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

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 elevating T-cell common gamma-chain cytokines like IL-7 and IL-15. However, lymphodepletion regimens have been identified as a risk factor for cytokine release syndrome (CRS) and infectious complications from opportunistic pathogens. 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. 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). A highly active moIL-2 was dosed for four weeks in mice. Thy1.1$^+$ OrthoPmel T cells were tracked by FACS and IHC systemically and in the tumor.

Orthogonal Cytokine + Cell Therapy: A Lock and Key System to Stimulate ACTs Selectively *In Vivo*

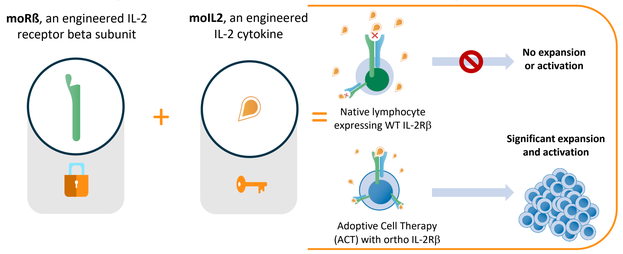


Figure 1. Orthogonal IL-2/IL-2Rβ schema. *Ortho*IL-2Rβ receptors (moRb) exhibit significant preference for their cognate ligand, moIL-2, a pegylated orthogonal IL-2. Therefore, engineered T cells expressing the *ortho* receptor will respond selectively to moIL-2, thereby allowing specific expansion and enhancement of engineered T cell activity.

moIL-2 does not expand NK cells or endogenous T cells compared to WT mIL-2 *in vivo*

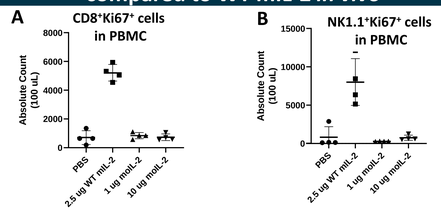


Figure 2. Analysis of PBMC CD8+ T cells and NK cells from WT mIL2 and moIL-2 treated mice. C57BL/6 non-tumor bearing mice were treated with PBS, 2.5 µg WT mIL-2, 1 µg moIL-2 or 10 µg moIL-2 q.o.d. PBMC were harvested on Day 6. **(A)** Cells were gated on Live/dead-CD3+NK1.1-CD8+KI67+ then absolute count determined. **(B)** Cells were gated on Live/dead-, CD3-, NK1.1+, KI67+.

moIL-2 enriches for moRβ transduced OrthoPmel T cells during *ex vivo* manufacturing

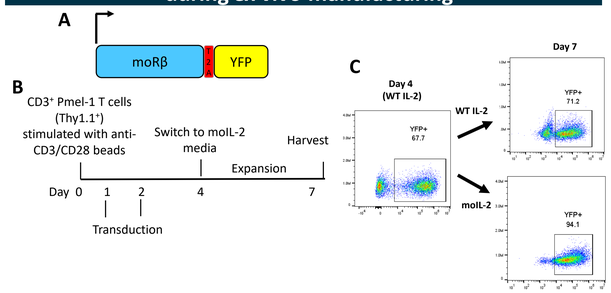
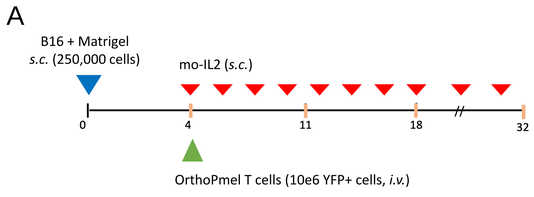


Figure 3. moRβ construct and OrthoPmel T cell manufacturing (A) Retroviral construct containing the mouse ortho receptor, the cleavage peptide T2A and YFP fluorescent marker expressed as a single mRNA **(B)** OrthoPmel T cell manufacturing schema **(C)** Flow cytometric analysis of moRβ-T2A-YFP transduction was performed on Day 4 and Day 7.

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



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

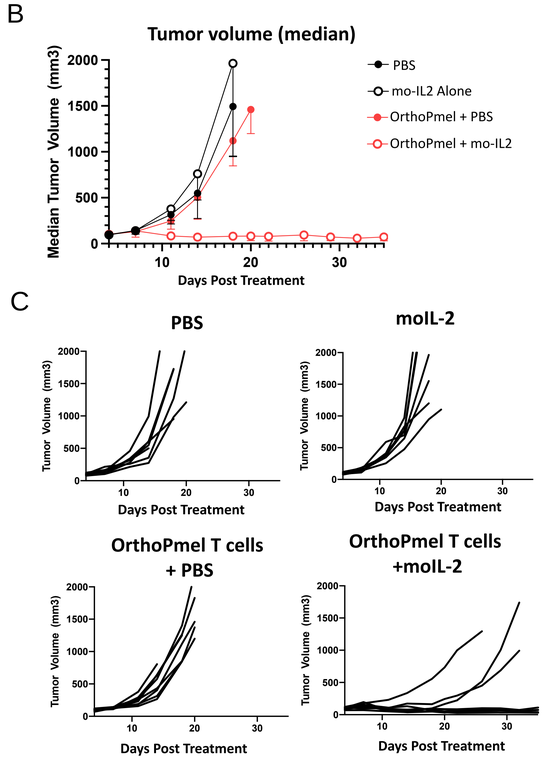


Figure 4. moIL-2 in combination with OrthoPmel T cells controls B16 tumors without lymphodepletion. (A) Treatment schedule for moIL-2 in combination with OrthoPmel T cells in a lymphoreplete subcutaneous B16 tumor model. **(B and C)** Tumor efficacy (median tumor volume) and individual mouse spider plots,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

moIL-2 expands and maintains a central memory phenotype of OrthoPmel T cells in the peripheral blood

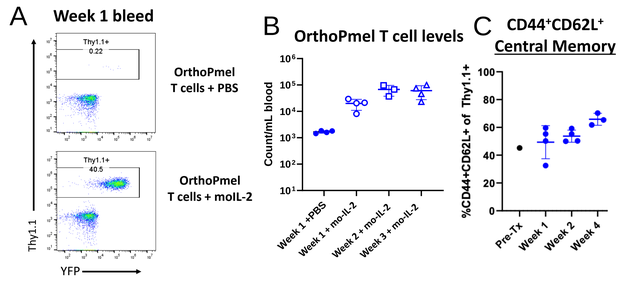
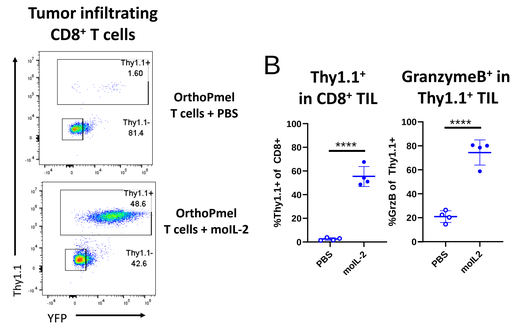
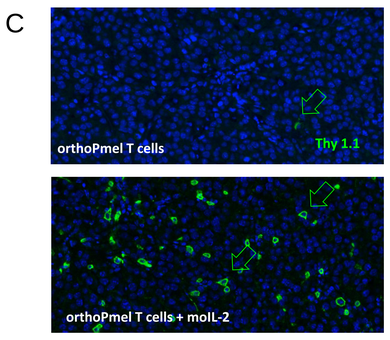


Figure 5. mo-IL2 expands OrthoPmel T cells in the blood without significantly affecting T cell differentiation phenotypes. (A) Flow cytometric analysis from week 1 post-ACT bleeds of mice treated with OrthoPmel T cells with or without moIL-2. Thy1.1$^+$ YFP$^+$ denotes OrthoPmel T cells. **(B)** Weeks 1-3 cells were gated on Live/dead-CD45+CD3+CD8+Thy1.1+ then absolute count determined. **(C)** Weeks 1-4 cells were gated on Live/dead-CD45+CD3+CD8+Thy1.1+ then %CD62L+CD44+ was determined.

moIL-2 increases numbers and activity of tumor infiltrating OrthoPmel T cells





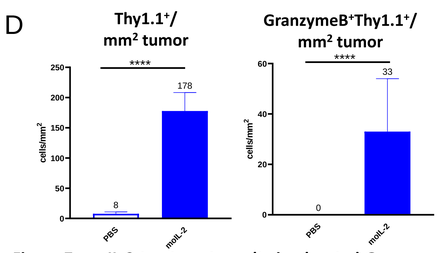


Figure 7. moIL-2 treatment results in elevated GranzymeB+ OrthoPmel T cells in B16 tumors. (**A**) Flow cytometric analysis of tumors taken down and dissociated on day 10. **(B)** Cells were gated on Live/dead-CD45+CD3+CD8+Thy1.1+ or Thy1.1+GranzymeB+. **(C, D)** IHC Analysis of Thy1.1 from tumors taken down on day 12. **** = P <0.0001

moIL-2 expands a central memory phenotype of tumor draining OrthoPmel T cells

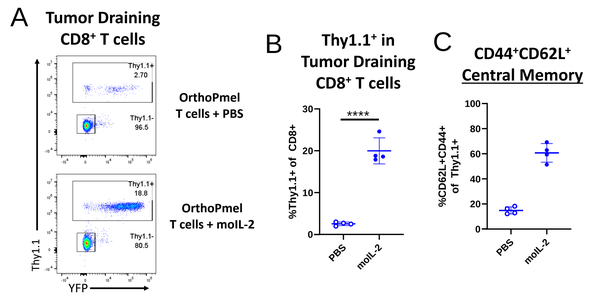


Figure 6. mo-IL2 expands OrthoPmel T cells in the draining lymph node (A) Flow cytometric analysis of tumor draining lymph nodes taken down and dissociated on day 10. Cells were gated on Live/dead-CD45+CD3+CD8+ **(B)** Flow cytometric analysis of the tumor draining lymph node on day 10, cells were gated on Live/dead-CD45+CD3+CD8+Thy1.1+. **(C)** Flow cytometric analysis of the tumor draining lymph node on day 10, cells were gated on Live/dead-CD45+CD3+CD8+Thy1.1+ then CD62L+CD44+ was determined. **** = P <0.0001

moIL-2 decreases expression of multiple checkpoints in tumor infiltrating and draining lymph node OrthoPmel T cells

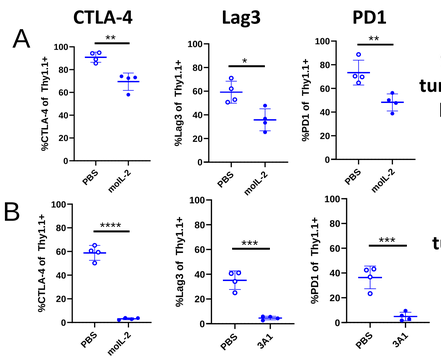


Figure 8. mo-IL2 treatment decreases checkpoint expression in B16 tumors and draining lymph nodes. (A) Flow cytometric analysis of tumors taken down and dissociated on day 10. Cells were gated on Live/dead-CD45+CD3+CD8+Thy1.1+ **(B)** Flow cytometric analysis of the tumor draining lymph node on day 10, cells were gated on Live/dead-CD45+CD3+CD8+Thy1.1+.
* = p <0.05, ** = p<0.01,*** = p<0.001, **** = P <0.0001

CONCLUSION

- **moIL-2 is selective for the moRβ transduced OrthoPmel T cells during *ex vivo* manufacturing and in *vivo* treatment.**
- **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 expanded and maintained OrthoPmel T cells systemically**
- **moIL-2 significantly expanded OrthoPmel T cells in the tumor draining lymph node, and intratumorally, accounting for greater than 20% and 60% of all CD8+ T cells, respectively**
- **moIL-2 expanded a central memory population of OrthoPmel T cells systemically and in the tumor draining lymph node**
- **OrthoPmel T cells had significantly greater Granzyme B expression and lower checkpoint expression in combination with moIL-2**

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