A cell-free DNA prognostic indicator for pathological response and recurrence in early triple negative breast cancer.

S. Kasimir-Bauer*, C. Zündorf*, A.-K. Bittner*, O. Hoffmann*, R. Kimmig*, J. Siveke#, S. Lueong#.

*Dep. of Gynecol. & Obstet., #Bridge Institute of Experimental Tumor Therapy (BIT), West German Cancer Center, both University Hospital of Essen, Germany.

Background

The addition of a checkpoint inhibitor (**ICI**) to platinum containing neoadjuvant therapy (**NACT**) has significantly improved pathological complete response (**pCR**), progression-free (**PFS**) and overall survival (**OS**) in early triple negative breast cancer (**eTNBC**). However, clinical management of this breast cancer (BC) subtype remains challenging, as only around 60% of patients achieve a pCR and the rest witness rapid disease recurrence. **Biomarkers** to accurately **predict pCR** and **recurrence**, therefore, constitute **unmet clinical needs**.

Purpose of the Study

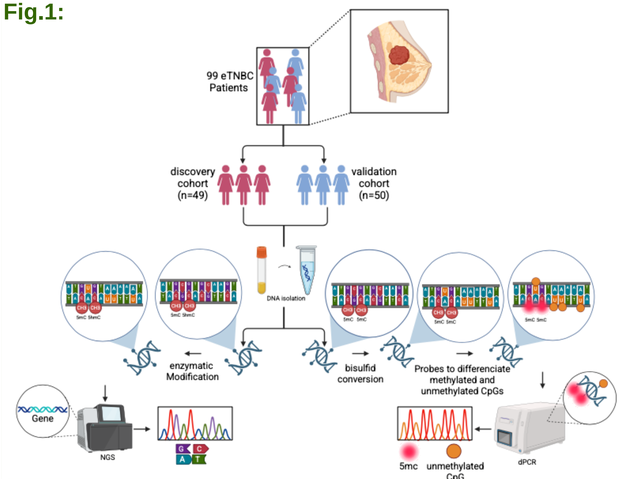
We herein deploy DNA-methylation patterns in tumor tissue as well as cell-free DNA (cfDNA) methylation before and after NACT for the identification of response and relapse biomarker in eTNBC.

Summary and Conclusion

- **We identified cfDNA methylation markers that clearly distinguish pCR from non-pCR cases. Several of these markers were not only predictive of response but also showed strong associations with recurrence risk, highlighting their broader clinical utility.**
- **The cfDNA methylation profiles closely mirrored tumor dynamics, suggesting that these circulating signals faithfully reflect biological changes occurring within the tumor during and after therapy.**
- **These findings support the premise that cfDNA methylation can serve as a minimally invasive surrogate for monitoring treatment-induced tumor remodeling.**
- **These efforts will advance the development of a clinically deployable cfDNA methylation assay capable of real-time monitoring of tumor dynamics, prediction of pCR, and early detection of recurrence.**

Workflow, Patients and Methods

Fig.1:

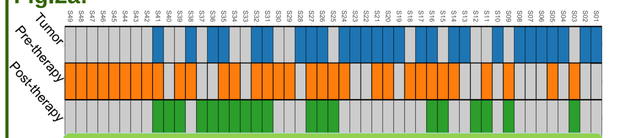


Experimental workflow for the discovery and validation arms of the study (created with biorender.com).

Blood and tumor tissue from **49 patients** (**discovery cohort**) presenting with first diagnosis of **eTNBC between Jan 2013 and Apr 2018** were evaluated by cfDNA methylation **before and after NACT lacking ICI treatment**. To this end, bead-based concentration and column isolation of cfDNA was achieved with the **QiaAmp Mini Elute cfDNA Mini kit**. Following fluorometric-based quantification, **cfDNA methylation sequencing libraries** were prepared with the **Enzymatic Methylseq kit (New England Biolabs)** followed by **hybrid capture** with an in-house panel. Libraries were sequenced on a **Nextseq 2000 P4 flow cell** and **data analyzed** with the **Bismark suite**. Missing values were imputed by K-Nearest Neighbors imputation (**KNN**). Differential methylation analyses was performed with the **RnBeads suit** and a penalized least absolute shrinkage and selection operator (**LASSO**) regression was used for biomarker identification. Maximal **log-rank statistic** was used for the determination of biomarker cut-off for all continuous variables.

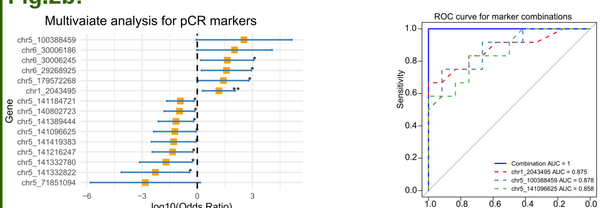
Results for the discovery cohort

Fig.2a:



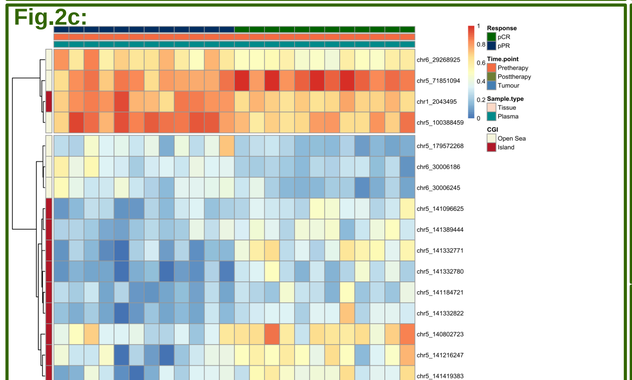
A binary plot summarizing sample distribution across different timepoints for all patients included in the discovery cohort. Grey represents missing samples

Fig.2b:



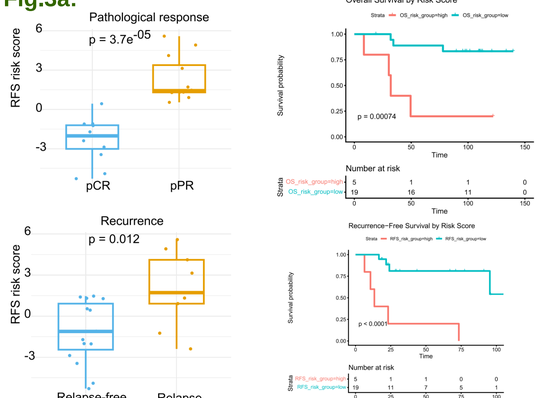
Multivariate regression analyses identify hypermethylation events associated with pCR and non-pCR (left). Combination of biomarker triplets improves predictive performance (right).

Fig.2c:



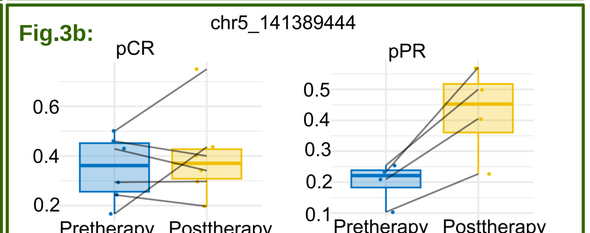
Pathological response is associated with distinct cfDNA methylation patterns for each marker site.

Fig.3a:



Response markers associated with pathological response are also associated with disease recurrence and show a significant prognostic impact on overall and recurrence-free survival.

Fig.3b:



Hypermethylated sites associated with pCR remain unchanged after NACT in cases of pCR meanwhile a sharp increase in methylation is observed in cases with non-pCR post NACT.

Perspectives: We are validating the identified markers in a larger, independent cohort to confirm their robustness and clinical applicability. We will expand analyses to include longitudinal sampling, enabling assessment of how these markers track treatment response over time and anticipate relapse.

Contact: Sabine.Kasimir-Bauer@uk-essen.de/Smiths-Sengkwawoh.Lueong@uk-essen.de