P473

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Adoptive Transfer of Deep IL-12 Primed™ T-cells Increases Sensitivity to PD-L1 Blockade for Superior Efficacy in Checkpoint Refractory Tumors

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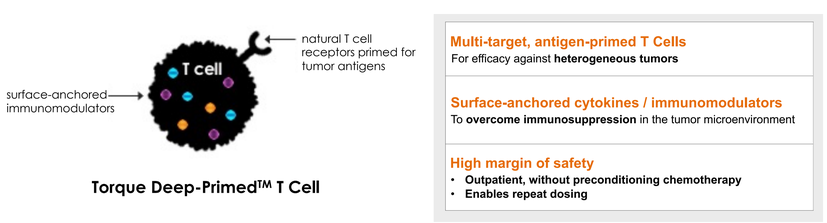
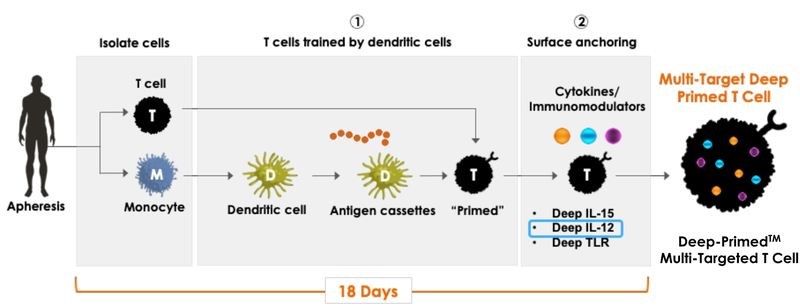
Background

While immune checkpoint blockade has revolutionized cancer care, many patients remain unresponsive to checkpoint inhibition. Converting checkpoint-refractory tumors into checkpoint-responsive tumors is a major challenge. Interleukin-12 (IL-12) is a potent cytokine that holds potential to reshape the immune environment in solid tumors. Its clinical utility, however, has been limited by severe toxicities both from systemic administration and from expression by adoptively transferred gene engineered T cells. We report here that adoptive cell transfer (ACT) of T cells carrying surface-tethered IL-12 (Deep IL-12) overcomes these challenges, enhances T cell therapeutic efficacy, activates immune cells in the tumor and overcomes resistance to checkpoint blockade. The combination Deep IL-12 Primed T cells and checkpoint blockade further improves anti-tumor efficacy

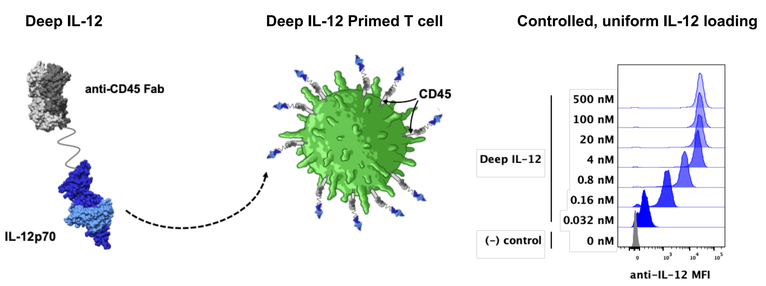
Torque platform is designed to overcome key limitations of current T cell therapies



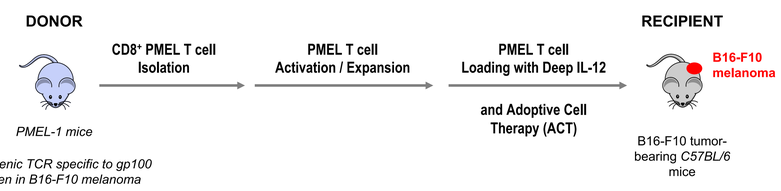
The Torque platform harnesses natural T cell biology in an *ex vivo* process



Deep IL-12 delivers controlled dose loading on the T cell surface



The PMEL T cell adoptive transfer mouse model



- Donor CD8+ PMEL T cells express a transgenic T cell receptor (TCR) directed against PMEL-17, an epitope of gp100, presented by MHC-I on B16-F10 melanoma cells.
- Model enables evaluation of antigen-specific T cell activity in fully immunocompetent mice
- Deep IL-12 loading on murine PMEL T cells and human multi-targeted T cells is comparable
- PMEL T cell *in vitro* expansion is comparable to that of human multi-targeted T cells

Results

Cell-tethered Deep IL-12 delivers superior efficacy

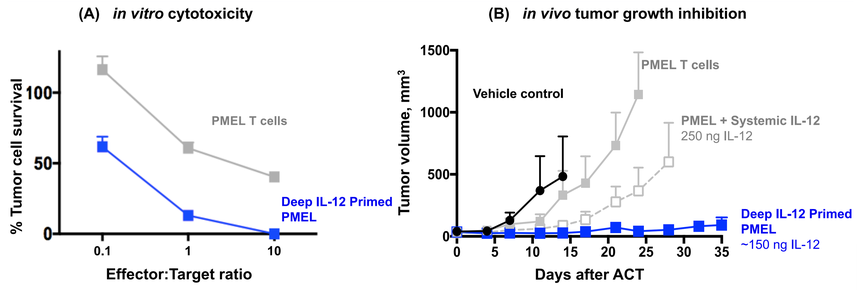


Figure 1. Anti-tumor activity of Deep IL-12 Primed T cells. We evaluated the ability of murine Deep IL-12 to augment adoptive cell therapy (ACT) for cancer using the PMEL/B16-F10 model. **(A)** Deep IL-12 enhanced cytotoxicity of PMEL T cells *in vitro*. **(B)** 3E6 Deep IL-12 Primed PMEL T cells deliver stronger tumor control than systemic coadministration of PMEL T cells and recombinant IL-12. Tumor growth curves are plotted until two mice in a given group reach tumor volume endpoint.

Cell-tethered Deep IL-12 drives activity specifically in the TME

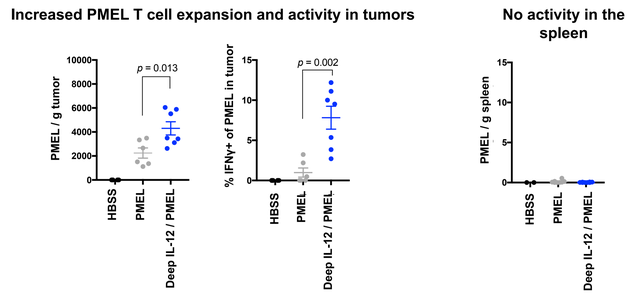


Figure 2. Effects of Deep IL-12 on PMEL T cell infiltration and IFNγ secretion in tumor and non-tumor tissue. PMEL infiltration and IFNγ secretion were assessed in tumor 4 days after ACT with 5E6 PMEL T cells alone or loaded with Deep IL-12. As a control for off-target, nontumor activity, spleens were also assessed. Deep IL-12 Primed PMEL T cells exhibited increased PMEL accumulation and activity in the tumor, but not in the spleen, demonstrating specificity of enhanced activity. .

Deep IL-12 induces immune activation in the tumor microenvironment (TME)

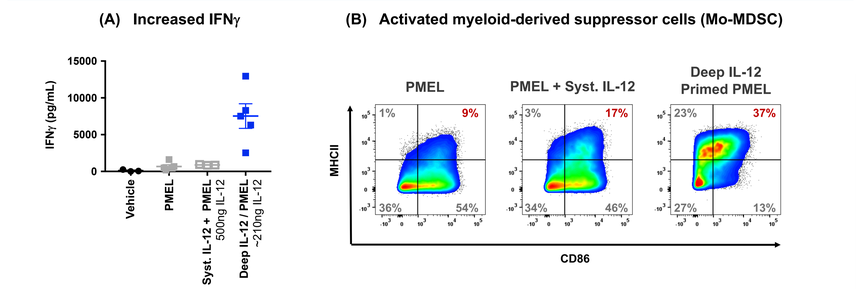


Figure 3. Immune activation in tumor microenvironment. IFNγ concentration and myeloid cell activation was assessed 4 days after ACT with 5E6 PMEL T cells alone or loaded with Deep IL-12. **(A)** Tumor IFNγ is elevated in response to Deep IL-12 Primed PMEL T cells. **(B)** Deep IL-12 (~210 ng IL-12) activates monocytic myeloid-derived suppressor cells (Mo-MDSC; $CD11b^{+}/CD11c^{-}/Ly6G^{Low}/Ly6C^{hi}$) more effectively than T cells alone or T cells co-administered with systemic IL-12 (500 ng).

Repolarized MDSC contribute to Deep IL-12 efficacy

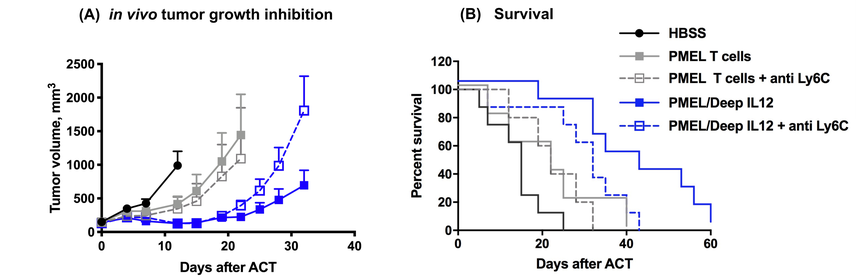
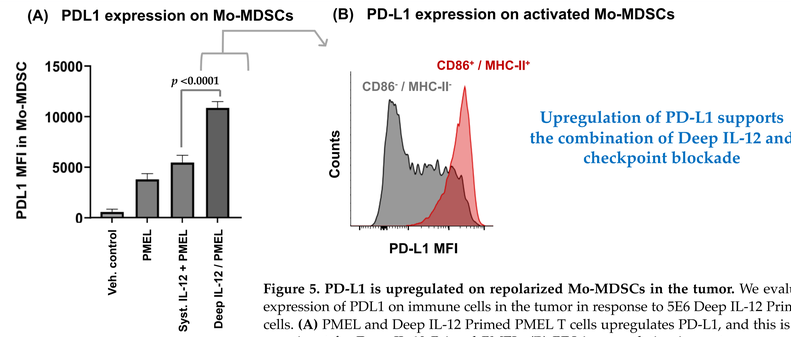


Figure 4. Depletion of Ly6C cells reduces the improved efficacy of Deep IL-12 Primed T cells. Ly6C depleting antibody was used to deplete Mo-MDSCs, a $Ly6C^{hi}$ cell population. The Ly6C depleting antibody attenuated the enhanced tumor growth inhibition **(A)** and survival **(B)** induced by 5E6 Deep IL-12 Primed PMEL T cells, but not of PMEL T cells alone.

Deep IL-12 upregulates PD-L1 expression on MDSC in the TME



Upregulation of PD-L1 supports the combination of Deep IL-12 and checkpoint blockade

Figure 5. PD-L1 is upregulated on repolarized Mo-MDSCs in the tumor. We evaluated expression of PDL1 on immune cells in the tumor in response to 5E6 Deep IL-12 Primed T cells. **(A)** PMEL and Deep IL-12 Primed PMEL T cells upregulates PD-L1, and this is most prominent for Deep IL-12 Primed PMEL. **(B)** PDL1 upregulation is most pronounced on the repolarized (CD86+ / MHC-II+) Mo-MDSCs.

Checkpoint blockade increases Deep IL-12 induced IFNγ expression in the tumor

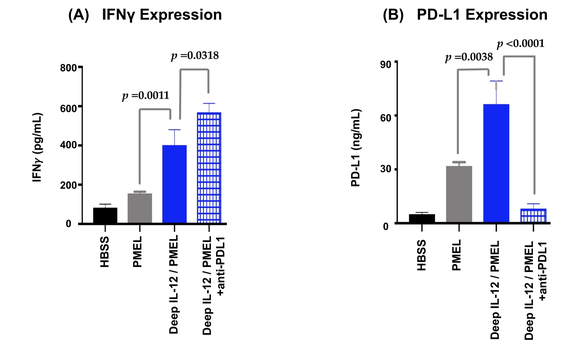


Figure 6. PD-L1 blockade enhances Deep IL-12 Primed T cell mediated secretion of IFNγ in the tumor microenvironment. IFNγ and PDL1 levels were assessed in tumor by ELISA 7 days after ACT with 5E6 PMEL T cells alone or loaded with Deep IL-12. **(A)** IFNγ expression is most strongly driven by Deep IL-12, and enhanced by PD-L1 blockade **(B)** PD-L1 expression is upregulated by PMEL and Deep IL-12 Primed PMEL. PD-L1 inhibition attenuates available ligand in tumor.

PD-L1 blockade in combination with Deep IL-12 prolongs survival

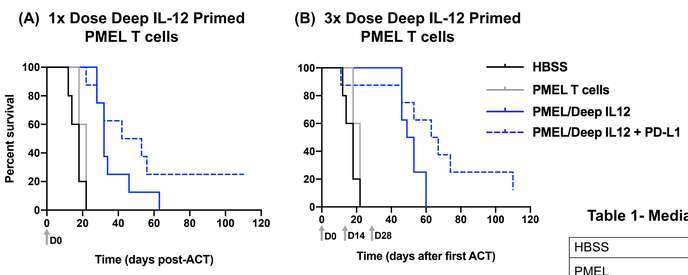


Figure 7. Deep IL-12 Primed cells in combination with PD-L1 blockade enhances overall survival. Mice were treated with 1 or 3 Deep IL-12 Primed T cell doses (5E6 T cells/dose) spaced two weeks apart with or without anti-PD-L1 checkpoint inhibitor. Lymphodepletion with cyclophosphamide was used one day prior to the first cell dose, while the second and third cell doses were given in the absence of additional lymphodepletion. PD-L1 checkpoint blockade enhanced median survival and durable responses induced by Deep IL-12 Primed PMEL T cells.

Table 1- Median Survival (Days)

HBSS	18
PMEL	22
PMEL/Deep IL-12 (1x)	32
PMEL/Deep IL-12 (1x) + anti-PD-L1	47.5
PMEL/Deep IL-12 (3x)	51
PMEL/Deep IL-12 (3x) + anti-PD-L1	65

Conclusions

- T cell-tethered Deep IL-12 overcomes key limitations of IL-12
  - Focuses inflammatory activity on the tumor microenvironment (TME)
  - Limits systemic effects
- Drives durable regression of established tumors
- Combination with PD-L1 blockade further improves efficacy
  - Deep IL-12 repolarizes Mo-MDSCs to activated APC-like phenotype, but also upregulates PD-L1 expression
  - Anti-PD-L1 antibody blocks PD-L1 activity in tumor and enhances survival

TORQUE