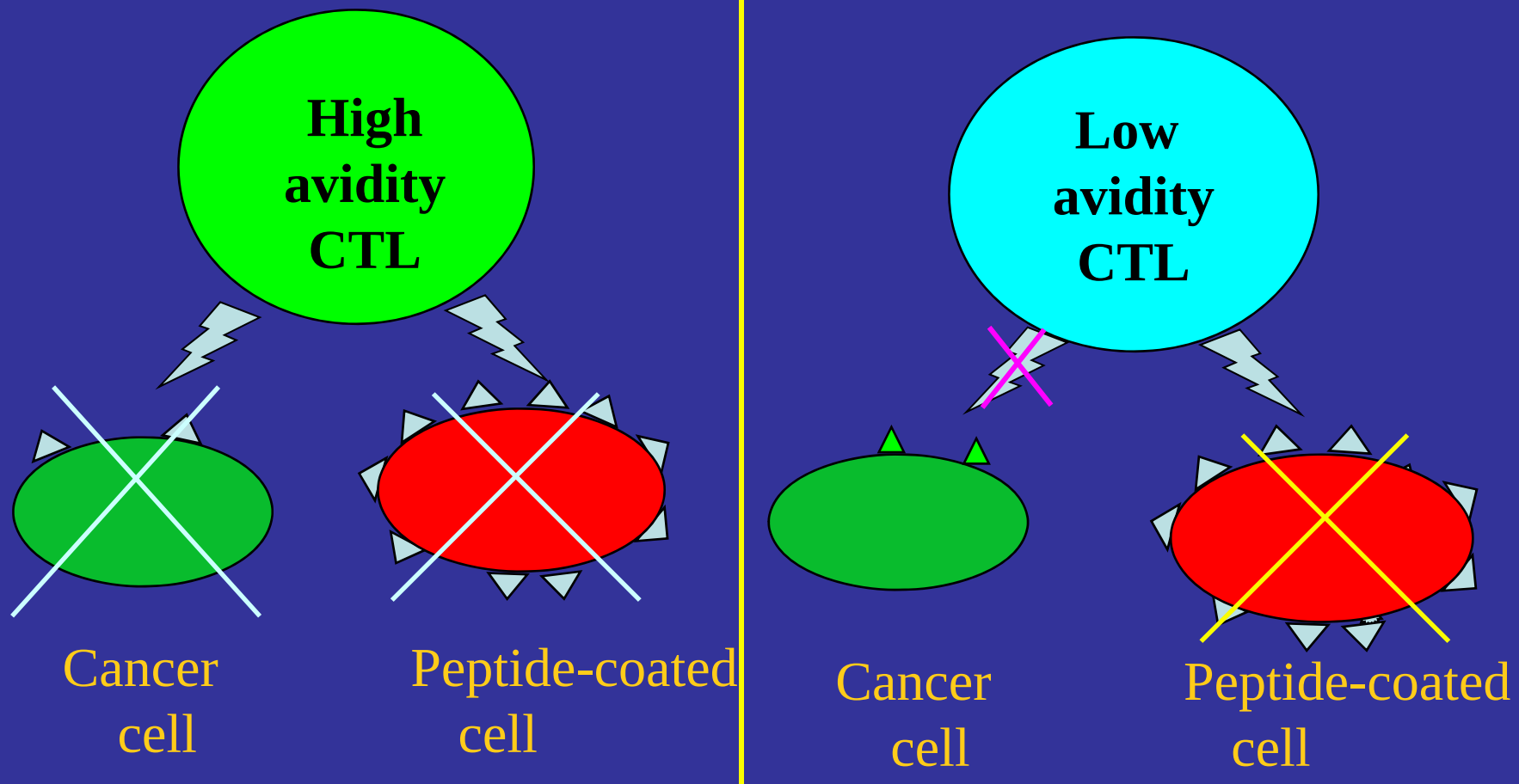
Use of IL-15 in the vaccine to induce high functional avidity CTL (recognizing low densities of peptide-MHC complexes on cells)

High, Intermediate, and Low **Functional Avidity** CTL Generated by Stimulation with Different Concentrations of Peptide Antigen



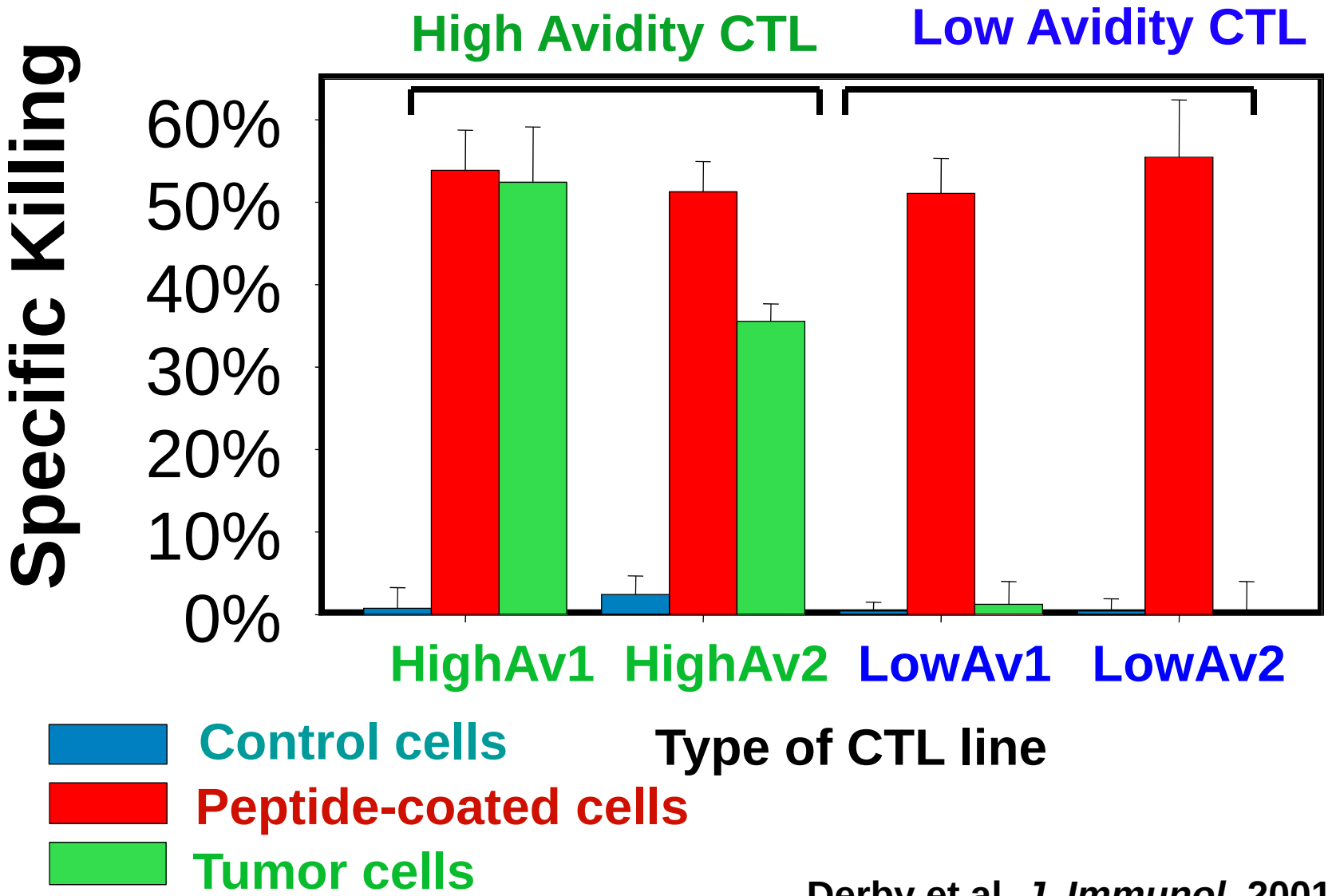
Alexander-Miller, Leggatt, & Berzofsky, *PNAS* 93: 4102, 1996

Hypothesis: high avidity CTL are more effective at killing tumor cells



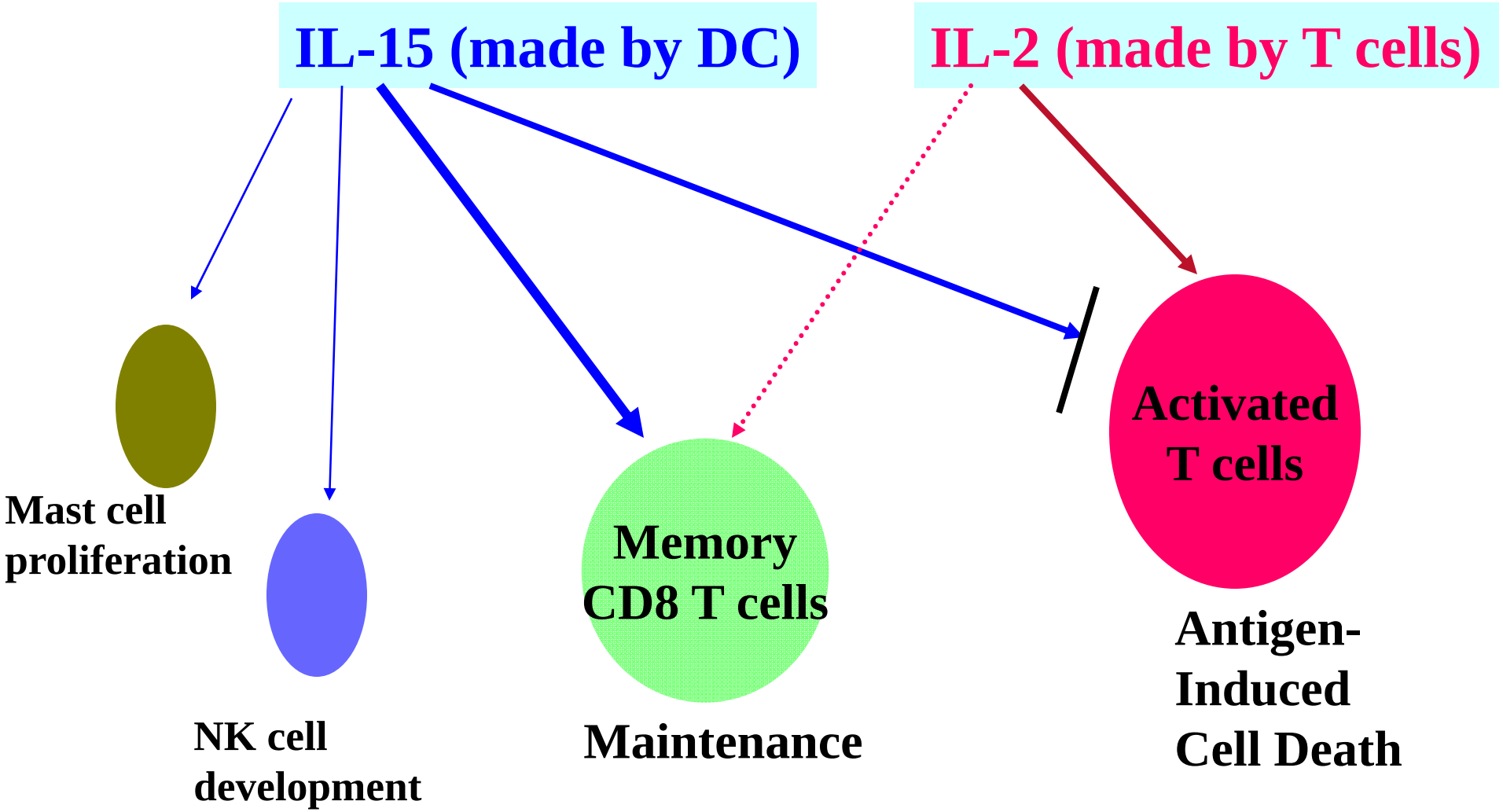
Alexander-Miller et al. *PNAS* 1996; Derby et al., *J. Immunol*. 2001; Belyakov et al, *Blood* 2006

Only HIGH AVIDITY CTL kill tumor cells

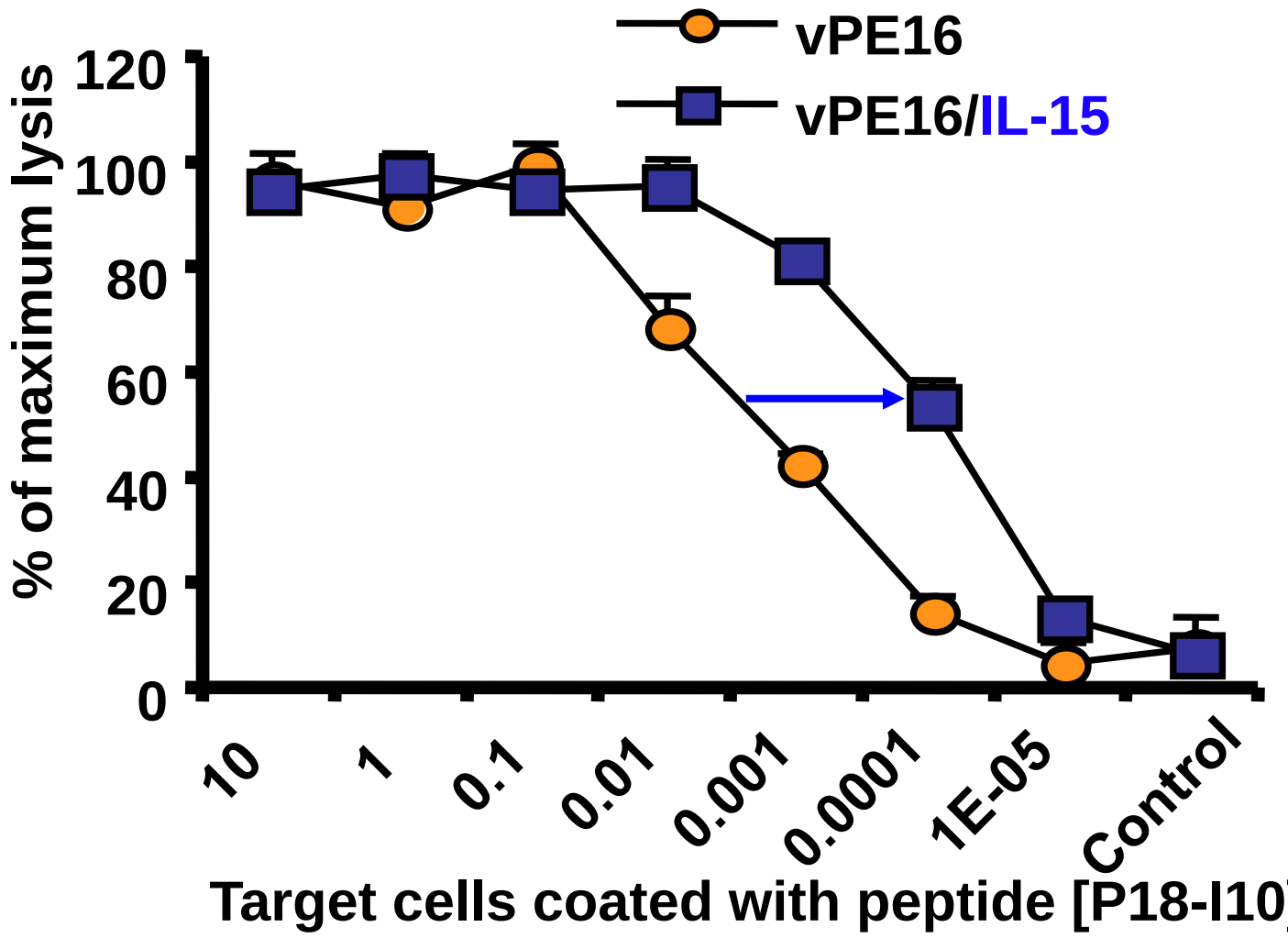


Derby et al, *J. Immunol.* 2001

IL-2 & IL-15: DISTINCT SOURCE & FUNCTIONS



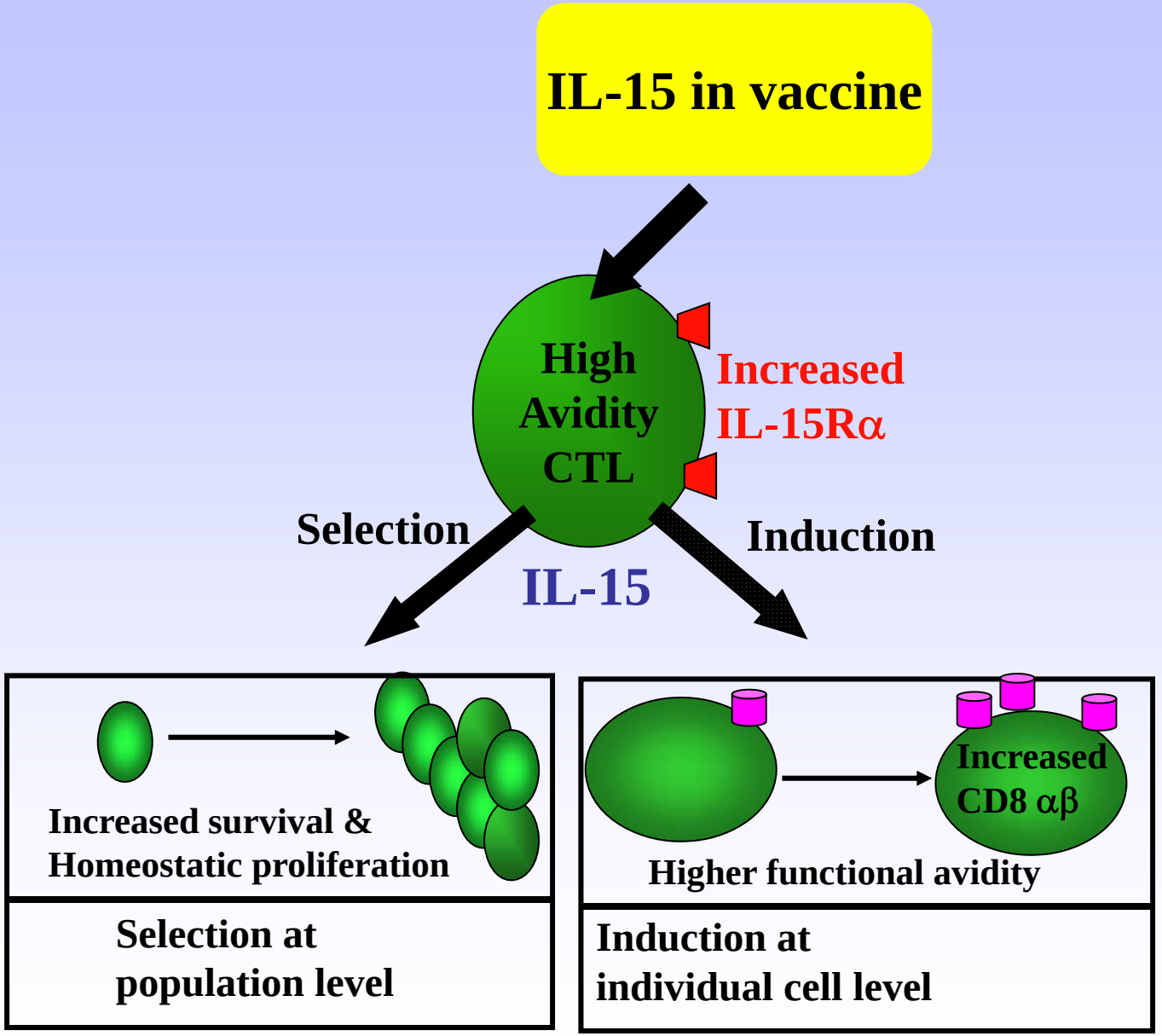
Immunization with antigen + IL-15 induces higher functional avidity memory CD8+ CTL



2 months after immunization

Oh et al., *PNAS* 2004

Complementary Mechanisms for IL-15 in CTL Avidity Maturation



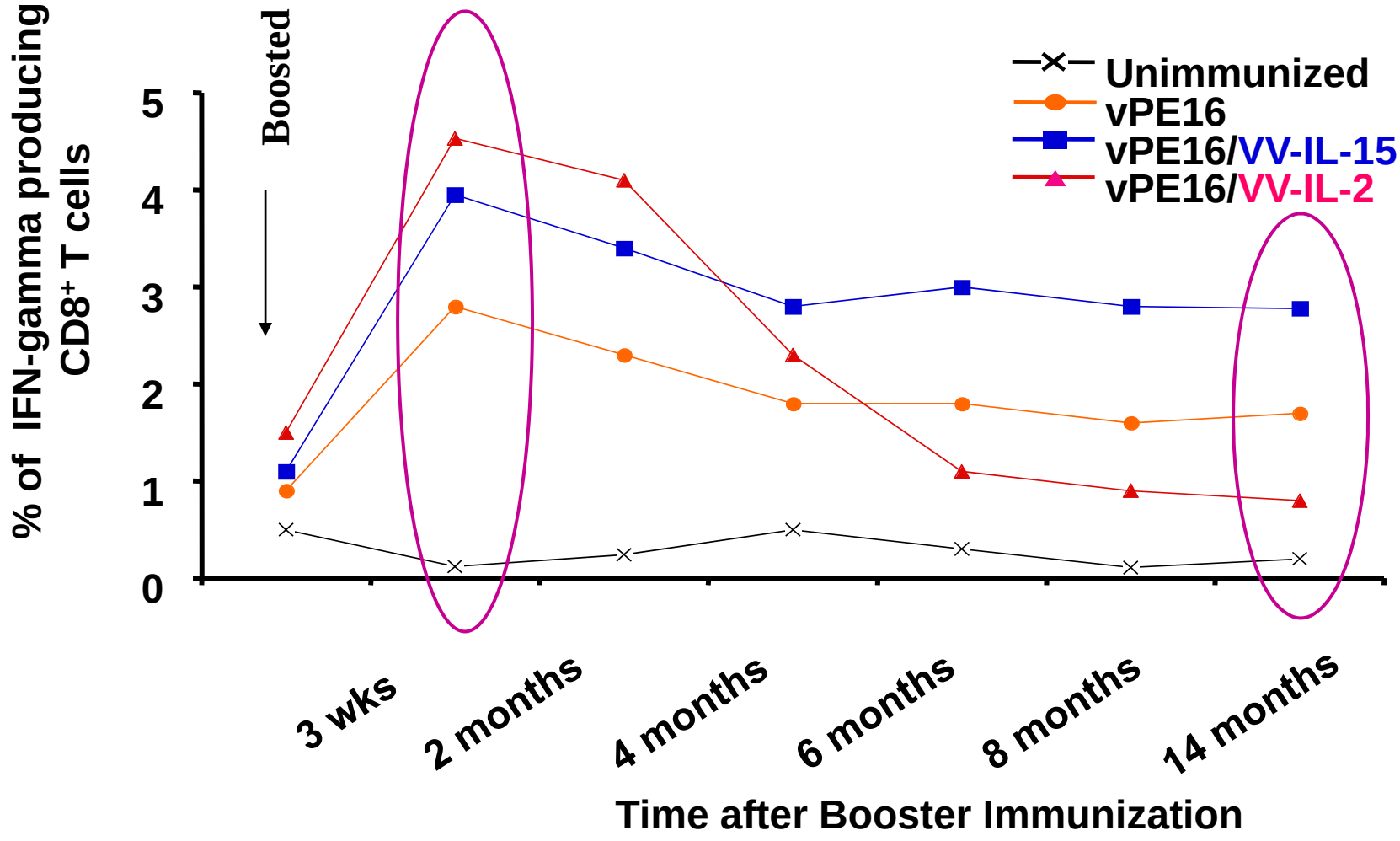
Oh et al. PNAS, 2004

Topics:

Use of IL-15 in the vaccine to induce high avidity CTL (recognizing low densities of peptide-MHC complexes on cells)

Improve CTL quality by using IL-15 to substitute for $CD4^+$ T cell help to induce long-lived memory CTL

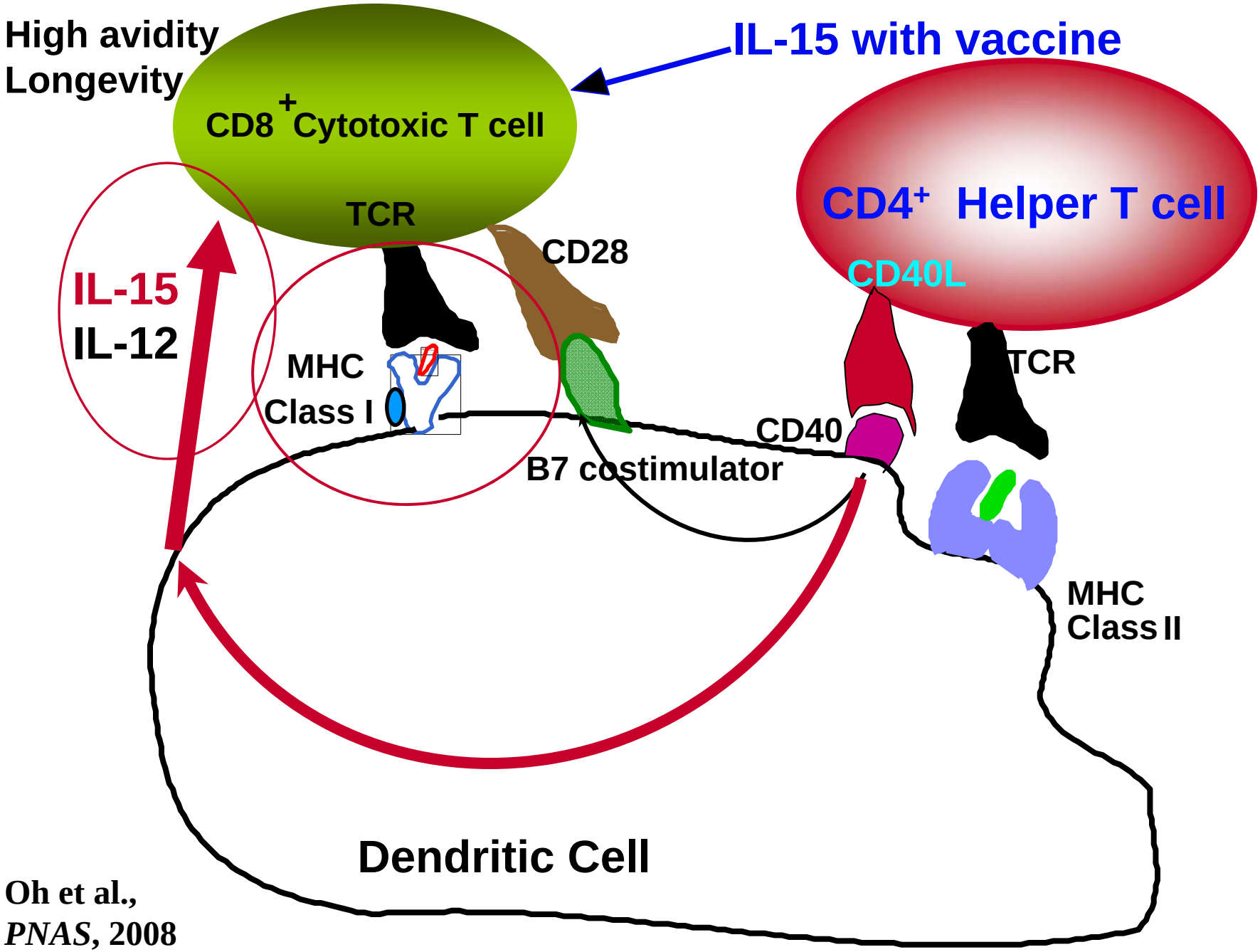
IL-15 expression by a vaccine vector induced longer-lived memory CD8+ CTL: IFN-gamma-producing cells



Explained by 1. Higher IL-15Rα expression
2. Greater homeostatic proliferation

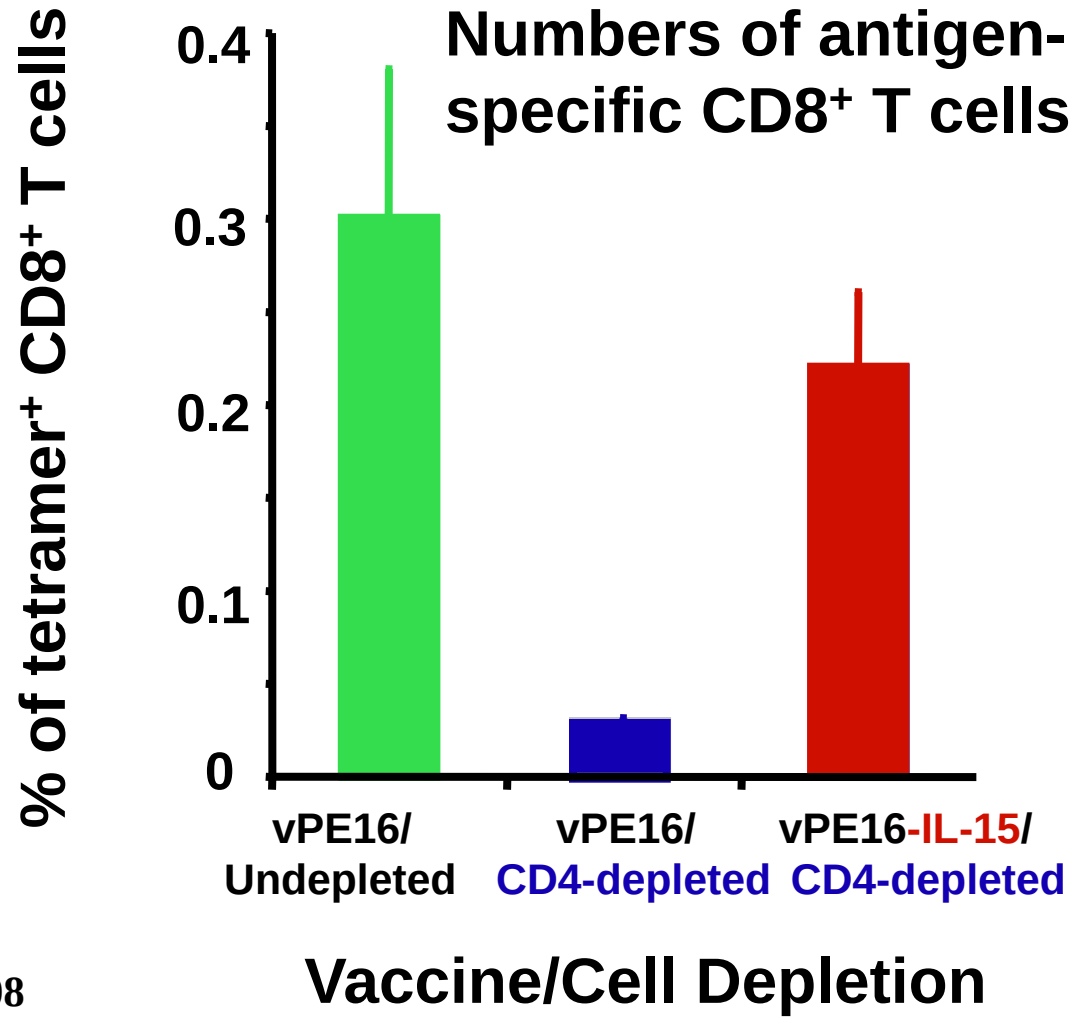
Oh et al., *PNAS* 2003

CD4+ T-cell Help for CD8+ CTL Mediated Through Activation of Dendritic Cell



Oh et al.,
PNAS, 2008

IL-15 during immunization substitutes for CD4$^+$ T cell help to induce long-lived memory CTL (One year after immunization)



Oh et al., *PNAS*, 2008

Conclusions for improving CTL quality

IL-15 in a vaccine:

- **Induces longer-lived memory CD8 CTL**
- **Induces higher avidity CD8 CTL**
- **Overcomes the need for CD4 T cell help to elicit prolonged CD8 T cell memory**
- **Is a critical natural mediator by which CD4 T help elicits long-lived CD8 memory T cells**

Thus IL-15 is a most promising candidate to enhance the efficacy of vaccines for use in HIV-infected or cancer patients with a deficiency of CD4 T cell help (including therapeutic vaccines for AIDS or cancer).

Topics:

Use of IL-15 in the vaccine to induce high avidity CTL (recognizing low densities of peptide-MHC complexes on cells)

Improve CTL quality by using IL-15 to substitute for $CD4^+$ T cell help to induce long-lived memory CTL

Remove the brakes by blocking negative regulation: A new NKT regulatory axis.

Cancer vaccines can induce CTL measured in vitro but much less often induce clinical tumor regression.

WHY?

TUMOR TOLERANCE

suppression by tumor
(anti-inflammatory Cytokines/STAT3 induc)

suppression by immune cells

absence of danger signals
(incorrect presentation);
Off-signals on T cells
(e.g. CTLA-4 or PD-1)

M2 macrophages
or tumor associated macrophages (TAM)

Myeloid-derived suppressor cells (MDSC),
Granulocyte suppressors

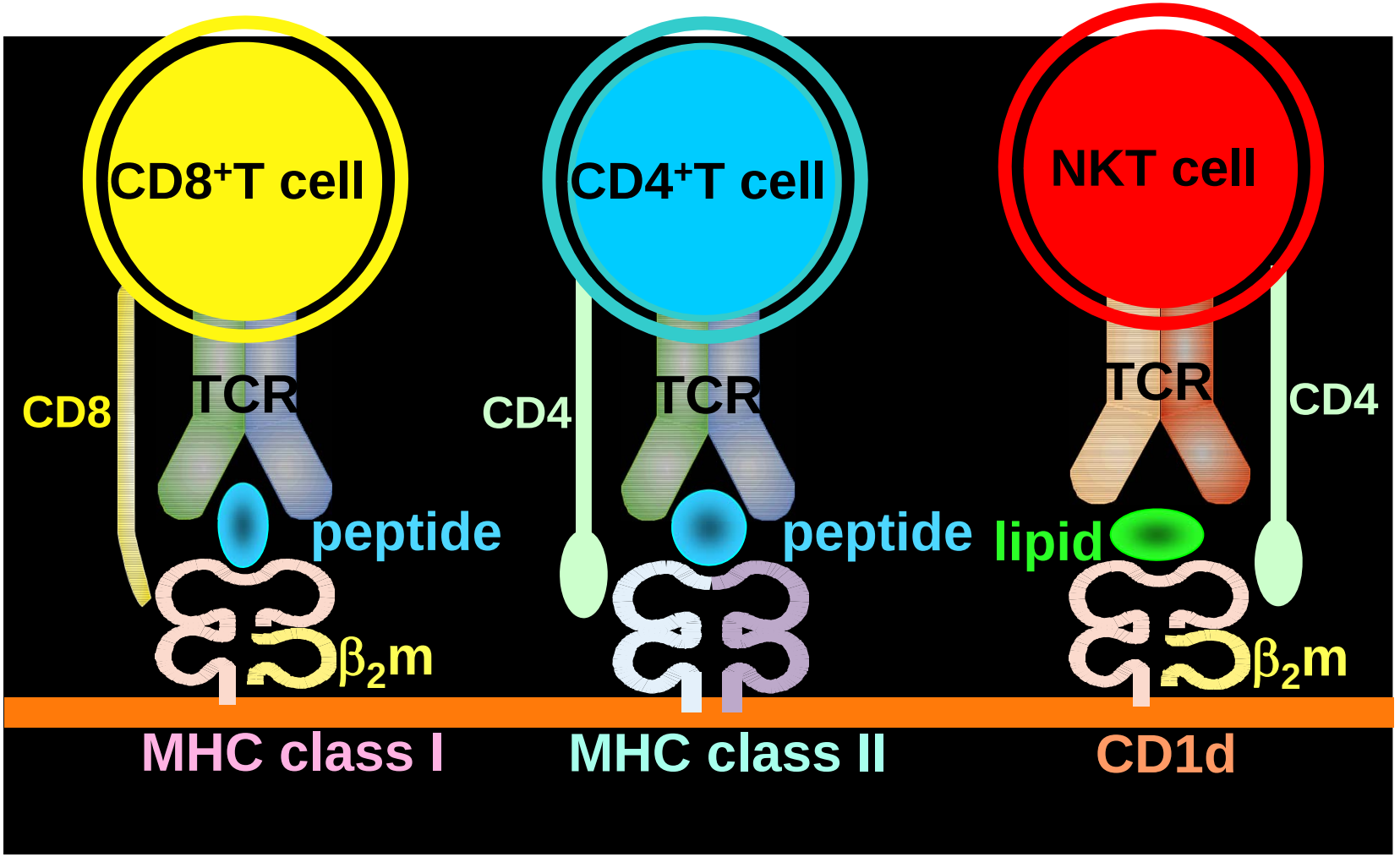
$CD4^+CD25^+$ T regulatory cells (Treg)

Natural Killer (NK) T cells

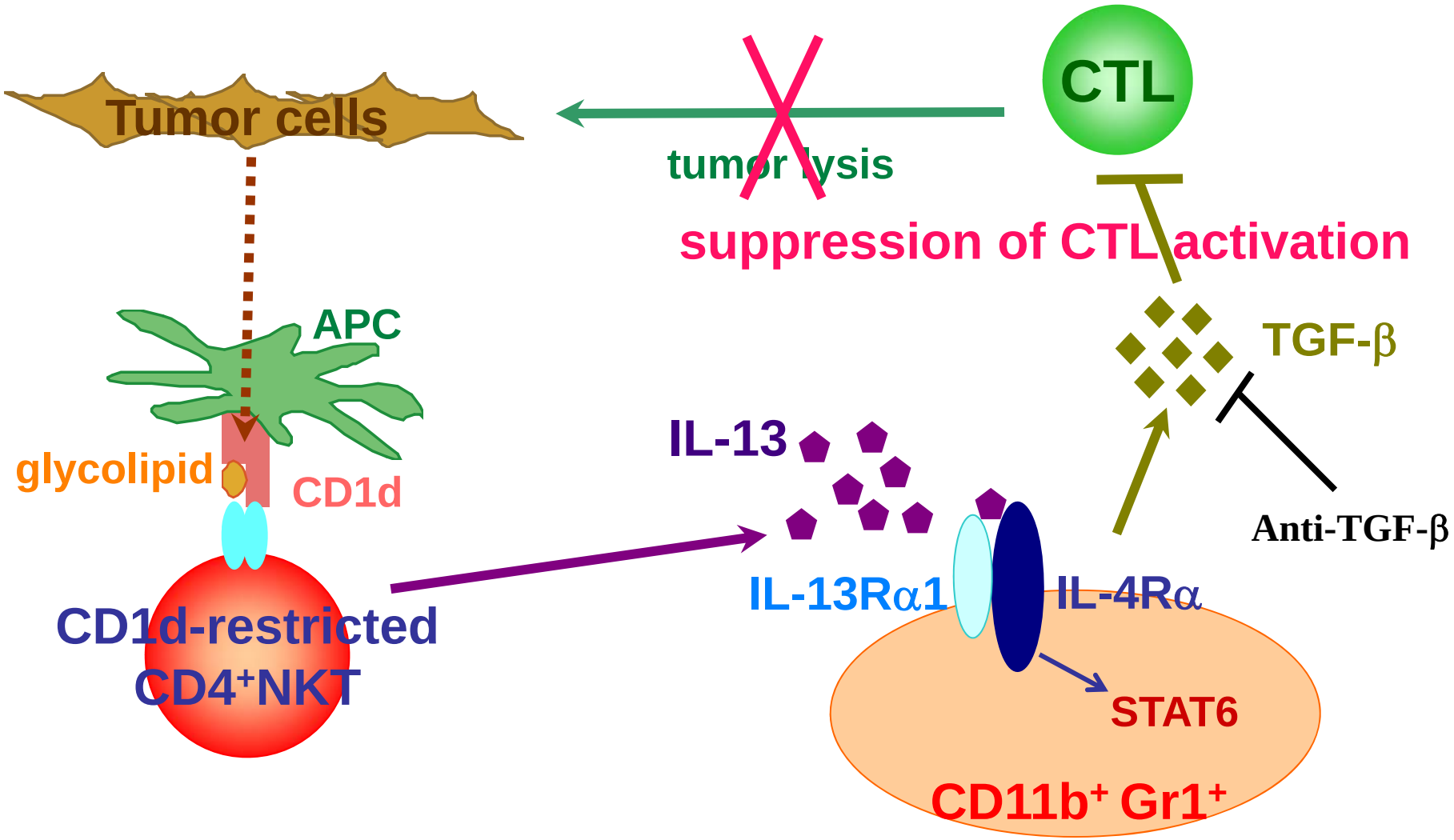
NKT cells

NKT cells

- Unlike NK cells, they express a TCR, but have unusual restriction to a nonclassical MHC molecule



NKT cells and IL-13 suppress CTL tumor immune surveillance though the IL-4R-STAT6 pathway to induce TGF-β production by CD11b^{+}Gr-1^{+} cells



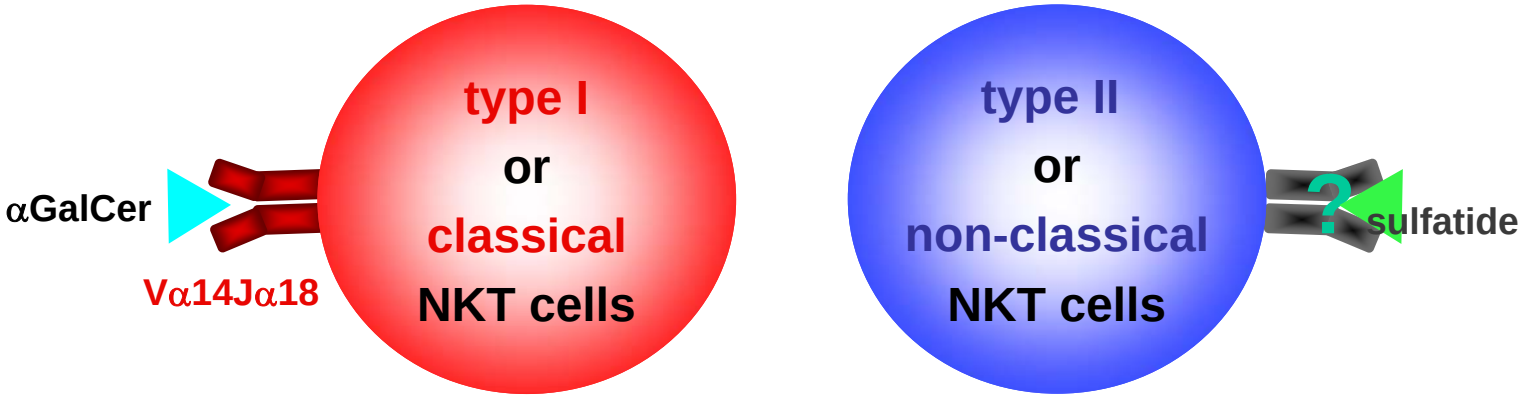
Terabe et al., *Nat Immunol*, 2000., Terabe et al., *J Exp Med*, 2003.

NKT cells

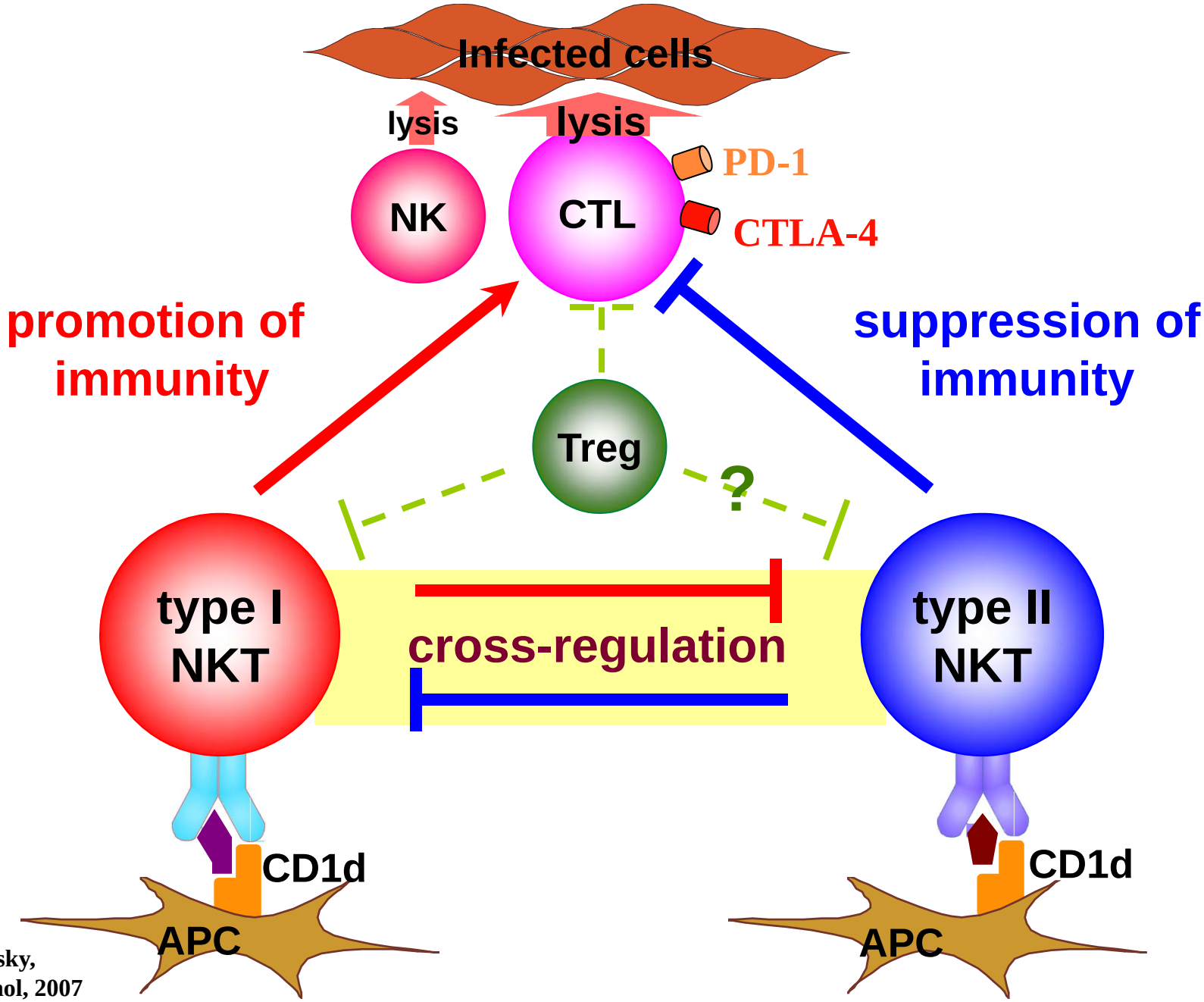
NKT CELLS ARE A HETEROGENEOUS CELL POPULATION

	Type I or Classical NKT cells	Type II or Non-classical NKT cells
CD1d-dependent	Yes	Yes
Glycolipid specificity	Alpha-GalCer, OCH	Sulfatide
TCR-α chain	Vα14-Jα18 (in mice)	diverse

(modified from: Godfrey D.I., Nat Rev Immunol 4: 231-237 (2004))

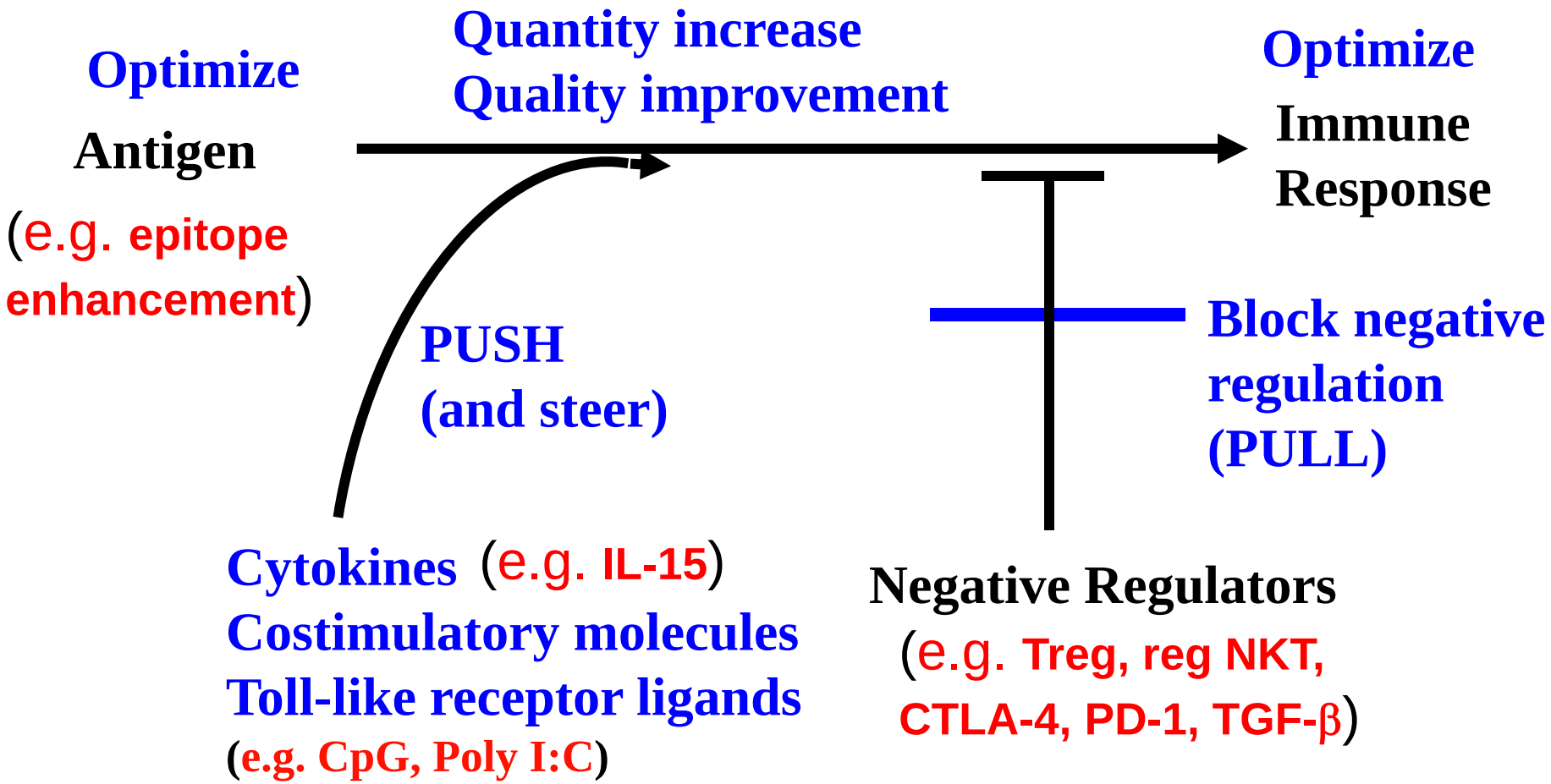


A new immunoregulatory axis



Terabe & Berzofsky,
Trends in Immunol, 2007

PUSH-PULL Approach to Optimizing Vaccine-induced T-cell Immunity



Key Collaborators

- Antibody-inducing Adeno-HER-2/neu vaccine: Jong-Myun Park, Masaki Terabe, Jason Steel, Yoshio Sakai, Guido Forni, John Morris
- Epitope enhancement: Jeff Ahlers, Takahiro Okazaki, Pablo Sarobe, SangKon Oh, Ira Pastan
- IL-15: SangKon Oh, Tom Waldmann, Liyanage Perera, Masaki Terabe, Don Burke
- Negative Regulation: Masaki Terabe, Elena Ambrosino, Jong Myun Park, Susanne Ostrand-Rosenberg, Mark Smyth, Dale Godfrey, Vipin Kumar, Takashi Yamamura