Unraveling the Basic Components of Cancer Immunotherapy

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Working Hypothesis

- Targeting missing immunostimulatory molecules to tumor can generate complete immune response with memory

- Deletion of natural immunosuppression can enable immunotherapy to be effective

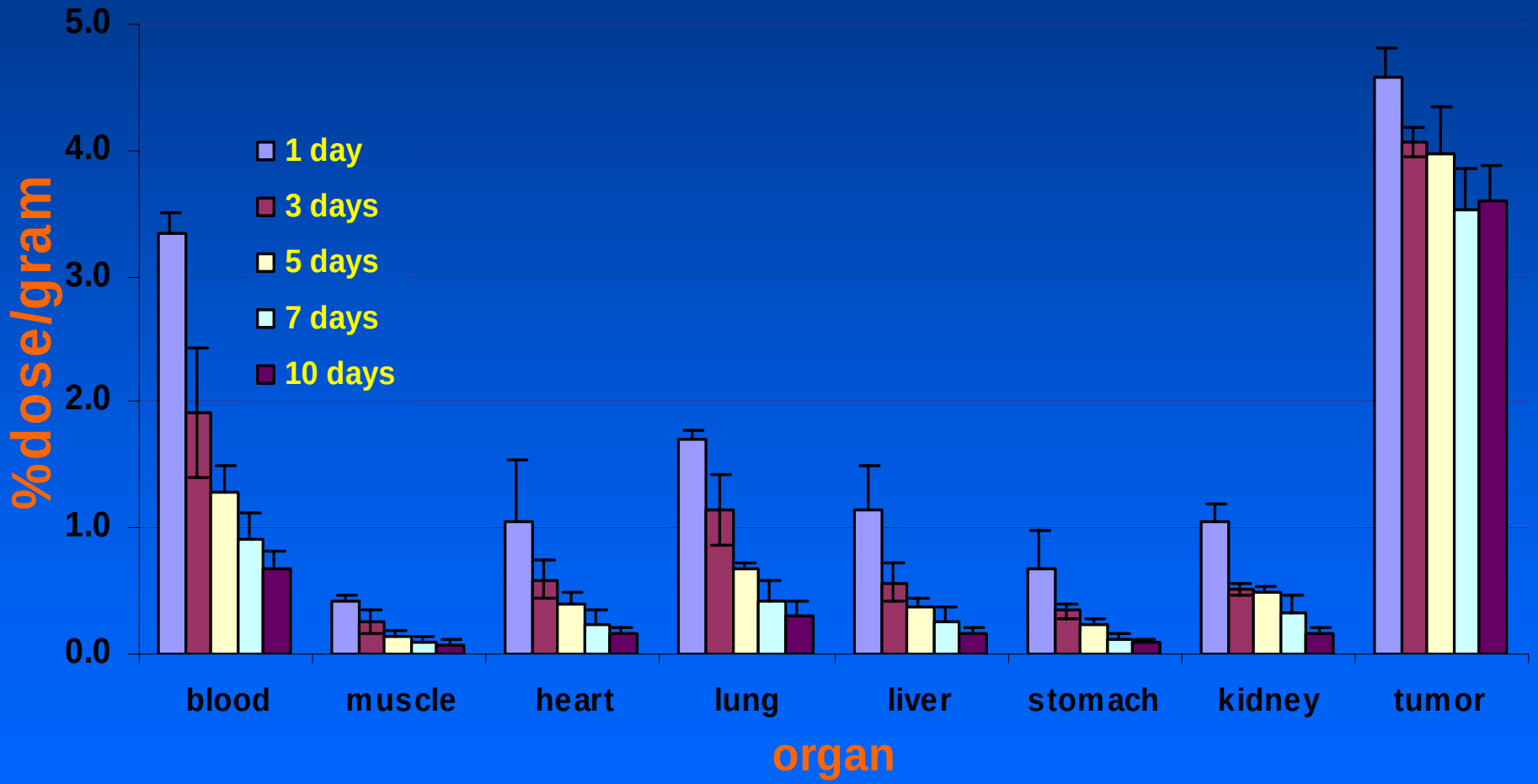
Targeting Tumor Necrosis with TNT Antibodies

USC

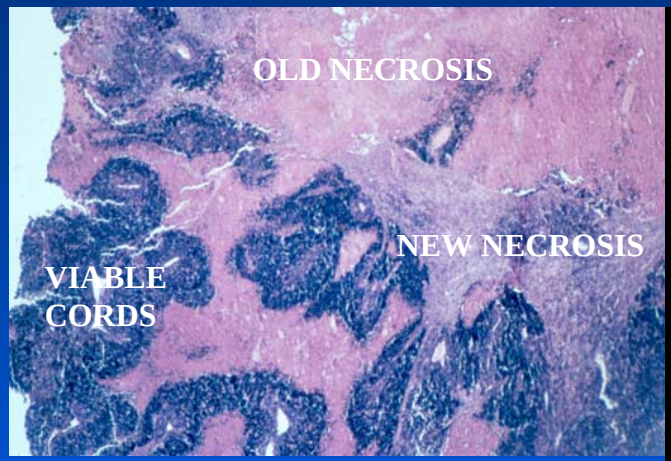
Major Characteristics of TNT Antibodies

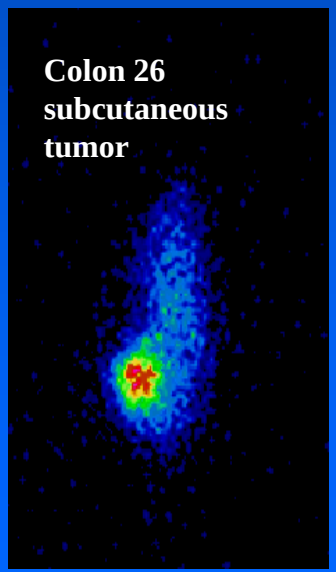
- Recognize abundant intranuclear antigens present in all cancers, all species
- Have long retention times in tumor
- Have enhanced uptake after cytoreductive therapies
- Localize to necrosis, a site rich in tumor antigens

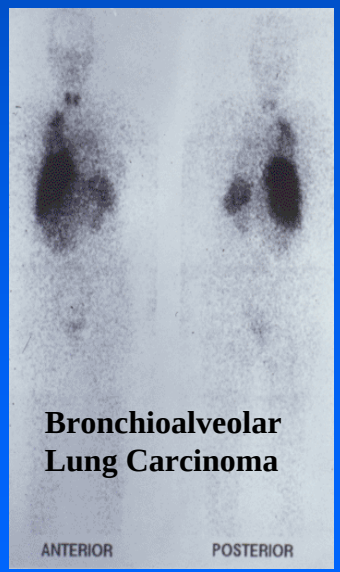
Tissue Biodistribution of I-125-chTNT-3/B in ME-180 Carcinoma-bearing Nude Mice

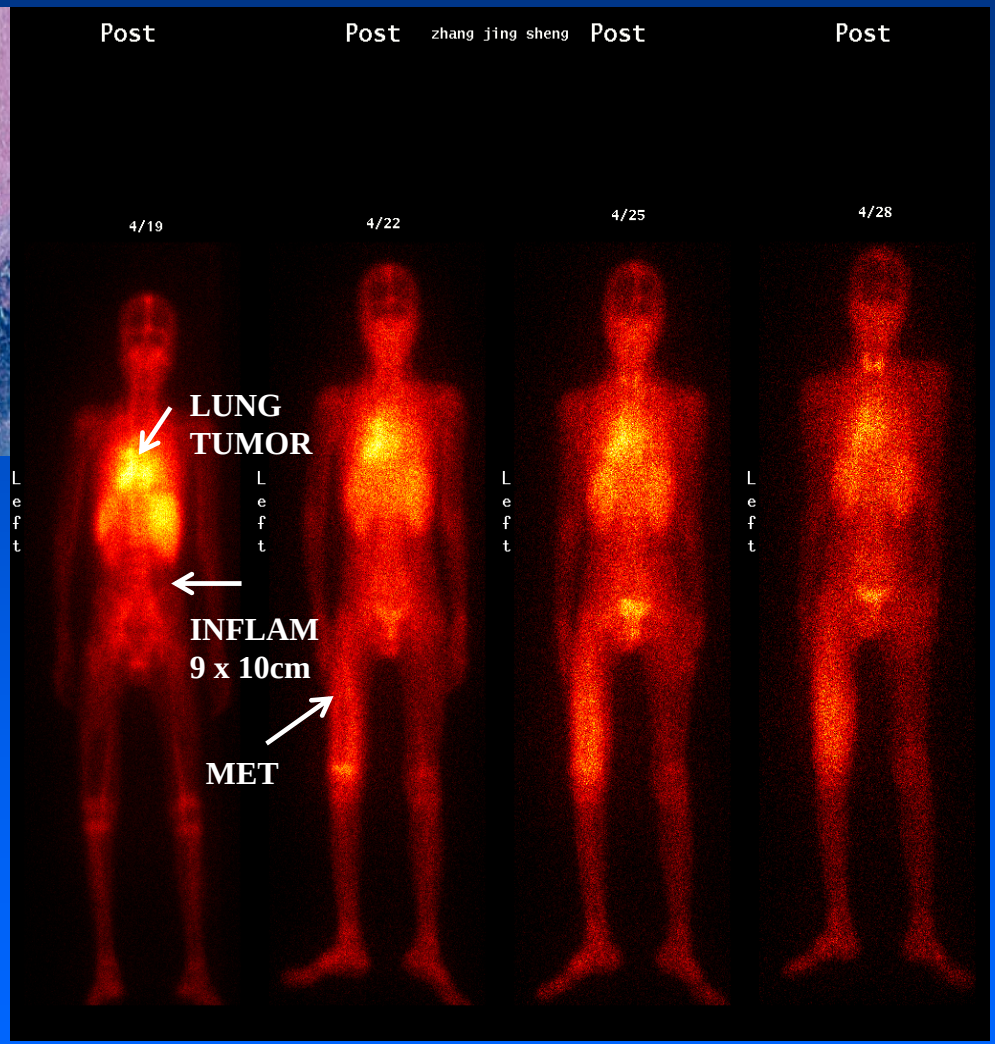


TNT Antibody Uptake in Tumor

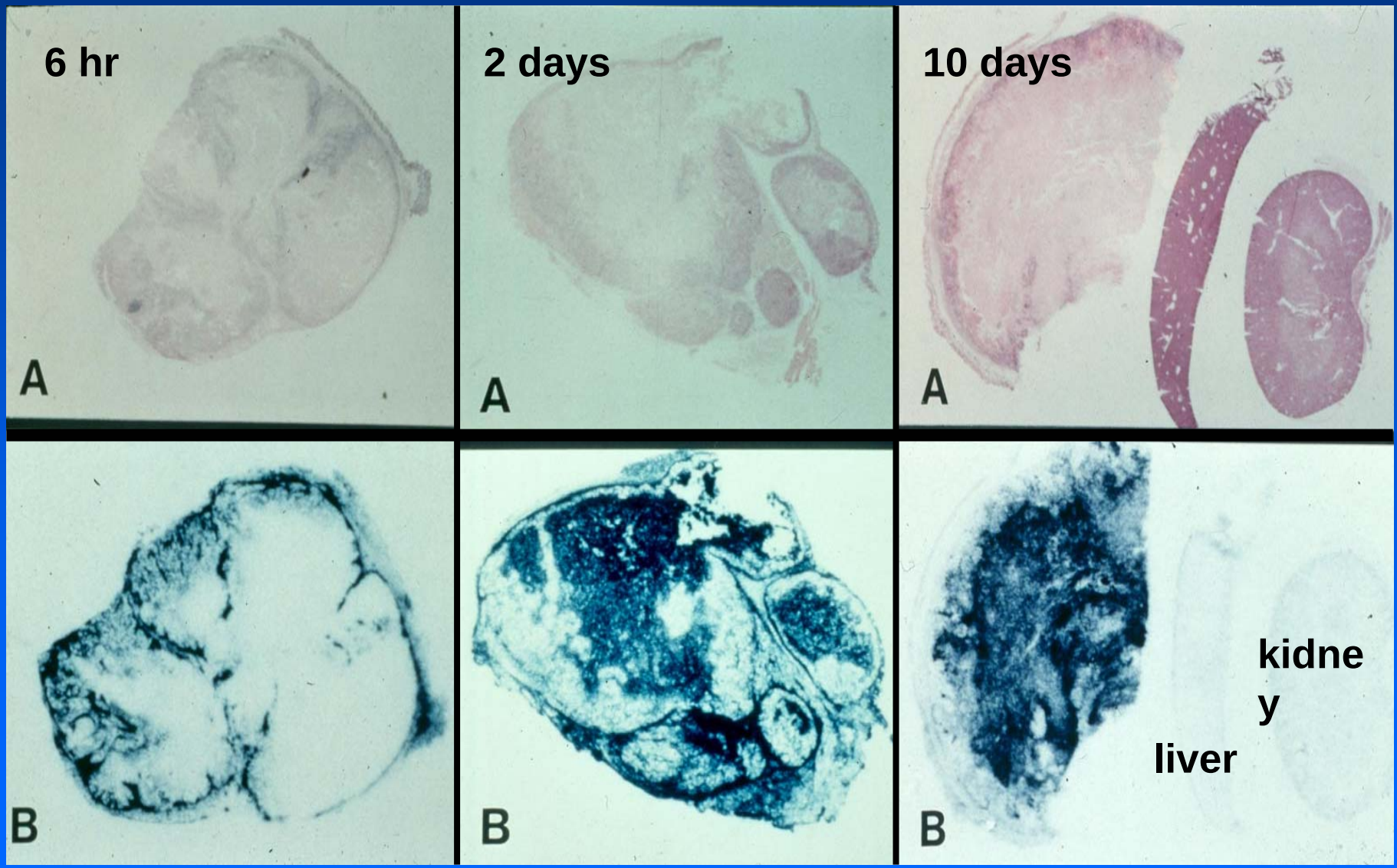




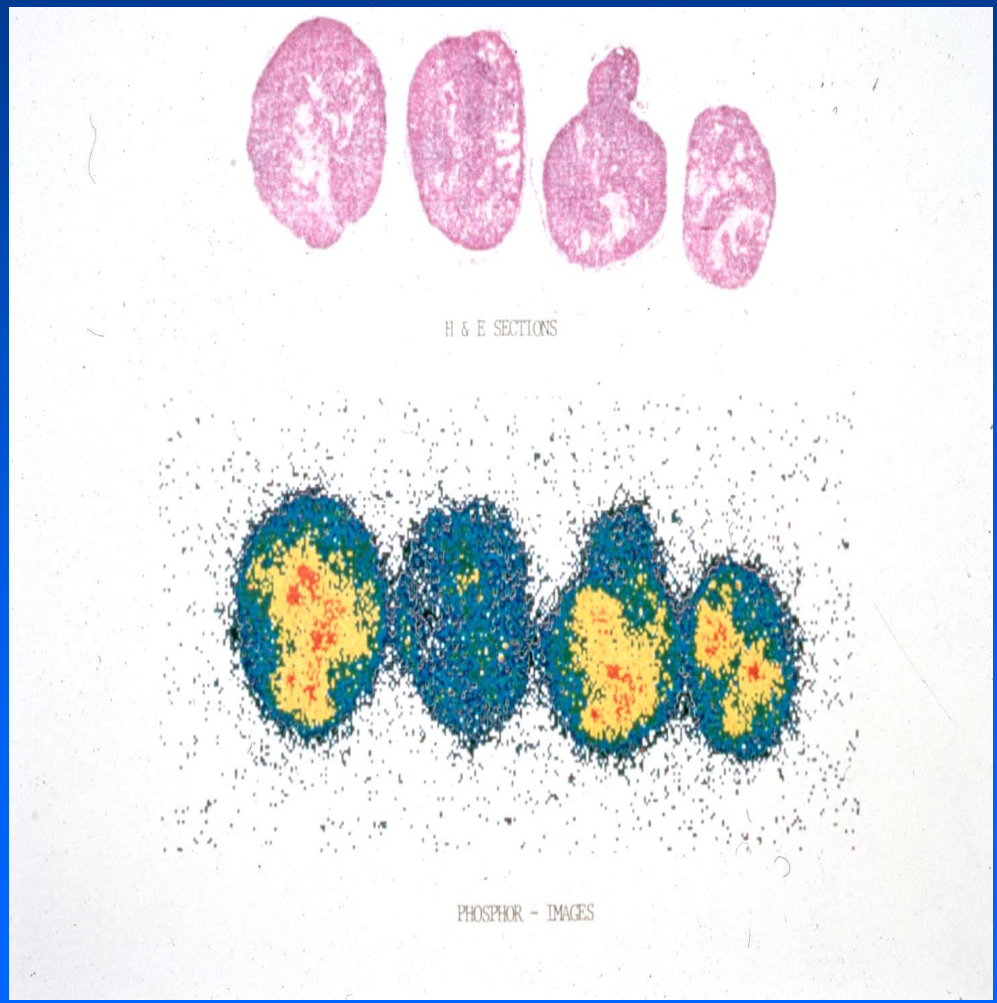


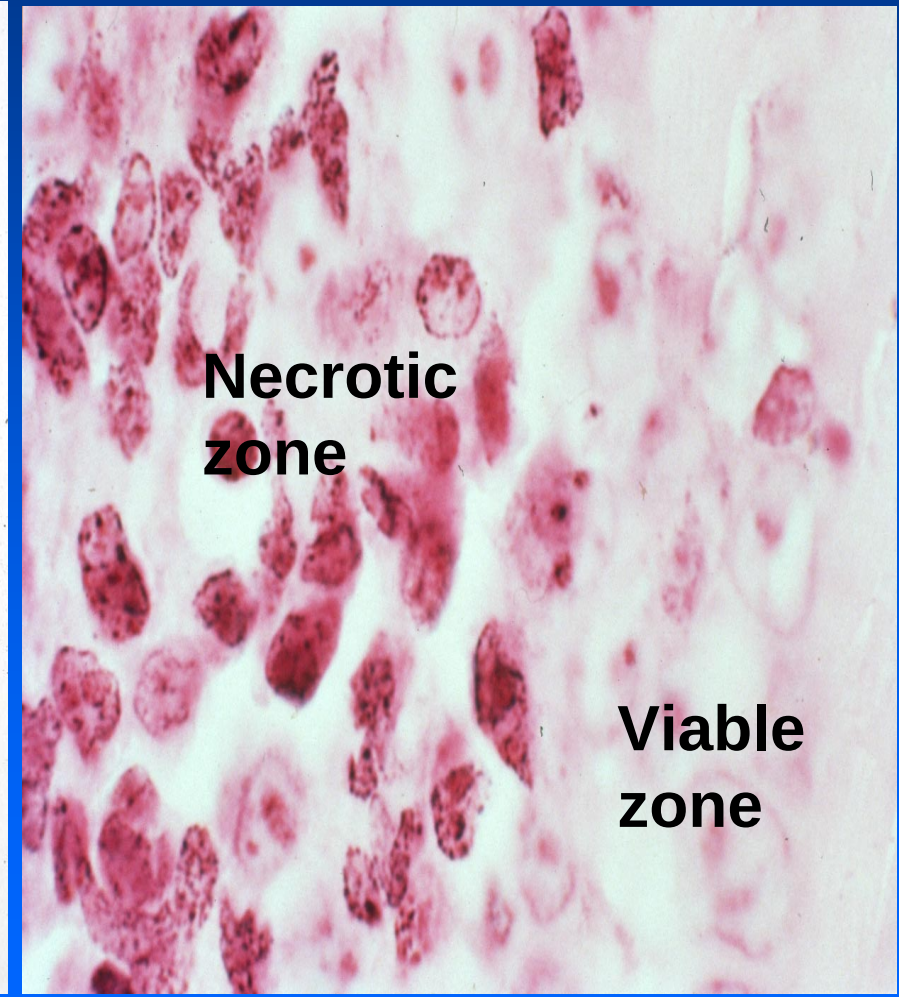


Macroautoradiography of ^{125}I-TNT-1 in ME-180 Human Cervical CA

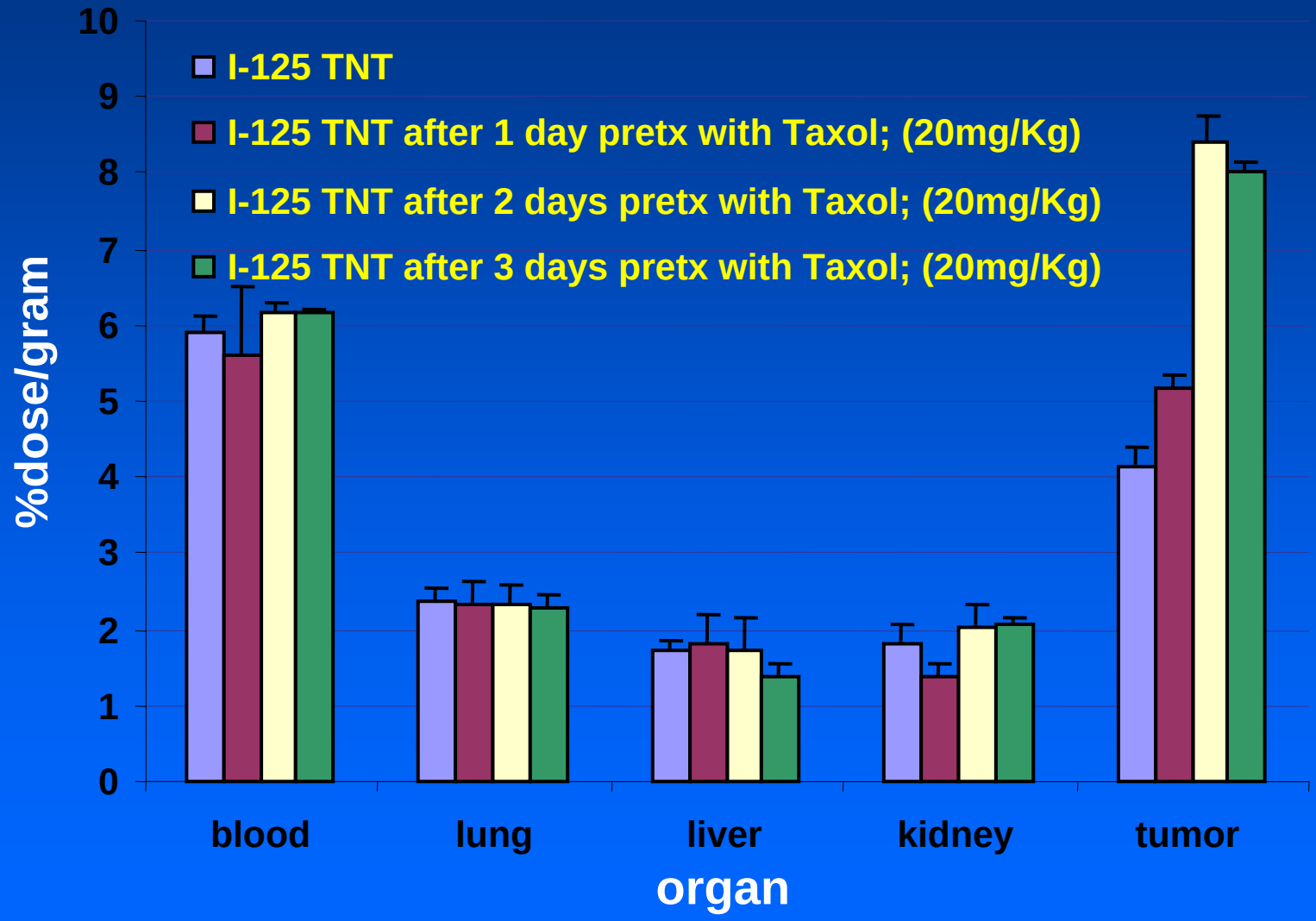


Macro and Microautoradiography of ^{125}I-TNT-3





Enhanced Uptake of TNT in Taxol Treated Colon 26 Tumors

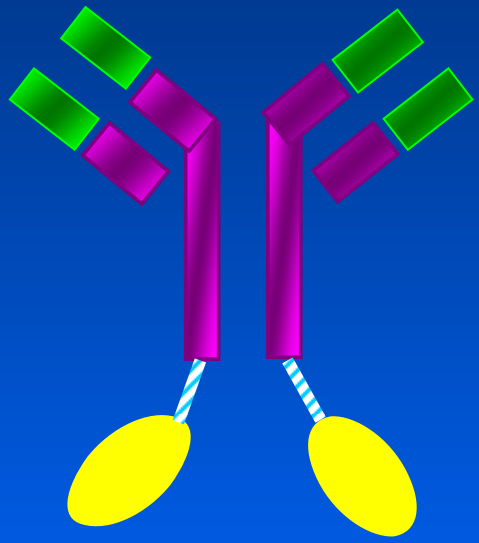


Methods of Immunotherapy

- Vaccines
- Cytokine Therapy
- Adoptive Transfer of Immunity
- Fusion Proteins
  - Targeted (MAb)
  - Untargeted (Fc)
- Genetic alteration of T-cells
- Immunomodulatory drugs

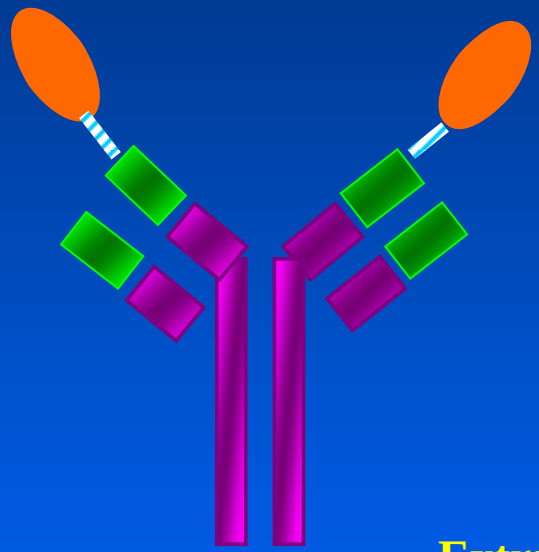
Targeted Fusion Proteins

C-Terminal Fusion



Cytokines, Type II costimulatory molecules

N-Terminal Fusion



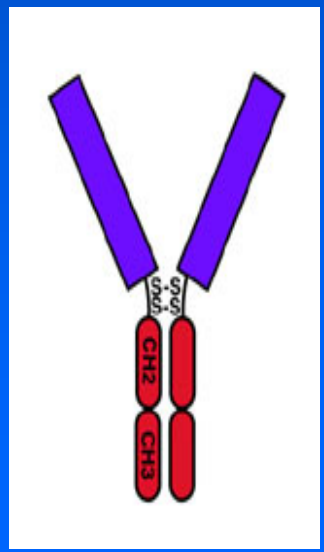
Chemokines, B7

Untargeted Fc

N-Terminal Fusion

Extracellular domains

Fc portion of human IgG1



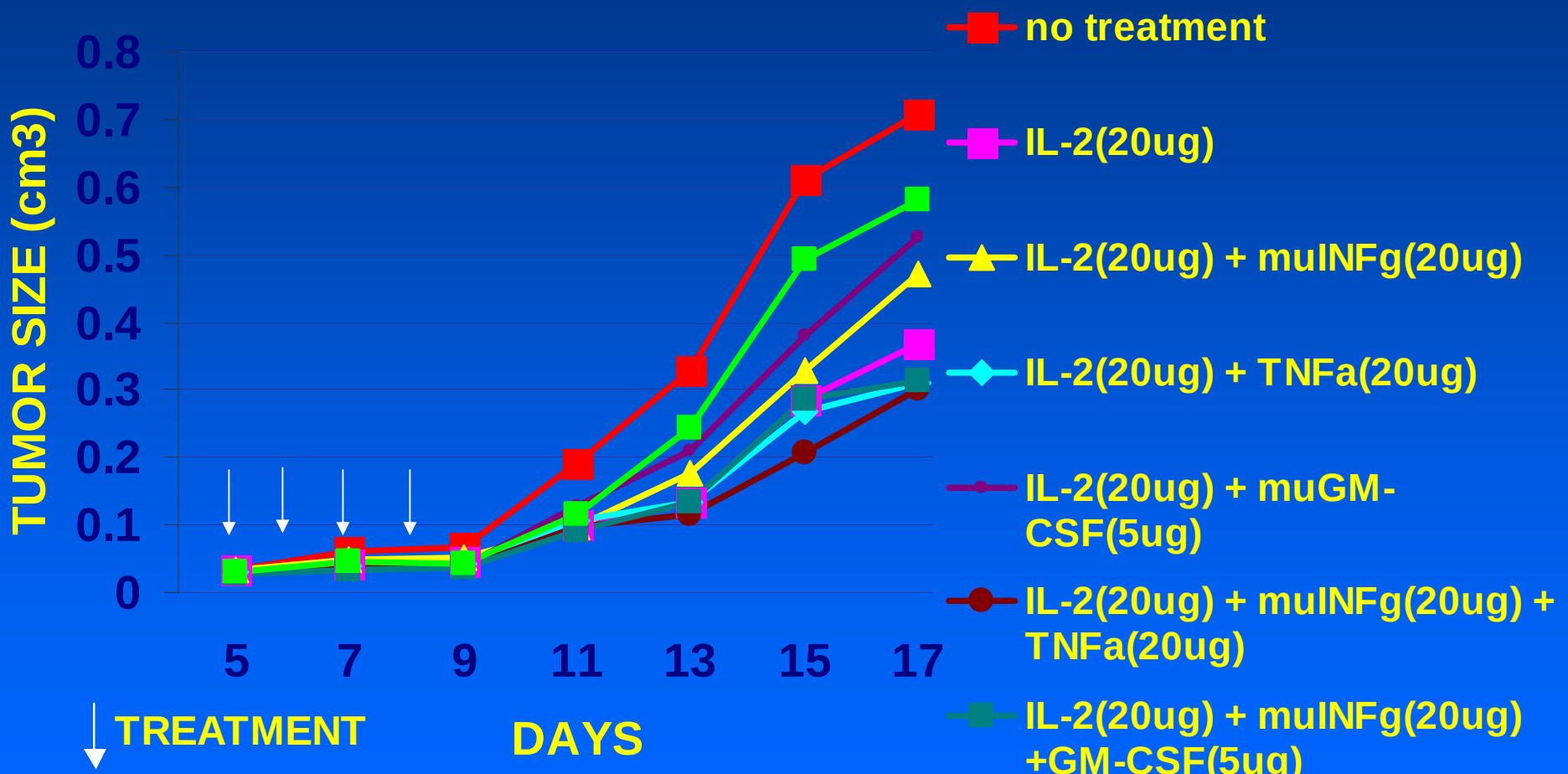
Cytokine Fusion Proteins

IL-2, IL-4, IL-12, TNFα, GM-CSF, IFNγ

Chemokine Fusion Protein

LEC

Immunotherapy of MAD 109 Lung CA Using Immunocytokine Fusion Proteins

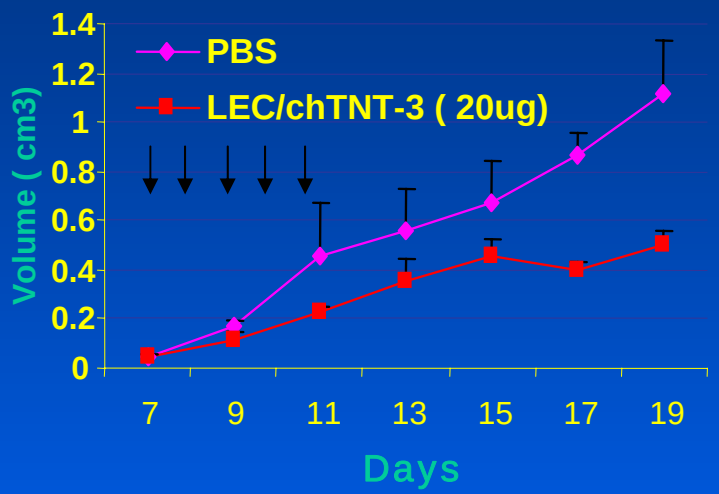


LEC Chemokine

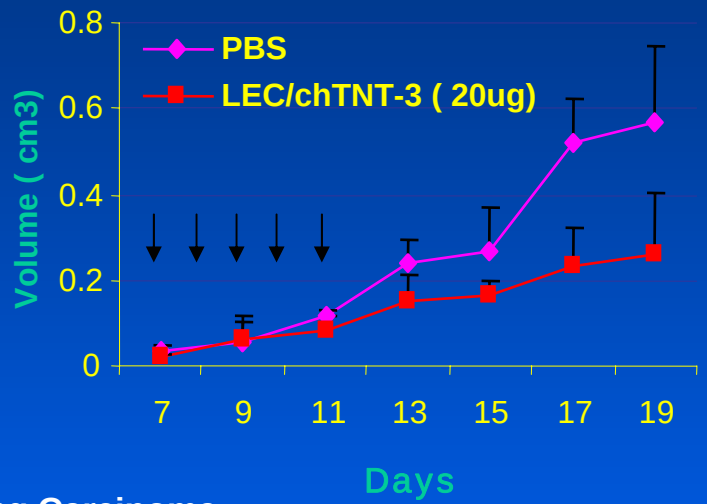
- Liver Expression Chemokine (LEC)
- A CC family (β family) chemokine (CCL16)
- Located on chromosome 17q in CC cluster
- Chemoattracts PMNS, monocytes, dendritic cells, and lymphocytes
- Interacts with CCR1, CCR5, and CCR8 receptors

LEC/chTNT-3 Immunotherapy in 3 Tumor Models of the BALB/c Mouse

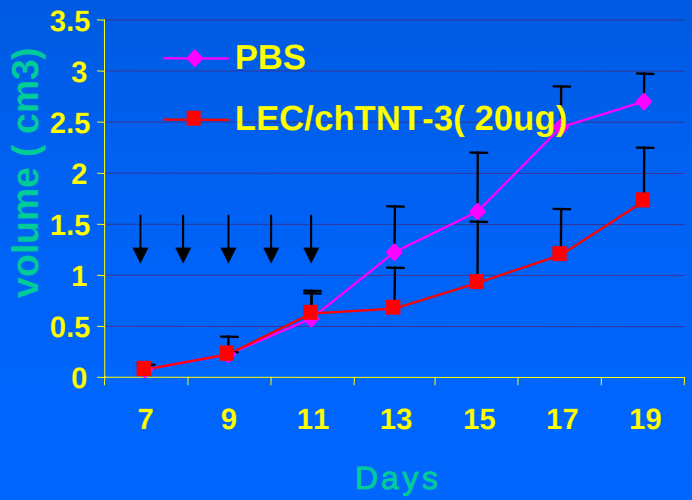
Colon 26 Colon Carcinoma



RENCA Renal Carcinoma



MAD 109 Lung Carcinoma

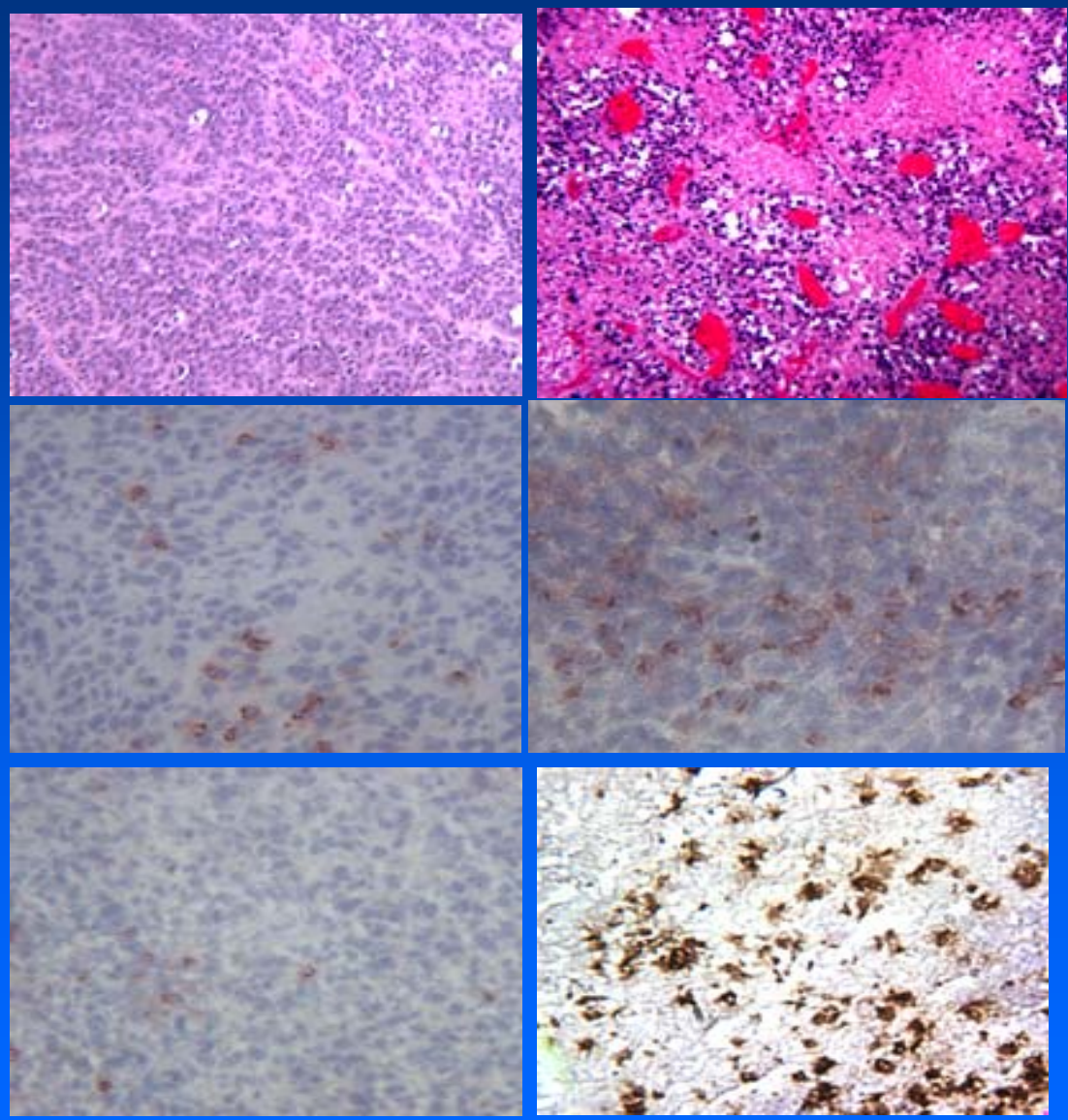


Histologic and IHC Analysis of Tumor Sections

H&E

Dendritic Cells

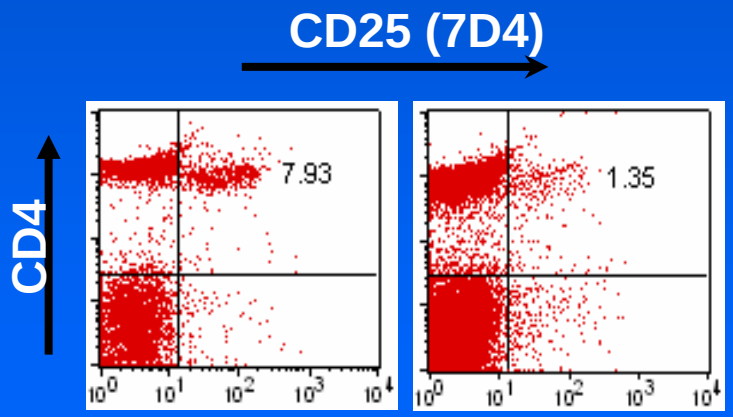
PMNS

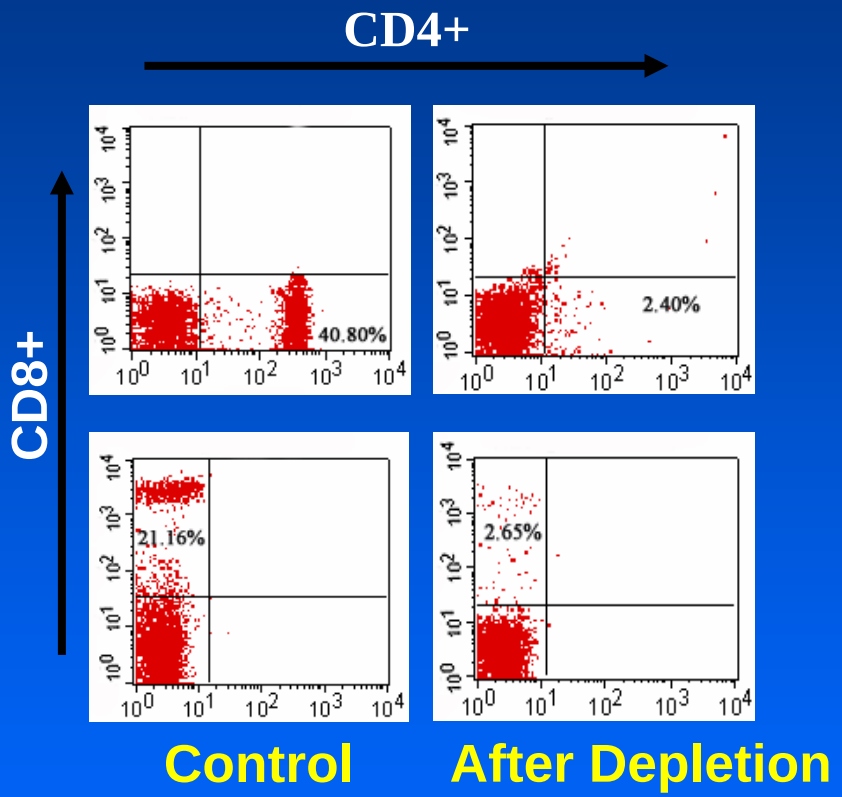


Control Treated LEC/chTNT-3 Treated

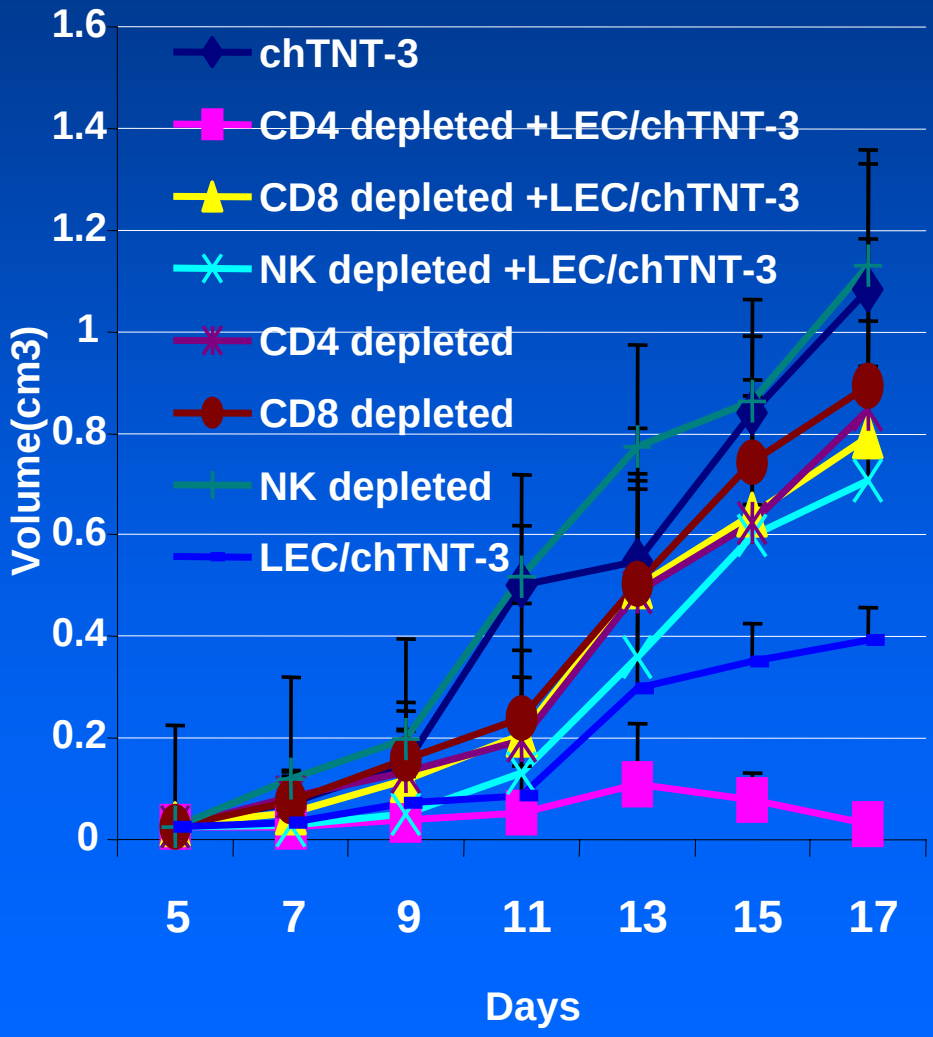
Lymphocyte Depletion Studies

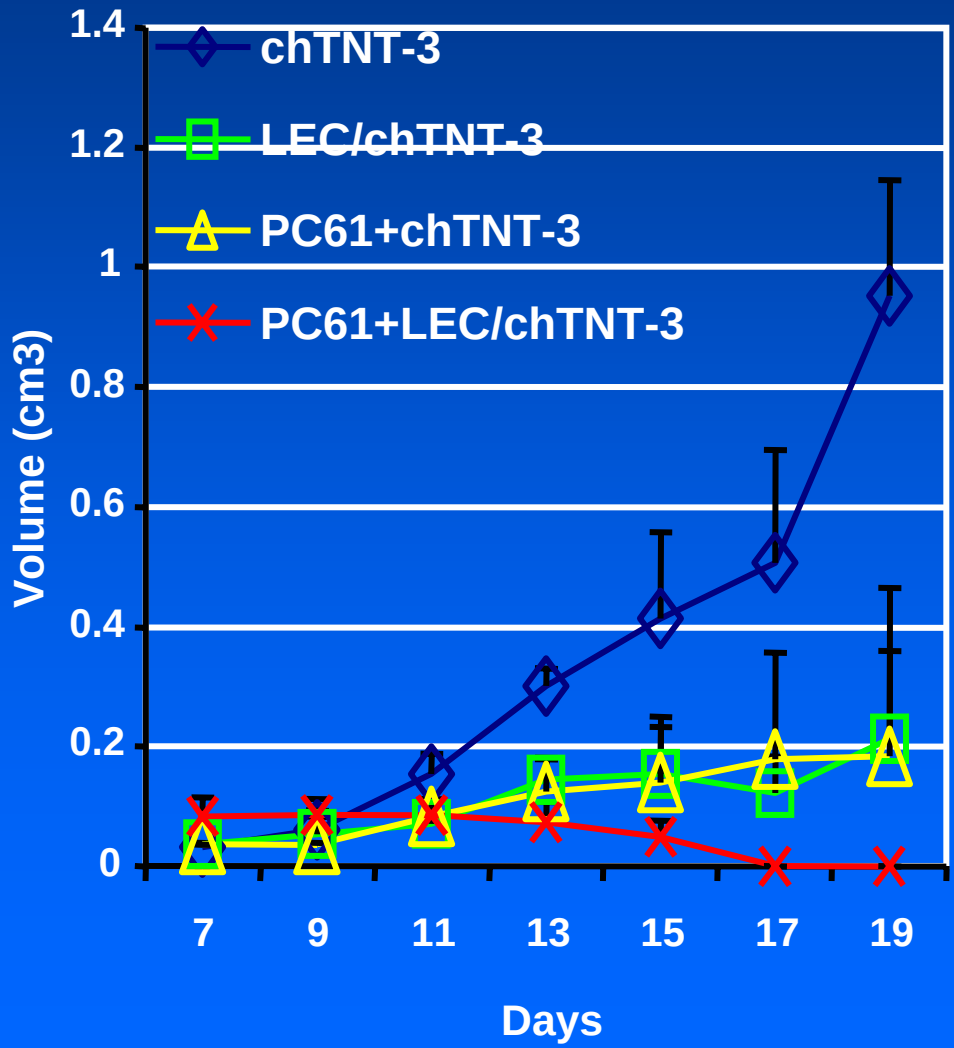
- CD4+ T cell depletion: GK1.5 (0.5mg ip q5 days)
- CD8+ T cell depletion:
- 2.43 (0.5 mg ip q5 days)
- NK depletion:
- anti-asilao GM1(0.35mg ip q5 days)
- CD4+CD25+ depletion: PC61 (0.5 mg ip Day 0)





T-cell Subset Depletion Studies in Colon 26





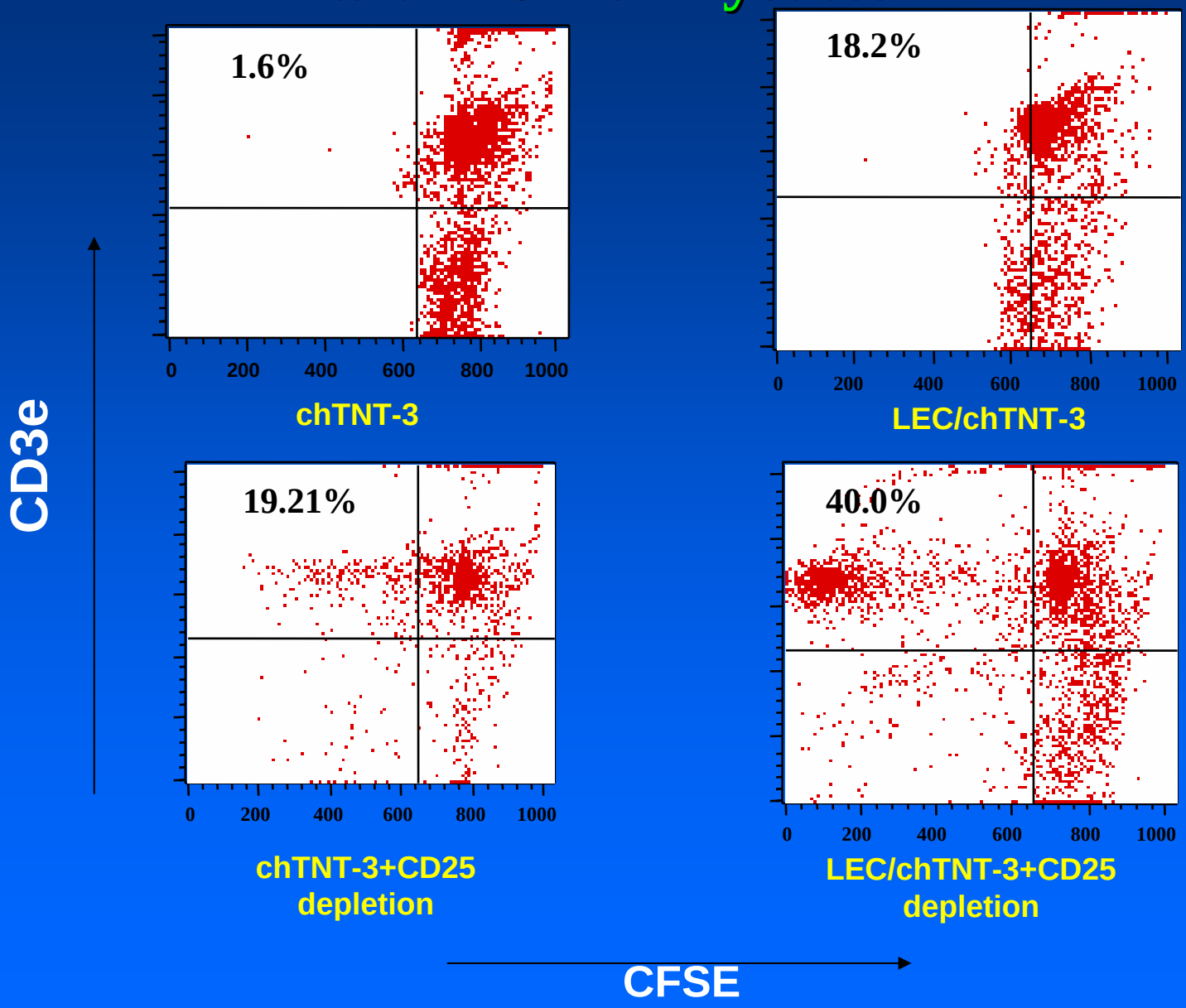
Control

CD4 depletion control

LEC/chTNT-3

LEC/chTNT-3+CD4 depletion

Cell Proliferation Assay of TDLN after Incubation with Tumor Lysates



Tumor Re-challenge Studies (3 months)

Colon 26

MAD109

Naïve Mice

Colon 26 Regressed Mice

Combination Cytokine or Chemokine Fusion Protein Immunotherapy and T-cell Subset Depletion in Colon 26

Immunotherapy[1]	T-cell Subset Depletion[2]	% Tumor Reduction (Day 19)
chTNT -3 (control)	-	0%
chTNT -3 (control)	$CD4^+$ depletion	33%
LEC/chTNT -3	-	60%
LEC/chTNT -3	$CD4^+$ depletion	100%
chTNT -3/IL -2	-	38%
chTNT -3/IL -2	$CD4^+$ depletion	64%
chTNT -3/IFN -γ	-	32%
chTNT -3/IFN -γ	$CD4^+$ depletion	33%
chTNT -3/TNF -α	-	10%
chTNT -3/TNF -α	$CD4^+$ depletion	33%

[1] Antibodies and fusion proteins (20 ug/dose) were injected iv for 5 consecutive days after tumors reached 0.5cm in diameter.

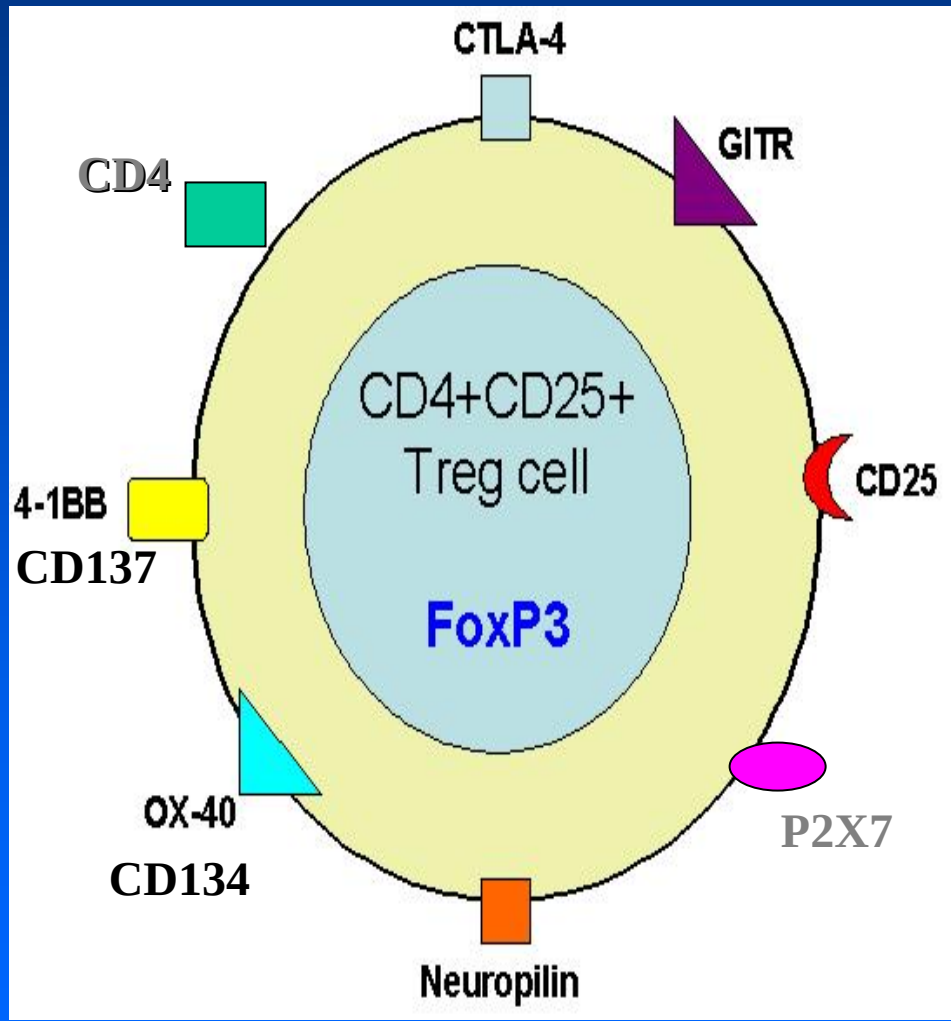
[2] $CD4^+$ depletion (0.5 mg/dose of GK1.5) was performed ip 1 day after tumor implantation and repeated every 5 days.

Treg Markers

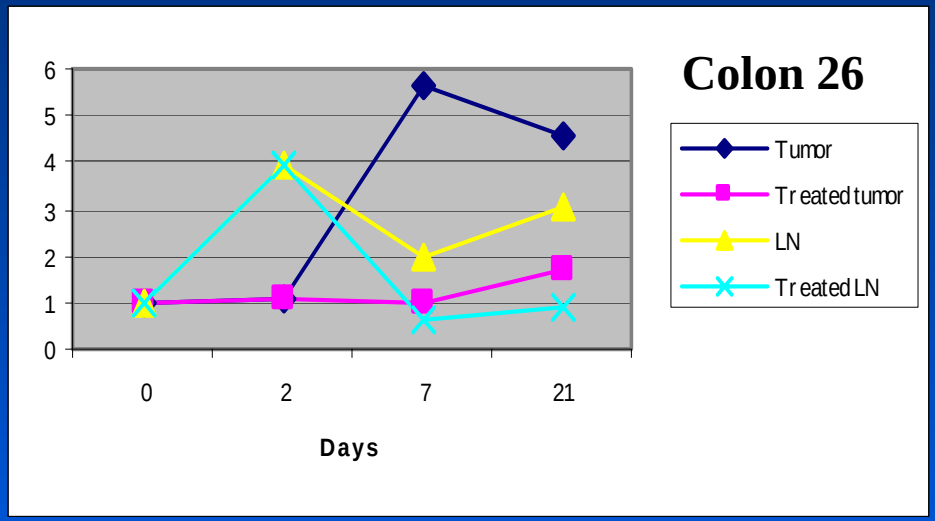
* The concept of suppressor T cells was elusive until: Sakaguchi et al identified a subpopulation (about 10%) of $CD4^+$ cells that express CD25.

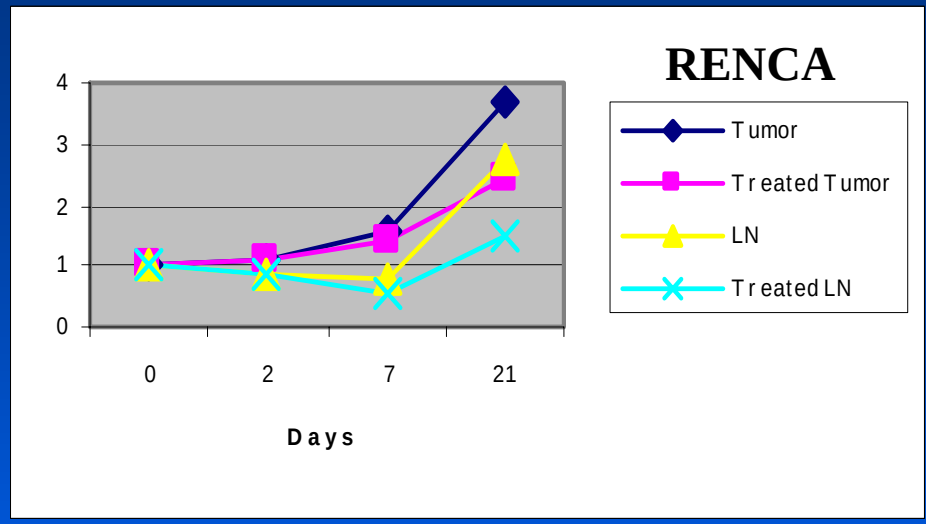
* Most cell markers for Treg cells are also expressed on $CD4^+CD25^-$ cells upon activation.

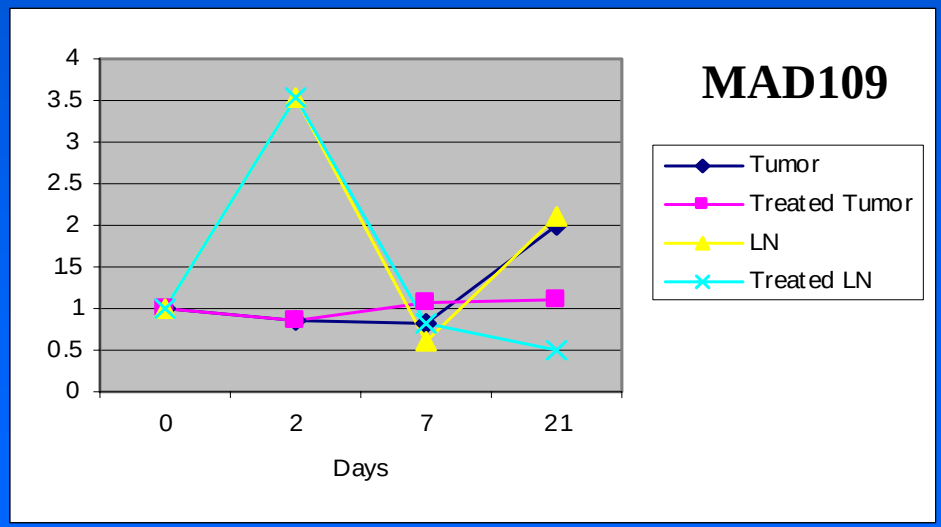
* None of the known cell surface markers appear to be responsible for $CD4^+CD25^+$ mediated suppression.

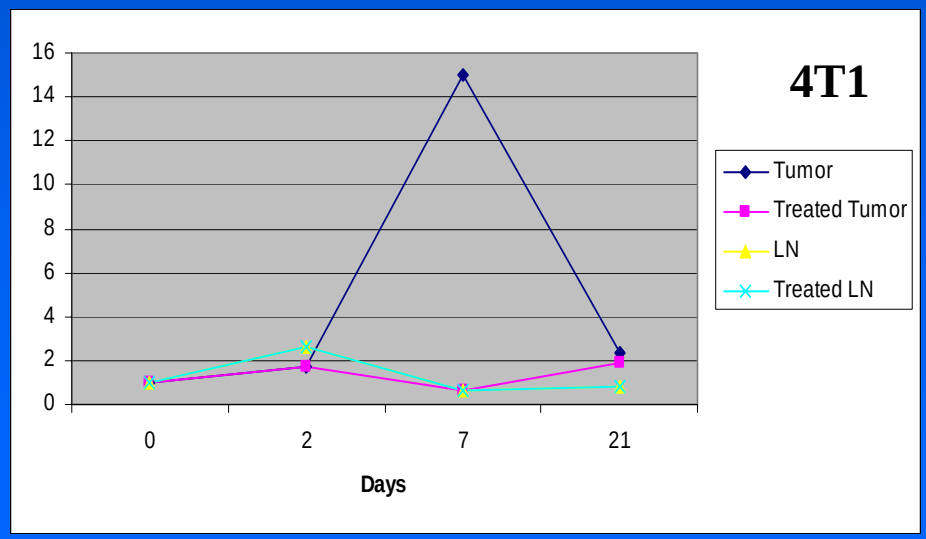


Real-Time PCR Analysis of Foxp3 in 4 Treated and Untreated Murine Tumor Models









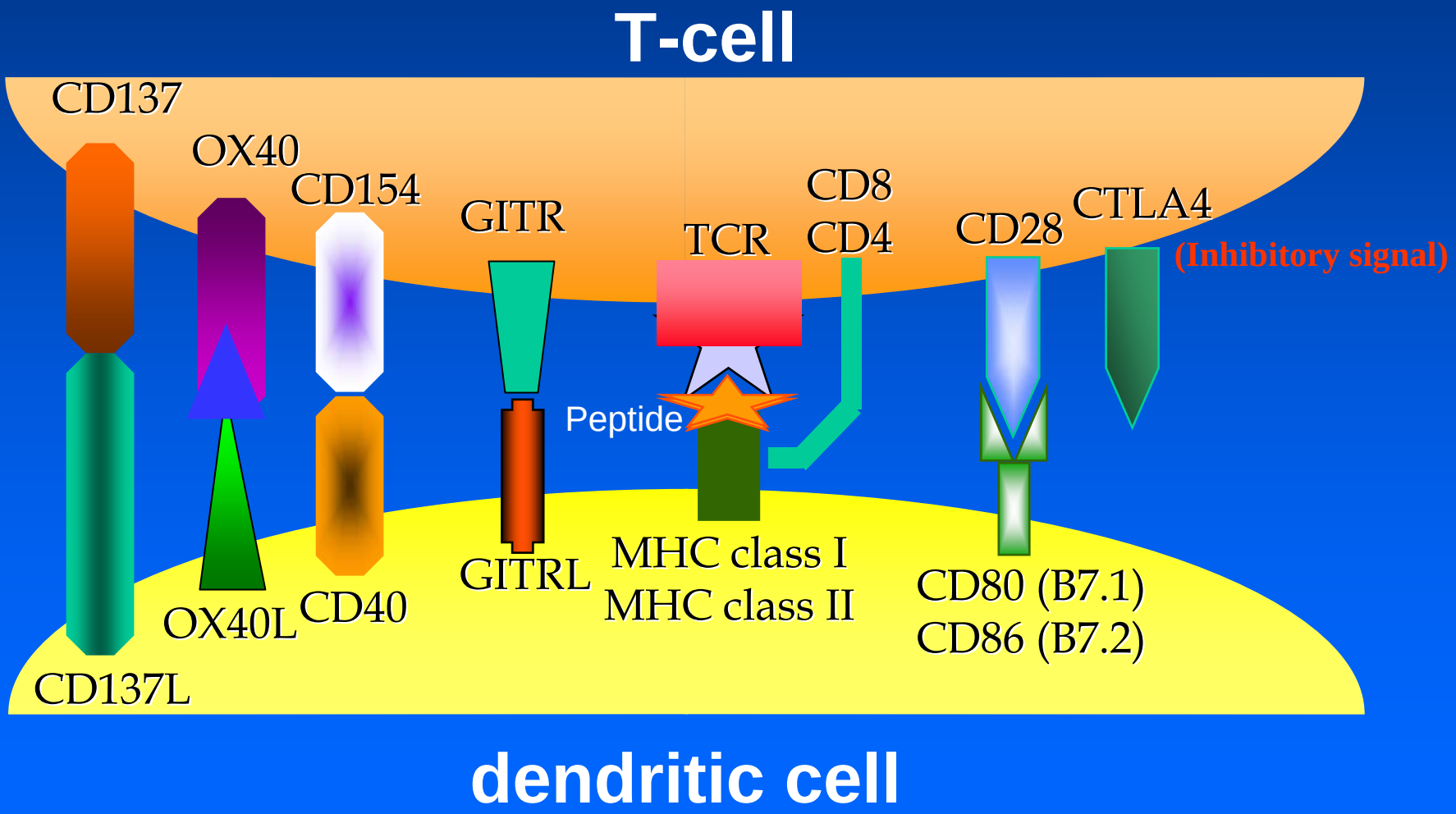
Y Axis: Fold Increase over control

Untargeted and Targeted Co-stimulation

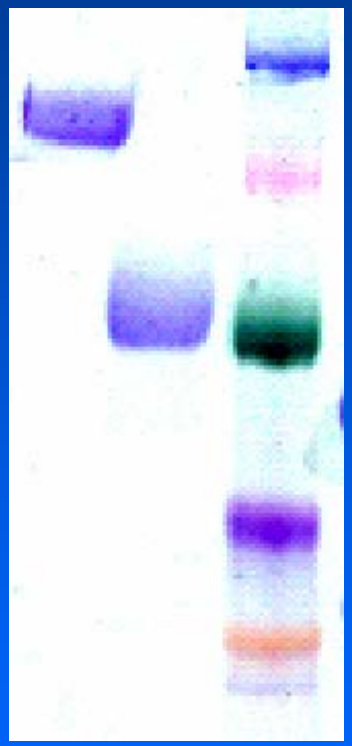
B7

GITRL

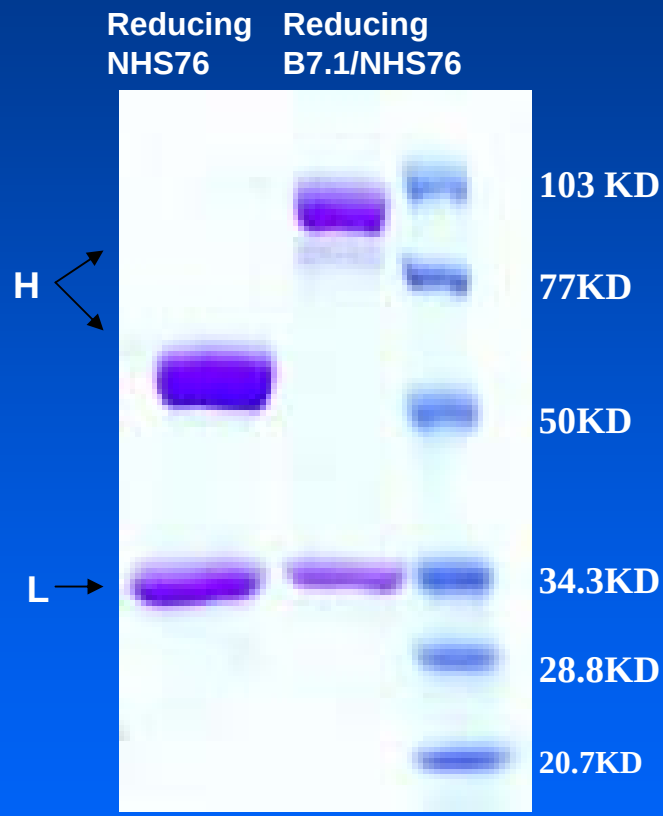
Co-stimulatory Molecules



B7.1-Fc



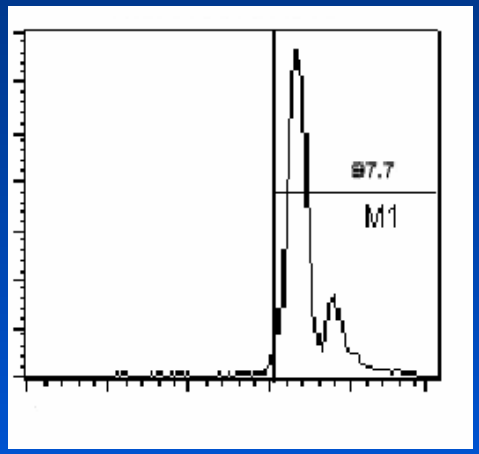
B7.1/NHS76



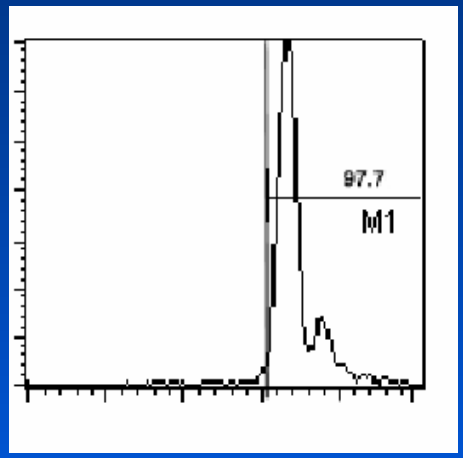
SDS PAGE

CFSE Proliferation Assay

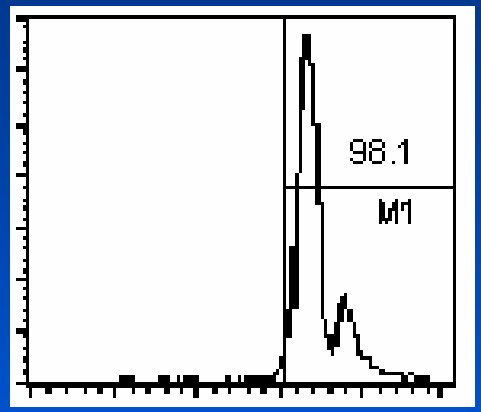
NHS76



B7.1/NHS76

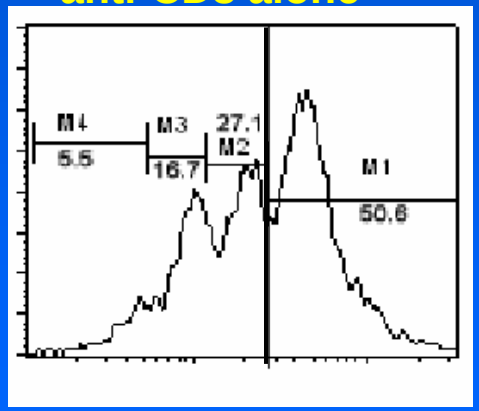


B7.1/Fc

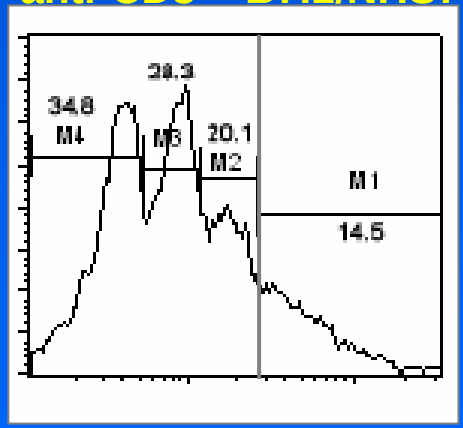


counts

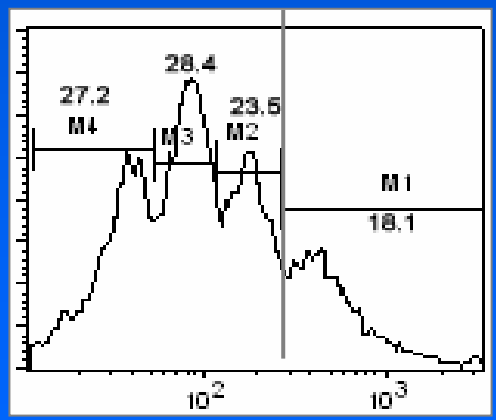
anti-CD3 alone



anti-CD3 + B7.1/NHS76

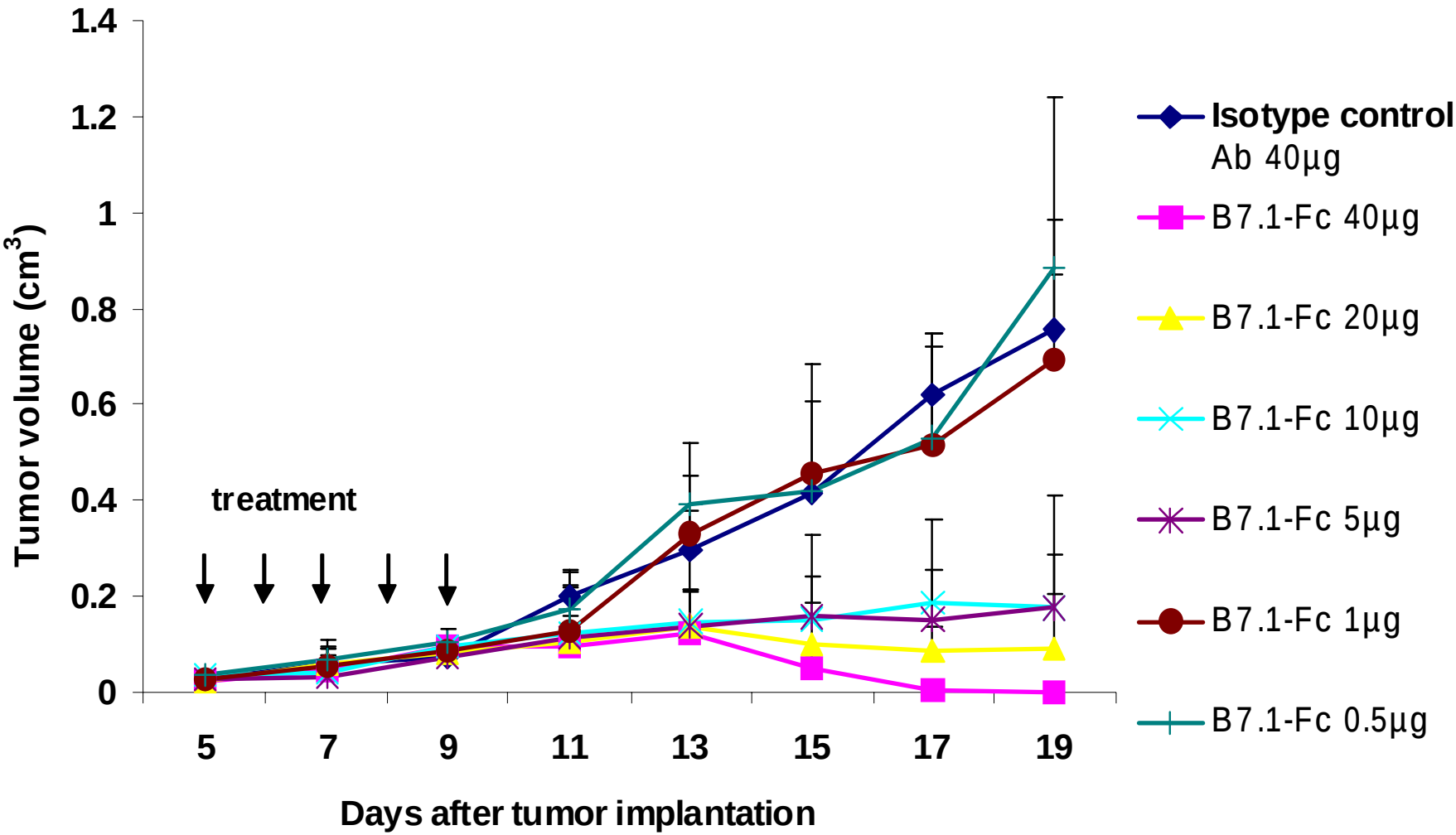


anti-CD3 + B7.1/Fc

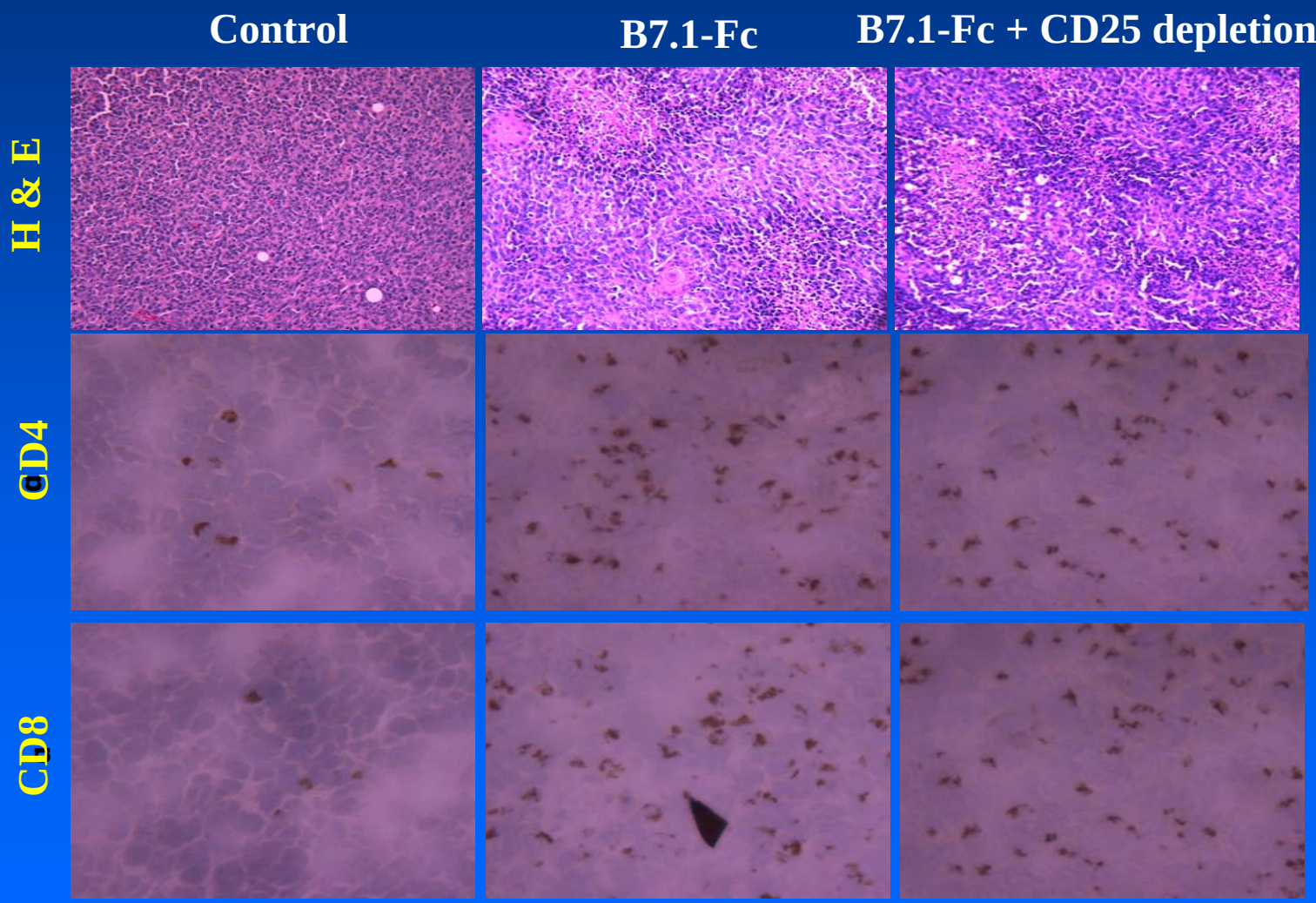


CFSE

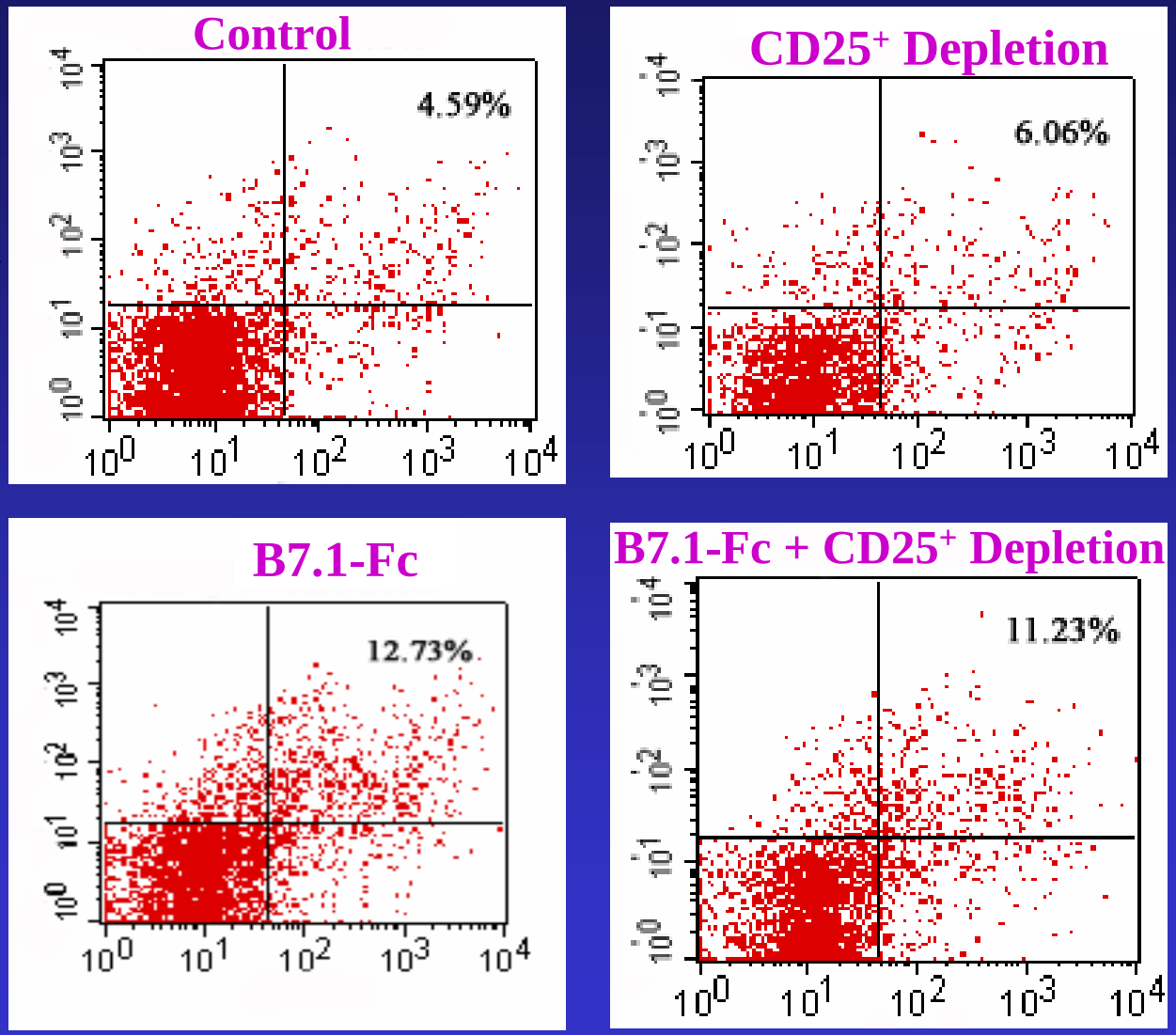
B7.1-Fc Dosing Study in Colon 26 Tumor Model



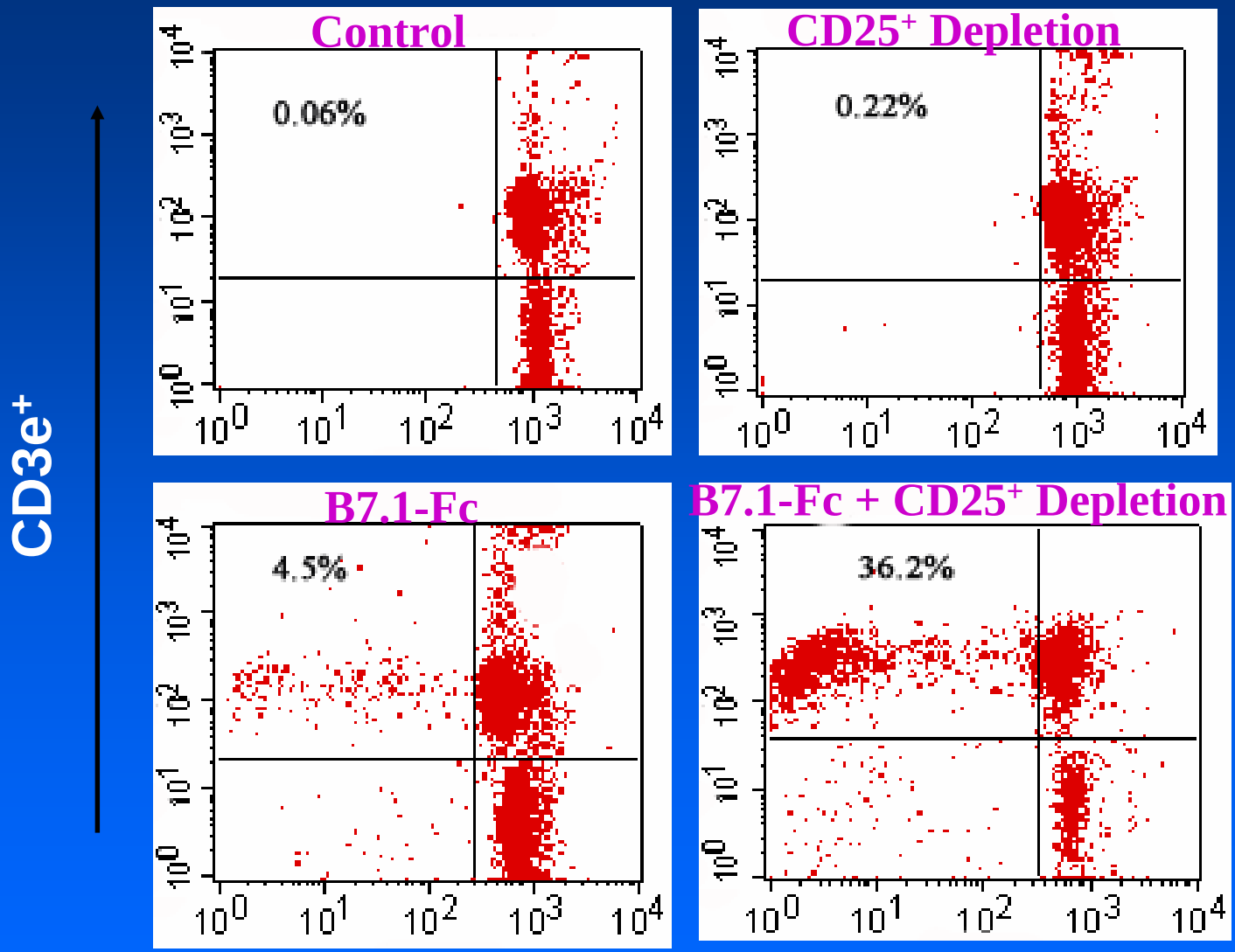
IHC of Control and Treated Colon 26



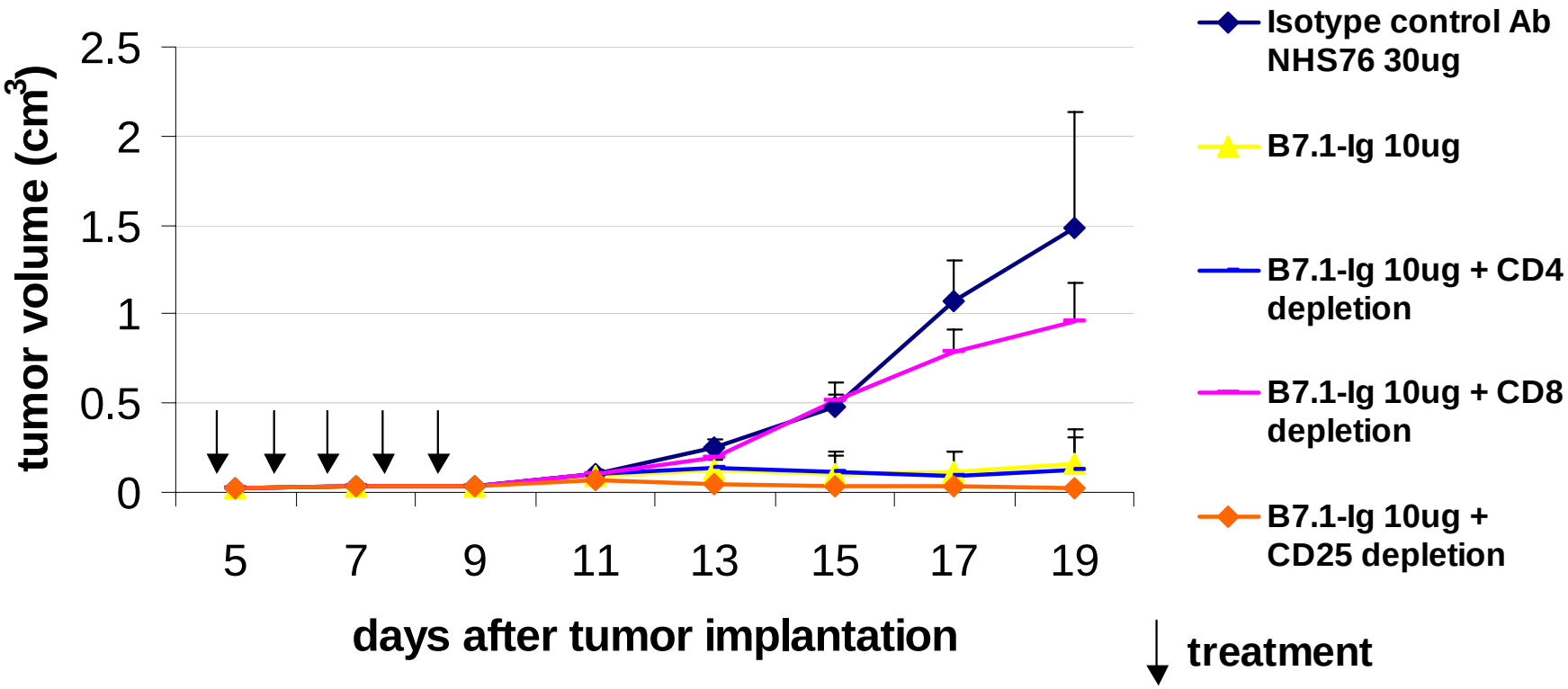
Tumor Infiltrating Lymphocytes (TIL)



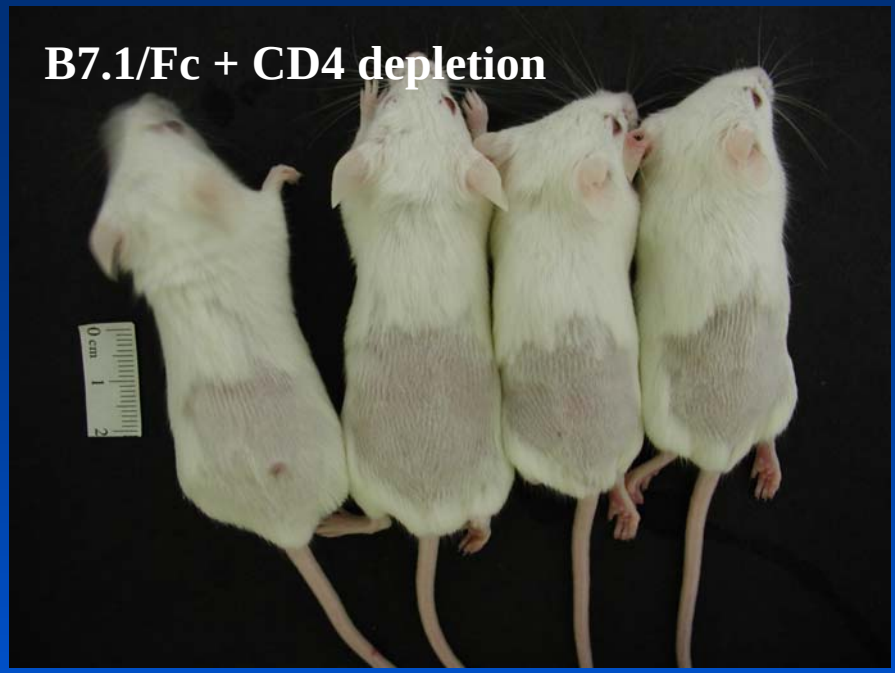
Activation of TIL With Tumor Lysate *In Vitro*



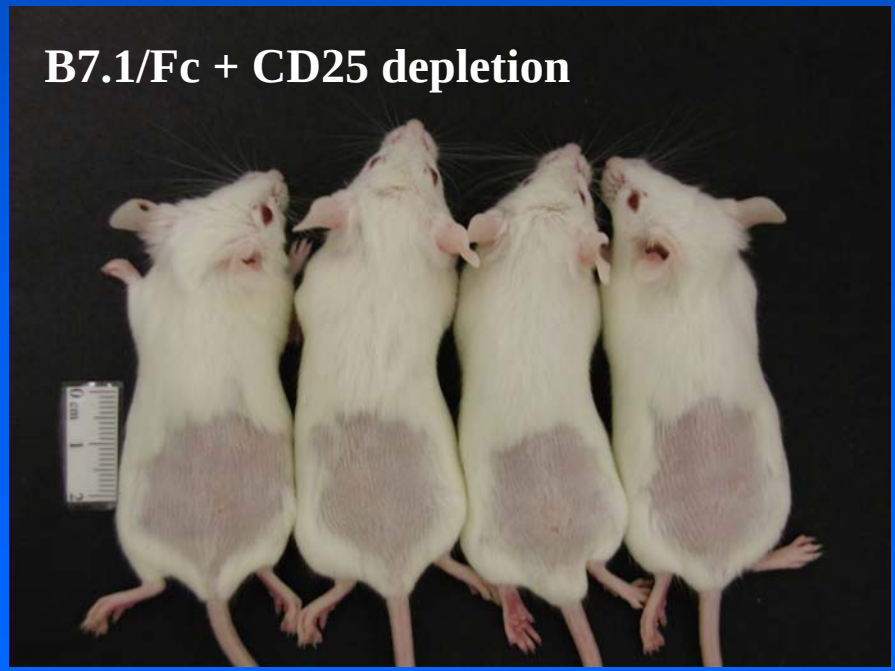
T-Cell Depletion Studies in B7.1-Fc Treated Colon 26-Bearing Mice




B7.1/Fc

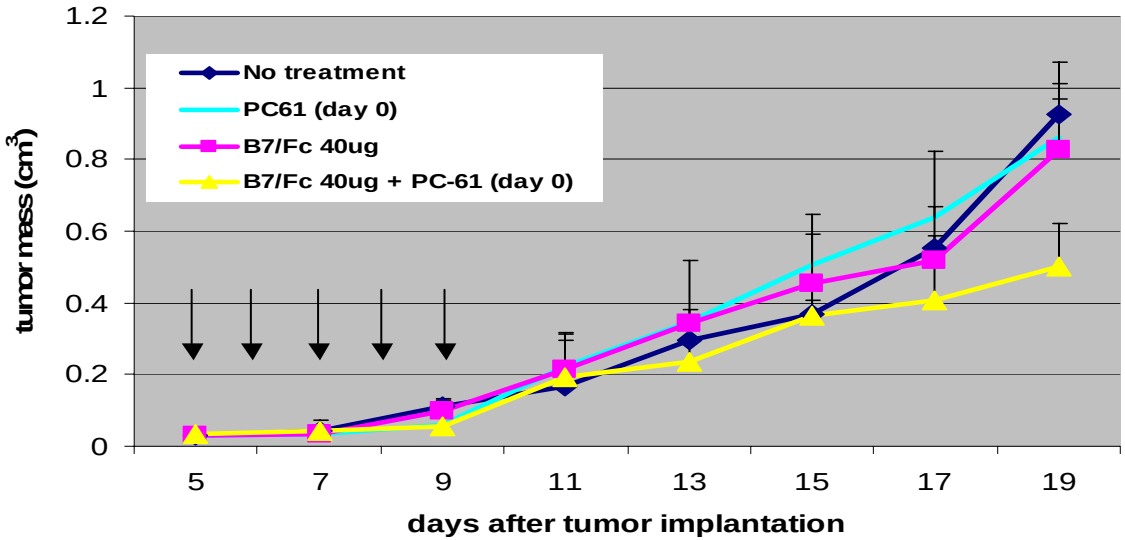

B7.1/Fc + CD4 depletion


B7.1/Fc + CD8 depletion

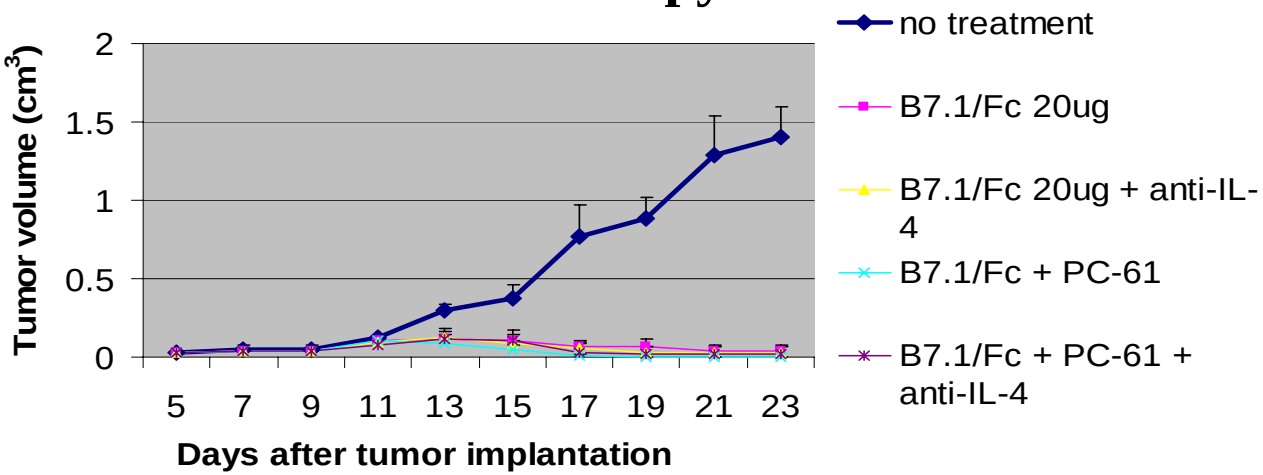

B7.1/Fc + CD25 depletion

Mechanism of Action Studies

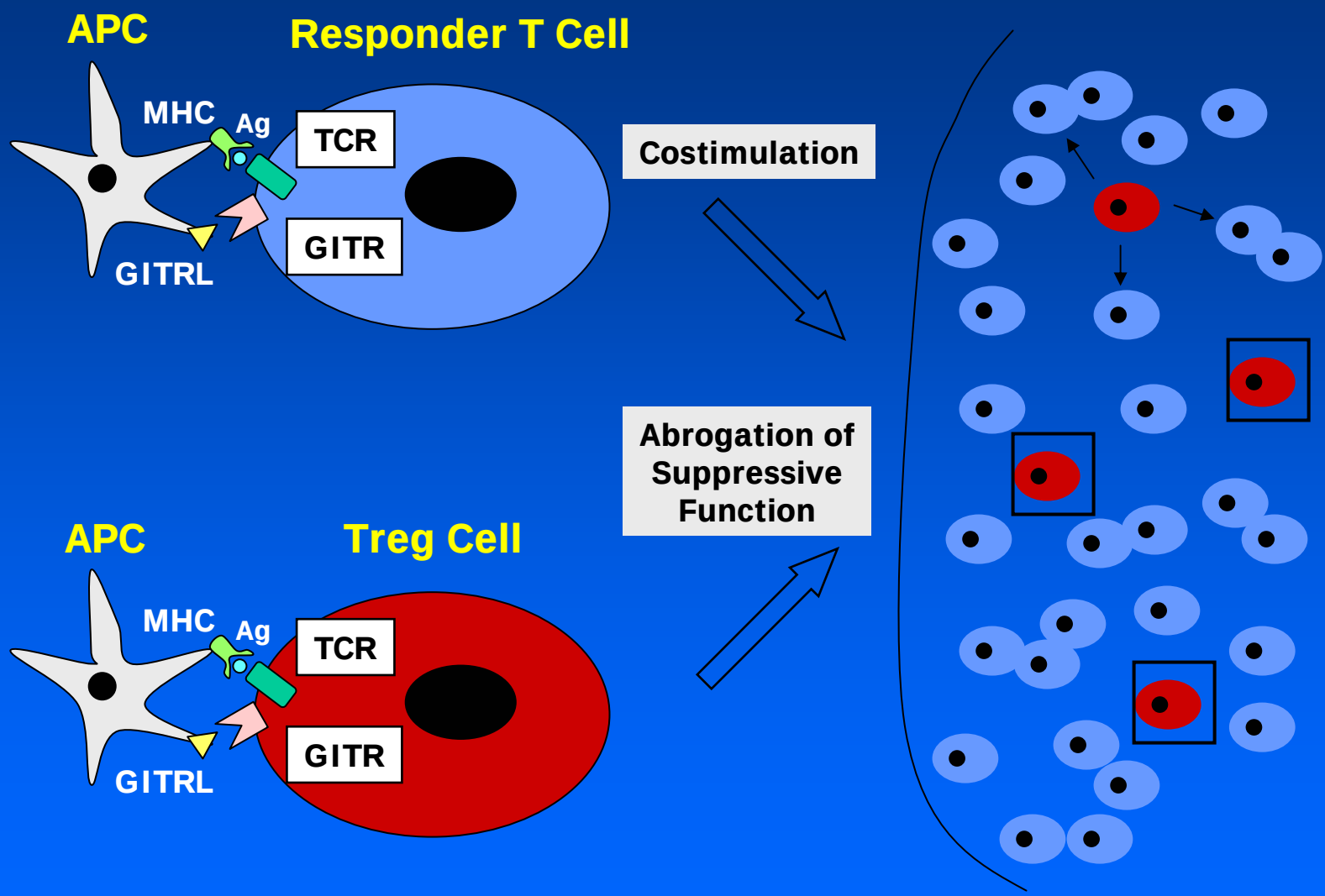
IFN-gamma Vital for B7.1 Therapy as Demonstrated in KO mice



Anti IL-4 Therapy Does Not Reverse B7.1-Fc Therapy

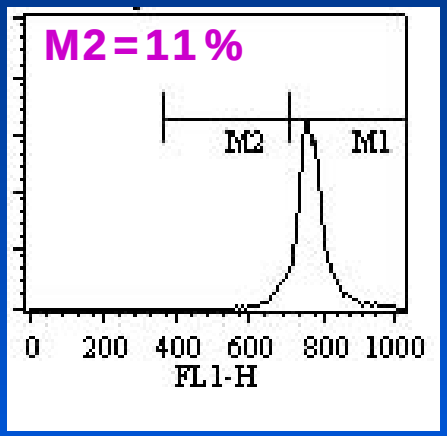


Dual Function of GITR

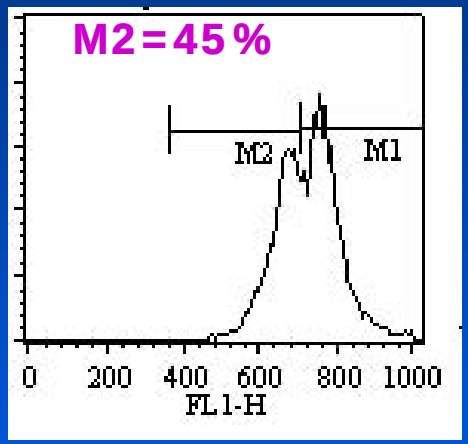


Activity Assay of GITRL Fusion Proteins at 48 Hours

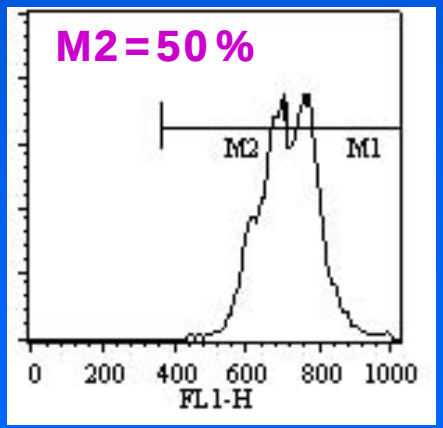
CD3 alone



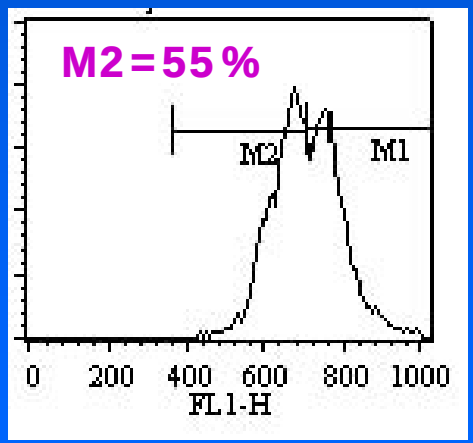
DTA-1



Fc-GITRL



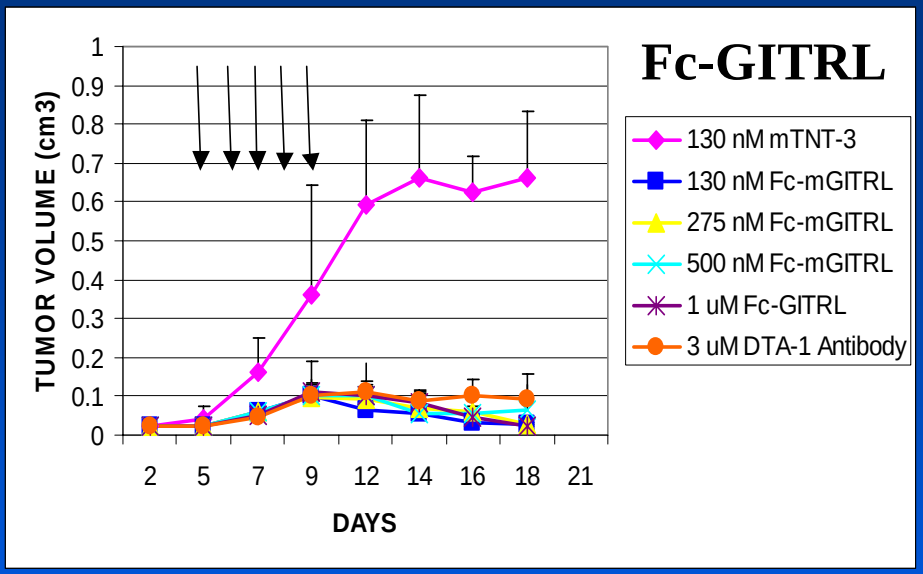
TNT3/GITRL

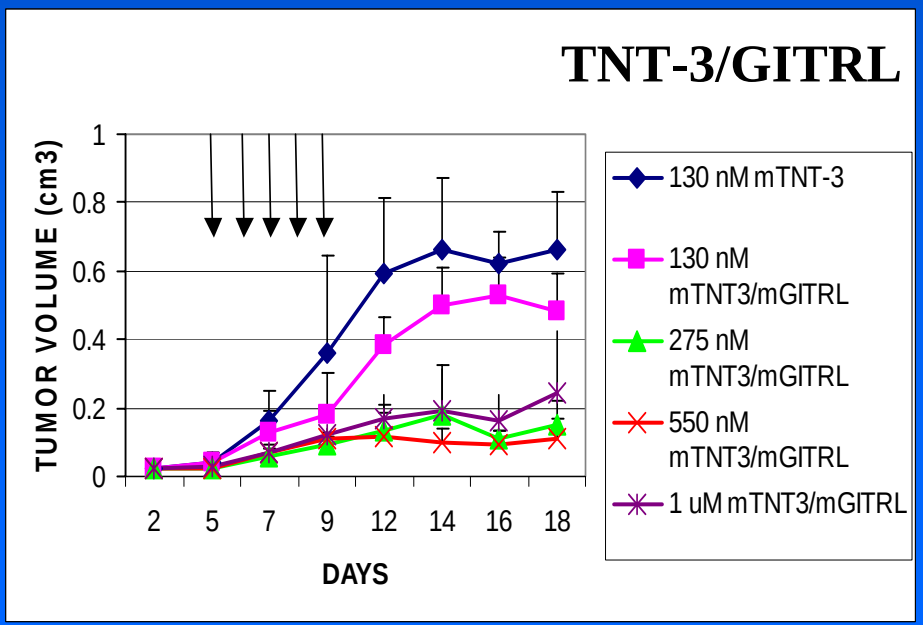


CFSE

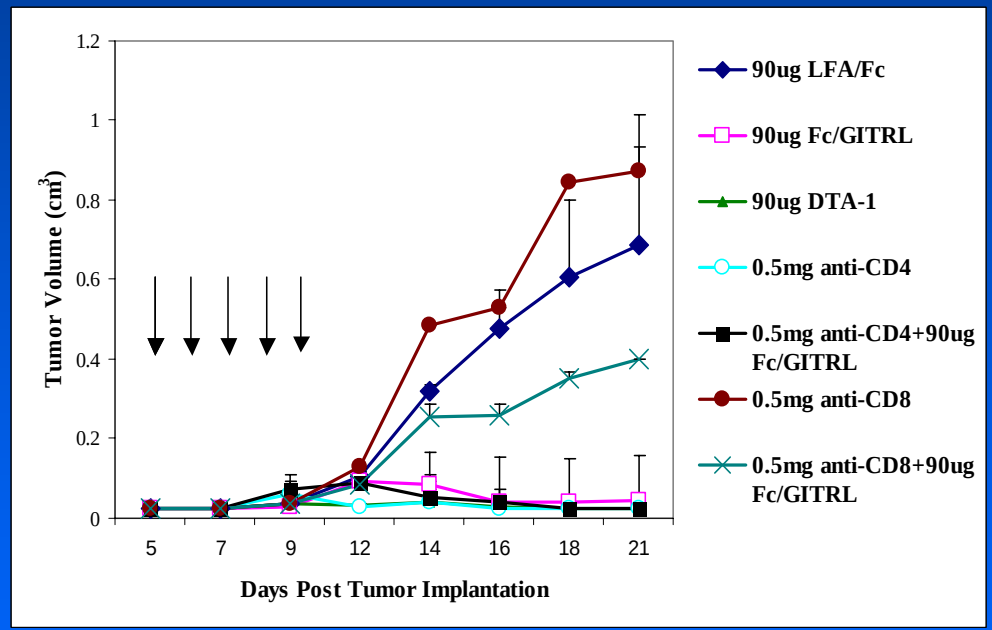
- **Performed on naïve splenocytes.**
- **2ug of protein was used for each sample.**
- **CFSE stained $CD4^+$ T cells**

Targeted and Non-targeted GITRL Dosing Studies in Colon 26 Tumor Model



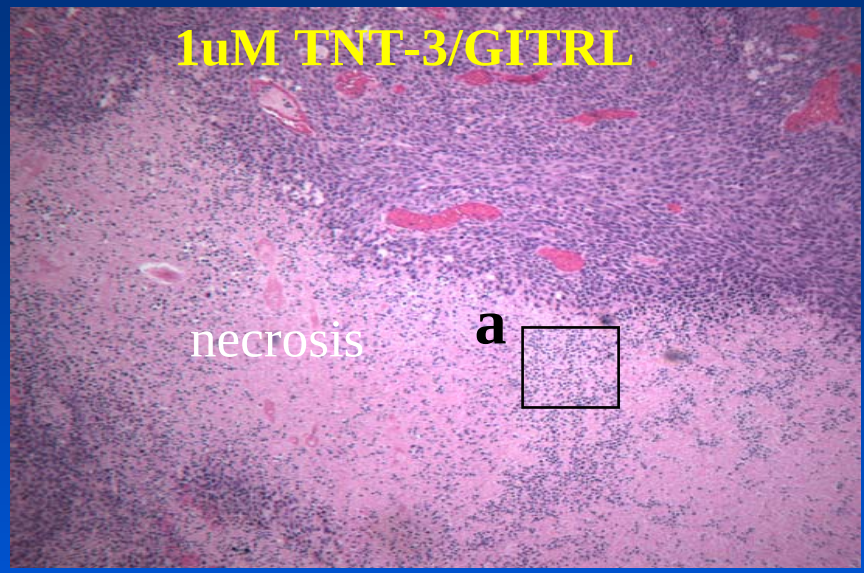


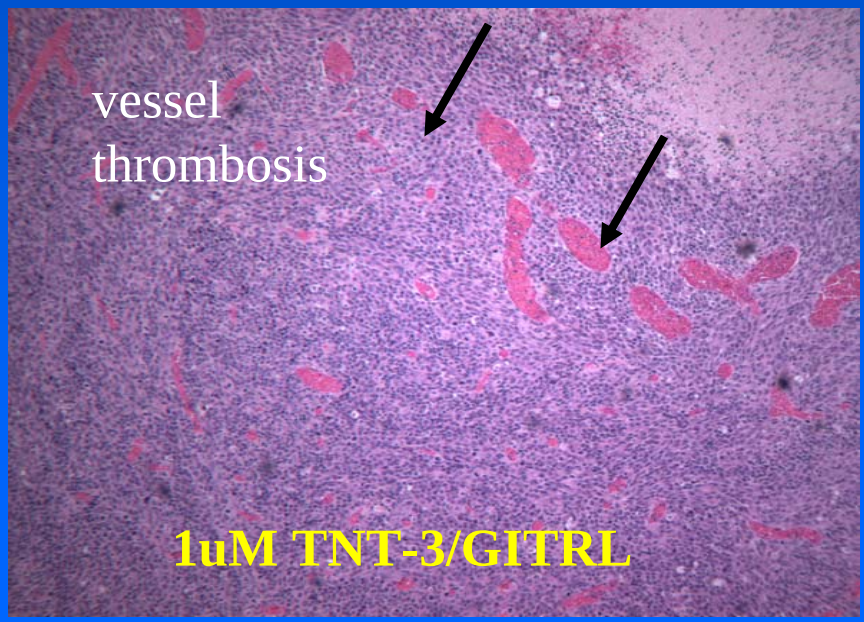
T-cell Subset Deletion Studies

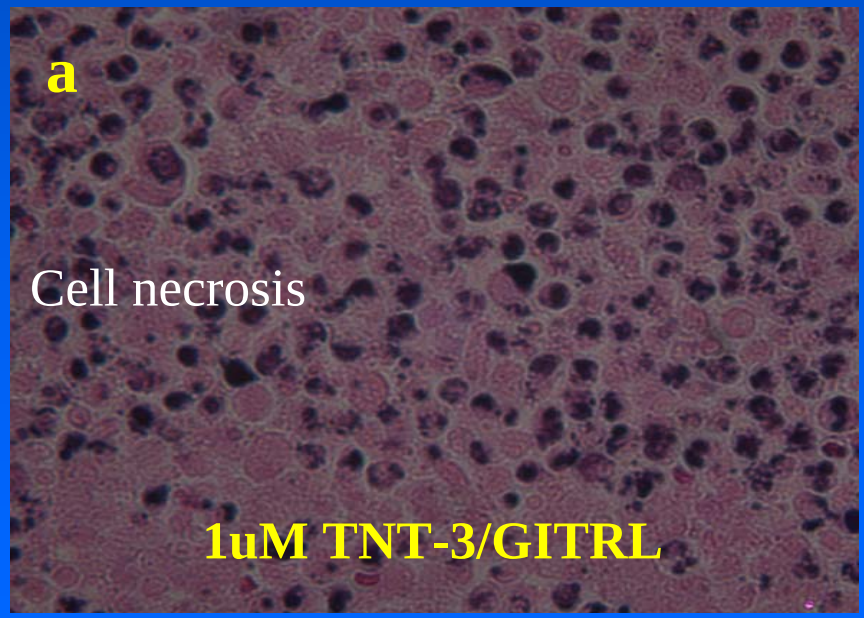


TREATMENT

H & E of GITRL Treated COLON-26 Bearing Mice





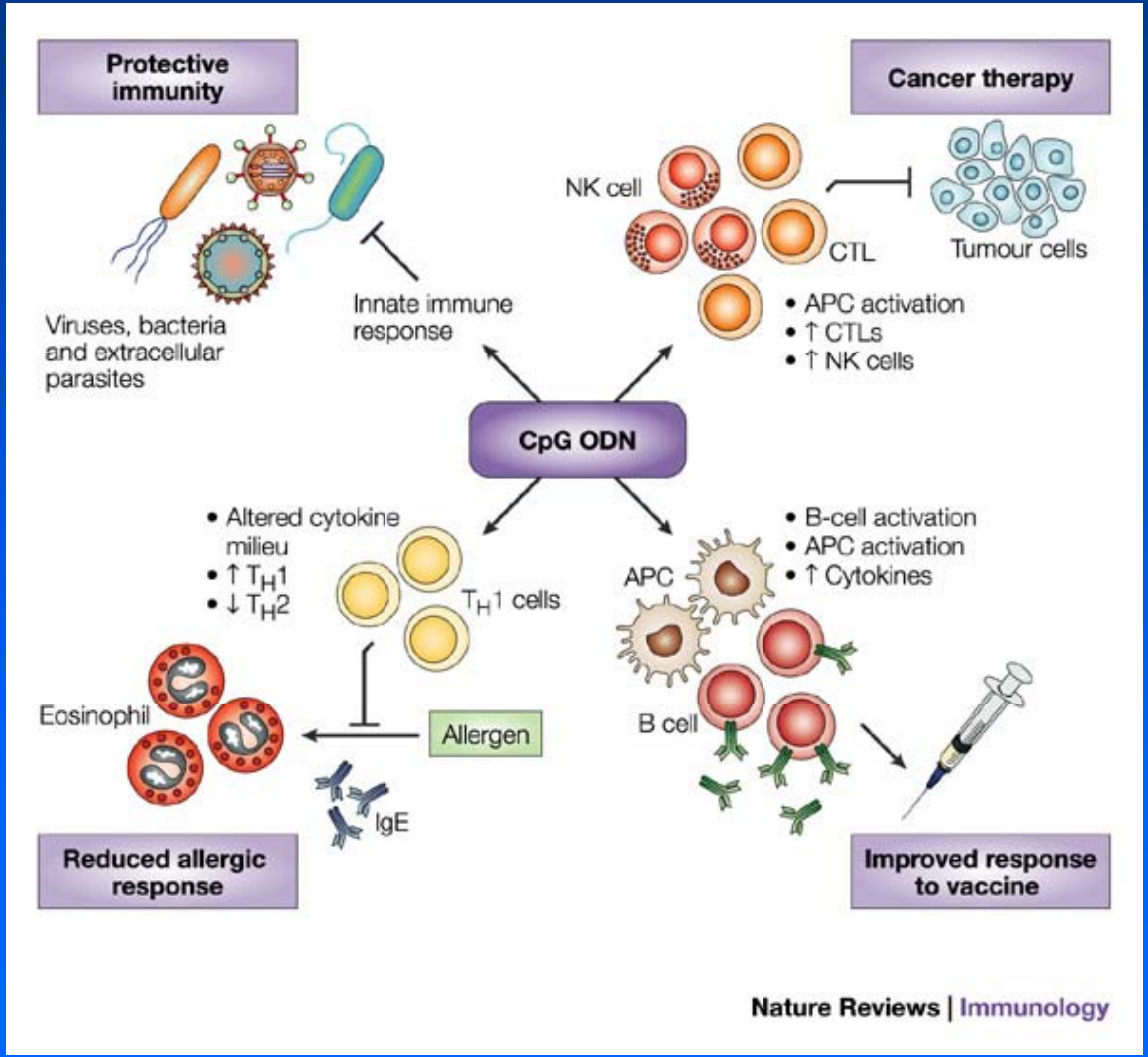


Targeting Innate Immunity

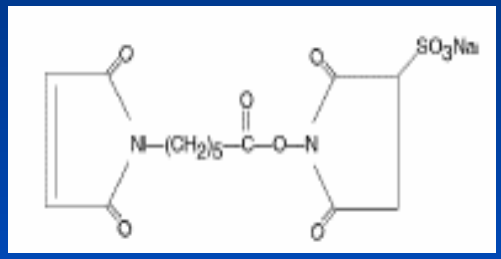
TNT-3/CpG

Multiple Functions of CpG

- **Potential for CpG ODNS**
  - **Protective Immunity**
    - **TLR9 detects CpG→ triggers ↑ response**
  - **Allergies**
    - **TH1 response**
  - **Vaccine Response**
    - **Th1 and pro-inflammatory cytokines→Improves APC function**
    - **Promotes induction of Ag-specific response**
  - **Cancer Therapy**
    - **↑ CTLs and NK cells**



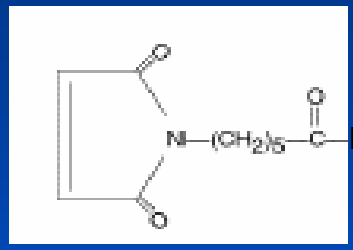
Heterobifunctional Linkage of CpG to Antibody



Sulfo - EMCS

+

MAb — NH_2 →



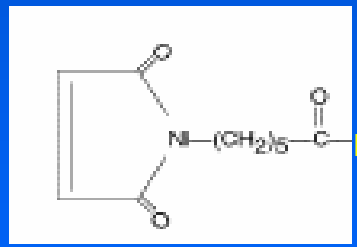
DMTrO—$(CH_2)_3$—S—S—$(CH_2)_3$—O—Succinyl—CpG

3'-Thiol-Modifier C3

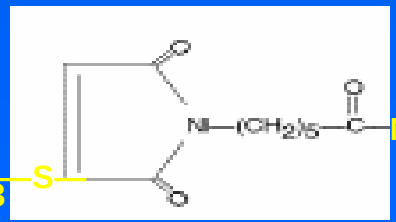
DTT →

CpG—succinyl—O—$(CH_2)_3$—S—

CpG—succinyl—O—$(CH_2)_3$—S— +



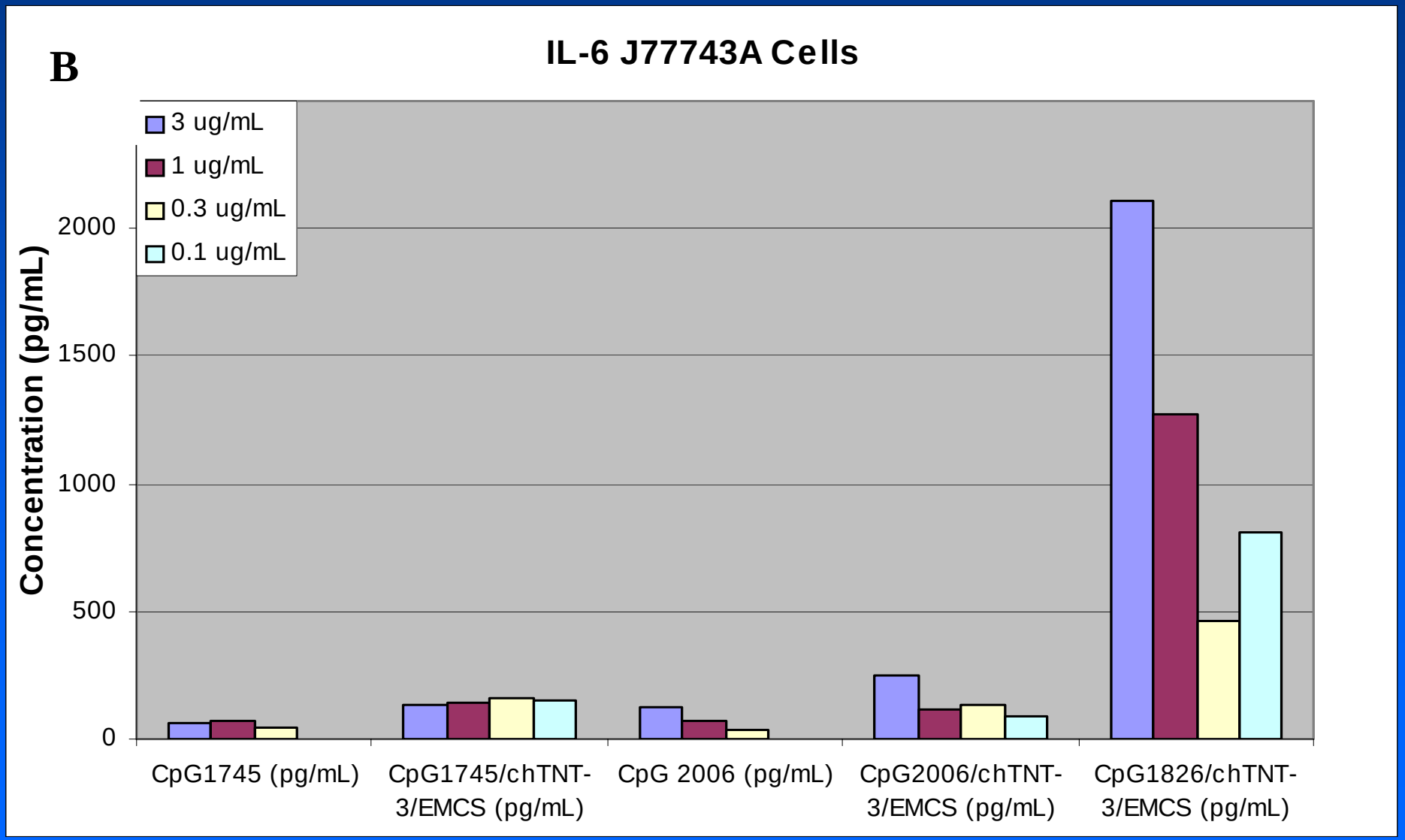
→



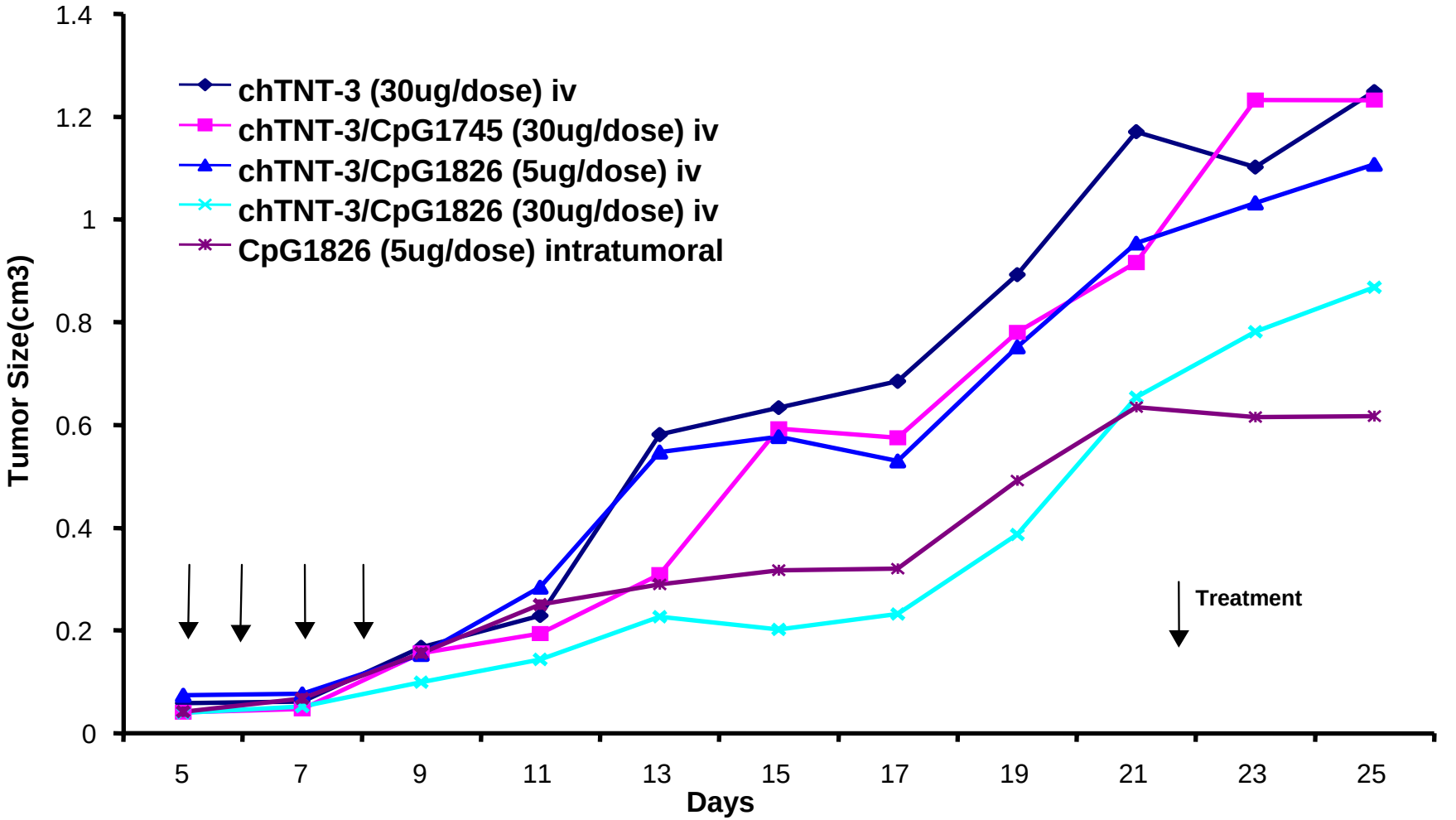
CpG—succinyl—O—$(CH_2)_3$—S

CpG/MAb

In Vitro Assay Demonstrating CpG Activity of Immunoconjugate



chTNT-3/CpG Immunotherapy



SUMMARY: Major Pathways of Immune Activation for Cancer Immunotherapy

- Chemotaxis (chemokines)
- Co-Stimulation (second signal)
- Combination T-cell activation and inhibition of Treg (GITRL)
- Activators of innate immunity (CpG)

SUMMARY: Major Inhibitory Mechanisms That Generate Tolerance to Tumors

- Treg cells
- T-cell death receptors (PD-1, 2)
- Soluble cytokines (IL-10, TGFβ)
- Inhibition of CD28 Co-stim (CTLA-4, B7.1-Fc?)
- IDO (Indoeamine 2,3-dioxygenase)
  - degrades tryptophan
- Loss or release of MHC class I molecules

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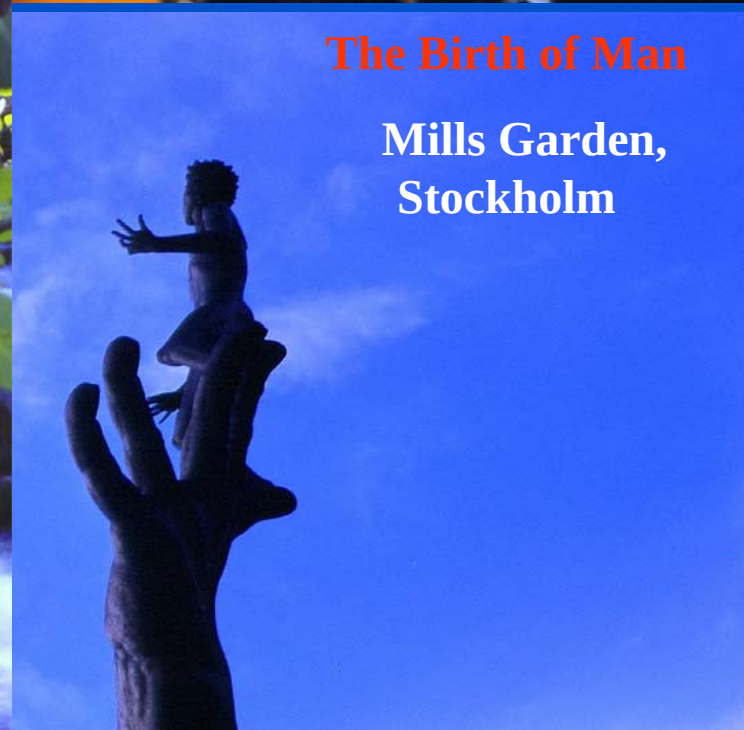
The Birth of Man
Mills Garden,
Stockholm