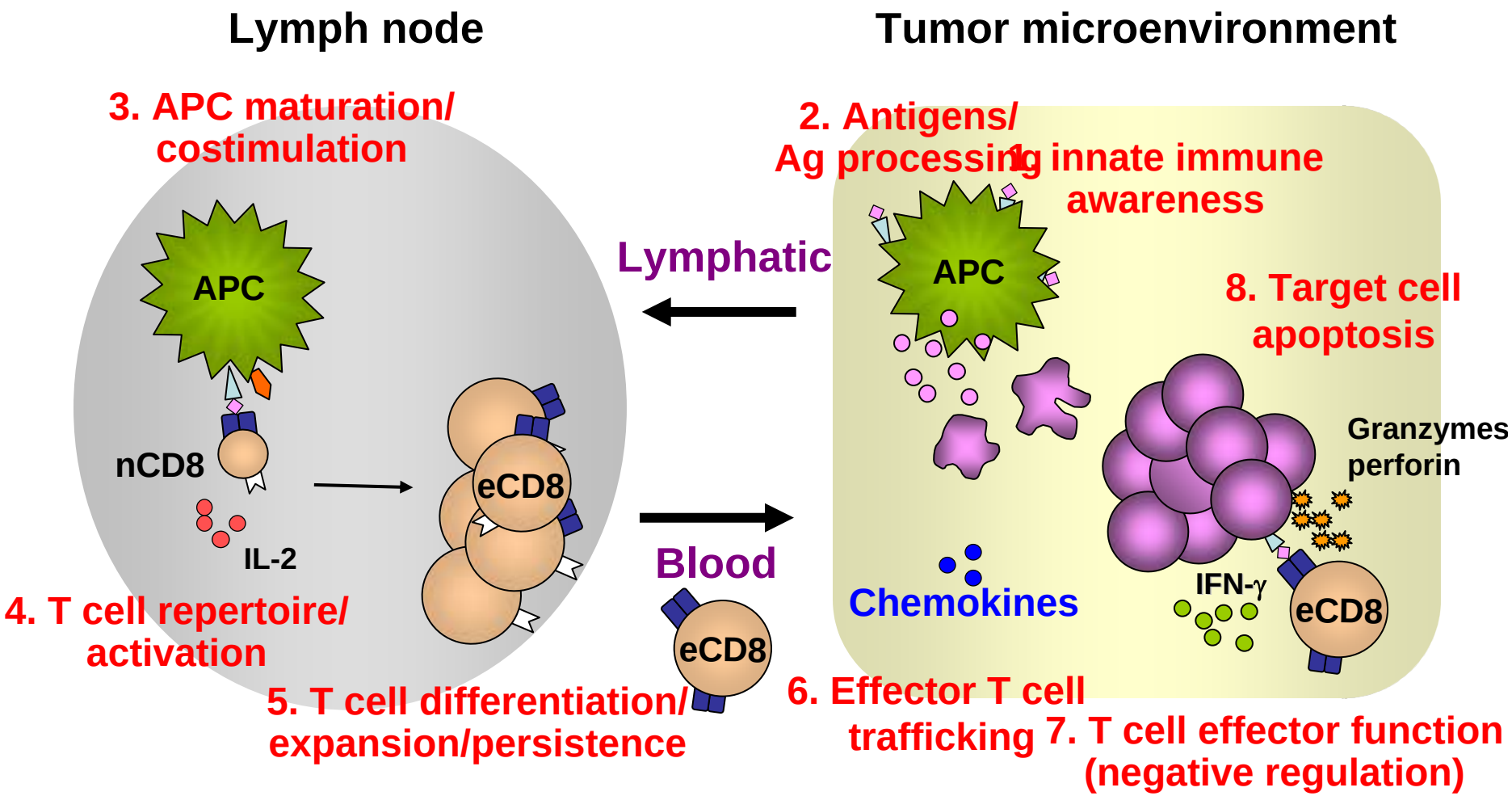
Immunotherapy/Immunotherapy combinations

Thomas F. Gajewski
Jon Wiggington
Lieping Chen
Glenn Dranoff

Rationale and foundation for discussion

- Important disclaimer: we are not suggesting that “vaccines don’t work” and therefore combinations of vaccines plus other therapies will automatically be required
- 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 T cell-based immunotherapy, the “drug” is not necessarily the product administered (e.g. vaccine)—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**

At what levels can a spontaneous anti-tumor T cell response fail?



Hypothetical barriers point towards strategies for intervention

Vaccines →

1. Innate immune awareness/Ag presentation/APC maturation
   - Are there “danger” signals to ensure productive antigen display?
2. T cell repertoire/initial activation
   - Repertoire may be restricted or of low avidity
   - Immune suppression may carry over to DLN compartment

Adoptive Tx →

3. T cell differentiation/expansion/persistence
   - Proper T cell phenotype might not be induced (Th1/CTL/memory)
   - Magnitude or duration of T cell response may be inadequate
4. T cell trafficking into tumor sites
   - Lack of proper chemokine receptors on T cells, or chemokines at tumor site
   - Signals for penetrating extracellular matrix?
5. Executing effector function in tumor microenvironment
   - Dominant negative regulatory pathways
   - Poor maintenance of effector function (e.g. regeneration of cytotoxic granules)
6. Tumor cell susceptibility to recognition and killing
   - Loss of antigens, processing machinery, MHC
   - Anti-apoptotic mechanisms: tumor cells can be resistant
   - Interface with tumor cell-intrinsic biology: oncogenic pathways orchestrating resistance

Candidate approaches to overcome these barriers

1. Innate immune awareness/Ag presentation/APC maturation
   - Innate immune cells and cytokines, TLR agonists, CD40 ligands, vaccination—novel Ag sources
2. T cell repertoire/initial activation
   - B7 and other costimulatory ligands
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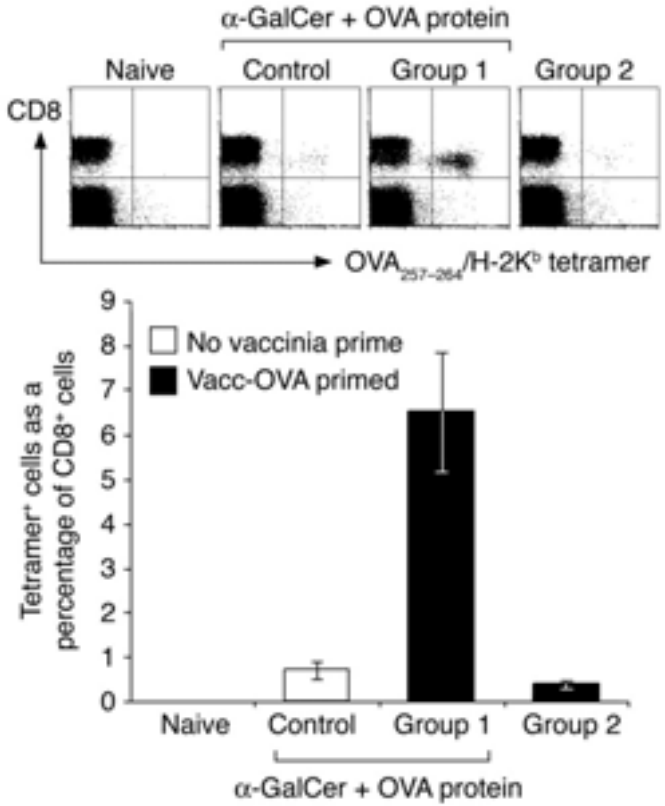
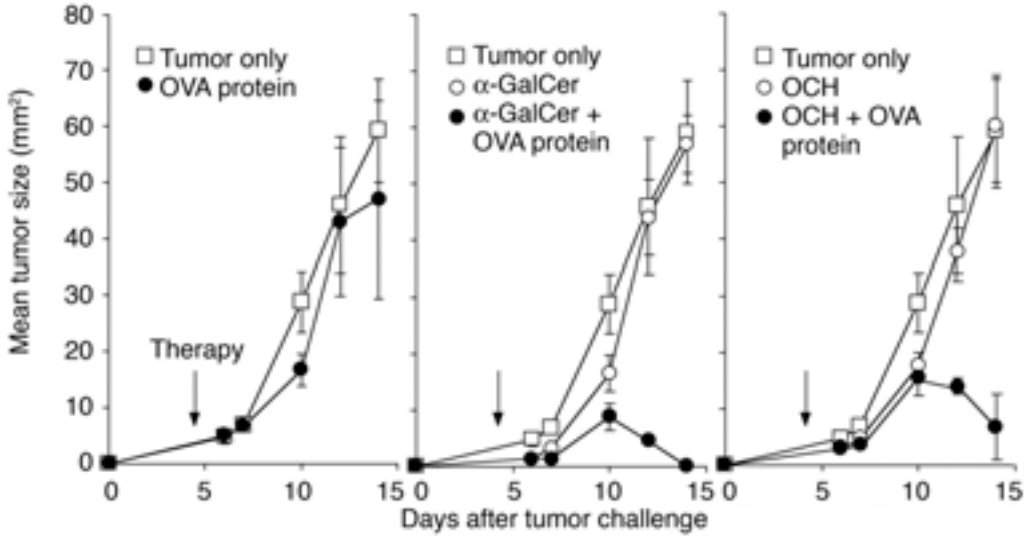
Example 1: α-GalCer

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





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

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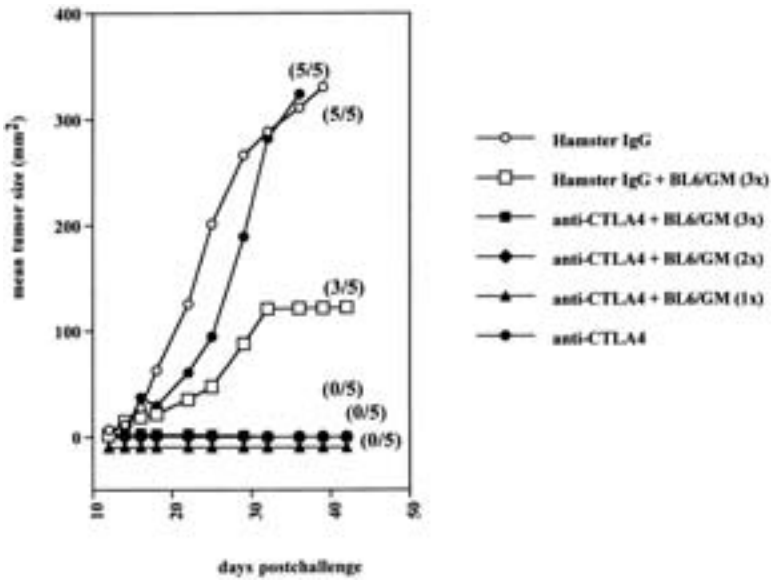
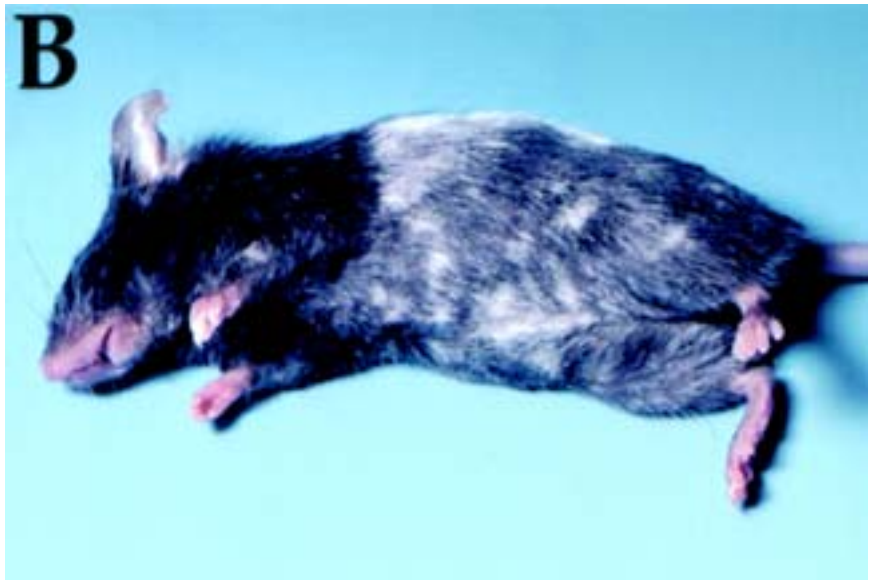
Example 2: CTLA-4

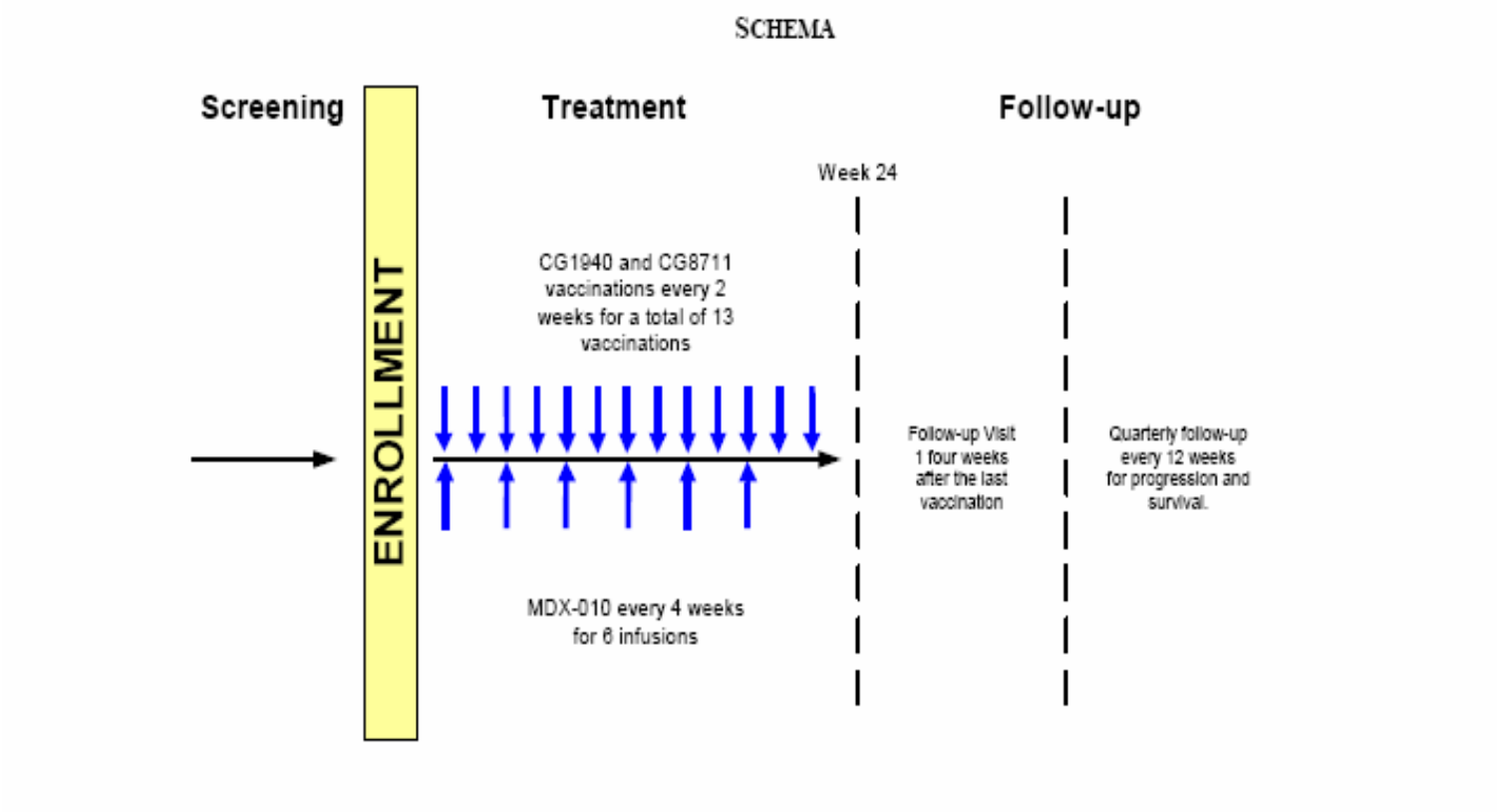
Anti-CTLA-4 mAb + GM-CSF-transduced B16 vaccine induces tumor rejection and leads to vitiligo





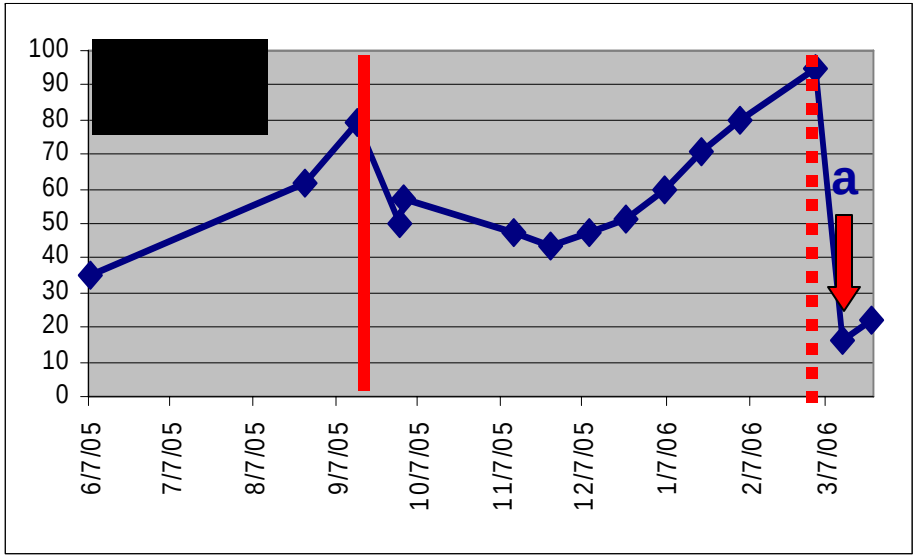
van Elsas, Allison et al. JEM 1999

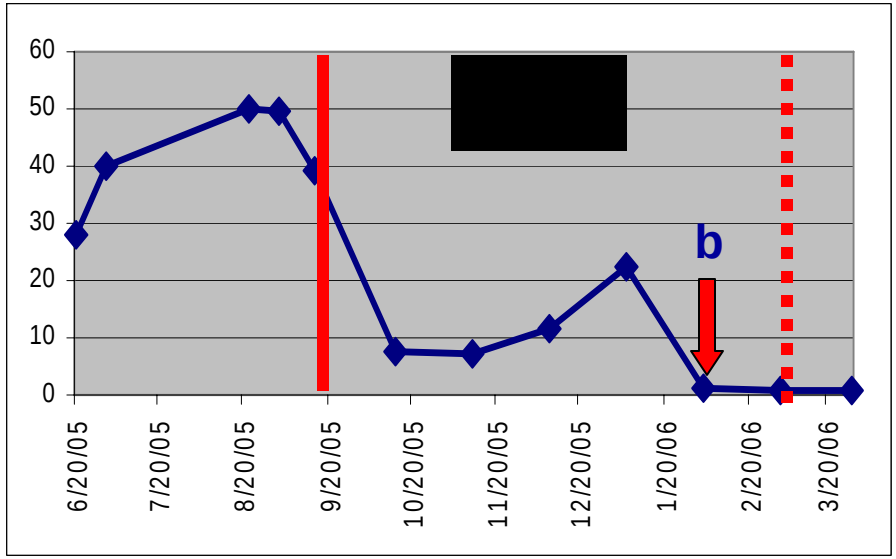
GVAX Immunotherapy (CG1940/CG8711) + Ipilimumab (MDX-010: anti-CTLA-4) for HRPC

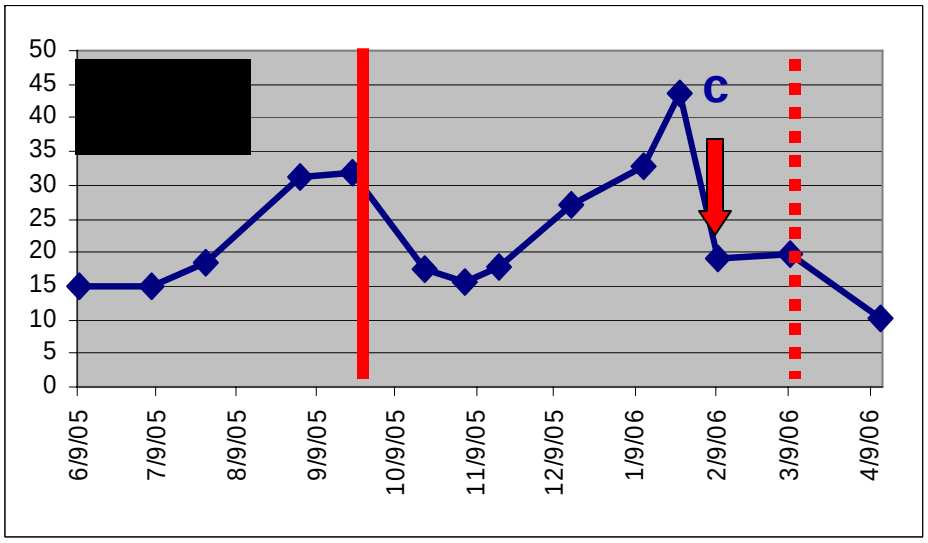


VUmc Cancer Center Amsterdam

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

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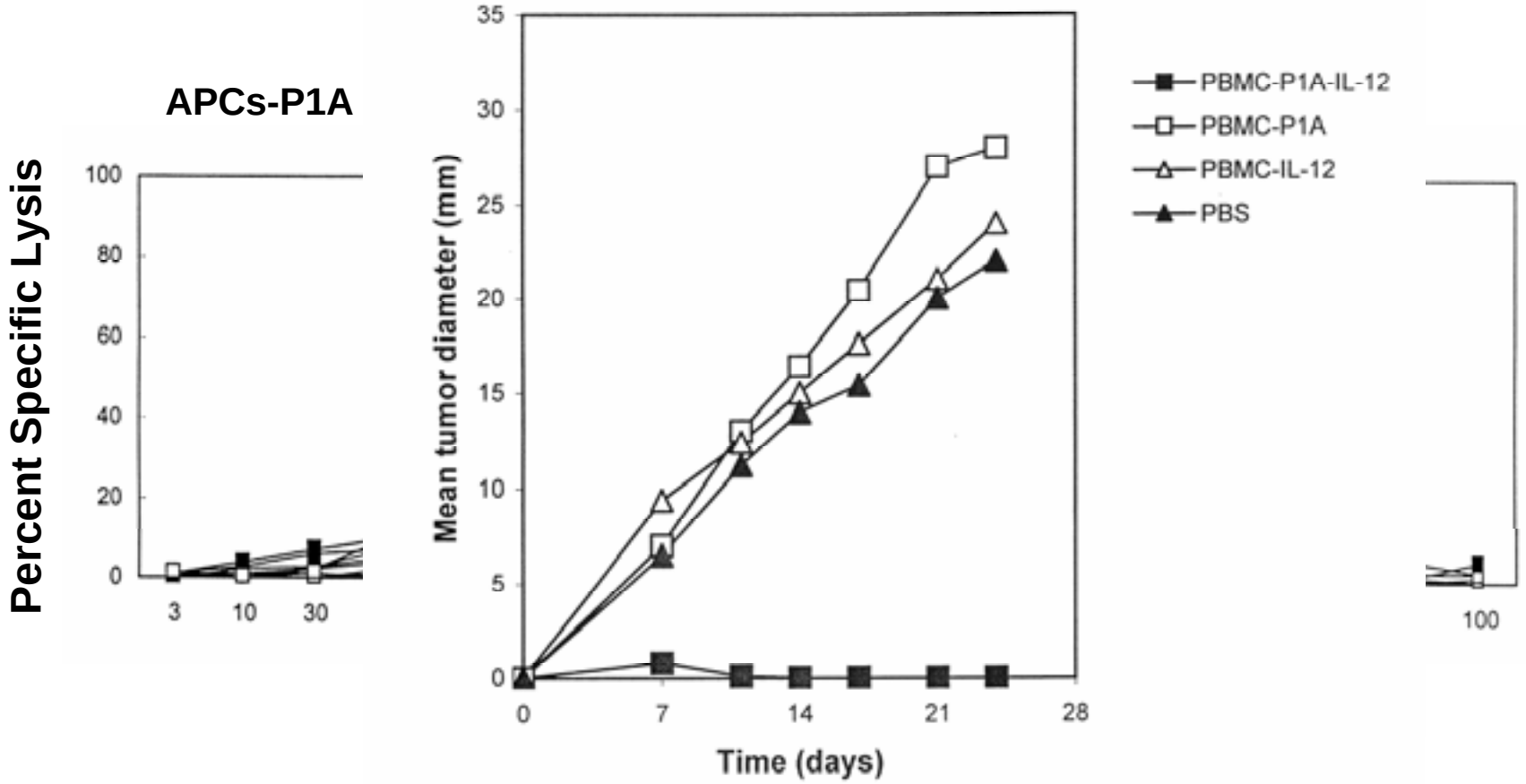
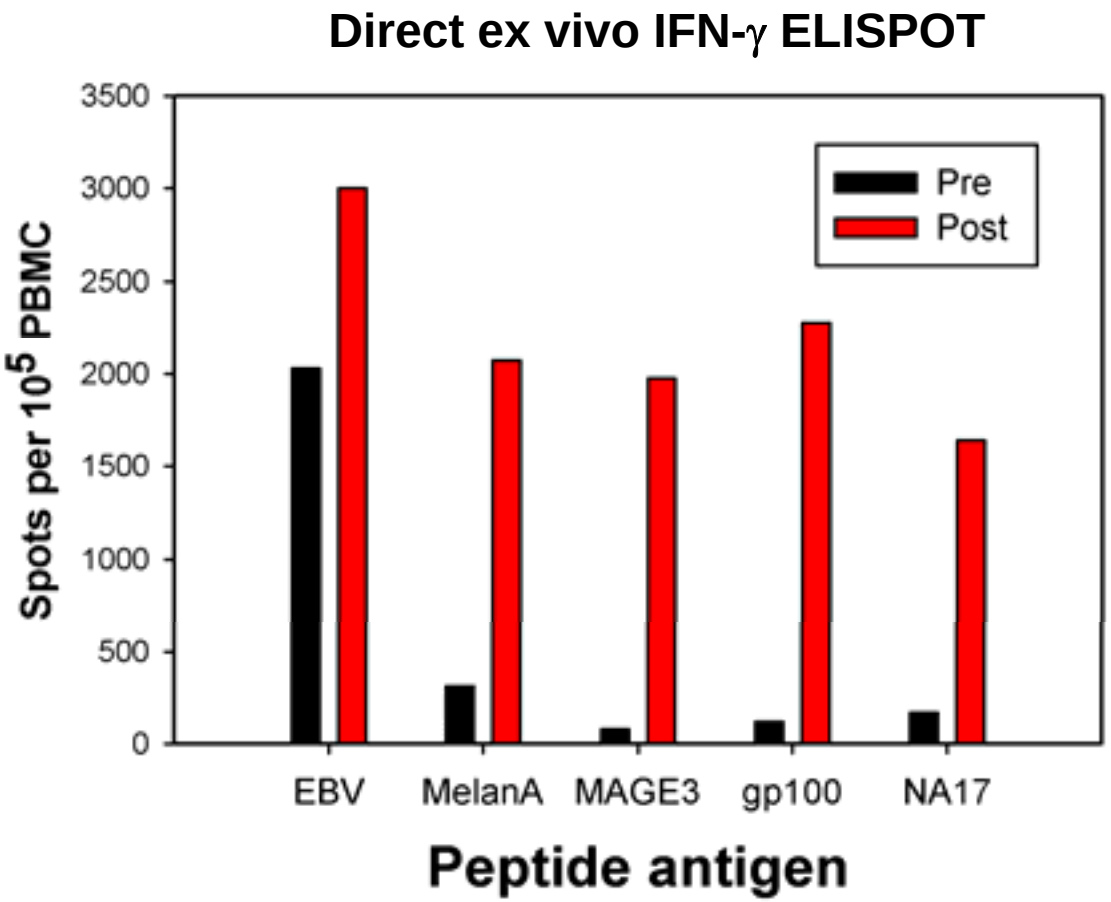
Example 3A: IL-12

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



Fallarino et al. Int. J. Cancer 1999

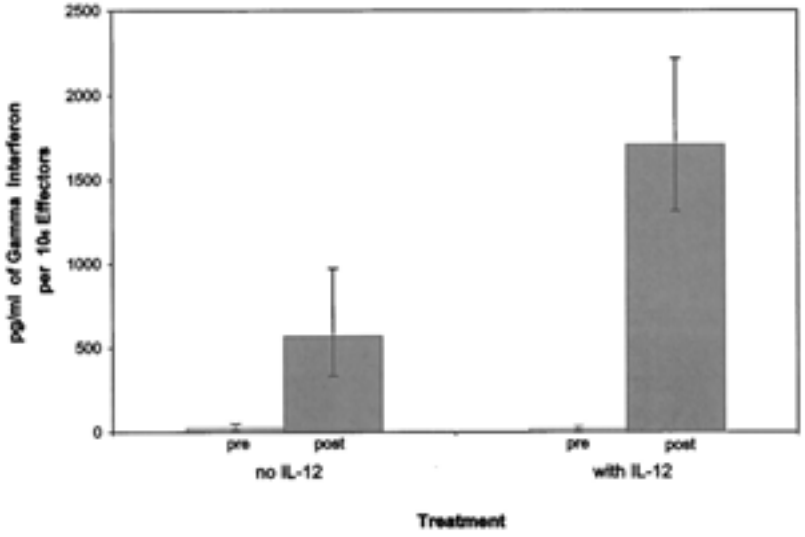
Potent T cell response against multiple antigens post-immunization of melanoma patients with peptide-pulsed PBMC + IL-12



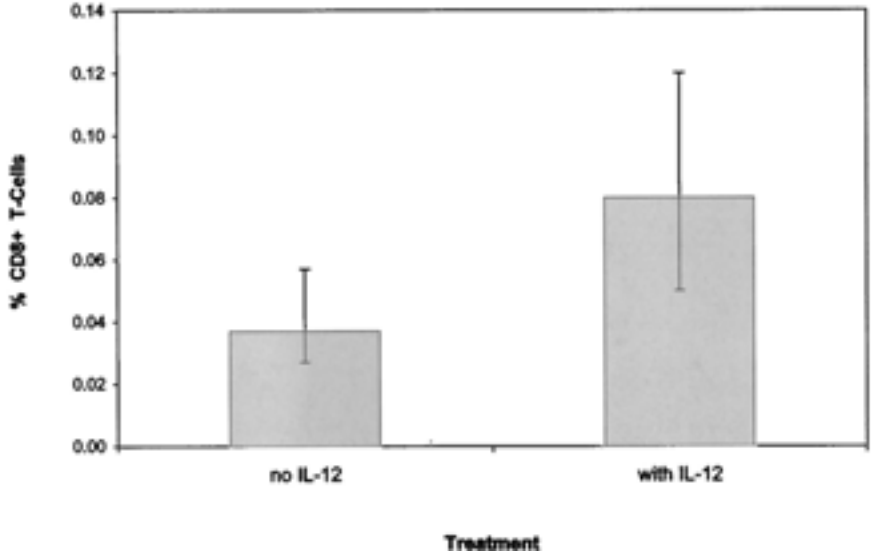
3 patients with CR post-vaccination

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

IFN-γ production



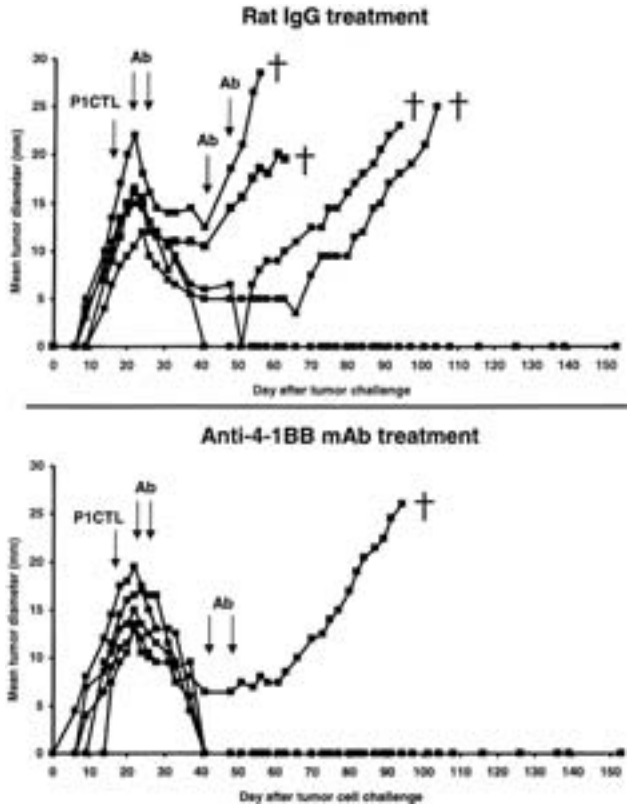
Tetramer staining

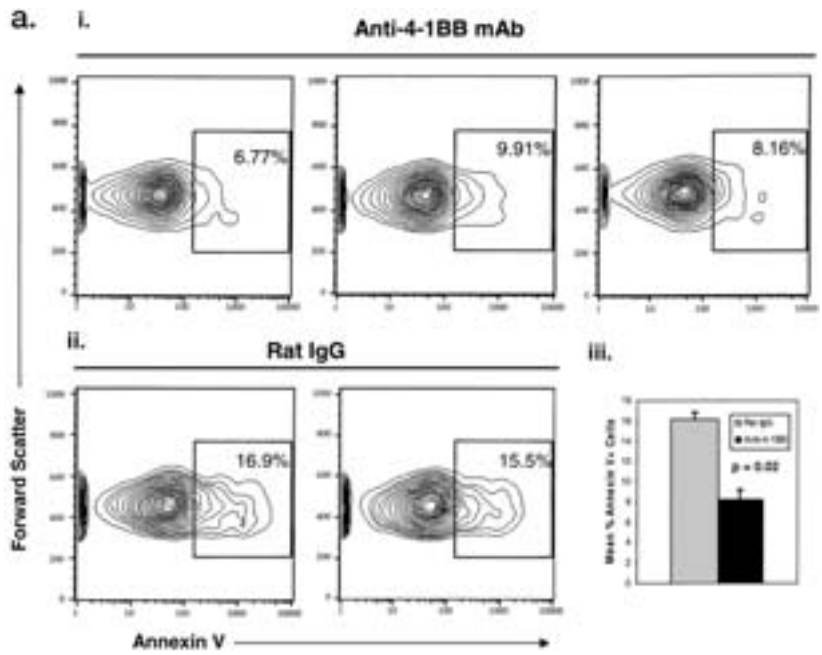


Lee, Weber et al. JCO 2001

Example 3B: 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

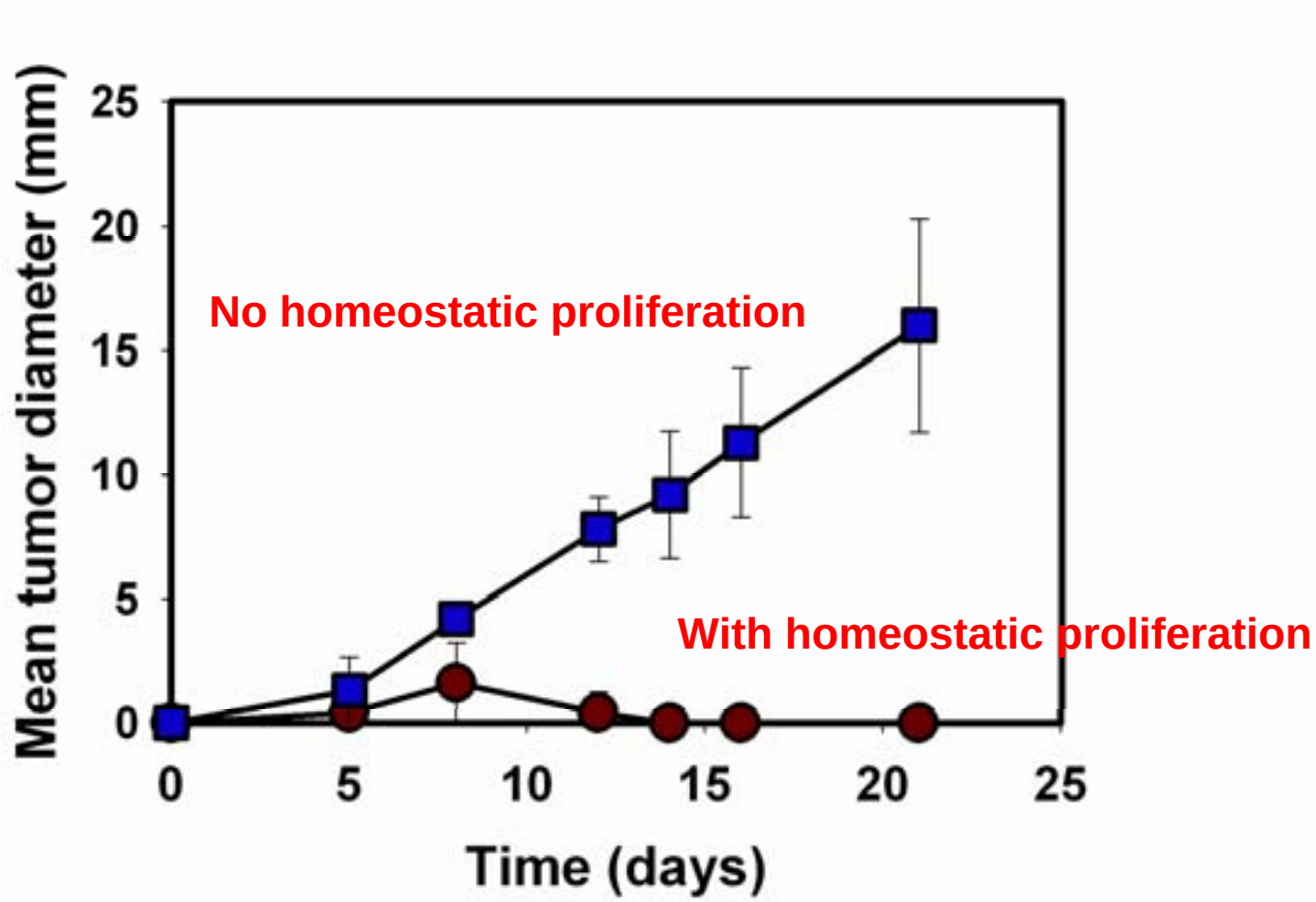




May, Liu et al. Cancer Res. 2002.

Example 3C: Homeostatic proliferation

Anergic 2C T cells reject tumors after homeostatic proliferation in RAG2$^{-/-}$ hosts



Brown et al., J. Immunol., 2006

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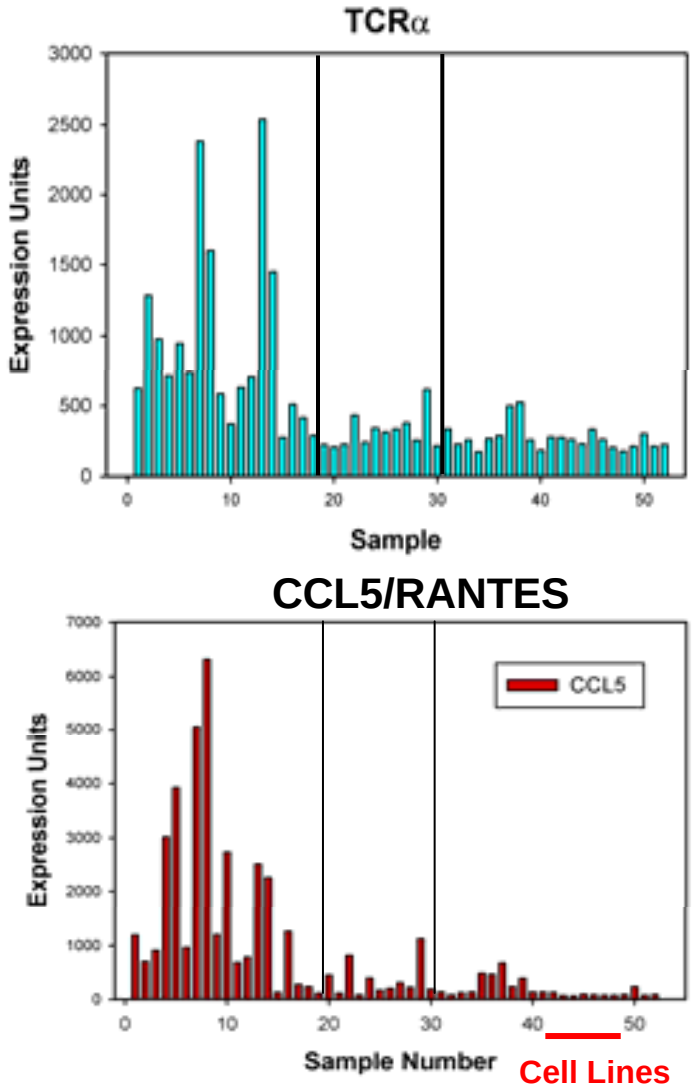
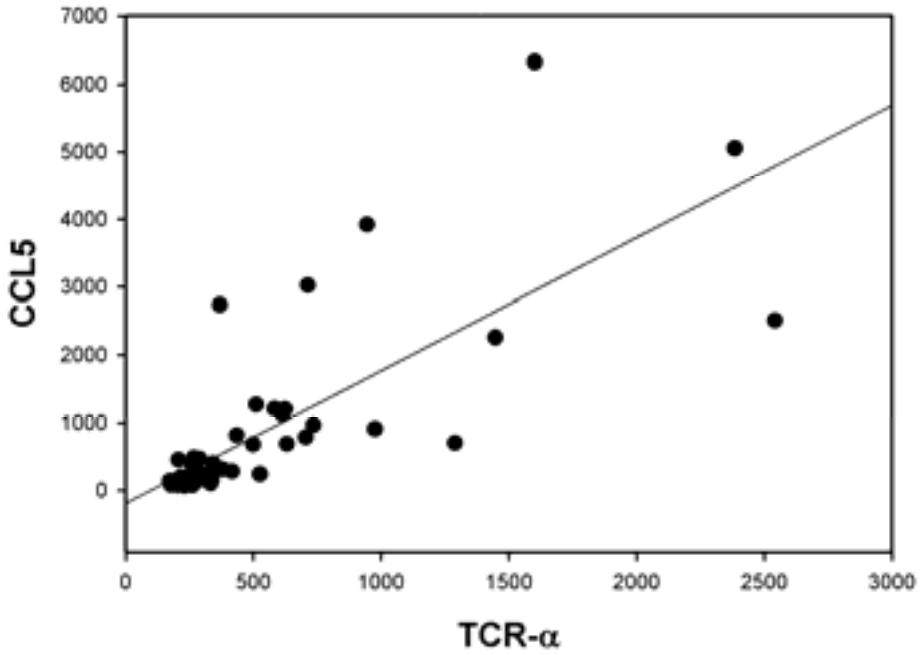
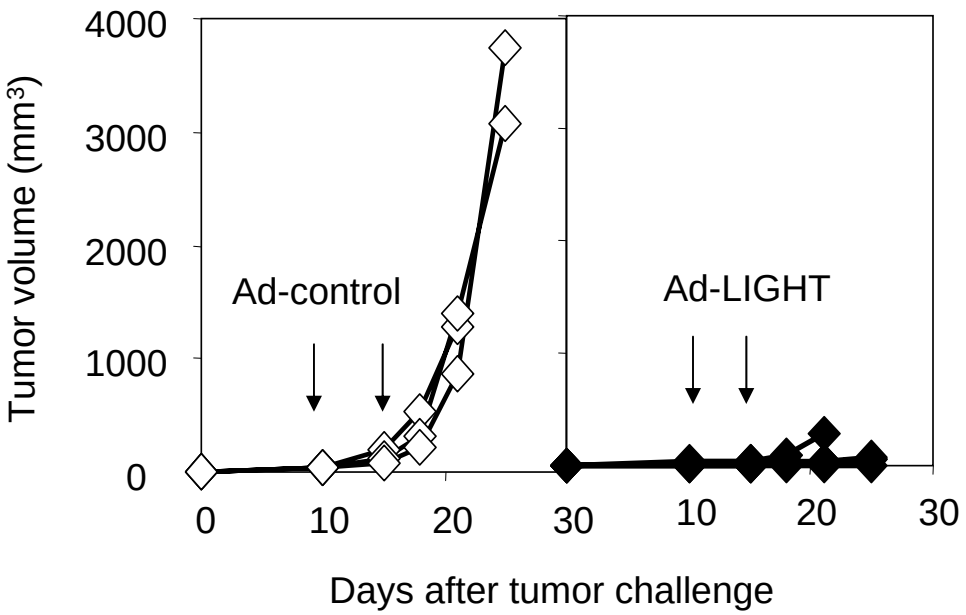
T cell transcripts in melanoma metastases are associated with expression of specific chemokine genes



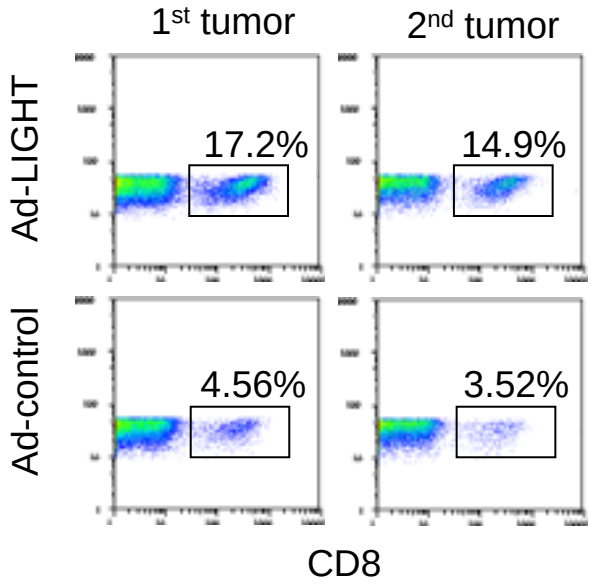


Example 4: Intratumoral LIGHT adenovirus in B16 melanoma promotes greater recruitment of CD8$^+$ T cells in primary tumor and leads to rejection of non-injected distant metastases

Tumor rejection



CD8$^+$ T cell infiltrate



Yu et al, J. Immunol. 2007

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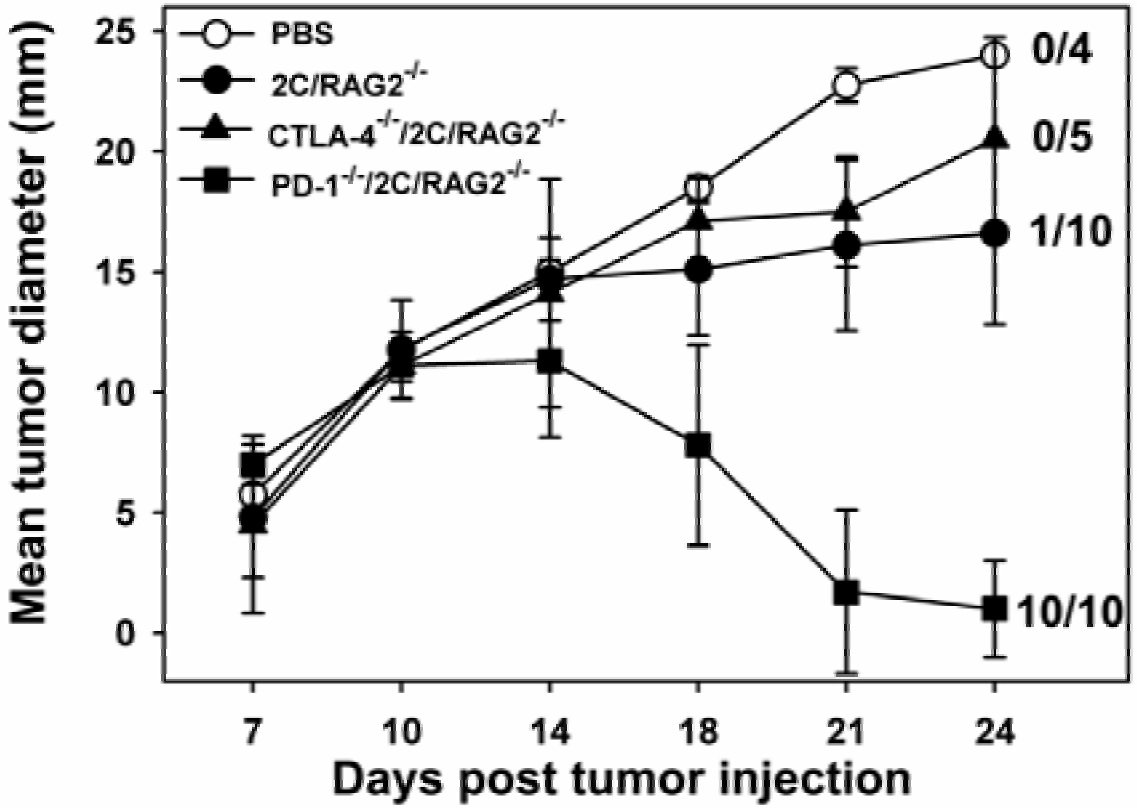
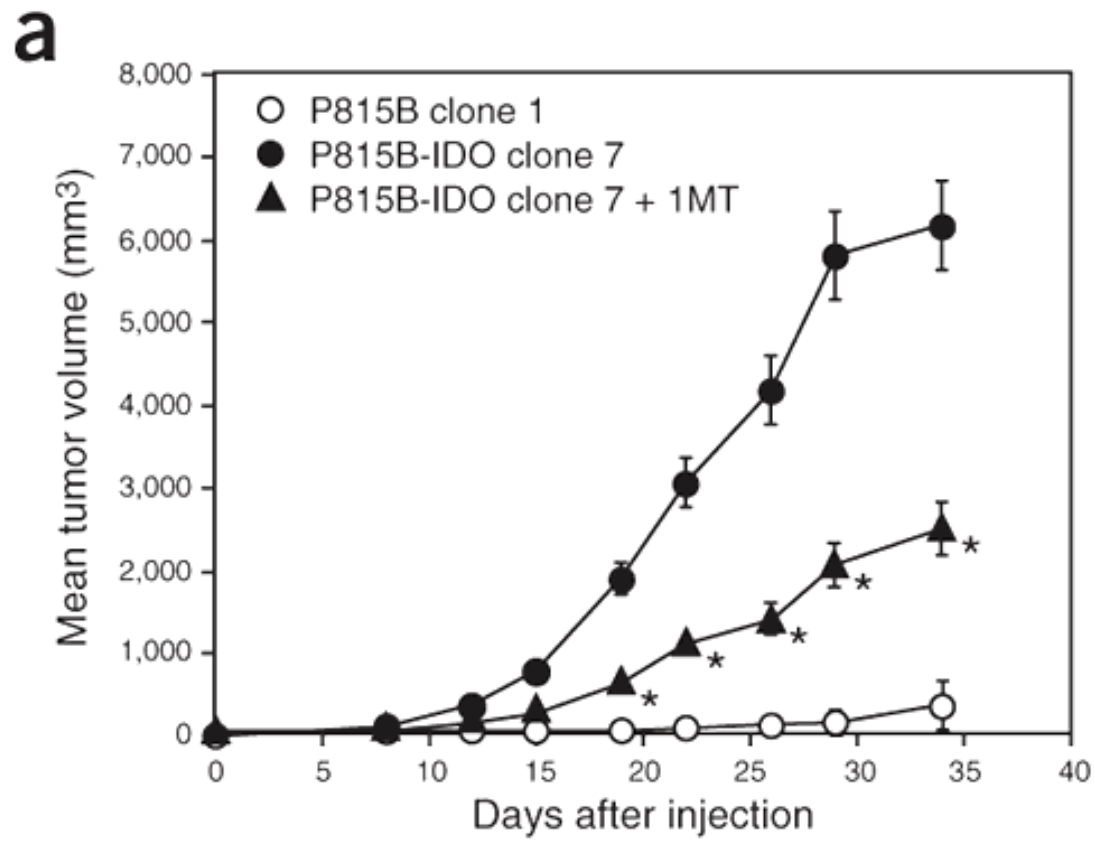
Example 5A: PD-1$^{-/-}$ 2C TCR Tg T cells are superior at tumor rejection in vivo



Blank et al, Cancer Research, 2004

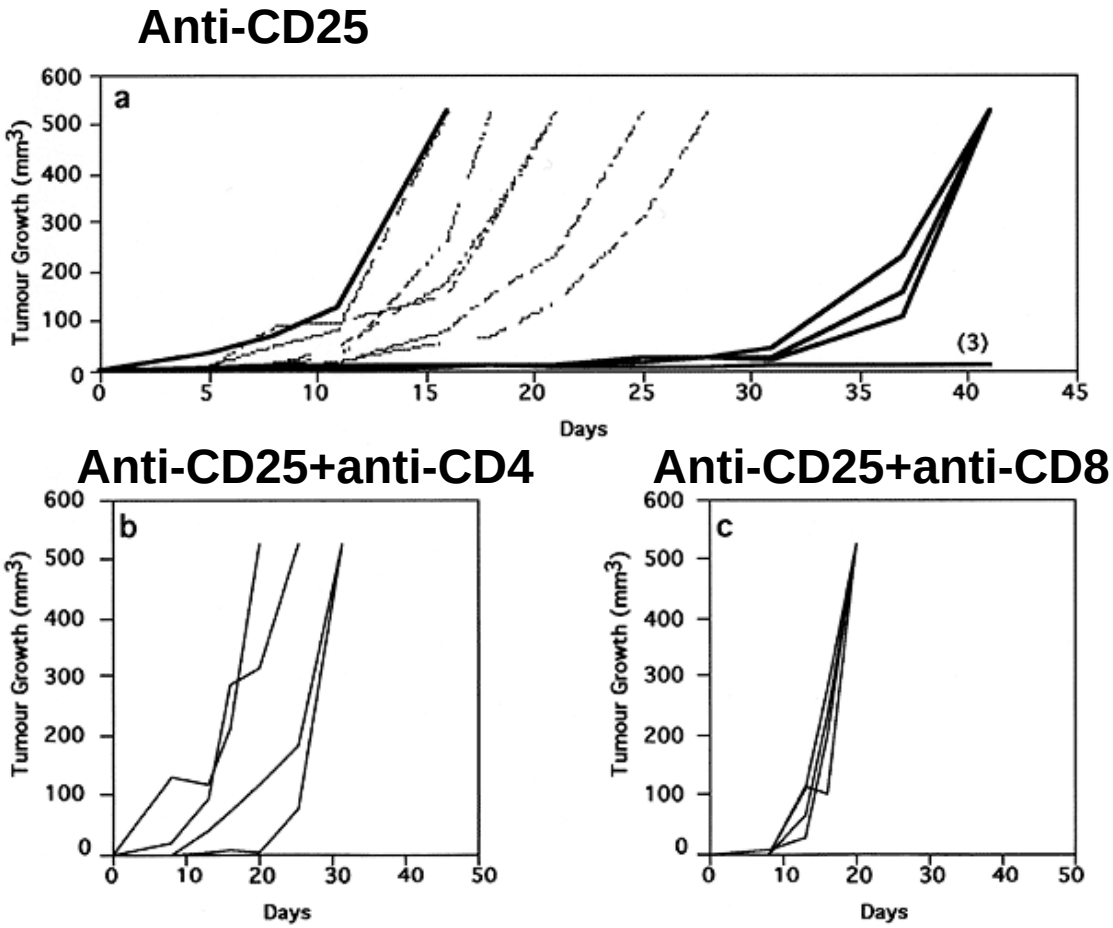
Example 5B: 1-methyltryptophan reverses immunosuppression by IDO and improves tumor control in vivo



Uyttenhove et al Nature Med. 9:1269, 2003

Example 5C: CD25^{+} Tregs

CD25 depletion can partially control B16 melanoma growth in vivo



Jones, Gallimore et al. Cancer Immunity 2002

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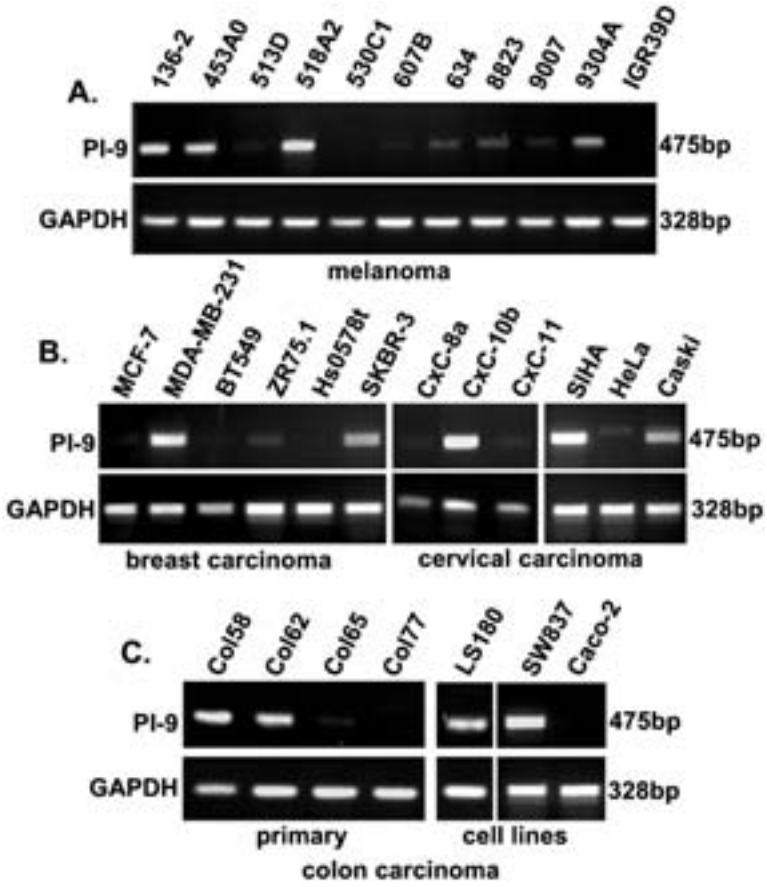
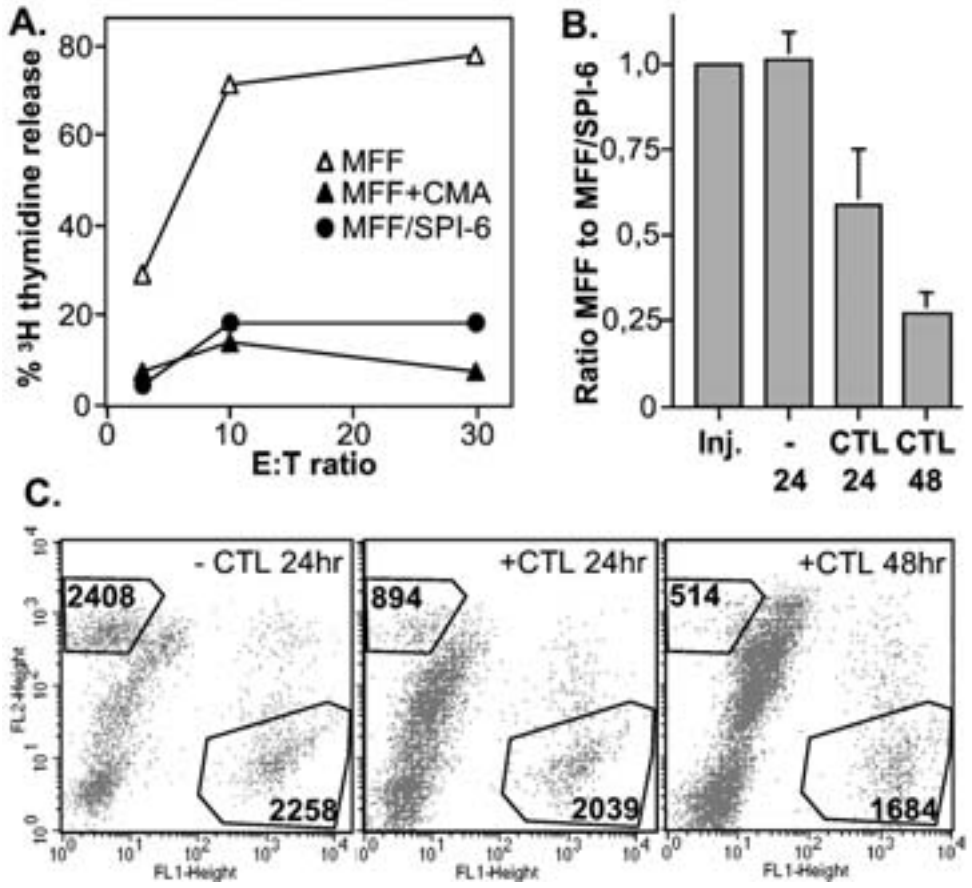
Example 6: PI-9/Spi6

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

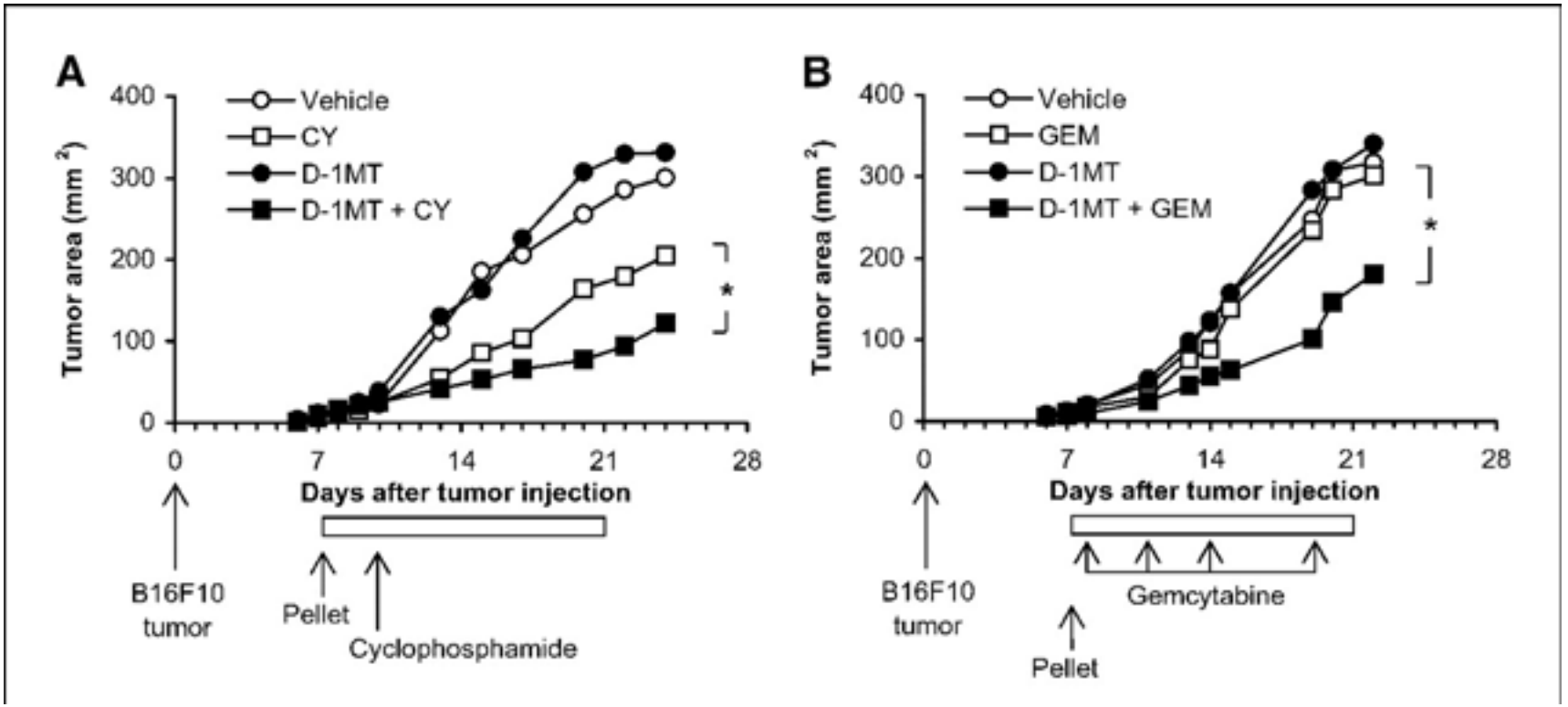


Multiple combinations:

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

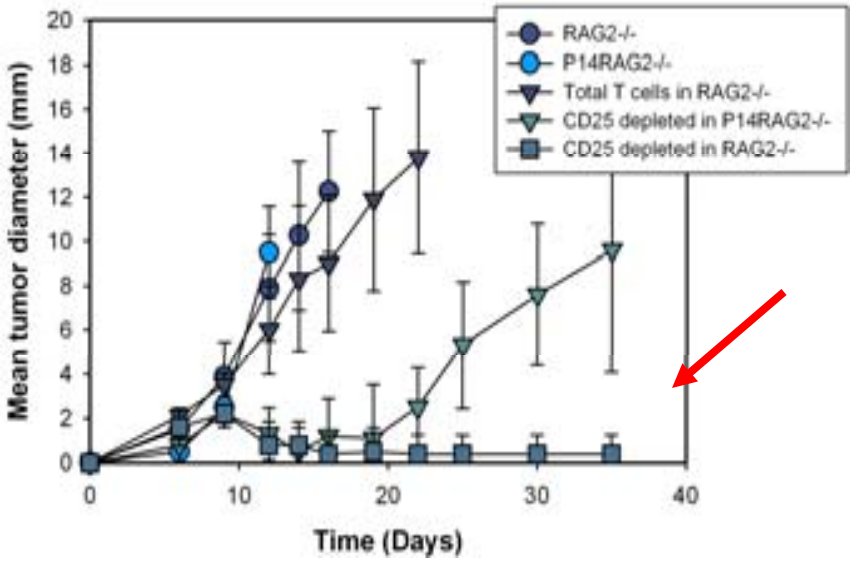
- 1-MT + lymphodepletion
- Anergy reversal + Treg-depletion
- Anti-4-1BB + anti-CTLA-4
- Anti-4-1BB + anti-PD-L1
- Anti-CTLA-4 + Treg depletion
- TLR agonist + Treg-depletion

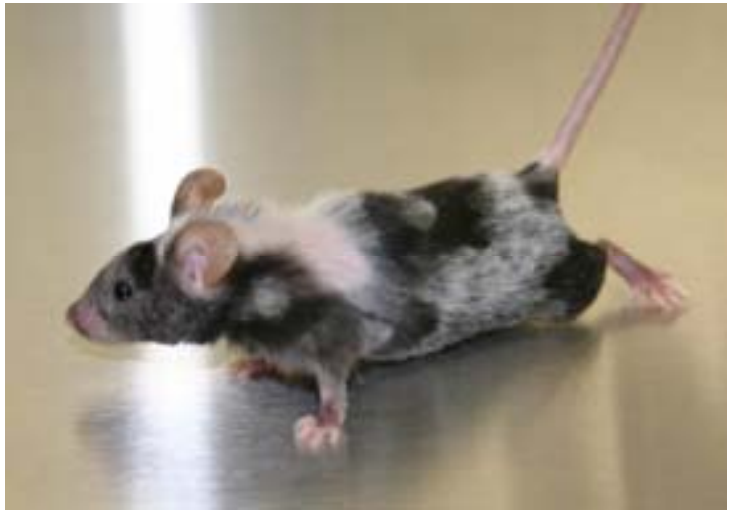
1-MT + lymphodepleting chemotherapy:
Partial control of B16 melanoma



Hou, Munn et al. Cancer Res. 2007

Treg depletion + anergy reversal CD25-depleted T cells transferred into lymphopenic hosts gives long-lived rejection of B16 melanoma and vitiligo

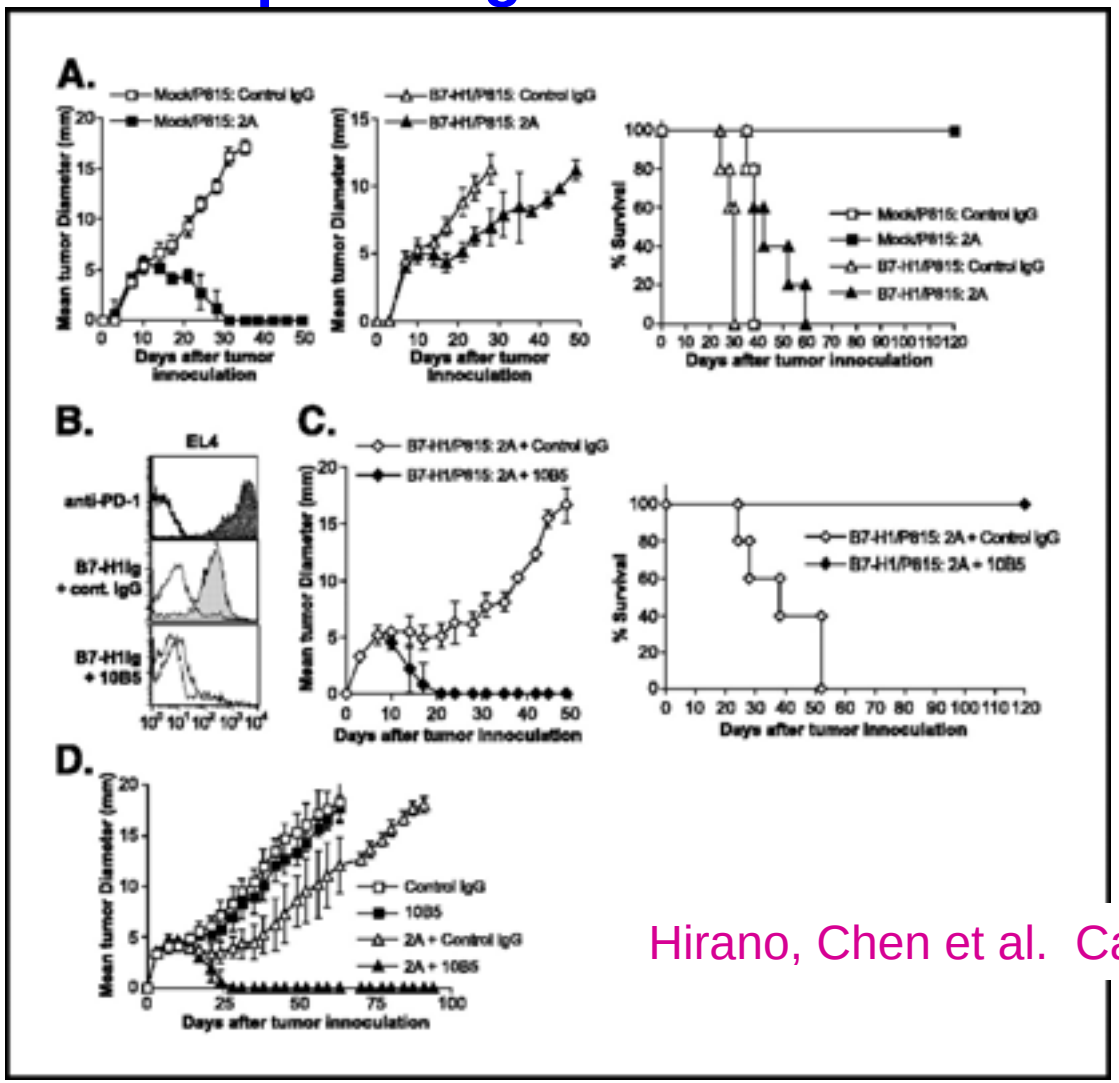




Kline et al. Submitted.

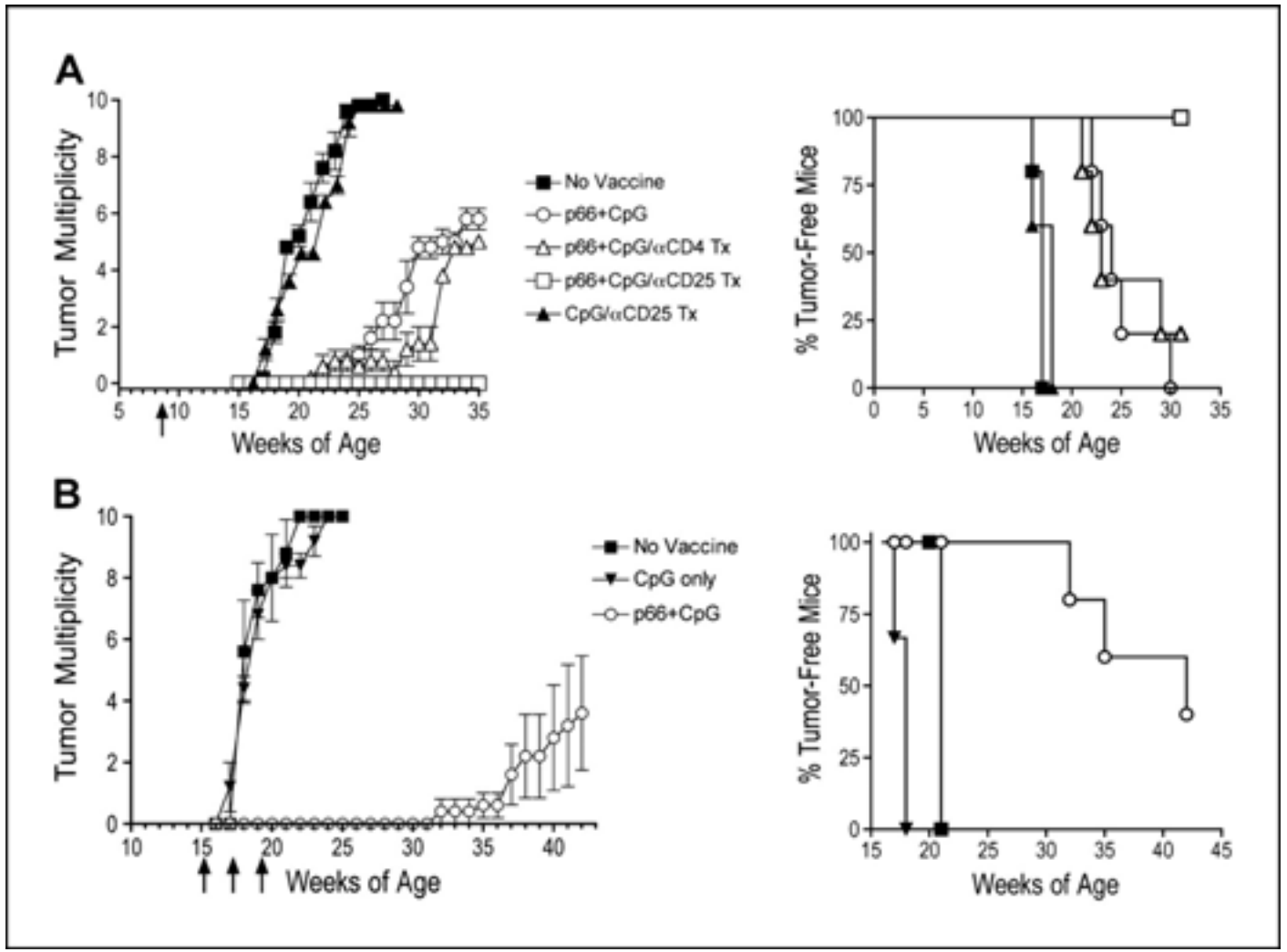
Anti-4-1BB + anti-PD-L1

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



Hirano, Chen et al. Cancer Res. 2005

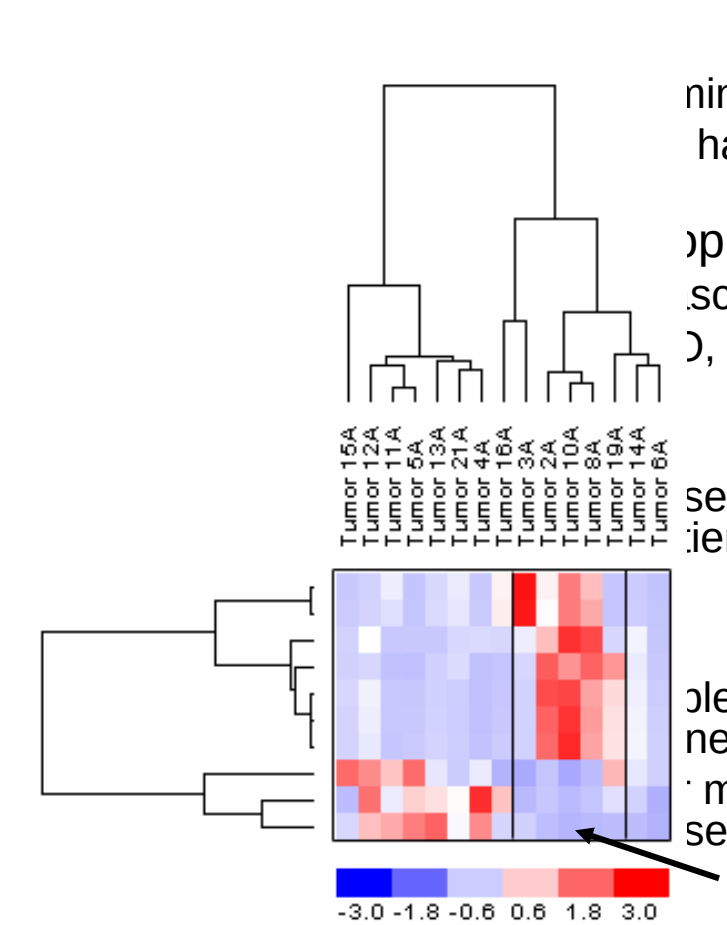
Vaccine + CpG + Treg depletion:
Control of mammary tumors in Neu Tg mice



Nava-Parada, Celis et al. Cancer Res. 2007

Additional issues

- Tumor hete
  - Differen
  - Differen barriers
- Opportuniti
  - Cellular
  - Molecul signalin
- How to pric
  - Many p of immu
  - Preclini
- Patient sel
  - Can we specific
  - Similarl effector immunc



ninant immunologic barriers
have distinct dominant immunologic

opment
sculature)
D, arginase, PD-1, LIGHT, T cell

sed on sound mechanistic analysis
ients with given cancer types

ble expandable tumor antigen-
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microenvironment that can support
se before enrolling on

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

6 mos SD or better

Conclusions

- Spontaneous anti-tumor T cell responses may fail at one of several levels
- Specific mechanisms of failure have identified new targets and strategies for intervention
- There is a strong scientific basis for combination therapies with the aim of overcoming specific barriers and immunologic checkpoints to increase the therapeutic efficacy of T cell-based immunotherapy of cancer
- Some agents are becoming available for clinical translation, but others need broad-based community support to be made available for clinical studies based on a sound rationale and preclinical data

Agents prioritized by scientific community for clinical development

Table 1. Final Rankings of Agents with High Potential for Use in Treating Cancer

Rank*	Agent	Agent Category
1	IL-15	T-Cell Growth Factor
2	Anti-Programmed Death-1 (PD1)and/or anti–B7-H1 (PD1 Ligand)	**T-Cell Checkpoint Blockade Inhibitor
3	IL-12	Vaccine Adjuvant
4	Anti-CD40 and/or CD40L	Antigen Presenting Cell Stimulator
5	IL-7	T-Cell Growth Factor
6	CpG	Vaccine Adjuvant
7	1-Methyl Tryptophan	Enzyme Inhibitor
8	Anti-CD137 (anti–4-1BB)	T-Cell Stimulator
9	Anti–TGF-beta	Signaling Inhibitor
10	Anti–IL-10 Receptor or Anti–IL-10	Suppression Inhibitor
11	Flt3L	Dendritic Cell Growth Factor/ Vaccine Adjuvant
12	Anti-Glucocorticoid-Induced TNF Receptor (GITR)	T-cell Stimulator
13	CCL21 Adenovirus	T-Cell Attracting Chemokine
14	Monophosphoryl Lipid A (MPL)	Vaccine Adjuvant
15	Poly I:C and/or Poly ICLC	Vaccine Adjuvant
16	Anti-OX40	T-Cell Stimulator
17	Anti–B7-H4	T-Cell Checkpoint Blockade Inhibitor
18	Resiquimod and/or 852A	Vaccine Adjuvant
19	LIGHT and/or LIGHT vector	T-Cell Stimulator
20	Anti–Lymphocyte Activation Gene-3 (LAG-3)	T-Cell Checkpoint Blockade Inhibitor

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/anti-CTLA4 trial Contributors

VU medisch centrum

Dept Medical Oncology

Tanja de Gruijl

Sinéad Lougheed

Helen Gall

Bob Pinedo

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

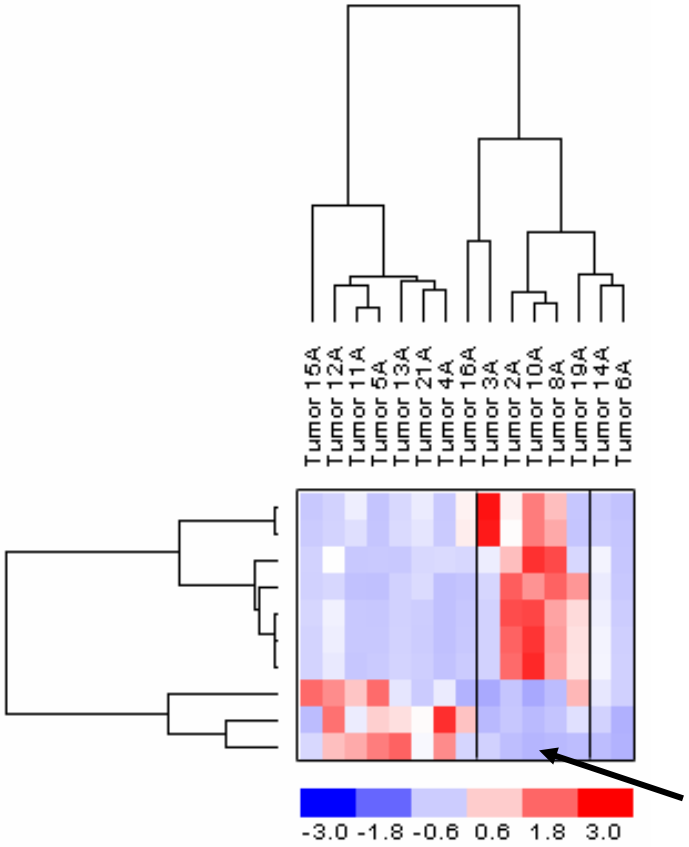
Steven Fischkoff

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



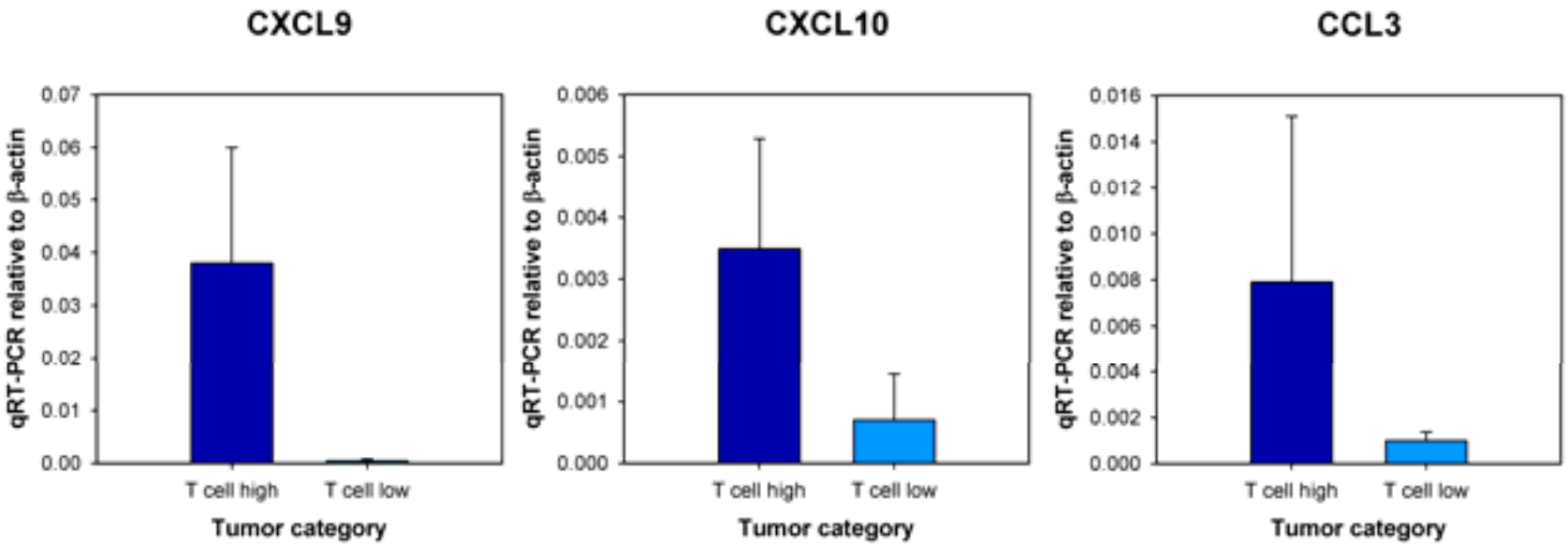
Represents only 7 genes:

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

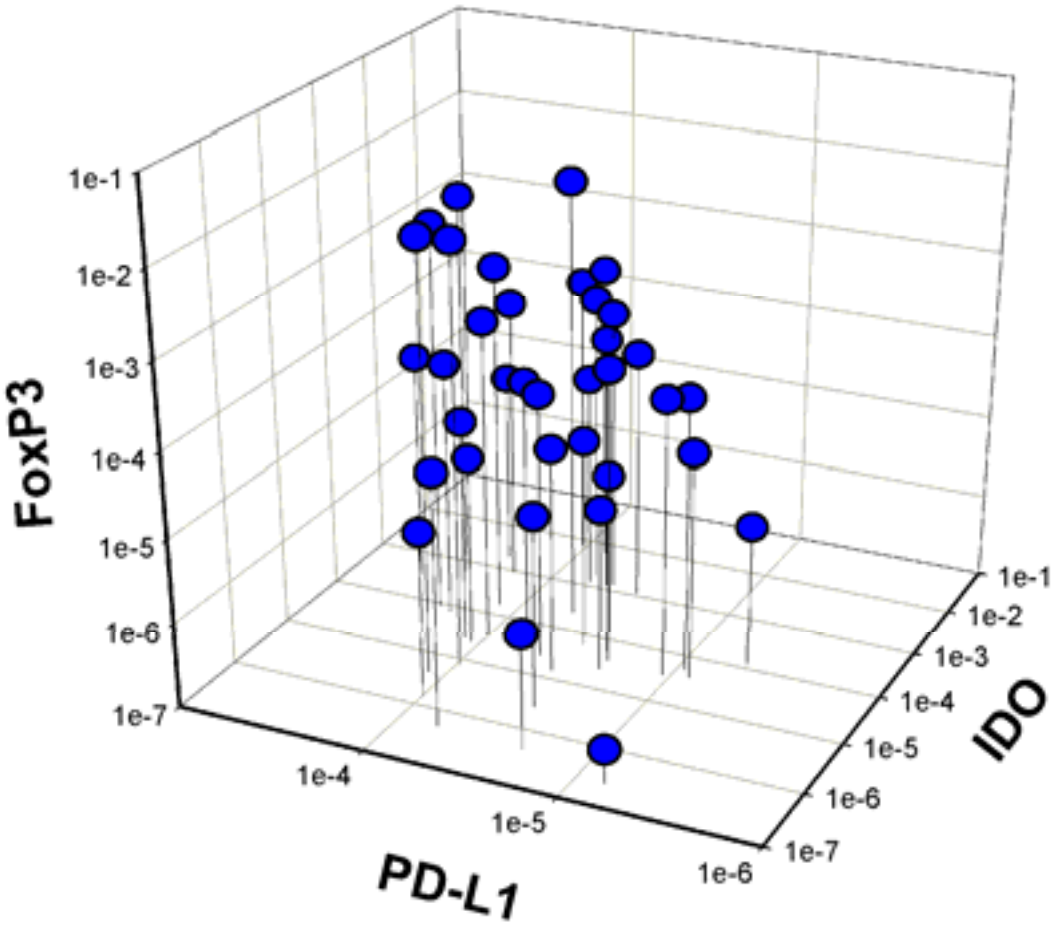
6 mos SD or better

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

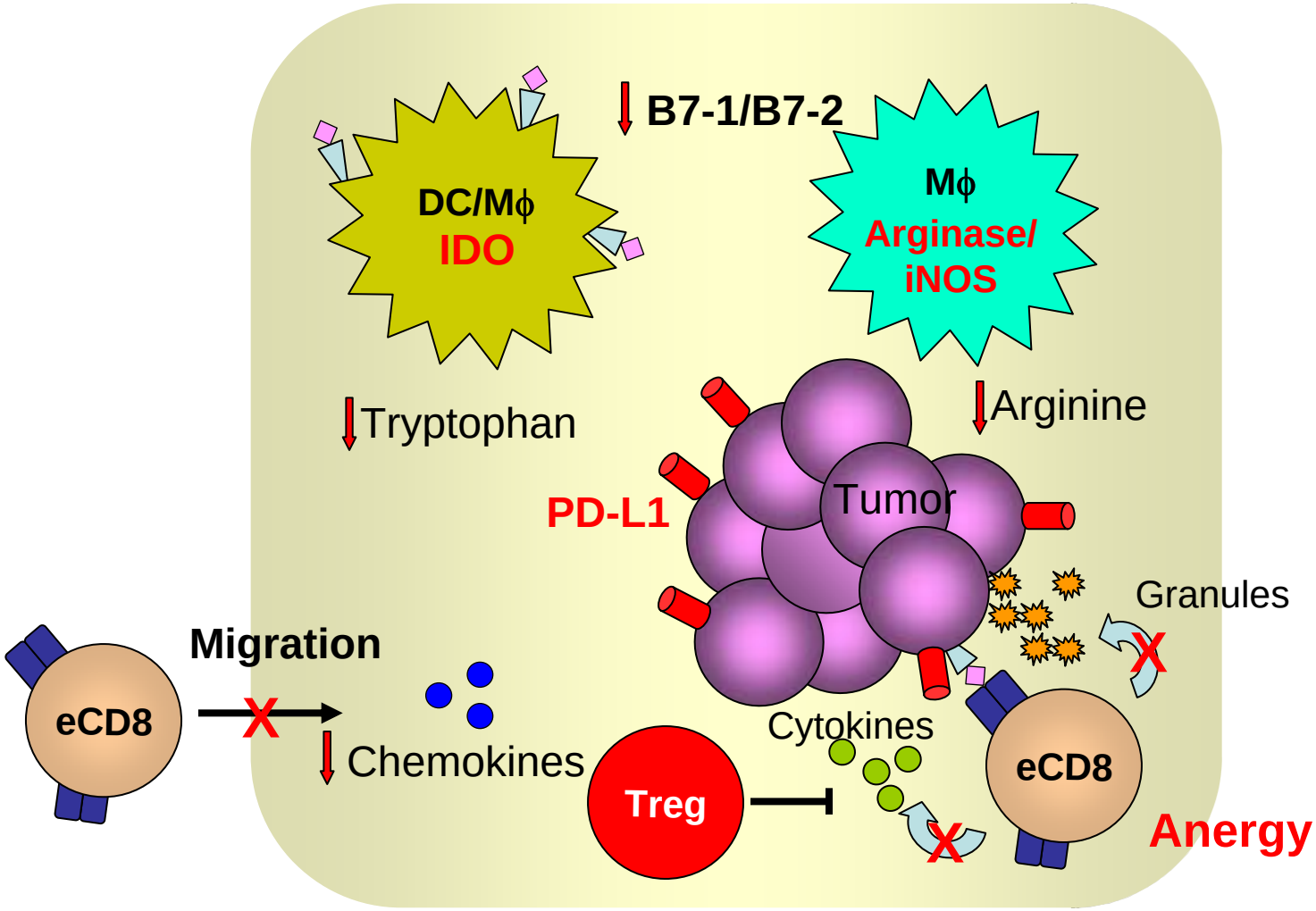
Differential chemokine expression in melanoma metastases with high versus low T cell transcripts



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

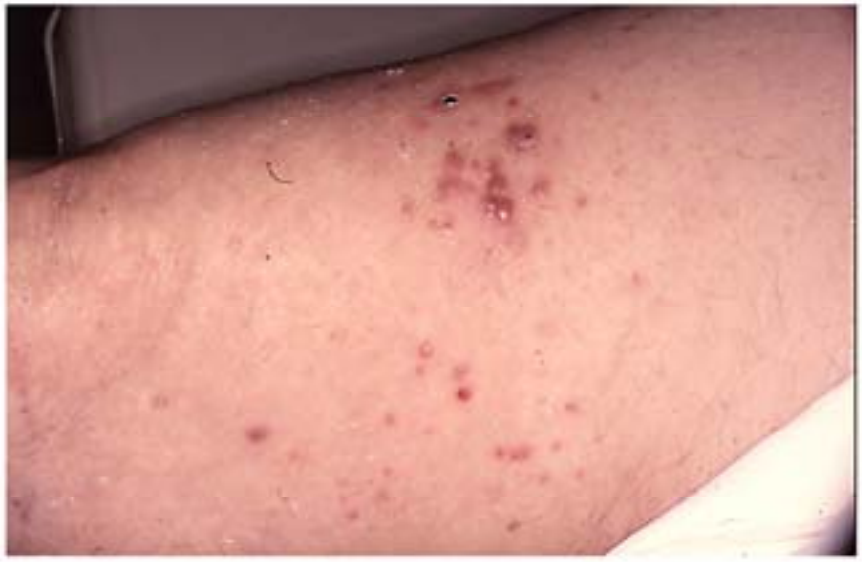


Summary of tumor microenvironment barriers: Need to promote T cell trafficking and overcome local immunosuppression

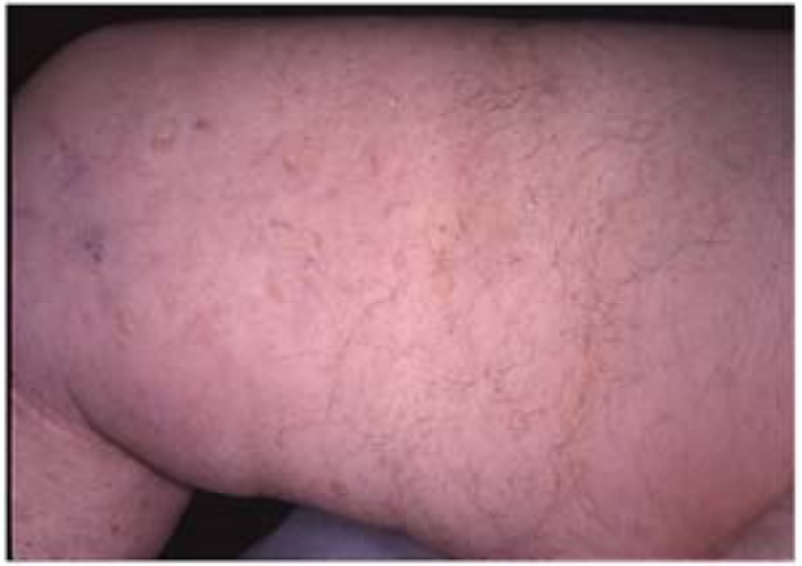


Resolution of cutaneous metastases following immunization with melanoma peptide-pulsed PBMC + rhIL-12

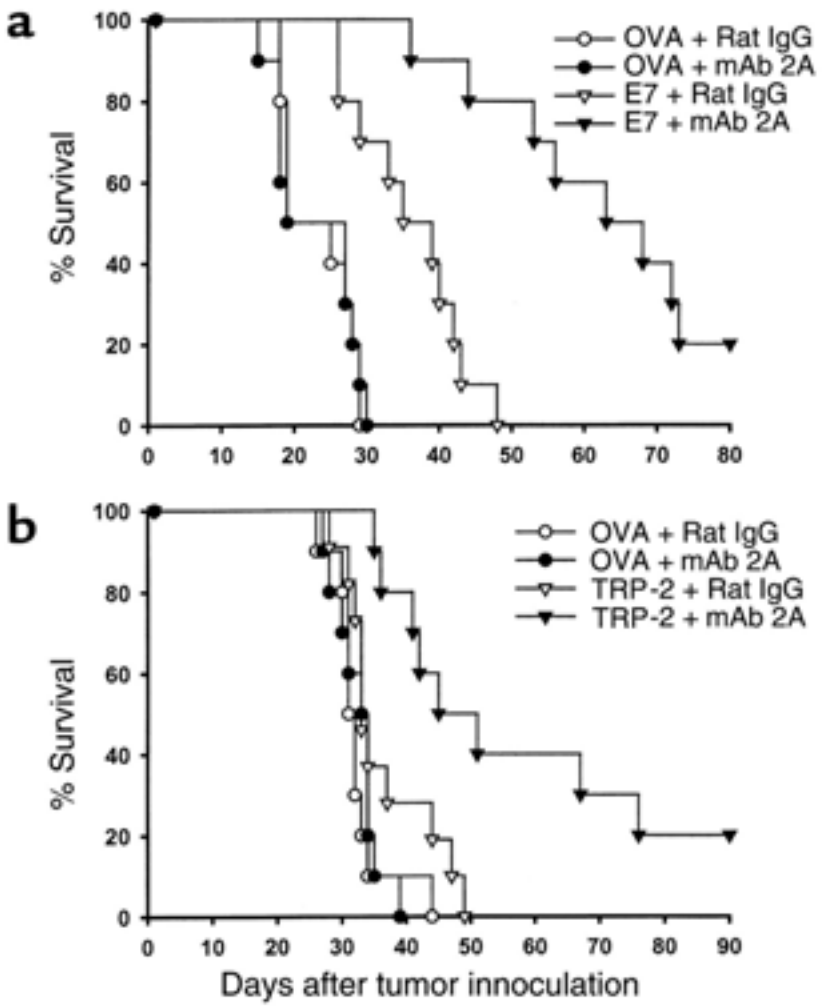
After 3 vaccines



After 9 vaccines

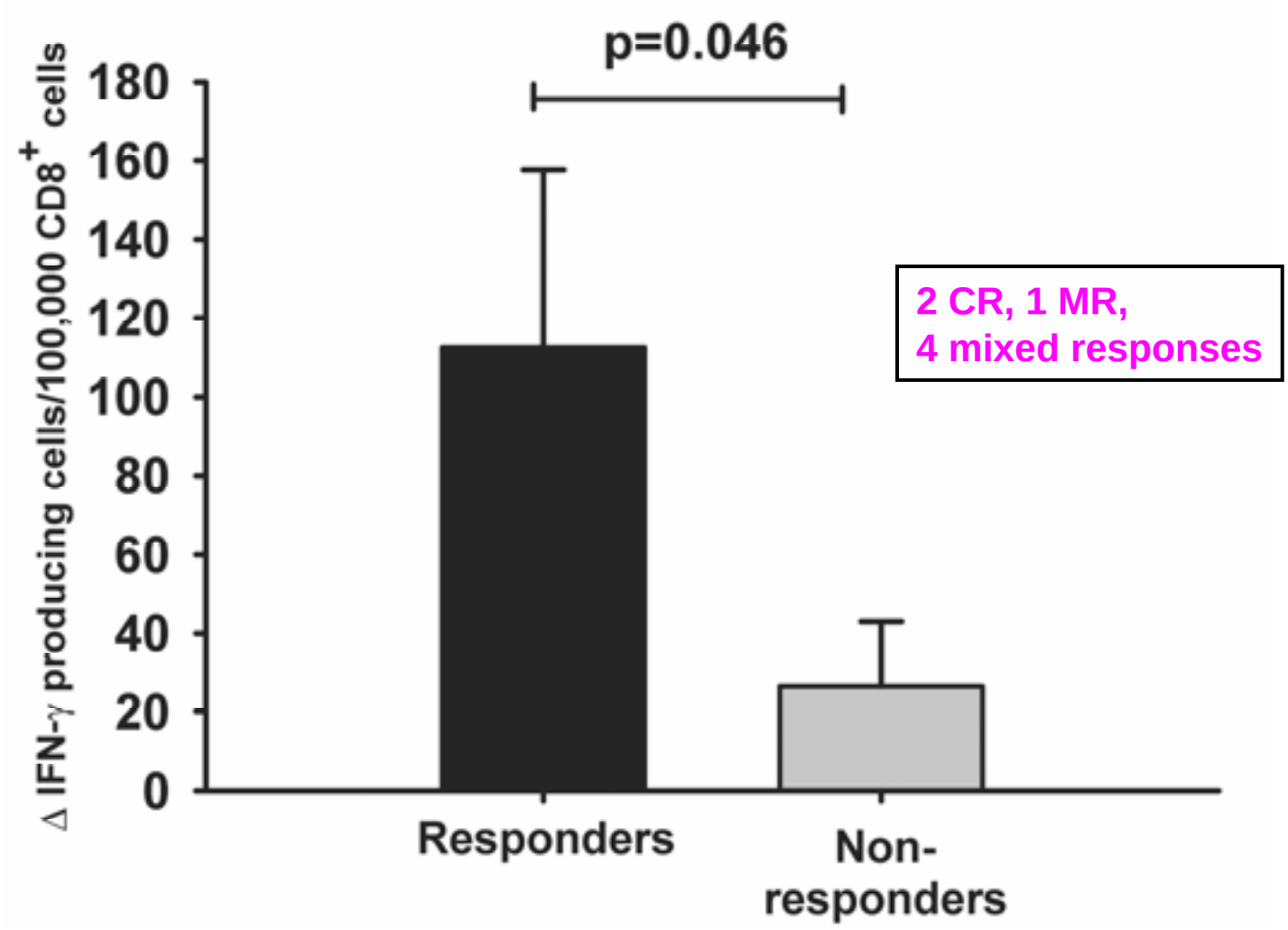


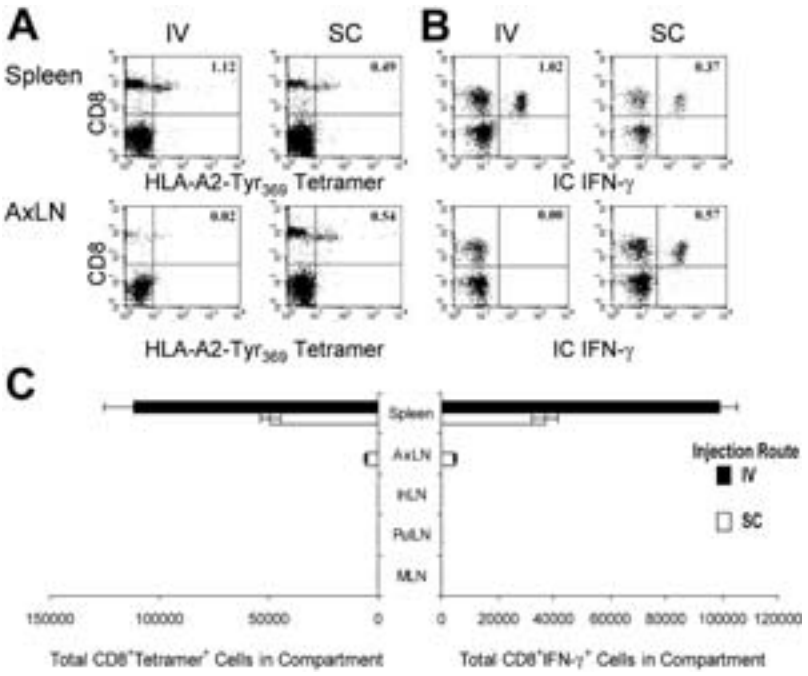
Peterson, Gajewski et al. JCO 2003.

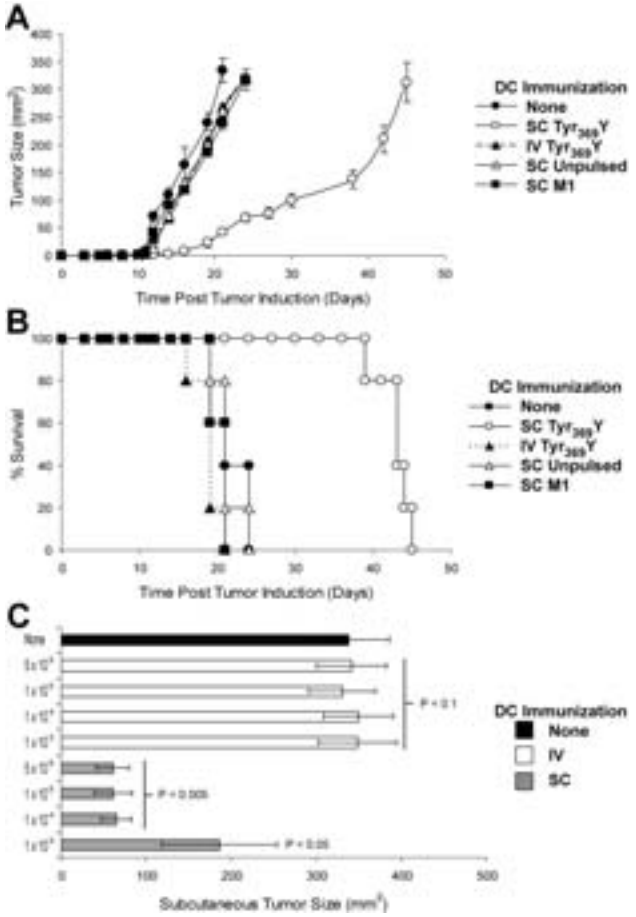


Wilcox, R. A. et al. J. Clin. Invest. 2002;109:651-659

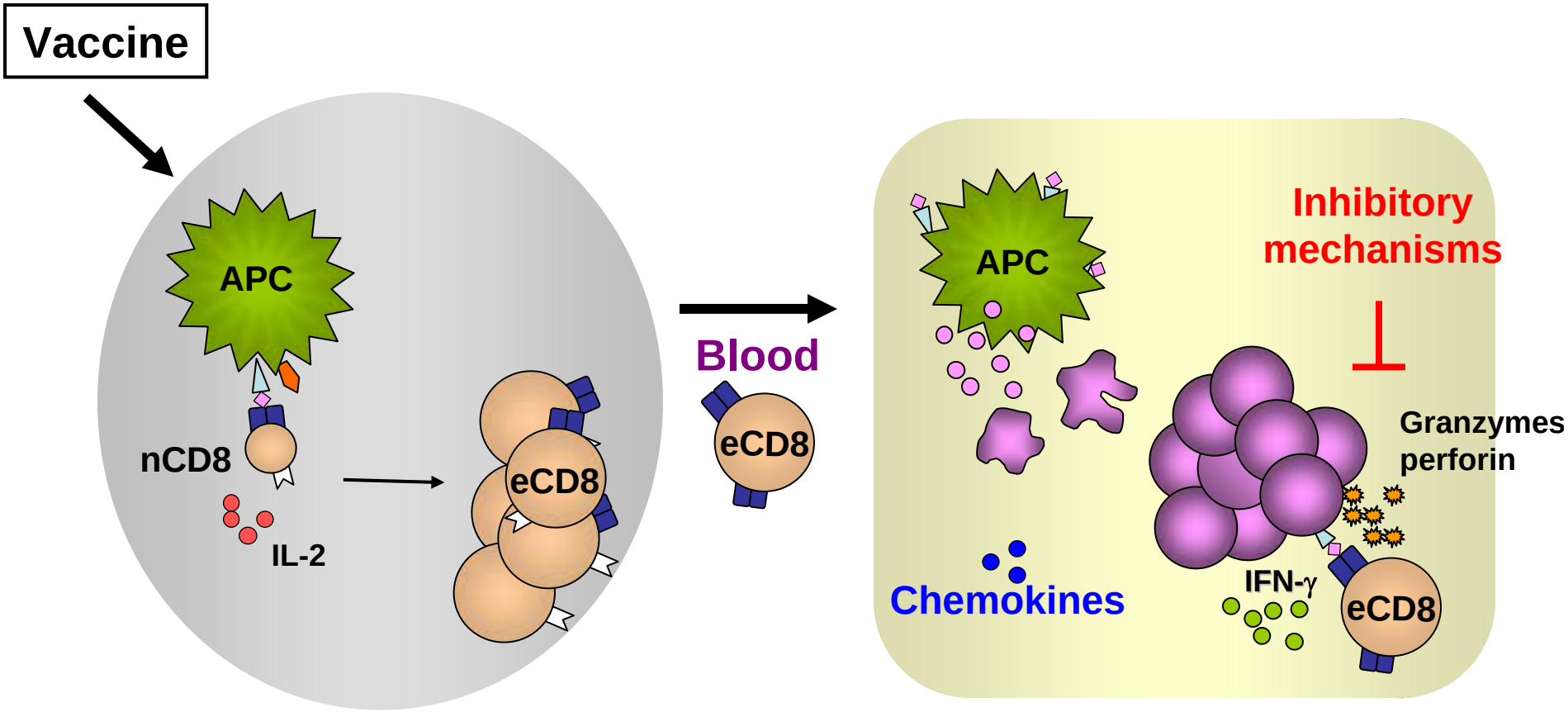
Greater increase in Melan-A-specific CD8$^+$ T cells in clinical responders




A
IV
SC
Spleen
CD8
1.12
0.49
HLA-A2-Tyr369 Tetramer
AxLN
0.82
0.54
HLA-A2-Tyr369 Tetramer
B
IV
SC
1.62
0.37
IC IFN-γ
0.00
0.57
IC IFN-γ
C
Spleen
AxLN
IHLN
PuLN
MLN
Injection Route
IV
SC
150000
100000
50000
0
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
P = 0.1
P = 0.005
P = 0.05
DC Immunization
None
IV
SC
Subcutaneous Tumor Size (mm²)

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

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)

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

III. T cell trafficking: considerations for combinations

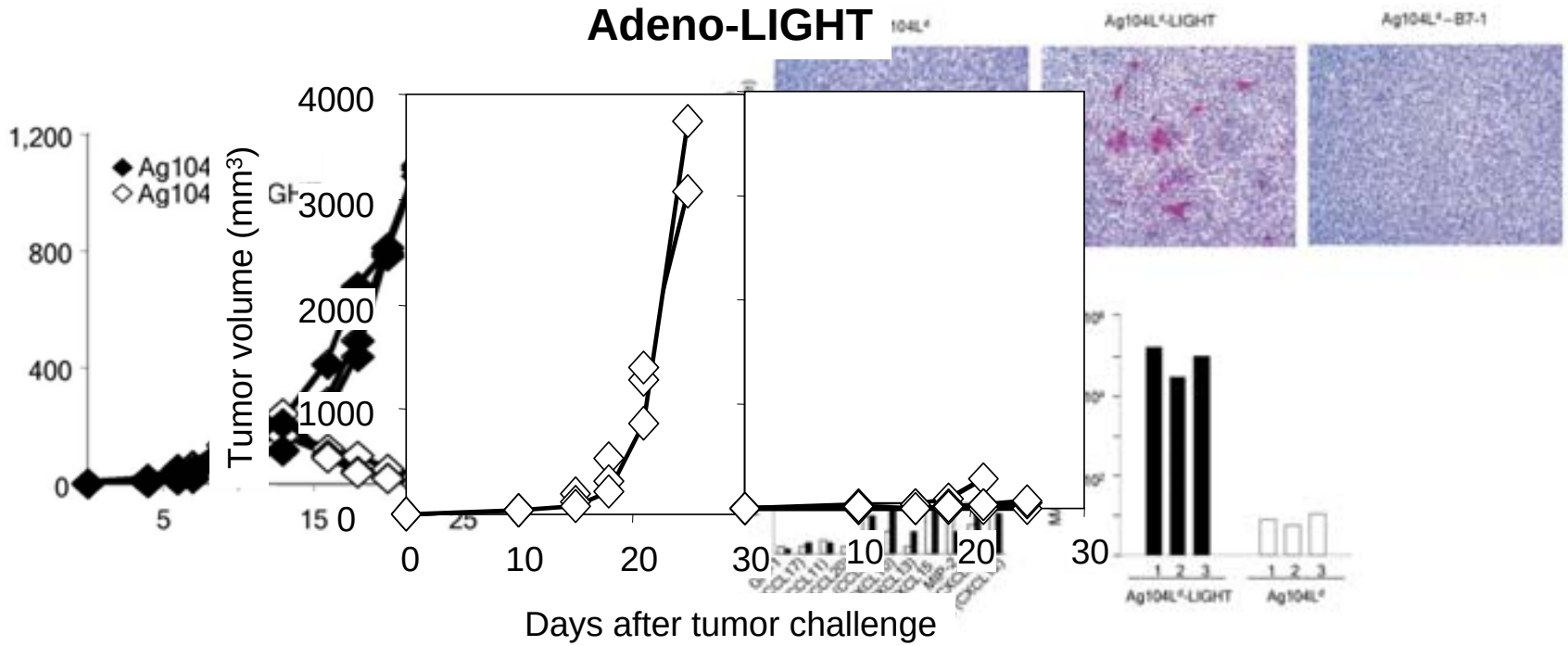
- Intratumoral chemokines
  - Mig, IP-10, MIP-1α
  - CCL21
  - (Blockade of TARC/MDC?)
- Intratumoral LIGHT
  - Promotes secondary generation of chemokines
- Homing receptors/adhesion molecules
  - Intratumoral ICAM-1 (component of TRICOM)
  - Immunizing via optimal route (tissue specific homing)
- Angiogenesis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

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

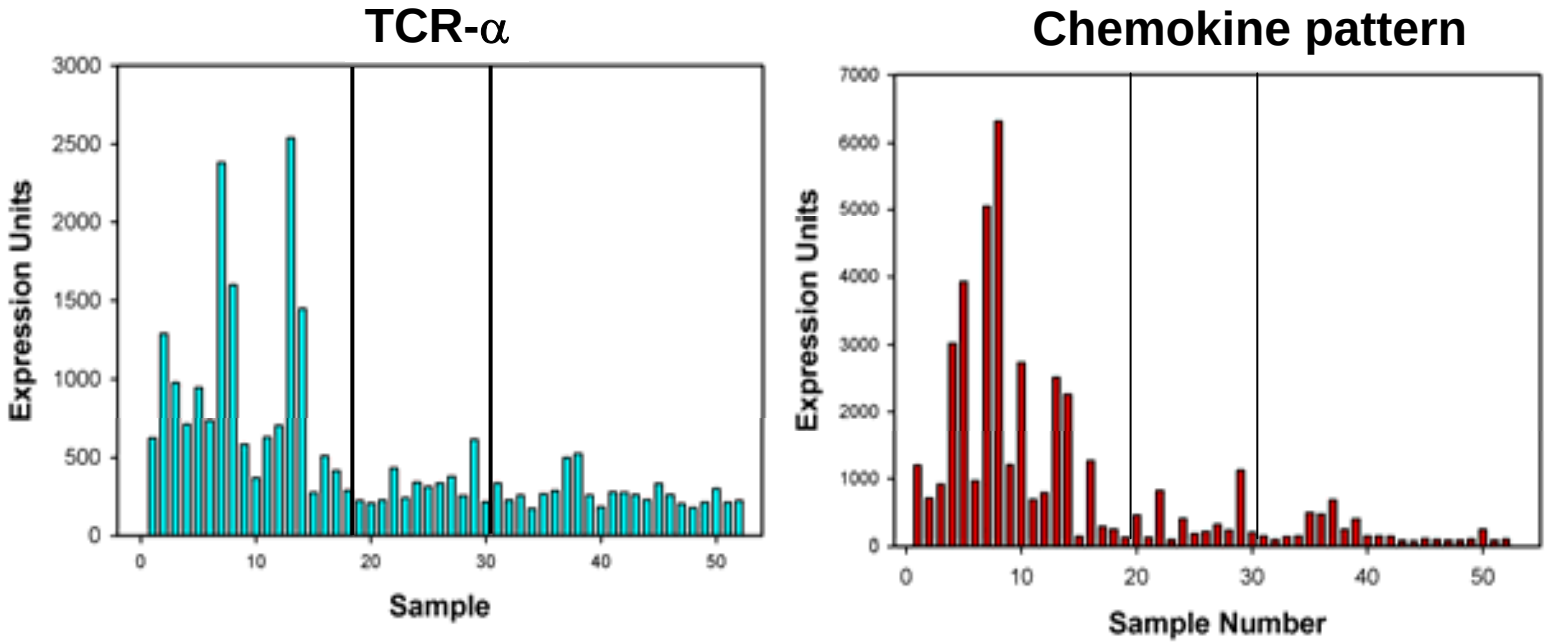
Example 4: LIGHT

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



Fu et al, submitted

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



Harlin, Gajewski et al. Manuscript in preparation.