# PRO-C3 as a Circulating Biomarker of Tumor Fibrosis in Breast Cancer: Functional Insights from Studying Cancer Associated Fibroblasts (CAFs) in vitro

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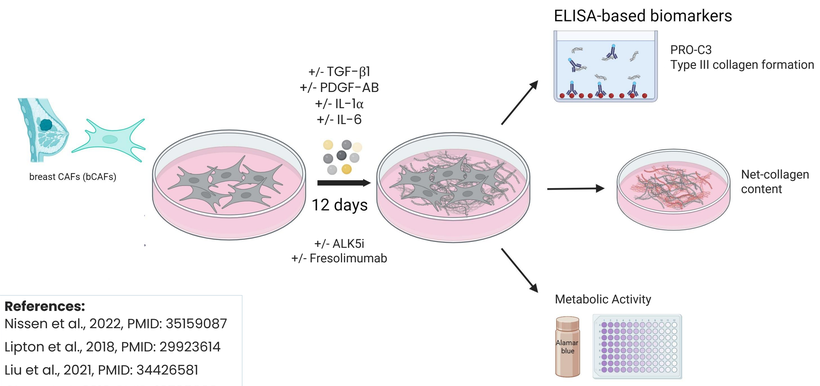
## BACKGROUND

- Extracellular matrix (ECM) remodeling is a hallmark of breast cancer tumor progression, driven in part by cancer-associated fibroblasts (CAFs).
- The N-terminal pro-peptide of type III collagen (PRO-C3), released during collagen formation, is a circulating biomarker reflecting active tumor fibrosis (Nissen et al., 2022).
- Elevated pre-treatment PRO-C3 levels have been associated with poor outcomes in metastatic HER2+ breast cancer treated with Trastuzumab (Lipton et al., 2018).
- In a Phase II trial of tetrathiomolybdate in high-risk triple-negative breast cancer, patients who remained disease-free exhibited lower PRO-C3 levels (Liu et al., 2021), suggesting a link between reduced collagen formation and improved outcomes.

## METHODS

To investigate the cellular origin and regulation of type III collagen production in breast cancer, we quantified PRO-C3 in conditioned medium (by competitive ELISA) using primary breast CAFs and primary benign breast fibroblast cultured in Ficoll-containing media with ascorbic acid, according to the *in vitro* Scar-in-a-jar fibroblast model system (Chen et al., 2019).

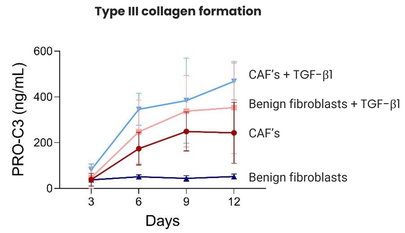
Breast fibroblasts were stimulated with profibrotic (TGF-β1, PDGF-AB) or inflammatory (IL-1α, IL-6) cytokines, and treated with antifibrotic agents (ALK5i, Fresolimumab) to assess potential changes in PRO-C3, collagen deposition and metabolic activity.

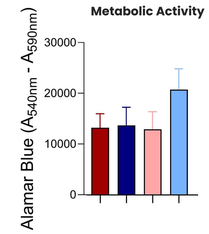



**References:**
Nissen et al., 2022, PMID: 35159087
Lipton et al., 2018, PMID: 29923614
Liu et al., 2021, PMID: 34426581
Chen et al., 2019, PMID: 19785660

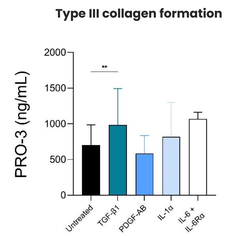
## RESULTS

### PRO-C3 is Elevated in CAFs and Induced by TGF-β1

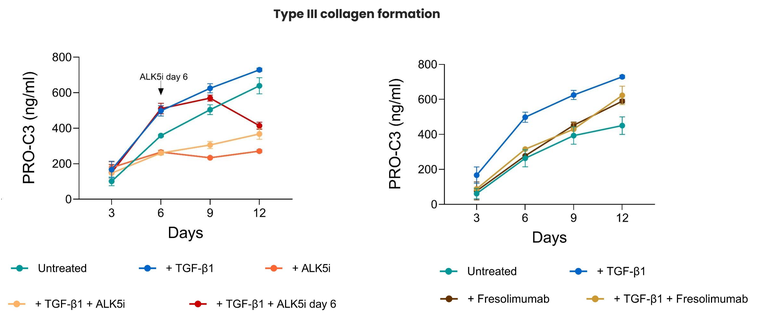


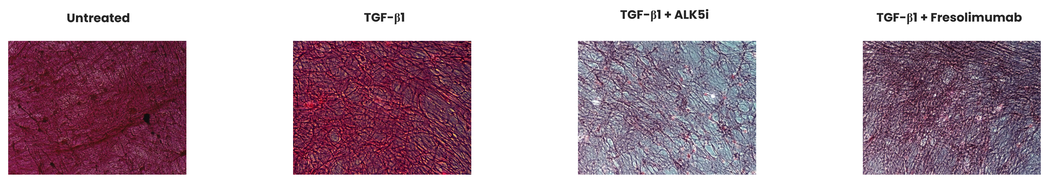

### Other Cytokines




### Inhibition of the TGF-β1 Pathway Alters The Fibrotic Activity (PRO-C3) in CAFs



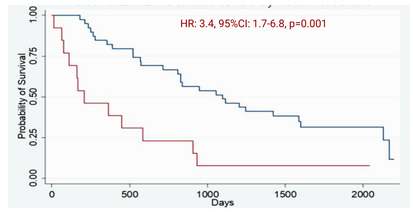

### Net-collagen content reflects PRO-C3 measurements



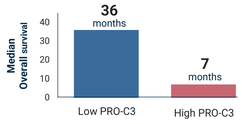

On day 12 of the SiaJ, the cultures were decellularized and stained with Picro Sirius Red to visualize the overall net-collagen content

### Supporting Published Clinical Data

**Overall survival in HER2+ metastatic patients treated with trastuzumab**

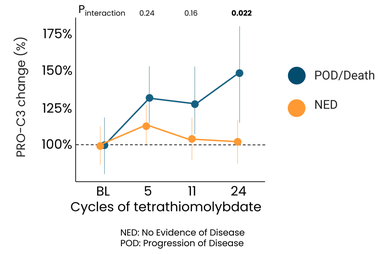



**Patients with low pretreatment PRO-C3 level are 5-times more likely to survive**




Lipton et al., 2018, PMID: 29923614

**PRO-C3 increases with progression/relapse in TNBC patients treated with tetrathiomolybdate (TM)-associated copper depletion**




Liu et al., 2021, PMID: 34426581

## CONCLUSIONS

- PRO-C3 reflects both intrinsic and inducible fibrotic activity in breast CAFs and serve as a surrogate marker of fibroblast activation.
- Our data support a fibroblast-derived origin of PRO-C3 and highlight TGF-β1 as a key upstream regulator.
- These findings underscore the need for individualized anti-fibrotic strategies in breast cancer management.
- Integration of PRO-C3 in clinical workflows enables non-invasive monitoring of fibroblast activity and fibrotic burden, informing prognosis and therapeutic response in patients with breast cancer.

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