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Androgen Receptor and *PIM1* Expression in Tumor Tissue of Patients With Triple-negative Breast Cancer

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Abstract. *Background/Aim: Effective targeted therapies for triple-negative breast cancer (TNBC) are limited. In a subset of TNBC, androgen receptor (AR) plays an important role, while the human proviral integration site for Moloney murine leukemia virus-1 (PIM1) overexpression is also implicated. PIM1 kinases phosphorylate AR, thus regulating its transcriptional activity, regardless of the presence or not of androgens. We evaluated the expression of AR and PIM1 and their prognostic significance in TNBC. Materials and Methods: AR and PIM1 transcripts were quantified by quantitative reverse transcription polymerase chain reaction in formalin-fixed paraffin-embedded tumor from 141 patients with TNBC. Results: AR was expressed in 38.3%, PIM1 in 10.6%, while co-expression of AR and PIM1 was detected in 7/141 cases (5.0%). No prognostic significance of AR or PIM1 was reached for overall or disease-free survival. Conclusion: Co-expression of AR and PIM1 exists in only in a small percentage of patients with TNBC. The implications of this finding in the therapeutic management of patients with TNBC should be investigated in larger patient cohorts.*

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Triple-negative breast cancer (TNBC) accounts for the 15-20% of breast cancer cases and is devoid of the expression of estrogen (ER) and progesterone receptors, and of human

epidermal growth factor receptor 2 (HER2) protein overexpression or amplification (1). It exhibits the poorest prognosis among other breast cancer subtypes, it is usually basal-like and of higher grade. These features combined with the lack of the common breast cancer targeted biomarkers render it a highly aggressive breast cancer subtype (2). Lehmann *et al*. were the first to classify TNBC into seven stable subtypes, indicating the high molecular heterogeneity of the disease (3).

Neoadjuvant chemotherapy is the current mainstay treatment option for patients with TNBC who intriguingly achieve improved pathological complete response compared to other breast cancer subtypes, in spite of its unfavorable prognosis (4, 5). However, disease in only a small subset of these patients is chemosensitive, while the majority are susceptible to treatment resistance (6). There are not only several established therapy protocols but also many ongoing clinical trials examining the efficacy of new targeted therapies or the combination of different regimens, based on distinct molecular alterations in TNBC (7). Nevertheless, the aggressive profile of TNBC and its heterogenous molecular landscape further underpin the necessity for determining new biomarkers that might enable selection of patients for targeted therapies.

Androgen receptor (AR) signaling plays an important role in breast cancer biology, albeit complicated due to the nuanced differences exhibited between AR-positive breast cancer in relation to the co-existence of other receptors (8). During the last years, the luminal androgen receptor subtype, which is characterized by the unique presence of AR, is in the forefront of research interest. This subtype is associated with better prognosis among other TNBC subtypes, is less responsive to chemotherapeutic regimens and presents lower pathological complete response after neoadjuvant treatment (9). The oncogenic functional role of AR in luminal androgen receptor TNBC is through maintaining cell proliferation (10). The prognostic role of AR in TNBC still remains controversial (11). A meta-analysis supports the favorable prognosis of AR-positive TNBC (12) in contrast to studies that support the opposite (13, 14), while others did not associate AR expression with prognosis at all (15, 16). Notwithstanding the equivocal results for the impact of AR expression on survival outcomes, targeted therapy for AR-positive TNBC may be beneficial. In the light of the prognostic and predictive role of AR in prostate cancer (PCa) and the availability of anti-androgen directed therapies, previous and ongoing clinical trials tested the efficacy of these drugs in AR-positive TNBC and presented some promising results (8, 17). In this rapidly evolving field of AR study, many other therapeutic targets involved in the AR pathway are also being examined (7).

The human proviral integration site for Moloney murine leukemia virus-1 (PIM1), a serine/threonine kinase, is normally implicated in cell-cycle progression, survival, proliferation, and apoptosis; on account of this, *PIM1* plays an important role in development, progression and maintenance of tumor (18, 19). PIM1 kinase was first associated with murine leukemia virus (MuLV)-induced lymphomas (20) and subsequent analyses in transgenic mice revealed its oncogenic role (21). Apart from leukemia (22), its up-regulation has been extensively studied in PCa and in many other types of cancer as well [reviewed in (23)]. Recent studies based on the collection of published datasets from clinical cohorts confirmed a significant up-regulation of *PIM1* in TNBC compared to the non-TNBC tumors and affiliated its aberrant expression with poor prognosis [reviewed in (24)]. The basal-like TNBC subtype exhibits the highest levels of *PIM1* expression, accompanied by copy-number gains and amplifications (25, 26). *In vitro* experiments using TNBC cell lines and xenografts showed that *PIM1* regulates cell proliferation and tumor growth by modulating anti-apoptotic proteins (BCL2 apoptosis regulator 2), cell-cycle proteins (p27) and the oncogene c-MYC, and that *PIM1* inhibition increased sensitivity in patients with TNBC treated with chemotherapy (25, 26). Moreover, based on previous evidence on PCa, PIM1 kinases phosphorylate AR, thus regulating its transcriptional activity, both in the presence and absence of androgens. This mechanism seems to play a crucial role during androgen deprivation therapy, and co-targeting of AR and PIM1 would exhibit improved therapeutic effects on resistant PCa (27).

In the present study, we evaluated *AR* and *PIM1* expression in TNBC clinical specimens using validated RT-qPCR assays (28, 29) and investigated their association with clinicopathological parameters and prognostic significance in terms of overall (OS) and disease-free (DFS) survival.

Materials and Methods

Patients. RNA was isolated from formalin-fixed paraffin-embedded (FFPE) tumor samples from 194 women with TNBC, and after evaluation of RNA quality, 141 such samples were finally included in the current analysis. Overall, 134/141 (95%) patients were treated for high-risk breast cancer in the context of Hellenic Cooperative Oncology Group's (HeCOG) adjuvant clinical trials (30-34). A total of 139 patients (98.6%) had received adjuvant chemotherapy, whereas one patient refused to receive any treatment, and for one additional patient there was no further information regarding the type of administered therapy. The type of adjuvant chemotherapy administered is shown in Table I. Adjuvant hormonal therapy was administered in 27 patients (19.1%). Among them, 15 patients were found to have ER/PgR-positive tumors in the local assessment based on low Histoscore (1-10) which, however, was not confirmed upon central re-evaluation of ER and PgR status. The remaining 12 patients were treated with luteinizing hormone-releasing hormone agonists only (four patients), tamoxifen only (in six), tamoxifen followed by aromatase inhibitor or aromatase inhibitor only (in one patient each) for hormonal manipulation, a practice used by some investigators at that time that has since been abandoned. The median

Table I. *Baseline patient and tumor characteristics*.

Characteristic		Total (N=141)
Age (N=141), years	Median (range)	52.5 (20.9-82.7)
Menopausal status (N=141), n (%)	Premenopausal	65 (46.1%)
	Postmenopausal	76 (53.9%)
Histology classification (N=141), n (%)	Inflammatory	1 (0.71%)
	Invasive ductal	119 (84.4%)
	Invasive lobular	4 (2.8%)
	Medullary with lymphocytic	11 (7.8%)
	Mixed	3 (2.1%)
	Other	3 (2.1%)
Histological grade (N=140), n (%)	1	2 (1.4%)
	2	23 (16.4%)
	3	113 (80.7%)
	4	2 (1.4%)
Tumor size (N=141), cm	Median (range)	2.7 (0.20-11.0)
	≤2 cm	45 (31.9%)
	2-5 cm	81 (57.4%)
	>5 cm	15 (10.6%)
Positive nodes (N=139), n	Median (range)	1.00 (0.00-40.0)
	0	47 (33.8%)
	1-3	50 (36.0%)
	≥4	42 (30.2%)
Type of adjuvant chemotherapy (N=139)	E-T-CMF	66 (47.5%)
	ET-CMF	22 (15.8%)
	E-CMF-wDoc	21 (15.1%)
	E-CMF-wT	21 (15.1%)
	Other	9 (6.5%)
Adjuvant hormonotherapy (N=141), n (%)	No	114 (80.9%)
	Yes	27 (19.1%)
Adjuvant radiotherapy (N=140)	No	29 (20.7%)
	Yes	111 (79.3%)
ER status (local assessment) (N=141), n (%)	Negative	128 (90.8%)
	Positive	13 (9.2%)
PR status (local assessment) (N=141), n (%)	Negative	126 (89.4%)
	Positive	15 (10.6%)
HER2 status (local assessment) (N=140), n (%)	No	131 (93.6%)
	Yes	9 (6.4%)
Basal-like (N=139)	Yes	124 (89.2%)
	No	15 (10.8)

ER: Estrogen receptor. PR: progesterone receptor. HER2: human epidermal growth factor receptor. E-T-CMF: 110 mg/m^2 epirubicin *q* 2 weeks × 3 followed by 250 mg/m^2 paclitaxel *q* 2 weeks × 3 followed by 840 mg/m^2 cyclophosphamide; 57 mg/m^2 methotrexate; 840 mg/m^2 fluorouracil (CMF) *q* 2 weeks × 3. ET-CMF: 83 mg/m^2 epirubicin + 187 mg/m^2 paclitaxel *q* 3 weeks × 4 followed by CMF *q* 2 weeks × 3; E-CMF-wDoc: 110 mg/m^2 epirubicin *q* 2 weeks × 3 followed by CMF *q* 2 weeks × 3 followed by weekly docetaxel 35 mg/m^2 × 9; E-CMF-wT: 110 mg/m^2 epirubicin *q* 2 weeks × 3 followed by CMF *q* 2 weeks × 3 followed by weekly 80 mg/m^2 paclitaxel × 9.

age at the time of breast cancer diagnosis was 53 (range=21-83) years. Table I summarizes the patient clinicopathological characteristics of the study population. Most patients were postmenopausal (53.9%) at the time of diagnosis and had invasive ductal tumors (84.4%) of higher grade. Of the 139 patients with data available for epidermal growth factor receptor and/or cytokeratin 5 protein expression, 124 (89.2%) had basal-like tumors. At a median follow-up of 12 years [95% confidence intervaI (CI)=11.0-13.3 years], a total of 50 DFS events were reported and 43 patients (30.5%) had died, while the median OS and DFS had not yet been reached at the time of the analysis.

Informed consent was obtained from all individuals included in this study. Ethical approval: The research related to human use complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration. Clinical protocols were approved by local regulatory authorities and were also included in the Australian New Zealand Clinical Trials Registry and allocated the following Registration Numbers: ACTRN12611000506998 (HE10/97), ACTRN12609001036202 (HE10/00), ACTRN1261000 0151033 (HE10/05) and ACTRN12615000161527 (HE10/08). The translational research protocol was approved by the Institutional Review Board of Papageorgiou Hospital.

Table II. *Associations between AR expression and selected clinicopathological parameters.*

		Negative (N=87)	Positive (N=54)	*p*-Value
Age, years	Median (range)	50.0 (29.0-82.7)	56.2 (20.9-72.2)	0.067[a]
Tumor size, cm	Median (range)	2.8 (0.20-11.0)	2.6 (1.3-10.0)	0.76[a]
	≤2 cm	27 (31.0%)	18 (33.3%)	0.62[b]
	2-5 cm	49 (56.3%)	32 (59.3%)	
	>5 cm	11 (12.6%)	4 (7.4%)	
Positive nodes, n	Median (range)	1.00 (0.00-40.0)	1.5 (0.00-31.0)	0.32[a]
	0	29 (34.1%)	18 (33.3%)	0.31[b]
	1-3	34 (40.0%)	16 (29.6%)	
	≥4	22 (25.9%)	20 (37.0%)	
Histological grade, n (%)	1-2	17 (19.8%)	8 (14.8%)	0.46[b]
	3-4	69 (80.2%)	46 (85.2%)	
Menopausal status, n (%)	Postmenopausal	40 (46.0%)	36 (66.7%)	0.017[b]
	Premenopausal	47 (54.0%)	18 (33.3%)	
Basal-like, n (%)	No	7 (8.1%)	8 (15.1%)	0.20[b]
	Yes	79 (91.9%)	45 (84.9%)	

[a]Wilcoxon rank-sum test; [b]Pearson's chi-square test.

RT-qPCR. Total RNA was isolated from FFPE tumor samples, followed by cDNA synthesis. Beta-2 microglobulin (*B2M*) was used as a reference gene for quality control, to ensure the presence of amplifiable material in all samples and to avoid false-negative results, as previously described (28, 29). RT-qPCR assays for the quantification of *AR* (28) and *PIM1* transcripts were performed as previously reported (29). To evaluate RT-qPCR specificity for each gene, we analyzed in exactly the same way matched samples from FFPE adjacent to tumor samples and ductal carcinoma in situ samples. In tumor samples, RT-qPCR data for *PIM1* and AR were normalized in respect to the expression of *B2M* reference gene and cut-off value was calculated by using the $2^{-\Delta\Delta Ct}$ approach, as previously described by Livak and Schmittgen (35). All single RT-qPCR reactions for *AR*, *PIM1* and *B2M* were performed in the COBAS z480 system (Roche Molecular System Inc, Pleasanton, CA, USA).

Statistical analysis. Frequencies with the corresponding percentages were used to describe categorical variables, while the median and range values were used to provide descriptive statistics of continuous variables. The chi-square and the Wilcoxon rank-sum tests were performed to evaluate the associations between AR expression and selected clinicopathological parameters. OS was defined as the time (in years) from diagnosis of breast cancer to death from any cause or last contact. Patients alive or lost to follow-up were censored at the date of their last contact. DFS was defined as the time from diagnosis to the first documented progression, death from any cause or last contact, whichever occurred first. Survival distributions were estimated using the Kaplan–Meier method and compared across groups with the log-rank test. The association of *AR* and *PIM1* expression with progression and mortality rates was assessed with univariate Cox regression models. All tests were two-sided at significance alpha level of 5%. No adjustment for multiple comparisons was performed since this study was exploratory and mainly hypothesis generating with predefined parameters. SAS version 9.3 (SAS Institute Inc., Cary, NC, USA) was used for statistical analysis.

Results

AR expression in TNBC samples. We evaluated the expression of *AR* using a previously developed and validated RT-qPCR assay (28) in 141 formalin-fixed paraffin-embedded tumor samples of patients with TNBC. In total, *AR* expression was detected in 54/141 patient samples (38.3%). The associations between selected clinicopathological parameters and *AR* expression are presented in Table II. Patients with positive *AR* expression were found to be less frequently of premenopausal status as compared to those with negative *AR* expression (chi-square p=0.017). No further significant associations were observed between *AR* expression and clinicopathological parameters.

Data on AR protein expression, as assessed by immunohistochemistry, were available for 31 patients (22.0%). Among them, positive *AR* gene expression was associated with positive AR protein expression (Fisher's p=0.001). *AR* mRNA expression did not show prognostic significance with respect to OS [hazard ratio (HR) for positive *AR* expression=0.98, 95% CI=0.53-1.82, p=0.95; Figure 1A). Similarly, significance for *AR* expression was not reached in terms of DFS (HR=1.07, 95% CI=0.60-1.89, p=0.82; Figure 1B). We further evaluated the prognostic significance of *AR* expression in the subgroup of patients with basal-like tumors (N=124). As in the entire cohort, *AR* expression was not found to be prognostic among patients with basal-like tumors for either OS (HR for positive *AR* expression=1.03, 95% CI=0.53-1.97, p=0.94; Figure 2A) or DFS (HR for positive *AR* expression=1.06, 95% CI=0.58-1.96, p=0.84; Figure 2B).

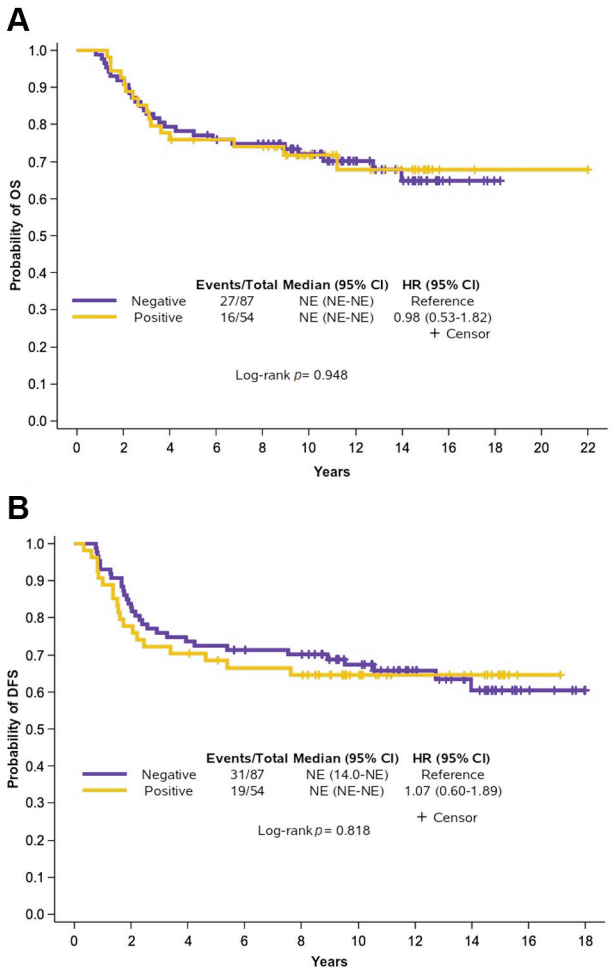

Figure 1. *Kaplan–Meier curves based on androgen receptor (AR) expression with respect to overall (OS) (A) and disease-free (DFS) (B) survival for the entire cohort. CI: Confidence intervaI; HR: hazard ratio; NE: not estimable.*

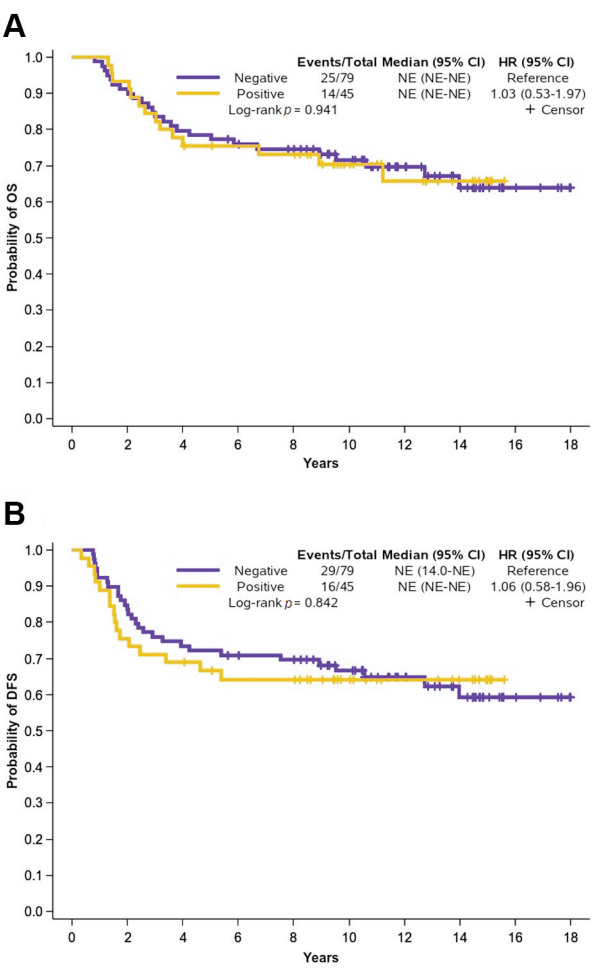

Figure 2. *Kaplan–Meier curves based on androgen receptor (AR) expression with respect to overall (OS) (A) and disease-free (DFS) (B) survival for patients with basal-like tumors. CI: Confidence intervaI; HR: hazard ratio; NE: not estimable.*

PIM1 expression in TNBC samples. Most patients had tumors negative for expression of *PIM1* (126 patients; 89.4%), whereas positivity of *PIM1* was detected in 10.6% of the study cohort. Seven patients (5.0%) carried tumors with positive expression of both *AR* and *PIM1*. *PIM1* expression was not correlated with OS (HR for positive *PIM1* expression=1.47, 95% CI=0.62-3.50, p=0.38; Figure 3A), nor with DFS even though the corresponding HR retained the direction observed for OS, suggesting an unfavorable effect for *PIM1* expression (HR=1.29, 95% CI=0.55-3.02, p=0.56; Figure 3B). We further evaluated the prognostic significance of *PIM1* expression in the subgroup of patients with basal-like tumors (N=124). As in the entire cohort, *PIM1* expression was not found to be prognostic among patients with basal-like tumors for either OS (HR for positive *PIM1* expression=1.57, 95% CI=0.66-3.76, p=0.31; Figure 4A) or DFS (HR for positive *PIM1* expression=1.38, 95% CI=0.58-3.26, p=0.46; Figure 4B).

Discussion

TNBC heterogeneity along with its aggressive features pose a puzzling problem for the identification of new potential biomarkers. Many molecular pathways implicated in TNBC biology remain to be explored. AR signaling has already been proven to play a crucial role in a subset of TNBC (7). Therapies based on AR expression have already been tested in PCa and to date are also being validated in patients with TNBC. Inferentially, most TNBC therapeutic approaches refer to AR-positive TNBC cases, leaving the AR-negative subset bereft of available treatment options. Nevertheless, quadruple-negative breast cancer (QNBC) should not be

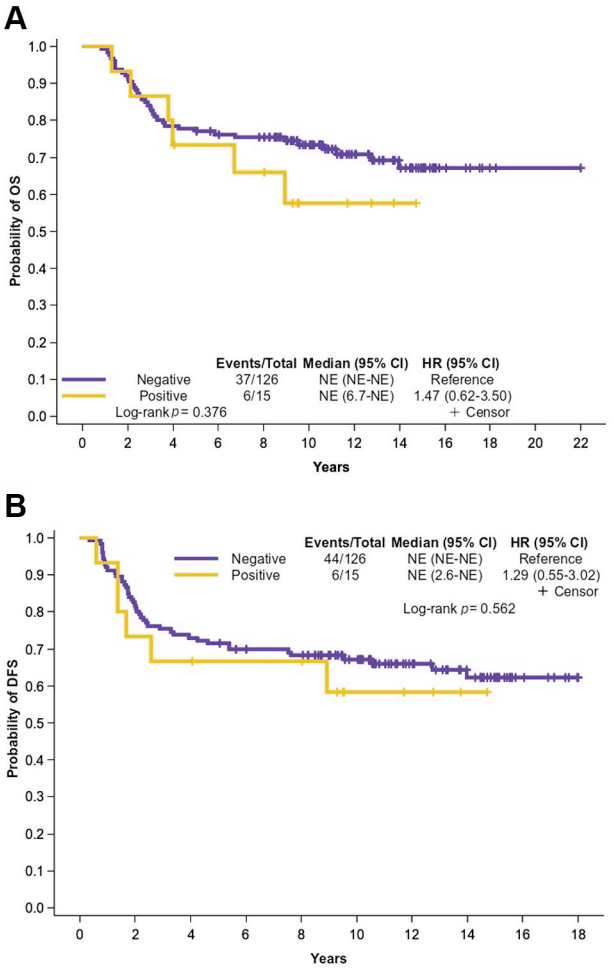

Figure 3. *Kaplan–Meier curves based on human proviral integration site for Moloney murine leukemia virus-1 (PIM1) expression with respect to overall (OS) (A) and disease-free (DFS) (B) survival for the entire cohort. CI: Confidence interval; HR: hazard ratio; NE: not estimable.*

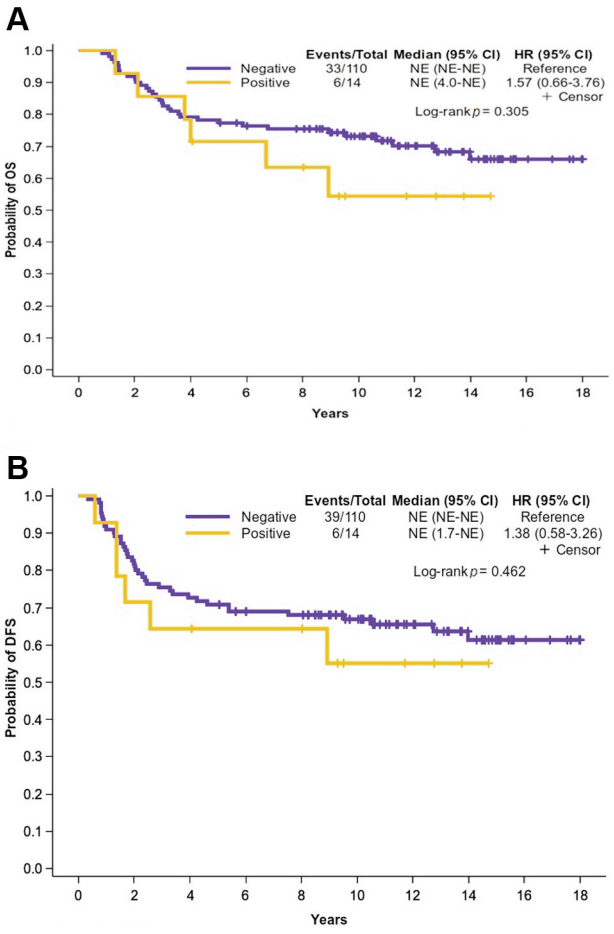

Figure 4. *Kaplan–Meier curves based on human proviral integration site for Moloney murine leukemia virus-1 (PIM1) expression with respect to overall (OS) (A) and disease-free (DFS) (B) survival for patients with basal-like tumors. CI: Confidence interval; HR: hazard ratio; NE: not estimable.*

disregarded as a unique molecular TNBC subtype with distinct features (36); it comprises 80% of TNBCs, mostly exhibits aggressive basal-like characteristics, and is often associated with worse prognosis in contrast to that of AR-positive TNBC (37, 38). In comparison to TNBC, molecular alterations between primary and metastatic paired samples during monitoring of disease revealed a more stable molecular profile of the QNBC subtype (39) and patients with QNBC seemed to be more sensitive to chemotherapy with long-term outcomes and longer DFS (37). However, treatment options are still restrictive and thus pathway proteins uniquely expressed in QNBC may serve as effective therapeutic targets (36).

Our study focused on the mRNA levels of *AR* in a cohort of 141 patients with early-stage TNBC. According to our results, 38.3% of TNBC cases were classified as AR-positive, a percentage in line with other studies (11, 40). We evaluated the possible associations between *AR* expression and selected clinicopathological features. The only significant association found pertained to menopausal status. In particular, the AR-positive rate was significantly higher in postmenopausal women, in agreement with results of a previous study (41). Another important clinicopathological characteristic which is also mentioned in previous published data is that a marginally higher percentage of basal-like tumors was found in AR-negative [79/87 (91.9%)] than in AR-positive [45/54 (84.9%)] cases (42).

Furthermore, we investigated the prognostic role of *AR* expression in this cohort of patients. *AR* expression did not show prognostic significance in terms of OS. Similarly, no prognostic significance was observed for DFS among the patients with early-stage TNBC. *AR* expression was further

evaluated in a subgroup of basal-like tumors in patients with early stage TNBC but no prognostic significance was found for OS and DFS.

With respect to AR expression and survival outcomes, our results are consistent with the conflicting evidence about the prognostic role of AR in TNBC (43). In a recent review and meta-analysis, Xu *et al*. extracted and statistically analyzed data from various studies on AR prognostic significance in TNBC and concluded that there was no correlation of AR status with any primary (OS or DFS) or secondary end point (distant DFS or recurrence-free survival) (44). Interestingly, a recent multi-institutional study revealed population-specific differences in prognostic patterns of AR positivity (45). In a study that compared AR expression between White women and African American women, the latter showed lower expression of AR (46). Both studies indicate that discrepancies in results might be attributed to different ethnic or racial characteristics.

In a smaller cohort of the studied patients with TNBC (n=31), we compared AR protein expression as assessed by immunohistochemistry with RT-qPCR results for *AR* mRNA expression and found significant correlation (Fisher's p=0.001). Despite the limited number of samples, we therefore suggest that RT-qPCR assay may successfully be used for the evaluation of *AR* expression in TNBC tumors.

We also evaluated the expression of *PIM1* in this cohort of patients with TNBC based on previous evidence that PIM1 plays a crucial role in TNBC biology (24). According to our results, only a small number of patients was found to be positive for *PIM1* expression (10.4%) and all of these had basal-like TNBC tumors, consistent with previous data which indicate increased *PIM1* expression in these breast cancer tumors (26). In terms of prognosis, no significant correlation was found between *PIM1* expression and OS or DFS either in the entire cohort or those with early-stage disease. On the contrary, according to published data, *PIM1* was a factor of poor prognosis in terms of diminished recurrence-free and distant metastasis-free survival in patients with hormone receptor-negative tumors and indirectly through its implication in c-MYC activity (25). Further studies on TNBC should clarify its prognostic role. Many studies based on *PIM1* functions indicate that *PIM1* might serve as a novel promising therapeutic target (23). *PIM1* inhibition in mouse models was already shown to have no significant effects on growth and reproduction, and in combination with other chemotherapeutic regimens enhanced chemosensitivity (25, 26), further enhancing the potential of PIM1 as a new targeted biomarker.

Based on previous evidence on PCa, PIM1 kinases phosphorylate AR, thus regulating its transcriptional activity, independently of androgens. This mechanism seems to play a crucial role during androgen deprivation therapy, thus co-targeting of AR and PIM1 could prove to be a promising therapeutic approach for resistant PCa (27). In our study, seven patients out of 141 (5.0%) carried tumors with positive expression of both *AR* and *PIM1*. However, since the number of these patients was very small, we cannot provide any evidence on these potential interacting partners in TNBC and therefore we suggest that extensive research should investigate their simultaneous activity, especially during therapy.

Of note, many discrepancies between the results of various studies regarding the prognosis can be attributed to staining and scoring methods, different AR antibodies used, small sample sizes and heterogeneous patient cohorts (39). An important limitation of our study was that *AR* and *PIM1* expression was tested on formalin-fixed paraffin-embedded tumor samples that are in most cases of poor RNA quality, as verified by the exclusion of 50 samples. We thus suggest further analysis of *AR* and *PIM1* expression in fresh frozen tissues of patients with TNBC.

There is still an unfilled gap in therapeutic options for TNBC since chemotherapy is the mainstay of treatment following surgery. Taking into consideration that patients with TNBC have unique molecular signatures, the discovery of novel biomarkers is important for molecular targeted therapies. With this in mind, in the present study, we studied *AR and PIM1* expression in TNBC, based on: a) the fact that therapies targeting *AR* expression are now being validated in patients with TNBC, b) evidence showing that *PIM1* plays a crucial role in TNBC biology, and c) interactions between AR and PIM1 play a crucial role during androgen deprivation therapy and thus co-targeting of AR and PIM1 might improve therapeutic effects. Our results indicate that co-expression of these biomarkers exists only in a small percentage of cases of TNBC. The implications of this finding in the therapeutic management of patients with TNBC should be investigated in larger patient cohorts.

Conclusion

AR expression defines a subgroup of TNBC that could benefit from AR-targeted therapies, thus the identification of AR levels in these patients is crucial. We suggest that further clinical trials based on validated assays should be held to provide reliable data correlated with the prognostic significance of AR in TNBC and that AR expression based on validated assays clearly discriminate patients with AR-positive TNBC from those with AR-QNBC and thus, contribute to clinician's therapeutic decisions. Furthermore, we suggest that extensive clinical trials should be carried out encompassing large cohorts of patients with TNBC to further test the utility of *PIM1* as a potential biomarker by providing additional data correlated with its diagnostic and prognostic significance.

Conflicts of Interest

There are no conflicts of interest to declare for all Authors in regard to this study.

Authors' Contributions

Conceptualization: EL, GF and VK. Methodology EL, VK, AN and AS. Validation: EL, AN and AS. Formal analysis: GAK. Investigation: AN and AS. Resources: FZ, DP, GP, CC, HG, CM, CP, PK, GA and GF. Data curation: AN and AS. Writing - Original Draft: AN and EL. Writing - Review and editing: All Authors. Supervision: EL, VK and GF. Funding Acquisition: EL, GF and VK.

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