Integrating clinicopathological and molecular data in the breast cancer patient : towards precision medicine

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Chapter 3

Tumor immune subtypes distinguish tumor subclasses with clinical implications in breast cancer patients

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Breast Cancer Res Treat. 2013 Nov;142(2):355-64

ABSTRACT

Introduction

There is strong evidence that the host's cellular immune response is linked to tumor progression, however its impact on patient outcome in breast cancer is poorly understood. The purpose of this study is to define tumor immune subtypes, focusing on cellular immune responses and investigate their prognostic effect in breast cancer patients.

Methods

Our training (n=440) and validation cohort (n=382) consisted of all early breast cancer patients primarily treated with surgery in our center between 1985 and 1996. Tumor tissue sections were immunohistochemically stained for CD8 (CTL) and PEN5 (NK cells). Tumor expression of classical and non-classical HLA class I, and tumor-infiltrating Tregs were previously determined. Tumor immune subtypes were constructed based on quantification of these markers and biological rationale.

Results

High, intermediate and low immune susceptible tumor immune subtypes were found in respectively 16%, 63% and 20% of patients in the training cohort and 16%, 71% and 13% in the validation cohort. The subtypes showed to be statistically significant prognostic in multivariate analyses for relapse free period (RFP) ($p<0.0001$, intermediate versus high: hazard ratio (HR) 1.95; low versus high HR 2.98) and relative survival (RS) ($p=0.006$, intermediate versus high HR 3.84; low versus high: HR 4.26). Validation of these outcome analyses confirmed the independent prognostic associations: RFP ($p=0.025$) and RS ($p=0.040$).

Conclusion

The tumor immune subtypes that we present represent a prognostic profile with solid underlying biological rationale and with high discriminative power confirmed in an independent validation cohort. Our results emphasize the importance of tumor immune surveillance in the control of tumor development and, therefore, in determining patient prognosis. Tumor immune subtype profiling is promising for prognosis prediction and the achievement of tailored treatment for breast cancer patients.

INTRODUCTION

Breast cancer is the most commonly diagnosed female cancer and is the leading cause of death from cancer in women in the western world [1]. Decisions regarding use of systemic therapy in primary non-metastasized breast cancer patients are mainly based on prognostic and predictive factors like lymph node status, tumor size, grade, hormone receptor and human epidermal growth factor receptor 2 (HER2) expression [2]. However, currently these do not provide optimal risk-stratification. Therefore, additional prognostic and predictive information is sought in order to improve tailored treatment for patients with breast cancer.

There is strong evidence that a host's cellular immune response is able to control tumor progression [3]. However, due to their intrinsic genetic unstable nature, tumor cells may acquire properties to escape from such immune recognition [4]. Various interactions underlie the balance between immune control and tumor escape (Figure 1). Cytotoxic T-lymphocytes (CTL) are capable of recognizing tumor-associated antigens presented by classical human leukocyte antigen (HLA) class I (HLA-A, HLA-B, HLA-C) on the tumor cell surface. In order to avoid immune recognition from CTL, cancer cells may lose expression of classical HLA class I [5]. However, this makes them prone to natural killer (NK) cell recognition [6]. Non-classical HLA class I molecules (HLA-E, HLA-G) play a crucial role in immune surveillance by NK-cells. Expression of these molecules on the cell surface causes an inhibitory effect on NK-cell attack [6-8]. Another tumor escape mechanism from immunosurveillance is attraction and induction of immunosuppressive regulatory T cells (Treg) in the tumor microenvironment [9].

A variety of immune reactions have been found to date in breast cancer. Studies have indicated that breast cancer is highly immunogenic and often shows high numbers of tumor-infiltrating lymphocytes [10;11]. However, as previously reported by our group and others, loss of classical HLA class I expression, upregulation of non-classical HLA-E and HLA-G expression [12-14] and induction and infiltration of Treg in the tumor microenvironment [13;15-17] are frequent events in breast cancer, indicating that breast tumors are also capable of evading immune recognition. Together, this suggests that complex interactions take place between breast tumor cells and cells from the immune system [18]. Therefore, to get a good perspective on the effects of the immune system on tumor progression and patient outcome, such interactions should be accounted for. Indeed, previous studies of our group and others showed interactions between classical HLA class I and Treg, where loss of HLA class I in combination with presence of Treg in the tumor microenvironment resulted in a worse patient's outcome [16;18]. This was also the case for classical HLA class I and HLA-E and HLA-G tumor expression, where HLA-E and HLA-G expression resulted in a worse patient outcome exclusively in patients with loss

of tumor expression of classical HLA class I [12]. Together, this emphasizes the importance of research on combinations of markers of immune surveillance together with markers of tumor immune escape.

We defined tumor immune subtypes, with focus on cellular immune responses, based on tumor expression of classical HLA class I, HLA-E and HLA-G, and tumor infiltration of CTL, NK cells, and Treg. The aim was to investigate the distribution and prognostic effect of the different immune subtypes in a large cohort of breast cancer patients and subsequently validate these effects on a second cohort of breast cancer patients.

PATIENTS AND METHODS

Patients and tumors

The total patient population comprised all retrospectively assessed primary non-metastasized breast cancer patients primarily treated with surgery in the Leiden University Medical Center between 1985 and 1996 (n=822). Patients with bilateral tumors or a prior history of cancer (other than basal cell carcinoma or cervical carcinoma *in situ*) were excluded. The following data were known: age, tumor grade, histological type, TNM stage, local and systemic therapy, time of locoregional/distant tumor recurrence, survival time, and expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) [19]. All tumors were graded according to current pathological standards by an experienced breast cancer pathologist. Approval for the study was obtained from the Leiden University Medical Center Medical Ethics Committee. All samples were handled in a coded fashion, according to national ethical guidelines ("Code for Proper Secondary Use of Human Tissue", Dutch Federation of Medical Scientific Societies). The REMARK criteria were respected for analyses of the immune subtypes and writing of this article [32]. No statistically significant differences were found in patient or tumor characteristics between the training cohort (1985-1990 (n=440)) and a validation set (1990-1996 (n=382)).

Immunohistochemistry

Mouse antibody against CD8 (ab17147 clone 144B: AbCam, UK) and PEN5 (IM2354, clone 5H10.21.5: Beckman Coulter, NL) were used for immunohistochemical staining of respectively CTL and NK cells in tissue sections cut from intra-operatively derived FFPE tumor material according to previously described standard protocols [16]. Previously described were immunohistochemical stainings for expression of classical HLA class (anti-HLA-A and anti-HLAB/C; Dr. J. Neefjes, Netherlands Cancer Institute, Amsterdam, the Netherlands, HLA-E (ab2216 clone MEM-E/02: AbCam, UK), HLA-G (kindly provided

by Prof. Dr. P.J. Van de Elsen) and Treg infiltration (FoxP3, ab20034 clone 236A/E7: AbCam, UK) [12;16].

Evaluation of immunostaining

Quantification of CD8-positive stained cells and PEN5-positive stained cells in microscopical fields containing tumor was performed by two independent observers in a blinded manner in both training and validation cohorts. CD8 tumor infiltration was classified in two groups: (1) low CTL infiltration, 0-100 CD8 tumor infiltrating cells/mm^2; (2) high CTL infiltration, 100-3000 CD8 infiltrating cells/mm^2. For PEN5, only few positive infiltrating cells were seen. Therefore, any versus none PEN5-positive infiltrating cell were considered as presence and absence of NK cell infiltration respectively. Expression of classical HLA class I, HLA-E and HLA-G and Treg infiltration were previously categorized respectively as loss versus expression, no expression versus expression and absent versus present infiltration [12;16].

Statistical analysis

Statistical analyses were performed using the statistical packages SPSS (version 16.0 for Windows, Spps Inc, Chicago, IL, USA) and Stata (version 10.0 for Windows, StataCorp, College Station, TX, USA). Cohen's kappa coefficient represented the inter-observer agreement. The χ^2 test evaluated associations between clinicopathological parameters and tumor immune subtypes. Relapse-free period was defined as the time from date of surgery until any recurrence and was reported as cumulative incidence function, after accounting for death as competing risk. The Kaplan–Meier method was used for survival plotting and log-rank test for comparison of curves. Cox proportional hazard analysis calculated univariate and multivariable analysis for relapse-free period. Relative survival was calculated by the Hakulinen method as the ratio of the survival observed among the cancer patients and the survival that would have been expected based on the corresponding (age, sex, and year) general population. National life tables were used to estimate expected survival. Relative excess risks of death were estimated using a multivariable generalized linear model with a Poisson distribution, based on collapsed relative survival data, using exact survival times. Hazard ratios and relative risks served as indications for respectively risk of relapse and relative risk of survival. Variables with a P-value of < 0.10 in univariate analysis were entered in multivariable analysis.

RESULTS

Patient and tumor characteristics

Tumor material was available of 86% (380/440) and 87% (334/382) of the patients in the training cohort and validation cohort respectively. For the training cohort the median age of patients was 58 years (range= 23-96 years) and the median follow-up was 19 years (range= 0.1-22). For the validation cohort the median age and follow-up of patients were respectively 58 years (range= 32-90) and 13 years (range= 0.2-17). Clinicopathological and treatment characteristics are shown in supplementary tables (training cohort table 1A, 1B; validation cohort table 2A, 2B).

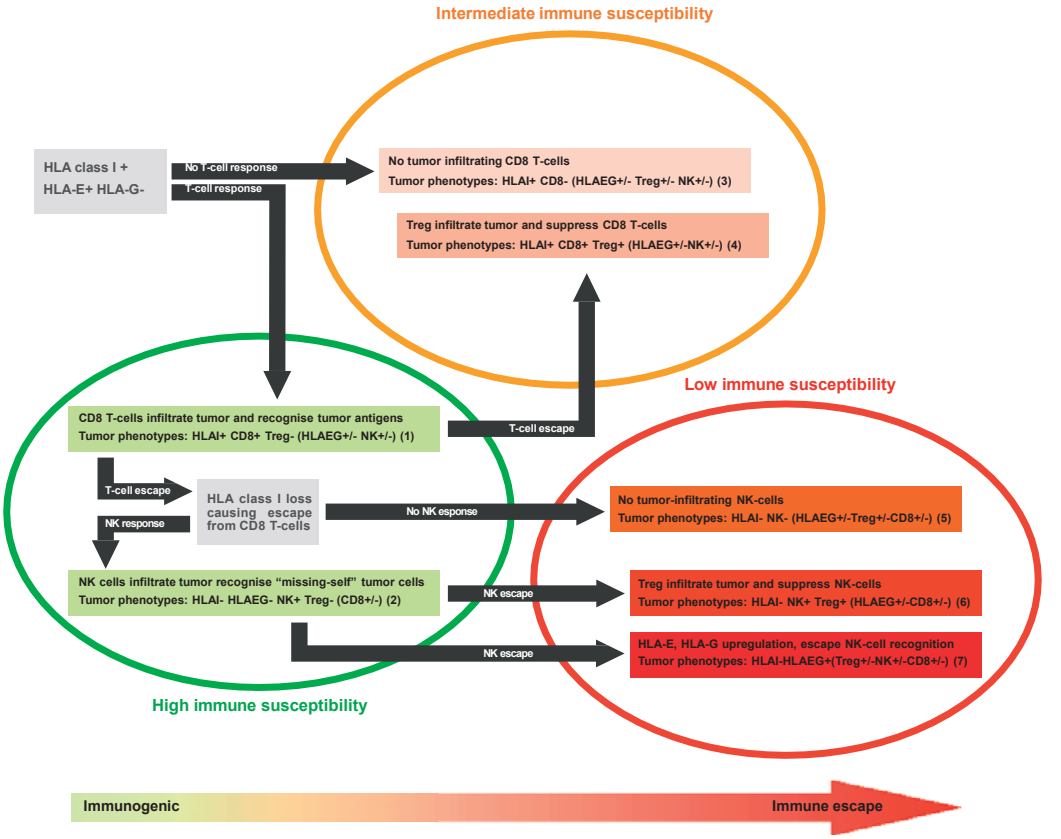

Figure 1: Tumor immune subtypes showing a schematic overview of different stages of immune surveillance and tumor immune escape classified into 7 tumor immune subtypes, graded from (1) to (7) in ascending order from highly immunogenic and therefore high immune susceptibility (green) to high immune escape and low immune susceptibility (red), concerning combinations of CTL infiltration, NK cell infiltration, Treg infiltration, classical HLA class I tumor expression and HLA-EG tumor expression. Tumor immune subtypes were clustered by combining from the original tumor immune subtypes groups as shown in by encircled groups (high immune susceptible) clustered (1) and (2)(green circle), (intermediate immune susceptible) clustered (3) and (4)(orange circle), (low immune susceptible) clustered (5), (6) and (7) (red circle).

Table 1: Cox univariate and multivariate analysis in the training cohort of breast cancer patients for recurrence free period and relative survival for tumor immune subtypes classified into 3 groups that are described in the results section.

Characteristic	N	Relapse Free Period						Relative Survival					
		Univariate analysis			Multivariable analysis			Univariate analysis			Multivariable analysis		
		HR	95% CI	*P*	HR	95% CI	*P*	RER	95% CI	*P*	RER	95% CI	*P*
Age													
<40	74	1.00		0.354				1.00		**0.048**	1.00		**0.031**
40-50	92	0.87	0.58-1.33					0.79	0.49-1.28		0.60	0.32-1.12	
50-60	81	1.24	0.82-1.88					1.51	0.96-2.38		1.49	0.83-2.65	
>60	133	0.95	0.64-1.42					1.20	0.71-2.03		1.05	0.54-2.05	
Grade													
I	53	1.00		**0.030**	1.00		0.293	1.00		**0.005**	1.00		**0.023**
II	186	1.38	0.86-2.22		1.30	0.73-2.31		1.74	0.82-3.68		0.62	0.30-1.30	
III	136	1.83	1.13-2.96		1.55	1.55-0.86		2.73	1.29-5.75		1.20	0.60-2.41	
Histological type													
Ductal	345	1.00		0.405				1.00		0.333			
Other	31	1.23	0.76-2.00					1.34	0.74-2.40				
Tumor stage													
pT1	127	1.00		**0.001**	1.00		**0.045**	1.00		**<0.001**	1.00		**0.003**
pT2	198	1.34	0.97-1.86		1.03	0.70-1.51		1.84	1.18-2.86		1.90	1.10-3.29	
pT3/4	45	2.56	1.51-3.69		1.75	1.06-2.88		3.69	2.18-6.24		3.40	1.68-6.89	
Nodal stage													
Negative	199	1.00		**<0.001**	1.00		**<0.001**	1.00		**<0.001**	1.00		**<0.001**
Positive	171	3.09	2.30-4.16		2.78	1.97-3.92		2.97	2.04-4.33		2.30	1.48-3.56	
ER status													
Negative	133	1.00		0.890				1.00		0.157			

Table 1: (continued)

Characteristic	N	Relapse Free Period						Relative Survival					
		Univariate analysis			Multivariable analysis			Univariate analysis			Multivariable analysis		
		HR	95% CI	*P*	HR	95% CI	*P*	RER	95% CI	*P*	RER	95% CI	*P*
Positive	229	1.02	0.76-1.38					0.77	0.54-1.10				
PGR status													
Negative	155	1.00		0.765				1.00		0.248			
Positive	201	1.05	0.78-1.41					0.81	0.56-1.16				
HER-2 status													
Negative	271	1.00		0.166				1.00		**0.004**	1.00		0.154
Positive	32	1.42	0.87-2.32					2.03	1.25-3.30		1.59	0.84-3.00	
Immune phenotype													
High immune susceptibility	48	1.00		**0.005**	1.00		**<0.001**	1.00		0.098	1.00		**0.006**
Intermediate immune susceptibility	186	1.80	1.06-3.05		1.95	1.13-3.39		1.95	0.98-3.98		3.84	1.62-9.09	
Low immune susceptibility	59	2.56	1.44-4.57		2.98	1.62-5.48		2.02	0.97-4.53		4.26	1.70-10.70	

Abbreviations: N: number of patients; HR: hazard ratio; 95%CI: 95% Confidence Interval; ER: estrogen receptor; PR: progesterone receptor; HER2: human epidermal growth factor receptor 2; ET: endocrine therapy; CT: chemotherapy

Table 2: Cox univariate and multivariate analysis in the validation cohort of breast cancer patients for recurrence free period and relative survival for tumor immune subtypes classified into 3 groups that are described in the results section.

Characteristic	N	Relapse Free Period						Relative Survival					
		Univariate analysis			Multivariable analysis			Univariate analysis			Multivariable analysis		
		HR	95% CI	*P*	HR	95% CI	*P*	RER	95% CI	*P*	RER	95% CI	*P*
Age													
<40	63	1.00		0.147				1.00		0.431			
40-50	83	0.62	0.38-1.03					0.58	0.30-1.10				
50-60	76	0.57	0.33-0.97					0.80	0.42-1.53				
>60	112	0.68	0.42-1.10					0.77	0.35-1.69				
Grade													
I	63	1.00		**0.001**	1.00		0.433	1.00		**0.026**	1.00		0.603
II	156	1.45	0.82-2.59		1.68	0.68-4.16		1.83	0.64-5.28		1.99	0.50-7.99	
III	108	2.54	1.43-4.52		1.86	0.72-4.79		3.27	1.16-9.21		1.69	0.40-7.14	
Histological type													
Ductal	293	1.00		0.298				1.00		0.300			
Other	35	1.35	0.77-2.35					1.46	0.71-3.01				
Tumor stage													
pT1	162	1.00		**<0.001**	1.00		0.171	1.00		**0.002**	1.00		0.227
pT2	130	2.18	1.46-3.23		1.78	0.98-3.26		2.57	1.34-4.90		1.96	0.85-4.52	
pT3/4	32	2.46	1.34-4.51		1.54	0.63-3.77		4.30	1.86-9.96		2.30	0.78-6.79	
Nodal stage													
Negative	182	1.00		**<0.001**	1.00		**0.01**	1.00		**<0.001**	1.00		0.208
Positive	142	2.81	1.93-4.08		2.06	1.19-3.57		3.09	1.73-5.13		1.59	0.77-3.25	

Table 2: (continued)

Characteristic	N	Relapse Free Period						Relative Survival					
		Univariate analysis			Multivariable analysis			Univariate analysis			Multivariable analysis		
		HR	95% CI	*P*	HR	95% CI	*P*	RER	95% CI	*P*	RER	95% CI	*P*
ER status													
Negative	155	1.00		**0.034**	1.00		0.889	1.00		**0.008**	1.00		0.488
Positive	164	0.67	0.46-0.97		1.04	0.60-1.82		0.44	0.24-0.81		0.78	0.39-1.57	
PGR status													
Negative	161	1.00		**0.006**	1.00		0.184	1.00		**0.028**	1.00		0.232
Positive	150	0.59	0.40-0.86		0.68	0.38-1.20		0.54	0.31-0.93		0.65	0.31-1.38	
HER-2 status													
Negative	249	1.00		**0.002**	1.00		0.934	1.00		**<0.001**	1.00		0.232
Positive	27	2.36	1.36-4.09		0.97	0.42-2.22		3.52	1.91-6.49		1.71	0.71-4.10	
Immune phenotype													
High immune susceptibility	34	1.00		**0.005**	1.00		**0.025**	1.00		0.089	1.00		**0.040**
Intermediate immune susceptibility	156	2.66	1.15-6.16		2.45	0.87-6.89		5.31	0.64-31.33		5.47	0.72-41.70	
Low immune susceptibility	29	4.72	1.83-12.18		4.73	1.48-15.06		11.12	1.12-55.41		10.95	1.31-91.63	

Abbreviations: N: number of patients; HR: hazard ratio; 95%CI: 95% Confidence Interval; ER: estrogen receptor; PR: progesterone receptor; HER2: human epidermal growth factor receptor 2; ET: endocrine therapy; CT: chemotherapy

Tumor immune subtypes

The Cohen's kappa coefficient for inter-observer agreement of CTL and PEN5 quantification all reached a coefficient of 0.82 or higher. Missing immunohistochemical data was due to tissue damage.

Tumor immune subtypes, representing tumor adaptive immune escape variants were constructed from available data (Figure1). The defined tumor immune subtypes were in ascending order from high immune susceptibility to low immune susceptibility: (1) CTL are able to recognize tumor-associated antigens (TAA) presented by classical HLA class I and anti-tumor immune reaction can take place: Tumors with expression of classical HLA class I, high infiltration of CTL and absence of infiltration of Treg; (2) Tumors with a lack of classical HLA class I expression can escape CTL recognition, but NK cells are able to recognize these cells and anti-tumor immune reaction can take place: Tumors with loss of expression of classical HLA class I, no expression of HLA-EG, present infiltration of NK cells and absent infiltration of Treg; (3) Classical HLA class I present TAA and could be recognized by CTL, but a low infiltration of CTL results in a limited anti-tumor immune reaction: Tumors with expression of classical HLA class I but low CTL infiltration; (4) Classical HLA class I present TAA and could be recognized by CTL, but immunosuppressive Treg weaken CTL function, resulting in a limited anti-tumor immune reaction: Tumors with expression of classical HLA class I, high infiltration of CTL, but also present infiltration of Treg; (5) Tumors with lack of classical HLA class I escape CTL recognition, but could be recognized by NK cells, which however are not present, resulting in failure of anti-tumor immune reaction: Tumors with loss of expression of classical HLA class I and absent NK cell infiltration; (6) Tumors with lack of classical HLA class I expression escape CTL recognition, but could be recognized by NK cells, however immunosuppressive Treg weaken NK cell function [19], resulting in failure of anti-tumor immune reaction: Tumors with loss of expression of classical HLA class I, present NK cell infiltration, but also present Treg infiltration; (7) Tumor with lack of classical HLA class I expression but expression of non-classical HLA-EG escape from both CTL recognition and NK cell recognition: Tumor with loss of expression of classical HLA class I and expression of HLA-EG.

A more simplified tumor immune subtype variable was constructed by joining together tumor immune subtypes: High (subtypes 1-2), intermediate (subtypes 3-4) and low (subtypes 5-7) immune susceptibility (Figure1, clustered groups shown by circles).

Associations between clinicopathological patient and tumor characteristics and tumor immune subtypes classified into 7 groups and into 3 groups are shown in supplementary tables 1A, B and 2A, B. No statistically significant validated association was found between patient and tumor characteristics and tumor immune subtypes classified into 7 groups or into 3 groups.

Tumor immune subtypes classified into 7 groups

Distribution in patient training and validation cohort

The tumor immune subtypes classified into 7 groups could be determined for patients with data available for all immune markers: 77% (293/380) of patients in the training cohort; 66% validation cohort. Distributions of immune subtypes and associations with known clinicopathological parameters are shown in supplementary tables (training cohort Table 1A; validation cohort Table 2A).

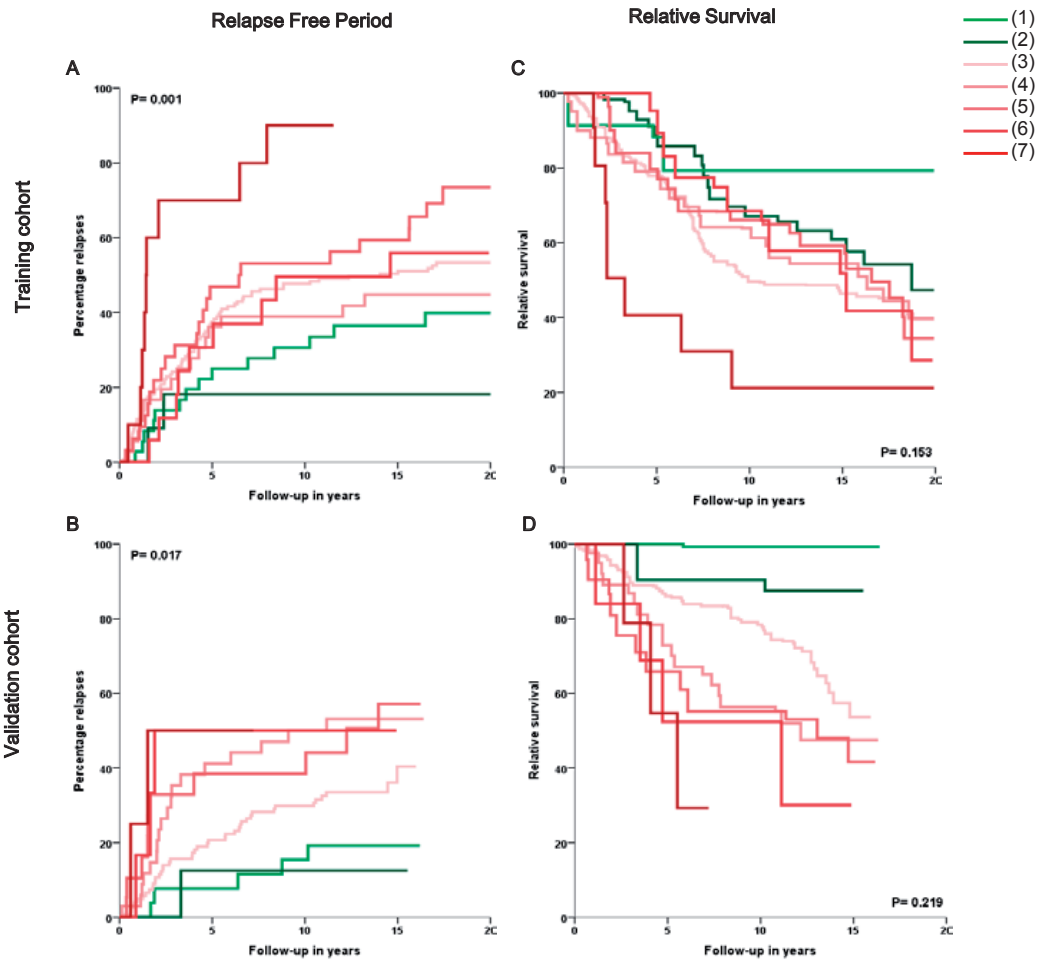

Figure 2: Outcome analyses by tumor immune subtypes for Relapse free period (RFP) (A, B) and relative survival (RS) (C, D) according to the 7 tumor immune subtypes that are described in the Results section for training cohort patients (A, C), and for validation cohort patients (B, D). Tumor immune subtypes representative for more tumor immune escape resulted in an unfavourable patient outcome concerning RFP and RS compared to more immunogenic tumor immune subtypes. Log-rank P-values are shown in each graph.

Prognostic associations with patient outcome

The association of tumor immune subtypes classified into 7 groups in the training cohort with relapse-free period and relative survival are shown in Figure 2. Analysis of relapse-free period showed a statistically significant association between the 7 tumor immune subtypes and clinical outcome of patient (RFP p=0.001, Figure 2 A). Tumors that were expected to show lower immune susceptibility resulted in more patient relapses over time compared to tumors that were expected to show higher immune susceptibility. A similar though not significant trend was seen for the association between the 7 immune subtypes and relative survival outcome of patients (RS p=0.153, Figure 2 C). Results for outcome analyses were confirmed in the validation cohort (RFP p=0.017, Figure 2B and RS p=0.219, Figure 2D). Multivariable analyses demonstrated that these 7 tumor immune subtypes were a statistically significant independent prognostic factor in breast cancer patients for both RFP and RS (supplementary Table 3). Though statistical significance was lost in multivariable analyses in the validation cohort, a statistical trend remained for the association between 7 tumor immune subtypes and patient outcome concerning RFP (p=0.055, supplementary Table 4).

Tumor immune subtypes classified into 3 groups

Distribution in patient training and validation cohort

The tumor immune subtypes, consisting of three groups as described above showed the following distribution in the training and validation cohort respectively: High immune susceptible, 16% (48/293) and 16% (34/219); Intermediate immune susceptible, 63% (186/293) and 71% (156/219); Low immune susceptible, 20% (59/293) and 13% (29/219). Associations with known clinicopathological parameters are shown in supplementary tables (training cohort Table 1B; validation cohort Table 2B).

Prognostic associations with patient outcome

The association of the tumor immune subtypes classified into 3 groups with relapse-free period and relative survival is shown in Figure 3. Analysis of relapse-free period showed a significant association between tumor immune subtype and clinical outcome of patients (RFP p=0.004, Figure 3A). Lower immune susceptible tumor subtypes, resulted in more relapses over time compared to higher immune susceptible tumor subtypes. Again, though not significant a similar associative trend was seen for relative survival outcome of patient and tumor immune subtype (RS p=0.146, Figure 3C). Results of outcome analyses in the validation cohort were similar to the results found in the training cohort (RFP p=0.003, Figure 3B and RS p=0.112, Figure 3D).

Multivariable analyses demonstrated that the tumor immune subtypes were a statistically significant independent prognostic factor in breast cancer patients for both RFP

(p<0.001, Table 1B) and RS (p=0.006, Table 1B) with high discriminative power; compared to patients with high immune susceptible tumors, patients with intermediate immune susceptible tumors showed an almost twice elevated risk (HR 1.95, 95%CI 1.13-3.39) for

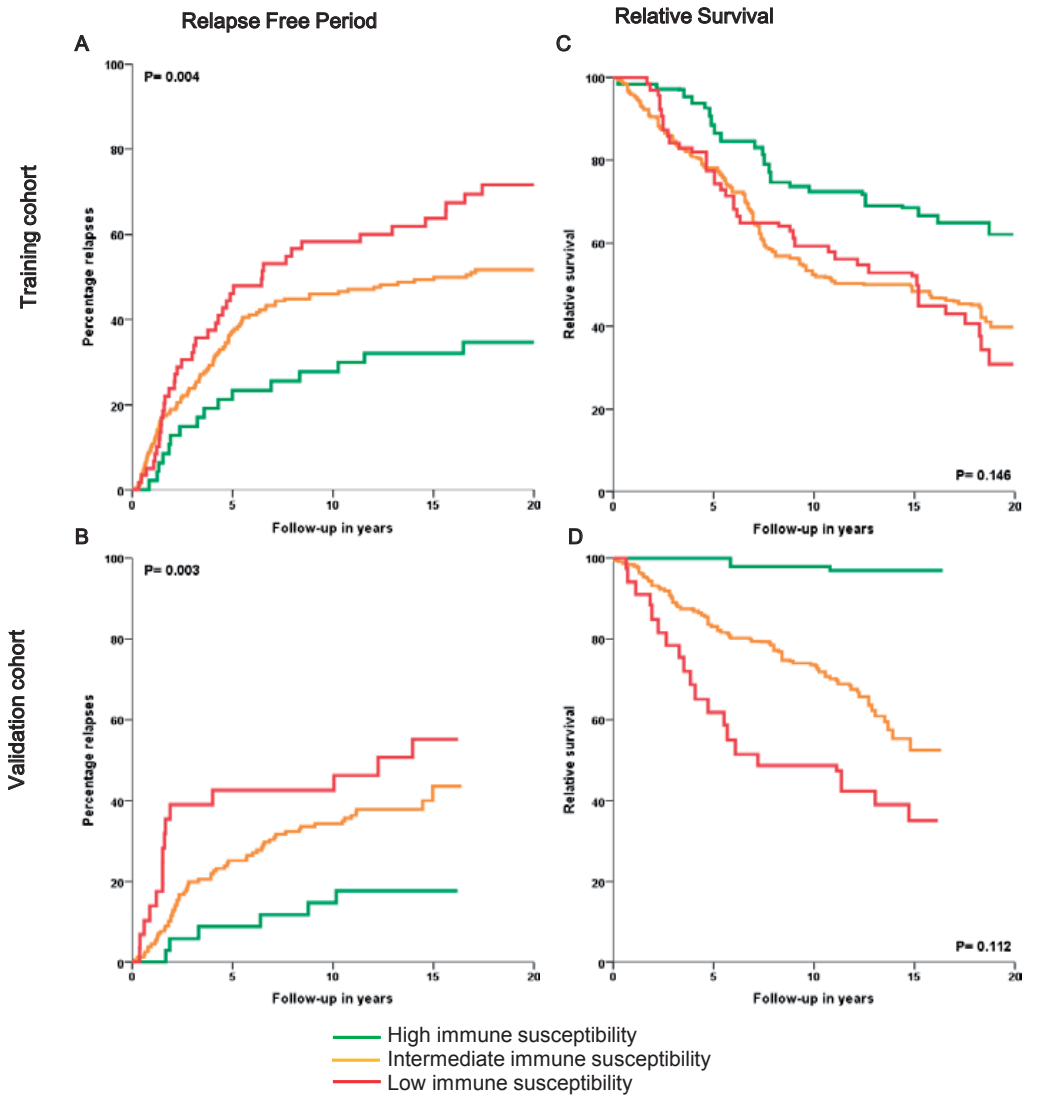

Figure 3: Outcome analyses by tumor immune subtypes for Relapse free period (RFP) (A, B) and relative survival (RS) (C, D) according to the 3 tumor immune subtypes that are described in the Results section for training cohort patients (A, C), and for validation cohort patients (B, D). Tumor immune subtypes representative for more tumor immune escape resulted in an unfavourable patient outcome concerning RFP and RS compared to more immunogenic tumor immune subtypes. Log-rank P-values are shown in each graph.

developing relapses over time and an almost four times higher relative risk for survival (RR 3.84, 95% CI 1.62-9.09), while patients with low immune susceptible tumors showed an almost three times elevated risk on relapses over time (HR 2.98, 95%CI 1.62-5.48) and

a more than four times higher relative risk for survival (RR 4.26, 95%CI 1.70-10.70) (Table 1B). Results of the validation cohort confirmed the associations found in multivariable analyses (RFP p=0.025, Table 2B and RS p=0.040, Table 2B)

DISCUSSION

The impact of the immune response and subsequent tumor immune evasion on tumor progression and patient outcome in breast cancer is poorly understood. Most studies focus on the effect of single parameters, like tumor expression of HLA class I or immune cell tumor infiltration, but separately these do not reflect the multifaceted interaction between immune cells and tumor cells. In order to get a good perspective on the processes involved in these interactions, we defined tumor immune subtypes. These subtypes were defined based on tumor susceptibility for cellular immune responses using expression of key factors in these responses that reflect local presence of CTL, NK cells, and Treg and tumor expression of classical HLA class I and HLA-E and -G. Outcome analyses of the immune subtypes revealed strong associations with patient outcome where tumors defined as being highly susceptible to immune system attack showed a favorable outcome for breast cancer patients compared to patients with tumors defined having a low immune susceptible profile. These prognostic effects were shown in this study to be independent of known clinicopathological prognostic parameters and were additionally validated in an independent breast cancer patient cohort confirming the high discriminative power on patient outcome stratification.

Prior studies by our group and others have focused on a cellular immune response and its effect on tumor progression and patient outcome in breast cancer [11-16]. DeNardo *et al.* even provides evidence that treatment response is in part regulated by the immune microenvironment [20], again urging the importance of comprehensive determination of the tumor immune status. High tumor infiltration of CD8+ lymphocytes, representative for CTL infiltration, has been found to result in a favorable patient prognosis in one study [11]. However, another study reported high CTL infiltration to be associated with a worse patient outcome [21]. Yet another study could not find a statistically significant prognostic effect for CTL [10]. High Treg infiltration resulted in an unfavorable prognostic factor in a variety of studies [10;15;22], while it did not show a statistically significant association with patient outcome in a previous study of our group [16]. Loss of expression of classical HLA class I showed to be a favorable [23] as well as an unfavorable [16] prognostic factor in two different studies and revealed no statistically significant associations with patient outcome in two other studies [24;25]. Concerning non-classical HLA-E and HLA-G, one study could not find a statistically significant relation with patient prognosis for HLA-G [13;25]

while a study of our group showed tumor expression of HLA-E and HLA-G resulted to be a statistically significant unfavorable prognostic parameter [12]. To our knowledge, the prognostic impact of NK cell infiltration has not been studied in breast cancer, but NK cell presence in the tumor microenvironment has been shown to result in a favorable patient outcome in colorectal cancer [26].

Taken together, these reports show contradictory results and, therefore, do not draw a clear picture of the interaction between breast cancer cells and the immune system. Our present study shows that this may be explained by the simple fact that a successful anti-tumor immune response depends not only on the level of expression of a single marker such as classical HLA class I, but on the variety of factors involved in the multifaceted immune response. Due to the complexity of the balance between immune surveillance and tumor immune escape, it is not a single marker that is able to reflect outcome of the interaction, but a set of key markers. In this study we analyzed a set of such crucial immune markers and defined tumor immune subtypes based on these markers. We demonstrated that a profile that represents tumors that may be more immune susceptible is predictive for a more favorable clinical outcome for patients with breast cancer. In addition, the prognostic impact with high discriminative power that we found for these tumor immune subtypes, suggests that previous single marker studies are understating or even confounding the impact of the immune system on tumor control. The results found for the tumor immune subtypes are not only concordant with prior evidence on tumor immune biology in breast cancer [4;18], but additionally join together the conclusions of prior studies by linking single tumor-immune markers to functional tumor-immune interaction. This is the first study providing detailed insight in tumor immune biology in breast cancer, showing that tumor immune surveillance is of crucial importance in the control of tumor progression and therefore in determining patient prognosis.

Many prognostic factors have been identified for breast cancer. Of these, the ASCO guidelines advised the use in clinical practice of urokinases plasminogen activator (uPA), plasminogen activator inhibitor-1 (PAI-1) and gene profiles detected with multiparameter gene expression assays [27]. The clinical value of microarray-based prognostic tools, like the MammaPrint, a 70-gene expression profile, and Oncotype DX, a 21-gene expression profile is currently being debated [28;29]. One major critique is that these gene prints were constructed using top-down analyses and were not defined based on a biological rationale. Therefore, it is unclear what tumor types are represented by the various patient risk-groups [30]. Contrary to these top-down analyses, the tumor immune subtypes we defined are based on well-founded biological hypotheses. Future research will further improve this function-based approach of prognostic profiling in breast cancer.

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SUPPLEMENTARY TABLES

Supplementary Table 1: Correlations between tumor immune subtypes into 7 groups that are described in the results section in the training cohort of patients (**A**) and well-established prognostic factors using chi-square test (**B**).

A	N	%	(1)		(2)		(3)		(4)		(5)		(6)		(7)		p-value
			N	%	N	%	N	%	N	%	N	%	N	%	N	%	
Age																	
<40	74	19.5	11	30.6	1	8.3	21	14.1	10	27.0	8	25.0	3	17.6	4	40.0	**0.047**
40-50	92	24.2	7	19.4	4	33.3	44	29.5	6	16.2	3	9.4	5	29.4	1	10.0	
50-60	81	21.3	6	16.7	2	16.7	29	19.5	6	16.2	13	40.6	2	11.8	4	40.0	
>=60	133	35.0	12	33.3	5	41.7	55	36.9	15	40.5	8	25.0	7	41.2	1	10.0	
Grade																	
I	53	14.1	4	11.1	4	36.4	17	11.4	1	2.7	6	18.8	4	23.5	3	30.0	**0.033**
II	186	49.6	16	44.4	5	45.5	78	52.3	19	51.4	17	53.1	10	58.8	1	10.0	
III	136	36.3	16	44.4	2	18.2	54	36.2	17	45.9	9	28.1	3	17.6	6	60.0	
Histological type																	
Ductal	345	91.8	31	86.1	10	90.9	141	94.6	33	89.2	29	90.6	16	94.1	10	100.0	0.578
Lobular	31	8.2	5	13.9	1	9.1	8	5.4	4	10.8	3	9.4	1	5.9	0	0.0	
T-status																	
T1	127	34.3	6	16.7	6	50.0	52	35.4	10	27.8	13	40.6	7	43.8	2	20.0	0.305
T2	198	53.5	25	69.4	5	41.7	70	47.6	22	61.1	14	43.8	9	56.2	6	60.0	
T3/4	45	12.2	5	13.9	1	8.3	25	17.0	4	11.1	5	15.6	0	0.0	2	20.0	
N-status																	
N0	199	53.8	20	57.1	6	50.0	83	57.2	16	44.4	16	51.6	10	58.8	2	20.0	0.321
N1-3	171	46.2	15	42.9	6	50.0	62	42.8	20	55.6	15	48.4	7	41.2	8	80.0	
ER-status																	

Supplementary Table 1: (continued)

A	N	%	(1)		(2)		(3)		(4)		(5)		(6)		(7)		p-value
			N	%	N	%	N	%	N	%	N	%	N	%	N	%	
Negative	133	36.7	18	50.0	5	41.7	58	39.2	14	37.8	5	16.1	4	23.5	6	60.0	0.057
Positive	229	63.3	18	50.0	7	58.3	90	60.8	23	62.2	26	83.9	13	76.5	4	40.0	
PGR-status																	
Negative	155	43.5	19	52.8	5	41.7	61	41.2	18	48.6	6	20.7	9	52.9	6	60.0	0.131
Positive	201	56.5	17	47.2	7	58.3	87	58.8	19	51.4	23	79.3	8	47.1	4	40.0	
HER-2-status																	
Overexpression -	271	89.4	26	86.7	11	100.0.0	105	85.4	28	93.3	25	96.2	16	100.0	8	100.0	0.206
Overexpression +	32	10.6	4	13.3	0		18	14.6	2	6.7	1	3.8	0	0.0	0	0.0	
Local Therapy																	
MAST-RT	132	34.7	11	30.6	6	50.0	55	36.9	15	40.5	11	34.4	7	41.2	1	10.0	0.714
MAST+RT	80	21.1	10	27.8	1	8.3	31	20.8	5	13.5	6	18.8	2	11.8	4	40.0	
BCS	168	44.2	15	41.7	5	41.7	63	42.3	17	45.9	15	46.9	8	47.1	5	50.0	
Systemic therapy																	
CT alone	78	20.5	11	30.6	1	8.3	35	23.5	9	24.3	2	6.2	2	11.8	4	40.0	0.273
HT alone	27	7.1	3	8.3	0	0.0	11	7.4	1	2.7	3	9.4	1	5.9	0	0.0	
CT&HT	4	1.1	0	0.0	0	0.0	1	0.7	1	2.7	2	6.2	0	0.0	0	0.0	
None	271	71.3	22	61.1	11	91.7	102	68.5	26	70.3	25	78.1	14	82.4	6	60.0	
Total	**380**	**100**	**36**	**100**	**12**	**100**	**149**	**100**	**37**	**100**	**32**	**100**	**17**	**100**	**10**	**100**	

B	N	%	High immune susceptibility		Intermediate immune susceptibility		Low immune susceptibility		p-value
			N	%	N	%	N	%	
Age									
<40	74	19.5	12	25.0	31	16.7	15	25.4	0.094
40-50	92	24.2	11	22.9	50	26.9	9	15.3	
50-60	81	21.3	8	16.7	35	18.8	19	32.2	
>=60	133	35.0	17	35.4	70	37.6	16	27.1	
Grade									
I	53	14.1	8	17.0	18	9.7	13	22.0	0.138
II	186	49.6	21	44.7	97	52.2	28	47.5	
III	136	36.3	18	38.3	71	38.2	18	30.5	
Histological type									
Ductal	345	91.8	41	87.2	174	93.5	55	93.2	0.332
Lobular	31	8.2	6	12.8	12	6.5	4	6.8	
T-status									
T1	127	34.3	12	25.0	62	33.9	22	37.9	0.534
T2	198	53.5	30	62.5	92	50.3	29	50.0	
T3/4	45	12.2	6	12.5	29	15.8	7	12.1	
N-status									
N0	199	53.8	26	55.3	99	54.7	28	48.3	0.669
N1-3	171	46.2	21	44.7	82	45.3	30	51.7	
ER-status									
Negative	133	36.7	23	47.9	72	38.9	15	25.9	0.058
Positive	229	63.3	25	52.1	113	61.1	43	74.1	
PGR-status									
Negative	155	43.5	24	50.0	79	42.7	21	37.5	0.437
Positive	201	56.5	24	50.0	106	57.3	35	62.5	
HER-2-status									
Overexpression -	271	89.4	37	90.2	133	86.9	49	98.0	0.081
Overexpression +	32	10.6	4	9.8	20	13.1	1	2.0	
Local Therapy									
MAST-RT	132	34.7	17	35.4	70	37.6	19	32.2	0.928
MAST+RT	80	21.1	11	22.9	36	19.4	12	20.3	
BCS	168	44.2	20	41.7	80	43.0	28	47.5	
Systemic therapy									
CT alone	78	20.5	12	25.0	44	23.7	8	13.6	0.508
HT alone	27	7.1	3	6.2	12	6.5	4	6.8	
CT&HT	4	1.1	0	0.0	2	1.1	2	3.4	
None	271	71.3	33	68.8	128	68.8	45	76.3	
Total	**380**	**100**	**48**	**100**	**186**	**100**	**59**	**100**	

Abbreviations: N: number of patients; %: percentage; ER: estrogen receptor; PR: progesterone receptor; HER2: human epidermal growth factor receptor 2; MAST: mastectomy; RT: radiotherapy; BCS: breast conservative surgery; ET: endocrine therapy; CT: chemotherapy

Supplementary Table 2: Correlations between tumor immune subtypes into 7 groups that are described in the results section in the validation cohort of patients (**A**) and well-established prognostic factors using chi-square test (**B**).

A	N	%	(1)		(2)		(3)		(4)		(5)		(6)		(7)		p-value
			N	%	N	%	N	%	N	%	N	%	N	%	N	%	
Age																	
<40	63	18.9	6	23.1	2	25.0	25	20.5	10	29.4	5	26.3	1	16.7	1	25.0	0.794
40-50	83	24.9	6	23.1	2	25.0	29	23.8	12	35.3	4	21.1	1	16.7	0	0.0	
50-60	76	22.8	5	19.2	3	37.5	25	20.5	5	14.7	4	21.1	3	50.0	2	50.0	
>=60	112	33.5	9	34.6	1	12.5	43	35.2	7	20.6	6	31.6	1	16.7	1	25.0	
Grade																	
I	63	19.3	6	24.0	2	25.0	25	20.7	2	6.1	2	11.1	1	16.7	1	33.3	0.420
II	156	47.7	8	32.0	4	50.0	62	51.2	14	42.4	9	50.0	2	33.3	1	33.3	
III	108	33.0	11	44.0	2	25.0	34	28.1	17	51.5	7	38.9	3	50.0	1	33.3	
Histological type																	
Ductal	293	89.3	22	88.0	8	100.0.0	111	91.7	29	87.9	13	72.2	4	66.7	3	100.0	0.109
Lobular	35	10.7	3	12.0	0		10	8.3	4	12.1	5	27.8	2	33.3	0	0.0	
T-status																	
T1	162	50.0	14	56.0	5	62.5	54	45.4	15	45.5	4	25.0	2	33.3	2	50.0	0.541
T2	130	40.1	10	40.0	2	25.0	52	43.7	5	45.5	8	50.0	2	33.3	2	50.0	
T3/4	32	9.1	1	4.0	1	12.5	13	10.9	3	9.1	4	25.0	2	33.3	0	0.0	
N-status																	
N0	182	56.2	17	68.0	5	62.5	61	51.3	18	54.5	9	50.0	3	50.0	1	33.3	0.779
N1-3	142	43.8	8	32.0	3	37.5	58	48.7	15	45.5	9	50.0	3	50.0	2	66.7	
ER-status																	
Negative	155	48.6	13	54.2	3	37.5	46	38.0	20	58.8	8	42.1	3	50.0	2	50.0	0.411
Positive	164	51.4	11	45.8	5	62.5	75	62.0	14	41.2	11	57.9	3	50.0	2	50.0	

Supplementary Table 2: (continued)

A	N	%	(1)		(2)		(3)		(4)		(5)		(6)		(7)		p-value
			N	%	N	%	N	%	N	%	N	%	N	%	N	%	
PGR-status																	
Negative	161	51.8	15	62.5	2	25.0	52	42.6	24	70.6	8	44.4	4	66.7	2	50.0	**0.046**
Positive	150	48.2	9	37.5	6	75.0	70	57.4	10	29.4	10	55.6	2	33.3	2	50.0	
HER-2-status																	
Overexpression -	249	90.2	15	83.3	6	100.0.0	99	92.5	28	90.3	15	93.8	4	66.7	4	100.0	0.316
Overexpression +	27	9.8	3	16.7	0		8	7.5	3	9.7	1	6.2	2	33.3	0	0.0	
Local Therapy																	
MAST-RT	153	45.8	13	50.0	4	50.0	55	45.1	14	41.2	9	47.4	3	50.0	3	75.0	0.807
MAST+RT	52	15.6	5	19.2	1	12.5	19	15.6	7	20.6	6	31.6	2	33.3	0	0.0	
BCS	129	38.6	8	30.8	3	37.5	48	39.3	13	38.2	4	21.1	1	16.7	1	25.0	
Systemic therapy																	
CT alone	49	14.7	2	7.7	1	12.5	18	14.8	6	17.6	7	36.8	1	16.7	1	25.0	0.594
HT alone	86	25.7	8	30.8	2	25.0	34	27.9	6	17.6	4	21.1	2	33.3	1	25.0	
CT&HT	23	6.9	0	0.0	1	12.5	11	9.0	5	14.7	0	0.0	0	0.0	0	0.0	
None	176	52.7	16	61.5	4	50.0	59	48.4	17	50.0	8	42.1	3	50.0	2	50.0	
Total	**334**	**100**	**26**	**100**	**8**	**100**	**122**	**100**	**34**	**100**	**19**	**100**	**6**	**100**	**4**	**100**	

B	N	%	High immune susceptibility		Intermediate immune susceptibility		Low immune susceptibility		p-value
			N	%	N	%	N	%	
Age									
<40	63	18.9	8	23.5	35	22.4	7	24.1	0.842
40-50	83	24.9	8	23.5	41	26.3	5	17.2	
50-60	76	22.8	8	23.5	30	19.2	9	31.0	
>=60	112	33.5	10	29.4	50	32.1	8	27.6	
Grade									
I	63	19.3	8	24.2	27	17.5	4	14.8	0.649
II	156	47.7	12	36.4	76	49.4	12	44.4	
III	108	33.0	13	39.4	51	33.1	11	40.7	
Histological type									
Ductal	293	89.3	30	90.9	140	90.9	20	74.1	**0.035**
Lobular	35	10.7	3	9.1	14	9.1	7	25.9	
T-status									
T1	162	50.0	19	57.6	69	45.4	8	30.8	0.148
T2	130	40.1	12	36.4	67	44.1	12	46.2	
T3/4	32	9.1	2	6.1	16	10.5	6	23.1	
N-status									
N0	182	56.2	22	66.7	79	52.0	13	48.1	0.253
N1-3	142	43.8	11	33.3	73	48.0	14	51.9	
ER-status									
Negative	155	48.6	16	50.0	66	42.6	13	44.8	0.740
Positive	164	51.4	16	50.0	89	57.4	16	55.2	
PGR-status									
Negative	161	51.8	17	53.1	76	48.7	14	50.0	0.901
Positive	150	48.2	15	46.9	80	51.3	14	50.0	
HER-2-status									
Overexpression -	249	90.2	21	87.5	127	92.0	23	88.5	0.691
Overexpression +	27	9.8	3	12.5	11	8.0	3	11.5	
Local Therapy									
MAST-RT	153	45.8	17	50.0	69	44.2	15	51.7	0.345
MAST+RT	52	15.6	6	17.6	26	16.7	8	27.6	
BCS	129	38.6	11	32.4	61	39.1	6	20.7	
Systemic therapy									
CT alone	49	14.7	3	8.8	24	15.4	9	31.0	0.104
HT alone	86	25.7	10	29.4	40	25.6	7	24.1	
CT&HT	23	6.9	1	2.9	16	10.3	0	0.0	
None	176	52.7	20	58.8	76	48.7	13	44.8	
Total	**334**	**100**	**34**	**100**	**156**	**100**	**29**	**100**	

Abbreviations: N: number of patients; %: percentage; ER: estrogen receptor; PR: progesterone receptor; HER2: human epidermal growth factor receptor 2; MAST: mastectomy; RT: radiotherapy; BCS: breast conservative surgery; ET: endocrine therapy; CT: chemotherapy

Supplementary Table 3: Cox univariate and multivariate analysis in the training cohort of breast cancer patients for recurrence free period and relative survival for tumor immune subtypes classified into 7 groups that are described in the results section.

Characteristic	Relapse Free Period							Relative Survival					
		Univariate analysis			Multivariable analysis			Univariate analysis			Multivariable analysis		
	N	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
Age													
<40	74	1.00		0.354				1.00		**0.048**	1.00		**0.006**
40-50	92	0.87	0.58-1.33					0.79	0.49-1.28		0.50	0.27-0.96	
50-60	81	1.24	0.82-1.88					1.51	0.96-2.38		1.52	0.84-2.72	
>60	133	0.95	0.64-1.42					1.20	0.71-2.03		1.00	0.49-2.04	
Grade													
I	53	1.00		**0.030**	1.00		0.384	1.00		**0.005**	1.00		**0.043**
II	186	1.38	0.86-2.22		1.35	0.76-2.41		1.74	0.82-3.68		0.59	0.28-1.24	
III	136	1.83	1.13-2.96		1.51	0.84-2.73		2.73	1.29-5.75		1.11	0.56-2.23	
Histological type													
Ductal	345	1.00		0.405				1.00		0.333			
Other	31	1.23	0.76-2.00					1.34	0.74-2.40				
Tumor stage							0.153						
pT1	127	1.00		**0.001**	1.00			1.00		**<0.001**	1.00		**0.002**
pT2	198	1.34	0.97-1.86		1.01	0.69-1.49		1.84	1.18-2.86		2.11	1.21-3.68	
pT3/4	45	2.56	1.51-3.69		1.57	0.94-2.61		3.69	2.18-6.24		3.62	1.77-7.41	
Nodal stage													
Negative	199	1.00		**<0.001**	1.00		**<0.001**	1.00		**<0.001**	1.00		**<0.001**
Positive	171	3.09	2.30-4.16		2.81	1.98-3.99		2.97	2.04-4.33		2.30	1.47-3.60	
ER status													
Negative	133	1.00		0.890				1.00		0.157			
Positive	229	1.02	0.76-1.38					0.77	0.54-1.10				

Supplementary Table 3: (continued)

Characteristic	Relapse Free Period							Relative Survival					
		Univariate analysis			Multivariable analysis			Univariate analysis			Multivariable analysis		
	N	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
PGR status													
Negative	155	1.00		0.765				1.00		0.248			
Positive	201	1.05	0.78-1.41					0.81	0.56-1.16				
HER2 status													
Negative	271	1.00		0.166				1.00		**0.004**	1.00		0.135
Positive	32	1.42	0.87-2.32					2.03	1.25-3.30		1.62	0.86-3.07	
Immune phenotype													
(1)	36	1.00		**0.002**	1.00		**0.010**	1.00		0.098	1.00		**0.002**
(2)	12	0.43	0.10-1.91		0.53	0.12-2.38		0.12	0.00-62.27		0.001	0-∞	
(3)	149	1.60	0.90-2.82		1.82	1.00-3.32		1.54	0.80-2.97		3.43	1.41-8.32	
(4)	37	1.34	0.65-2.75		1.40	0.67-2.94		1.26	0.54-2.92		2.40	0.86-6.67	
(5)	32	2.15	1.11-4.18		2.45	1.20-4.99		1.39	0.62-3.13		2.33	0.84-6.51	
(6)	17	1.48	0.64-3.41		2.18	0.91-5.22		1.03	0.33-3.21		4.26	1.28-14.15	
(7)	10	5.09	2.19-11.82		4.41	1.83-10.62		3.68	1.44-9.40		11.84	3.86-36.34	

Abbreviations: N: number of patients; HR: hazard ratio; 95%CI: 95% Confidence Interval; ER: estrogen receptor; PR: progesterone receptor; HER2: human epidermal growth factor receptor 2; ET: endocrine therapy; CT: chemotherapy

Supplementary Table 4: Cox univariate and multivariate analysis in the validation cohort of breast cancer patients for recurrence free period and relative survival for tumor immune subtypes classified into 7 groups that are described in the results section.

Characteristic	Relapse Free Period							Relative Survival					
		Univariate analysis			Multivariable analysis			Univariate analysis			Multivariable analysis		
	N	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Age													
<40	63	1.00		0.147				1.00		0.431			
40-50	83	0.62	0.38-1.03					0.58	0.30-1.10				
50-60	76	0.57	0.33-0.97					0.80	0.42-1.53				
>60	112	0.68	0.42-1.10					0.77	0.35-1.69				
Grade													
I	63	1.00		**0.001**	1.00		0.61	1.00		**0.026**			
II	156	1.45	0.82-2.59		1.55	0.62-3.89		1.83	0.64-5.28				
III	108	2.54	1.43-4.52		1.62	0.61-4.30		3.27	1.16-9.21				
Histological type													
Ductal	293	1.00		0.298				1.00		0.300			
Other	35	1.35	0.77-2.35					1.46	0.71-3.01				
Tumor stage													
pT1	162	1.00		**<0.001**	1.00		0.113	1.00		**0.002**			
pT2	130	2.18	1.46-3.23		1.93	1.04-3.56		2.57	1.34-4.90				
pT3/4	32	2.46	1.34-4.51		1.79	0.73-4.39		4.30	1.86-9.96				
Nodal stage													
Negative	182	1.00		**<0.001**	1.00		**0.014**	1.00		**<0.001**			
Positive	142	2.81	1.93-4.08		2.03	1.16-3.56		3.09	1.73-5.13				
ER status													
Negative	155	1.00		**0.034**	1.00		0.728	1.00		**0.008**			
Positive	164	0.67	0.46-0.97		1.11	0.62-1.97		0.44	0.24-0.81				

Supplementary Table 4: (continued)

Characteristic	Relapse Free Period							Relative Survival					
		Univariate analysis			Multivariable analysis			Univariate analysis			Multivariable analysis		
	N	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
PGR status													
Negative	161	1.00		**0.006**	1.00		0.243	1.00		**0.028**			
Positive	150	0.59	0.40-0.86		0.70	0.39-1.27		0.54	0.31-0.93				
HER2 status													
Negative	249	1.00		**0.002**	1.00		0.815	1.00		**<0.001**			
Positive	27	2.36	1.36-4.09		1.11	0.46-2.66		3.52	1.91-6.49				
Immune phenotype													
(1)	26	1.00		**0.031**	1.00		0.055	1.00		0.219			
(2)	8	0.58	0.07-4.94		0.77	0.08-7.67		$5.2^{E}5$	0-∞				
(3)	122	2.10	0.83-5.31		2.04	0.61-6.89		$1.5^{e}6$	0-∞				
(4)	34	3.45	1.28-9.28		3.06	0.85-10.97		$2.5^{e}6$	0-∞				
(5)	19	4.09	1.39-12.01		3.67	0.91-1479		$2.6^{e}6$	0-∞				
(6)	6	3.82	0.91-16.02		4.16	0.81-21.44		$3.7^{e}6$	0-∞				
(7)	4	5.91	1.14-30.67		13.4	2.12-84.86		$6.5^{e}6$	0-∞				

Abbreviations: N: number of patients; HR: hazard ratio; 95%CI: 95% Confidence Interval; ER: estrogen receptor; PR: progesterone receptor; HER2: human epidermal growth factor receptor 2; ET: endocrine therapy; CT: chemotherapy