Abstract Poster # P227

Presented at
SITC Annual Meeting 2018
November 7-11, 2018
Washington, D.C.

Tethering IL-12 to the surface of T cells induces broad immune activation and potent anti-tumor activity in mice without inducing systemic toxicities

De-Kuan Chang, Gulzar Ahmad, Jonathan D. Nardozzi, Katharine L. Sackton, Thomas L. Andresen, Douglas S. Jones
Torque Therapeutics, Cambridge, MA

Abstract

Introduction

T cell-based immunotherapy has shown dramatic efficacy in some hematologic malignancies but translating these successes to solid tumors has been limited. A key challenge has been overcoming the immunosuppressive tumor microenvironment, which inhibits T cell activity and survival. Interleukin-12 (IL-12) holds strong potential for reshaping the anti-inflammatory environment in solid tumors. Its clinical utility, however, has been limited by severe toxicities both from systemic administration, or from its constitutive production by genetically engineered tumor-specific T cells. To overcome these obstacles, we developed the Deep Primed™ platform, which anchors potent immune modulators such as IL-12 on the surface of adoptively transferred T cells to support immune activation in the tumor microenvironment. Our approach is versatile and enables tunable loading and persistence of IL-12 on the T cell surface to avoid systemic exposure and overt toxicity.

Materials and experimental design

Safety and efficacy profile of our Deep IL-12 cytokine was evaluated in an immune-competent adoptive T cell therapy model. Briefly, we utilized CD8 T cells from Pmel mice, which express a transgenic T cell receptor specific for the gp100 antigen expressed in B16-F10 melanoma cells. Deep IL-12 was loaded *ex vivo* onto Pmel T cells which were then adoptively transferred into C57BL/6J mice bearing B16-F10 tumors. Here we present the effects of Pmel T cells with or without Deep IL-12 Priming on tumor growth inhibition, cytokine secretion, toxicity biomarkers, blood and tissue chemistry, and immune cell activity.

Results

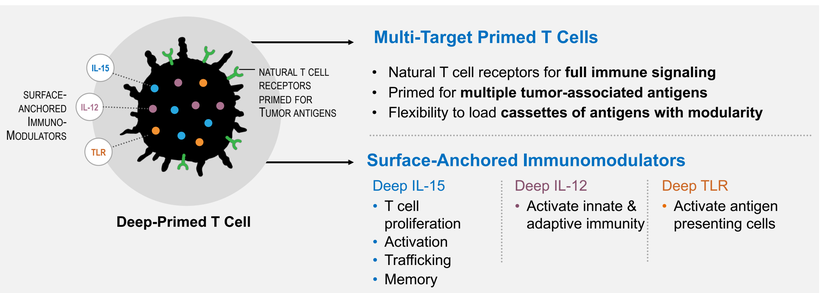
Deep IL-12 significantly improved the anti-tumor efficacy of adoptively transferred Pmel T cells against established B16-F10 tumors compared with Pmel T cells alone or with systemic co-administration of IL-12. Multiple doses of Deep IL-12 Primed Pmel T cells further improved anti-tumor activity. This contrasts with multiple doses of Pmel T cells alone, which provided minimal anti-tumor activity. Deep IL-12 increased peak expansion and long-term engraftment of Pmel T cells without expanding circulating NK cells, which are believed to be a key mediator of IL-12 toxicity. There were no observed overt toxicities in the form of body weight loss, liver, or kidney toxicity despite a modest, transient increase in circulating IFNγ. IFNγ induction in tumors, by comparison, was both more elevated and sustained than in circulation. This shows Deep IL-12 Priming delivers strong pro-inflammatory activity in the tumor microenvironment.

Conclusions

Our data demonstrates that tethering IL-12 to the T cell surface with Deep IL-12 dramatically improves the efficacy of tumor-targeted immunotherapy and mitigates the systemic toxicity of IL-12.

Deep-Primed T Cells

Directed immune activation deep within the tumor microenvironment



Tethering IL-12 to surface of tumor-specific T cells

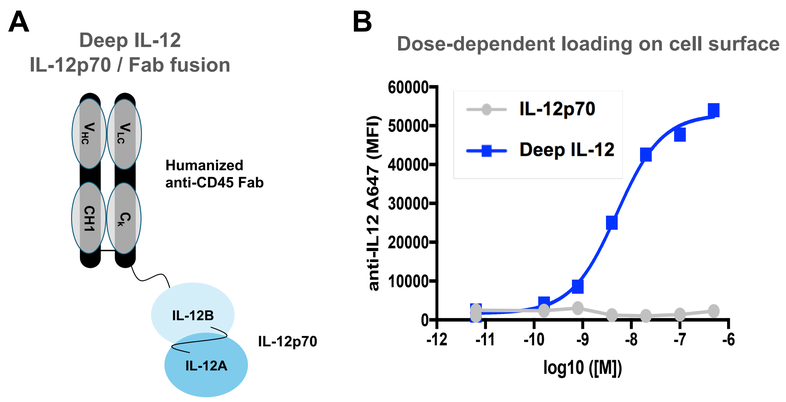


Figure 1. Antibody-mediated tethering of IL-12 to an abundant cell surface receptor allows concentration-dependent cell loading. (A) Schematic of Deep IL-12, comprising a fusion of IL-12 with a high-affinity anti-CD45 antibody Fab fragment. (B) Deep IL-12 – but not native IL-12 – allows concentration-dependent loading of IL-12 on the T cell surface.

Results

Deep IL-12 Priming improves T cell therapy better than systemic IL-12

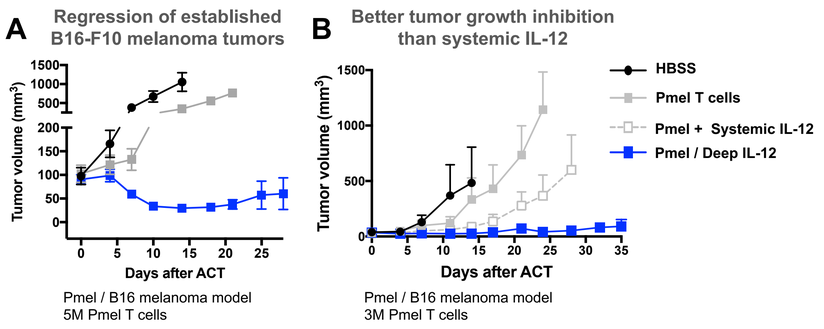


Figure 2. (A) Deep IL-12 Primed Pmel T cells – but not Pmel T cells alone – induced regression of established orthotopic B16-F10 melanoma tumors (100 mm³ starting volume). Pmel T cells were administered one day after lymphodepletion with cyclophosphamide. (B) Deep IL-12 Primed Pmel T cells deliver stronger tumor control than systemic co-administration of Pmel T cells and recombinant IL-12 (250 ng). Tumor growth curves are plotted until two mice in a given group die.

Multiple Deep IL-12 Primed T cell doses further improve tumor control and survival

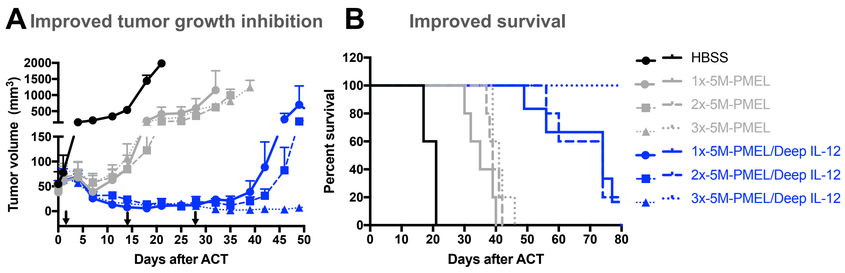


Figure 3. Mice were treated with 1 to 3 T cell doses spaced two weeks apart. Lymphodepletion with cyclophosphamide was applied one day before the first T cell dose. Multiple doses of tumor-specific T cells carrying Deep IL-12 – but not multiple doses of tumor-specific T cells alone – further augmented anti-tumor efficacy (A) and survival (B).

No observed liver or kidney toxicities from Deep IL-12 Primed T cell therapy

No observed liver toxicity

	AST (U/L)	ALT (U/L)	GGT (U/L)	Bilirubin (mg/dL)
HBSS	187.3±11.0	53.33±5.86	2±2.65	0.23±.005
Pmel	144.4±23.3	51.8±10.13	1.4±0.55	0.22±0.04
Pmel/Deep IL-12	158.5±28.6	59±1.83	1.75±1.5	0.225±0.05

No observed kidney toxicity

	BUN (mg/dL)	Creatinine (mg/dL)	Lipemia Index
HBSS	28.33±3.0	0.13±0.12	Normal
Pmel	25.8±4.3	0.2	Normal
Pmel/Deep IL-12	26.25±2.6	0.125±0.05	Normal

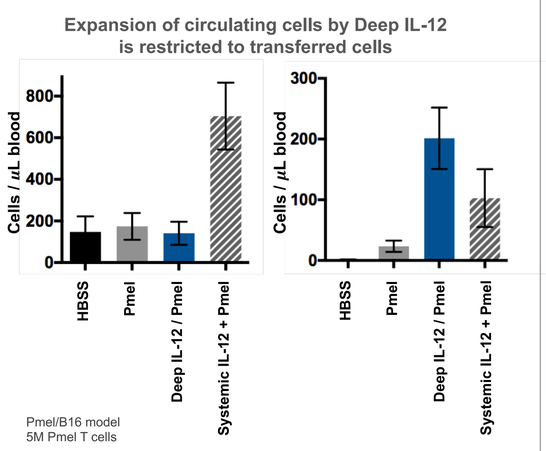
Pmel/B16 model
5M Pmel T cells

Table 1. Liver and kidney circulating biomarkers were assessed four days after adoptive transfer of Deep IL-12 Primed Pmel T cells into B16 tumor-bearing mice. No significant changes in liver or kidney toxicity biomarkers were observed.

Tethering IL-12 to T cell surface avoids activation of circulating immune cells

Figure 4. NK cells have been implicated as a key mediator of IL-12 toxicity in patients. Tethering Deep IL-12 to Pmel T cells increased their peak expansion about 10-fold relative to Pmel T cells alone (right), but did not affect the number of circulating NK cells (left).

Conversely, systemic co-administration of 250 ng recombinant IL-12 increased circulating NK cells and had more modest effects on Pmel T cells.



Results

Only transient induction of circulating cytokines

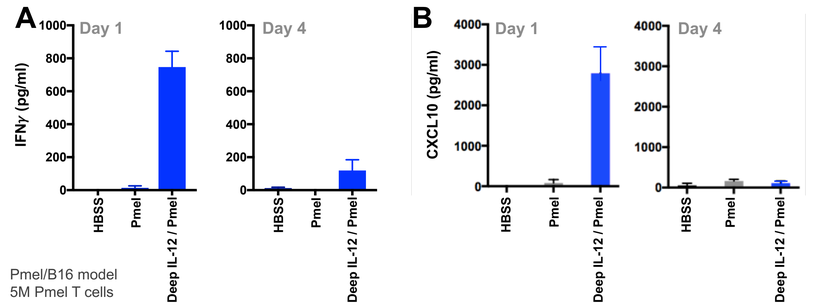


Figure 5. Elevation of systemic IFNγ (A) and CXCL10 (B) levels was modest one day following ACT with Deep IL-12 Primed Pmel T cells and had returned to within four days.

Deep IL-12 drives sustained IFNγ production in the tumor microenvironment

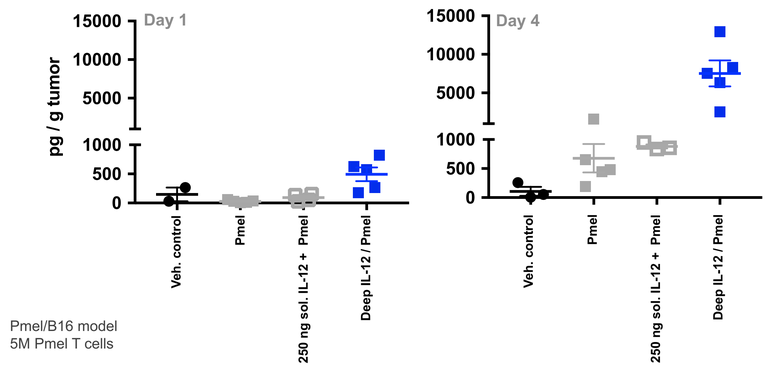


Figure 6. Strong and sustained IFNγ induction in the tumor – including and time-points where circulating IFNγ has returned to baseline.

Specific activation and expansion in the tumor microenvironment

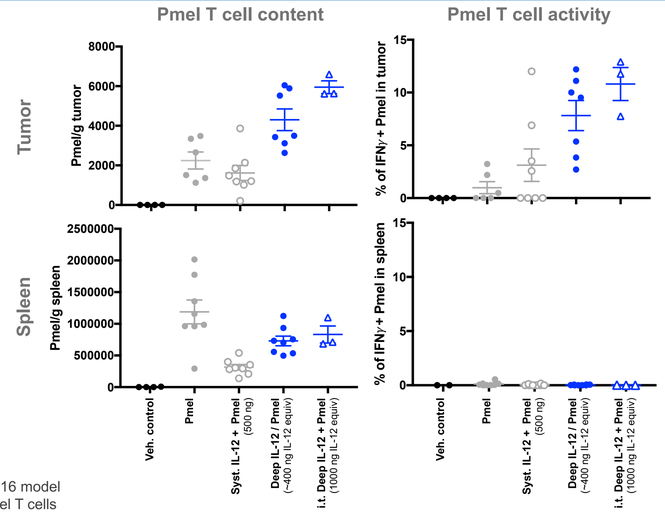


Figure 7. Deep IL-12 Priming increased Pmel T cell accumulation and activity in tumors, but not in nontumor tissue. Cell-anchored Deep IL-12 approaches the maximal intra-tumoral activity and specificity achieved via intratumor (i.t.) injection of Deep IL-12. Co-administration of systemic IL-12 failed to augment Pmel T cell accumulation or activity in tumors.

Deep IL-12 Key Findings

Superior efficacy and T cell activity in TME for Deep IL-12 vs. systemic IL-12

Deep IL-12 Priming overcomes obstacles limiting systemic IL-12 or gene-engineered IL-12

- Elevated and durable induction of IFNγ in tumor vs transient induction in circulation
- Increased activity of adoptively transferred T cells specifically in TME
- Limited systemic exposure and no observed systemic toxicity

No genetic engineering required

Readily incorporated into other cell therapies for cancer

- CAR-T cells, TCR-T cells, tumor-associated antigen-specific cells, and NK cells

Biologic manufacturing and IND-enabling toxicology studies have been initiated for a Deep IL-12 clinical candidate

TORQUE