Tumor-educated platelet transcriptomics as a potential tool to monitor therapy in luminal breast cancer- 1037

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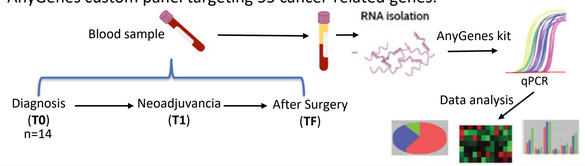
Introduction

Platelets, traditionally known for their roles in hemostasis, thrombosis, and wound healing, are now recognized as key players in cancer progression—particularly in metastasis. Their interaction with tumor cells triggers activation and modifies their RNA profile.

Platelets can internalize and store tumor-derived RNAs—including mRNA, circular, long non-coding, and mitochondrial species—resulting in a highly dynamic RNA repertoire with strong potential for cancer diagnosis. These tumor-educated platelets (TEPs) show distinct RNA and protein profiles compared with healthy individuals, enabling up to 96% accuracy in detecting multiple tumor types and 71% accuracy in identifying their primary origin.

Methods and Materials

The objective of this study was to investigate whether longitudinal analysis of TEPs-RNA can predict treatment response in patients with luminal breast cancer. Fourteen luminal breast cancer patients from Arnau de Vilanova Hospital (Lleida) were recruited, and serial blood samples were collected at diagnosis, after neoadjuvant therapy, and post-surgery. Platelet RNA, isolated after double low-speed centrifugation, was analyzed by real-time PCR using an AnyGenes custom panel targeting 55 cancer-related genes.



Results

Platelet transcriptomic profiling of 14 luminal breast cancer patients across treatment stages (diagnosis, post-neoadjuvant, and post-surgery) revealed distinct clustering patterns in PCA, driven by genes such as TP53, CCND2, CACNB1, APC, VEGFA, and NCOA3 after treatment, and L1CAM, CACNA2D1, and CTLA4 at baseline. Differential expression analysis identified therapy-modulated genes associated with pathways involved in cell cycle regulation, immune response, and signal transduction. Functional enrichment confirmed significant alterations in biological processes linked to platelet activation and tumor–immune interactions. A logistic regression model using baseline expression of L1CAM, CTLA4, and CACNA2D1 showed predictive capacity for axillary node involvement (AUC = 0.917), high tumor reduction (AUC = 0.725), and pathological complete response (AUC = 0.725). These exploratory findings support the potential of tumor-educated platelet RNA signatures as minimally invasive biomarkers to monitor therapeutic response in luminal breast cancer.

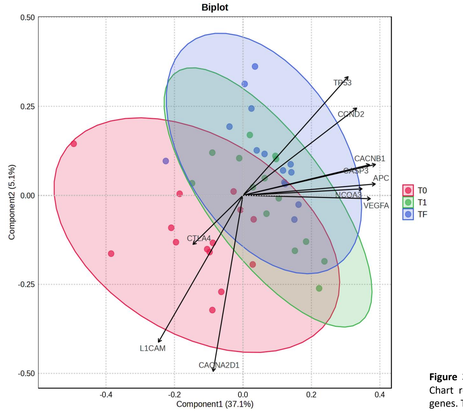


Figure 1. PCA biplot of platelet gene expression in luminal breast cancer across treatment stages. PCA of platelet RNA from 14 luminal breast cancer patients at diagnosis (T0, red), after neoadjuvant therapy (T1, green), and after surgery (TF, blue). Ellipses show 95% confidence intervals.



Figure 3. Functional enrichment analysis of biological processes and reactome pathways. Chart represents the enriched biological processes associated with differentially expressed genes. The vertical axis indicates biological process terms (Gene Ontology), while the horizontal axis shows the proportion of genes in each term. The size of the bubbles reflects the number of genes involved in each process, and the color indicates the FDR (False Discovery Rate), with lighter tones representing greater statistical significance. This analysis highlights the most relevant biological processes in the studied genes.

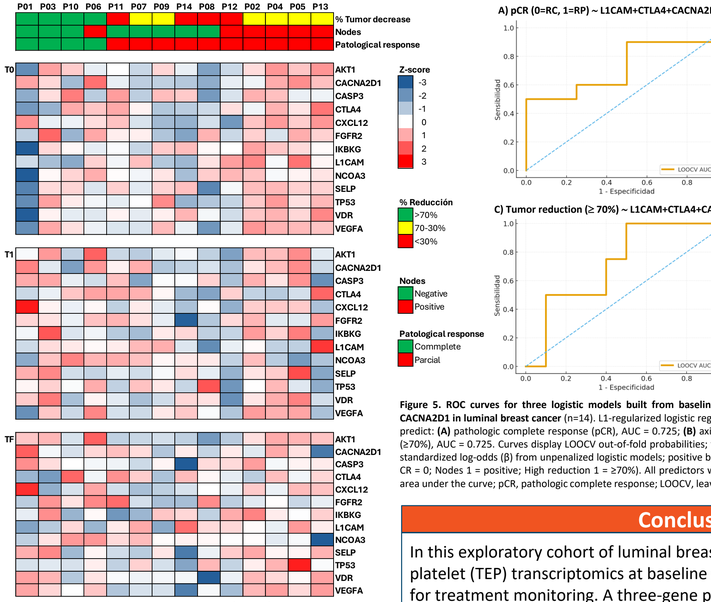


Figure 4. Heatmaps of gene expression changes (Z-score) in luminal breast cancer patients across treatment stages. Thirteen genes selected for their predictive potential (AUC > 0.7 and high VIP scores) are shown. Patients are ordered by pathological response (pR). Legends indicate Z-score values, % tumor decrease categories (>70%, 30–70%, <30%), nodal involvement (positive/negative), and pR status (complete/partial).

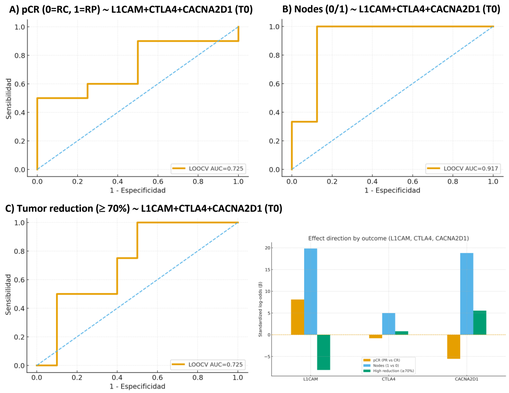


Figure 5. ROC curves for three logistic models built from baseline (T0) platelet (TEP) gene expression of L1CAM, CTLA4, and CACNA2D1 in luminal breast cancer (n=14). L1-regularized logistic regression with leave-one-out cross-validation (LOOCV) was used to predict: **(A)** pathologic complete response (pCR), AUC = 0.725; **(B)** axillary node positivity, AUC = 0.917; and **(C)** high tumor reduction (≥70%), AUC = 0.725. Curves display LOOCV out-of-fold probabilities; the diagonal shows no-skill performance. **(D)** Direction of effect: standardized log-odds (β) from unpenalized logistic models; positive bars indicate increased odds of the outcome (pCR coded PR = 1 vs CR = 0; Nodes 1 = positive; High reduction 1 = ≥70%). All predictors were z-standardized.ROC, receiver operating characteristic; AUC, area under the curve; pCR, pathologic complete response; LOOCV, leave-one-out cross-validation; T0, diagnosis/baseline.

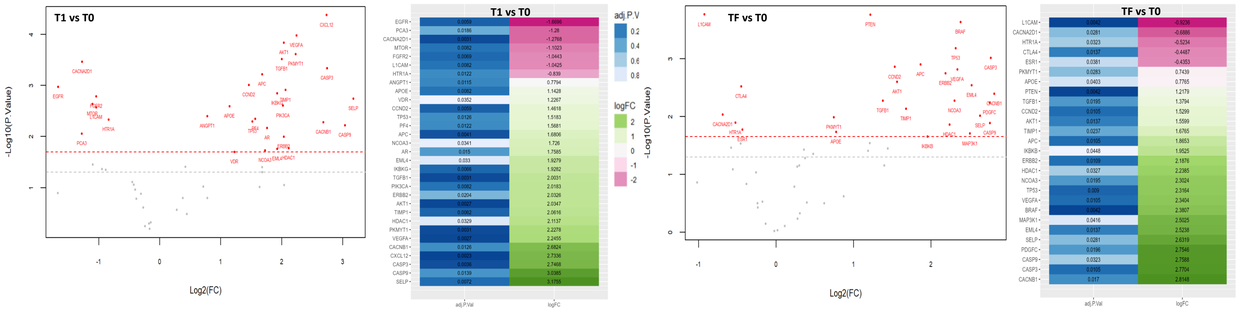


Figure 2. Differentially expressed genes in TEPs RNA from luminal breast cancer patients after neoadjuvant therapy (T1 vs T0) and post-surgery (TF vs T0). Volcano plot of gene expression, for adjusted p-values. The line vertical corresponds to the 0.05 p-value threshold and the horizontal lines to the (0.025, 0.975) fold change quantile. All genes whose expression is significantly changed between conditions show up as red dots. Analysis performed using the limma package (Ritchie et al., 2015) for the identification of differentially expressed genes .

Conclusions

In this exploratory cohort of luminal breast cancer (n=14), tumor-educated platelet (TEP) transcriptomics at baseline showed measurable predictive signal for treatment monitoring. A three-gene panel (L1CAM, CTLA4, CACNA2D1) achieved good discrimination for axillary node status (LOOCV AUC = 0.917) and moderate discrimination for pCR and high tumor reduction (≥70%) (AUC = 0.725 each), supporting the biological and clinical relevance of TEP-RNA.

Although overall calibration and precision are limited by the small sample size, the findings demonstrate the feasibility and potential utility of TEP RNA as a minimally invasive, blood-based biomarker to complement standard clinico-pathologic factors and enable longitudinal response assessment. These results warrant validation in larger, independent cohorts and motivate targeted qPCR panels for scalable implementation and prospective testing.

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