# Orthogonal IL-2/IL-2Rβ signaling in adoptively transferred T cells controls tumor growth without the need for lymphodepletion in a B16 tumor model

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## ABSTRACT

**Background**

Multiple adoptive T-cell therapy modalities (ACT) have delivered promising clinical responses in cancer patients. However, challenges including poor T cell effector function, lack of proliferation, and limited persistence have prevented ACTs from reaching their full curative potential. In addition, ACTs typically require lymphodepletion to aid cell engraftment. Lymphodepletion has been shown to improve persistence and efficacy of ACTs by several mechanisms including eradication of immunosuppressive cells and elimination of homeostatic cytokine sinks, thereby elevating T-cell common gamma-chain cytokines like IL-7 and IL-15. However, lymphodepletion also carries risk for the patient, potentially causing toxicities such as cytopenias, infections, and secondary malignancies. IL-2, another common gamma-chain cytokine, is a potent stimulator of T cells, making it an attractive cytokine to support ACT and potentially bypass the need for lymphodepletion. However, therapeutic use of IL-2 is limited by systemic toxicity due its promiscuous activation of immune cells.

**Methods**

To facilitate selective delivery of an IL-2 signal to engineered T cells and avoid signaling in bystander T cells and NK cells, we developed a mouse orthogonal receptor/ligand system consisting of a mutated IL-2 Receptor Beta (moRβ) and a pegylated, IL-2 mutein (moIL-2) that does not significantly activate the wild type IL-2β receptor but does activate moRβ.

T cells from pmel-1 T cell receptor-transgenic mice, recognizing gp100 on B16 melanoma cells were transduced with moRβ (orthoPmel). moIL-2 was continuously dosed for four weeks in mice. Thy1.1$^+$ orthoPmel T cells were tracked by FACS and IHC systemically and in the tumor.

**Results**

During orthoPmel manufacturing, moIL-2 specifically enriched orthoPmel compared to mouse WT IL-2. OrthoPmel in combination with moIL-2 controlled tumor growth in lymphoreplete mice bearing established B16 tumors while neither component alone inhibited tumor growth. moIL-2 significantly expanded orthoPmel systemically and intratumorally, with orthoPmel ultimately accounting for greater than 40% or 80% of all peripheral and intratumoral T cells, respectively. Systemic orthoPmel maintained a consistent central memory and effector memory mix throughout the four-week moIL-2 treatment course. moIL-2 also induced the expression of activation markers, CD25 and Granzyme B, in intratumoral orthoPmel.

**Conclusions**

These findings validate that an orthogonal IL-2/IL-2Rβ platform can enhance efficacy of ACTs without peripheral expansion or activation of NK cells or non-tumor specific T cells and the toxicities typically associated with high dose IL-2 therapy[1,2]. Importantly, these results demonstrate the potential of this platform to overcome the requirement of lymphodepletion in adoptive cell therapies.

**References**

1- Aspuria et al. AACR Annual Meeting 2021

2- Aspuria et al. Science Translational Medicine (In Press)

## Orthogonal mouse IL-2/IL-2Rβ pair for selective engineered T cell enhancement *in vivo*

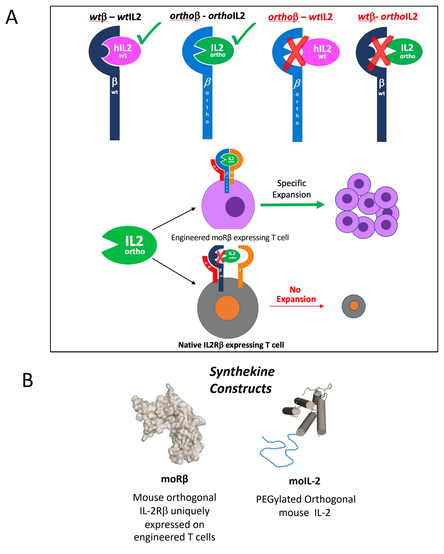



**Figure 1. Orthogonal IL-2/IL-2Rβ schema. (A)** Wild type (WT) and *ortho*IL-2Rβ receptors (moRβ) exhibit significant preference for their cognate ligand, WT and *ortho*IL-2, respectively. Therefore, engineered T cells expressing the *ortho* receptor will respond selectively to *ortho*IL-2, thereby allowing specific expansion and enhancement of engineered T cell activity. **(B)** Synthekine constructs. moRβ can be engineered into any adoptive T-cell therapy modality for expression in a desired target cell. moIL-2 is a pegylated orthogonal IL-2 for *subcutaneous* administration.

## moIL-2 enriches for moRβ transduced orthoPmel T cells during ex vivo manufacturing

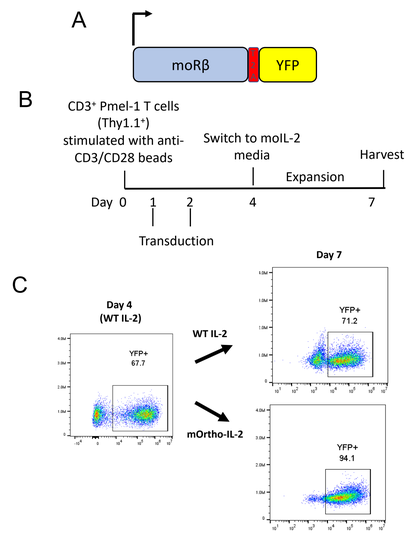



**Figure 2. moRβ construct and orthoPmel T cell manufacturing (A)** Retroviral construct containing the mouse ortho receptor, the cleavage peptide T2A and YFP fluorescent marker are expressed as a single mRNA **(B)** orthoPmel T cell manufacturing schema **(C)** Flow cytometric analysis of moRβ-T2A-YFP transduction was performed on Day 4 and Day 7.

## moIL-2 and WT mIL-2 grown orthoPmel T cells have equivalent transcriptomes

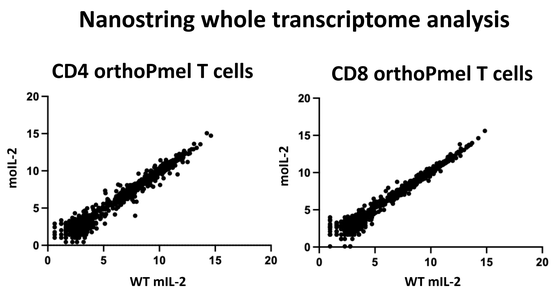



**Figure 3. Nanostring analysis of orthoPmel T cells.** OrthoPmel T cells grown in moIL-2 or WT mIL-2 were sorted for CD4 YFP$^+$ or CD8 YFP$^+$. RNA was harvested and subjected to nanostring analysis. Transcript abundance of each gene is displayed as a dot whereby the y-axis = expression in moIL-2 orthoPmel T cells and the x-axis = expression in WT mIL-2 ortho Pmel T cells. No statistically significant changes in any transcripts detected were found between the two conditions.

## moIL-2 increases the number and activity of tumor infiltrating orthoPmel T cells

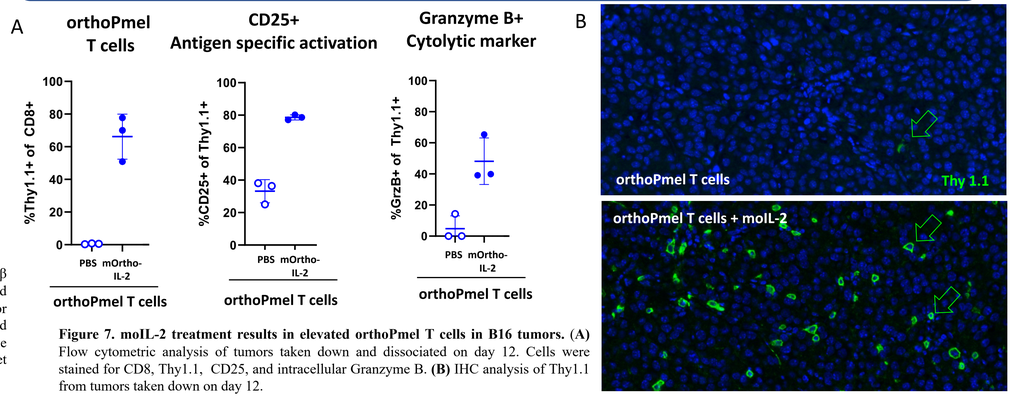
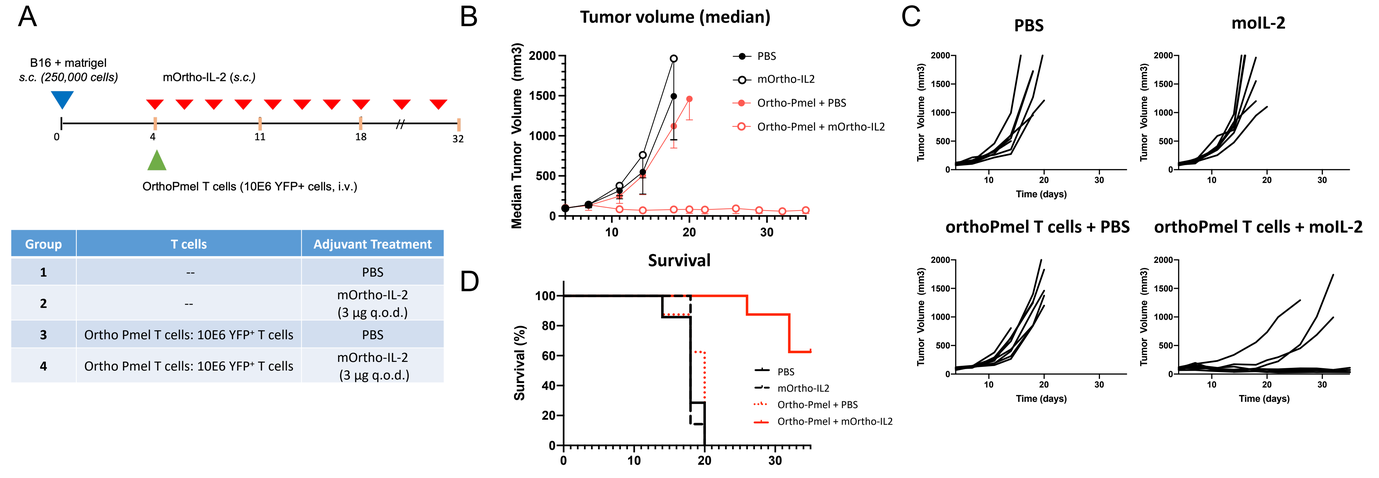



**Figure 7. moIL-2 treatment results in elevated orthoPmel T cells in B16 tumors. (A)** Flow cytometric analysis of tumors taken down and dissociated on day 12. Cells were stained for CD8, Thy1.1, CD25, and intracellular Granzyme B. **(B)** IHC analysis of Thy1.1 from tumors taken down on day 12.

## moIL-2 + OrthoPmel T cells control established B16 tumors without the need for lymphodepletion




| Group | T cells | Adjuvant Treatment |
|---|---|---|
| 1 | -- | PBS |
| 2 | -- | mOrtho-IL-2 (3 µg q.o.d.) |
| 3 | Ortho Pmel T cells: 10E6 YFP$^+$ T cells | PBS |
| 4 | Ortho Pmel T cells: 10E6 YFP$^+$ T cells | mOrtho-IL-2 (3 µg q.o.d.) |

**Figure 4. moIL-2 in combination with orthoPmel T cells controls B16 tumors without lymphodepletion. (A)** Treatment schedule for moIL-2 in combination with orthoPmel T cells in a lymphoreplete subcutaneous B16 tumor model. **(B and C)** Tumor efficacy (median tumor volume) and individual mouse spider plots, respectively. **(D)** Survival of mice with subcutaneous B16 tumors over the course of the study.

## moIL-2 + orthoPmel T cells treatment of B16 tumors results in a decrease of cancer serum biomarkers

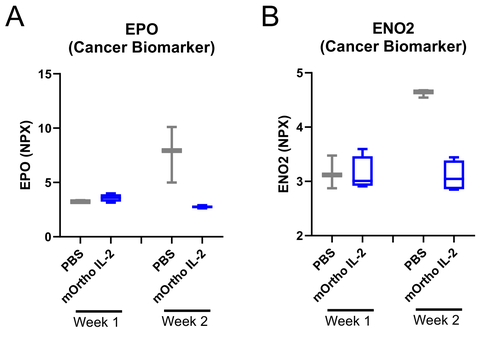



**Figure 5. O-link biomarker analysis from mouse serum reveals changes in cancer biomarkers when treated with moIL-2 and orthoPmel T cells. (A)** Erythropoietin (EPO) and **(B)** Enolase 2 (ENO2) were found to be decreased 2 weeks post-ACT in the orthoPmel T cell + moIL-2 cohort relative to orthoPmel + PBS treated mice.

## moIL-2 expands and maintains the memory phenotypes of orthoPmel T cells in the peripheral blood

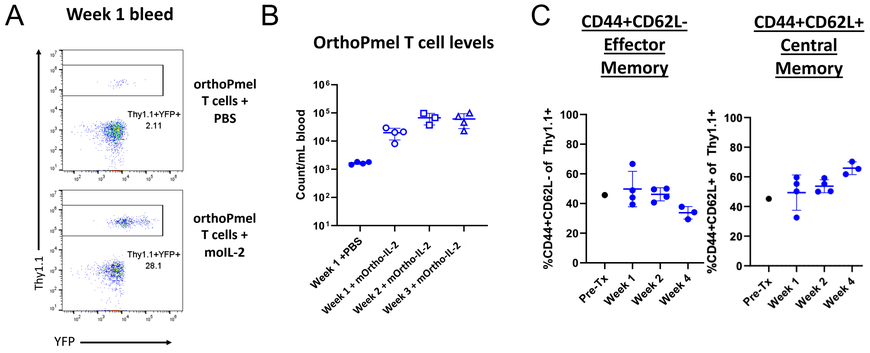



**Figure 6. mo-IL2 significantly expands orthoPmel T cells in the blood without significantly affecting T cell differentiation phenotypes. (A)** Flow cytometric analysis from week 1 post-ACT bleeds of mice treated with orthoPmel T cells with or without moIL-2. Thy1.1$^+$ YFP$^+$ denotes orthoPmel T cells. **(B)**

## moIL-2 + orthoPmel T cells treatment of B16 tumors shows evidence of epitope spreading and less exhausted endogenous host TILs

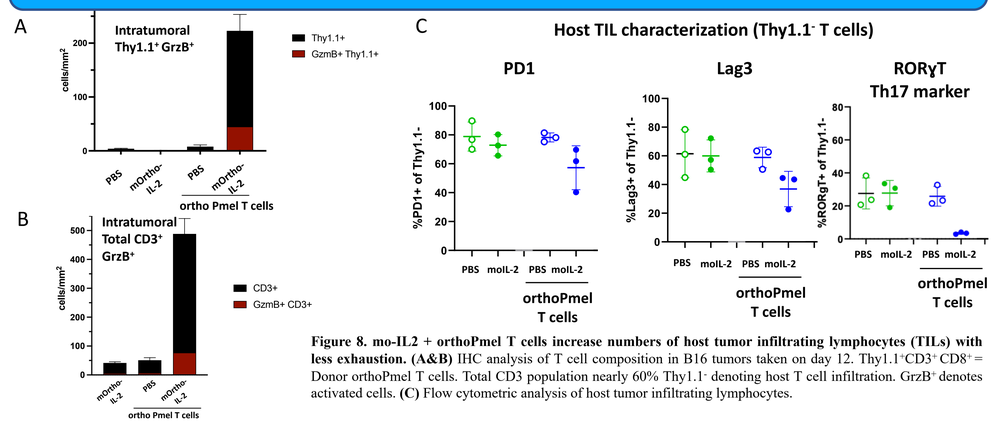



**Figure 8. mo-IL2 + orthoPmel T cells increase numbers of host tumor infiltrating lymphocytes (TILs) with less exhaustion. (A&B)** IHC analysis of T cell composition in B16 tumors taken on day 12. Thy1.1$^+$CD3$^+$ CD8$^+$ = Donor orthoPmel T cells. Total CD3 population nearly 60% Thy1.1$^-$ denoting host T cell infiltration. GrzB$^+$ denotes activated cells. **(C)** Flow cytometric analysis of host tumor infiltrating lymphocytes.