T-cell Delivery Of Interleukin-12 To The Tumor Microenvironment Triggers Potent Endogenous Anti-tumor Responses

Sid Kerkar, M.D.

Outline

- Background/Objective
- Adoptive cell transfer experiments with murine tumor-specific pmel-1 CD8+ T cells engineered to secrete IL-12
- The importance of T cell receptor specificity for delivery of IL-12 directly to the tumor
- Mechanisms for enhanced tumor treatments

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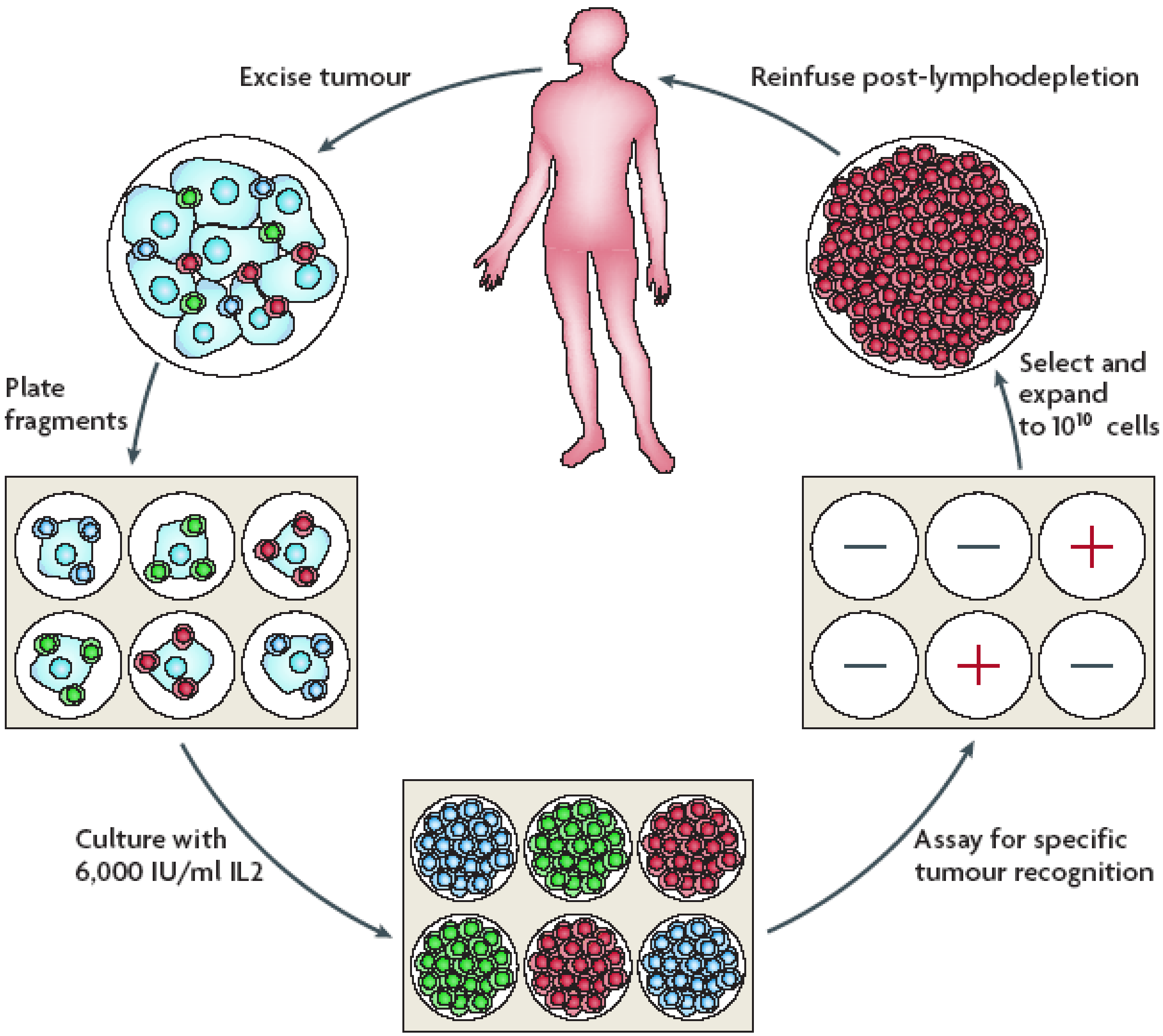
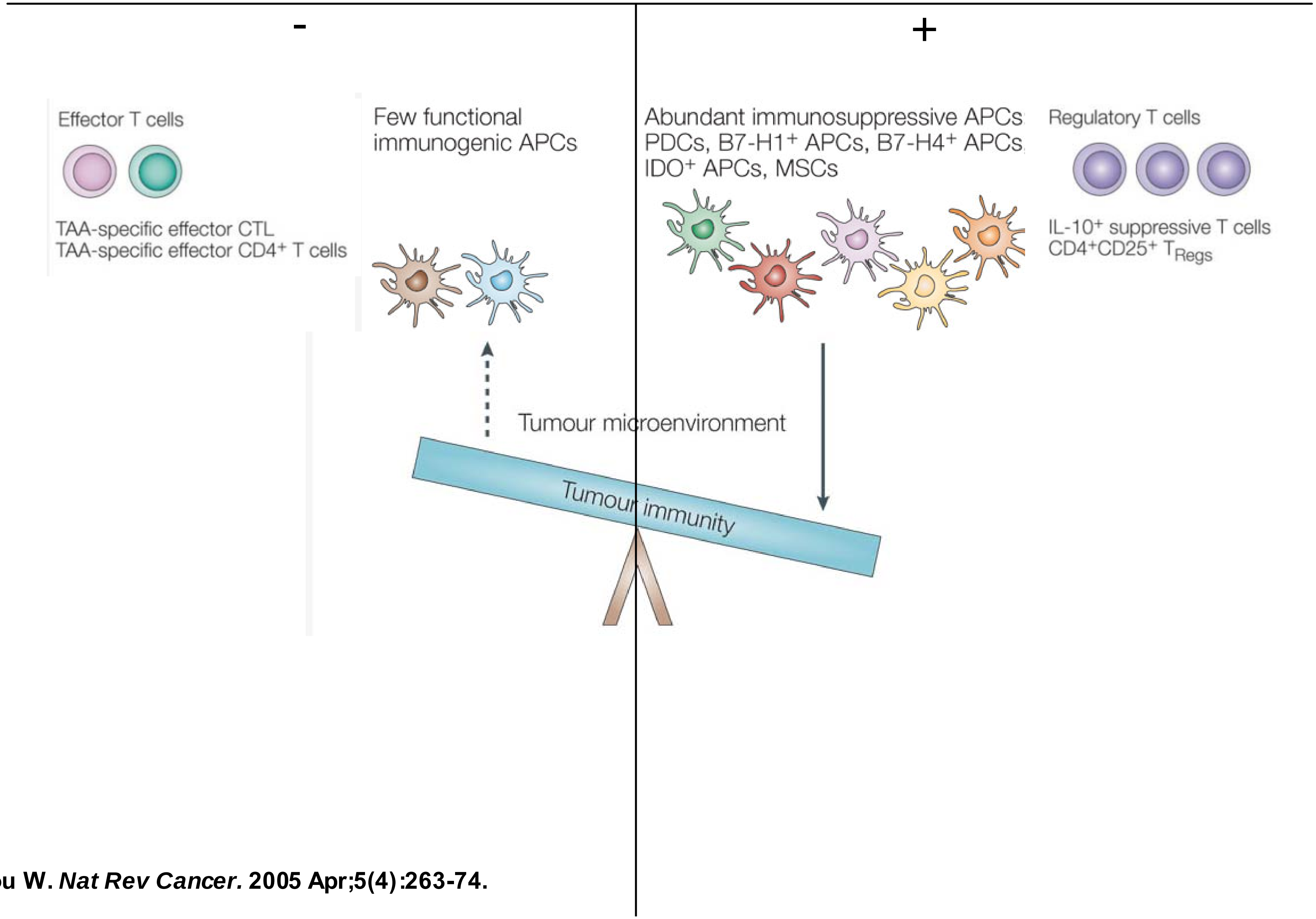
Adoptive Cell Transfer



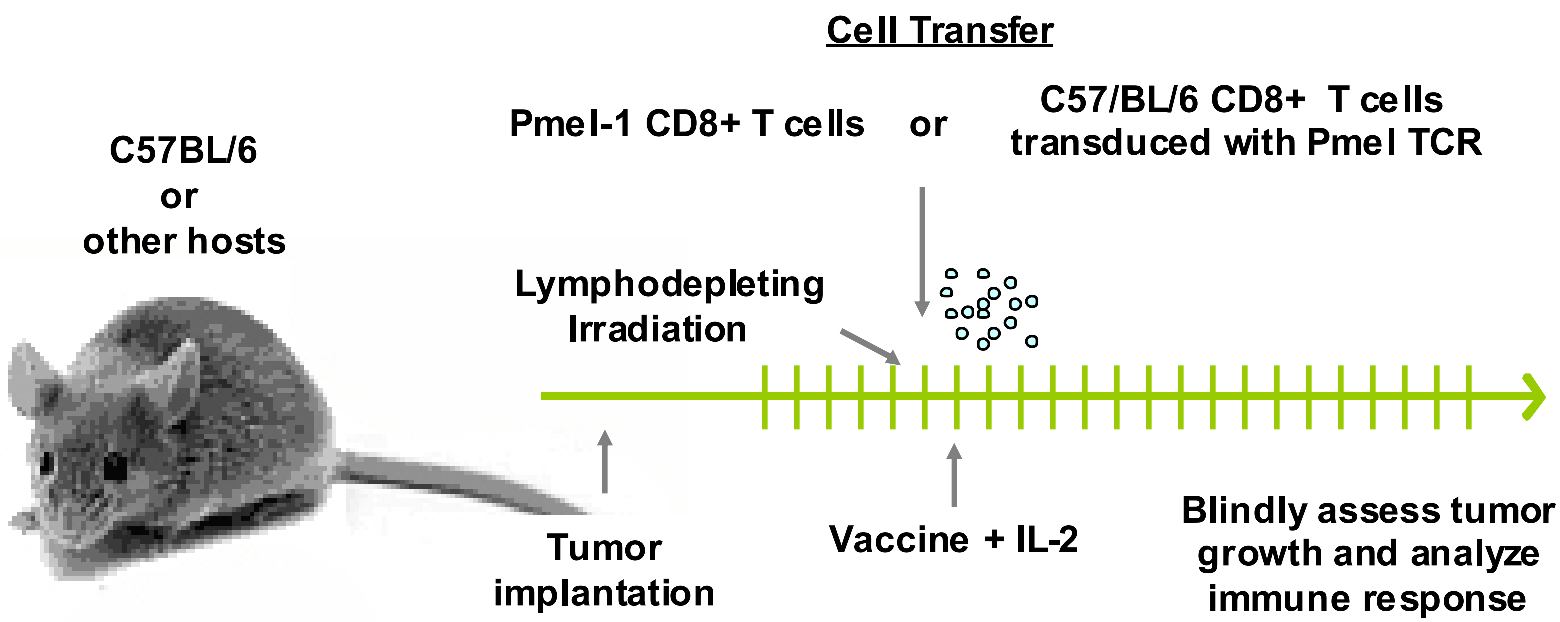
Rosenberg SA et al. Nat Rev Cancer. 2008; 8:299-308

Immunosuppressive Tumor Microenvironment



Zou W. *Nat Rev Cancer.* 2005 Apr;5(4):263-74.

Mouse B16/Melanoma Model of Adoptive Cell Transfer



A tripartite regimen is effective:

i) 1 million Pmel-1 CD8+ cells

ii) 2 x 10^7 plaque-forming units of rVV encoding hgp100

iii) administration of high-dose IL-2: 600,000 IU BID for 3 days

Overwijk WW et al. J Exp Med. 2003 Aug 18;198(4):569-80

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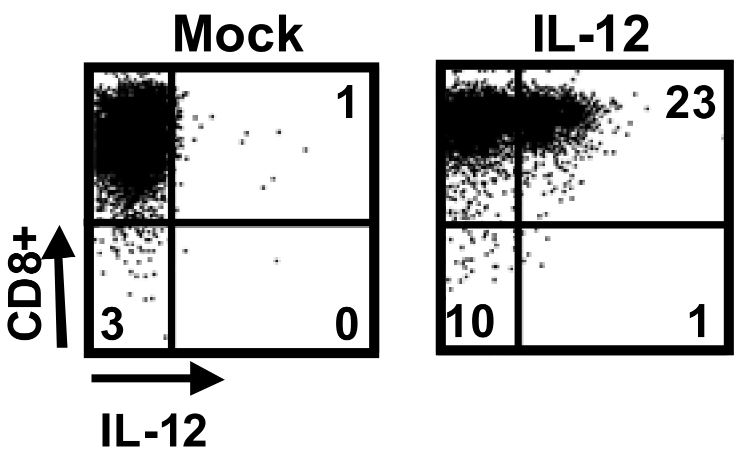
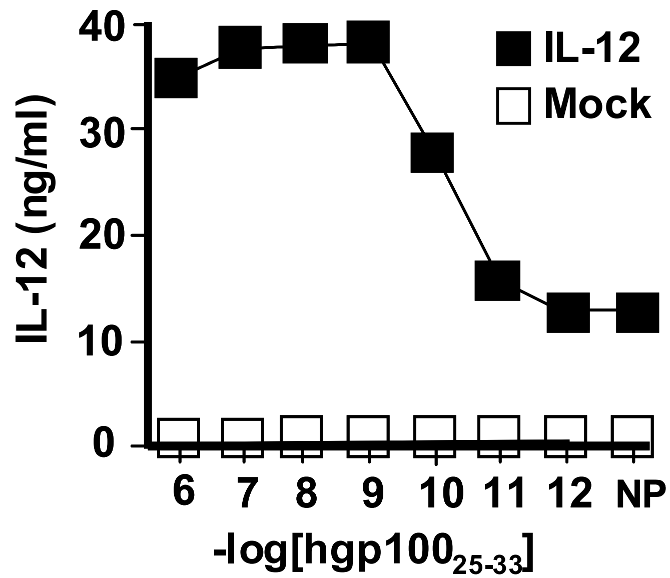
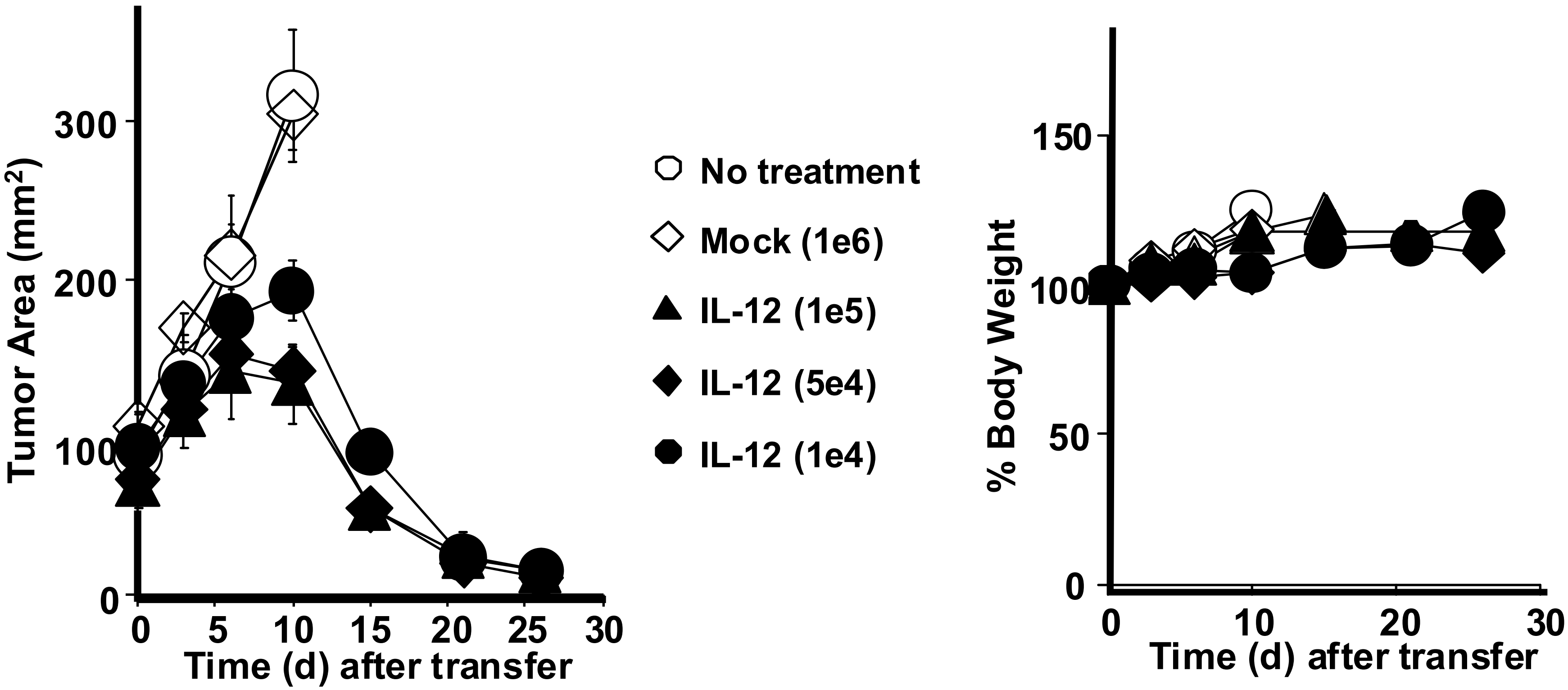
Tumor Specific Pmel-1 CD8+ T cells Engineered to Secrete IL-12

- We chose to genetically engineer tumor-antigen specific CD8+ T cells to secrete the heterodimeric cytokine IL-12 because of its capacity to augment the functionality of multiple end effectors in adaptive immunity

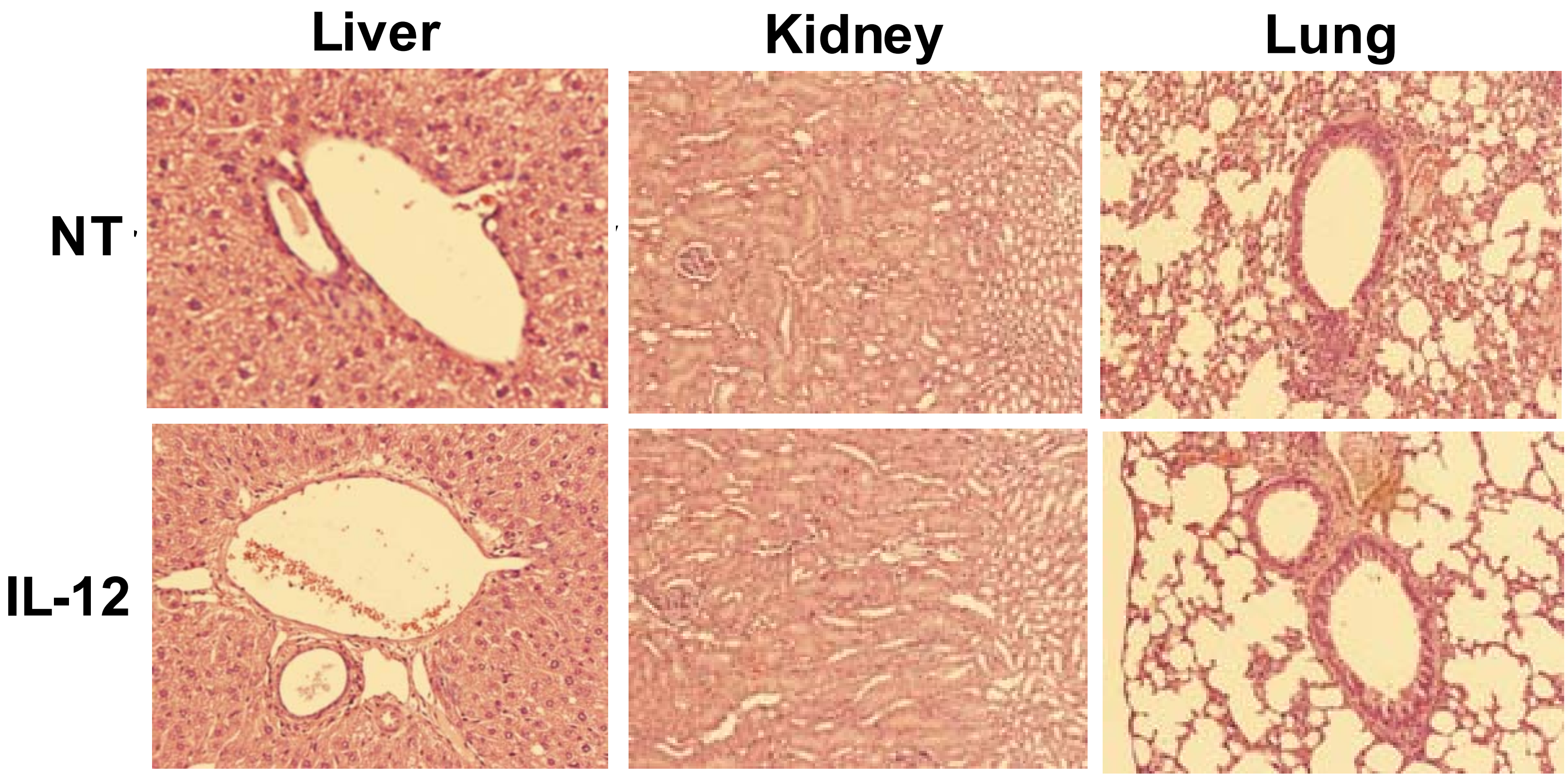




Tiny Numbers of pmel-1$^{IL\text{-}12TD}$ cells cause Regression of Large Established B16 Melanomas



Tiny Numbers of pmel-1$^{IL-12TD}$ cells are safe to adoptively transfer in mice



An Example of a Complete Response

No Treatment

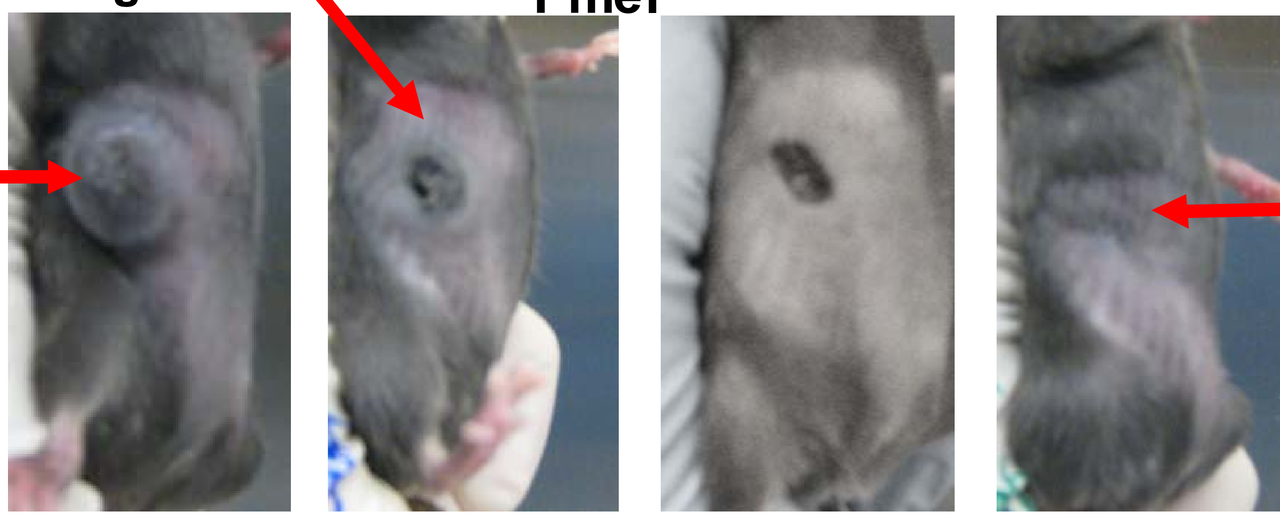
B16 Melanoma

Day 0 Day 14

Pmel$^{IL-12TD}$

Regressing Lesion

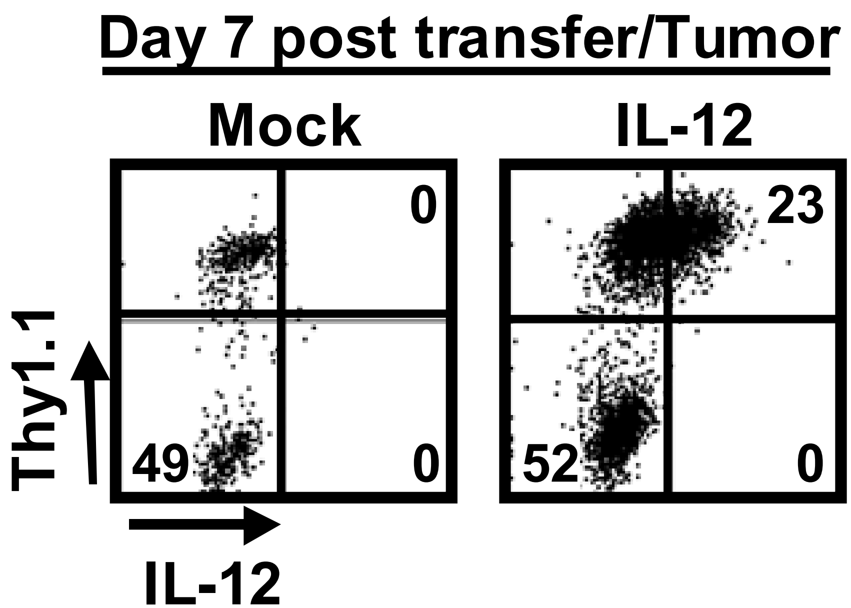
B16 Melanoma



Complete Response

Day 0 Day 9 Day 14 Day 21

Pmel-1$^{IL\text{-}12TD}$ Cells Secrete High Levels Of IL-12 Directly Into The Tumor Microenvironment



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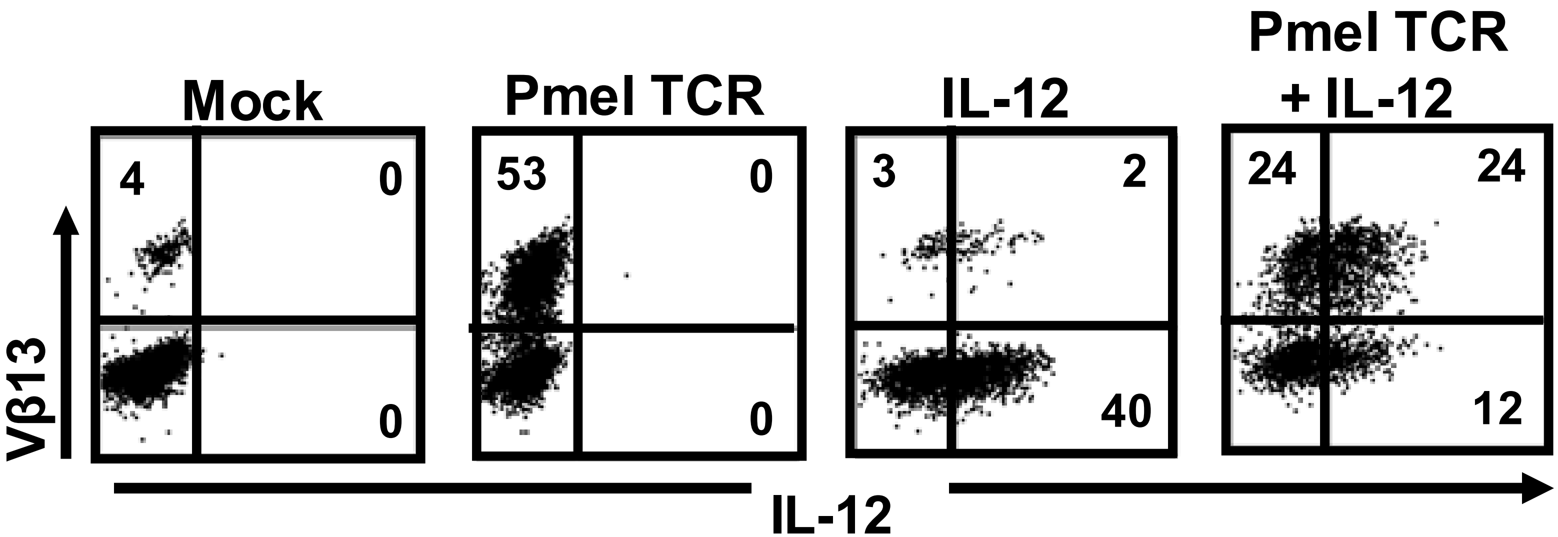
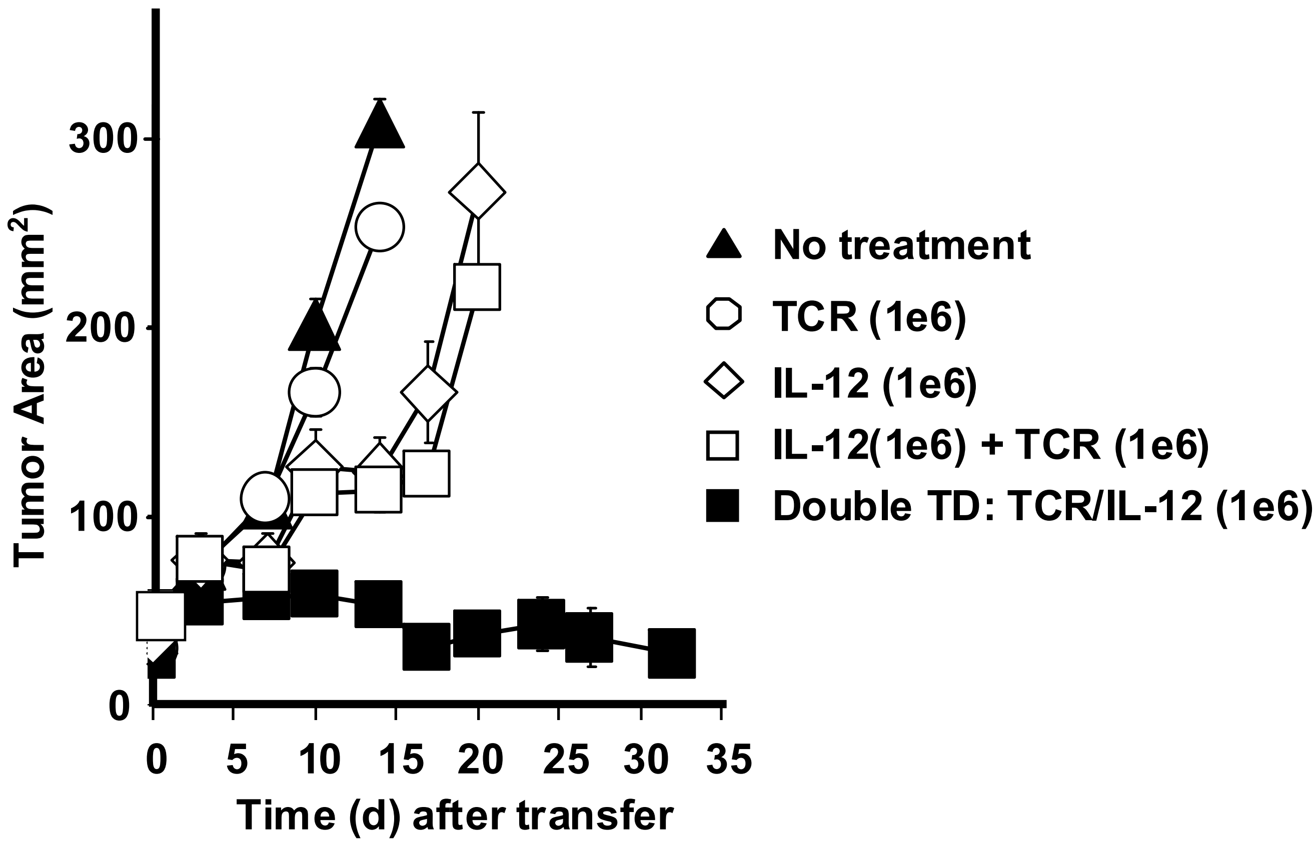
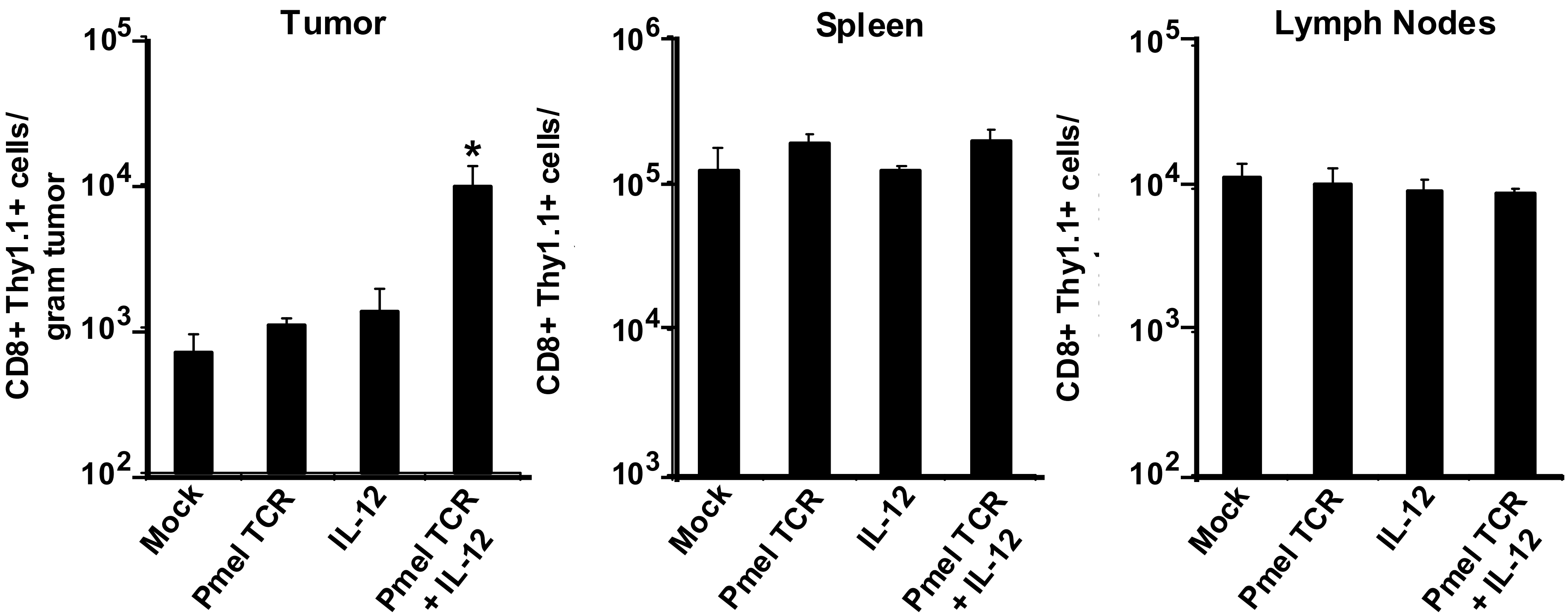
Generation of CD8+ T cells expressing a) the pmel-1 TCR alone; b) IL-12 alone, or c) both the pmel-1 TCR and IL-12



Only CD8+ T cells Expressing both IL-12 and the Pmel-1 TCR Destroy Tumor

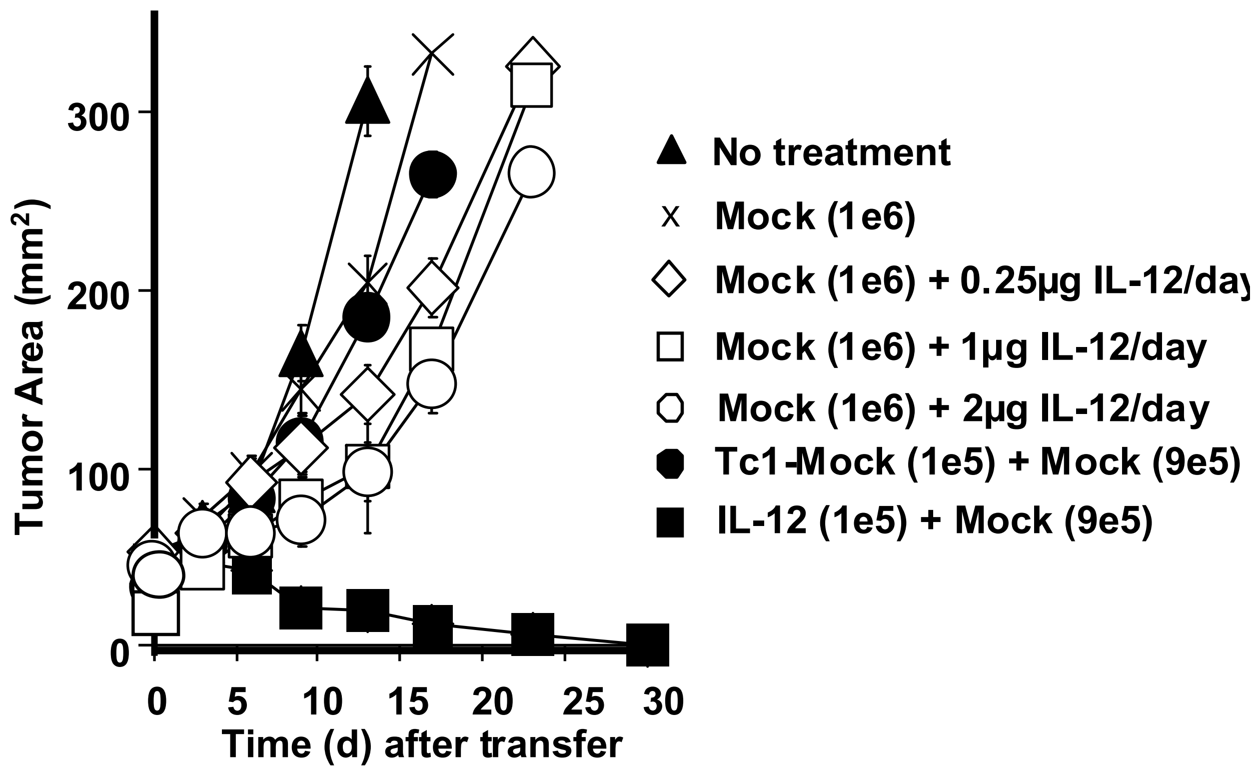


TCR specificity enhances delivery of IL-12 directly to the tumor site



* $p = 0.037$

Exogenous IL-12 is less effective than T cell produced IL-12 on anti-tumor activities of pmel-1 CD8+ T cells



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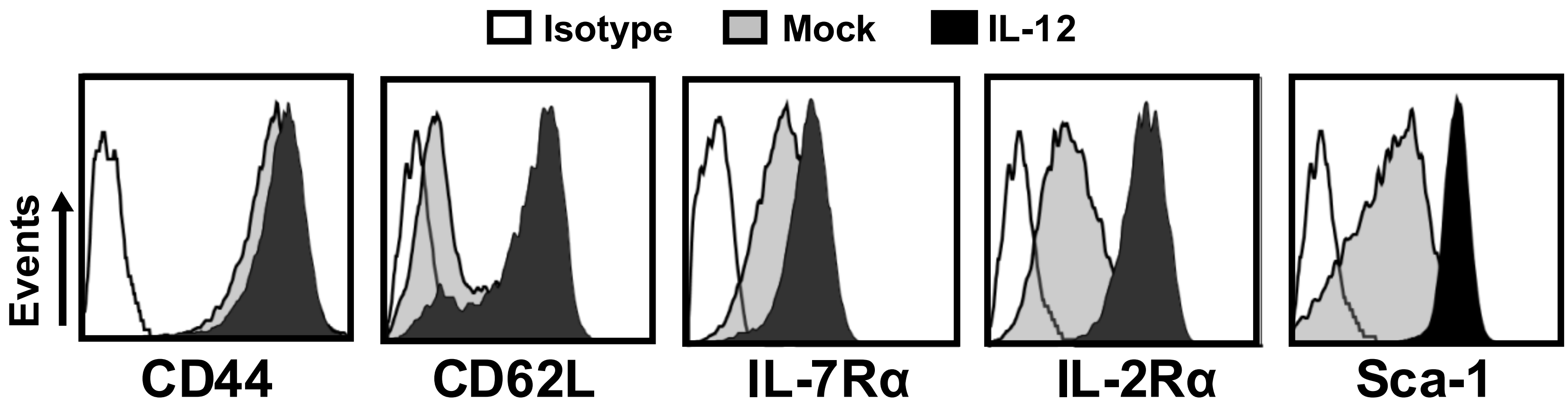
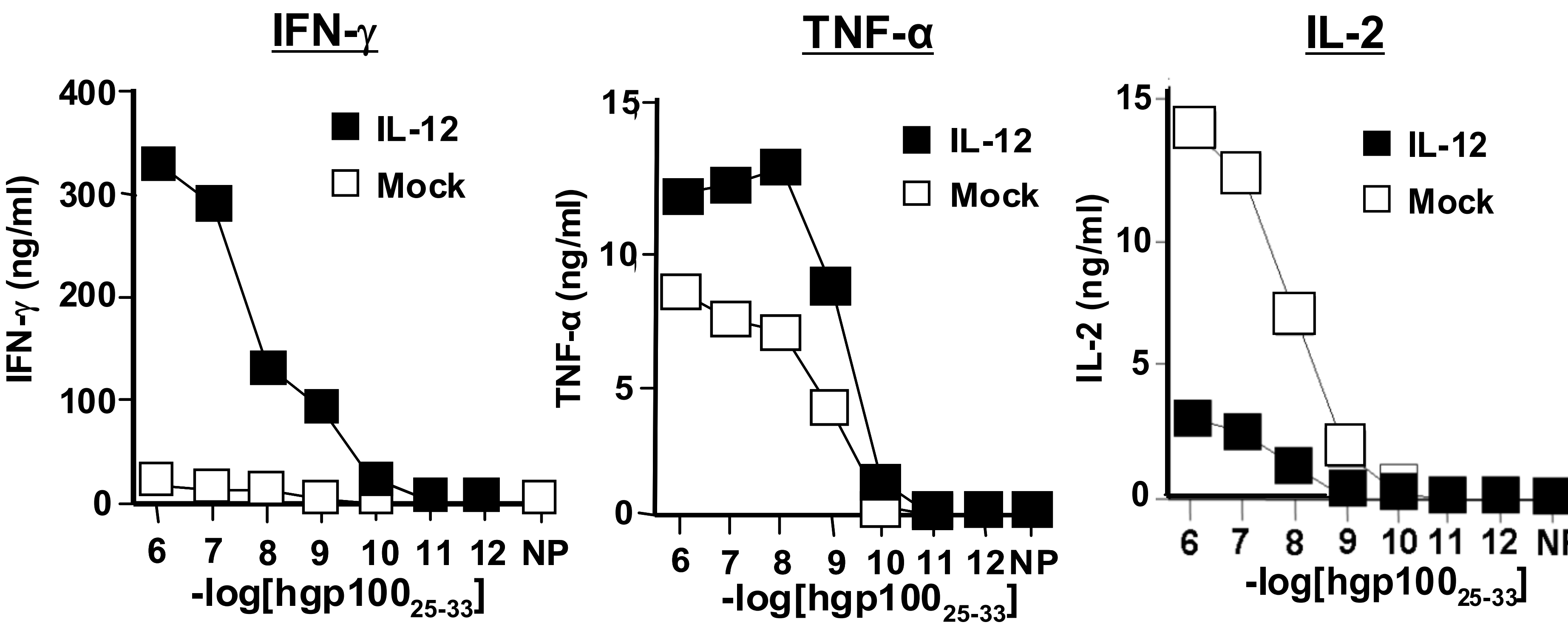
IL-12 Transduced Pmel-1 CD8+ T cells Express a Distinct Phenotype

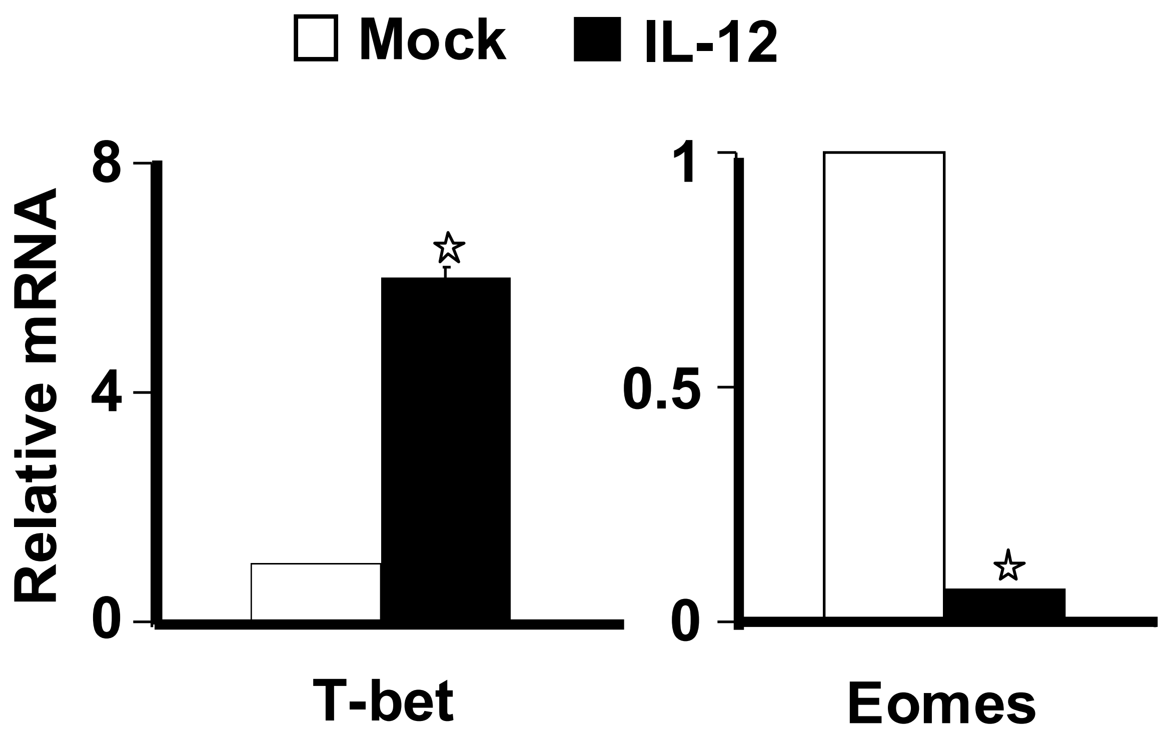
We hypothesized that the phenotype and function of tumor-specific T cells transduced with IL-12 were altered in ways that increased their anti-tumor activities



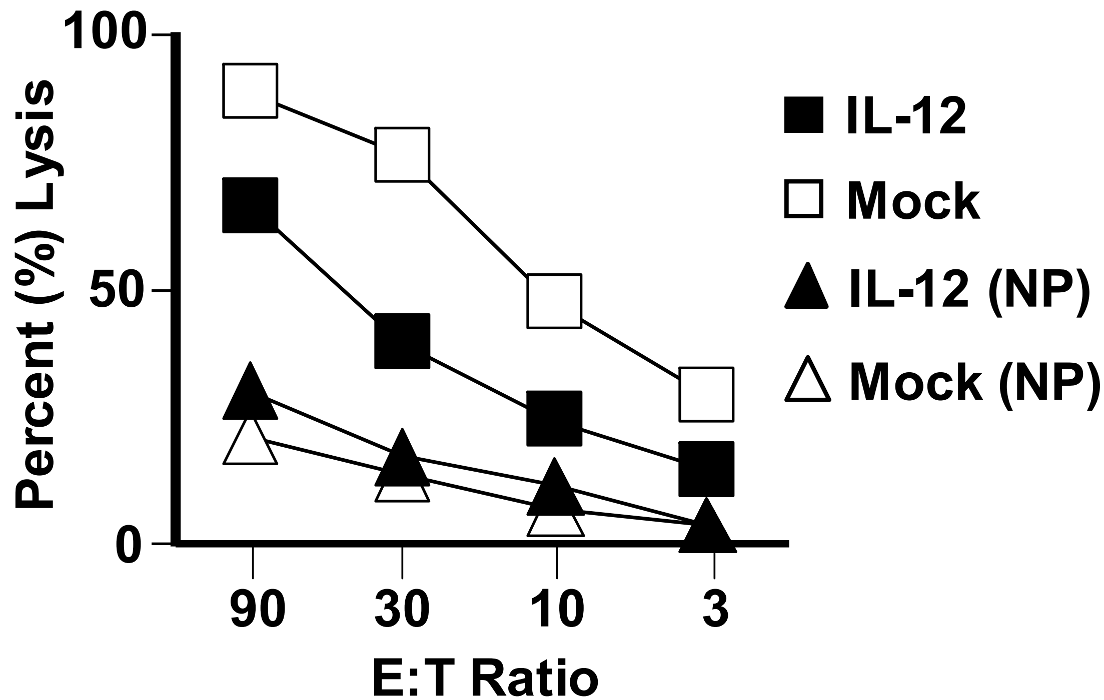
IL-12 Engineered Pmel-1 Cells Secrete More IFN-γ and TNF-α, but Less IL-2



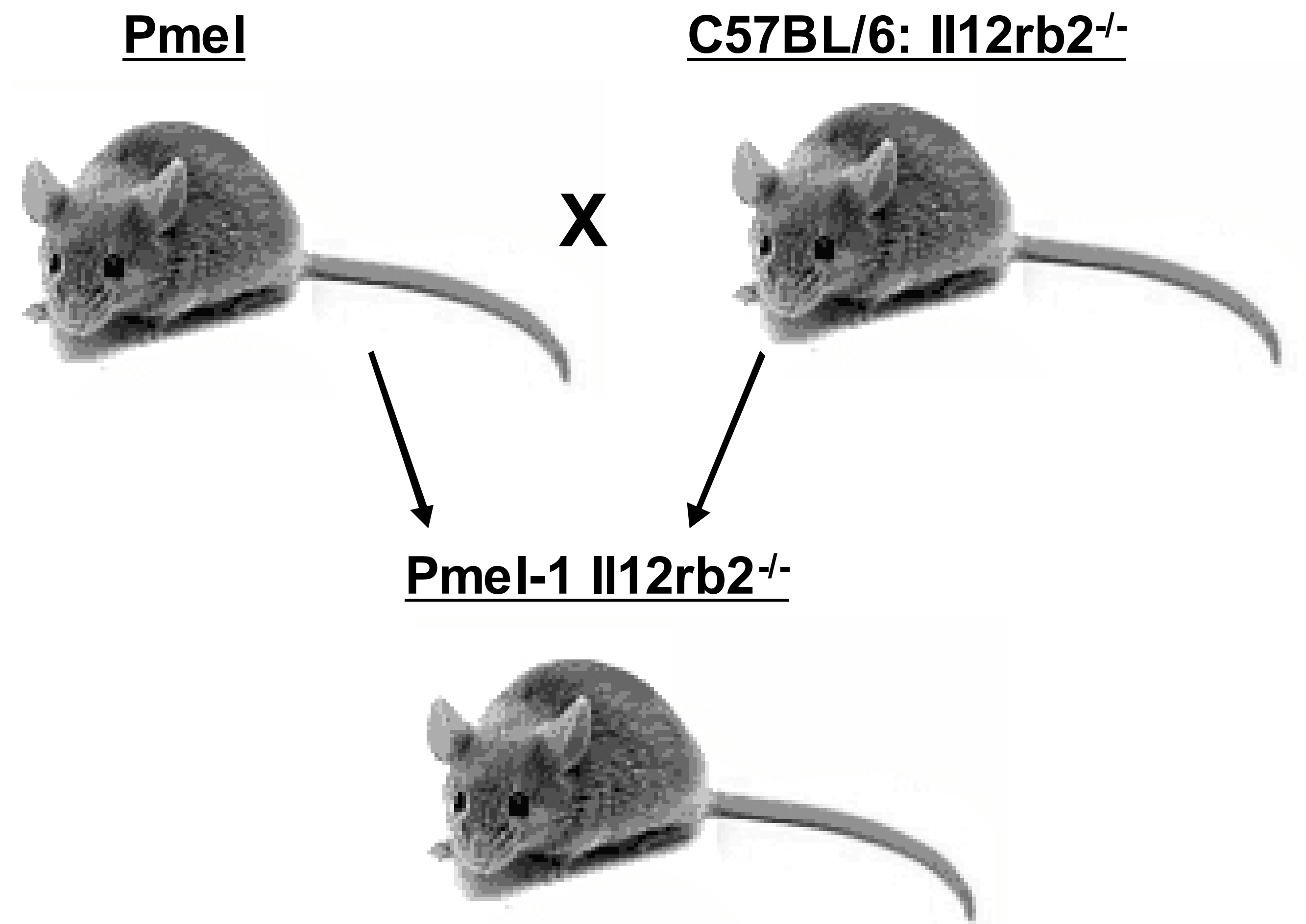
IL-12 Engineered Pmel-1 Cells have Increased Expression of T-bet and Down Regulation of Eomesodermin



Pmel$^{IL\text{-}12TD}$ Cells are Less Lytic Against Peptide Pulsed Targets *In Vitro*

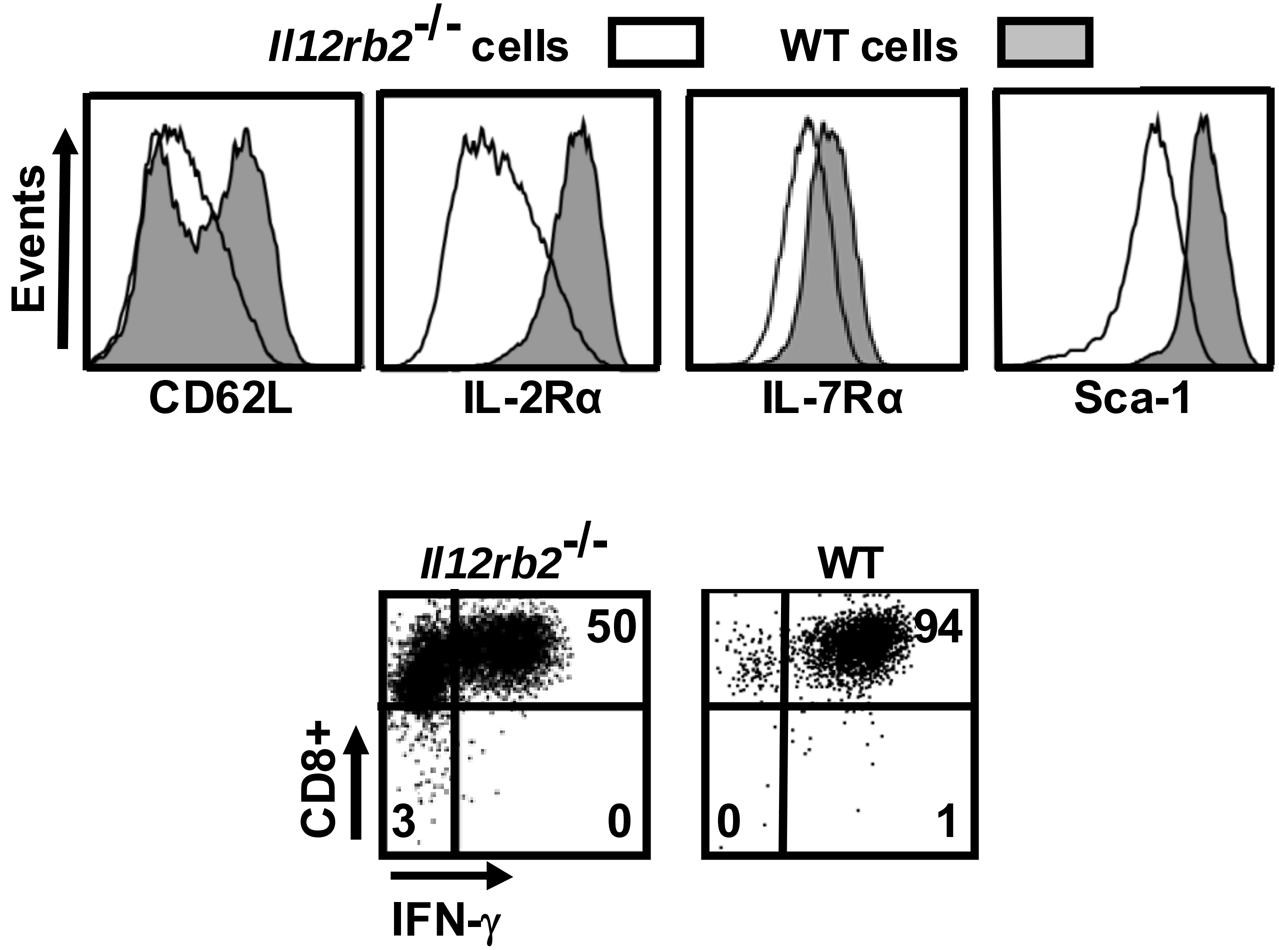


IL-12rβ2$^{-/-}$ Mice Were Used to Examine the Functional Importance of Cell-Intrinsic Characteristics

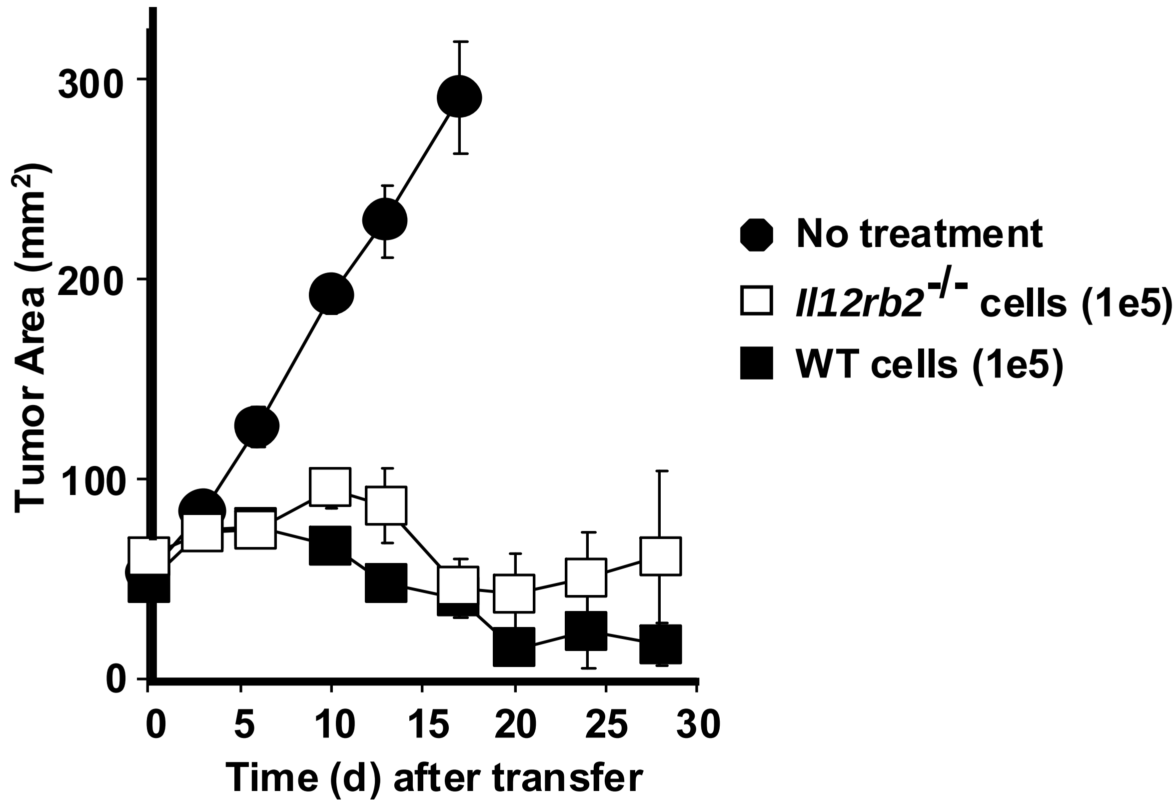


- Confirmed no differences in CD3+, CD4+, and CD8+ T cells in the spleen

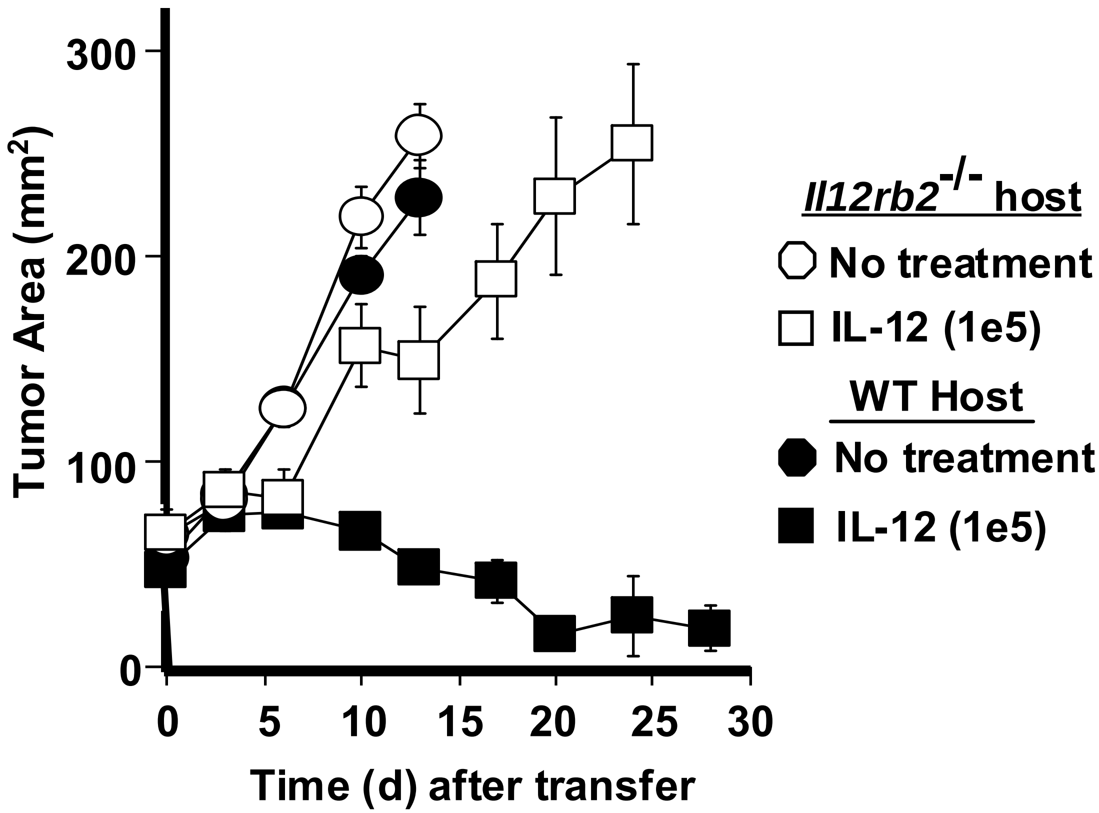
$Il12r\beta2^{-/-}$ Pmel-1$^{IL\text{-}12TD}$ Cells do not Display the Distinct Phenotype and Function of Wild Type (WT) Pmel-1$^{IL\text{-}12TD}$ Cells



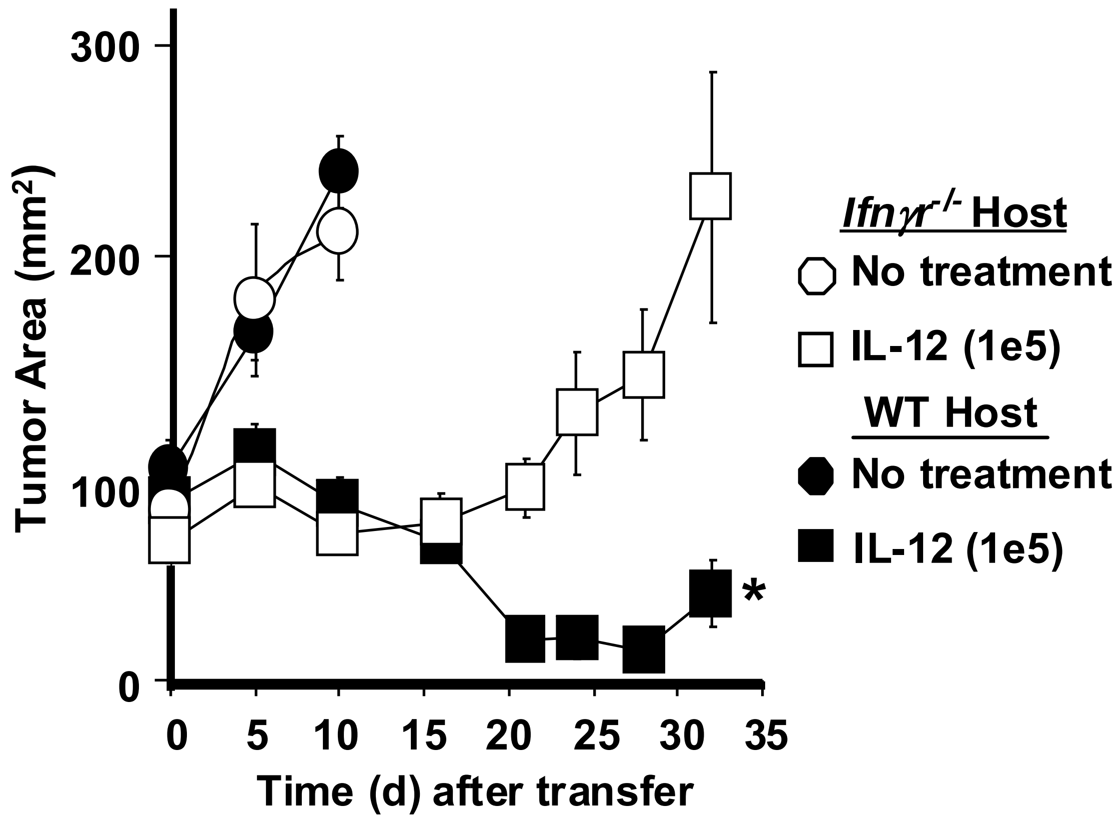
Il12rβ2$^{-/-}$ Pmel-1$^{IL\text{-}12TD}$ can Treat Tumors Similar to Wild Type (WT) Pmel-1$^{IL\text{-}12TD}$ Cells



Pmel-1$^{IL-12TD}$ are Less Effective in Il12rβ2$^{-/-}$ Mice Bearing Tumors

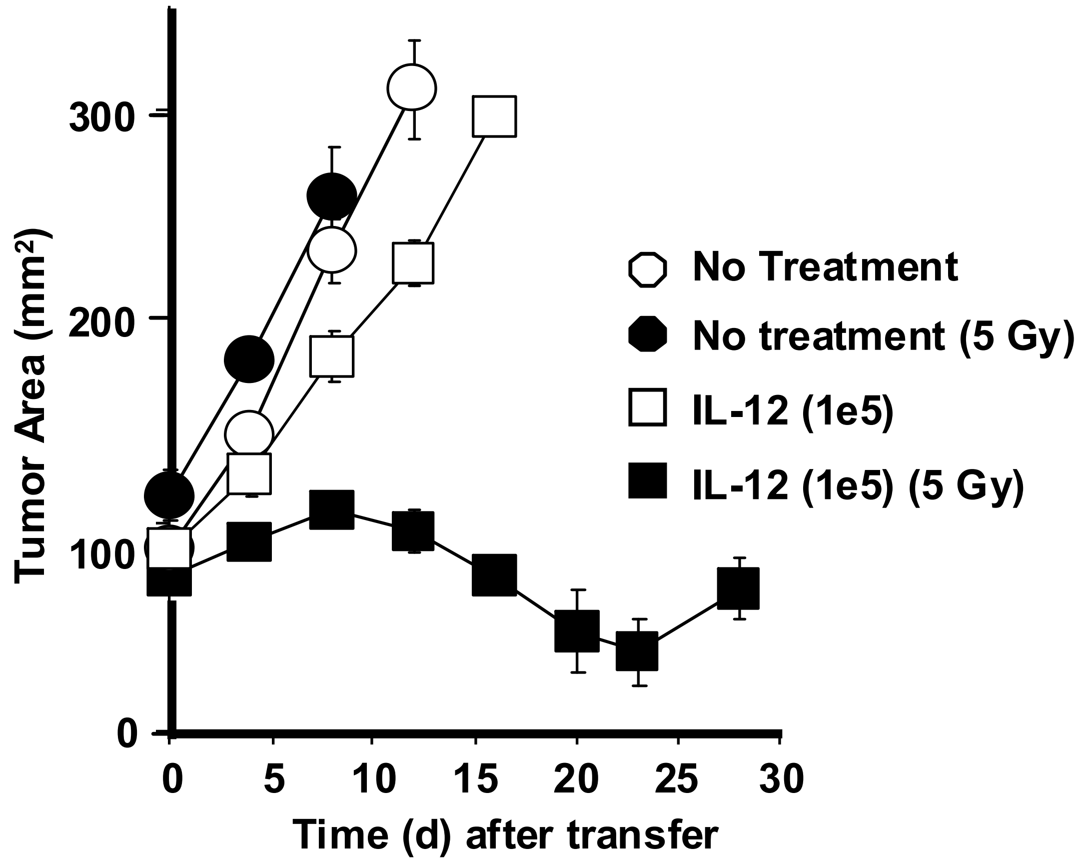


Pmel-1$^{IL-12TD}$ are Less Effective in IFN-γR$^{-/-}$ Mice Bearing Tumors

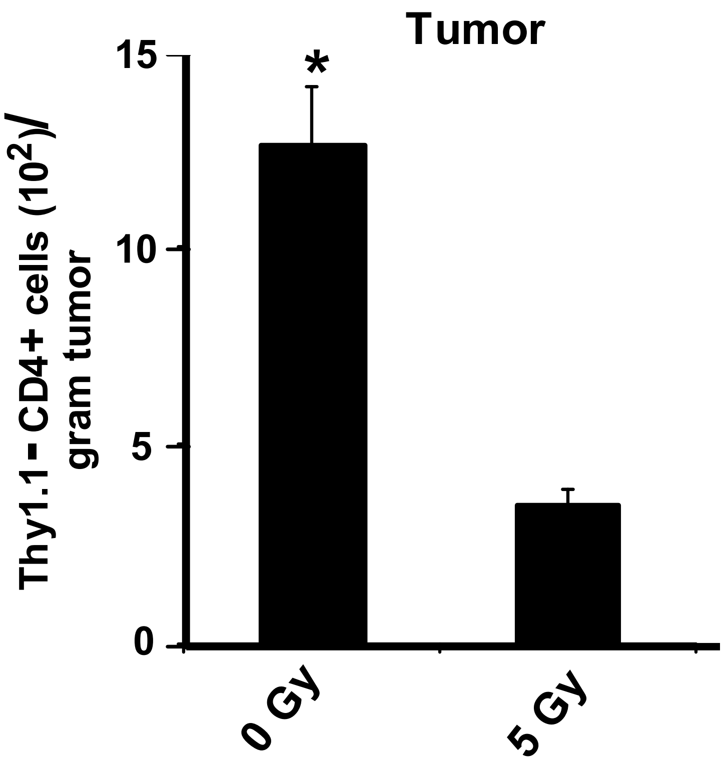


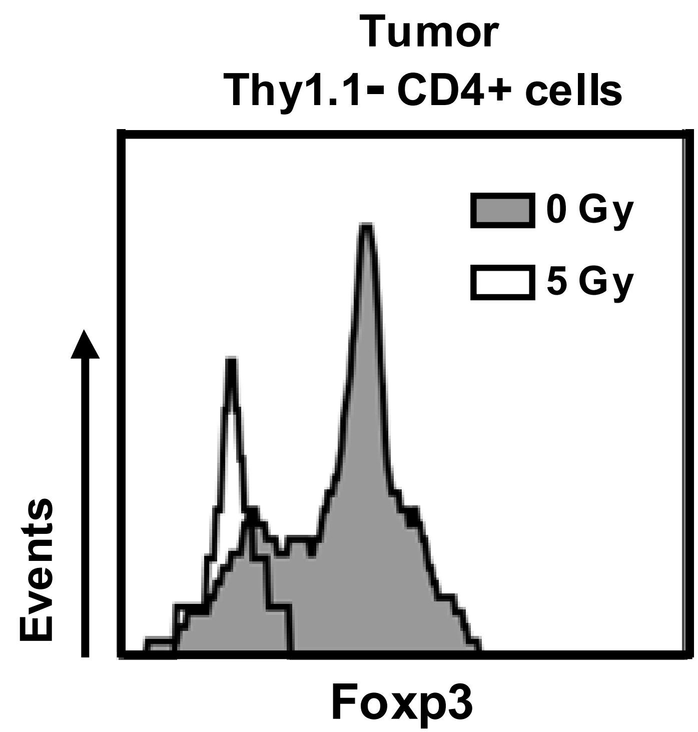
* p = 0.014; Wilcoxon rank sum test

Pmel-1$^{IL-12TD}$ are more effective following lymphodepletion



Decreased Numbers of Intra-tumoral CD4+ Foxp3+ cells following 5 Gy Irradiation





Conclusion

- Tiny numbers of tumor-specific CD8+ T cells engineered to produce IL-12 triggered the destruction of large vascularized tumors
- The expression of a tumor antigen-specific T cell receptor allowed for preferential delivery of IL-12 directly to the tumor microenvironment and was required for successful tumor treatments
- Host anti-tumor immunity triggered by IL-12 at the tumor site were critical for treatment responses

Clinical Applicability

- Gene Engineering lymphocytes with IL-12 may dramatically improve cellular therapies against metastatic cancer
- Decreasing the number of cells required for adoptive transfer may help facilitate the widespread acceptance of a ACT
- These findings may open possibilities for delivering other molecules to the tumor microenvironment designed specifically to elicit endogenous anti-tumor immunity

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