CD103 and Intratumoral Immune Response in Breast Cancer

Zhi-Qiang Wang1,4, Katy Milne1, Heather Derocher1,2, John R. Webb1,2, Brad H. Nelson1,2,3, & Peter H. Watson1,4

1Trev & Joyce Deeley Research Centre, British Columbia Cancer Agency, Victoria, British Columbia, Canada; 2Department of Biochemistry and Microbiology, University of Victoria, Victoria, British Columbia, Canada; 3Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada; 4Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada.

Running title: Prognostic Significance of CD103 TIL in Breast Cancer

Keywords: CD103, CD8, TIL, breast cancer

Correspondence:
Peter H. Watson
Trev and Joyce Deeley Research Centre
BC Cancer Agency, Vancouver Island Centre
2410 Lee Ave, 3rd Floor Research
Victoria, BC, Canada, V8R 6V5
Telephone: 250-519-5700; Fax: 250-519-2040
Email: pwatson@bccancer.bc.ca

Disclosure of Potential Conflicts of Interest: The authors declare no conflict of interest.

Grant Support: This study was sustained by a grant to P.H.W from the Cancer Research Society of Canada (#702375).

Word count: 3592

Total numbers of Figures and Tables: three figures, two tables, two supplementary figures and four supplementary tables.

Statement of Translational Relevance: Tumor infiltrating lymphocytes (TIL) have potential as diagnostic, prognostic, and predictive biomarkers in breast cancer. CD8 T cells have been the most consistently associated with clinical factors and outcomes. As a member of the integrin family (αEβ7), CD103 is expressed by subsets of T cells and is considered as a marker of antigen educated tumor-specific CD8 TIL. In this study, we identified that intra-epithelial TIL with CD103+ status, alone or in combination with CD8+ status, is a prognostic marker for outcome specifically within the Basal-like subtype of breast cancer. CD103 is therefore a promising biomarker of Basal-like tumors in which the immune response is relevant to outcome and potentially most responsive to cancer immunotherapies.
Abstract

**Purpose:** CD103 is expressed in several immune cell types but in the context of the intratumoral immune response may be most important as a marker of antigen activated CD8 T cells. **Methods:** We have examined the prognostic significance of CD103 TIL in breast cancer by immunohistochemistry in a cohort of 424 breast cancer patients. **Results:** CD103 TIL were present in all subtypes but were more abundant in ER negative tumors where CD103 TIL were preferentially localized to the intra-epithelial compartment. CD103 was associated with tumor size, tumor grade, and ER/PR status (p < 0.05). CD103 TIL density and the epithelial to stromal ratio was highest in the Basal-like tumors. Intra-epithelial CD103 but not intra-stromal CD103 was associated with better relapse free and overall survival in Basal-like subtype tumors (Hazard ratio = 0.28, 95% CI = 0.17 - 0.72, p = 0.0047 and Hazard ratio = 0.25, 95% CI = 0.17 - 0.66, p = 0.0017 respectively). CD8 status showed similar but less significant associations, but the combination of dual CD103+CD8+ TIL status was the most strongly prognostic combination for relapse free and overall survival (Hazard ratio = 0.10, 95% CI = 0.07 - 0.62, p = 0.006 and Hazard ratio = 0.09, 95% CI = 0.07 - 0.57, p = 0.003 respectively). **Conclusions:** CD103 TIL are indicative of a good prognosis specifically within Basal-like subtype breast cancer.

Introduction

The value of evaluating tumor infiltrating lymphocytes (TIL) as a diagnostic, prognostic, and predictive biomarker in breast cancer has now been shown by a range of studies (1-5). In many of these studies, the evaluation of TIL was based on H&E stained sections and recommendations around the conduct of this effective and simple ‘immunoscore’ may improve the standardization for clinical implementation (6). Consideration of factors such as TIL location, type, and functional status encompassed by what has been termed the ‘immunocontexture’ (7) also have the potential to enhance the value of the ‘immunoscore’ (8). Amongst the many TIL types distinguishable by immunohistochemistry, CD8 T cells have been the most consistently associated with clinical factors and outcomes. We and others have shown that CD8 TIL are not only prognostic but also predictive of response to anthracycline chemotherapy in Her2 and triple negative breast cancer phenotypes (9). We have also shown that CD8 TIL may be a biomarker to distinguish between true recurrence and new primary tumors in the clinical setting of ipsilateral breast tumor relapse (2). These observations are consistent with the central role of CD8 cytotoxic T cells in mediating tumor cell destruction following differentiation and maturation stimulated by antigen presenting dendritic cells and CD4 T cells. However, it is probable that some intra-tumoral CD8 T cells are only bystanders that are not active participants in the immune response to the tumor while other CD8 T cells are cells that have recently engaged antigen (cytotoxic effector T cells) or previously engaged antigen (Trm T cells) and that are functionally relevant to the anti-tumor response (10).

CD103 is a member of the integrin family (αEβ7) that is expressed by subsets of T cells (11). It was first identified in association with intraepithelial CD8 T cells and has been
shown to be upregulated in CD8 T cells following antigen stimulation, but can also be expressed by regulatory FoxP3 positive CD4 T cells and by activated dendritic cells (12). Nevertheless, our studies in ovarian cancer show that the majority of CD103 positive cells are CD8 T cells and similar findings were reported in lung, colorectal and bladder cancer (13-16). CD103 expression on CD8 T cells is upregulated in response to simultaneous TGF-β1 stimulation and antigen recognition (17). Based on these findings, we have proposed that CD103 is a marker of an antigen educated tumor-specific CD8 TIL and therefore may be a more relevant TIL subset to analyze as a biomarker of the anti tumor immune response (18). In this study, our objective was to confirm and expand on our previous preliminary finding that CD103+CD8+ TIL are present and preferentially localized within epithelium in breast tumors (15). We also sought to explore the prognostic significance of intra-epithelial as compared to intra-stromal TIL.

Patients and Methods

Case Cohort
A cohort of 424 breast cancer cases was studied representing primary tumors collected by the Manitoba Breast Tumor Bank at time of diagnosis and initial surgical intervention. Age at diagnosis, tumor grade, size, nodal status, and outcomes in terms of relapses, and deaths were recorded (19). All tumors were histologically classified and graded by one pathologist (PHW). The time of diagnosis and accrual by the bank (1988-1995) predated current biomarker assays. Therefore immunohistochemistry (IHC) was previously performed by the Bank using an auto-immunostainer (Discovery Staining Module, Ventana Medical Systems, AZ, USA) on TMA sections from the cohort for ER, PR, Ki67, CK5/6, EGFR and Her2 biomarkers. ER, PR, and Her2 were scored and positive status assigned according to ACP guidelines (20, 21). Ki67, CK5/6 and EGFR were also scored and positive status assigned as >14% (Ki67) or any positive tumor cell staining (CK5/6 and EGFR). On the basis of the IHC determined expression of these five biomarkers the cohort was classified by the Bank into five intrinsic molecular subtypes: Luminal A (ER+/Ki67-/Her-), Luminal B (ER+/Ki67+/Her-), Her2 (Her2+), Triple Negative Non-Basal (TNNB, ER-/PR-/Her-/CK5/6-/EGFR-), and Basal-like (ER-/PR-/Her- and either CK5/6+ and/or EGFR+ (22, 23). The Bank operates with approval of the University of Manitoba Biomedical Research Ethics Board and this research study was conducted under approval from the BC Cancer Agency Research Ethics Board. A report concerning the source of the biospecimens and data used according to the BRISQ guidelines (24) is provided in Supplemental Table 1.

Tissue microarray (TMA) construction
Primary tumors were represented in tissue microarrays (TMAs) compiled by the Tumor Bank. To construct a TMA, all cases were initially selected from the database and then sections were re-reviewed to confirm and select areas for coring of corresponding blocks. Duplicate tissue cores (0.6 mm diameter) were taken from central cellular areas of each tumor with a tissue arrayer instrument (Beecher Instruments, Silver Spring, MD, USA). The original cohort of 636 cases was arrayed across 7 blocks. Prior utilization of these blocks and exhaustion of individual cores meant that the final interpretable cohort for this study was reduced to 424 cases.
Immunohistochemistry and TMA scoring

CD103 and CD8, CD4, CD68, and MHCII staining was performed on deparaffinized sections from TMAs using a Biocare Medical Intellipath FLX autostainer using reagents from Biocare (Concord, CA) unless otherwise noted. Slides were deparaffinized manually through xylene and graded alcohols then antigen retrieval performed in Biocare’s decloaking chamber using Diva decloaking solution for 125°C for 30 seconds. Slides were loaded into the Intellipath FLX, subjected to non-specific blocking with Peroxidased-1 and background sniper then incubated with either CD103 (clone EPR4166 (2), Epitomics, US, 1/1000), MHCII (clone CR3/43, Affinity Bioreagents, Golden, CO, 1/500), CD8 (clone C8/144B, Cell Marque, Rocklin, CA, 1/250), CD4 (clone EPR6855, Abcam, 1/250) or CD68 (clone SP251, Spring Biosciences, Pleasanton, CA, 1/150) in Da Vinci Green diluents for 30 minutes at room temperature. The slides were then incubated with either Rabbit- (CD103, CD68, CD4)-HRP or Mach2 Mouse- (MHCII, CD8) polymer for 30 minutes at room temperature and then detected with IP DAB for 5 minutes followed by counterstaining with a 1:10 dilution of CAT hematoxylin, air drying and coverslipping with Ecomount.

IHC scoring was performed in a blinded fashion by an experienced breast pathologist (PHW). Immunostained TMA sections were initially assessed at low magnification to select the core with the highest density of positive cells. The two types of biomarker TIL markers (indicating immune cell subsets) and MHCII (expressed by tumor epithelial cells and indicating potential for tumor antigen presentation) and were assessed. CD103, CD8, CD4, and CD68 tumor infiltrating leucocytes (TIL) were assessed as described previously (2) by direct counting up to 20 cells or by estimation when in excess of this number (IHC score, range 0–100) within the selected core area. The area of the entire core occupied by tumor epithelium versus stroma was then assessed followed by estimation of the proportion of positive TIL that were intra-epithelial or intra-stromal (intra-epithelial localization, was defined as lymphocytes within tumor cell nests and/or adjacent to and in direct contact with tumor cells). Intra-epithelial and intra-stromal TIL density per core was then calculated for each type of TIL and for each case. Only MHCII staining within tumor cells was scored and this signal was relatively diffuse and so was assessed by assigning an expression score on a 4 point scale (0 to 3+) with 0 = absent, 1 = weak intensity/less than 10% cells, 2 = moderate intensity/10-50% cells, 3 = strong intensity/50-100% cells.

Statistical analysis

Associations between CD103 expression and clinical-pathological features were evaluated using chi-square test and Fisher’s exact test. Assessment of the correlation between CD103 and other immune markers was performed using a nonparametric Spearman correlation. Comparisons between intra-epithelial and stromal IHC scores of CD8 and CD103 were performed using Kruskal-Wallis test, and comparisons between intra-epithelial/stromal ratios of CD8 and CD103 were performed using Mann-Whitney test. Survival was calculated using the Kaplan-Meier method and curves were compared with the log-rank test. Multivariate survival analyses were done using Cox regression analysis. All statistical tests were two-sided with significance established at p-values less
than 0.05. Statistical analyses were performed using Graphpad Prism 6.0 (GraphPad, La Jolla, CA, USA) and SPSS statistics 17 (SPSS, Chicago, IL, USA).

Results

Cohort clinical characteristics
A total of 424 patients with primary breast cancer diagnosed in the period 1988-1995 were studied. Follow-up outcomes data was available, with mean of 52 months (range 2 to 185 months). There were 174 breast specific deaths (mean time 26.5 months from diagnosis) and 250 survivors (mean time to last follow-up date 90 months). Primary therapy included surgical resection in all cases followed by adjuvant hormone, radiation, and chemotherapy in 319 (75%), 157 (37%), 87 (21%) cases respectively and 28 (7%) did not receive any form of systemic therapy. The clinical-pathological characteristics of the population are provided (Table 1).

Expression of CD103 in breast tumors
We and others have shown that in most tumors the majority of CD103 cells are a subset of CD8 TIL, and so we assessed the distribution of CD103 TIL along with that of CD8 TIL (Figure 1). CD103 TIL were present in 369/424 (87%) assessable tumors with an overall mean (+/-SD) score of 54 (+/-77) cells/mm$^2$. By comparison, CD8 TIL were present in a similar proportion of tumors with a higher mean (+/-SD) score of 75 (+/-112) cells/mm$^2$. However, as we have previously shown in ovarian tumors and a small cohort of breast tumors (15), whereas CD8 TIL densities were similar overall within intra-epithelial and stromal areas, CD103 TIL were relatively more numerous within intra-epithelial areas. Across the entire cohort, the ratio of intra-epithelial to stromal density was significantly higher for CD103 as compared to CD8 (Figure 2).

When we compared TIL densities within molecular intrinsic subtypes, IHC scores of CD103 and CD8 TIL within both epithelial and stromal compartments were lowest in Luminal A subtype tumors and incrementally higher in Luminal B, Her2, and Triple Negative Non-Basal (TNNB), and Basal-like subtype tumors ($p < 0.0001$) (Figure 2A). Furthermore, the epithelial to stromal ratio of CD103 and CD8 TIL was also different and followed a similar pattern between subtypes, with the most significant difference seen for CD103 TIL within Luminal A, Luminal B and Basal-like subtype tumors (Figure 2B).

We further analyzed the relationship between CD103 and other markers of active intra-tumoral immune response including tumor cell epithelial MHCII expression (eMHCII) and CD8, CD4 and CD68 TIL within the 98 tumors belonging to the two triple negative tumor subtypes (TNNB and Basal-like). Spearman’s correlation analyses showed that intra-epithelial CD103 (eCD103) strongly correlated with tumor cell expression of eMHCII and with intra-epithelial and intra-stromal CD8 in both TNNB and Basal-like subtypes. CD103 also showed additional correlations with CD68 and CD4 mostly in Basal-like subtype tumors (Supplemental Table 2).

Expression of CD103 and association with clinical-pathological features
We next examined the association between CD103 density and clinical-pathological features using a cutoff value at the 90th percentile to delineate low from high density. CD103 in both epithelial and stromal compartments was associated with tumor size, tumor grade, and ER/PR status (Supplemental Table 3). CD103 positive TIL status within molecular intrinsic subtype classes was also significantly different, with high levels of eCD103 present in a small proportion (< 5%) of Luminal A and B subtype tumors but significantly higher proportions (11% - 23%) of Her2, TNNB, and Basal-like subtype tumors (Supplemental Table 3). We also looked for correlations between CD103 and tumor type (ductal vs lobular) since E-cadherin expression has been implicated with CD103 but observed no significant relationship (data not shown).

Association of CD103 with outcomes
Univariate analysis of standard prognostic factors in the entire cohort confirmed tumor size, nodal status, ER status and PR status as highly significant and patient age and high tumor grade as significant prognostic factors for both RFS and OS (Supplemental Table 4). The upper quartile of CD8 TIL-immunoscore density scores were used to classify low vs. high TIL status. eCD103, sCD103, eCD8 and sCD103 were not prognostic for relapse free survival (RFS) or overall survival (OS) in the overall cohort (Supplemental Table 4), and sCD103 and sCD8 were not prognostic in any of the molecular subtypes (data not shown). However, eCD103 was associated with RFS and OS within the Basal-like subset (Hazard ratio = 0.28, 95% CI = 0.17 - 0.72, p = 0.0047 and Hazard ratio = 0.25, 95% CI = 0.17 - 0.66, p = 0.0017 respectively) (Figure 3 and Supplemental Table 4). eCD8 positive status within the Basal-like subset showed a similar pattern for RFS and was significant for OS (Hazard ratio = 0.43, 95% CI = 0.21 - 0.87, p = 0.021) (Figure 3 and Supplemental Table 4). In multivariate analysis of eCD103 and eCD8 with clinical prognostic factors within the whole cohort, tumor size, nodal status, ER status, PR status and eCD8 were prognostic and significant for both RFS and OS (Supplemental Table 4).

While sCD103 was not prognostic in the Basal-like subset, high levels of sCD8 showed a trend towards better outcomes (Supplemental Figure 1). We therefore also assessed the TIL immunoscore (corresponding to non-specific intra-stromal TIL discernable on H&E slides of which CD8 cells are a major component) within the Basal-like tumors according to the standardized approach described in recent recommendations (6). The TIL immunoscores ranged from low (0 - 10%) in 57% (37/65), intermediate (15 - 40%) in 28% (18/65), and high (50 - 75%) in 15% (10/65) tumors. While not statistically significant, tumors with high TIL immunoscores (above the median score of 10%) showed a similar trend (p = 0.062) to that shown by sCD8 towards better outcomes in the Basal-like tumor subset (Supplemental Figure 1).

Furthermore, univariate analysis within the Basal-like subset showed that eCD8+eCD103+ TIL status was strongly associated with RFS and OS as compared to tumors with mixed dual negative eCD8 eCD103 status (Hazard ratio = 0.10, 95% CI = 0.07 - 0.62, p = 0.006 and Hazard ratio = 0.09, 95% CI = 0.07 - 0.57, p = 0.003 respectively) (Figure 3 and Table 2). Multivariate analysis of CD103 with clinical prognostic factors within the Basal-like subset showed that only combined eCD103+/eCD8+ status was independently prognostic and significant for both RFS and
OS (Table 2). Finally, we performed univariate analysis of outcomes within the Basal-like subset in relation to eCD103 and eCD8 alone vs. eCD103/eCD8 combined TIL, and found that eCD103+ status and eCD8+ status alone were associated with better OS as compared to tumors with dual negative eCD8 eCD103 status (Hazard ratio = 0.22, 95% CI = 0.09 - 0.54, p = 0.001 and Hazard ratio = 0.39, 95% CI = 0.16 - 0.83, p = 0.017 respectively) (Supplemental Figure 2).

Discussion

We have shown that CD103 is a biomarker of good prognosis in Basal-like subtype breast cancer. We also confirm that CD103 strongly correlates with CD8 but in contrast to the distribution of CD8 positive tumor infiltrating leucocytes (TIL) within the tumor, CD103 TIL are preferentially localized to the intra-epithelial compartment and only intra-epithelial CD103 positive TIL were prognostic in this cohort.

Host immunity plays an important role in tumorigenesis and tumor progression (25). Breast cancers are often immunogenic, stimulating antigen-specific anti-tumor lymphocyte responses that are reflected by TIL (26-28). Recent work has shown that at least some of the TIL is response to specific epitopes created by tumor mutation events (1, 29, 30). These observations explain and are consistent with the fact that TIL have now been shown to be prognostic and predictive markers in breast cancer (2-5). One approach to evaluating TIL is to enumerate immune-like cells on H&E stained sections to derive an ‘immunoscore’ (TNM-I) (31). However, tumors are complex and several technical issues related to scoring TIL need to be better defined to refine and standardize this approach to implement TNM-I in different tumor types (8). For breast cancer an international working group has published recommendations for scoring on H&E sections. These recommendations (6) propose that all mononuclear cells within the intra-stromal compartment should be scored. The main basis for advocating stromal TIL assessment for the basic immunoscore is that TIL tend to be present in lower numbers and are more difficult to observe within intra-epithelial compartments whereas they can be more accurately delineated within stroma when only an H&E stain is used. Stromal and epithelial TIL also correlate and both have been shown to be predictive of outcome in different studies. However, in some tumor types the stromal compartment is difficult to delineate. Also while there are merits to the simplicity of basic assessment on H&E stained sections, consideration of ‘immunecontexture’ factors may enhance the value of the immunoscore. Such factors include more precise distinction of TIL location and TIL subtypes and functional status through the use of immunohistochemical markers (8).

Amongst the many TIL distinguishable by immunohistochemistry, CD8 T cells have been the most consistently associated with outcomes. However, while the CD8 marker is predominantly expressed by T cells it can also be expressed on NK cells and dendritic cells. Amongst CD8 positive T cells the marker delineates a range of T cell classes encompassing naïve, antigen-educated and activated effector T cells, and memory T cells (central, effector, and tissue resident classes) and a range of functional states that have been correlated with additional biomarkers including TIA1 and granzyme B. CD103 is mostly expressed by T cells in solid tumors, but is also expressed by subsets of CD4, NK,
and dendritic cells. T cells require both antigen stimulation and TGFβ signaling to upregulate CD103, and as we have previously proposed CD103 may therefore be a marker of antigen educated effector T cells that become resident within tissues (tissue-resident memory (Trm) T cells (11, 12). CD103+ Trm cells appear to have less proliferative potential and greater cytokine production capacity (32) and CD103 is involved in functional interactions between T cells and epithelial tumor cells (33-35). Our findings here are consistent with these observations in which CD103 was most closely correlated with CD8 but the CD103 expressing subset of TIL were predominantly intra-epithelial and more strongly associated with outcomes than CD8.

Furthermore, only CD103 intra-epithelial TIL were prognostic and then only within the Basal-like subset. By contrast, stromal TIL (as assessed by either TIL immnoscore, sCD8, and sCD103) was not prognostic. However, the trend seen with all three stromal TIL assessments suggests that significance is just weaker than intra-epithelial TIL and prognostic significance of intra-stromal TIL might be attained with a larger cohort of Basal-like tumors (36). One explanation for this might be that intra-stromal TIL are not as functionally relevant and/or include a higher proportion of irrelevant ‘bystanders’ (e.g. T cells that lack tumor antigen specificity).

It is now fairly well established that the intra-tumoral immune response is most closely associated with and most relevant to outcome of breast tumors within the Basal-like category. Our findings here correlate with this and confirm a different distribution of TIL within triple negative type tumors with higher epithelial/stromal ratios of CD8+ and CD103+ TIL. We and others have previously made similar observations in ovarian cancer. CD103 TIL was found to be highest in the high grade serous subtype relative to mucinous, endometrioid, and clear cell types, and only consistently prognostic in the former subtype. Furthermore, Bosmuller et al (37) have also shown that while high numbers of both intra-epithelial CD3 and CD103 were prognostic in high grade serous ovarian tumors, the combined CD3+/CD103+ TIL conferred the strongest prognostic significance. Dual staining and flow sorting approaches have established that the majority of CD103+ TILs in ovarian tumors lung, colorectal and bladder tumors are CD3+CD8+ T lymphocytes. Minor subsets of CD103+ TILs are CD56+ NK cells and CD4 T cells (14-16). This is consistent with the finding that CD103 correlates most closely with CD8 and MHCII expression on tumor cells (as a possible marker of an active immune response) (38) in the epithelial compartment but is only weakly correlated with CD8 in the stromal areas where the relative proportions of CD4 and other immune cells are higher.

In conclusion, interpretation of our findings should be qualified by the relatively small numbers of cases within molecular subsets in the study cohort. However, overall these observations are consistent with the view that CD103+ TIL are predominantly localized within intra-epithelial areas of breast tumors and are indicators of an intra-tumoral immune response that is associated with a better prognosis in Basal-like breast tumors.

Authors' Contributions
Conception and design: P.H. Watson, Z.Q. Wang
Development of methodology: K. Milne, H. Derocher
Acquisition of data: P.H. Watson, Z.Q. Wang
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Z.Q. Wang, P.H. Watson
Writing, review, and/or revision of the manuscript: P.H. Watson, Z.Q. Wang, B.H. Nelson, J.R. Webb, K. Milne
Study supervision: P.H. Watson

Acknowledgments
Clinical specimens were provided by the Manitoba Breast Tumor Bank, associated with the Canadian Tissue Repository Network (CTRNet). We also thank Michelle Parisien and Dr Leigh Murphy for assistance in compiling the case cohort from the Manitoba Breast Tumor Bank and Sonya Laan for assistance with immunostaining.
References
15. Webb JR, Milne K, Watson P, Deleeuw RJ, Nelson BH. Tumor-infiltrating lymphocytes expressing the tissue resident memory marker CD103 are associated


Figure legends

Figure 1. Immunohistochemical staining showing the different epithelial and stromal distribution of CD103 and CD8 positive TIL. Representative images showing the same region within a single breast tumor in semi-serial sections stained by hematoxylin and eosin (A), and immunohistochemistry for CD103 (B) and CD8 (C). Magnification ×400. Bar 200 um.

Figure 2. Associations between CD103 and CD8 density and localization within the entire cohort and molecular subtypes. A. CD8 and CD103 density IHC scores within all tumors and subsets. B. CD8 and CD103 epithelial/stromal ratios within the cohort and subsets. The mean ratio for each group is indicated by a horizontal line. NS = no significance, *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001.

Figure 3. Prognostic impact of CD103 and CD8 in breast cancer. Kaplan–Meier plots representing the probability of overall survival (OS) in entire cohort (left column) and Basal-like subgroup (right column) stratified according to the expression status of intra-epithelial eCD103 (panels A and B) and eCD8 (panels C and D) and eCD103/eCD8 combined TIL (panels E and F). The log-rank test was used to compare curves and p-values less than 0.05 were considered significant. NS = no significance.
Figure 1

A. H&E  
B. CD103  
C. CD8
Figure 2

A. IHC scores

- CD8
- CD103
- All cases
- Lum A
- Lum B
- Her2
- TNNB
- Basal-like

B. Localization (epithelial to stromal ratio)

- CD8
- CD103
- All cases
- Lum A
- Lum B
- Her2
- TNNB
- Basal-like

NS

****

***

*NS*
Figure 3

A. All

- eCD103
- Overall survival rate
- Time (months)
- High, n = 43
- Low, n = 381
- NS

B. Basal-like

- eCD103
- Overall survival rate
- Time (months)
- High, n = 18
- Low, n = 45
- p = 0.0017

C. eCD8

- Overall survival rate
- Time (months)
- High, n = 209
- Low, n = 190
- NS

D. eCD8

- Overall survival rate
- Time (months)
- High, n = 27
- Low, n = 25
- p = 0.021

E. eCD103/eCD8

- Overall survival rate
- Time (months)
- Both high, n = 28
- Either high, n = 75
- Both low, n = 259
- NS

F. eCD103/eCD8

- Overall survival rate
- Time (months)
- Both high, n = 9
- Either high, n = 19
- Both low, n = 20
- p = 0.009, p = 0.003
Table 1. Demographic and clinical-pathological characteristics of patients in the study cohort.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Status</th>
<th>Cases</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis</td>
<td>≤35 years</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>&gt;35 years</td>
<td>412</td>
<td>97</td>
</tr>
<tr>
<td>Tumor size</td>
<td>T1a/b</td>
<td>2</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>T1c</td>
<td>68</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>268</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>59</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>27</td>
<td>6</td>
</tr>
<tr>
<td>Nodal status</td>
<td>Positive</td>
<td>209</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>191</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>24</td>
<td>6</td>
</tr>
<tr>
<td>Tumor grade</td>
<td>1</td>
<td>66</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>256</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>101</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>1</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>ER^b</td>
<td>Positive</td>
<td>234</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>190</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PR^b</td>
<td>Positive</td>
<td>202</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>222</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Molecular subtypes</td>
<td>Luminal A</td>
<td>178</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>Luminal B</td>
<td>50</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Her2</td>
<td>64</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>TNNB^c</td>
<td>35</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Basal-like</td>
<td>63</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Unclassified</td>
<td>34</td>
<td>8</td>
</tr>
</tbody>
</table>

^aTumor size: 0.1cm<T1a/b<1cm; 1cm ≤ T1c<2cm; 2cm ≤T2<5cm; 5cm ≤T3.
^bER negative defined as <10 fmol/mg protein and PR negative as ≤15 fmol/mg protein (ligand binding assay).
^cTNNB=Triple-Negative-Non-Basal.
Table 2. Univariate and multivariate analyses of associations between clinical parameters and eCD103/eCD8 combined status and either relapse free survival or overall survival in the Basal-like subgroup.

<table>
<thead>
<tr>
<th>A/ Recurrence-free survival</th>
<th>Parameter</th>
<th>Comparison</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>HR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Age at diagnosis (yrs)</td>
<td>&gt;35 vs ≤35</td>
<td>0.99 (0.24-4.13)</td>
<td>0.709</td>
<td>0.87 (0.18-4.14)</td>
</tr>
<tr>
<td>Tumour size (cm)</td>
<td>&gt;2cm vs ≤2cm</td>
<td>2.12 (0.72-4.74)</td>
<td>0.204</td>
<td>0.67 (0.16-2.75)</td>
</tr>
<tr>
<td>Nodal status</td>
<td>pos vs neg</td>
<td>1.43 (0.63-3.24)</td>
<td>0.398</td>
<td>1.03 (0.41-2.57)</td>
</tr>
<tr>
<td>Tumor grade</td>
<td>2 vs 1</td>
<td>1.61 (0.27-9.53)</td>
<td>0.598</td>
<td>0.48 (0.06-3.97)</td>
</tr>
<tr>
<td></td>
<td>3 vs 1</td>
<td>1.14 (0.17-7.84)</td>
<td>0.892</td>
<td>1.15 (0.44-3.03)</td>
</tr>
<tr>
<td>eCD103 + eCD8 expression</td>
<td>high vs low</td>
<td>0.10 (0.07-0.62)</td>
<td>0.006</td>
<td>0.08 (0.01-0.74)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B/ Overall survival</th>
<th>Parameter</th>
<th>Comparison</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>HR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Age at diagnosis (yrs)</td>
<td>&gt;35 vs ≤35</td>
<td>0.98 (0.23-4.12)</td>
<td>0.979</td>
<td>0.72 (0.15-3.44)</td>
</tr>
<tr>
<td>Tumour size (cm)</td>
<td>&gt;2cm vs ≤2cm</td>
<td>1.51 (0.60-3.82)</td>
<td>0.379</td>
<td>0.43 (0.12-1.53)</td>
</tr>
<tr>
<td>Nodal status</td>
<td>pos vs neg</td>
<td>1.39 (0.64-3.01)</td>
<td>0.418</td>
<td>1.03 (0.41-2.57)</td>
</tr>
<tr>
<td>Tumor grade</td>
<td>2 vs 1</td>
<td>1.48 (0.24-8.94)</td>
<td>0.672</td>
<td>0.42 (0.05-3.46)</td>
</tr>
<tr>
<td></td>
<td>3 vs 1</td>
<td>1.32 (0.23-7.67)</td>
<td>0.761</td>
<td>1.47 (0.56-3.90)</td>
</tr>
<tr>
<td>eCD103 + eCD8 expression</td>
<td>high vs low</td>
<td>0.09 (0.07-0.57)</td>
<td>0.003</td>
<td>0.07 (0.01-0.57)</td>
</tr>
</tbody>
</table>

*eCD103 = intra-epithelial CD103; eCD8 = intra-epithelial CD8.
Clinical Cancer Research

CD103 and Intratumoral Immune Response in Breast Cancer


Clin Cancer Res  Published OnlineFirst June 7, 2016.

Updated version  Access the most recent version of this article at: doi:10.1158/1078-0432.CCR-16-0732

Supplementary Material  Access the most recent supplemental material at: http://clincancerres.aacrjournals.org/content/suppl/2016/06/07/1078-0432.CCR-16-0732.DC1

Author Manuscript  Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.