Transcriptomic Profiling Reveals PMSB8-AS1 and LINC00996 as Potential Prognostic Biomarkers in Triple-Negative Breast Cancer Treated with Neoadjuvant Docetaxel–Carboplatin.

S. López-Tarruella[1,2], C. Polo[1], E. Alvarez[1], Y. Jerez[1], I. Echavarria[1], M. del Monte-Millán[1], B. Herrero[1], P. Jara[1], F. Moreno Antón[3], J. García-Sáenz[3], C. Bueno[4], M. Bringas[1], I. Márquez-Rodas[1], A. Ballesteros[6], N. Jiménez-Alduán[1], A. López de Sa[3], H. Gómez Moreno[7], H. Fuentes[7], A. Barnadas[8,9], A. Prat[10], B. Peláez-Lorenzo[11], R. Martín Lozano[1], A. Sánchez de Torre[12], M. Cebollero[9, 13], T. Massarrah[1,2,9], I. Ocaña[1], A. Arias[1], L. Villarejo[1], V. Cañadilla[1], F. Ayala de la Peña[1], M. Martín[1,2]

[1]Medical Oncology, Hospital General Universitario Gregorio Marañón. IiSGM, Madrid, SPAIN. [2]Universidad Complutense, Madrid, SPAIN. [3] Medical Oncology, Hospital Universitario Clínico San Carlos. IdISSC, Madrid, SPAIN. [4]Medical Oncology, Hospital Infanta Cristina y FIB H.U. Puerta de Hierro, Madrid, SPAIN. [6]Medical Oncology, Hospital General Universitario La Princesa, Madrid, SPAIN. [7]Department of Medical Oncology, Clinic., INEN, Lima, PERU. [8] Medical Oncology, Medical Oncology Department, Hospital de la Santa Creu I Sant Pau de Barcelona, CIBERONC-ISCIII, Barcelona, SPAIN. [9] GEICAM, Madrid, SPAIN. [10]IDIBAPS, Hospital Clínic of Barcelona, Barcelona, SPAIN. [11]Medical Oncology, Hospital Clínico de Valladolid, Valladolid, SPAIN. [12]Medical Oncology, Hospital U. Infanta Cristina (Parla), Madrid, SPAIN. [13]Anatomical pathology, Hospital General Universitario Gregorio Marañón. IiSGM, Madrid, SPAIN.

Introduction

Triple-negative breast cancer (TNBC) accounts for approximately 10% of all breast cancer cases but is responsible for a great number of disease relapses. Its aggressive clinical behaviour and lack of expression of estrogen receptor (ER), progesterone receptor (PR), and HER2 limit the number of effective targeted treatment options available[1].

Thus, the development of predictive biomarkers in early-stage TNBC is a key research priority. Several lncRNAs have emerged as potential biomarkers[2,3] due to their regulatory roles in gene expression and are currently being evaluated in ongoing clinical trials. However, further evidence is still needed to establish their prognostic relevance and clinical utility in TNBC.

Methods and Materials

At diagnosis



FFPE biopsy of 140 responders (pCR)

FFPE biopsy of 141 non-responders (RCB I, II, III)

Bulk RNA-Seq (Illumina)

Annotation: STAR (v.2.7.9a) and SALMON (v.1.6.0)

Differential expression: Wald test (statistical correction using FDR)

Figure 1. Transcriptomic analysis over diagnostic biopsies (threshold of tumoral area = 2mm^2) from TNBC patients obtained before neoadjuvant systemic treatment with 6 cycles of docetaxel-carboplatin (NACT).

Results

Transcript biotype analysis revealed that TNBC tumors contain a high proportion of non-coding transcripts, including lncRNAs. Furthermore, patients with residual disease (RCB I, II, III) showed a pronounced decrease in immunoglobulin (IG) and T-cell receptor (TCR) transcript expression compared with those achieving pCR, suggesting differences in immune-related transcriptional activity (Chart 1).

Differential expression analysis identified 166 lncRNAs significantly deregulated between groups, among which PMSB8-AS1 emerged as a candidate prognostic biomarker (higher level of expression in patients with a better prognosis), showing a significant association with event-free survival (EFS) in Kaplan–Meier analysis ($p < 0.05$) (Chart 2). However, stratified analysis demonstrated a significant association with EFS and distant recurrence-free survival (DRFS) only in the RCB I subgroup according to Symmans' classification[4]. Additionally, in a Cox proportional hazards model, PMSB8-AS1 showed an independent protective effect regardless of pCR status per Symmans' classification ($p = 0.04$).

Furthermore, PMSB8-AS1 and LINC00996 expression correlated with immune-related genes ITK, ITGAL, GPR174, and SASH3 (Chart 3). Among these, ITK and GPR174 are involved in T-cell activation, ITGAL encodes CD11A, and SASH3 is also lymphocyte-expressed (function not fully characterized). These associations were independent of the basal-like subtype. ITGAL, GPR174, and SASH3 showed significant associations with EFS (Chart 2) and DRFS (data not shown, similar results to EFS), while ITGAL was additionally associated with overall survival (OS, data not shown).

In multivariate clinicopathological analysis (covariates: cT, cN, Ki67, PAM50 subtype, stromal TILs[5] as continuous variable, and pathological response as binary variable), PMSB8-AS1 remained significantly associated with EFS ($p < 0.05$) (Table 1). Therefore, the effect of PMSB8-AS1 is independent of the value of the aforementioned clinical covariates, and its protective effect appears to be cumulative. Finally, the combined lncRNA signature (PMSB8-AS1 + LINC00996) was associated with both EFS and OS ($p < 0.05$) and showed a trend toward significance for DRFS ($p = 0.08$) (Table 1).

Table 1. Multivariate analysis where significant values (p-value) indicate that the corresponding lncRNA provides additional information to the clinical-pathological variables indicated for the prediction of EFS, DRFS, and OS. The hazard ratio (HR) has been calculated with 95% CI.

	EFS		DRFS		OS	
	HR	P-value	HR	P-value	HR	P-value
LINC00996	0.90 (0.61–1.33)	0.6	0.91 (0.59–1.41)	0.66	0.99 (0.61–1.59)	0.96
PSMB8-AS1	0.67 (0.46–0.98)	**0.04**	0.77 (0.51–1.16)	0.21	0.70 (0.45–1.09)	0.11
Signature	2.75 (1.26–6.01)	**0.01**	2.20 (0.89–5.45)	0.09	2.72 (1.08–6.85)	**0.03**

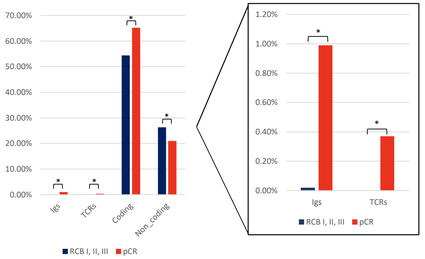


Chart 1. Percentage of biotype according patient groups (pCR vs. RCB I, II, III). * p-value ≤ 0.001

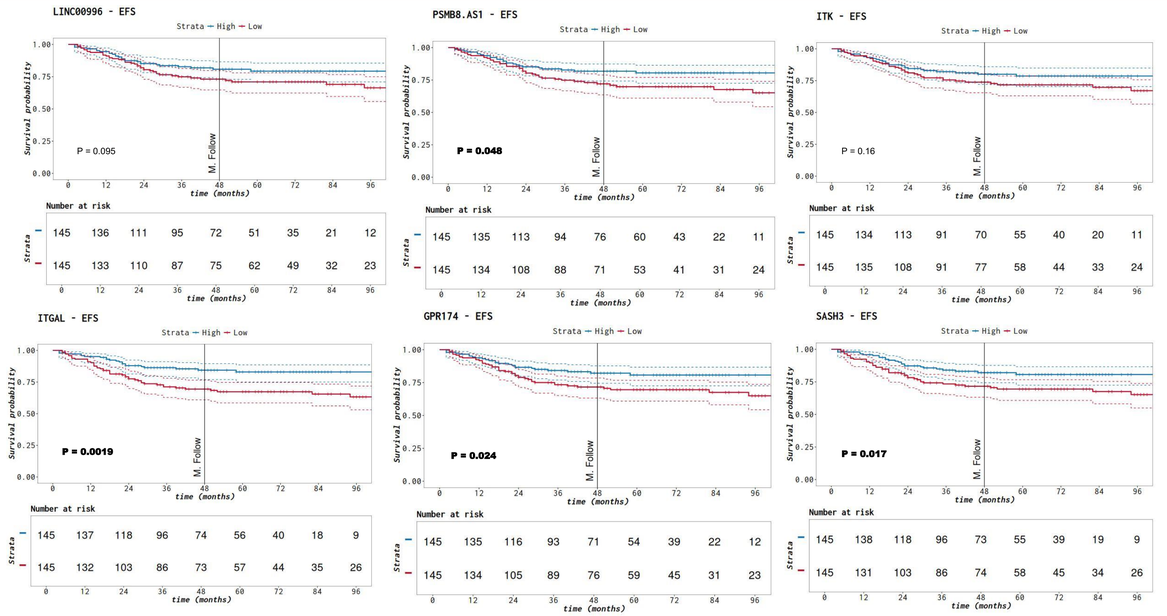


Chart 2. Kaplan-Meier analysis of lncRNA PMSB8-AS1 and LINC00996 and the genes ITK, ITGAL, GPR174 and SASH3 regulated by them.

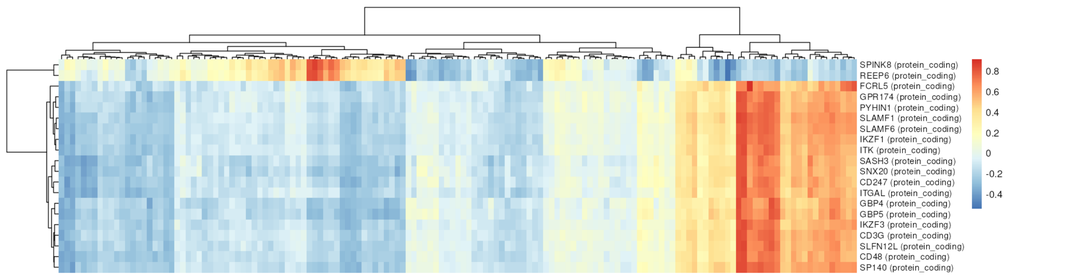


Chart 3. Gene expression (Y-axis) regulated by two or more lncRNAs (X-axis). Red indicates expression in the same direction; blue indicates expression in opposite directions.

Conclusions

Our findings suggest that PMSB8-AS1 may serve as an early prognostic biomarker of EFS in TNBC and may reflect immune and T-cell activation (regardless of the level of stromal TILs) through its association with ITK, ITGAL, GPR174, and SASH3 expression, consisting of the reduced IgG and TCR transcript levels before NACT observed in non-responders.

Moreover, the combined lncRNA signature (PMSB8-AS1 + LINC00996) improves risk stratification for EFS and OS when integrated with clinicopathological features (cT, cN, Ki67, PAM50 subtype, TILs, and pathological response). Further functional studies are required to validate the biological role of these lncRNAs in immune modulation, as well as *in silico* validation in other patient cohorts.

Contact

Sara López-Taruella MD, PhD
Medical Oncology Department, Hospital General Universitario Gregorio Marañón
Instituto de Investigación Sanitaria Gregorio Marañón (IiSGM)
Universidad Complutense, Madrid, Spain
Email: slopeztarruella@gmail.com

Acknowledgments:

Funded by Instituto de Salud Carlos III (ISCIII) PI15/00117, PI18/01775, PI22/01346, and co-funded by the European Union "A way to make Europe/Investing for your future").
We thank to all patients who participated in this study.

References

1. Zagami P, Carey LA. Triple negative breast cancer: Pitfalls and progress. NPJ Breast Cancer. 2022 Aug 20;8(1):95. doi: 10.1038/s41523-022-00468-0.
2. Ito M, Miyata Y, Okada M. Current clinical trials with non-coding RNA-based therapeutics in malignant diseases: A systematic review. Transl Oncol. 2023 May;31:101634. doi: 10.1016/j.tranon.2023.101634. Epub 2023 Feb 23.
3. Coan M, Haefliger S, Ounzain S, Johnson R. Targeting and engineering long non-coding RNAs for cancer therapy. Nat Rev Genet. 2024 Aug;25(8):578-595. doi: 10.1038/s41576-024-00693-2. Epub 2024 Feb 29.
4. Symmans WF, Peintinger F, Hatzis C, et al. Measurement of residual breast cancer burden to predict survival after neoadjuvant chemotherapy. J Clin Oncol. 2007 Oct 1;25(28):4414-22. doi: 10.1200/JCO.2007.10.6823. Epub 2007 Sep 4.
5. Salgado R, Denkert C, Demaria S, et al; International TILs Working Group 2014. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. Ann Oncol. 2015 Feb;26(2):259-71. doi: 10.1093/annonc/mdu450. Epub 2014 Sep 11.

This presentation is the intellectual property of the author/presenter. Contact them at slopeztarruella@gmail.com for permission to reprint and/or distribute.

© Poster Template by Genigraphics® 1.800.790.4001 www.genigraphics.com