Investigation of a potential protein biomarker signature that may predict clinical benefit of NT-I7 and pembrolizumab in patients with cold gastrointestinal tumors

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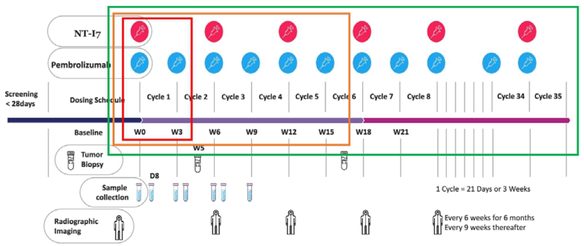
BACKGROUND

Microsatellite-stable colorectal (MSS-CRC) and pancreatic cancer (PDAC) are immunologically cold tumors with null response to checkpoint inhibitors (CPI). NT-I7, a long-acting IL-7, in combination with pembrolizumab (pembro) has shown to significantly increase intratumoral T cell infiltration and elicit some tumor control in these hard-to-treat gastrointestinal indications (visit the poster for abstract #2621 for updated clinical data). However, while a limited set of patients achieve objective response, the disease control rate and the duration of response and stable disease point to a larger subset obtaining clinical benefit. To identify novel predictive biomarkers, we analyzed baseline peripheral and biopsy samples from patients based on treatment duration.

STUDY DESIGN

- This is an open-label Phase 2a study in subjects with relapsed/refractory CPI-naïve MSS-CRC and PDAC; NT-I7 1200 µg/kg IM every 6 weeks (Q6W), pembro 200 mg IV Q3W. Antitumor activity is assessed by RECIST 1.1/iRECIST.
- Subjects were grouped by treatment duration, measured as NT-I7 doses administered before treatment discontinuation for any cause:
  - Short (ST): 1 dose before treatment discontinuation (in study <6 weeks)
  - Medium (MT): 2-3 doses before treatment discontinuation (in study <18 weeks)
  - Long (LT): 4 or more doses before treatment discontinuation (in study >18 weeks)



- Correlative studies in peripheral samples included proteomics, T cell receptor sequencing (TCRseq), and single cell RNA sequencing (scRNAseq)
- Correlative studies in biopsy samples included genomics, transcriptomics, and TCRseq.

CONCLUSIONS

- LT patients, who remained in treatment the longest, had increased activation of the TPEX compartment. This may suggest that preserved tumor-specific TPEX activity could be required for NT-I7 and pembrolizumab activity.
- Correlative analysis shows that there are 3 potentially predictive protein biomarkers that may help identify a patient subset experiencing clinical benefit in response to treatment with NT-I7 and pembrolizumab.
- Patients with a negative biomarker signature showed prolonged survival on treatment with NT-I7 and pembrolizumab.

ACKNOWLEDGMENTS

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RESULTS

Patient characteristics

- As of 02 Oct 2023, 53 evaluable patients had completed or discontinued treatment (5 still on followup)
- All tumor biopsies were confirmed MSS with low tumor mutational burden (TMB)
- All groups had similar age (ST vs LT, p=0.740) but LT patients had lower sum of target lesions (ST vs LT, p=0.022)
- Patients with LT had significantly better tumor control and higher median PFS and OS; all partial responders were also in this group

Table 1. Group characteristics

Parameter	ST (n=21)	MT (n=22)	LT (n=10)
Age (years); median [IQR]	59 [53 – 71.5]	59.5 [53.5 – 64.8]	66 [47.3 – 73.5]
Treatment duration (months); median [IQR]	1.41 [1.41 – 1.41]	2.8 [2.8 – 4.1]	14.7 [6.8 – 19.7]
Sum of target lesions at baseline; median [IQR]	81 [64 – 109]	75 [54 – 135]	58 [32 – 77]
Best tumor % change (iRECIST); median (min-max)	19.1 (5.2 – 87.0)	23.3 (-23.9 – 57.7)	-31.2 (-78.7 – 8.3)
RECIST PFS (weeks); median [IQR]	5.9 [5.7 – 6.4]	6.0 [5.6 – 11.4]	28.8 [10.6 – 43.6]
iRECIST iPFS (weeks); median [IQR]	6.0 [5.7 – 7.1]	6.9 [5.8 – 12.2]	47.4 [30.9 – 71.7]
OS (weeks); median [IQR]	14.0 [7.9 – 53.4]	27.7 [16.6 – 39.1]	113.9 [74.5 – 120]

iPFS = progression-free survival by iRECIST; IQR = interquartile range; OS = overall survival, PFS = progression-free survival by RECIST 1.1

LT subjects show upregulated pathways related to immunity

- Tumor samples from subjects in the LT group had 317 differentially expressed genes (p≤0.05) at baseline (Fig. 1).
- Although subjects' tumors were confirmed cold, these pathways were consistent with higher immune activity (Fig. 1), suggesting that some immune response remains in responders to NT-I7 + pembro treatment.

KEGG "library"	Biological processes	Cellular processes
Antigen processing and presentation (hsa04612)	Regulation of lymphocyte activation (GO:0051249)	T cell receptor complex (GO:0042101)
Hematopoietic cell lineage (hsa04640)	Antigen receptor-mediated signaling pathway (GO:0050851)	Plasma membrane signaling receptor complex (GO:0098802)
Intestinal immune network for IgA production (hsa04672)	Regulation of T cell activation (GO:0050863)	Immunoglobulin complex (GO:0019814)
Cell adhesion molecules (hsa04514)	Positive regulation of lymphocyte activation (GO:0051251)	Receptor complex (GO:0043235)
Th1 and Th2 cell differentiation (hsa04658)	Immune response-regulating cell surface receptor signaling pathway (GO:0002768)	Intermediate filament (GO:0005882)
Chemokine signaling pathway (hsa04062)	Lymphocyte differentiation (GO:0030098)	Keratin filament (GO:0045095)
B cell receptor signaling pathway (hsa04662)	Leukocyte cell-cell adhesion (GO:0007159)	Intermediate filament cytoskeleton (GO:0045111)
Th17 cell differentiation (hsa04659)	Mononuclear cell differentiation (GO:1903131)	Cornified envelope (GO:0001533)
Viral protein interaction with cytokine and cytokine receptor (hsa04061)	Immunoglobulin production (GO:0002377)	External side of plasma membrane (GO:0009897)
T cell receptor signaling pathway (hsa04660)	Production of molecular mediator of immune response (GO:0002440)	IgG immunoglobulin complex (GO:0071735)
Cytokine-cytokine receptor interaction (hsa04060)	**Molecular function**	
Osteoclast differentiation (hsa04380)	Antigen binding (GO:0003823)	
Wnt signaling pathway (hsa04310)		

Figure 1. Upregulated pathways related to immunity in LT subjects. Whole transcriptome sequencing was performed on biopsy samples from each group. The lists above highlight the most relevant and significantly upregulated genes in the LT group, compared to the ST group, at baseline.

LT groups show increased CD8 activation and proliferation patterns

- Stem-like T cells (both TSCM and TPEX) have specific gene activation patterns in the subjects from the LT group.
- Pathways strongly related with CD8 T cell activation and proliferation are enriched in the TPEX population in the LT group. However, those same pathways are enriched in the memory and effector populations in subjects with ST.
- This suggests that preserved stemness may be necessary to elicit a response to NT-I7 + pembro.

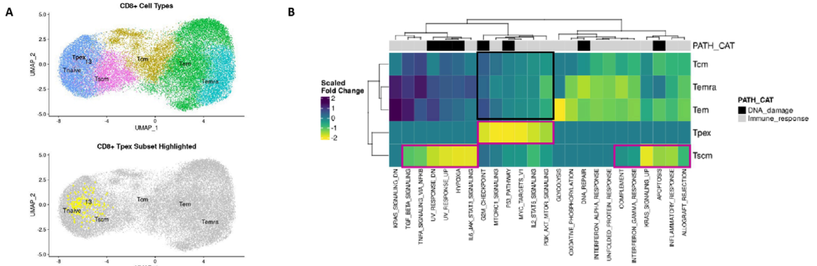

Figure 2. CD8 activation and proliferation patterns in LT subjects. scRNAseq analysis was used to identify peripheral CD8+ cell subsets and assess gene expression patterns within each subset. **A)** UMAP showing the different identified subsets. **B)** Differential gene expression patterns in the LT group compared to the ST group, revealing that the T_{PEX} subset in this group is enriched for genes related to CD8 T cell activation and proliferation.

Lower levels of tumor growth-related proteins in peripheral blood may represent potential biomarkers of treatment response

- Soluble proteins that could reflect the tumor and/or immune status described by the transcriptomic analyses were explored.
- Out of 45 cytokines, chemokines, and growth factors investigated at baseline, only biomarkers related to tumor growth were lower in the LT group.
- This may suggest that the preserved TPEX population identified in Figure 2 may be exerting pressure on the tumor in a manner that could be necessary for NT-I7 + pembro efficacy.

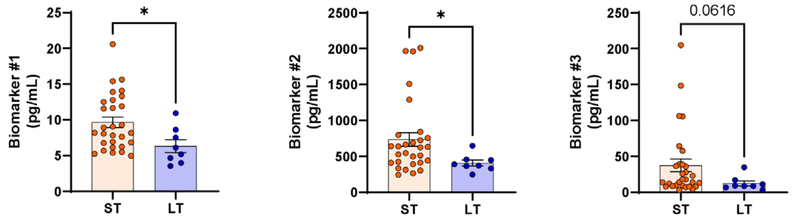

Figure 3. Soluble protein analysis in peripheral blood of ST and LT subjects at baseline. A panel of 48 cytokines, chemokines and growth factors was used to identify potential biomarkers among soluble proteins in peripheral blood. The panels above show that the LT group has lower levels of certain soluble proteins compared to the ST group; these proteins are related to tumor growth.

Correlation between survival and biomarker signature

- Based on the level of the peripheral biomarkers described in Figure 3, subjects were divided into positive and negative signatures.
  - POSITIVE: Subjects with 2 or 3 values above the median
  - NEGATIVE: Subjects with 0 or 1 value above the median
- Subjects with a NEGATIVE signature at baseline had significantly higher overall survival (OS), regardless of group assignment (13.2 vs 8.9 months, p = 0.0305).

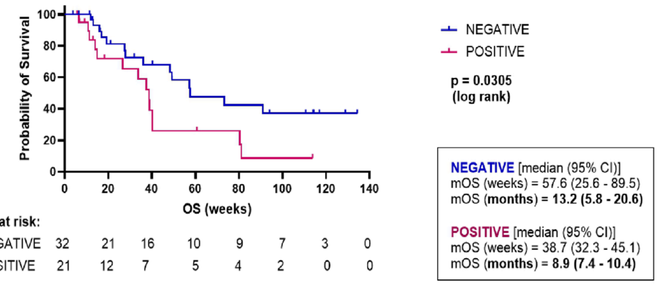

Figure 4. Comparison of overall survival in subjects with positive vs negative biomarker signatures at baseline. The survival curves of subjects with negative (blue) versus positive (pink) biomarker signatures, show a significant improvement in survival in subjects with a negative signature, regardless of group assignment.

These data are preliminary, resulting from the analysis of 53 patients. Further verification of the potentially predictive nature of this signature in independent cohorts is ongoing.

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