3117. Validation of an Immunomodulatory Gene Signature Algorithm to Predict Response to Neoadjuvant Immunochemotherapy in Patients with Primary Triple-Negative Breast Cancer

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Background

- We previously established a 101-gene algorithm as a molecular subtyping method for triple-negative breast cancer (TNBC); this algorithm includes an immunomodulatory (IM) subtype based on genes active in immune cell processes.
- We now have narrowed the IM subtype to a 27-gene signature algorithm and established its predictive ability for immunotherapy response in lung cancer.

Objective

To validate the accuracy of IM subtype determined by the 27-gene algorithm for predicting pathological complete response (pCR) in TNBC patients treated with neoadjuvant immunochemotherapy.

Methods

1. We obtained RNA-sequencing data from pretreatment core needle biopsy in 55 patients with stage I-III primary TNBC who received neoadjuvant immunochemotherapy (**Figure 1**).
2. We used the 27-gene algorithm to determine IM positivity using a cutoff point previously validated from biopsies from 71 lung cancer patients treated with immunotherapy (**Figure 2**).
3. We compared the 27-gene algorithm, 101-gene algorithm, and PD-L1 immunohistochemistry (IHC, antibody: SP263) predictive accuracy for pCR after neoadjuvant immunochemotherapy (**Figure 3**).
4. In cases positive for the IM subtype and pCR, we analyzed the immune microenvironment by deconvoluting the immune infiltration using a computational algorithm.

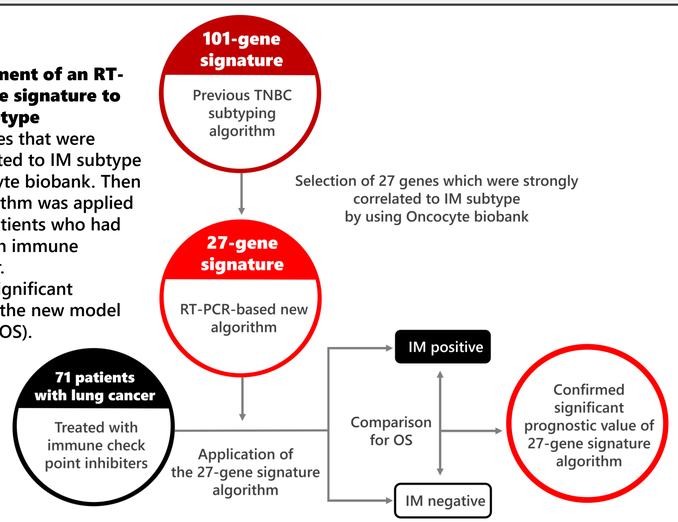

Figure 2. Development of an RT-PCR-based 27-gene signature to distinguish IM subtype
We selected 27 genes that were significantly correlated to IM subtype by using the Oncocyte biobank. Then the new gene algorithm was applied to 71 lung cancer patients who had been treated with an immune checkpoint inhibitor.
We confirmed the significant prognostic value of the new model for overall survival (OS).

- The 27-gene algorithm that defines IM subtype is a possible predictive marker for response to neoadjuvant immunochemotherapy for patients with early TNBC.
- Antigen-presenting immune cells may have a crucial role in immunochemotherapy response.
- Our results justify further study of the 27-gene algorithm in early TNBC.

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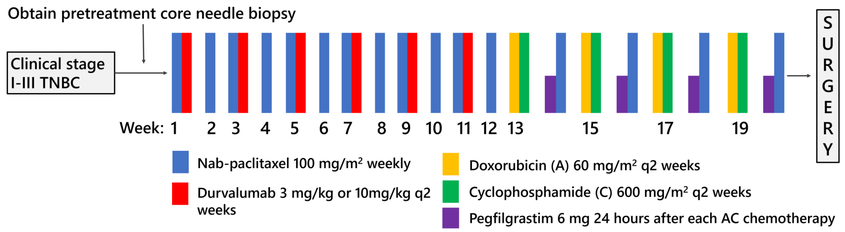


Figure 1. The overview of neoadjuvant immunochemotherapy clinical trial (NCT02489448)
Durvalumab was administered with weekly nab-paclitaxel followed by dose-dense AC and nab-paclitaxel in a phase I/II trial

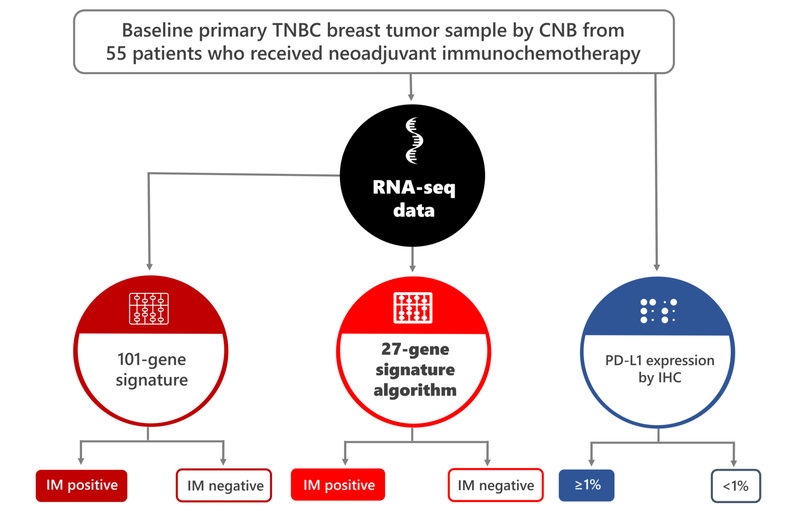


Figure 3. Validation study for TNBC patients treated with neoadjuvant immunochemotherapy
Baseline core needle biopsy samples from TNBC patients were obtained from the neoadjuvant immunochemotherapy trial. Gene data were obtained by RNA sequencing. Predictive accuracy was evaluated by diagnostic indicators. In cases positive for the IM subtype and pCR, we analyzed the immune microenvironment by deconvoluting the immune infiltration using a computational algorithm.

Results

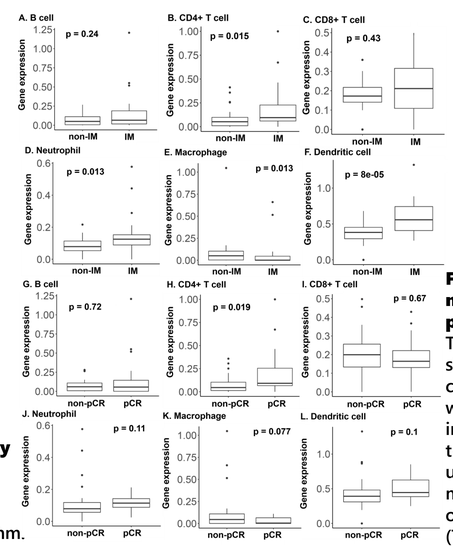
Table 1. Patient characteristics

Characteristics		Number of patients (%)	Characteristics		Number of patients (%)
All patients		55 (100)	Tumor size	cT1	18 (33)
Age (years)	≤40	11 (20)		cT2	29 (53)
	41-50	18 (33)		cT3	7 (13)
	51-69	26 (47)		Unknown	1 (2)
	>70	0 (0)	Nodal status	cN0	28 (51)
Race	White	37 (67)		cN1	22 (40)
	Black	10 (18)		cN2	1 (2)
	Asian/American indian	4 (7)		cN3	3 (5)
	Unknown	4 (7)		Unknown	1 (2)
			pCR	Yes	25 (45)
				No	30 (55)

Table 2. Evaluation of diagnostic indicators by applying several cutpoints

Subtyping method	Cutpoint	OR	95% CI lower	95% CI upper	p-value	Sensitivity (%)	Specificity (%)	PLR	NLR	PPV	NPV
101-gene signature (Centering)	0.17	3.143	0.979	10.9	0.054	64.7	63.2	1.76	0.56	44.0	80.0
	0.195	3.143	0.979	10.9	0.054	64.7	63.2	1.76	0.56	44.0	80.0
	0.10	3.033	0.978	10	0.054	63.2	63.9	1.75	0.58	48.0	76.7
27-gene signature	0.09	4.125	1.36	13.5	0.012	65.2	68.8	2.09	0.51	60.0	73.3
IHC PD-L1 expression	1%	2.631	0.817	9.21	0.106	73.9	48.1	1.43	0.54	55	68

We tested various cut points for the 101-gene signature (0.17, 0.195, and 0.10) and compared results with the 27-gene algorithm. The 27-gene algorithm had superior accuracy for predicting pCR (odds ratio, 4.125; 95% CI, 1.36-13.47; P = 0.012). The 101-gene signature used "Centering" as a subtyping method as follows: the mean expression for each gene was determined across the samples, and these were centered and scaled around zero by the following formula: (GE(x) - GE(mean))/GE(sd).
Abbreviations: PLR, positive likelihood ratio; NLR, negative likelihood ratio; PPV, positive predictive value; NPV, negative predictive value.



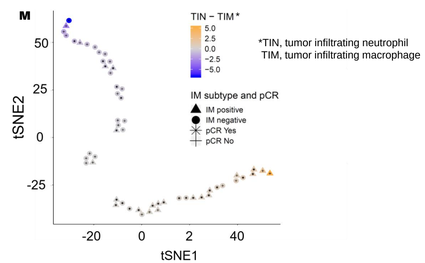


Figure 4. Association between baseline tumor microenvironment constituents, IM subtype, and pCR. (**A**-**L**) Baseline immune-cell constituents by TIMER method. **B** and **F**, CD4+ & dendritic cells were significantly increased among the baseline immune cell population in IM-positive patients. (**M**) The cases with macrophage-enriched microenvironment included more non-IM subtype and showed less pCR. t-distributed stochastic neighbor embedding (tSNE) using TIMER scores separated into 2 groups: macrophage-enriched and neutrophil-enriched based on the ratio between tumor-infiltrating neutrophils (TIN) and tumor-infiltrating macrophages (TIM).