Interferon Signaling and Outcomes in Triple-negative Breast Cancer (TNBC) in FinXX, CALGB 40603 (Alliance) and Real-World Clinico-Genomic Data

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Abstract# 569

Abstract

Background: Several studies established the prognostic role of both the amount and locations of tumor-infiltrating lymphocytes (TILs) in TNBC. Three distinct immunotypes were described based on the amount and locations of TILs: immune enriched (IN), immune excluded, and immune desert. Using single-cell spatial transcriptomic analysis in the Mayo Clinic TNBC cohort, our previous studies showed the central role of interferon (IFN) signaling in IN phenotype. Herein, we evaluated the association between IFN and outcomes in TNBC in 3 independent datasets.

Methods: NanoString IO360 and Breast Cancer 360 were performed in 114 samples from FinXX (NCT00114816) to generate 22-gene IFNα and 33-gene IFNγ signatures. RNA sequencing was performed in 388 samples from CALGB 40603 (NCT00861705). 3038 TNBC samples were tested by WTS (NovaSeq; Caris Life Sciences, Phoenix, AZ). Median values were used as cut-offs for high vs low IFNγ RNA expression and 18-gene IFNγ signature scores. Caris Life Science CODEai was used to evaluate real-world overall survival (OS) obtained from insurance claims and calculated from tissue collection to last contact using Kaplan-Meier estimates. Chi-square, Mann-Whitney U, ANOVA, and Cox regression were used.

Results: A high 22-gene IFNα signature score was associated with significantly improved recurrence-free survival (RFS) in FinXX (hazard ratio [HR] 0.32, 95% confidence interval [CI] 0.14-0.74, p 0.007) and OS (HR 0.28, 95%CI 0.12-0.66, p 0.003). Similar findings were observed with 33-gene IFNγ signature with significant improvement in RFS (HR 0.21, 95%CI 0.09-0.51, p < 0.001) and OS (HR 0.18, 95%CI 0.08-0.44, p < 0.001). Furthermore, in CALGB 40603, both IFNα and IFNγ scores were positively associated with pathologic complete response (pCR: IFNα p 0.019 and IFNγ p 0.007) and residual cancer burden (RCB: IFNα p 0.044 and IFNγ p 0.013). Using the Caris data platform to further validate, we identified 2899 TNBC patients (pts) with genomic and clinical outcome data. High IFNγ expression was associated with significant improvement in OS (25.95 vs 17.43 months; HR 0.65, 95% CI 0.59 – 0.72, p < 0.001). Similarly, pts with high IFNγ signature scores had significant improvement in median OS (25.79 vs 16.22 months; HR 0.66, 95% CI 0.6 – 0.73, p < 0.001).

Conclusions: This study underscores the pivotal role of IFN signaling in TNBC. High IFNα and IFNγ signatures were consistently associated with improved RFS, OS, higher pCR rates, and lower RCB across clinical trial cohorts and real-world data. These findings signify IFN signaling as a potential key biomarker and therapeutic target in TNBC.

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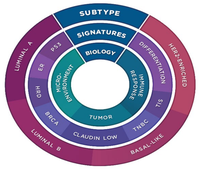
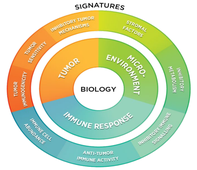
Methods

RNA isolation and NanoString Breast Cancer 360™ (BC360) and PanCancer IO 360™ (IO360) nCounter analysis

- RNA was extracted from FFPE tissue sections and analyzed using NanoString nCounter system with the Breast Cancer 360™ NanoString panel
- BC360 gene expression panel includes a total of 776 genes across 33 biologically relevant signatures in breast cancer.
- IO360 includes a total of 770 genes involved in the complex interplay between the tumor microenvironment and immune response in cancer.
- Additional 30 customized genes reported related to capecitabine and fluorouracil absorption, distribution, metabolism and elimination were included.
- Signature scores were generated using prespecified algorithms developed by NanoString Technologies and analyzed by nSolver™ software.
- Cox proportional hazard ratio was used to determine the association of each gene signature with recurrence free survival as a primary endpoint.





Outcomes in Real-World Clinico-Genomic Data

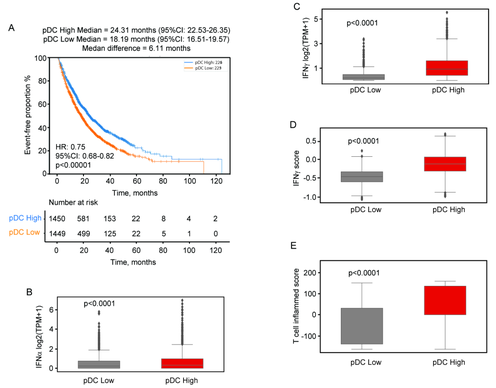


Figure 1: Within the CODEai data platform, we identified 2,899 patients with TNBC. **A.** Using a transcriptomic deconvolution approach to estimate plasma cytoid dendritic cells (pDC) abundance from whole transcriptome sequencing (WTS) data (NovaSeq; Caris Life Sciences, Phoenix, AZ), patients with higher pDC levels had significant improved event free survival of 24.31 months (95% CI: 22.53–26.35), compared to 18.20 months (95% CI: 16.51–19.57), reflecting a median difference of approximately 6.11 months (hazard ratio [HR] 0.75, 95% CI: 0.68–0.82, p < 0.00001) **B.** Tumors with high level of pDC had significantly higher IFNα expression **C.** IFNγ expression **D.** IFNγ score and **E.** T cell inflamed score.

IFN Signaling and Outcomes

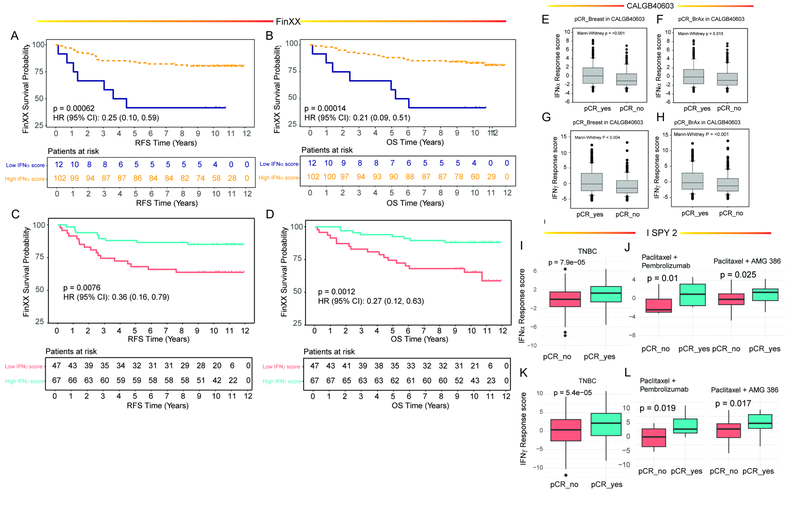


Figure 2: A. The Kaplan-Meier curve of patients with TNBC in the FinXX trial. A high IFNα signature score was significantly associated with improved recurrence-free survival (RFS; hazard ratio [HR] = 0.25, 95% confidence interval [CI]: 0.10–0.59, p = 0.0006) and **B.** overall survival (OS; HR = 0.21, 95% CI: 0.09–0.51, p < 0.001). **C.** Similarly, a high IFNγ signature score correlated with significantly better RFS (HR = 0.36, 95% CI: 0.16–0.79, p = 0.0076) and **D.** OS (HR = 0.27, 95% CI: 0.12–0.63, p = 0.0012). **E.** In CALGB 40603 trial, high IFNα signature score is associated with higher pCR in the breast, and **F.** in both the breast and axilla. **G.** High IFNγ signature score is associated with higher pCR in the breast, and **H.** in both the breast and axilla. **I.** In the I-SPY 2 trial, a high IFNα signature score is associated with higher pCR in the TNBC, and **J.** in patients with TNBC treated with pembrolizumab or AMG386. **K.** A high IFNγ signature score is associated with higher pCR in the TNBC, and **L.** in patients with TNBC treated with pembrolizumab or AMG386.

Conclusions

- High IFNα and IFNγ signatures were consistently associated with improved RFS, OS, higher pCR rates, and lower RCB across clinical trial cohorts and real-world clinico-genomic data.
- High IFNα and IFNγ signatures were associated with higher levels of plasmacytoid dendritic cells and improved event-free survival.
- These findings signify IFN signaling as a potential key biomarker and therapeutic target in TNBC.