NT-I7 (efineptakin alfa), a long-acting IL-7, in combination with pembrolizumab improves T cell fitness in heavily pretreated subjects with gastrointestinal tumors

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BACKGROUND

Individuals with cancer include various populations with underlying immune dysfunction, including those who have received transplants, have chronic infections, have been heavily-pretreated, are receiving concurrent chronic steroid therapy, are elderly, are pregnant, or have poor performance status. Immune dysfunction curtails efficacy of immunotherapy. NT-I7 (efineptakin alfa), a long-acting IL-7, is a potent T cell amplifier that increases systemic stemness as monotherapy or in combination with checkpoint inhibitors (CPIs).

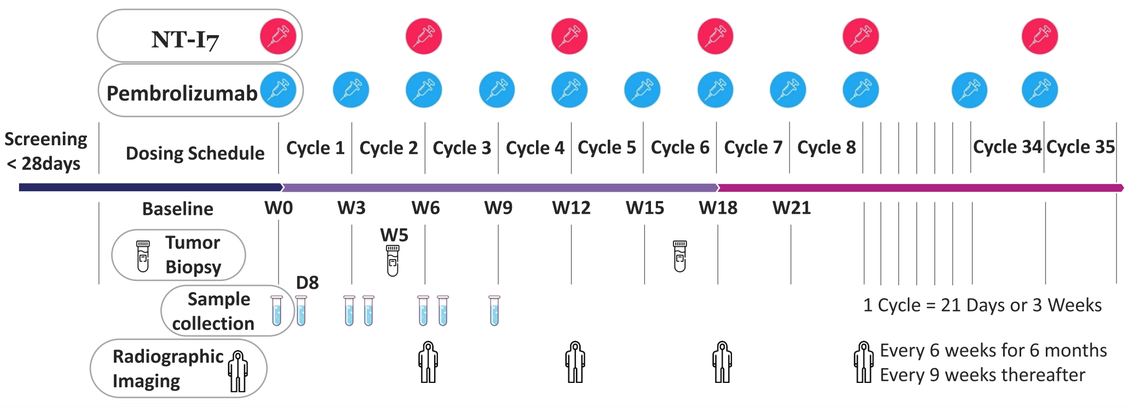
Here, we explore the systemic beneficial effects of NT-I7 on the T cell fitness of subjects with immune dysfunction when combined with pembrolizumab.

METHODS

Correlative studies included single cell RNA (scRNAseq) and T cell receptor (scTCRseq) sequencing (n=27) and immunophenotyping (n=53) of longitudinal peripheral blood samples. Gene counts and single cell TCR repertoires were generated from 10X GEX/TCR libraries using CellRanger 7.0.1. Downstream processing was done using the Seurat v4 framework and vegan v2.6-4 in R v4. Gene scoring was performed using Ucell v2.5.0. Flow cytometry data was analyzed using FlowJo v10.8.1. Visualization and statistical tests were performed using Seurat and Prism v9.

STUDY DESIGN

- Open-label, Phase 2a study (NCT04332653)
- Arms: relapsed/refractory CPI-naïve microsatellite-stable colorectal cancer (MSS-CRC) and pancreatic cancer (PC)
- NT-I7 1200 µg/kg IM every 6 weeks (Q6W), pembrolizumab 200 mg IV Q3W



RESULTS

Subject demographics, disposition and disease history

- 61 subjects were enrolled and 53 subjects were evaluable as of 04-Nov-2022
- 41 evaluable subjects (77.4%) had received 3 or more prior anticancer therapies

Table 1. Subject characteristics.

	MSS-CRC (N=29)	PC (N=32)	Total (N=61)
Age (years); median [min, max]	54.7 [35 – 81]	65.1 [31 – 81]	60.5 [31 – 81]
Sex (male); n (%)	19 (65.5%)	16 (50.0%)	35 (57.4%)
Race (white); n (%)	22 (75.9%)	27 (84.4%)	49 (80.3%)
Stage at diagnosis; n(%)			
1-3	10 (34.5%)	19 (59.4%)	29 (47.5%)
4	18 (62.1%)	13 (40.6%)	31 (50.8%)
Unknown	1 (3.4%)	0 (0.0%)	1 (1.6%)
Presence of liver metastasis at baseline; n(%)	23 (79.3%)	25 (78.2%)	48 (78.7%)
Sum of target lesions at baseline ≤ 100 mm; n (%)	17 (58.6%)	26 (81.3%)	43 (70.5%)
Number of prior anti-cancer therapies; n (%)			
1	1 (3.4%)	3 (9.4%)	4 (6.6%)
2	3 (10.3%)	7 (21.9%)	10 (16.4%)
3	6 (20.7%)	11 (34.4%)	17 (27.9%)
≥ 4	19 (65.6%)	11 (34.4%)	30 (49.2%)
Safety analysis set; n (%)	29 (100%)	32 (100%)	61 (100%)
Efficacy evaluable set; n (%)	27 (93.1%)	26 (81.3%)	53 (86.9%)
Treatment disposition; n (%)			
On treatment	1 (3.4%)	1 (3.1%)	2 (3.3%)
Complete the treatment	1 (3.4%)	0 (0.0%)	1 (1.6%)
Discontinued from treatment	27 (93.1%)	31 (96.9%)	58 (95.1%)
Reason for treatment discontinuation; n (%)			
Adverse event	7 (24.1%)	4 (12.5%)	11 (18.0%)
Death	0 (0.0%)	0 (0.0%)	0 (0.0%)
Progressive disease	17 (58.6%)	22 (68.8%)	39 (63.9%)
Other (including physician decision and withdrawal by subject)	3 (10.3%)	5 (15.6%)	8 (13.1%)

Distribution of peripheral immune subsets shifted toward T cells on-treatment

- Increase in the absolute lymphocyte count (ALC), especially the T cell compartment, is the main pharmacodynamic marker of NT-I7 biological activity.
- The proportion of monocytes and dendritic cells in peripheral blood mononuclear cells (PBMC) decreases significantly on-treatment with NT-I7 + pembrolizumab.
- A concomitant increase in the proportion of CD4 and CD8 T cells suggests that NT-I7 + pembrolizumab treatment favors a more inflammatory immune compartment.

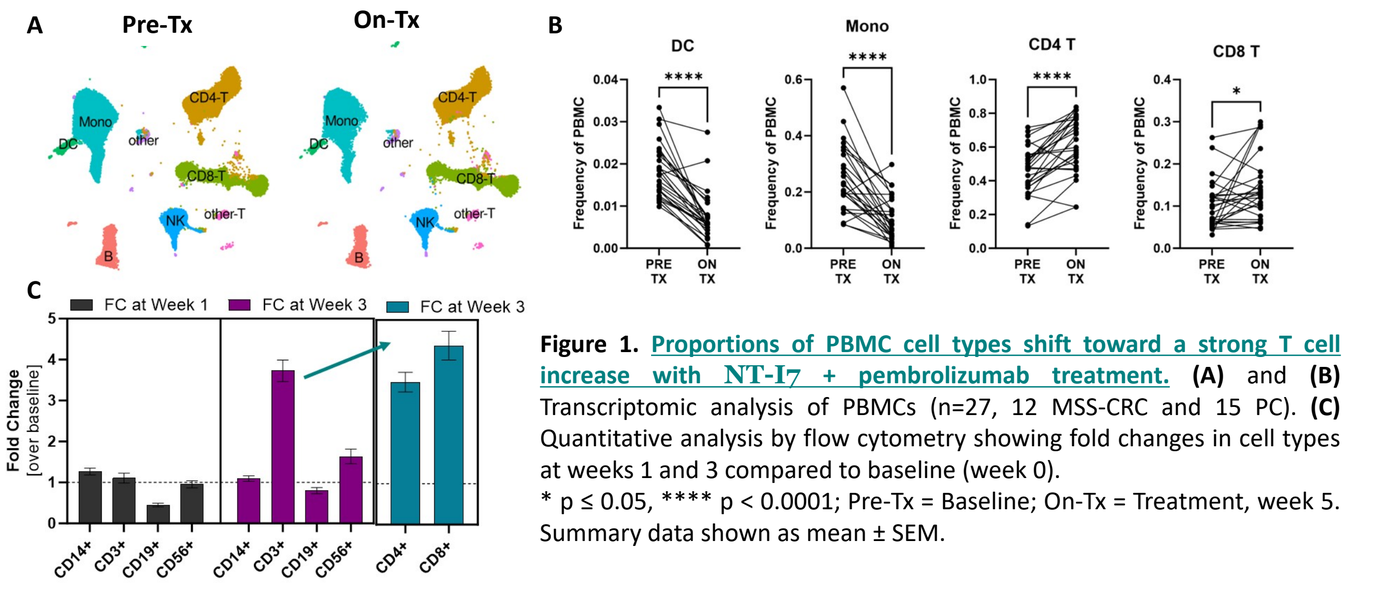


Figure 1. Proportions of PBMC cell types shift toward a strong T cell increase with NT-I7 + pembrolizumab treatment. **(A)** and **(B)** Transcriptomic analysis of PBMCs (n=27, 12 MSS-CRC and 15 PC). **(C)** Quantitative analysis by flow cytometry showing fold changes in cell types at weeks 1 and 3 compared to baseline (week 0).
* p ≤ 0.05, **** p < 0.0001; Pre-Tx = Baseline; On-Tx = Treatment, week 5. Summary data shown as mean ± SEM.

NT-I7 and pembrolizumab treatment is able to counteract immune dysfunction

- Less-differentiated CD8 subsets and Tpex express high levels of IL-7Rα at baseline and will be more responsive to NT-I7 while regulatory T cells (T_{REG}) express low levels and were not expected to respond (Fig. 2A).
- Treatment led to a transient decrease in IL-7Rα in all subsets and a concomitant increase in Ki67 expression that was stronger in subsets with higher baseline IL-7Rα (Fig. 2A-B).
- Baseline immune dysfunction, with higher levels of effector and terminally differentiated subsets, was alleviated on-treatment. At week 9, all CD8 T cell subsets showed similar percentages, with a significant decrease in T_{REG} (Fig. 2C & Table 2).
- Similar results were observed in CD4 T cells.

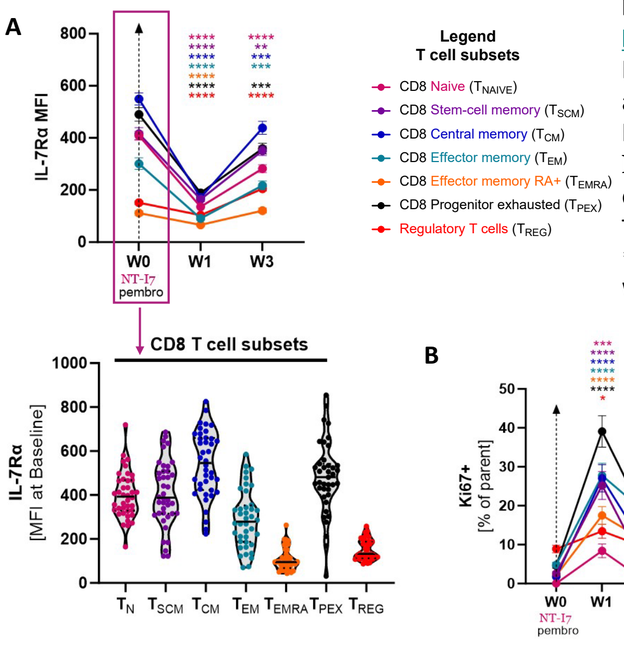


Figure 2. Less differentiated T cells show significant increases over baseline upon NT-I7 + pembrolizumab treatment. Peripheral blood flow cytometry was performed on 50 subjects (25 MSS-CRC and 25 PC). **(A)** IL-7Rα median fluorescence intensity (MFI) at baseline across T cell subsets. Longitudinal changes, in response to NT-I7 and pembrolizumab treatment, in **(A)** IL-7Rα and **(B)** Ki67 expression. **(C)** Longitudinal assessment of the distribution of CD8 T cell subsets from week 0 to week 9.
* p ≤ 0.05, ** p< 0.01, *** p< 0.001, **** p < 0.0001;
WX = week of treatment. Summary data shown as mean ± SEM.

- Sample integration of scRNAseq data was performed to eliminate processing artifacts while preserving biological information.
- Fourteen distinct cell clusters were found after unsupervised clustering in Seurat with a resolution of 0.8.
- Preferential increase of the cluster with stem-like properties was observed, while clusters associated with terminal differentiation significantly decreased.

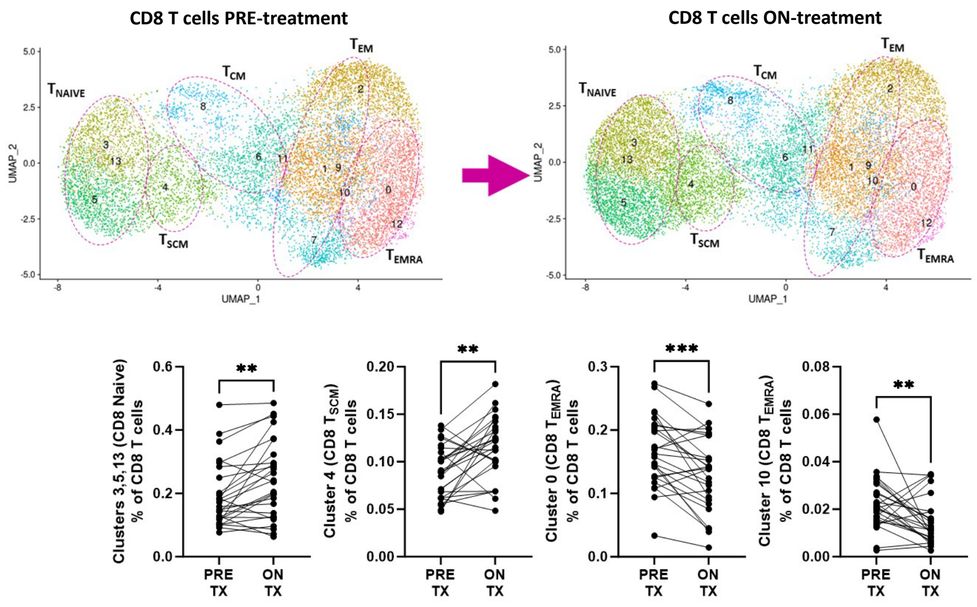


Figure 3. CD8 T cell population dynamics shift toward less-differentiated cell types in response to NT-I7 + pembrolizumab treatment. scRNAseq analysis of CD8 T cells pre-treatment and on-treatment with NT-I7 and pembrolizumab (n=27, 12 from MSS-CRC, 15 from PC). ** p< 0.01, *** p< 0.001; Pre-Tx = Baseline; On-Tx = Treatment, week 5.

Table 2. T cell subsets dynamics

Subset	Markers	Week 0 (%)	Week 3 (%)	Week 9 (%)	Subset	Markers	Week 0 (%)	Week 3 (%)	Week 9 (%)
CD8 $T_{Naïve}$	**CD45RA+CCR7+**CD95-	19.3 ± 19.0	7.9 ± 7.2	22.6 ± 21.3	CD8 T_{EM}	CD45RA-CCR7-	30.1 ± 14.8	24.1 ± 11.4	20.7 ± 11.9
CD8 T_{SCM}	**CD45RA+CCR7+CD95+**	**5.2 ± 6.0**	**38.8 ± 16.5**	**20.0 ± 14.9**	CD8 T_{EMRA}	CD45RA+CCR7-	30.0 ± 21.4	16.9 ± 15.0	20.1 ± 17.3
CD8 T_{CM}	CD45RA-CCR7+	14.7 ± 10.4	12.1 ± 7.9	16.9 ± 10.9	CD8 T_{REG}	CD4+CD25+FoxP3+	**4.9 ± 2.7**	**3.2 ± 2.8**	**2.4 ± 1.3**

Frequency of each T cell subset at the indicated time points is shown as mean ± standard deviation (SD)

NT-I7 + pembrolizumab restore immune system fitness, increasing TCR diversity and costimulatory receptor expression while decreasing T cell exhaustion

- Treatment significantly increased TCR diversity (18/26, p=0.0102) (Fig. 4A)
- Frequency of costimulatory receptors increased in the CD8 T cell compartment (Fig. 4B) and, especially, in the effector populations (Fig. 4C), while the frequency of exhaustion markers was decreased (Fig. 4C)

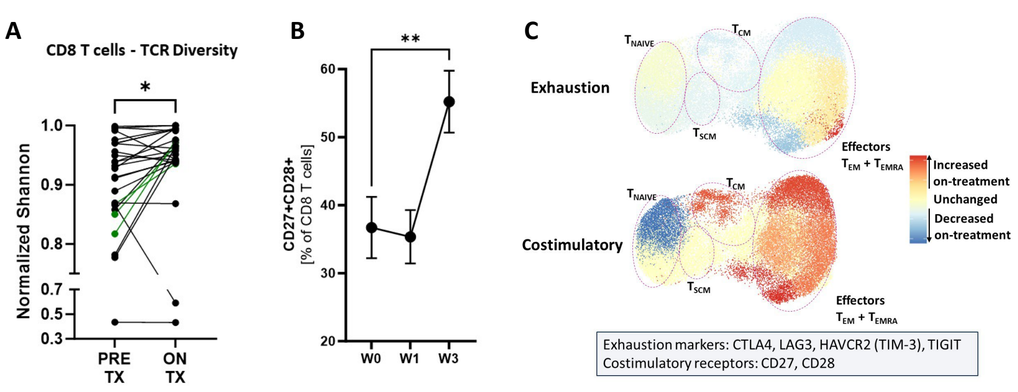


Figure 4. Treatment increases TCR diversity and costimulatory molecule expression, and decreases exhaustion markers. **(A)** scTCRseq from 26 subjects (11 from MSS-CRC and 15 from PC) including in subjects who achieved partial response (PR, shown in green). An MSS-CRC subject was not reportable due to QC issues. **(B)** Flow cytometry analysis for costimulatory receptors CD27 and CD28 from baseline to week 3 post-treatment in CD8+ T cells (n=53, 27 from MSS-CRC and 26 from PC). **(C)** Single-cell RNAseq analysis for exhaustion markers and costimulatory receptors (n=27, 12 from MSS-CRC and 15 from PC).* p ≤ 0.05, ** p < 0.01. Pre-Tx = Baseline; On-Tx = Treatment, week 5; WX = week of treatment. Summary data shown as mean ± SEM.

- Treatment increased early activation markers on naïve and effector memory T cell subsets
- Treatment significantly increased late activation markers only for effector subsets
- Cytotoxicity markers are maintained for effector subsets, but decreased for naïve and central memory cells

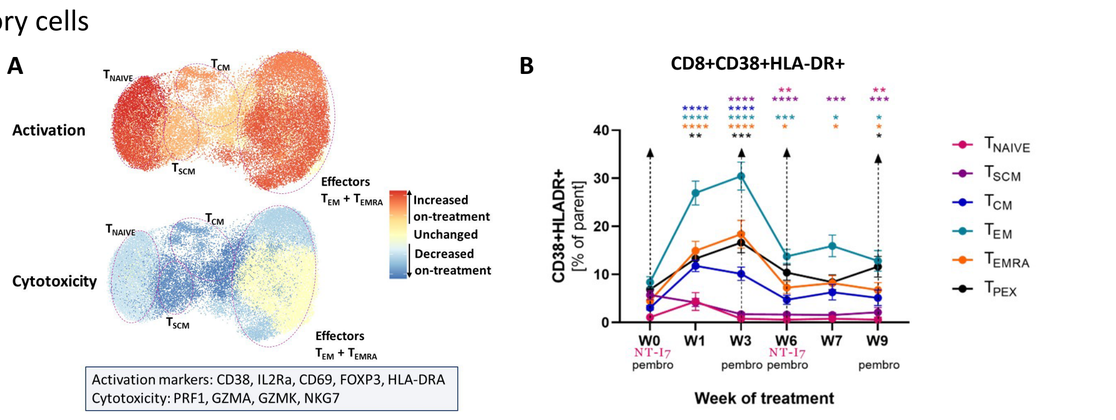


Figure 5. NT-I7 + pembrolizumab favors activation and cytotoxicity of effector cells. **(A)** scRNAseq of activation and cytotoxicity markers (n=27, 12 from MSS-CRC and 15 from PC). **(B)** Flow cytometric analysis of T cell subsets for late-phase activation markers CD38 and HLA-DR across T cell subsets (n=50, 25 from MSS-CRC and 25 from PC). * p ≤ 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001 Pre-Tx = Baseline; On-Tx = Treatment, week 5; WX = week of treatment. Summary data shown as mean ± SEM.

CONCLUSIONS

- NT-I7-driven T cell expansion, when combined with the anti-PD-1 agent pembrolizumab, promotes stemness and restores T cell fitness in heavily pretreated subjects showing signs of immune dysfunction.
- Combining NT-I7 with immunotherapy or other anticancer agents has added systemic benefits that could impact long-term clinical response in these subjects.

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