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Presented at the 4th CRI-CIMT-EATI-AACR International Cancer Immunotherapy Conference

September 30–October 3, 2018
New York, NY

T cell priming with Deep™ IL-15 improves preclinical safety compared to systemic IL-15, and increases in vivo persistence and activity

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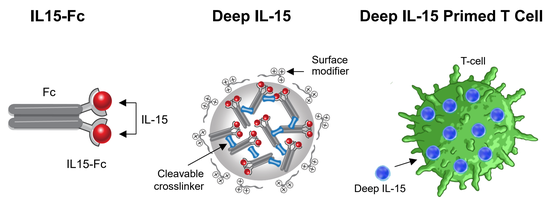
Introduction

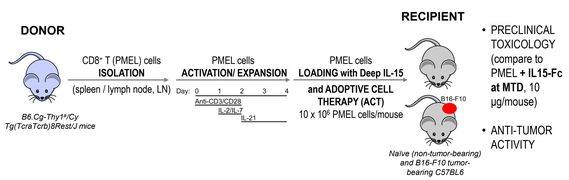
Interleukin-15 (IL-15), provides strong activation of both CD8⁺ T cells and NK cells, without regulatory T cell activation, making it an attractive immune modulator in cancer therapy. Systemic delivery of IL-15 to patients has revealed dose-limiting toxicities resulting primarily from expansion of NK cells. Preclinical data suggest that IL-15 immunotoxicity is mediated by hyperproliferation and activation of NK cells (Guo Y, J Immunol 2015). In this study, we investigated safety and efficacy of T cells loaded with Deep IL-15 (Deep IL-15 Primed T cells), in a syngeneic mouse model. Deep IL-15 is a multimer of chemically crosslinked IL-15/IL-15 Rα/Fc heterodimers (IL15-Fc) that is designed to be surface-anchored to T cells prior to adoptive cell transfer with the aim of improving the therapeutic window by autocrine signaling to the primed cells without causing the immunotoxicological effects normally associated with IL-15. Deep IL-15 is loaded on the T cells and, upon crosslinker cleavage, releases IL15-Fc to stimulate the primed cell. This novel T cell-based therapeutic approach enables autocrine T cell activation and expansion, and limits systemic exposure to IL15-Fc, thus reducing associated toxicities.

Deep IL-15 Provides Autocrine Cytokine Stimulation



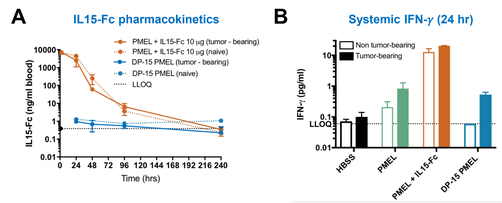
PMEL model: surrogate for human Deep IL15 Primed multi-targeted T cells

- Donor CD8⁺ PMEL T cells express a transgenic T cell receptor (TCR) directed against PMEL-17, an epitope of gp100, expressed on B16-F10 melanoma tumor cells.
- Model enables evaluation of antigen-specific T cell activity in a fully immunocompetent mouse
- Deep IL-15 loading on mouse PMEL cells and human multi-targeted T cells is comparable
- PMEL cells show in vitro expansion comparable to multi-targeted T cells



Results

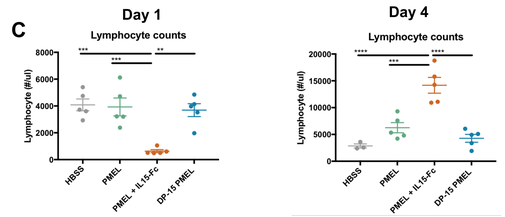
Deep IL-15 results in lower exposure to IL15-Fc and lower IFN-γ release compared to soluble IL15-Fc



PMEL + IL15-Fc (24 hr post-dose):

- > 3,000-fold higher IL15-Fc systemic concentration than DP-15 PMEL
- > 41-fold higher IFN-γ release compared to DP-15 PMEL

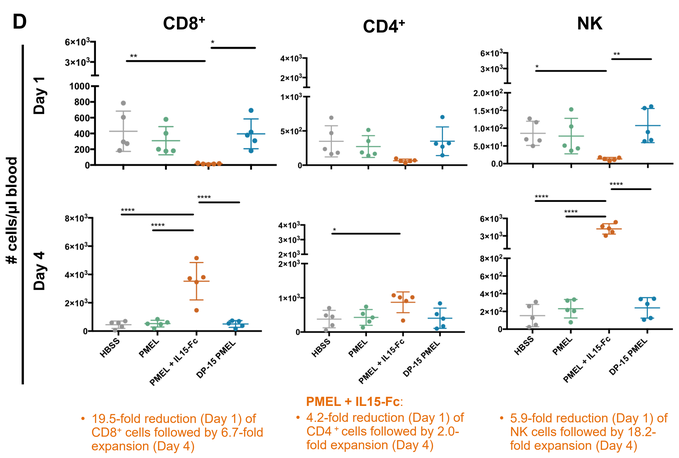
In contrast to IL15-Fc, Deep IL-15 does not affect leukocyte counts



PMEL + IL15-Fc:

- 6.2-fold reduction (Day 1) in lymphocyte counts followed by a 2.3-fold expansion (Day 4)
- Neutrophils similarly affected

Deep IL-15 does not expand endogenous CD8⁺, CD4⁺ and NK cells



PMEL + IL15-Fc:

- 19.5-fold reduction (Day 1) of CD8⁺ cells followed by 6.7-fold expansion (Day 4)
- 4.2-fold reduction (Day 1) of CD4⁺ cells followed by 2.0-fold expansion (Day 4)
- 5.9-fold reduction (Day 1) of NK cells followed by 18.2-fold expansion (Day 4)

Figure 1. PMEL cells were activated ex vivo with anti-CD3/anti-CD28 coated plates, loaded with HBSS (PMEL, 10 x 10⁶) or Deep IL-15 (DP-15-PMEL, 10 x 10⁶), and injected into naïve or B16-F10 tumor-bearing C57B6 mice. HBSS and PMEL cells (10 x 10⁶) co-administered with IL15-Fc (10 µg/mouse) were injected as negative and positive controls. Blood was drawn at 2, 24, 48, 96 and 240 hrs for quantification of IL15-Fc (ELISA) (**A**) and IFN-γ (Luminex) (**B**), for Complete Blood Counts (CBCs) evaluation (**C**), and for enumeration of endogenous CD8⁺ and NK cells (**D**) Flow Cytometry).

Deep IL-15 PMEL cells result in minimal/mild histopathology findings

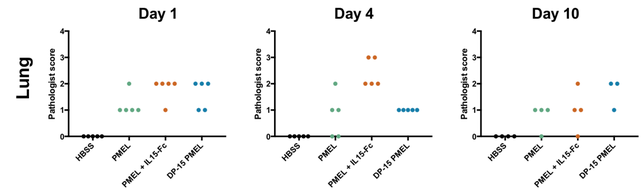


Figure 2. Formalin-fixed paraffin-embedded (FFPE) tissues (liver, lung, spleen, kidney, heart and brain) were stained with hematoxylin and eosin (H&E) and scored in a blinded fashion by a board-certified veterinary pathologist (score 0 to 4). *0 = no change; 1 = minimal; 2 = mild; 3 = moderate; and 4 = marked*. The results are shown for the lungs "Round Cell Infiltrate" around the pulmonary vein.

Deep IL-15 improves persistence of transferred PMEL cells across multiple tissues

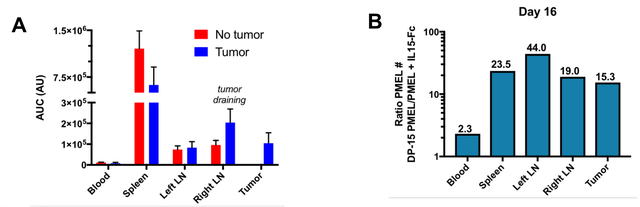
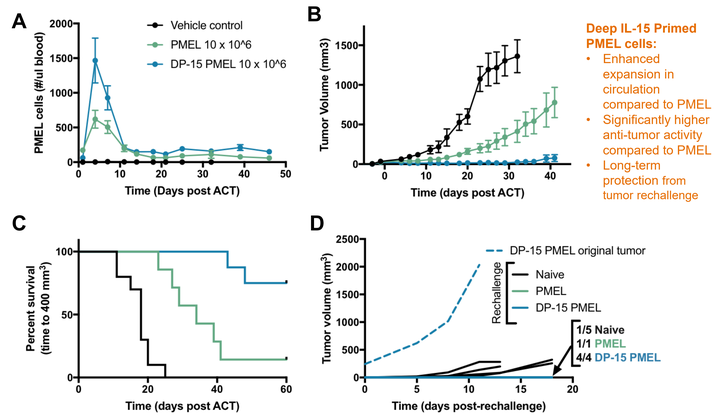


Figure 3. Deep IL-15 Primed PMEL cells (CD90.1⁺) in blood, spleen, left and right lymph nodes (LN) were enumerated by flow cytometry. (**A**) Biodistribution of PMEL cells in naïve and tumor - bearing mice treated with Deep IL-15 Primed PMEL cells (10 x 10⁶). The Area Under the Curve (AUC, Day 1 to Day 10) for the individual tissues was calculated in Prism. (**B**) Ratios of the numbers of PMEL cells in Deep IL-15 Primed PMEL cells or PMEL + IL15-Fc treated mice for the individual tissues at the end of the study (Day 16).

Deep IL-15 loading improves expansion and anti-tumor activity compared to PMEL



Deep IL-15 Primed PMEL cells:

- Enhanced expansion in circulation compared to PMEL
- Significantly higher anti-tumor activity compared to PMEL
- Long-term protection from tumor rechallenge

Figure 3. C57BL6 mice were inoculated with B16-F10 melanoma tumor cells (0.2 x 10⁶, intradermal). When tumors reached an average volume of 29 mm³, mice were treated with cyclophosphamide (4 mg/mouse, Day -1), followed by administration of vehicle control (HBSS), PMEL cells (10 x 10⁶) or Deep IL-15 Primed PMEL cells (DP-15 PMEL, 10 x 10⁶) (Day 0). Blood was drawn at designated time points for enumeration of transferred and endogenous cells by Flow Cytometry (**A**). Tumor volume (**B**) and percent survival (time to 400 mm3) (**C**) were monitored over time. 60 days post dose, remaining mice on study were rechallenged with B16-F10 (**D**).

Summary

- Deep IL-15 Primed PMEL cells:
  - Preferentially expand CD8+ T cells in circulation and intratumorally
  - Do not increase bystander circulating neutrophils or lymphocytes, including NK cells, which are associated with the immunotoxicity for systemic IL-15
  - Result in dramatically lower systemic exposure to IL15-Fc
  - Do not induce significant systemic cytokine release
  - Do not result in significant body weight loss
  - Result in histopathology findings of lower severity compared to IL15-Fc across multiple organs
- Deep IL-15 Primed PMEL cells do show different biodistribution in naïve vs tumor – bearing mice:
  - Reduced accumulation in spleen of tumor – bearing mice
  - Enhanced accumulation in tumor-draining LN compared to contralateral LN
- Loading of PMEL cells with Deep IL-15 results in increased persistence of PMEL cells in circulation as well as in the periphery and at the tumor site.
- Deep IL-15 primed PMEL cells show improved in vivo expansion and anti-tumor activity compared to PMEL.
- Clinical trials with Deep IL-15 Primed multi-target T cells (TRQ15-01) are expected to start in 2018.

References

1. Guo Y et al., J Immunol (2015), **IL-15 Superagonist-Mediated Immunotoxicity: Role of NK Cells and IFN-γ.**
2. Tang L et al Nat. Biotechnol. (2018) **Enhancing T cell therapy through TCR-signaling-responsive nanoparticle drug delivery.**

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