Therapeutic efficacy of MUC1-specific CTL and CD137 co-stimulation in a spontaneous mammary cancer model

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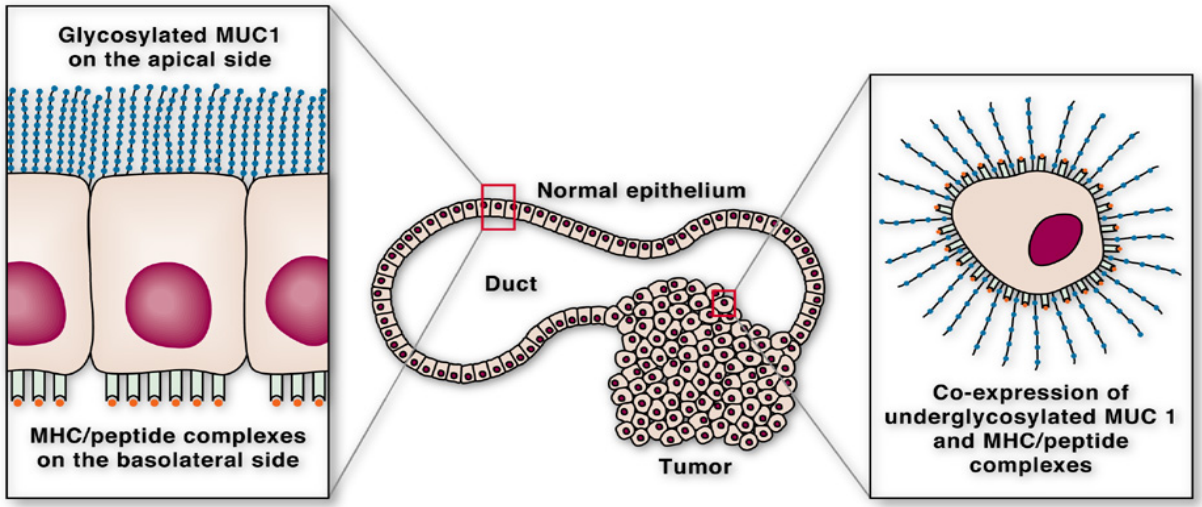
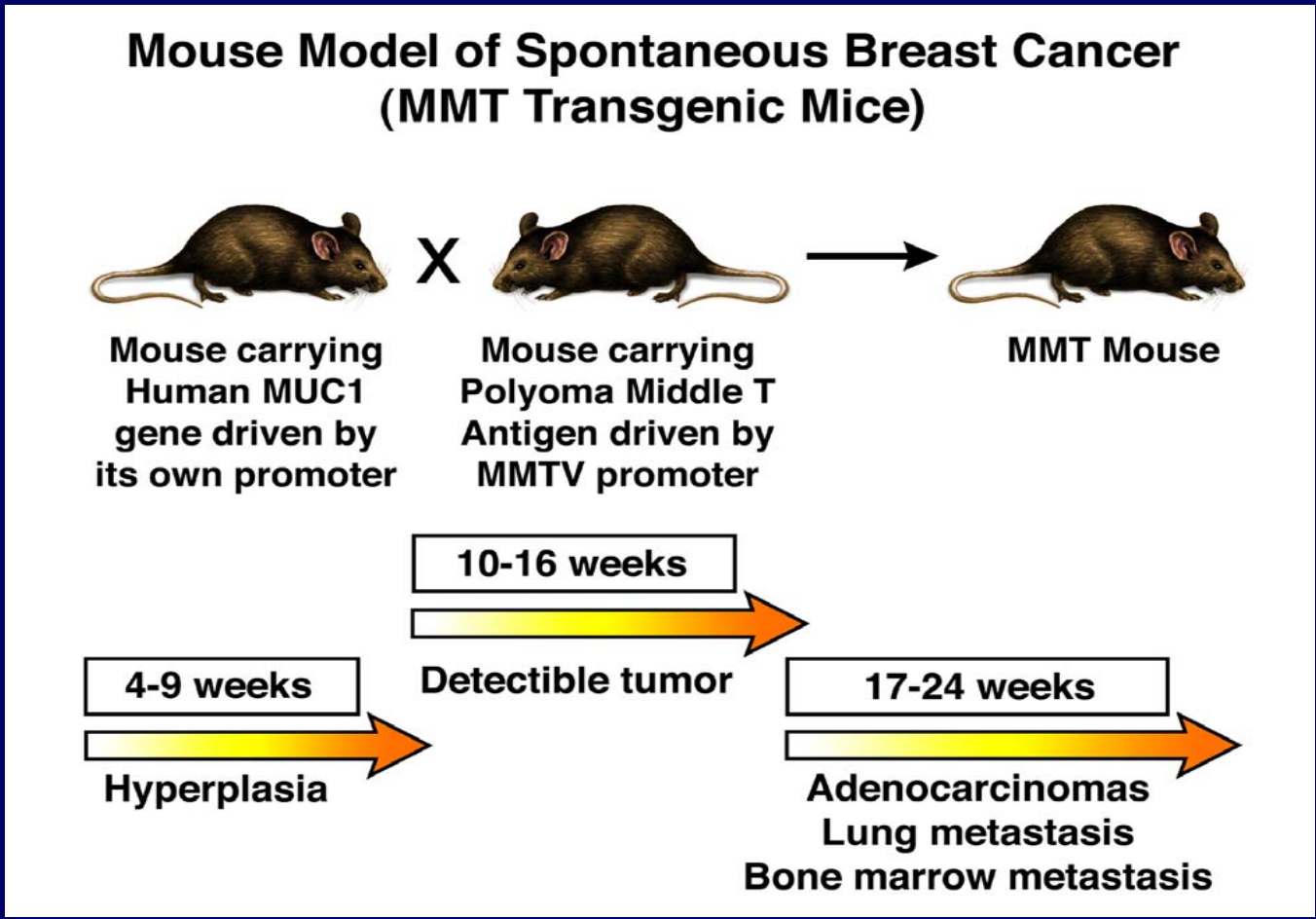
Goal of Immunotherapy

- Boosting the low level anti-tumor immune response
- Generate long lasting strong anti-tumor CTL and helper response
- Avoid tumor-induced immune suppression

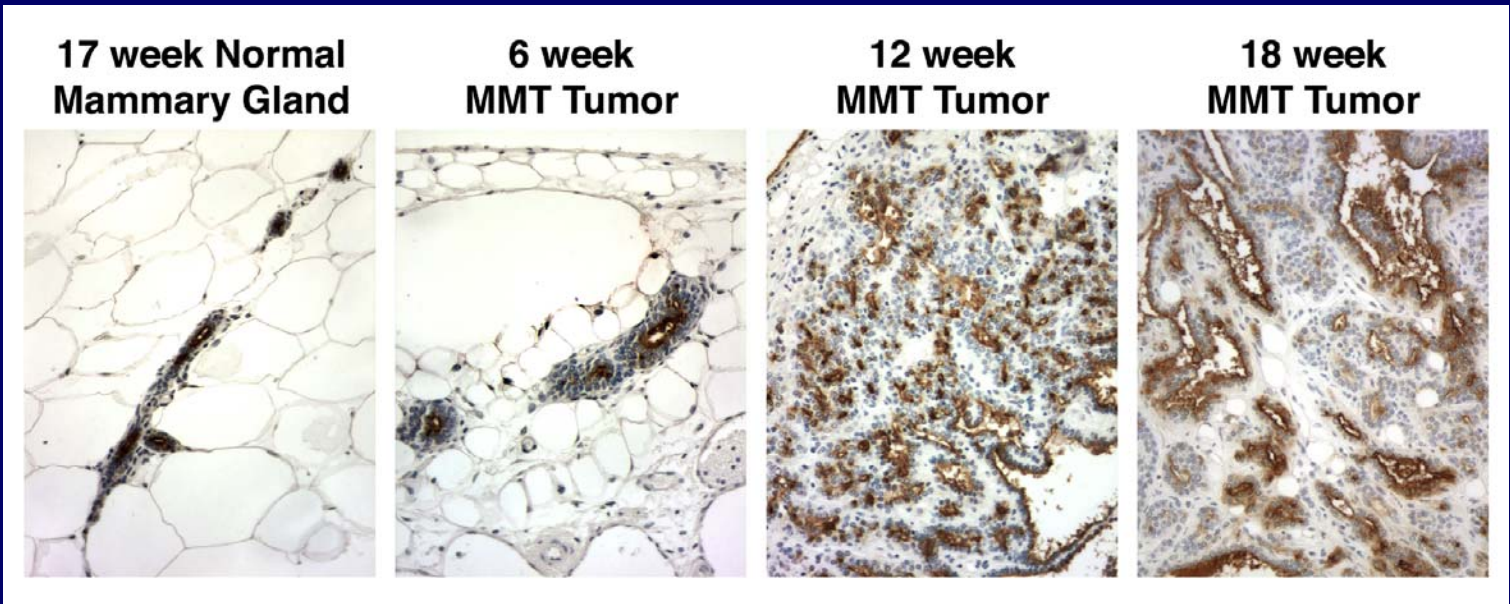
MUC1 is a target for Immunotherapy



Schematic representation of a mouse model of spontaneous metastatic breast cancer and approximate time-line of tumor progression from hyperplasia to adenocarcinomas and metastasis.



Increased MUC1 expression as tumors progress



Immunotherapy (Preclinical Trials)

Single tumor antigen-based vaccine

Dendritic cells pulsed with liposomal-MUC1 peptide (JI 2000, JIT 2003, and Glycoconjugate J. 2003)

Tumor – based vaccine

Dendritic cells fused with primary resectable tumor cells (JI 2003, Immunol 2004, and JS 2002)

Dendritic cells fed with whole tumor cell lysate + co-stimulatory factors (41BB, OX40, CD40 antibody)

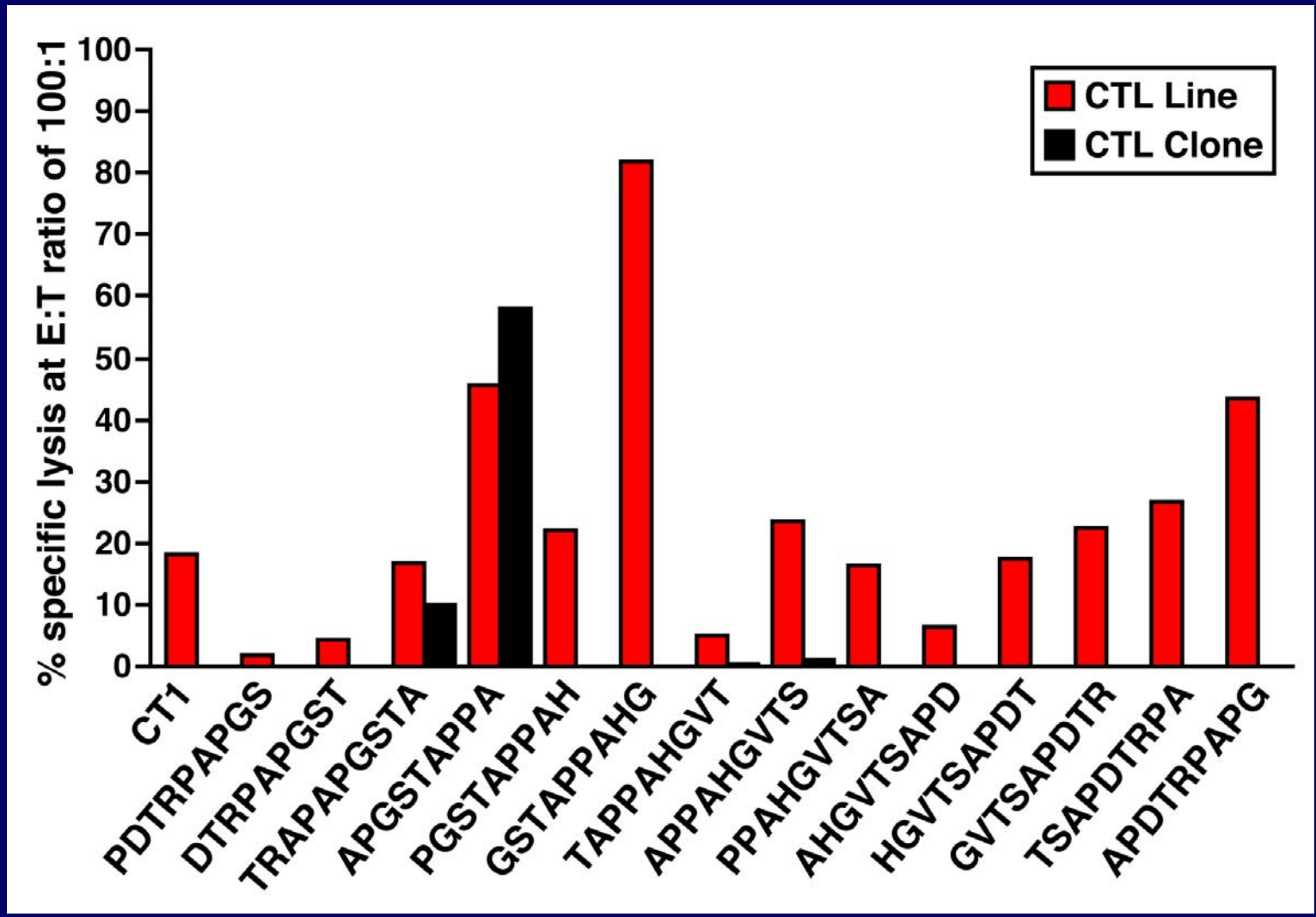
★Cytotoxic T cell adoptive therapy

MUC1-specific Cytotoxic T lymphocytes isolated from a pancreatic cancer model

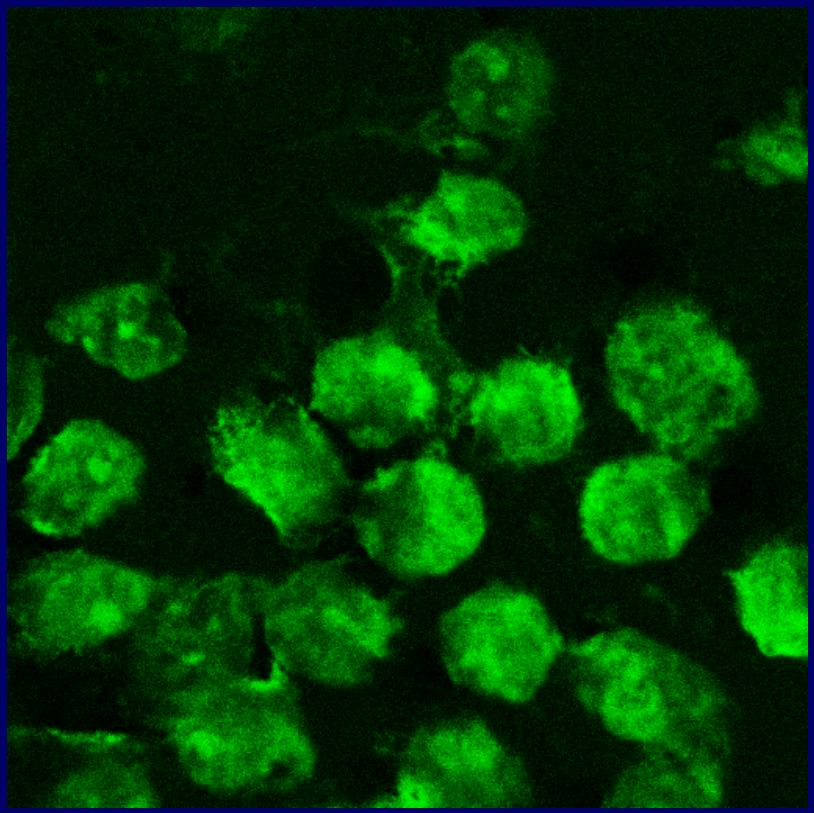
- MET mice naturally develop MUC1-specific CTLs as the pancreas tumor progresses

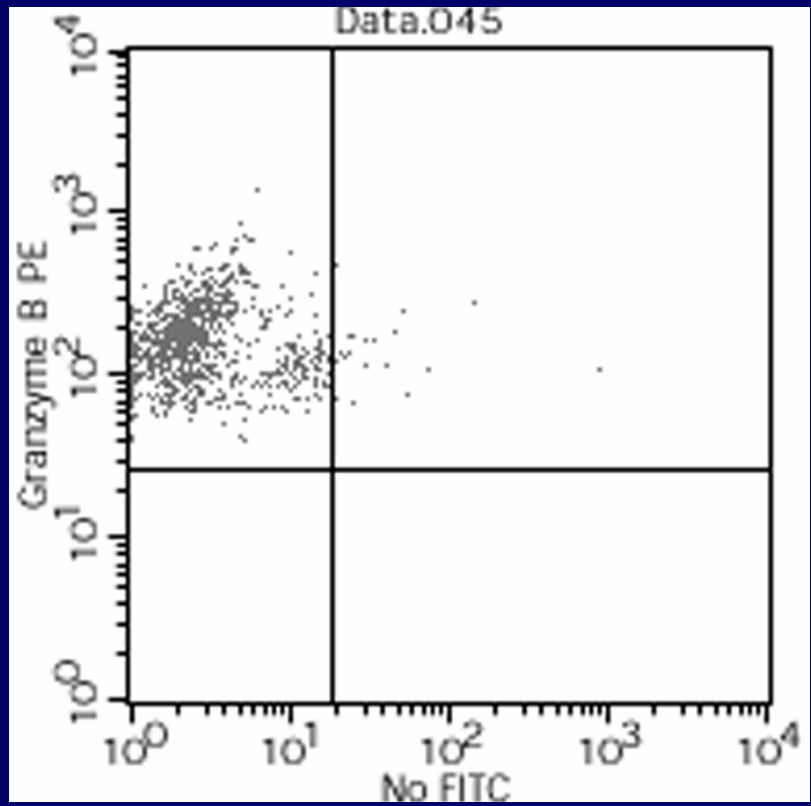
- Several clones of MUC1-specific CTLs were isolated from these MET mice and characterized

Epitopes recognized by MUC1-specific CTL line and clone.



CTL clone expresses high levels of perforin and granzyme B

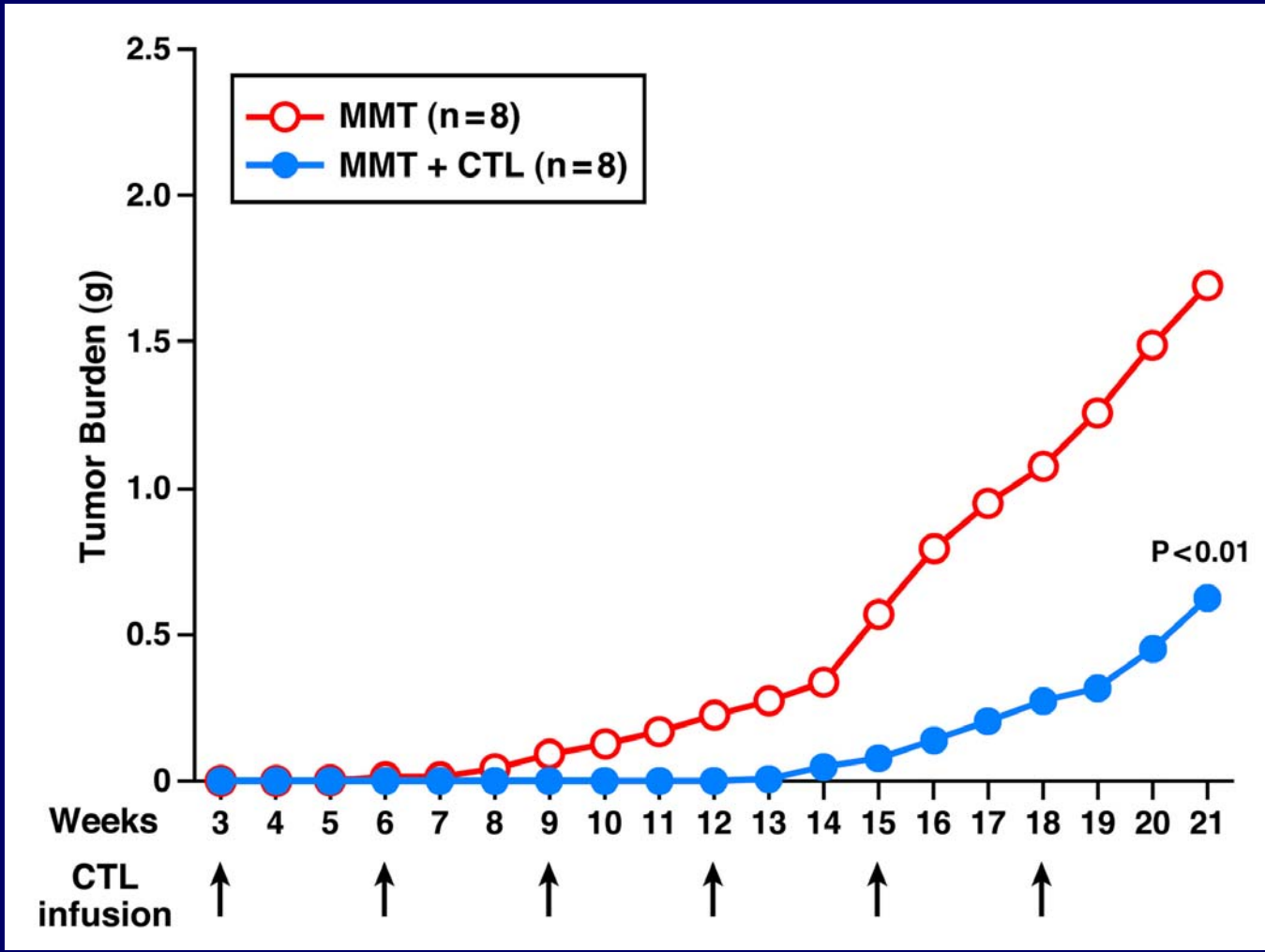




Perforin and Granzyme A and B are components of the cytolytic granule of cytotoxic T cells and NK cells that mediates lymphocyte-dependent killing

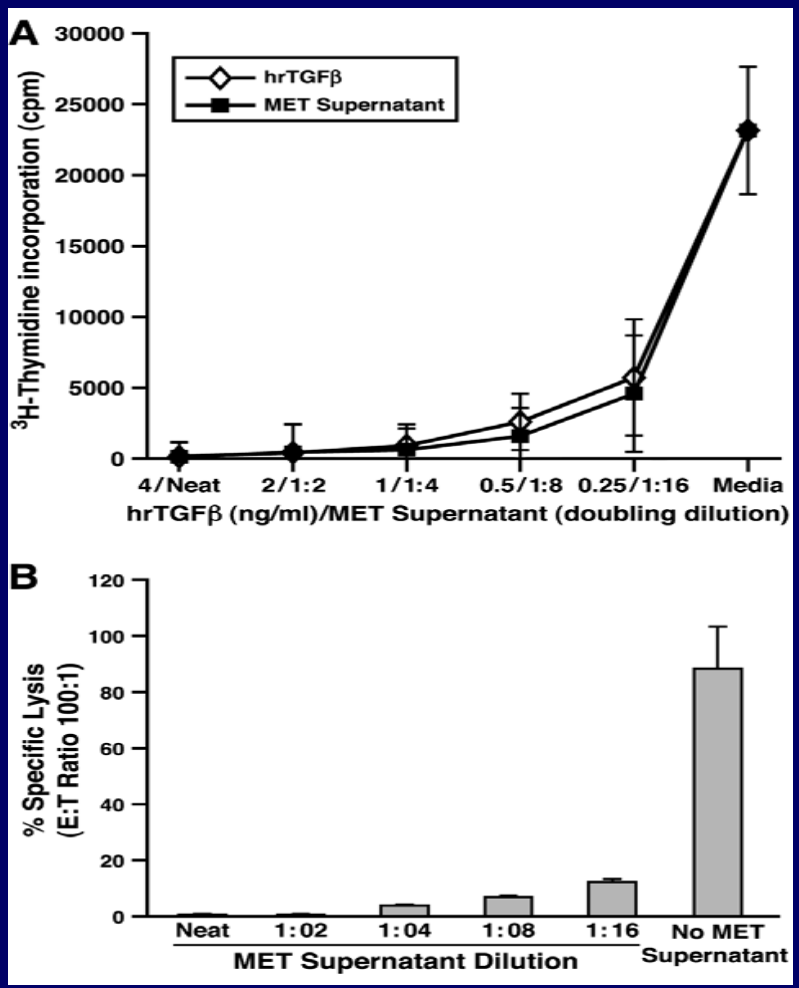
Adoptive transfer of MUC1-specific CTLs eradicates injected tumors that express MUC1 and generates a strong memory response

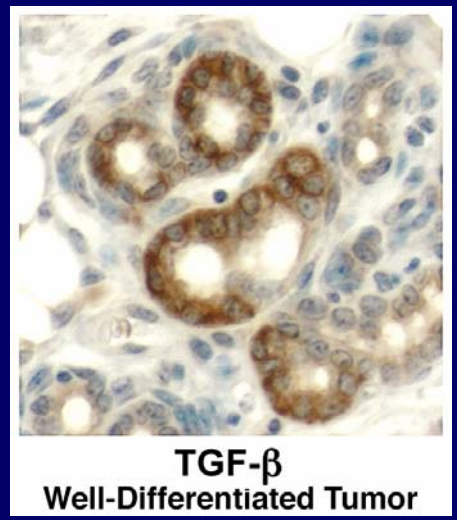
Adoptively transferred MUC1-specific CTL clone inhibits tumor progression in MMT mice.



Immunosuppressive factor (TGF-β) is expressed and secreted in the tumor microenvironment

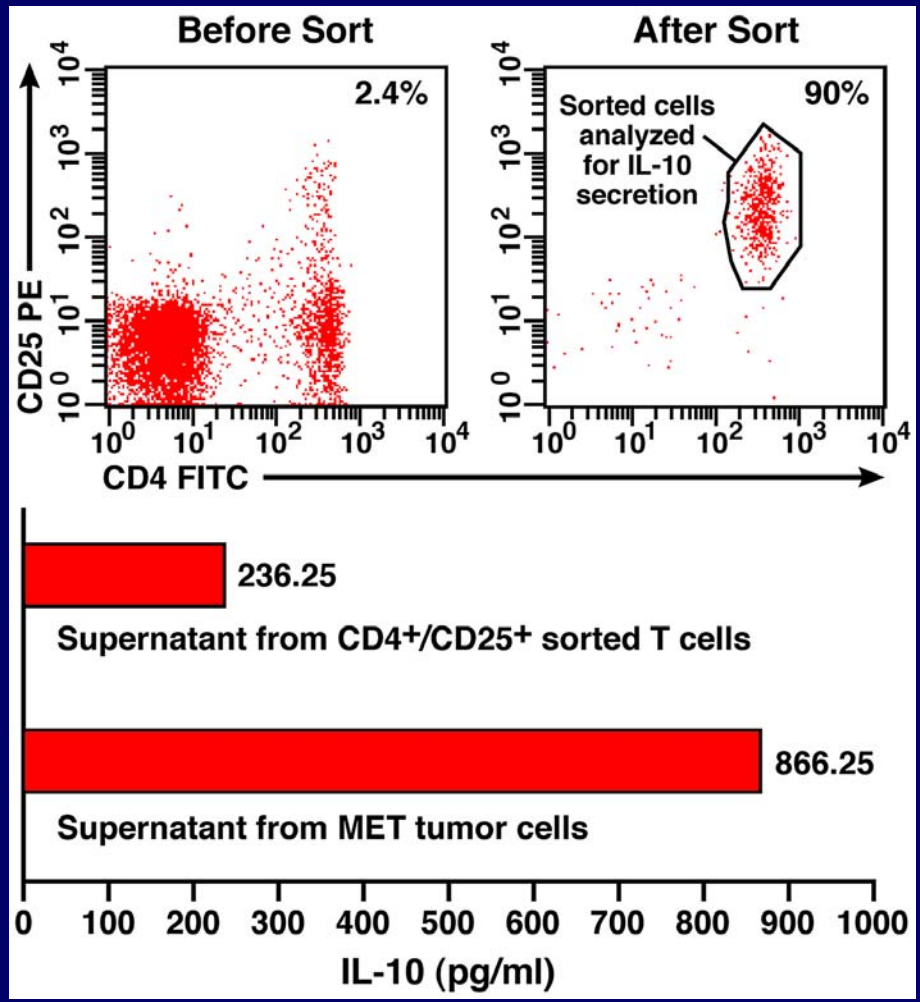
A.Growth inhibition of MvlLu cells that are sensitive to TGF-β inhibition
B. Inhibition of CTL cytolytic function



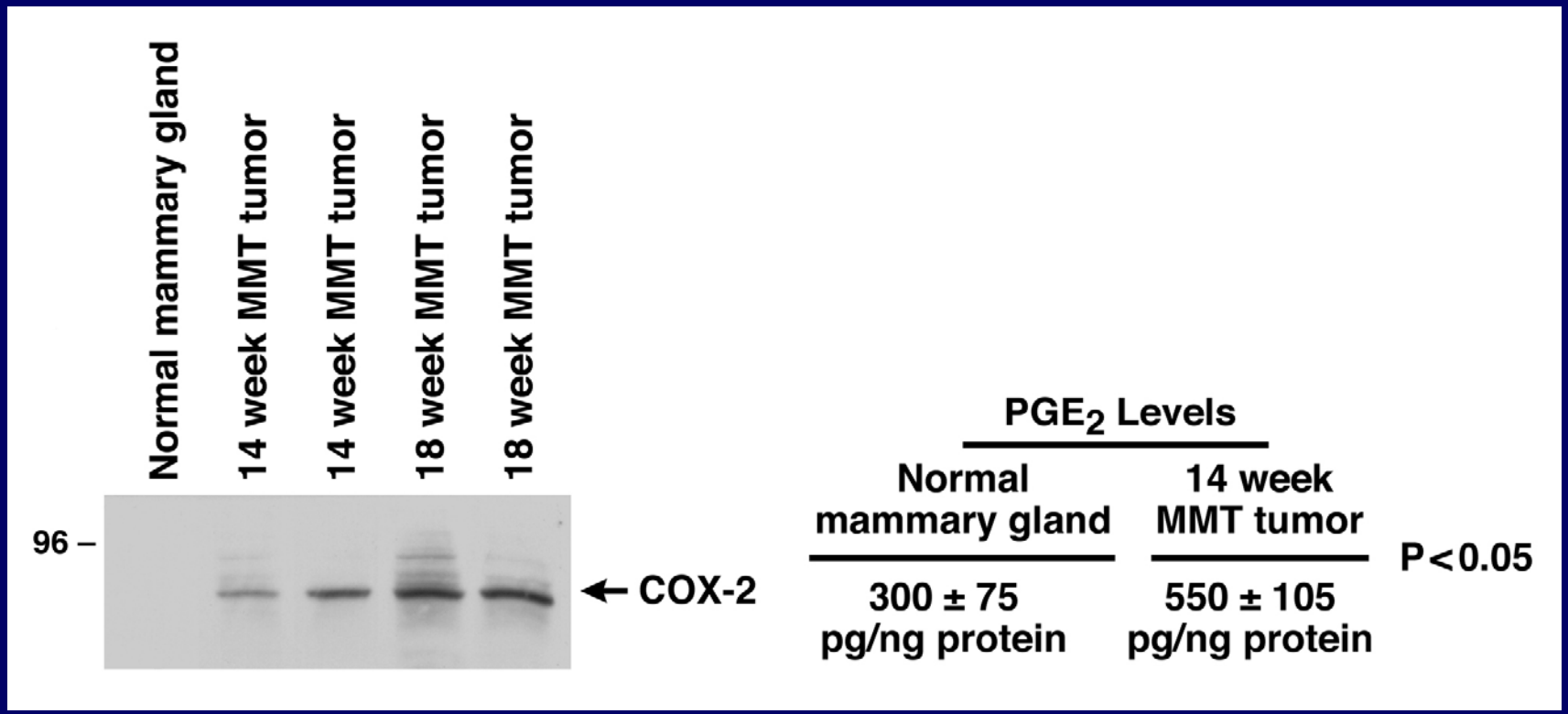


- **Bioactive form of TGF-β is secreted by MET tumor cells in culture.**
- **Supernatant derived from MET tumor cells inhibits cytolytic activity of the CTL clone.**

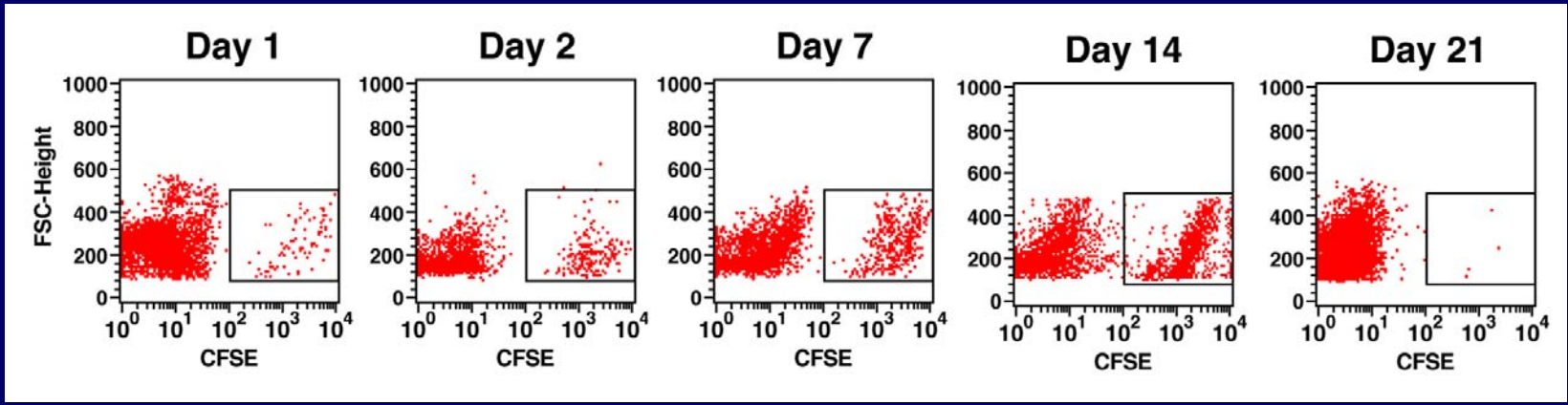
Immunosuppressive Factor (IL-10) is secreted in the tumor microenvironment



COX-2 and PGE_2 is present in the mammary tumor microenvironment



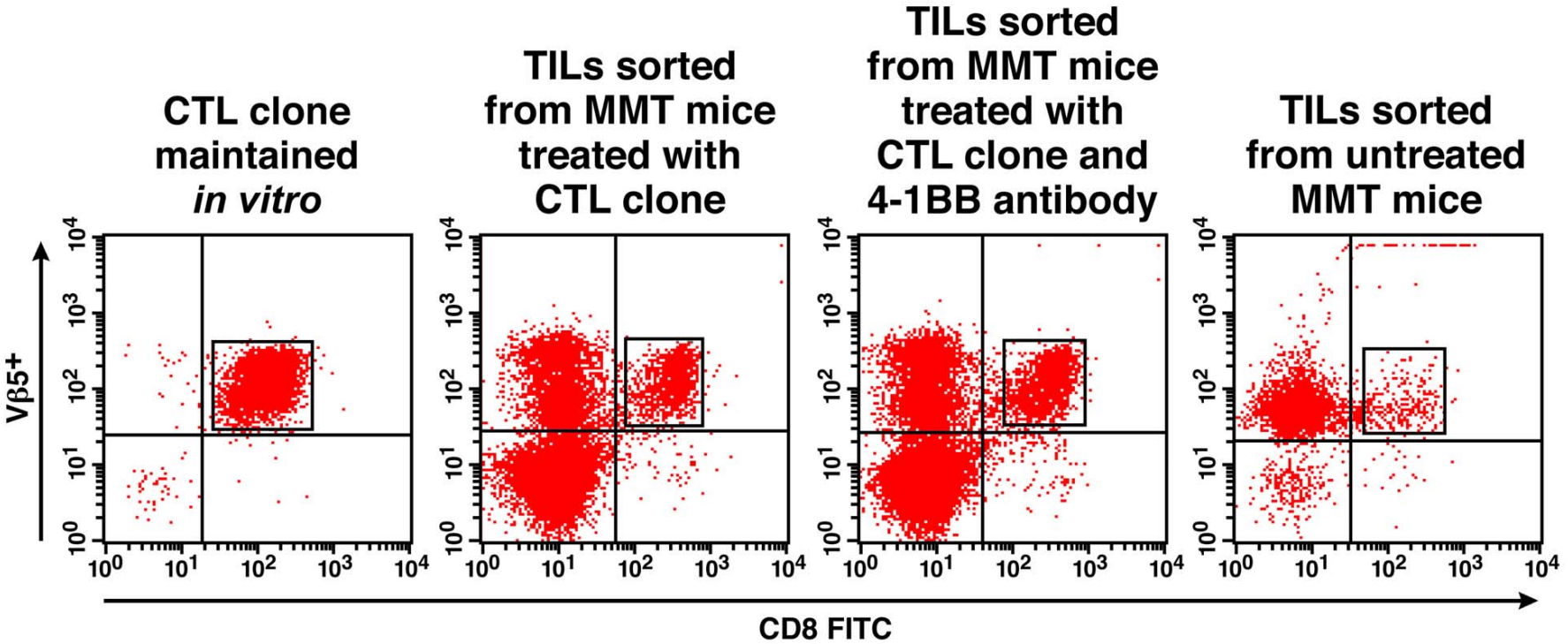
Adoptively transferred CTLs home and divide within the tumor microenvironment



CD137

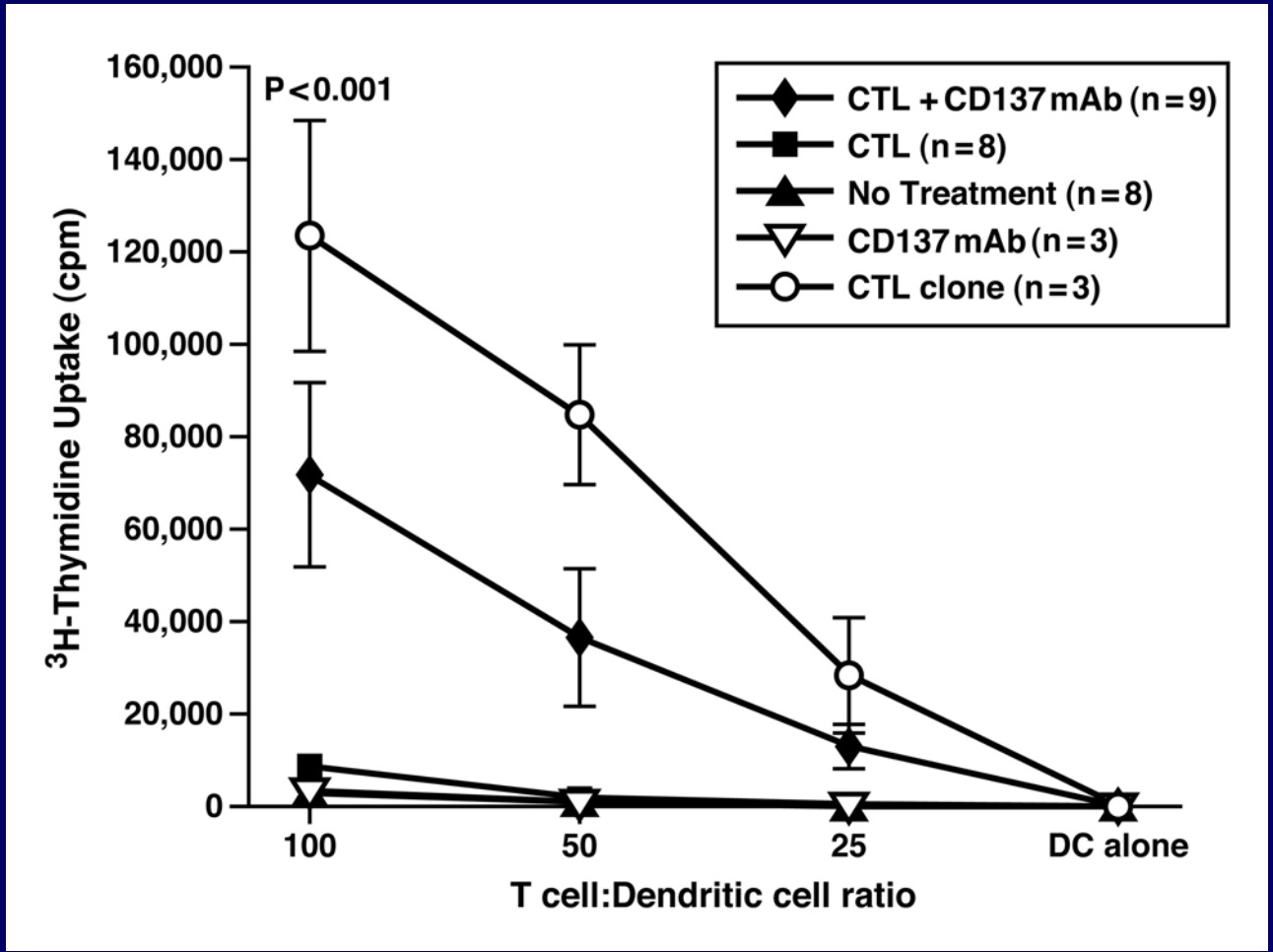
- **Member of the tumor necrosis factor receptor superfamily expressed on primed but not on naïve $CD4^+$ and $CD8^+$ T cells**
- **Binds to a high affinity ligand expressed on APCs and delivers a mitogenic signal for T cell activation and proliferation**
- **CD137 monoclonal antibodies can amplify T cell-mediated immune responses and can eradicate established tumors**
- **Postulated to reverse T cell tolerance induced by tumor cells**
- **Tested efficacy of CD137 antibody therapy in reversing tolerance in the *in vivo* breast cancer model**

Flow cytometric profile of TCR Vβ5^{+}/CD8^{+} T cells sorted from TILs by flow cytometry

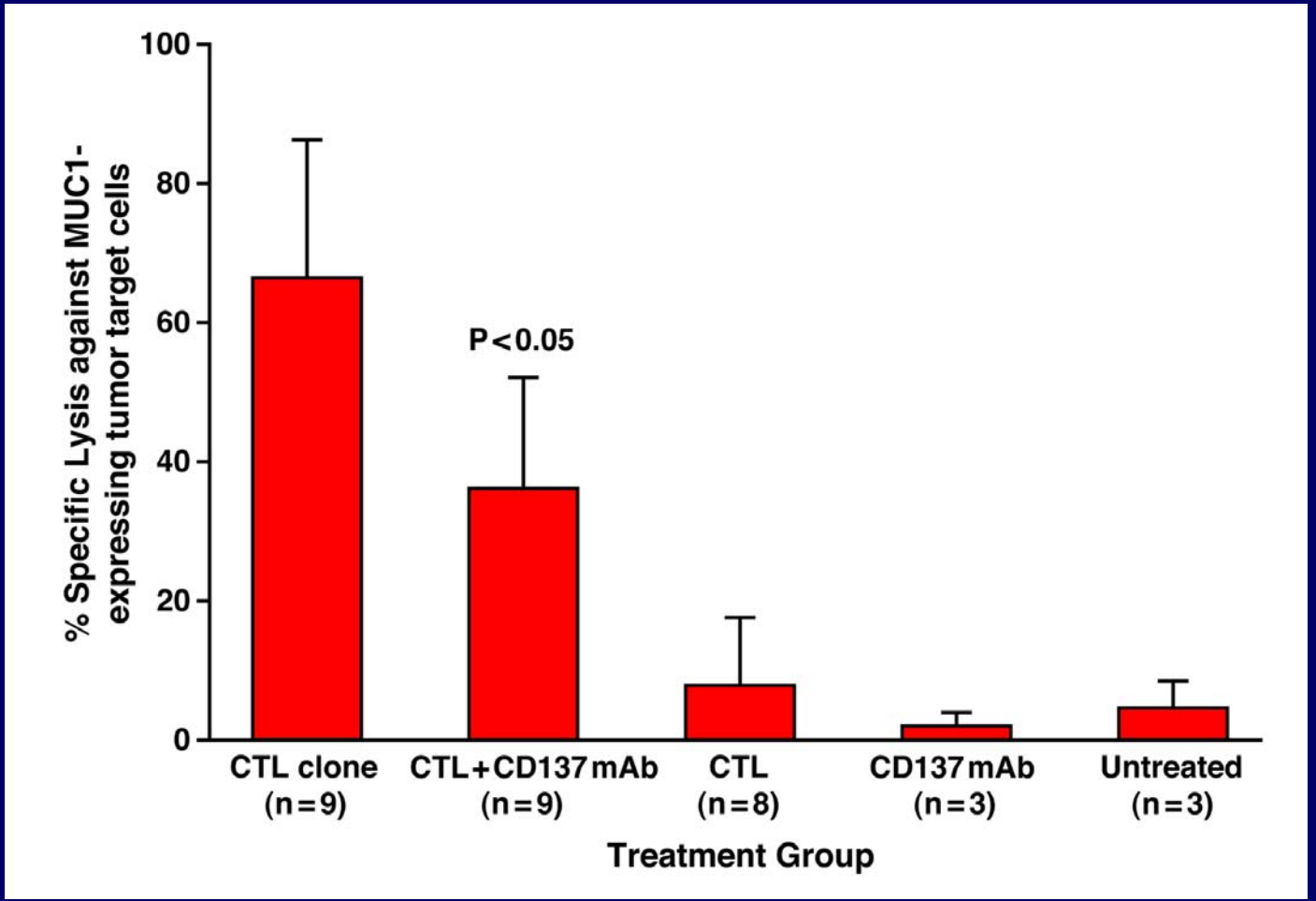


TILs were isolated from tumors of MMT mice that received adoptively transferred MUC1-specific CTL clone

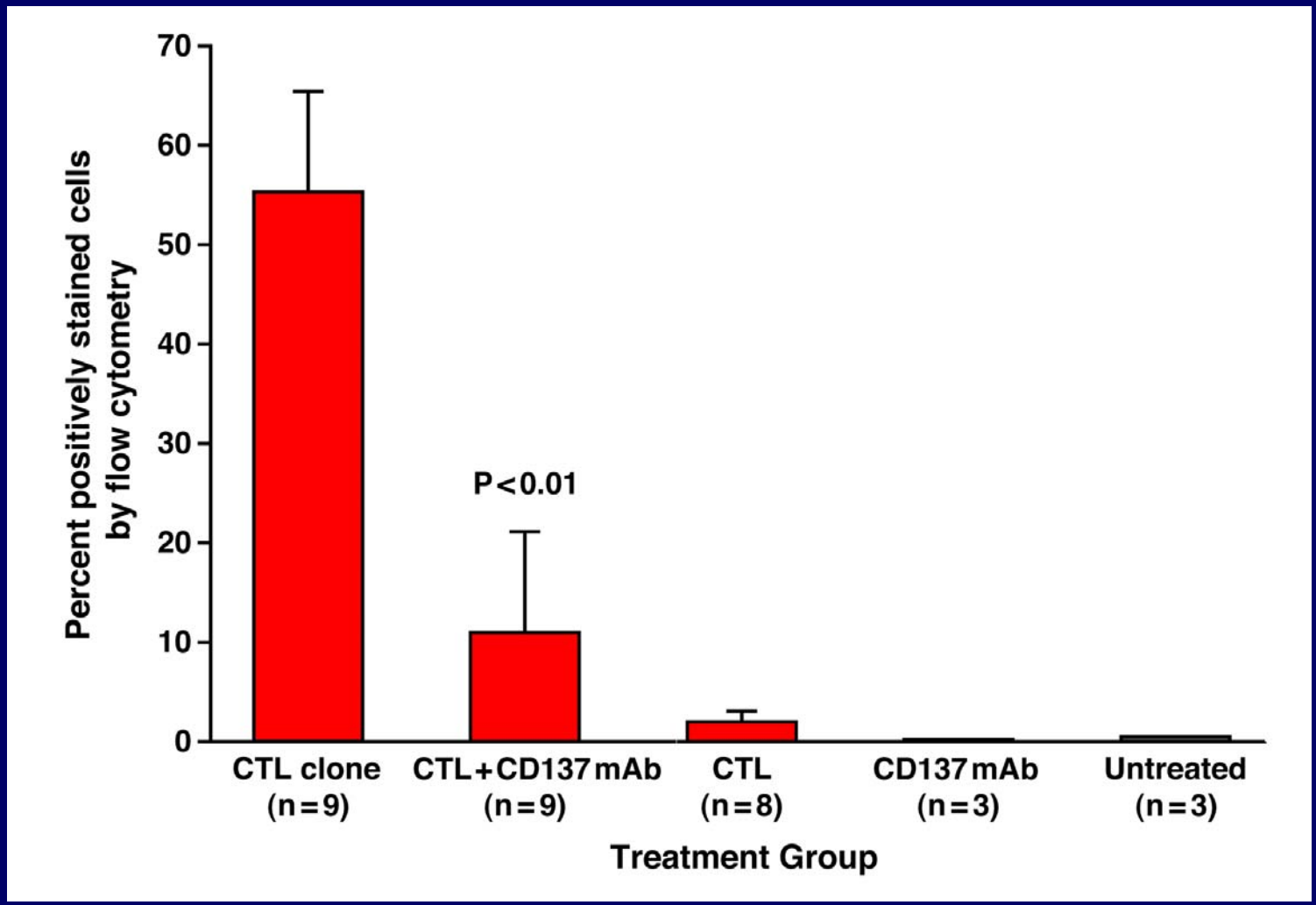
Adoptively transferred CTL become tolerant to MUC1 antigen within the tumor microenvironment



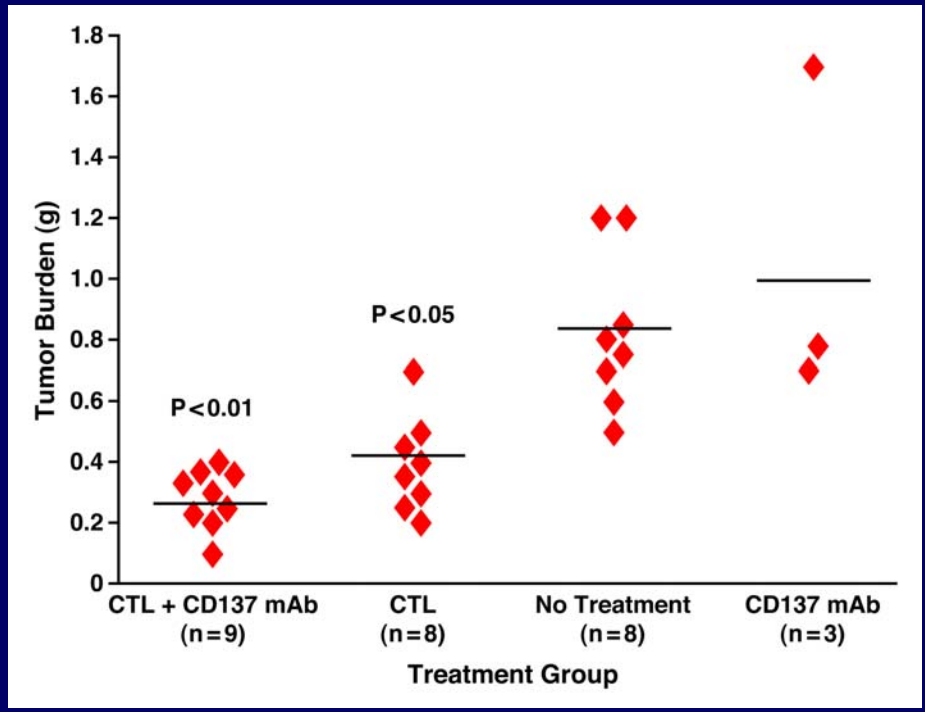
Adoptively transferred CTL are cytolytically inactive within the tumor microenvironment.

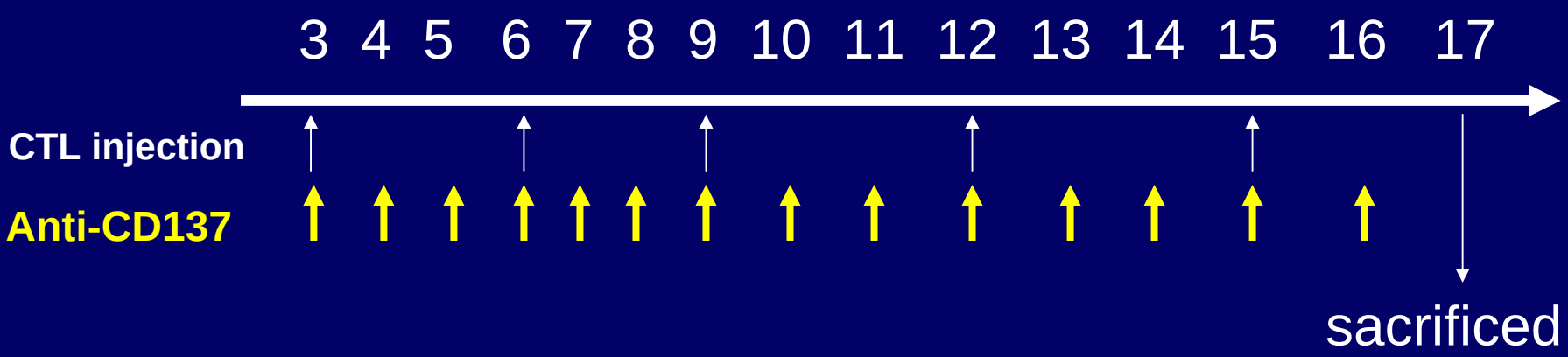


Adoptively transferred CTL are negative for granzyme expression within the tumor microenvironment.



Anti 4-1BB in combination with MUC1-specific CTL therapy is more efficient in reducing tumor burden than CTL therapy alone





Conclusions

- **Inefficiency of MUC1 CTLs to affect tumor burden and survival in spontaneous tumor models is due to the immunosuppressive tumor microenvironment that renders the infiltrating CTLs inactive**
- **Adoptively transferred CTL become tolerant to MUC1 and are cytolytically inactive after encounter with tumor cells.**
- **Anti-CD137 antibody can reverse tolerance in MMT mice and has synergistic anti-tumor affect when combined with MUC1-specific CTL therapy.**

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