12-chemokine gene expression score in breast cancer patients treated with neoadjuvant chemotherapy

Hatem Soliman[1], Sangeetha Prabhakaran[2], Marilin Rosa[1], Charles Cox[3], Pat Whitworth[4], Sahra Uygun[5], Heather M. Kling[5], Erin B. Yoder[5], and William Audeh[5]

1. Moffitt Cancer Center, Tampa, FL; 2. University of New Mexico Comprehensive Cancer Center, Albuquerque, NM; 3. University of South Florida, Tampa, FL; 4. Nashville Breast Center, Nashville, TN; 5. Agendia Inc., Irvine, CA

BACKGROUND

Studies demonstrating the presence of immunoregulatory gene activation[1] and tumor-infiltrating lymphocytes in the breast tumor microenvironment suggest the importance of an effective anti-tumor immune response. Chemokines, which act as trafficking signals for immune cells, influence the spatial organization of the host immune response and the formation of organized extranodal lymphoid follicles, also known as ectopic lymph node-like structures (ELNs), in response to invading pathogens, chronic inflammation, and in solid tumors[2-4]. These structures likely attract T cells and activated B cells in response to tumor antigens. ELNs have been implicated in improved clinical outcomes in several types of cancer, including breast cancer[5], and may represent a therapeutic target[6] and/or a predictive biomarker for responses to immune checkpoint inhibitors[7].

Previously, a 12-chemokine gene expression signature was identified in colorectal cancer[8] and in melanoma[9]. This gene signature has been associated with pathologic responses to durvalumab and overall survival in lung and bladder cancer[7]. High chemokine score (CS) also predicts presence of tumor-localized ELNs in patients with invasive breast cancer[10]. Compared with low CS cases, patients with a high CS have high risk clinical features, such as high grade, ER/PR-negative, and/or HER2-positive tumors; however, these patients also have significantly better recurrence free survival and overall survival[10].

In the current study, we investigate the association of CS with 70-gene signature (70-GS, MammaPrint/MP) for risk of distant metastasis, 80-gene signature (80-GS, BluePrint/BP) molecular subtype, and pathologic complete response (pCR) rate in early stage breast cancer patients who received neoadjuvant therapy.

METHODS

Patients: Tumor specimens (FFPE) used in this retrospective analysis (n=92) were from breast cancer patients enrolled in either MINT (NCT0151487) or NBRST (NCT01479101) neoadjuvant registry trials from 2011 to 2016. Clinical data were captured with informed consent. Neoadjuvant therapy was selected at the discretion of the physician. 91/92 patients received neoadjuvant chemotherapy; one patient received neoadjuvant endocrine therapy, and was excluded from pCR analyses. 28/31 HER2+ patients received neoadjuvant HER2-directed therapy. pCR was defined as absence of invasive carcinoma in the resection specimen. Residual disease (RD) includes all other responses (partial, stable, progressive).

Genomic classification: MP, BP, and full transcriptome data were generated by Agendia, Inc. MP stratified tumors into Low Risk (LR), High Risk (HR), and Ultra High Risk (UH). HR and UH are similar to MP High1 (MP1) and MP High2 (MP2), respectively, reported in the I-SPY2 trial, which has demonstrated superior chemosensitivity and pCR rates in tumors classified as UH/MP2[11]. BP classified tumors as Luminal, HER2, or Basal type.

ELN assessment: Hematoxylin and eosin (H&E) stained tissue sections from core needle biopsies were evaluated for the presence of tumor-localized ELNs.

Chemokine score and statistical analysis: Gene expression data were quantile normalized using R limma package. Principal component analysis (PCA) was performed on the normalized dataset using R princomp package. Chemokine score (CS) was defined as the first principal component values resulting from PCA. CS were compared using Mann-Whitney test. High and low CS were defined as greater or less than the median CS in the group that achieved pCR. Rates of pCR and clinical factors were compared between groups using chi-square or Fisher's Exact test.

RESULTS

Table 1. Clinical Characteristics

Clinical Characteristics (*unknowns excluded*)	All Patients (n=92)	Low CS (n=46)	High CS (n=46)	P value
Age, years				
Median	53	54	53	
Mean	54	55	53	0.49
Menopausal Status				
Pre or Peri	38 (43%)	18 (41%)	20 (45%)	0.83
Post	50 (57%)	26 (59%)	24 (55%)	
Ethnicity				
Caucasian/white	62 (67%)	31(68%)	31(68%)	
African American/Black	21 (23%)	9 (20%)	12 (26%)	0.49
Hispanic	6 (7%)	3 (6%)	3 (6%)	
Asian	2 (2%)	2 (4%)	0 (0%)	
Native American	1 (1%)	1 (2%)	0 (0%)	
Tumor Stage				
cT1	2 (2%)	0 (0%)	2 (4%)	
cT2	55 (60%)	26 (56%)	29 (65%)	0.33
cT3	28 (31%)	17 (37%)	11 (24%)	
cT4	6 (7%)	3 (7%)	3 (7%)	
Nodal Stage				
N0	8 (9%)	5 (11%)	3 (7%)	
N1	75 (82%)	36 (78%)	39 (87%)	0.73
N2	6 (7%)	4 (9%)	2 (4%)	
N3	2 (2%)	1 (2%)	1 (2%)	
Grade				
G1	4 (5%)	2 (5%)	2 (5%)	
G2	31 (36%)	22 (51%)	9 (21%)	*0.05*
G3	51 (59%)	25 (44%)	32 (74%)	
Tumor Type				
IDC	81 (88%)	39 (85%)	42 (91%)	
ILC	5 (5%)	4 (9%)	1 (2%)	0.41
Mixed IDC/ILC	4 (4%)	1 (2%)	3 (7%)	
Other	2 (2%)	2 (4%)	0 (0%)	

Table 2. Molecular and Pathology Characteristics

Molecular and Pathology Results	All Patients (n=92)	Low CS (n=46)	High CS (n=46)	P value
MammaPrint Result				
Low Risk	15 (16%)	14 (31%)	1 (2%)	***0.004***
High Risk	24 (26%)	13 (28%)	11 (24%)	
UltraHigh Risk	53 (58%)	29 (41%)	34 (74%)	
BluePrint subtype				
Luminal-type	36 (39%)	22 (48%)	14 (30%)	***0.03***
HER2-type	24 (26%)	14 (30%)	10 (22%)	
Basal-type	32 (35%)	10 (22%)	22 (48%)	
ER status (IHC)				
Positive	52 (57%)	31 (69%)	21 (46%)	***0.03***
Negative	39 (43%)	14 (31%)	25 (54%)	
PR status (IHC)				
Positive	44 (48%)	27 (60%)	17 (37%)	***0.04***
Negative	47 (52%)	18 (40%)	29 (63%)	
HER2 status (IHC/FISH)				
Positive	31 (37%)	16 (36%)	15 (37%)	
Negative	51 (60%)	26 (59%)	25 (61%)	0.87
Equivocal	3 (3%)	2 (5%)	1 (2%)	

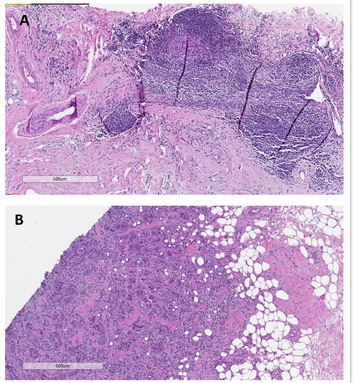

Figure 1: Tumor ELNs. Tumor ELNs were scored in H&E stained tumor sections from the top 10th and bottom 10th CS percentile. ELNs were found in 3/10 specimens with high CS (**A**) and 0/10 specimens with low CS (**B**).

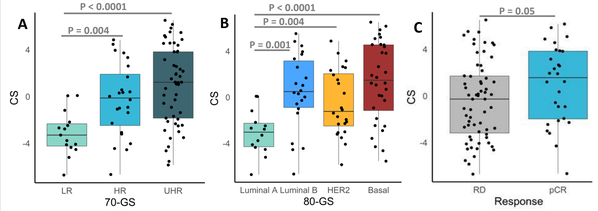

Figure 2: CS in relation to MP risk group, BP subtype, and pCR. CS was significantly higher in MP HR and UH compared with LR tumors (**A**) and in BP Basal, HER2, and Luminal B type tumors compared with Luminal A type tumors (**B**). CS was significantly higher in tumors from patients who achieved pathologic complete response (pCR) following neoadjuvant chemotherapy (**C**).

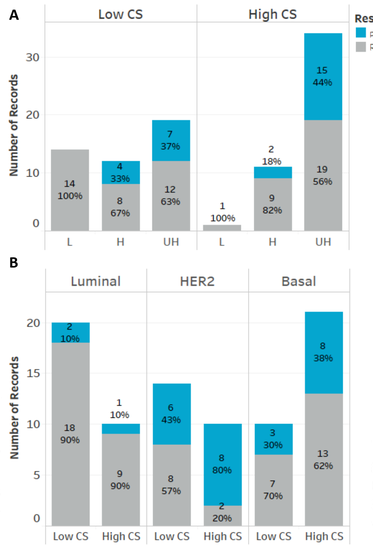

Figure 3

Groups	Residual Disease	pCR	P value
CS group			
Low CS	34 (76%)	11 (24%)	0.26
High CS	29 (63%)	17 (36%)	
MP Risk Group			
Low Risk	15 (100%)	0 (0%)	***0.008***
High Risk	17 (74%)	6 (26%)	
Ultrahigh Risk	31 (58%)	22 (42%)	
BP subtype			
Luminal	32 (91%)	3 (9%)	
HER2	10 (42%)	14 (58%)	***<0.001***
Basal	21 (66%)	11 (34%)	

Table 3 and Figure 3: Rates of pCR in CS, MP, and BP groups. MP and BP were significantly associated with pCR (**Table 3**). When combined with MP (**Fig. 3A**) or BP (**Fig. 3B**), CS demonstrated enhanced capacity to select groups with high pCR rate.

CONCLUSIONS

- In support of previous studies[10], high CS was associated with more aggressive clinical features, such as high histopathologic grade and lack of ER and PR expression.
- Tumor ELNs were only found in three specimens, likely due to the small tissue sample available from core needle biopsies; however, all of the tumor specimens with ELNs had high CS.
- The current study demonstrated a significantly higher CS in MP High Risk tumors and a relationship with MP index. 74% of high CS tumors were MP Ultra High Risk, suggesting an immunogenic phenotype within this group.
- Higher CS in BP Basal, HER2, and Luminal B tumors, suggests greater prevalence of ELNs, compared with Luminal A tumors. CS may be particularly relevant in HER2 type tumors, which supports previous studies indicating a favorable outcome associated with presence of ELNs in HER2+ breast cancer[5].
- Higher mean CS was associated with pCR following neoadjuvant therapy; however, MP and BP were more predictive of pCR. These data suggest that prediction of pCR using CS may be improved by combining with MP and/or BP.

FUTURE DIRECTIONS

- The current study was limited by the small sample size and limited available tissue for ELN assessment; future studies will evaluate CS in a larger dataset of neoadjuvant breast cancer patients.
- Recent studies have demonstrated association of CS with response to durvalumab[7]; future studies may further explore the utility of this score in predicting responses to immunotherapies in breast cancer patients.

References

1. Ascierto et al. 2012 *BCRT*
2. Carragher et al. 2008 *Seminars in Immunol*
3. Coppola and Mule 2008 *JCO*
4. Timmer et al. 2007 *Arthritis and Rheum.*
5. Liu et al. 2017 *The Oncologist*
6. Johansson-Percival et al. 2017 *Nature Immunology*
7. Sridhar et al. 2020 AACR
8. Coppola et al. 2011 *Am J of Pathology*
9. Messina et al. 2012 *Scientific Reports*
10. Prabhakaran et al. 2017 *Breast Cancer Res*
11. van 't Veer et al. 2018 EORTC-NCI-AACR Symposium

ASCO 2020 #591

For more information: Hatem.Soliman@moffitt.org