NT-I7 plus pembrolizumab combination treatment enhances infiltration of PD-1+ T cells and provides a more immunogenic tumor microenvironment. Biomarker data from the NIT-110 study.

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BACKGROUND

Checkpoint (CPI) monotherapy is ineffective in immunologically cold malignancies with low T cell infiltration. Previously, we have shown that NT-I7 (efineptakin alfa) increases both stem-cell memory CD8 T cells (T_{SCM}) alone (1) and in combination with pembrolizumab (2) and tumor lymphocyte infiltration (TIL), as measured by manual TIL count of hemotoxylin and eosin (H&E)-stained biopsy samples (2). This study aims to explore in greater depth the immune-associated changes in the tumor microenvironment (TME) after NT-I7 plus pembrolizumab treatment.

STUDY DESIGN

Patients (pts) with CPI-naïve microsatellite-stable colorectal cancer (MSS-CRC), pancreatic cancer (PaC) and ovarian cancer (OC) received NT-I7 at 1200 µg/kg intramuscular (IM) Q6W + pembrolizumab at 200 mg intravenous (IV) Q3W. Pre- and on-treatment biopsies were collected for analysis of tumor immune infiltrates. Response data was only included for patients with at least 2 independent tumor assessments post-treatment. Analyses included:

- Flow cytometry for immunophenotyping of peripheral blood. For t-Distributed Stochastic Neighbor Embedding (t-SNE) analysis 1000 down-sampled CD8$^+$ events from each FCS file were concatenated into a single file for each timepoint. Files containing less than 1000 CD8$^+$ events were excluded from analysis. t-SNE was then performed on each concatenated file in FlowJo, excluding upstream markers of CD8$^+$ gating. Manually gates for T cell subsets (Naïve, CM, EM, etc.) were then overlaid on the resulting t-SNE plots to visualize the clustering of these subsets on each t-SNE plot at each timepoint.
- Immunofluorescence (mIF) and flow cytometry analysis of tumor tissue biopsy samples.
- Genomic analyses including whole transcriptome sequencing (WTS) of tissue biopsy samples and TCR sequencing (TCRseq) of paired peripheral blood mononuclear cells (PBMC) and tissue biopsy samples. TCR sequences were characterized from RNA using the Archer Dx platform. Downstream analysis was performed with MiXCR and R. Clonotypes were defined by CDR3 amino acid sequence.

CONCLUSIONS

- The combination of NT-I7 and pembrolizumab induces infiltration of CD8 T cells into the tumor microenvironment in over 80% (22/27) of analyzed samples.
- Over 50% of on-treatment tumor biopsies had a significant, over 5-fold, CD8 T cell increase after only one dose of NT-I7 + pembrolizumab.
- The magnitude of the CD8+ T cell infiltration was positively associated with increased frequencies of circulating stem-like memory T_{SCM} CD8 T cells.
- Infiltrating CD8 T cells express PD-1+ and have increased clonality, suggesting that tumor-specific CD8 T cells are successfully infiltrating in these subjects.
- Treatment with NT-I7 and pembrolizumab increases the immunogenicity of the TME.
- Treatment-induced reduction of the tumor volume is associated with the magnitude of CD8 (but not CD4) T cell infiltration and a higher on-treatment tumor CD8-to-Treg ratio.

The combination of NT-I7 and pembrolizumab has promising clinical efficacy in very cold and immunosuppressive indications (3-4). These results suggest that this clinical efficacy may be mediated, at least in part, by enhanced tumor-specific CD8 T cell infiltration into the tumor microenvironment.

ACKNOWLEDGMENTS

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RESULTS

NT-I7 + pembrolizumab increase T cell infiltration into the tumor microenvironment

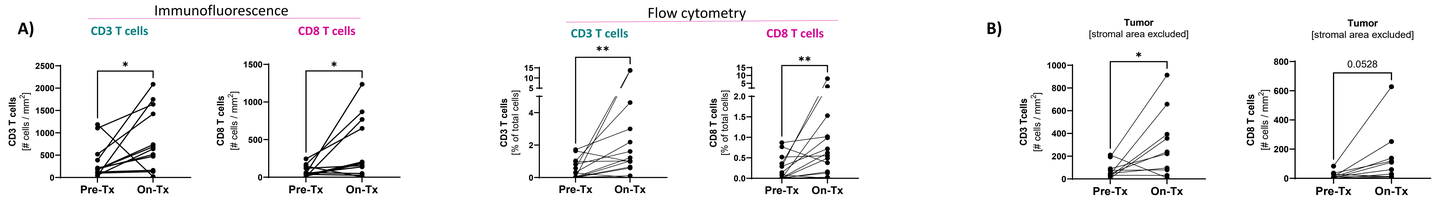

Figure 1. Combination treatment with NT-I7 and pembrolizumab leads to T cell infiltration in the TME and tumor. **(A) TME:** Pre- (Pre-Tx, W0) and on-treatment (On-Tx, W5) matched tissue biopsy samples were analyzed by immunofluorescence (mIF, n=12) and by flow cytometry (n=15). Remarkably, **91.7%** (11/12; average 79 → 373 cells/mm^2) and **73.3%** (11/15; average 0.2 → 1.1% of total cells) of samples analyzed showed increased T-cell infiltration upon treatment with NT-I7+pembrolizumab. 55.5% (15/27) of all samples analyzed showed at least a 5-fold increase in CD8 T cells on-treatment, even in these notoriously cold tumor indications. **(B) Tumor only:** T cell infiltration also increased significantly in the tumor area, as demonstrated by mIF on tumor tissue without inclusion of the stroma.

Stem-like memory CD8 T cells (T_{SCM}) may be the source of the T cell infiltration

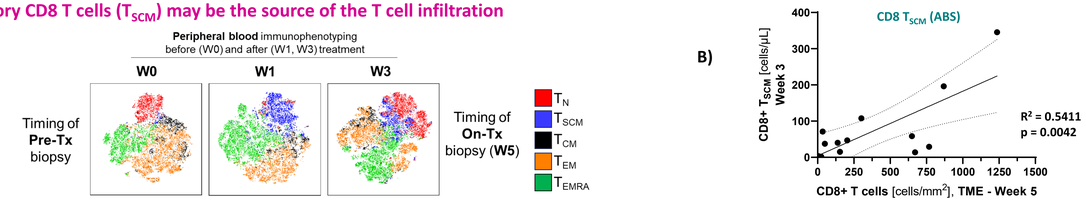

Figure 2. CD8 T_{SCM} cells increase following combination treatment, in direct association with the increase in intratumoral CD8 T cells. **(A)** As previously reported, NT-I7 plus pembrolizumab increase circulating CD8 T_{SCM}. tSNE plots show the peripheral blood CD8 T cell population dynamics before and after biopsy collection (n=27). T_N = naïve T cells, T_{SCM} = stem-cell memory T cells, T_{CM} = central memory T cells, T_{EM} = effector memory T cells, T_{EMRA} = effector memory T cells expressing CD45A. **(B)** The absolute number of peripheral CD8 T_{SCM} cells at week 3 (W3) is directly associated with the absolute number of intratumoral CD8 T cells at week 5 (W5; n=12). This association is not maintained when total T cells, regardless of differentiation subset, are analyzed.

Analysis suggests infiltrating lymphocytes may include tumor-specific CD8 T cells

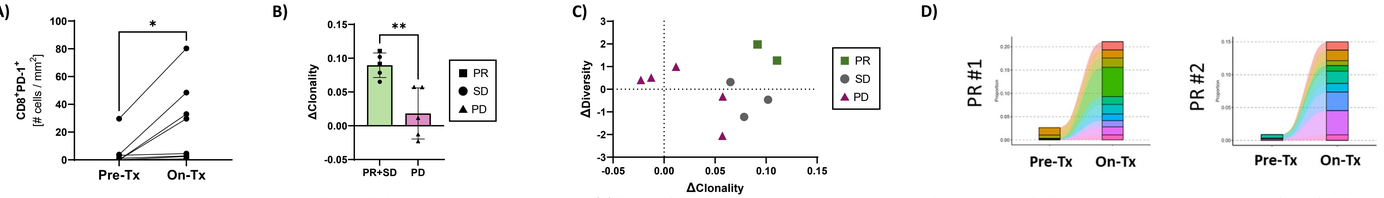

Figure 3. PD-1 expression and clonotype analysis suggest that infiltrating CD8 T cells are tumor-specific. **(A)** Tumor-infiltrating CD8 T cells express PD-1, suggesting that tumor-specific T cells, not only bystander cells, successfully infiltrate the TME (n=12). **(B)** Pts with disease control (PR + SD) show a greater increase in intratumoral clonality on-treatment than PD pts (p = 0.0079), suggesting there is selective expansion of certain clonotypes, possibly tumor-specific, in patients with favorable clinical outcomes (n=10). **(C)** Increase in intratumoral clonality on-treatment in the 2 PR pts was uniquely accompanied by an increase in intratumoral diversity (Shannon Entropy) (n=10). **(D)** In most pts, the repertoire proportions of the top 10 on-treatment clonotypes were >2x larger on-treatment than pre-treatment. Data from the 2 PR pts are shown; each color segment represents a TCR clonotype.

NT-I7 + pembrolizumab increases the immunogenicity of the tumor microenvironment

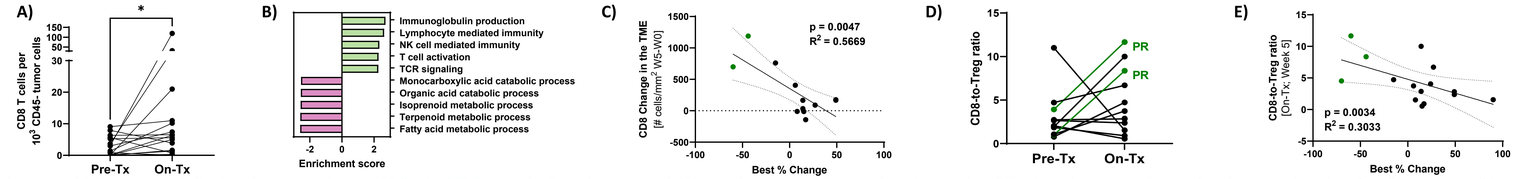

Figure 4. Multiple parameters indicating a hot TME are increased following NT-I7 + pembrolizumab treatment. **(A)** Flow analysis on tumor biopsies shows increased T cell density in the tumor, with a significantly increased number of T cells per tumor cell (n=15). **(B)** Gene set enrichment analysis (GSEA) showed increased gene expression in immune response and lymphocyte activation gene sets on-treatment (n=10). All FDR q-values < 0.0001. **(C)** Magnitude of CD8 T cell infiltration, but not CD4 T cell infiltration, is directly associated with the best percentage of change in the tumor volume. Pts with partial response (PR) are highlighted (n=12). **(D)** Treatment significantly increased the intratumoral CD8-to-Treg ratio; the increase in the CD8-to-Treg ratio was particularly remarkable in pts with partial response (PR) (n=12). **(E)** The on-treatment CD8-to-Treg ratio at week 5 is significantly associated with the best percentage of tumor change (n=12).

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