Combination Immunotherapies

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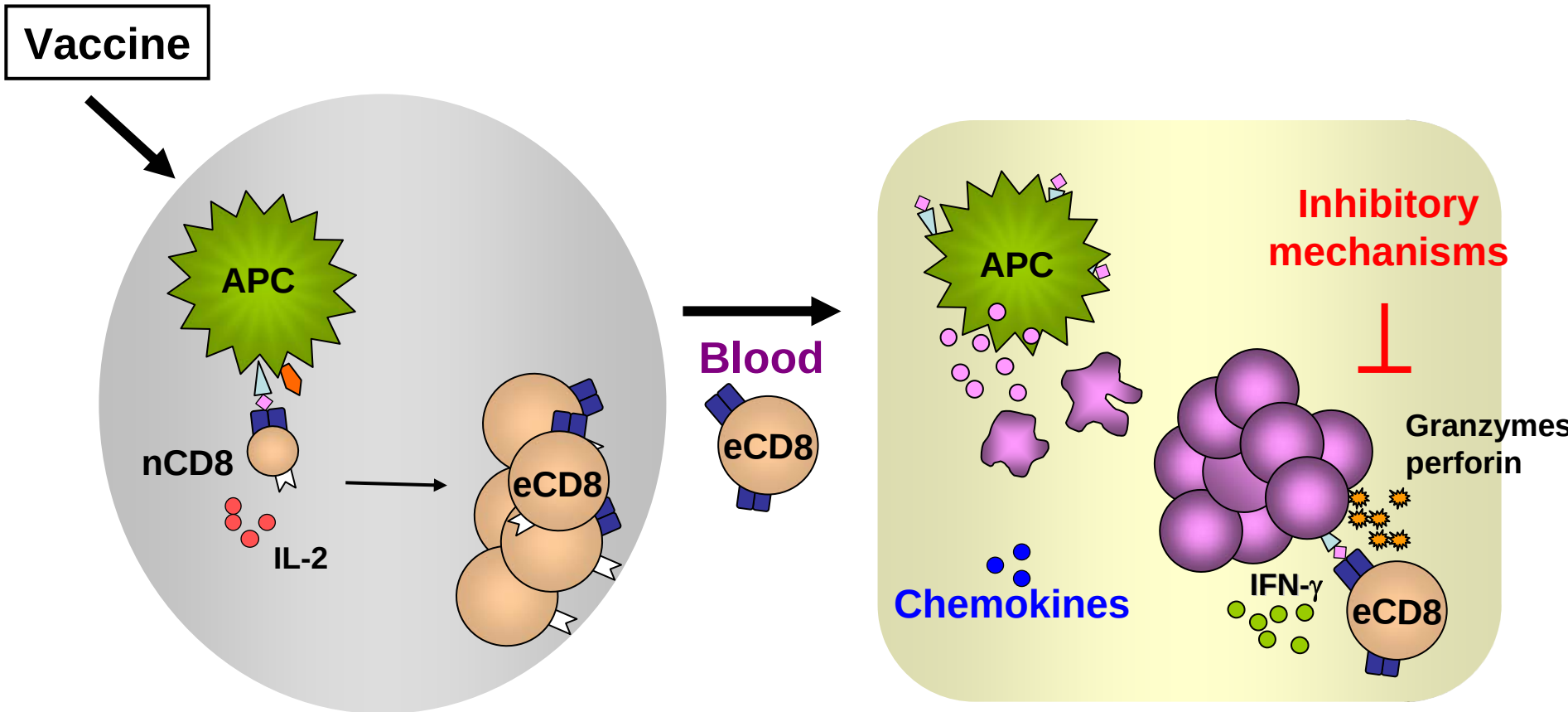
Rationale and foundation for discussion

- Important disclaimer: we are not suggesting that “vaccines don’t work” and therefore combinations of vaccines plus other therapies may show synergy
- Rather, our view is that an anti-tumor immune response is a complex and multi-stage process that can become dysregulated at several levels in the context of a growing tumor
- Overcoming each of these defects may require a distinct intervention, and therefore combination therapies may be important in order to translate immune responses into tumor regression
- Another way to look at it: with cancer vaccines, the “drug” is not the vaccine itself—rather, the therapeutic entity is the properly generated tumor **antigen-specific effector T cell population** that has **penetrated the tumor microenvironment** and **maintained effector function there**

An effective anti-tumor immune response is a multistep process

1. Priming phase (vaccination)
   - Magnitude and breadth of T cell response
   - Qualitative aspects of T cell differentiation (effector functions)
   - Issue: limitations of available repertoire
2. Expansion and persistence
   - Survival of effector cells in periphery
   - Immunologic memory
3. Trafficking to tumor sites
   - Chemokines and homing receptors
4. Executing effector function
   - Overcoming negative regulatory pathways
     - Anergy, CTLA-4, PD-1, IDO, Tregs
   - Maintenance of effector function
     - Regeneration of cytotoxic granules
5. Tumor cell susceptibility to recognition and killing
   - Expression of antigens, processing machinery, MHC
   - Overcoming anti-apoptotic mechanisms
   - Interface with tumor cell-intrinsic biology

Complexities of anti-tumor immune responses: Taking into account the effector phase



Lymph node
(Priming phase)

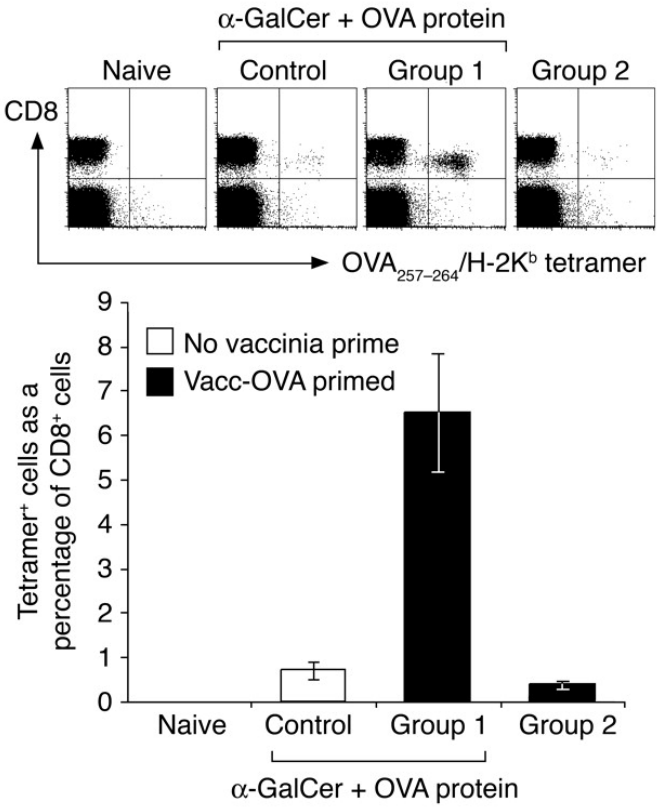
Tumor microenvironment
(Effector phase)

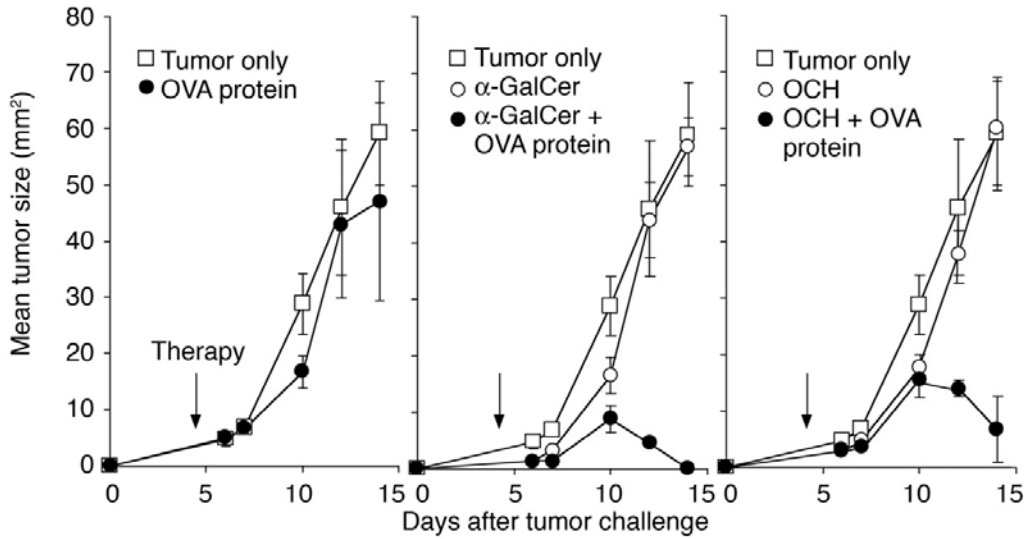
I. Priming phase/vaccine: considerations for combinations

- Antigen choice(s)
  - Peptides, protein, DNA, RNA, bulk tumor cells
  - Type of antigen (e.g. necessary for malignant phenotype)
  - Class I MHC, class II MHC, non-classical (glycolipids)
- Adjuvant components
  - Emulsions in oil-based formulations
  - TLR agonists (LPS/MPL + CpG)
  - Cytokine additions—differentiation promoters
  - Microbial vectors
  - Dendritic cell-oriented
- Dose, schedule, route of administration
  - Issue of tissue-specific homing of T cells

Example 1: α-GalCer

Administration of protein and α-GalCer can synergistically expand CD8+ T cells

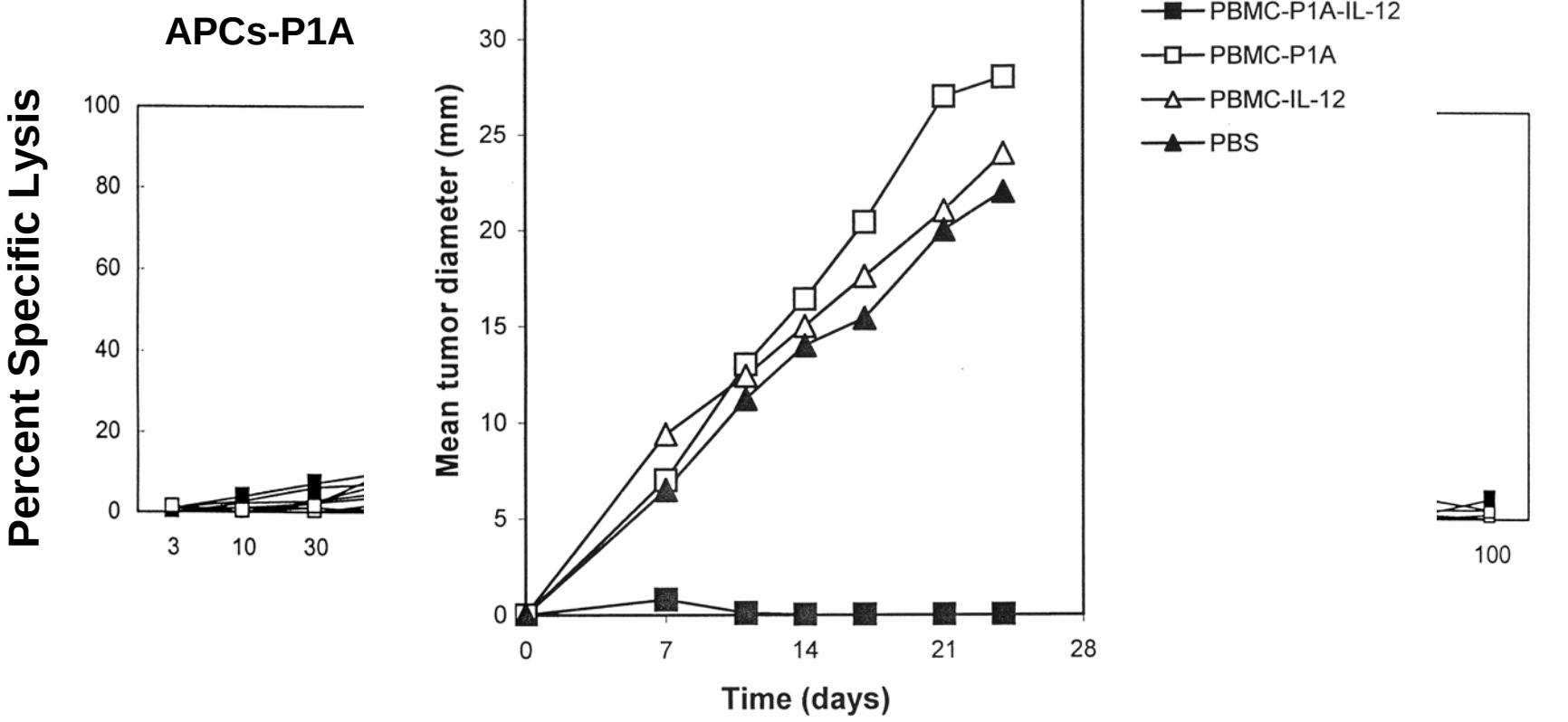




Silk, Cerundolo et al. J. Clin. Invest. 2004

Example 2: IL-12

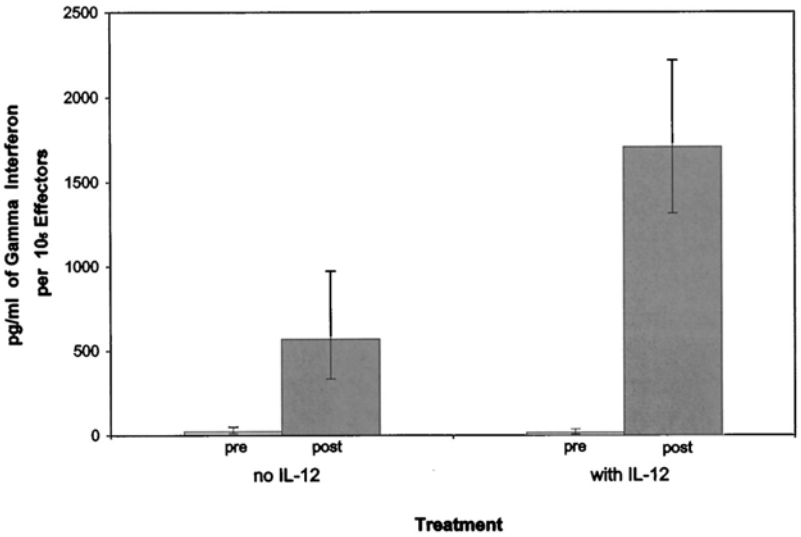
Superior induction of specific CTL responses in mice using peptide-loaded APCs + IL-12



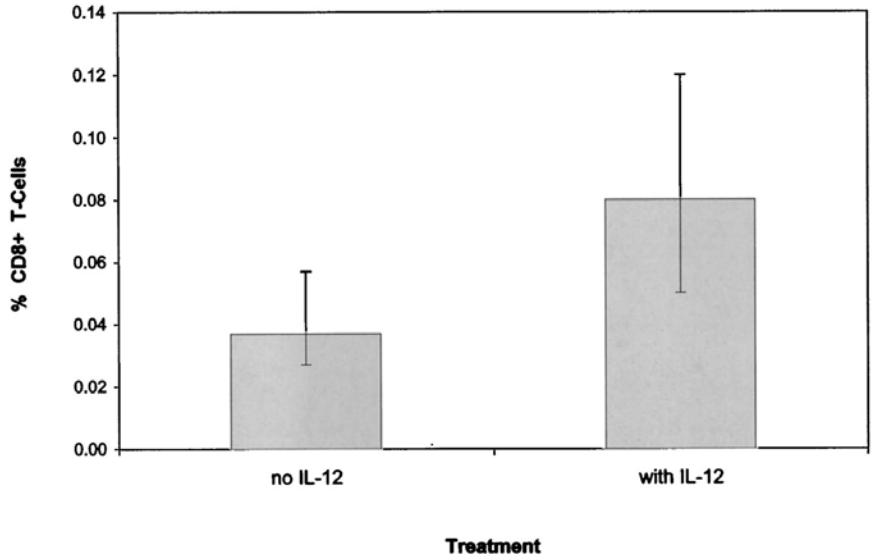
Fallarino, Gajewski et al. Int. J. Cancer 1999.

Superior immune responses with IL-12 + peptides in Montanide in patients with melanoma

IFN-γ production

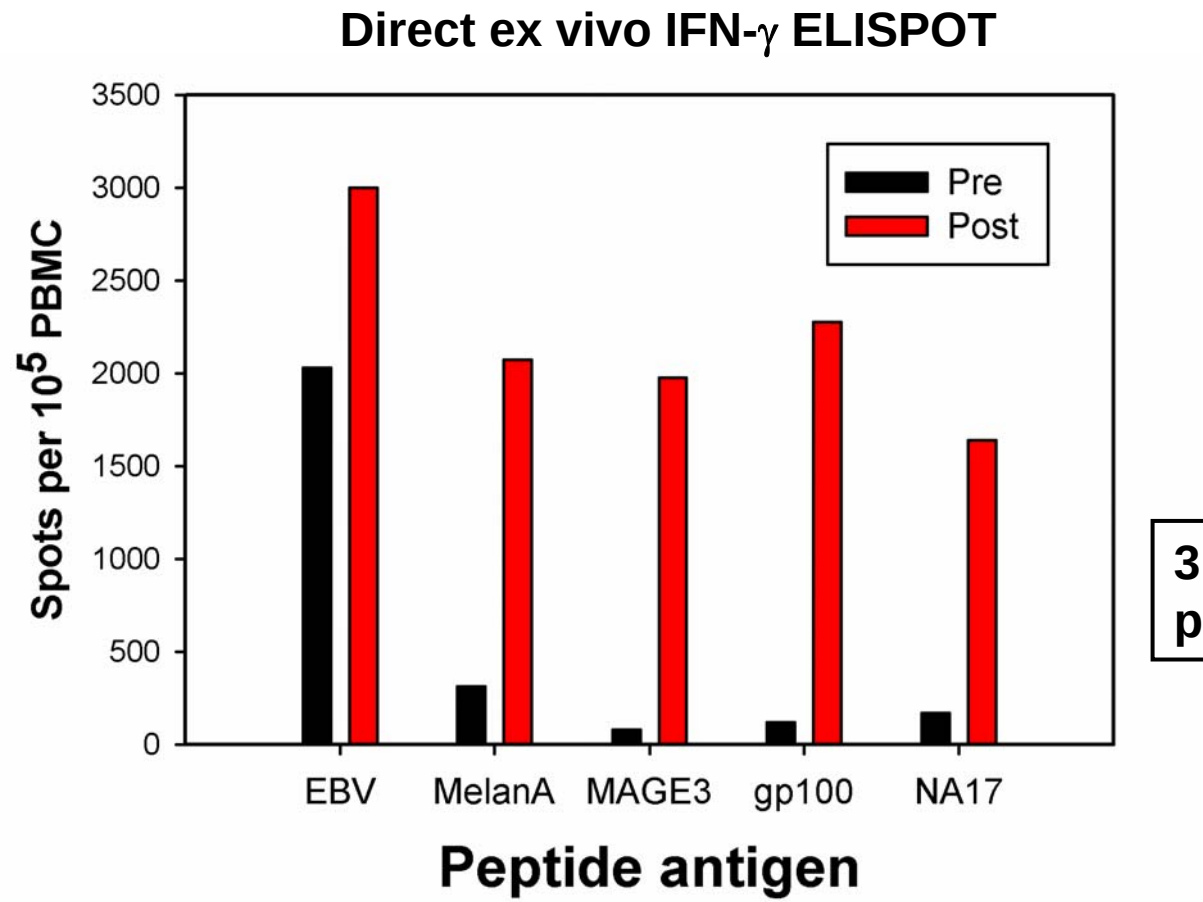


Tetramer staining



Lee, Weber et al. JCO 2001.

Potent T cell response against multiple antigens post-immunization of melanoma patients with peptide-pulsed PBMC + IL-12



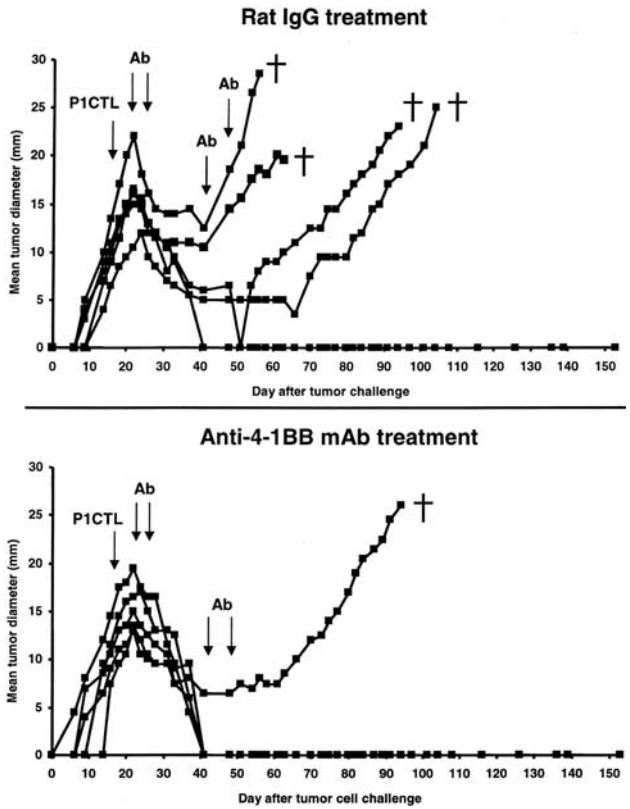
3 patients with CR post-vaccination

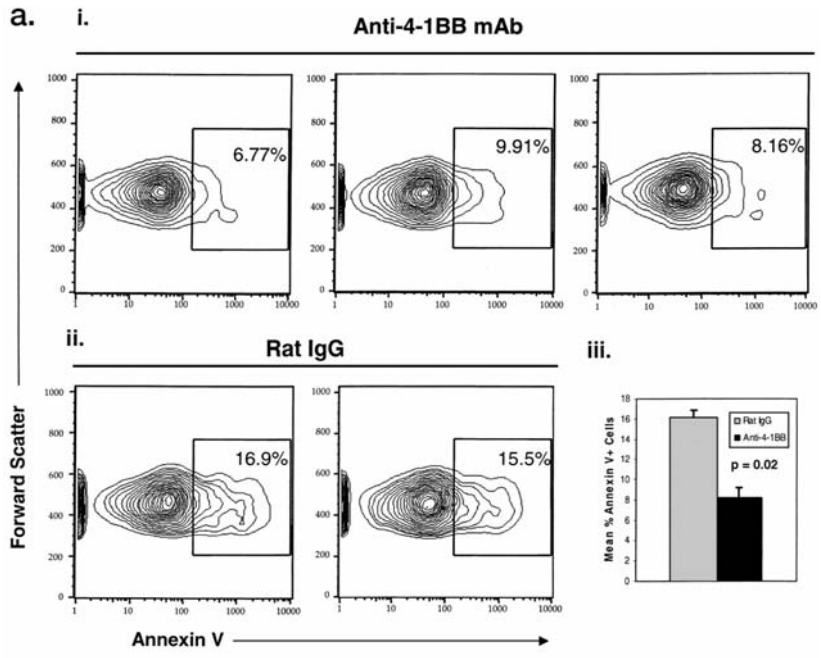
II. T cell expansion and persistence: considerations for combinations

- Survival/homeostatic cytokines
  - IL-7
  - IL-15
  - IL-21
- Costimulatory receptors
  - B7 family members
  - 4-1BB
  - Other TNFR family members

Example 3: Anti-4-1BB

Co-administration of anti-4-1BB mAb with adoptively transferred T cells induces superior tumor rejection and T cell survival in mice



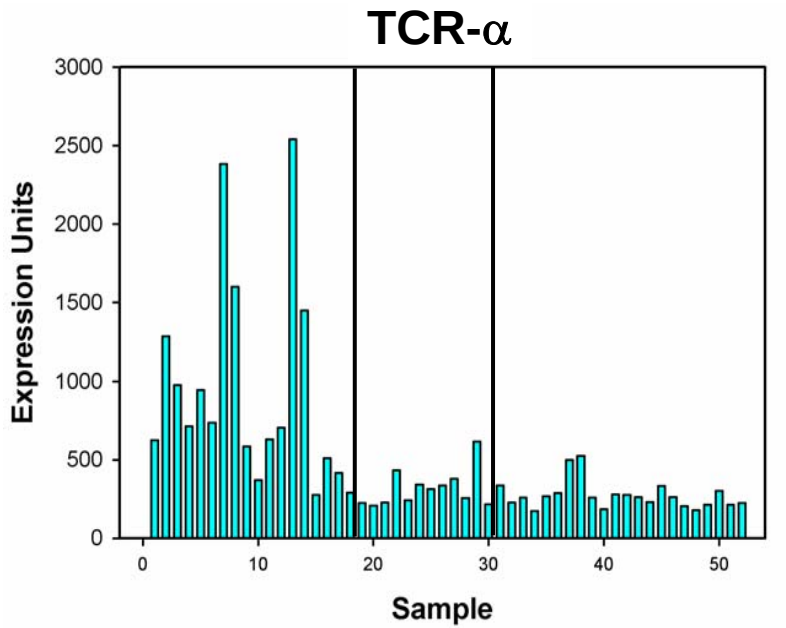


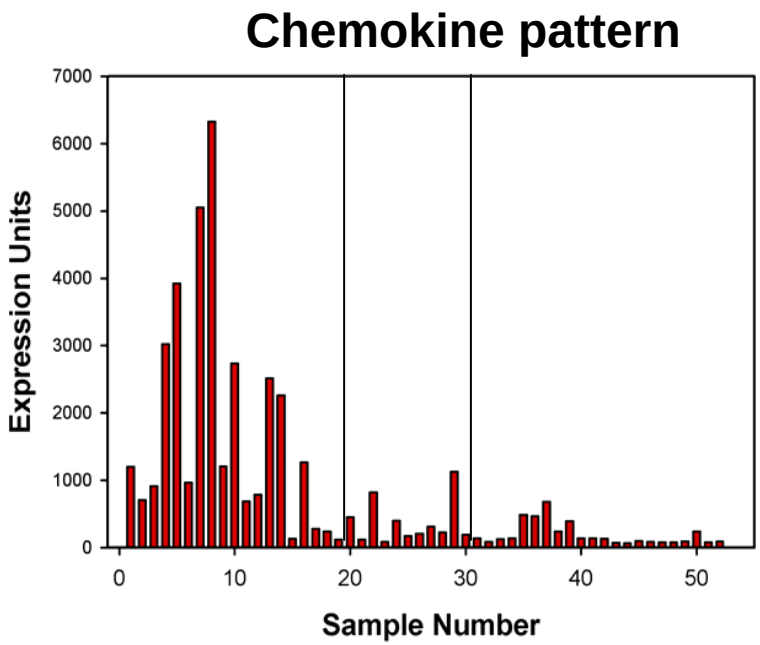
May, Liu et al. Cancer Res. 2002.

III. T cell trafficking: considerations for combinations

- Intratumoral chemokines
  - Mig, IP-10, MIP-1α
  - CCL21
  - (Blockade of TARC/MDC?)
- Intratumoral LIGHT
  - Promotes secondary generation of chemokines
- Homing receptors/adhesion molecules
  - Intratumoral ICAM-1 (component of TRICOM)
  - Immunizing via optimal route (tissue specific homing)
- Angiogenesis targeting

Only a subset of melanoma metastases appear to have the appropriate signature for T cell recruitment

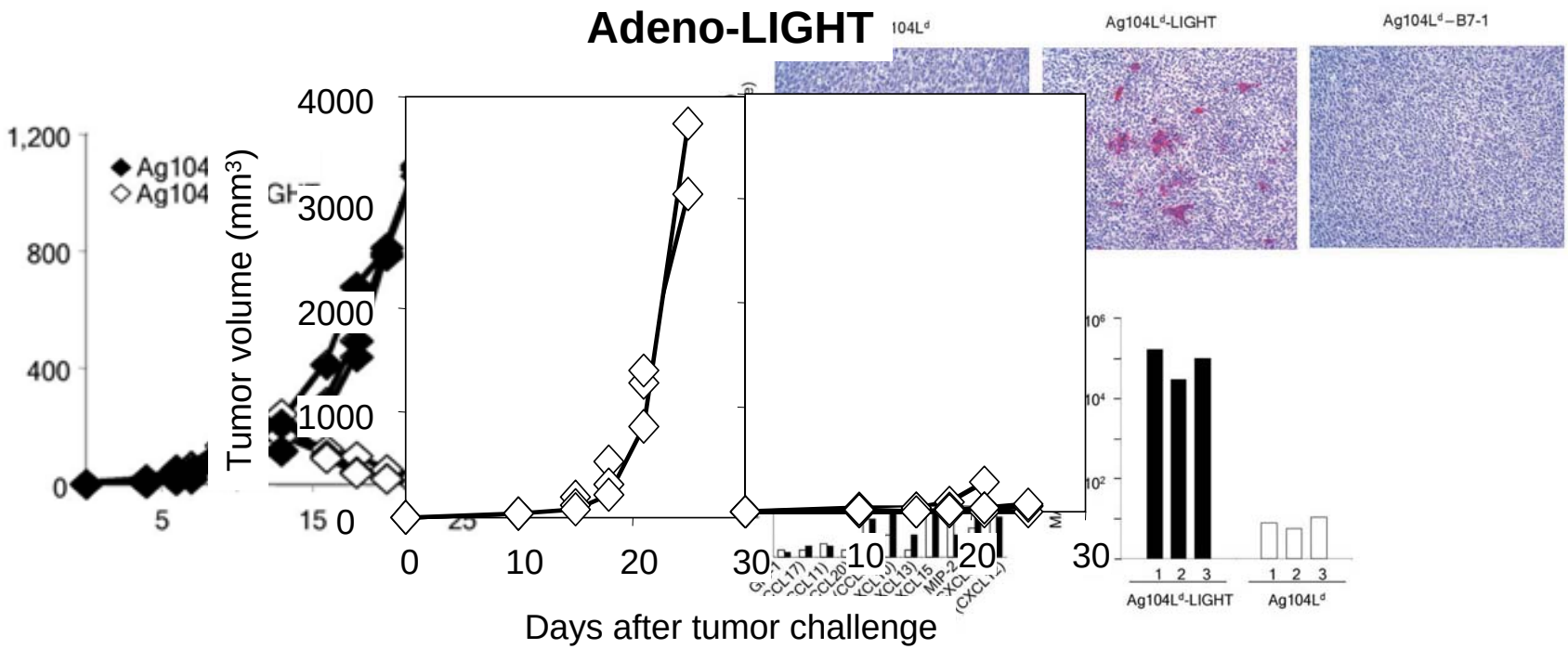




Harlin, Gajewski et al. Manuscript in preparation.

Example 4: LIGHT

Intratumoral LIGHT can induce T cell recruitment and tumor rejection in multiple tumor models



Fu et al, submitted

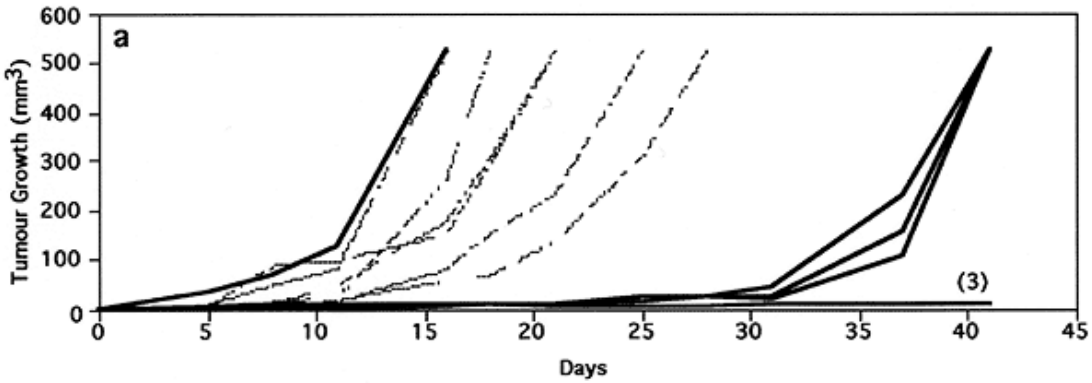
IV. Negative regulatory pathways: considerations for combinations

- Inhibitory receptors on T cells
  - CTLA-4
  - PD-1
  - KIRs
- Inhibitory cytokines
  - TGF-β
  - IL-10
- Inhibitory cell populations
  - CD4+CD25+FoxP3+ Tregs
  - Other Tregs
  - Myeloid suppressor cells
  - B cells
- Metabolic regulation
  - IDO
  - Arginase
  - Nutrient deprivation (glucose)

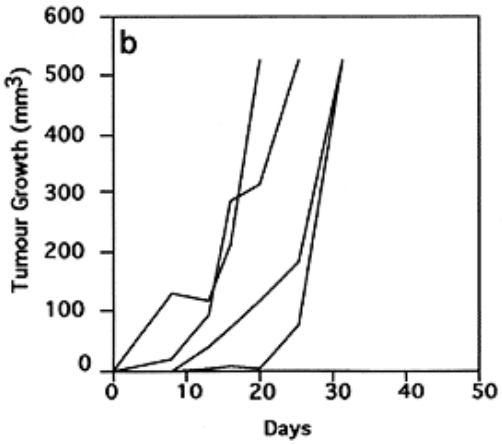
Example 5: CD25^{+} Tregs

CD25 depletion can partially control B16 melanoma growth in vivo

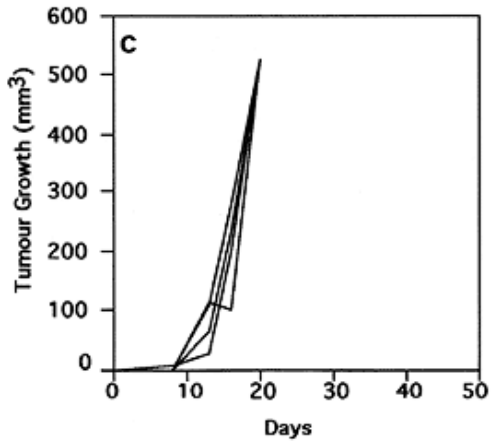
Anti-CD25



Anti-CD25+anti-CD4



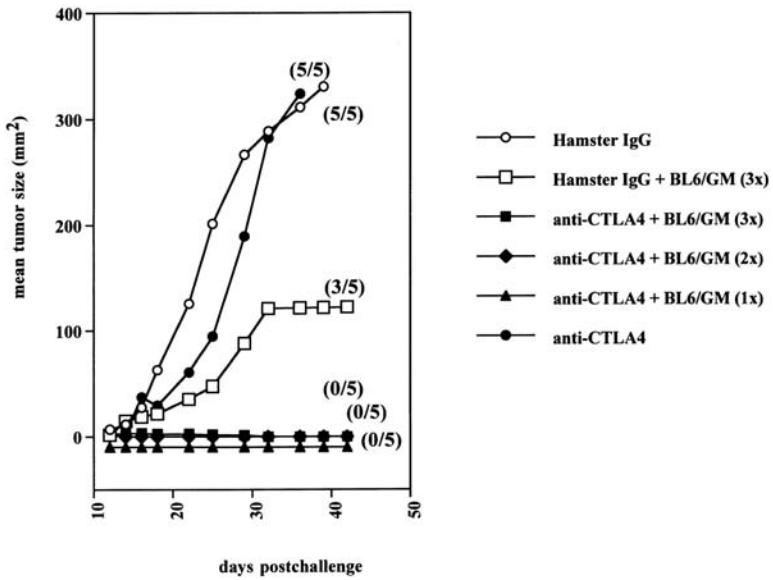
Anti-CD25+anti-CD8

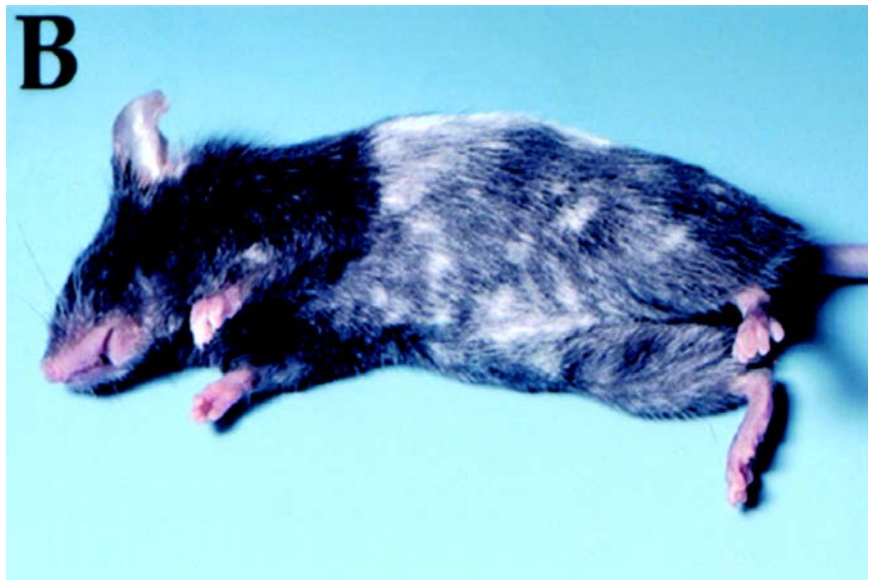


Jones, Gallimore et al. Cancer Immunity 2002.

Example 6: CTLA-4

Anti-CTLA-4 mAb + GM-CSF-transduced B16 vaccine induces tumor rejection and leads to vitiligo



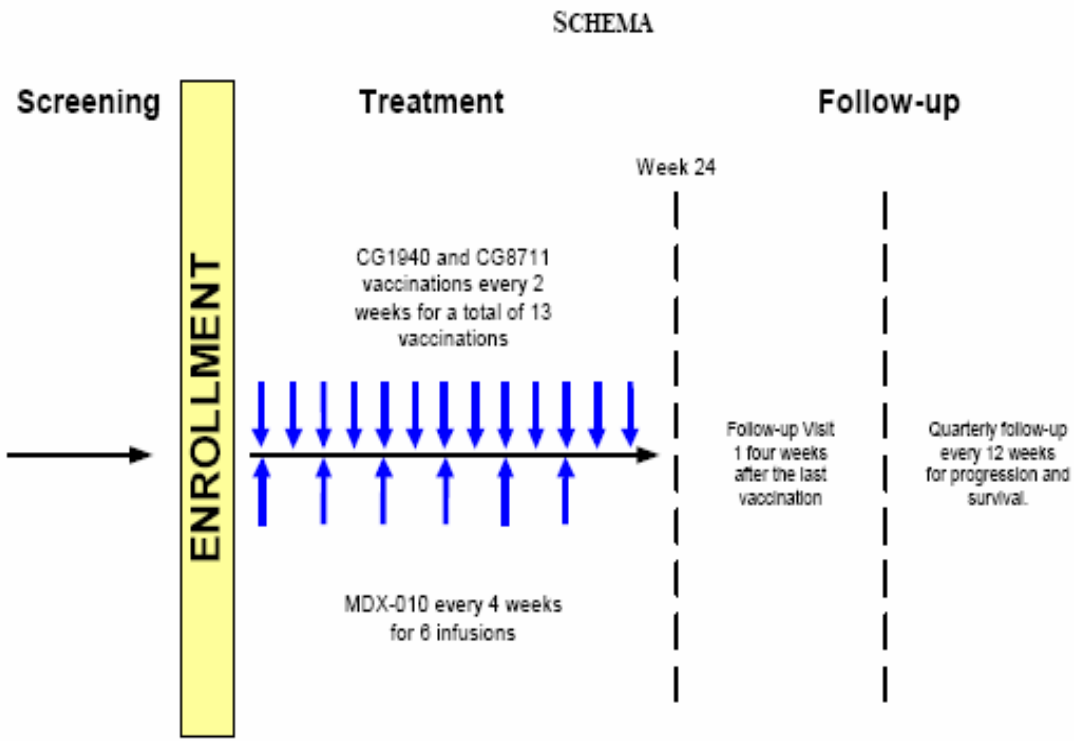


van Elsas, Allison et al. JEM 1999

Clinical development of anti-CTLA-4 mAb: Example of MDX-010 (Ipilumumab)

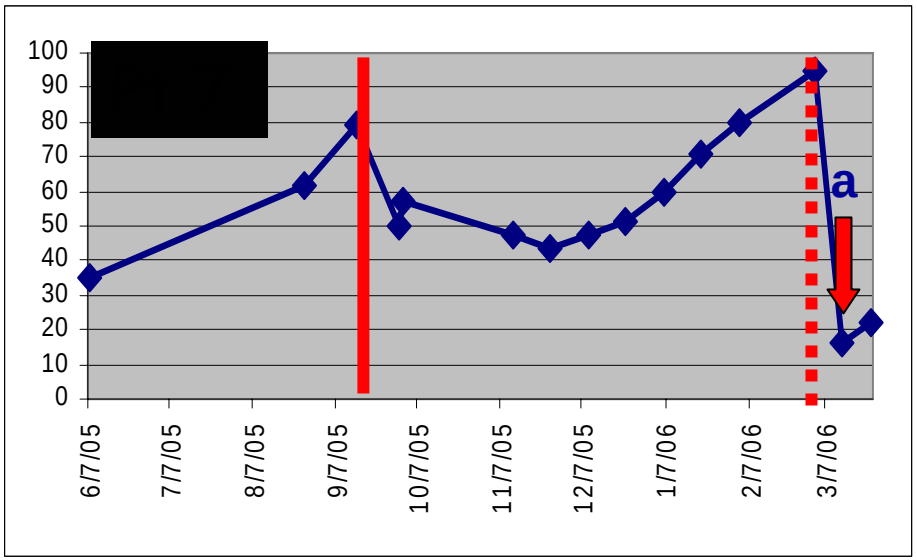
- Fully human IgG1 monoclonal antibody to human CTLA-4 created by Medarex
- Blocks binding of CTLA-4 to CD80 and CD86
- Augments immune responses in primate models
- Co-developed by Medarex and Bristol-Myers Squibb in multiple cancer indications
  - Phase III study in metastatic melanoma ongoing
  - Phase II studies in renal cell carcinoma, prostate cancer, ovarian cancer, and others

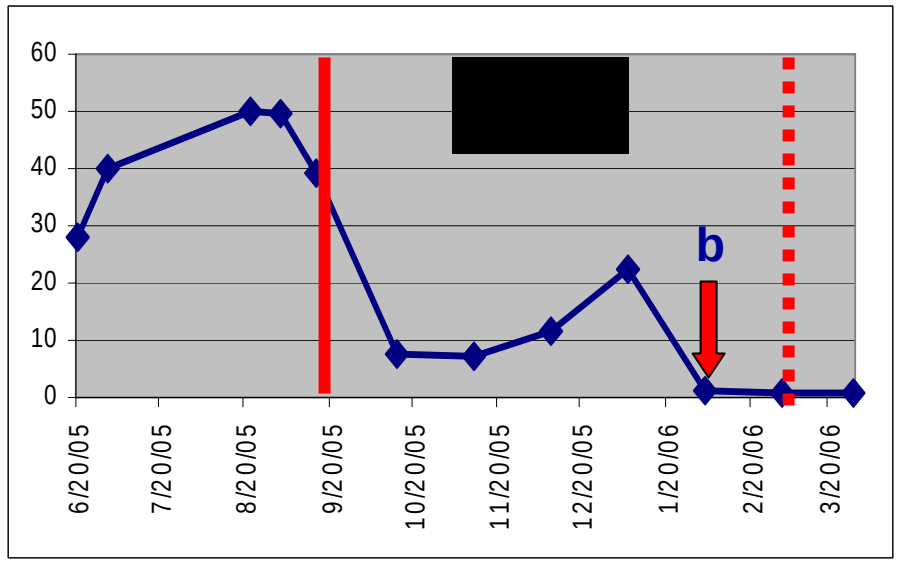
GVAX Immunotherapy (CG1940/CG8711) + Ipilimumab (MDX-010: anti-CTLA-4) for HRPC

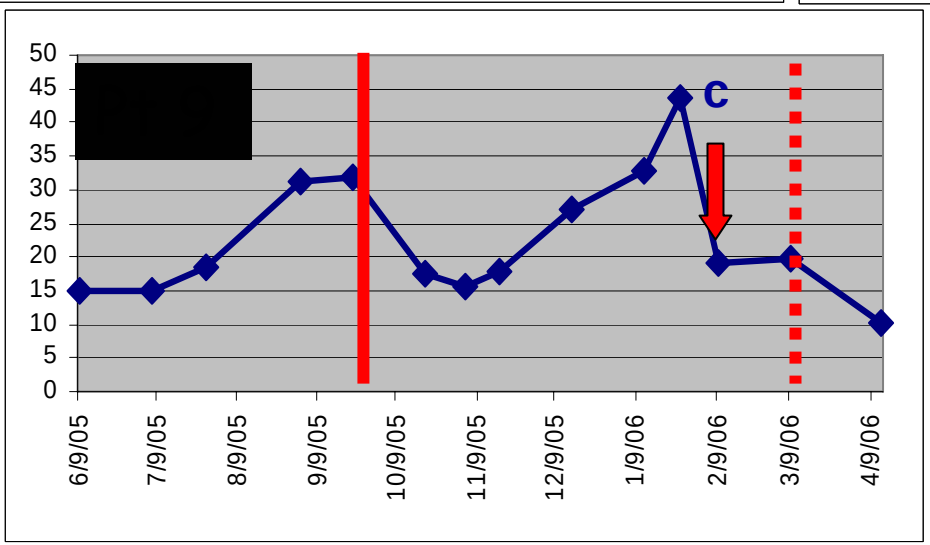


VUmc Cancer Center Amsterdam

GVAX + anti-CTLA-4 in prostate cancer: PSA curves – Dose Level 3 (3 mg/kg)







a : 13Mar06: SAE -Hypophysitis (7 mo)

b: 03Feb06: Hypophysitis (5 mo)

c: 09Feb06: SAE – Hypophysitis (5 mo)

Gerritsen et al. ASCO 2006

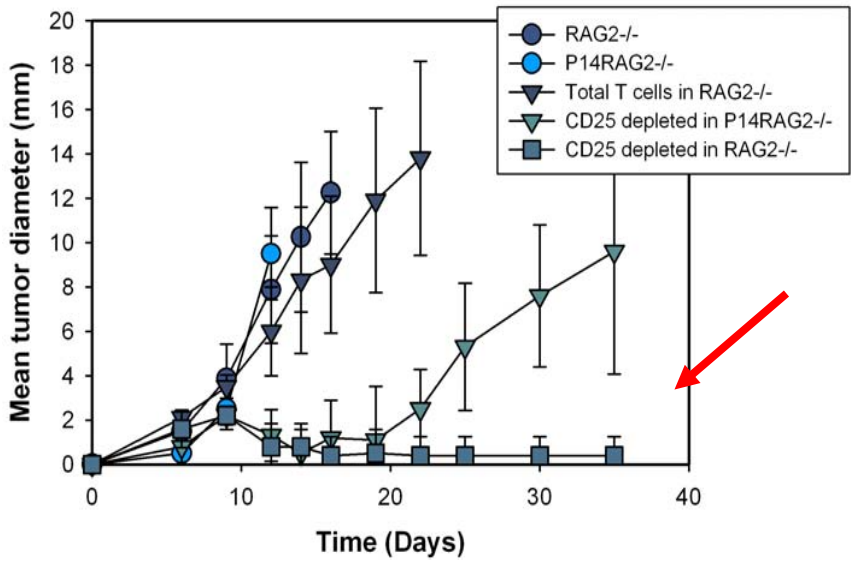
Multiple combinations:

Another layer of complexity and excitement through combined manipulation of regulatory checkpoints

- Anergy reversal + Treg-depletion
- Anti-4-1BB + anti-CTLA-4
- Anti-4-1BB + anti-PD-L1
- Anti-CTLA-4 + Treg depletion

Example 8: Treg depletion + anergy reversal

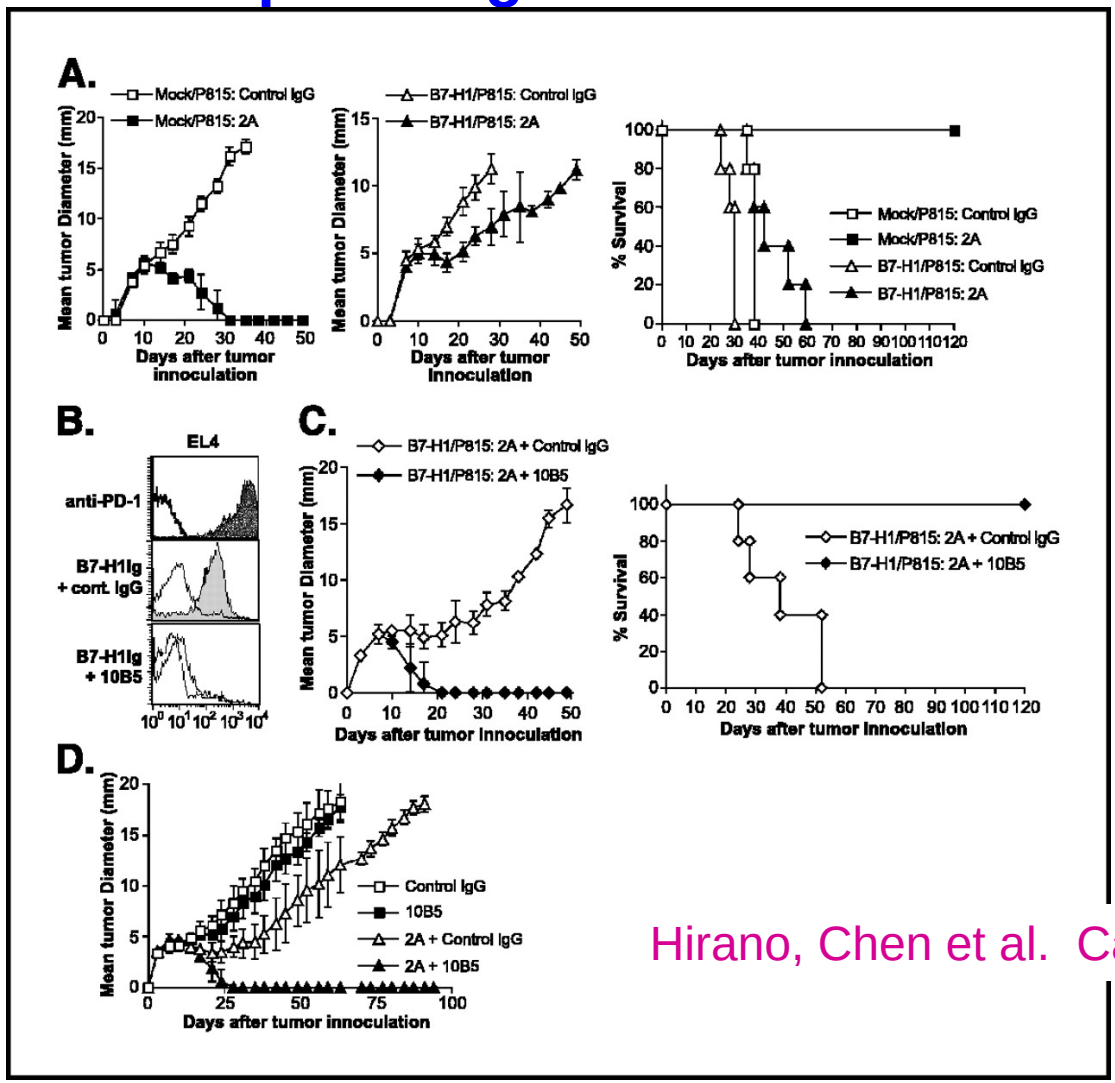
CD25-depleted T cells transferred into lymphopenic hosts gives long-lived rejection of B16 melanoma and vitiligo



Brown, Gajewski et al. J. Immunol. In Press.
Kline, Gajewski et al. Submitted.

Example 9: Anti-4-1BB + anti-PD-L1

Combination induces induces rejection of PD-L1-expressing tumors in vivo



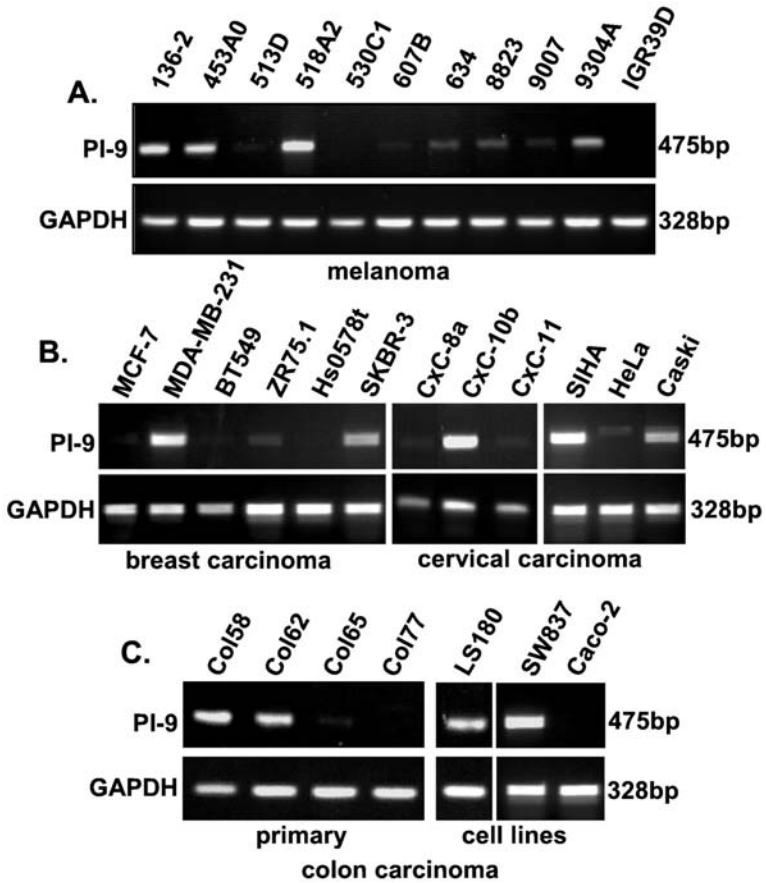
Hirano, Chen et al. Cancer Res. 2005

V. Tumor cell susceptibility : considerations for combinations

- Expression of “signal 1”
  - Antigens
  - Antigen processing machinery
  - MHC, β2M
- Overcoming anti-apoptotic mechanisms
  - Survivin
  - Bcl2-family members
  - Serine protease inhibitors
- Interface with tumor cell-intrinsic oncogenes
  - Ras/MAP kinase pathway & DC activation
  - Stat3 pathway and chemokines
  - Notch pathway and survival, immune gene expression

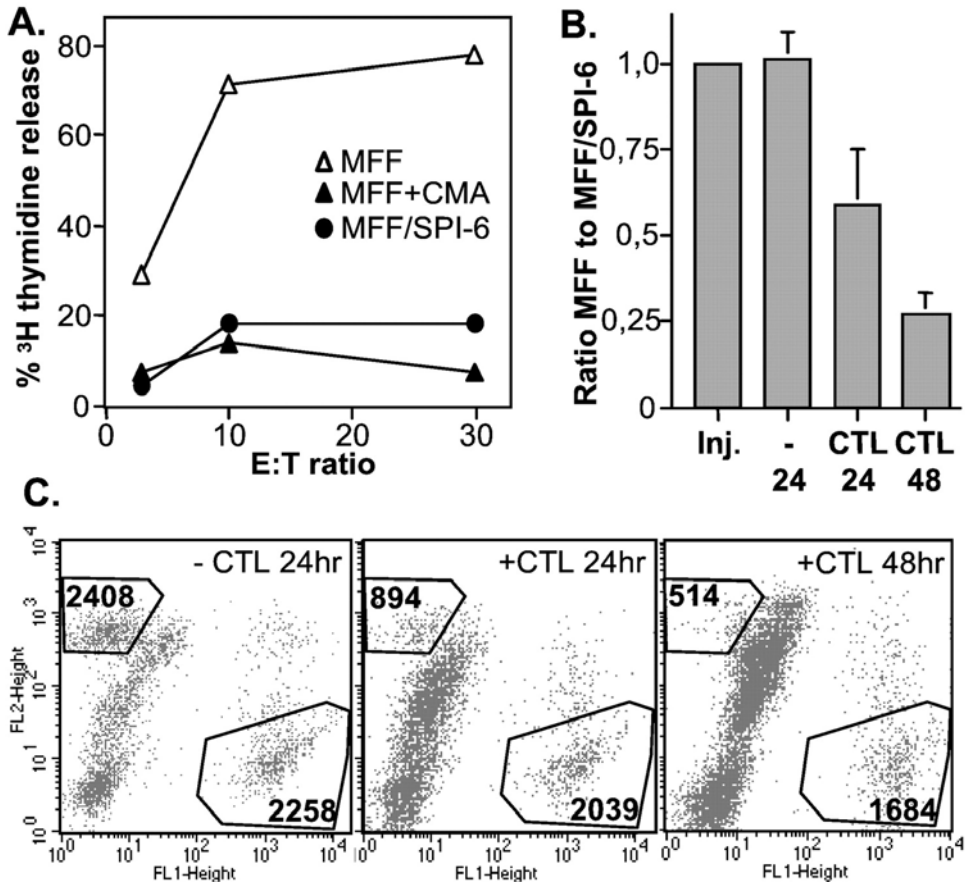
Example 10: PI-9/Spi6

Serine protease inhibitor PI-9 is frequently expressed in human cancers



Medema, J. P. et al. Proc. Natl. Acad. Sci. USA 2001

Introduction of the murine equivalent Spi6 into tumor cells decreases susceptibility to T cell-mediated lysis in vitro



Additional issues

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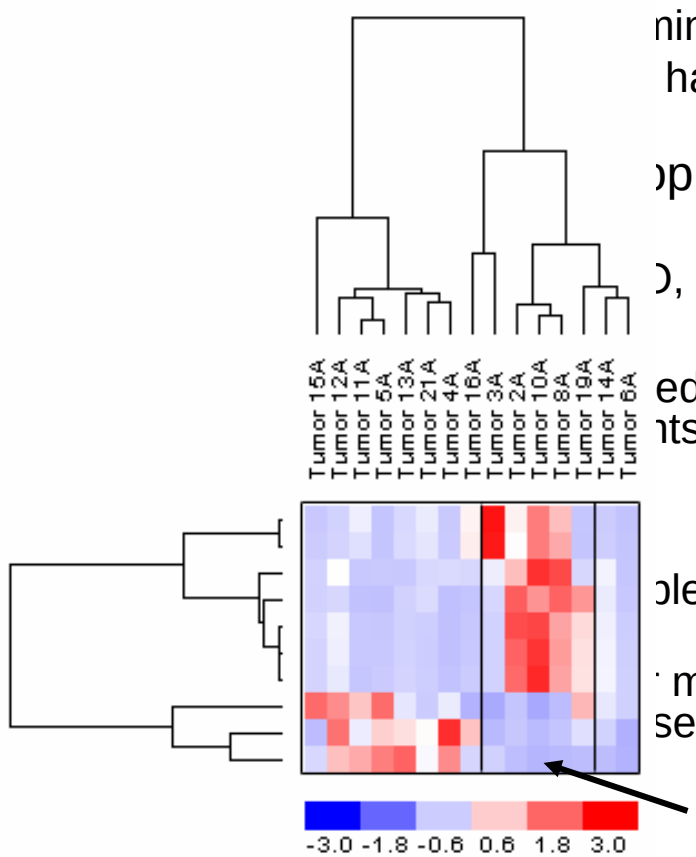

Represents only 7 genes:
- **4 upregulated**
- **3 downregulated**

6 mos SD or better

Additional issues

- Tumor heterogeneity
  - Different cancer types may have distinct dominant immunologic barriers
  - Different patients with the same cancer may have distinct dominant immunologic barriers
- Opportunities for drug discovery and development
  - Cellular targets (e.g. Tregs, MSCs)
  - Molecular targets (FoxP3, LAG-3, GITR, IDO, arginase, PD-1, LIGHT)
- How to prioritize combinations?
  - Too many choices => Ideally, should be based on sound mechanistic analysis of immunologic barriers in populations of patients with given cancer types
  - Preclinical models should show synergy
- Patient selection
  - Can we identify patients who have measurable expandable CTL precursors before enrolling on vaccine trials?
  - Similarly, can we identify patients with tumor microenvironment that can support effector phase of anti-tumor immune response before enrolling on immunotherapy trials?
- The hurdles seem great—why keep exploring this area?
  - Elegant specificity of immune response
  - Memory—what other cancer therapeutic persists like an immune response?

Acknowledgments

- James Allison, Memorial Sloan Kettering
- Drew Pardoll, Johns Hopkins University
- Jon Wiggington, NCI➔Merck

GVAX/anti-CTLA4 trial Contributors

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Helen Gall

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Beppe Giaccone

Winald Gerritsen

Fons van den Eertwegh

Dept Pathology

Saskia Santegoets

Anita Stam

Petra Scholten

Erik Hooijberg

Mary von Blomberg

Rik Scheper

CELL GENESYS

Natalie Sacks

Kristen Hege

Shirley Clift

Karin Jooss

David Rhodes

Sayeh Morali

MEDAREX

Israel Lowy

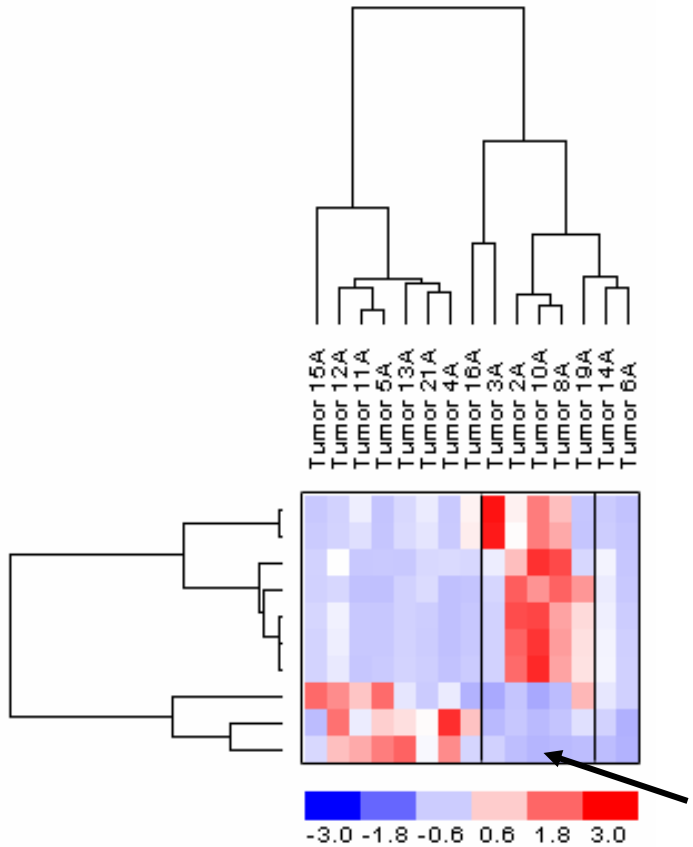
Steven Fischkoff

Elizabeth Levy

Prostate Cancer Foundation

KWF KANKER BESTRIJDING

Affymetrix gene array analysis of pre-treatment biopsies from patients on melanoma vaccine sorted by clinical outcome



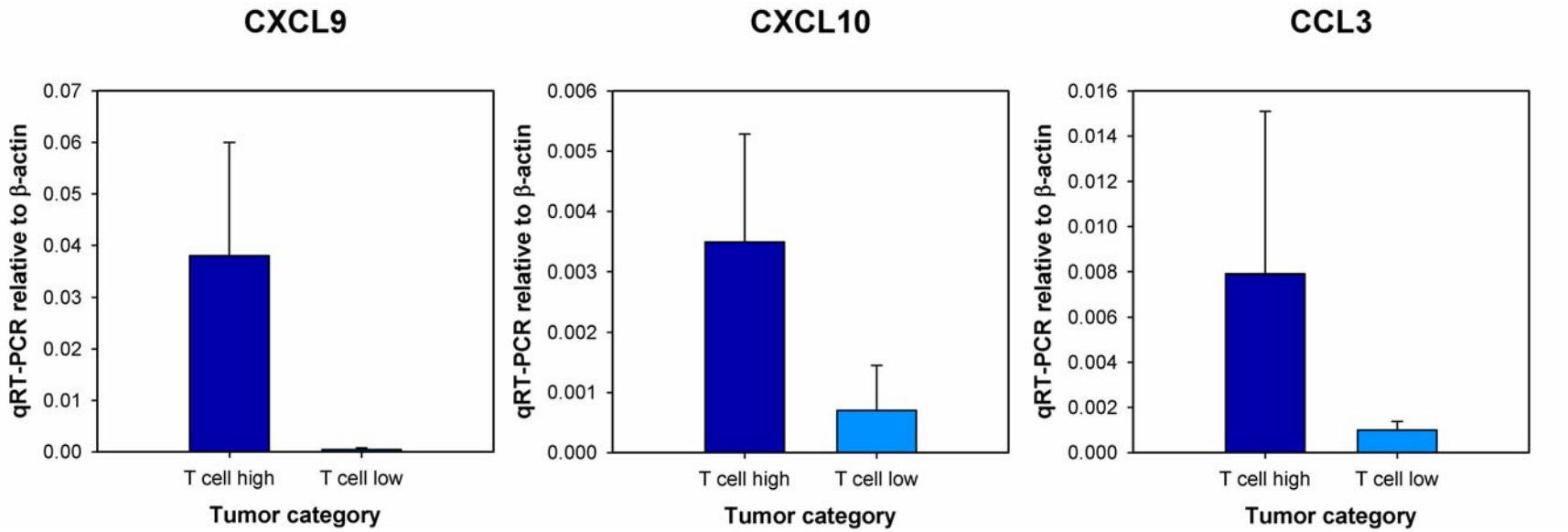
Represents only 7 genes:

- **4 upregulated**
- **3 downregulated**

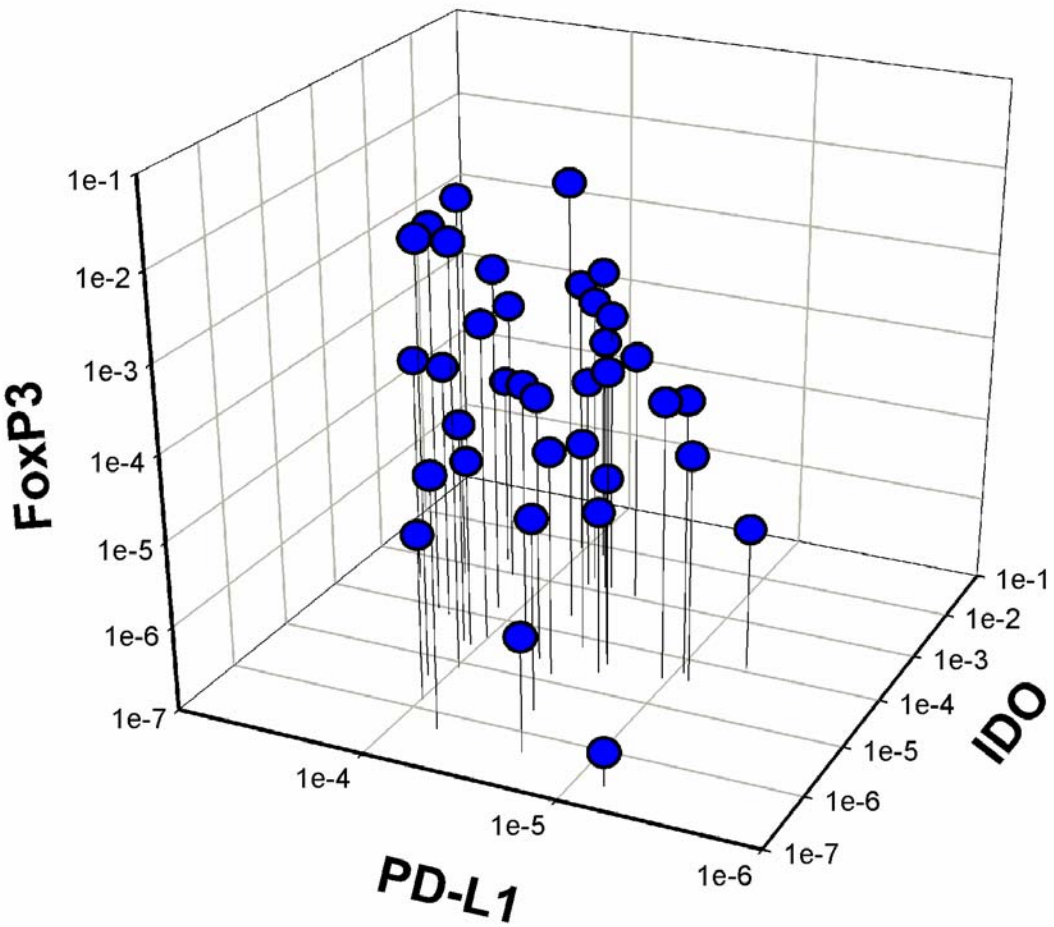
6 mos SD or better

Has implications for patient selection on vaccine trials, and understanding biology

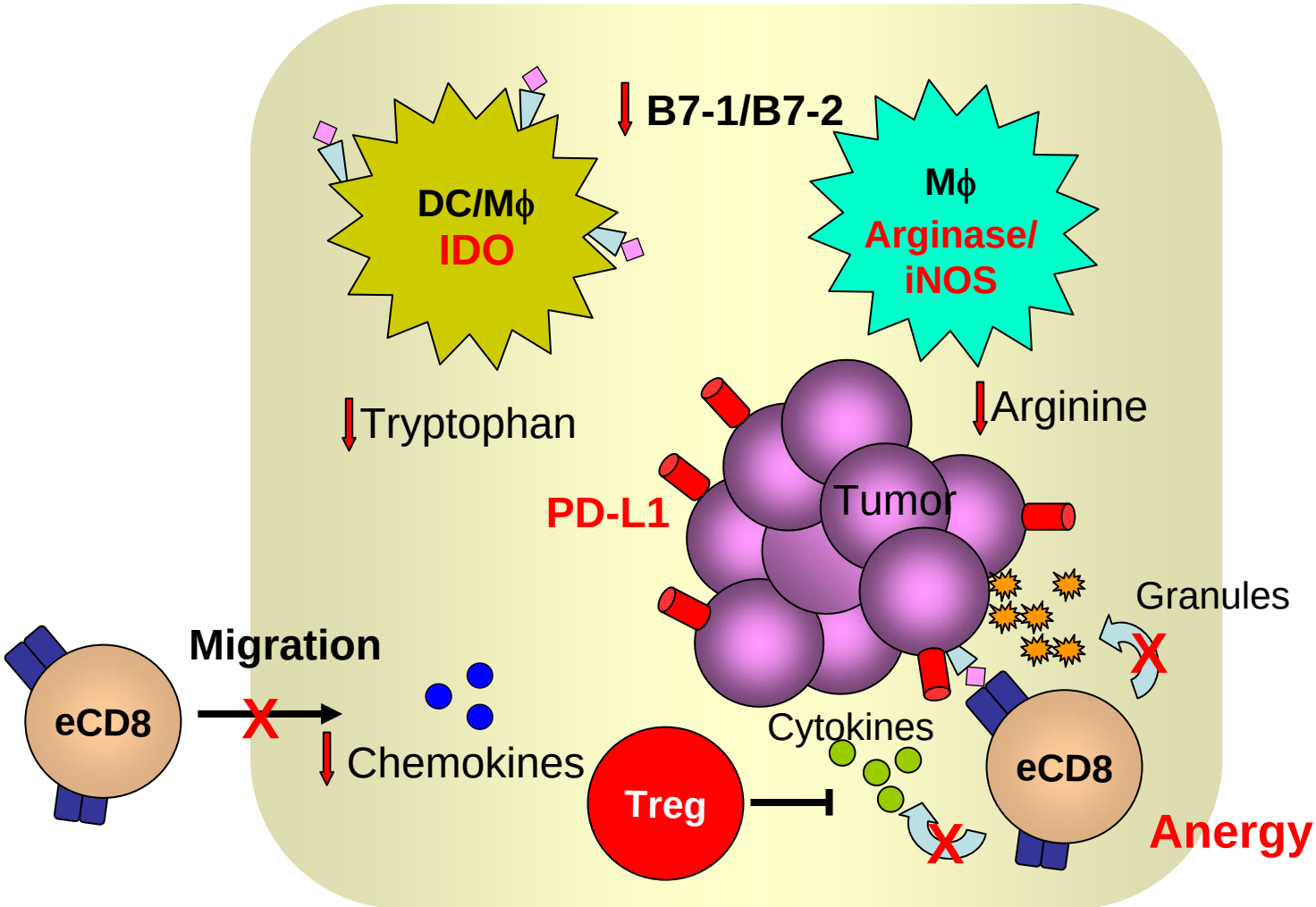
Differential chemokine expression in melanoma metastases with high versus low T cell transcripts



Co-expression of IDO, PD-L1, and FoxP3 transcripts in individual tumors

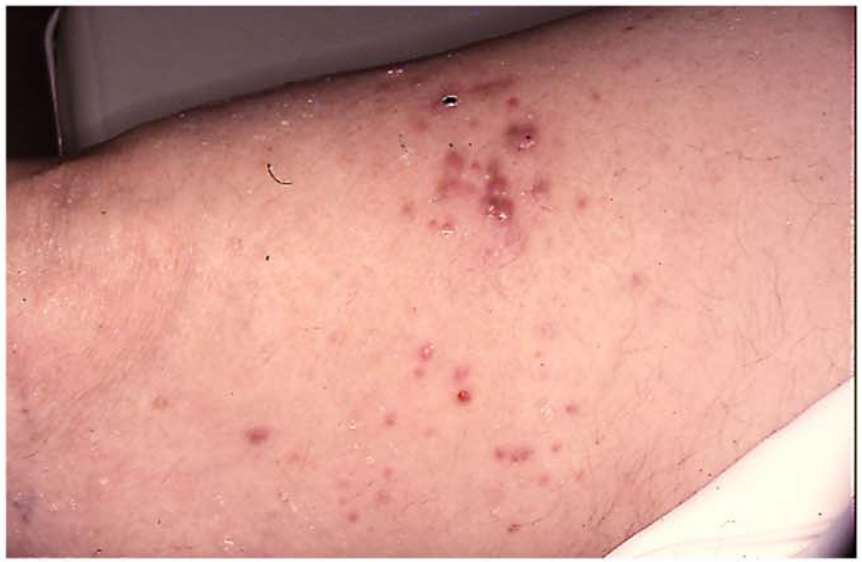


Summary of tumor microenvironment barriers: Need to promote T cell trafficking and overcome local immunosuppression

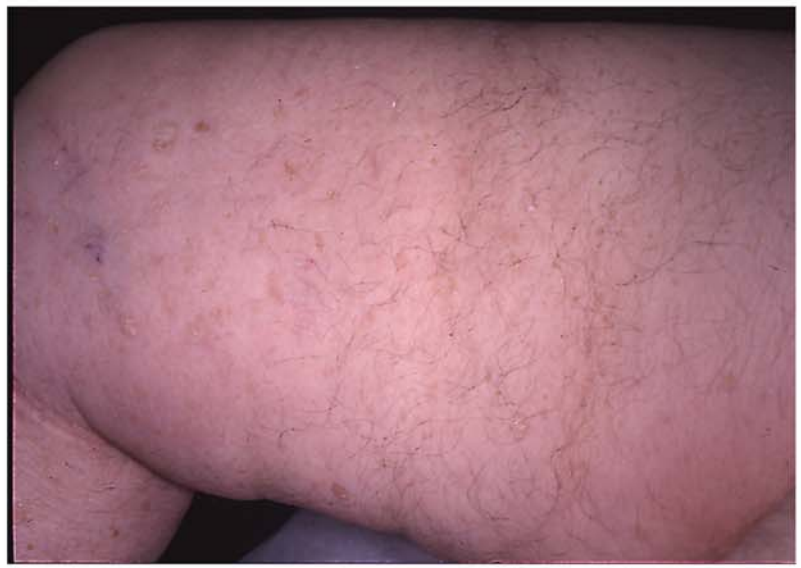


Resolution of cutaneous metastases following immunization with melanoma peptide-pulsed PBMC + rhIL-12

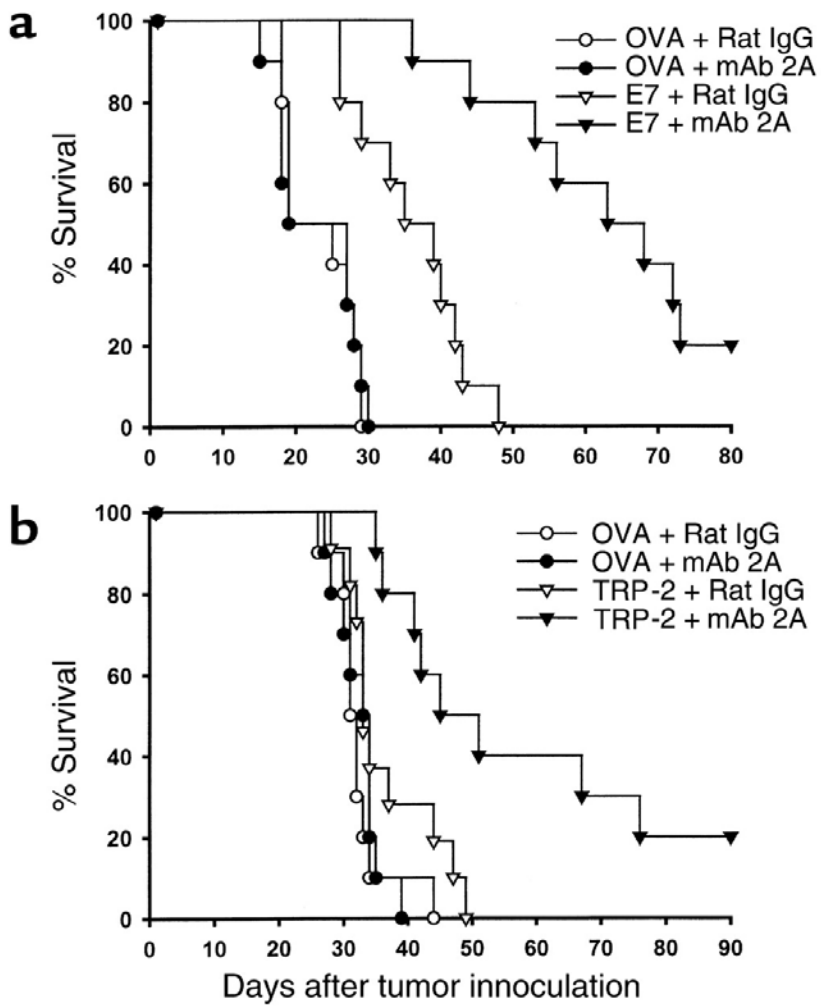
After 3 vaccines



After 9 vaccines

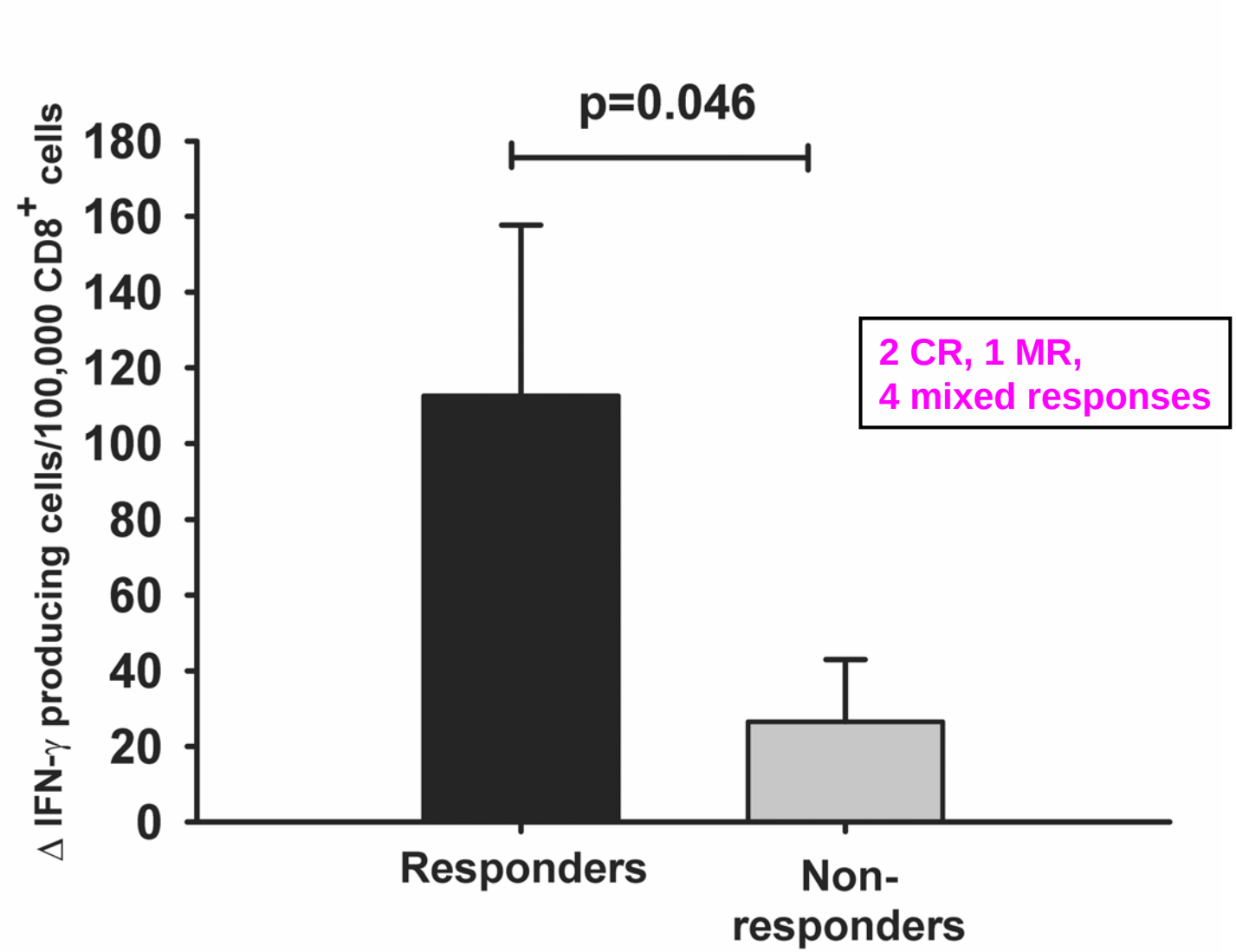


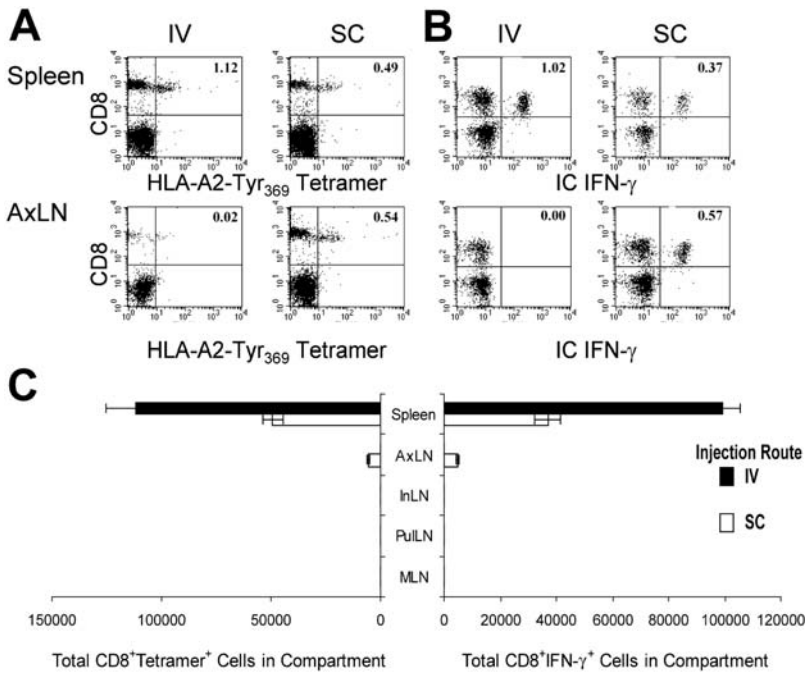
Peterson, Gajewski et al. JCO 2003.



Wilcox, R. A. et al. J. Clin. Invest. 2002;109:651-659

Greater increase in Melan-A-specific CD8$^+$ T cells in clinical responders




A
IV
SC
Spleen
AxLN
CD8
1.12
0.49
0.02
0.54
HLA-A2-Tyr369 Tetramer
B
IV
SC
1.02
0.37
0.00
0.57
IC IFN-γ
C
Spleen
AxLN
InLN
PuLN
MLN
Injection Route
IV
SC
150000
100000
50000
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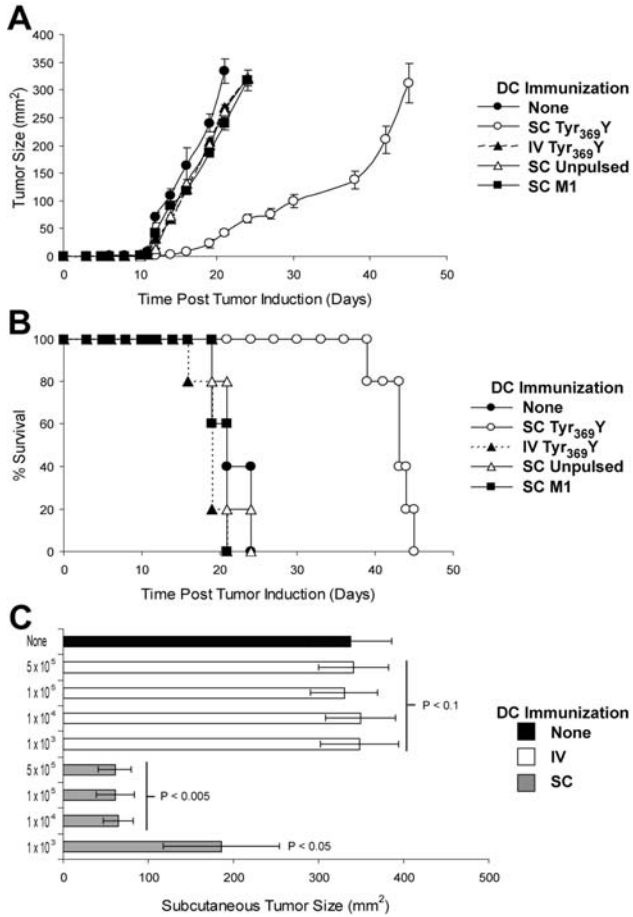
0
20000
40000
60000
80000
100000
120000
Total CD8+Tetramer+ Cells in Compartment
Total CD8+IFN-γ+ Cells in Compartment


A
Tumor Size (mm2)
Time Post Tumor Induction (Days)
DC Immunization
None
SC Tyr369Y
IV Tyr369Y
SC Unpulsed
SC M1
B
% Survival
Time Post Tumor Induction (Days)
DC Immunization
None
SC Tyr369Y
IV Tyr369Y
SC Unpulsed
SC M1
C
None
5 x 10-5
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P < 0.05
DC Immunization
None
IV
SC
Subcutaneous Tumor Size (mm2)