Dana-Farber Cancer Institute

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Transcriptomic Profiling of the Tumor Microenvironment Indicates Differential Response To Neoadjuvant Chemotherapy With and Without Pembrolizumab in Early TNBC

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HARVARD MEDICAL SCHOOL

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BACKGROUND

- Neoadjuvant chemotherapy (CT) with pembrolizumab (P) has significantly improved pathologic complete response (pCR) and survival outcomes compared to CT alone in patients (pts) with stage II-III TNBC.[1-3]
- Predictive biomarkers of response to the addition of P to CT are lacking to help identify pts who are more likely to benefit from the combination and those in whom the added toxicity of P could be spared.
- Here we aim to compare transcriptomic features in pre- and post-treatment tumor samples of pts diagnosed with early-stage TNBC who participated in the DFCI Multicenter TNBC Registry and received neoadjuvant CT with or without P.

STUDY DESIGN AND METHODS

Primary Objective: To describe differences in tumor microenvironment (TME) composition and signatures in response to neoadjuvant chemotherapy (CT) with or without pembrolizumab (P) based on timepoint (baseline, BL vs residual disease, RD), neoadjuvant treatment (CT vs CT-P), and RCB status (pCR vs RD) in patients diagnosed with TNBC.

Cohort:

- Patients who received neoadjuvant CT or CT-P were identified from the prospective DFCI Multicenter TNBC Registry (DF/HCC IRB #18-617) from 5/2019-1/2024.
- Samples collected were FFPE research or archival BL biopsies and surgical samples (if RD) after neoadjuvant treatment.

Methods:

- **RNA-seq.** After review for tumor content, RNA expression was quantified using whole transcriptome sequencing of isolated RNA. RNA expression was quantified using *STAR* and *RSEM*. TME cell type abundance was estimated using the deconvolution methods included in TIMER3. RNA signatures (Hallmark, 3CA) were calculated using ssGSEA. Based on RNA expression, the intrinsic subtype of tumors was obtained using research-based PAM50. Comparison between groups was performed using linear models with tumor abundance as a covariate and paired samples as a random effect intercept.
- **Survival Analysis.** Associations with recurrence-free (RFS) and overall survival (OS) used a Cox proportional hazards model adjusted for stage and pCR/RD (if analysis included pts with pCR and RD) and a likelihood ratio test.

Cohort Characteristics:

Patients	CT-P (n=70)	CT (n=42)	Total (n=112)
Stage			
I	5 (7.1%)	11 (26.2%)	16 (14.3%)
II	49 (70.0%)	27 (64.3%)	76 (67.9%)
III	16 (22.9%)	4 (9.5%)	20 (17.9%)
gBRCA1/2			
gBRCA1*	8 (11.4%)	4 (9.5%)	12 (10.7%)
gBRCA2	5 (7.1%)	1 (2.4%)	6 (5.4%)
Negative	47 (67.1%)	32 (76.2%)	79 (70.5%)
Unknown	10 (14.3%)	5 (11.9%)	15 (13.4%)
qPALB2			
Positive	1 (1.4%)	1 (2.4%)	2 (1.8%)
Negative	55 (78.6%)	31 (73.8%)	86 (76.8%)
Unknown	14 (20.0%)	10 (23.8%)	24 (21.4%)
Follow-up			
Median (mo.)	27	48	31
95% CI	(9-42)	(18-70)	(11-70)

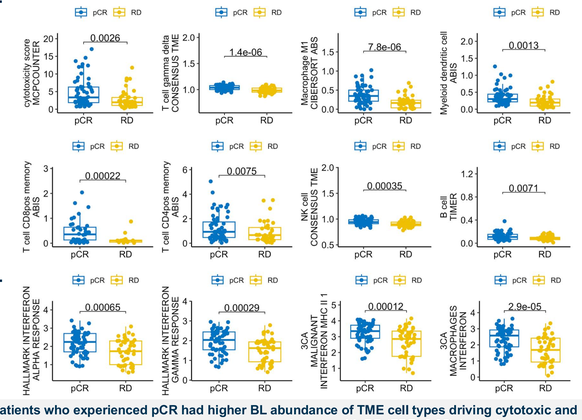
*Includes 2 patients with gBRCA2 status unknown.

Neoadjuvant treatment	CT-P (n=70)	CT (n=42)	Total (n=112)
Anthracycline			
Y	59 (84.3%)	35 (83.3%)	94 (83.9%)
N	11 (15.7%)	7 (16.7%)	18 (16.1%)
Taxane			
Y	68 (97.1%)	42 (100.0%)	110 (98.2%)
N	2 (2.9%)	0 (0.0%)	2 (1.8%)
Platinum			
Y	67 (95.7%)	7 (16.7%)	74 (66.1%)
N	3 (4.3%)	35 (83.3%)	38 (33.9%)
Pathologic response			
pCR	39 (55.7%)	20 (47.6%)	59 (52.7%)
RD	31 (44.3%)	22 (52.4%)	53 (47.3%)

Samples	CT-P (n=94)	CT (n=53)	Total (n=147)
Baseline	67 (71.3%)	37 (69.8%)	104 (70.7%)
Surgery (RD)	27 (28.7%)	16 (30.2%)	43 (29.3%)

RESULTS

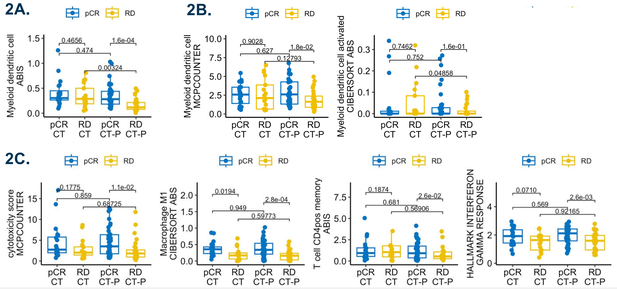
Figure 1. Association between BL TME composition and pCR.



A. Patients who experienced pCR had higher BL abundance of TME cell types driving cytotoxic and adaptive anti-tumor immune responses compared to pts with RD (FDR<0.05), indicating that presence of an immune-enriched TME is associated with a higher likelihood of pCR.

B. RNA signatures showed higher proinflammatory processes at BL in pts with pCR vs RD (FDR<0.05). Interferon signatures at BL were amongst the top processes upregulated in those pts with pCR vs RD (IFN alpha and gamma, top 2 signatures in Hallmark; IFN programs, top 4 signatures in 3CA).

Figure 2. Association between BL TME composition and pCR in pts who received neoadjuvant CT-P vs CT alone.



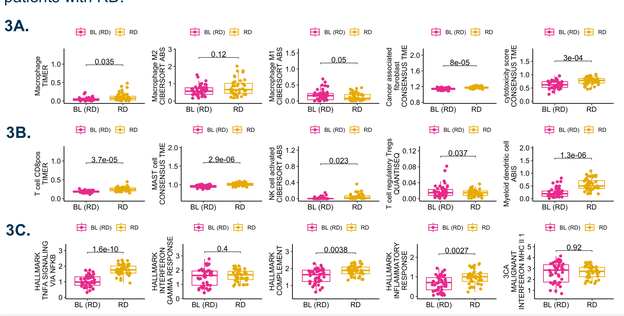
A. Higher dendritic cells at BL were associated with pCR in pts receiving CT-P (p=1.6e-04) but not in those receiving CT (p=0.47). Dendritic cells at BL were higher in pts with RD after CT vs CT-P (p=0.0032, FDR <0.20), while there was no difference between pts with pCR after CT vs CT-P (p=0.47).

B. Dendritic cell estimation from other methods showed a similar pattern of higher dendritic cells at BL in patients with pCR to CT-P but not CT, and higher dendritic cells at BL in pts with RD after CT vs CT-P (p<0.05).

C. Other TME cell types at BL that differed between pts who experienced pCR and RD (see Fig. 1; e.g., anti-tumor TAMs) did not show significant differences between CT-P and CT groups (FDR>0.20).

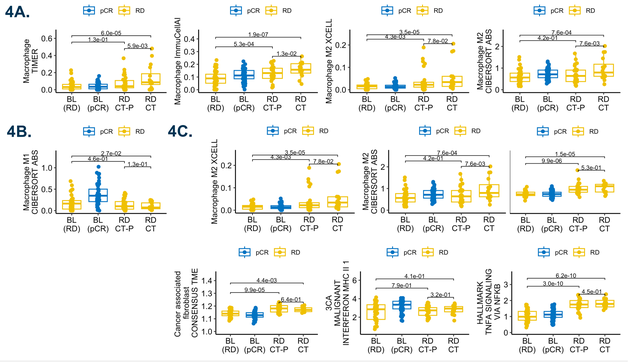
RESULTS (CONT.)

Figure 3. Differences in TME composition between BL and surgical (RD) samples in patients with RD.



(A-B) Among pts with RD (N=43, 35 with paired BL-RD samples), the **RD TME was characterized by a wound-healing response** (Panel A: higher total and suppressive TAMs, cancer-associated fibroblasts [CAFs], mast cells; lower antitumor-like TAMs) **and activated anti-tumor lymphocytes** (Panel B: higher CD8 T cells, cytotoxic cells, activated NK cells; lower Tregs) compared to BL samples (FDR<0.15).
(C) Inflammatory processes (e.g., TNF-α signaling via NF-κB, complement, and inflammatory response) **were upregulated in the RD TME compared to BL samples** among pts with RD (FDR<0.05). Notably, 10/11 interferon signatures/programs tested were not different between RD and BL (p>0.05), with 1/11 (not shown) being higher in RD vs BL (p=0.020, FDR>0.20), in contrast to the comparison of BL samples, in which they were the top significant signatures enriched in pts with pCR vs RD (Fig. 1).

Figure 4. Differences in RD TME in pts who received neoadjuvant CT-P vs CT alone.



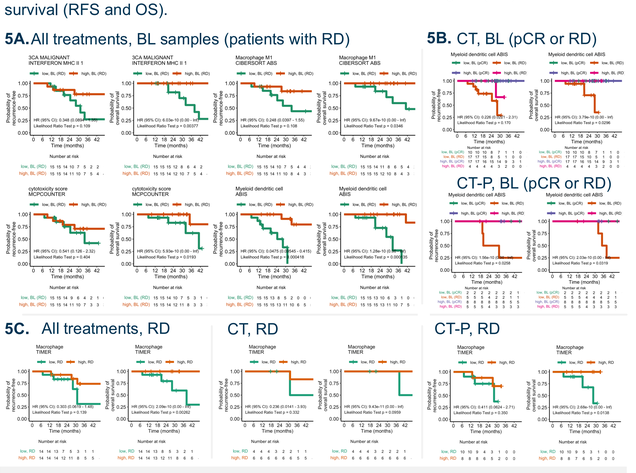
A. Total TAMs and suppressive TAMs abundances were lower in RD samples after CT-P vs those after CT alone (FDR<0.20). Comparing RD vs BL samples by neoadjuvant treatment, an increase in total and suppressive TAMs was observed in RD samples, with a stronger signal after CT (FDR<0.05) than after CT-P (FDR<0.05).

B. Anti-tumor TAM abundances based on the CIBERSORT ABS gene set shows a numerical trend opposite to that of total TAMs and suppressive TAMs: **higher in the RD samples after CT-P vs CT** (p=0.1), and lower in RD vs BL samples (CT, p=0.03; CT-P, p=0.5).

C. Abundances of other TME cell types that changed between BL and RD were not significantly different in between CT-P vs CT RD samples (FDR>0.20).

RESULTS (CONT.)

Figure 5. Association between TME composition in BL and surgical (RD) samples and survival (RFS and OS).



A. Anti-tumorigenic immune cell types and processes enriched at BL in pts with pCR were also associated with better prognosis when detected at BL in pts with RD (p<0.05).

B. Higher dendritic cells at BL were associated with better prognosis with CT-P (RFS, p=0.026; OS, p=0.032) and a similar though not-significant trend with CT alone (RFS, p=0.17; OS, p=0.030).

C. Higher total TAMs in RD samples was associated with a favorable prognosis trend in RFS (p=0.1) and improved OS (p=0.003), which was significant for CT-P (p=0.01) and not CT (p=0.1)

CONCLUSIONS

- Proinflammatory TME in TNBC at BL was associated with pCR to neoadjuvant therapy (FDR<0.05)
- Higher dendritic cells at BL identified pts who were more likely to experience pCR with CT-P (but not CT) and better RFS and OS in CT-P (p<0.05), with a similar trend in CT (p<0.09).
- Combined anti-tumor (higher cytotoxic cells, lower Tregs) and immunosuppressive (higher CAFs and suppressive TAMs) changes were observed in the TME after treatment. Lower suppressive TAMs after CT-P suggests presence of a more favorable TME compared to RD after CT alone.
- Whole genome, single-nucleus RNA and methylation profiling are ongoing to further identify markers of response to neoadjuvant CT with and without P.

REFERENCES

1. Schmid P et al. N Engl J Med. 2020;382(9):810-821. 2. Schmid P et al. N Engl J Med. 2022;386(6):556-567. 3.Schmid P et al. N Engl J Med. 2024;391(21):1981-1991.

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