Enhanced expansion and tumor targeting of adoptively transferred T cells with NKTR-214

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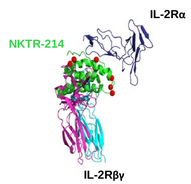
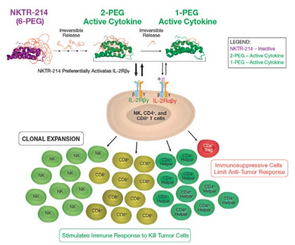
Background

- The adoptive cell transfer (ACT) of genetically engineered T cells expressing cancer-specific TCR in combination with high dose Interleukin-2 (IL-2) is able to induce effective anti-tumor response. However, tumors frequently relapse after an effective initial response and the known toxicities of IL-2 further limit use of this therapy.
- IL-2 is a cytokine that activates and expands tumor killing lymphocytes, but also potently activates suppressive T regulatory cells (Tregs) by binding to the heterotrimeric IL-2Rαβγ.
- NKTR-214 is a CD122-biased cytokine agonist conjugated with multiple releasable chains of polyethylene glycol (PEG), designed to provide sustained signaling through the IL-2Rβγ pathway to preferentially activate and expand effector CD8+ T and NK cells over Tregs in the tumor.
- NKTR-214 is being evaluated in an outpatient setting in a Phase 2 expansion trial. NKTR-214 has a favorable safety and tolerability profile.

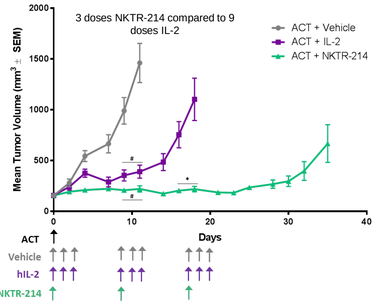


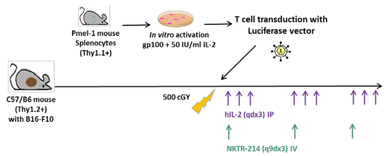


Here we evaluated the tumor immunology, biodistribution of CD8 T cells by immuno-imaging and anti-tumor activity of NKTR-214 in the pre-clinical model of pmel-1 ACT in the B16F10 tumor melanoma model.

Results

NKTR-214 provides significant anti-tumor growth delay in combination with ACT compared to IL-2 + ACT

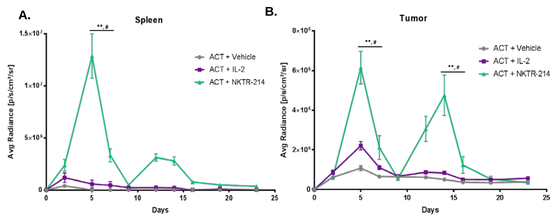




C57/BL6 mice were implanted subcutaneously with B16F10 syngeneic murine melanoma cell line on D-7 and lymphodepleted on D-1. On D0, mice were treated with the combination of ACT (pmel-1 gp100 TCR transgenic T lymphocytes) and NKTR-214 (0.8 mg/kg, q9dx3, i.v.) or IL-2 (0.4 mg/kg, qdX3 every 9 days for 3 cycles, i.p.), or vehicle.

* p<0.0001 compared to ACT+IL-2; # p<0.0001 compared to vehicle (pair-wise comparison using Bonferroni test).

Increased T cell expansion in the spleen and homing to and persistence in the tumor is associated with NKTR-214 treatment

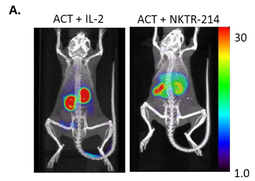


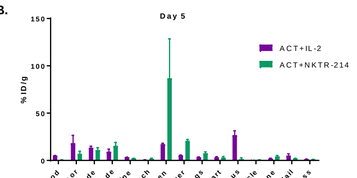
In vivo bioluminescence imaging (BLI) of adoptively transferred pmel-1 transgenic T cells expressing luciferase. Quantification of serial images in the region of interest (ROI) of spleen (**A.**) and tumor (**B.**).

(** p<0.0001 compared to IL-2+ACT, # p<0.0001 compared to ACT+vehicle, pair-wise comparison using Tukey test, n =5, mean ± SE).

Results

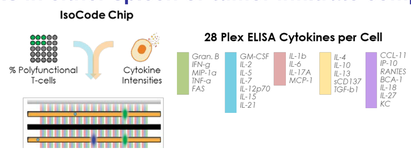
Immuno-PET imaging using cys-diabody (cDb) targeting CD8 *in vivo* shows significant T cells expansion in spleen for ACT+NKTR-214-treated mice



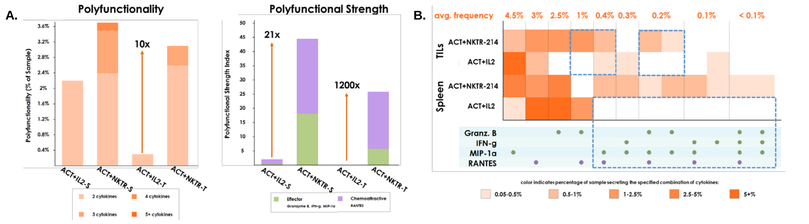


A. Representative CD8 cys-diabody immuno-PET/CT images acquired on D5 after treatment with ACT+IL-2 or ACT+NKTR-214 (n=3/group). I.V. injection of Zr-89 labeled anti-CD8 cDb was performed 24h prior imaging of C57/BL6 mice. **B.** *Ex-vivo* biodistribution analysis. %ID/g: injected dose per gram. The diabody and residualizing radionucleotide undergo renal clearance, hence signals from the kidney are not plotted. These values are mean ID%/g: 72.5 and 12.09 for ACT+NKTR-214 and ACT+IL-2, respectively.

NKTR-214 elicits a marked upregulation of polyfunctionality in Thy1.1 specific T cells in either spleen or tumor infiltrate compared to IL-2

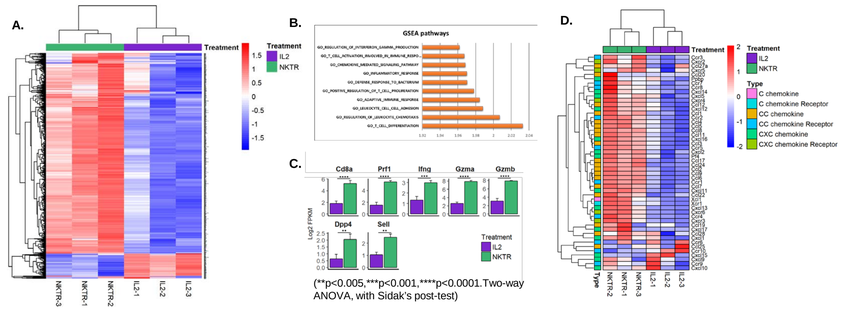


Single cell measure of T cell polyfunctionality and strength. Sorted antigen-specific CD8+ T cells Thy1.1+ from tumors and spleen D7 after ACT+NKTR-214 or ACT+IL-2 (3 mice/group) were stimulated and stained with anti-mouse CD8. The cells were loaded onto an IsoPlexis IsoCode chip containing ~12000 microchambers pre-patterned with a 28-plex antibody array to the secreted cytokines (left). Polyfunctionality is defined as secretion of 2+ cytokines per cell and evaluated by Isoplexis' software.



A. Polifunctionality and Polyfunctional Strength are considerably increased in samples treated with ACT+NKTR-214 vs ACT+IL-2. *Polyfuctionality: co-secretions of 2+ cytokines per cell; ** PSI: percentage of polyfunctional cells in the sample, multiplied by the intensities of the secreted cytokines. **B.** Heatmap reveals that NKTR-214 elicits more polyfunctional cell subsets in the adoptively transferred TCR-T cells in spleen and tumor compared to IL-2.

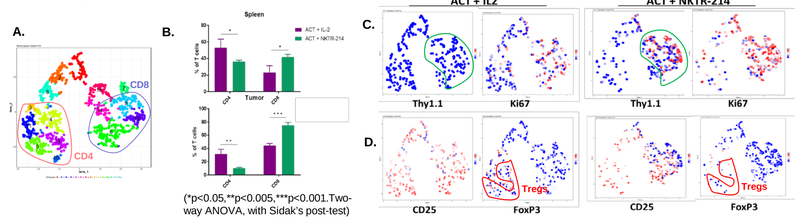
NKTR-214 increases expression of genes associated with T cell cytotoxicity and memory



(**p<0.005,***p<0.001,****p<0.0001.Two-way ANOVA, with Sidak's post-test)

Tumors were harvested at D12, after the second cycle of NKTR-214 or IL-2 administered at D9. RNA-Seq was performed on tumor samples to assess gene expression changes. **A.** Heatmap representing hierarchical clustering of most variable genes (~1500 genes) in the ACT+NKTR-214 treated group vs. ACT+IL-2 (3 replicates/group). Data show that ACT+NKTR-214 treatment induced far greater gene expression in tumor samples. **B.** Gene set enrichment analysis of log2 fold-change of ACT+NKTR-214 vs ACT+IL-2 samples, representing the top-10 biological pathways with significant gene induction. **C.** Bar charts of select cytotoxic (upper panel) and memory (lower panel) T cell genes. **D.** Clustering of chemokines and their receptors, showing a general trend of increased expression in the ACT+NKTR-214 group.

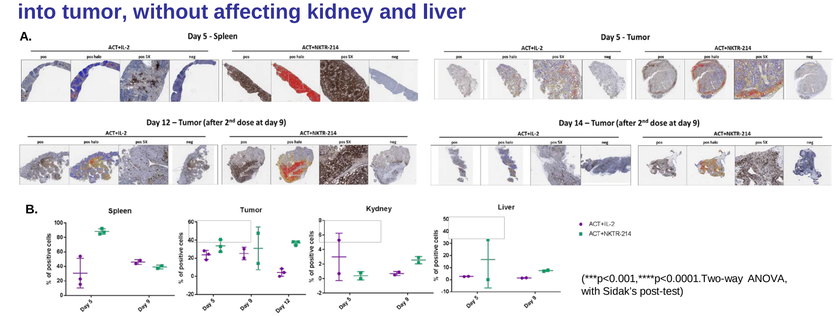
NKTR-214 promotes persistent proliferation of tumor-specific Thy1.1 T cells, but not Tregs in tumor



(*p<0.05,**p<0.005,***p<0.001.Two-way ANOVA, with Sidak's post-test)

Mass cytometry to comprehensively profile immune cells was performed on spleen/tumor at D14 after cycle 2 of NKTR-214 or IL-2 (D9). Manually gated singlet viable CD45+ events were imported into Cytofkit and analyzed by viSNE. **A.** PhenoGraph analysis of spleen and tumor. **B.** Percentage of T-cell clusters shows a marked increase in CD8/CD4 ratio in tumors of the ACT+NKTR-214 group which is less pronounced in peripheral tissue (spleens) **C.** t-SNE plots showing persistence of specific (Thy1.1) and proliferating (Ki67) TILs in ACT+NKTR-214 group only. **D**. t-SNE plots showing higher regulatory T cells (CD25+ FoxP3+) tumor infiltration in the ACT+IL-2 group compared to ACT+NKTR-214.

NKTR-214 triggers rapid expansion in spleen and persistent CD8 T cell infiltration into tumor, without affecting kidney and liver



(***p<0.001,****p<0.0001.Two-way ANOVA, with Sidak's post-test)

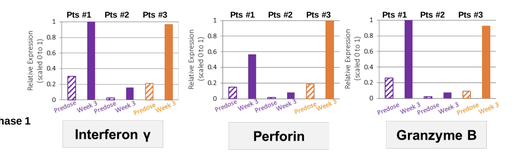
CD8+ staining of FFPE from spleen, tumor, liver and kidney at indicated time point from mice treated with ACT+NKTR-214 or ACT+IL-2. Quantitative analysis of CD8+ cells performed with HALO software. **A.** Representative images, spleen or tumor (n=3/group). **B.** Graphical representation from HALO quantification. NKTR-214 promotes significantly higher CD8 in spleen at day 5 and provides durable CD8 tumor infiltration which persists at D14. Very little infiltration was observed in liver and kidney.

Translation to clinic: NKTR-214 is well-tolerated, administered in an out-patient setting and increases cytotoxic and memory T cells in tumor biopsies

RNA was extracted from tumor biopsy pre-dose and 3 weeks post-dose of NKTR-214 for gene expression analysis (Nanostring).

- Purple: 0.003 mg/kg (n=2 pts)
- Orange: 0.006 mg/kg (n=1 pts)

Datasource: Nektar Therapeutics, ASCO 2017, Phase 1 dose escalation of NKTR-214



Conclusion

- ACT+NKTR-214 is well tolerated and provides a robust anti-tumor response in the aggressive B16F10 model.
- Treatment with NKTR-214+ACT mobilizes antigen-specific CD8+ T cells into the tumor but not regulatory T cells. The CD8+ T cells in tumor durably persist. Peripheral tissue (kidney, liver) are relatively spared of CD8+ T cells.
- NKTR-214 administration leads to significant upregulation of genes associated with T cells activation, proliferation and function in the tumor.
- NKTR-214 increases the polyfunctionality of antigen-specific T cells which secrete Granzyme B, IFN-γ, MIP-1α and RANTES that are associated with anti-tumor immunity.
- The persistent and tumor-specific induction of cytotoxic T cells provided by NKTR-214 supports its combination with cell-based therapies.
- In the clinic, NKTR-214 is dosed administered in an out-patient setting and provides an activated TIL phenotype in human tumors, similar to these observations in the mouse modeling.

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