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Correlation of HLA-A and HLA-B/C Expression With PD-1 and PD-L1 Expression in Patients With Metastatic Breast Cancer as a Potential Prognosticator of Favorable Survival

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Abstract

Background/Aim: Class 1 human leukocyte antigen (HLA) ensures that cytotoxic T lymphocytes (CTLs) attack tumor cells. As part of tumor de-differentiation, the expression of HLA on the tumor cell surface may decrease, which can facilitate tumor growth. Therefore, reduced expression of HLA is generally negatively associated with overall survival (OS). The reverse is true for programmed cell death protein (PD-1) and programmed death ligand 1 (PD-L1). The presence of PD-1 and PD-L1 on the surface of cancer cells inhibits immune defense mechanisms against cancer cells. Therefore, one would expect that increased PD-1/PDL-1 expression would result in decreased 5-year survival. The aim of this study was to correlate the expression levels of HLA-A and HLA-B/C with the expression levels of PD-1 and PDL-1 to evaluate the reliability of their prediction of 5-year OS.

Materials and Methods: This study retrospectively examined patients upon the start of a new therapy line for metastatic breast cancer (MBC). The pilot cohort fulfilling very demanding inclusion criteria consisted of 34 patients. The diagnostics were primarily carried out using RT-qPCR.

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Results: The expression of HLA-A, HLA-B/C, PD-1, and PD-L1 is not significantly associated with OS.
Conclusion: This pilot study found no significant association between HLA-A, HLA-B/C, PD-1, or PD-L1 expression and OS in MBC, indicating limited prognostic value for these biomarkers in this cohort.

Keywords: Breast cancer, HLA-A, HLA-B/C, PD-1, PD-L1, prognosticator, survival.

Introduction

Breast cancer is one of the most common cancer in women worldwide and is responsible for most cancer-related deaths in women (1, 2). The incidence rates continue to rise, especially among young women (3, 4). The prognosis, especially in the metastatic situation, remains unsatisfactory, despite scientific and therapeutic progress, particularly with regard to biological non-cytotoxic therapies and earlier and comprehensive diagnostics including nation-wide screening programs (5-8).

This study aimed to identify an approach to improve prognosis estimation and develop a diagnostic tool that can better predict overall survival (OS) in women with metastatic breast cancer. For this purpose, the expression levels of HLA-A, HLA-B/C, PD-1, and PD-L1 were examined for their possible association with the patients' OS to determine whether a combination of these parameters could enable valid prediction of OS.

HLA are surface proteins whose task is to recruit cytotoxic T lymphocytes (CTLs) through the presentation of tumor antigens, which in turn can attack and destroy tumor cells (9-13). The higher the HLA expression, the more CTLs are recruited, thereby inhibiting tumor progression (10, 14). However, the processes of tumor de-differentiation, clonal progression, and/or tumor intrinsic phenotype heterogeneity can lead to a reduction in HLA on the tumor surface, or even complete loss of HLA expression (15-17). PD-1 and PD-L1 are also surface proteins found on tumor cells (18). They also regulate the immune response, but in the opposite way to HLA (19). Increased expression of PD-1/PD-L1 promotes tumor growth by inhibiting the immune response (20-22). With increased PD-1/PD-L1 expression one would expect a negative association with OS.

Materials and Methods

This is a retrospective subgroup analysis of the initial prospective, double-blind cohort study in which 34 patients who were treated for metastatic breast cancer at the German National Center for Tumor Diseases (NCT) at the University of Heidelberg, Germany, between March 2010 and May 2015 were analyzed.

RT-qPCR technology was used in order to determine the expression levels of HLA-A, HLA-B/C, PD-1, and PD-L1 in breast cancer cells, while utilizing *CALM2* as the house-keeping gene, according to established protocols (16). The measurements were taken in distant metastatic tissue. Receiver operating characteristic (ROC) curves were constructed to assess the sensitivity and specificity of the analyzed parameters in relation to their predictive value for patients' OS. To this end, Youden J points were calculated for the ROC curves with consecutive determination of sensitivity and specificity at the respective J points. All statistics were calculated using R (R Foundation for Statistical Computing, Vienna, Austria, version 3.1.2). Additionally, Kaplan-Meier curves were determined in order to evaluate the impact of individual parameters on OS. The relative gene expression was presented as 40-ΔΔCT with higher values corresponding to higher mRNA counts in the tissue biopsy sample. Details on progression restaging regimen, double-blinding, utilization of electronic health records and patient demographics have been extensively reported on in recent publications of our collaborative group (23-26).

Results

A total of 34 patients were included in the study. The median age of the patients at initial diagnosis was 50 years and at inclusion in the study it was 55 years. Distant

Table I. *Patient characteristics.*

Patient characteristics	N	Value
Age at initial breast cancer diagnosis, median (range), years	34	50 (34-73)
Age at study enrolment, median (range), years	34	55 (42-80)
OS after breast cancer diagnosis, median (range), months	34	114 (2-260)
OS after study enrolment, median (range), months	34	33.5 (1-156)
OS longer than 3 years, frequency (%)	34	16 (47.1%)
OS longer than 5 years, frequency (%)	33	10 (30.3%)
Death until end of study or loss to follow-up, frequency (%)	34	29 (85.3%)

OS: Overall survival.

Table II. *Tumor characteristics.*

Tumor characteristic	N	Value
Age at initial BC diagnosis, median (range), years	34	50 (34-73)
Age at study enrolment, median (range), years	34	55 (42-80)
Metastasis site		
Bone, frequency (%)	34	7 (20.6%)
Liver, frequency (%)		5 (14.7%)
Lung, frequency (%)		2 (5.9%)
Brain, frequency (%)		2 (5.9%)
Other, frequency (%)		18 (52.9%)
Tumor grade		
Grade 1, frequency (%)	34	16 (47.1%)
Grade 2, frequency (%)		9 (26.5%)
Grade 3, frequency (%)		0 (0%)
Unknown, frequency (%)		9 (26.5%)
Tumor subtype		
Luminal A, frequency (%)	34	12 (35.3%)
Luminal B, frequency (%)		13 (38.2%)
Triple negative, frequency (%)		7 (20.6%)
Unknown, frequency (%)		2 (5.9%)
PD-L1 40-ΔΔCT, median (range)	34	30.4 (19-33.8)
PD-1 40-ΔΔCT, median (range)	34	34.7 (15-36.8)

BC: Breast cancer; PD-L1: programmed death ligand 1; PD-1: programmed cell death protein.

metastases were most commonly found in the bones, followed by the liver, lungs and brain. The most common mRNA-based intrinsic tumor subtype was luminal B, followed by Luminal A and triple negative breast cancer. The median OS after study enrolment was 33.5 months, and over 47% of patients had OS of more than three years. Over 85% of the patients died by the end of the study or were lost to follow-up. Table I and Table II provide a detailed list of the patients and tumor characteristics, respectively.

Figure 1 shows the Kaplan-Meier curves for the OS based on PD-1, PD-L1, HLA-A, and HLA-B/C, showing no association with OS. Figure 2 shows the ROC curves for HLA-A, HLA-B/C, PD-1, and PDL-1. The ROC curves provide a graphical representation of the diagnostic value of the expression of the individual parameters in relation to OS. The effective values for the sensitivity and specificity of the individual parameters are listed separately in Table III.

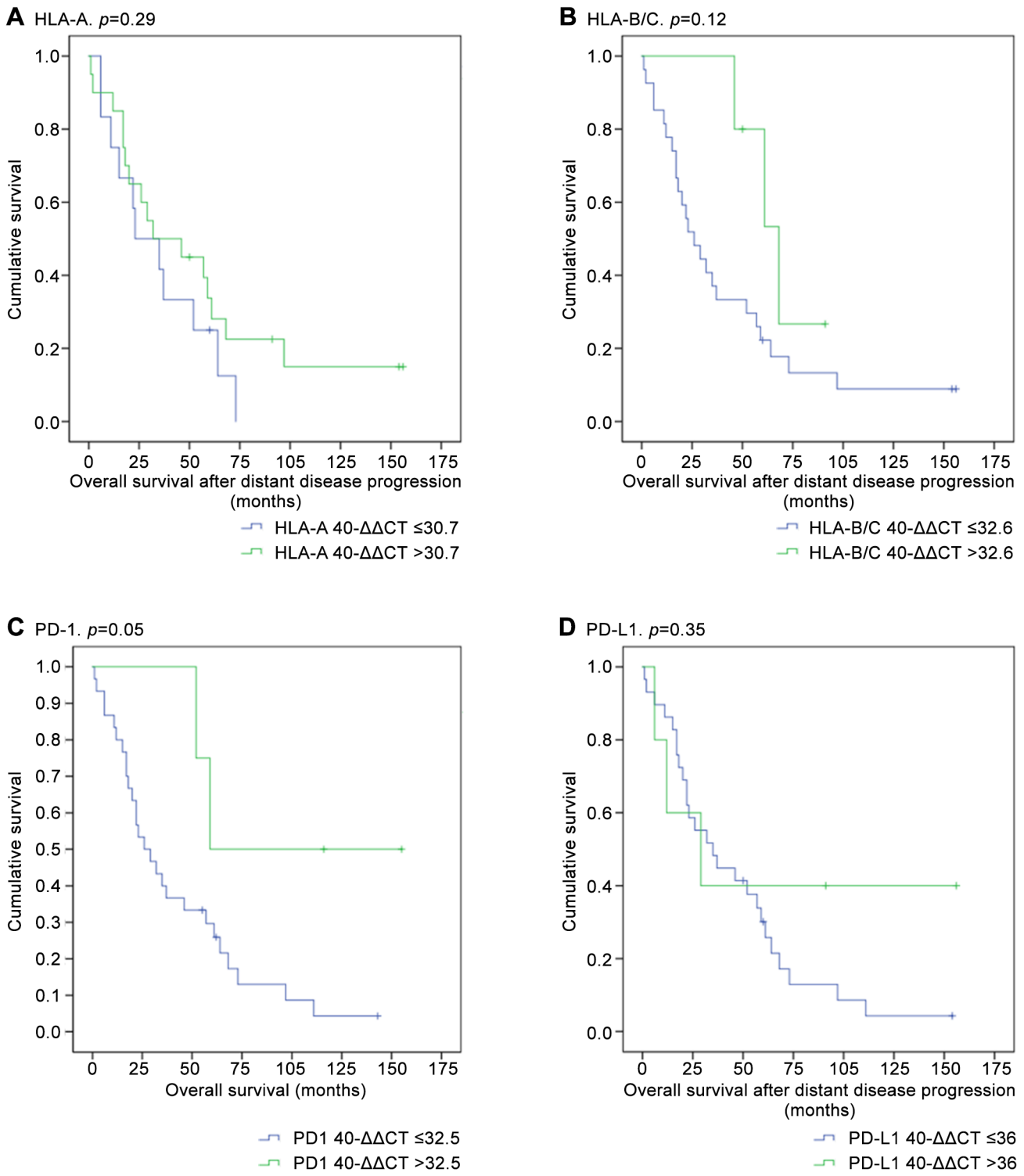

Figure 1. *Kaplan-Meier analysis of overall survival after study enrolment based on mRNA expression of: A) Class 1 human leukocyte antigen (HLA)-A, B) HLA-B/C, C) programmed cell death protein 1 (PD-1), and D) programmed death ligand 1 (PD-L1).*

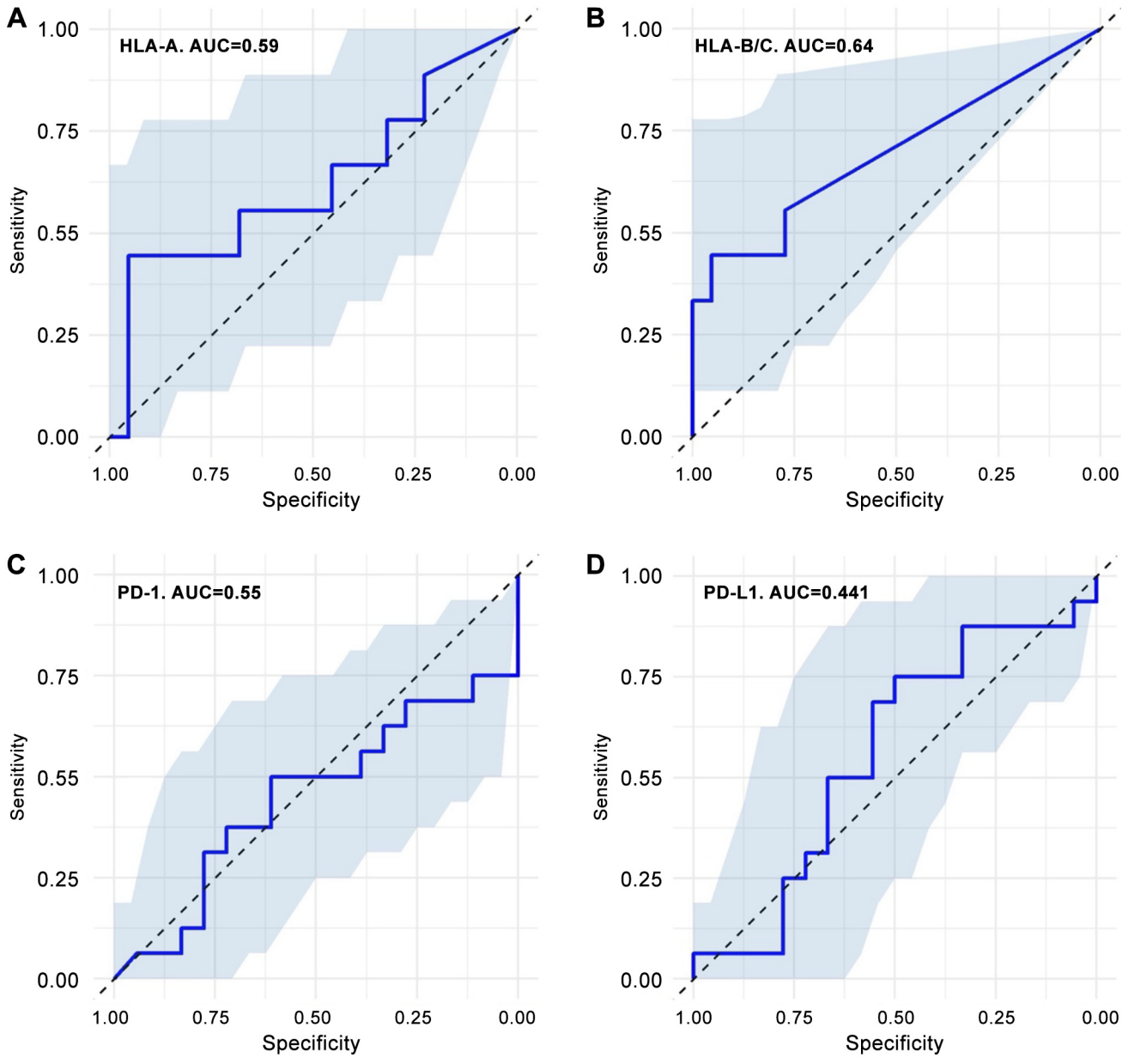

Figure 2. *Receiver operating characteristic (ROC) curves for detection of overall survival (OS) post study enrolment representing the sensitivity and specificity of A) Class 1 human leukocyte antigen (HLA)-A (5-year-OS; n=31), B) HLA-B/C (5-year-OS; n=31), C) programmed cell death protein 1 (PD-1) (3-year-OS; n=34), and D) programmed death ligand 1 (PD-L1) (3-year-OS; n=31) expression. The light blue areas signify the 95% confidence interval.*

Discussion

It is well known that the expression of HLA-A and HLA-B/C on tumor cells promotes the destruction of tumor cells by cytotoxic T cells through the presentation of tumor antigens. This is a key process in controlling tumor growth. The reduction of HLA expression to the point of complete loss as part of tumor de-differentiation can lead

Table III. *The effective values of sensitivity and specificity for HLA-A, HLA-B/C, PD-1, and PD-L1.*

Cut-off value determination strategy	Cut-off value (40-ΔΔCT)	Sensitivity	Specificity
PD-1 (3-year OS)			
Youden J point	30.3	50%	61.1%
Optimized for maximum sensitivity	32.5	75.5%	5.5%
Optimized for maximum specificity	23.7	6.3%	94.4%
PD-L1 (3-year OS)			
Youden J point	34.9	75%	50%
Optimized for maximum sensitivity	36	93.8%	5.6%
Optimized for maximum specificity	32	6.3%	94.4%
HLA-A (5-year OS)			
Youden J point	33.6	42.9%	95.5%
Optimized for maximum sensitivity	30.7	71.4%	31.8%
Optimized for maximum specificity	33.6	42.9%	95.5%
HLA-B/C (5-year OS)			
Youden J point	32.6	42.9%	95.5%
Optimized for maximum sensitivity	32.6	42.9%	95.5%
Optimized for maximum specificity	32.6	42.9%	95.5%

OS: Overall survival; HLA: human leukocyte antigen; PD-L1: programmed death ligand 1; PD-1: programmed cell death protein.

to exponential tumor growth because the cytotoxic T cells can no longer adequately attack the tumor cells. It was therefore previously assumed that reduced expression of HLA on tumor cells had a negative influence on tumor development and overall outcome (9-12).

In the case of HLA-A, HLA-B/C, PD-1, and PDL-1, no statistically significant association was found with OS in this cohort. The aim of this study was to examine HLA-A, HLA-B/C, PD-1 and PD-L1 for their association with OS and, if there is a statistically significant association, to correlate the parameters and generate an improved forecast estimate than each parameter alone can do. Since the examined parameters do not appear to have a statistically significant influence on OS in this collective, a combination of those parameters does not make sense, as this cannot lead to an improved prognostic assessment of OS. It remains unclear whether the lack of association between the examined parameters and OS reflects a true absence of connection or is distorted by the small cohort size, leading to non-significant results. Therefore, for the time being it remains to be noted that HLA-A and HLA-B/C as well as PD-1 and PD-L1 could be a useful tool for prognosis assessment. This leads to the limitations of this study. Basically, the main weakness of this study is the very small cohort of 34 patients. However, the methods applied in this study are resource-consuming, including mRNA-based determination of intrinsic tumor subtypes and HLA molecules of several classes. Another limitation of this study is the heterogeneous patient population, especially the fact that the patients were in different stages of therapy, which could potentially lead to a bias regarding OS. The distribution of the different therapy stages in the overall cohort has already been described elsewhere and can be transferred to this subgroup analysis (24). This is a subgroup analysis of a prospective, double-blind study, but to obtain fully reliable results it will be necessary to carry out a large-scale multi-center study that examines a significantly larger number of patients, which not least allows for a more differentiated subgroup analysis according to the therapy stage in which the patients are, because due to the small cohort in this study, a subgroup analysis divided according to the therapeutic stage in which the patients were was not possible. In addition, the combination of HLA with markers other than PD-1 and PD-L1 should be considered to improve prediction.

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Conclusion

This proof-of-concept study found no significant associations between HLA-A, HLA-B/C, PD-1, and PDL-1 with OS in metastatic breast cancer. Whether this reflects a true lack of influence or is due to the small cohort size and demanding methods remains unclear, warranting further investigation.

Conflicts of Interest

R. W. is an employee of Stratifyer Molecular Pathology GmbH. All other Authors have nothing to disclose in relation to this study.

Authors' Contributions

Conception and design of the study: J.H., S.S., M.W.; Data collection: R.W., A.S., T.M.D.; Data analysis & interpretation: U.K., S.S., A.S., L.G.; Statistical analysis: U.K., L.G.; Manuscript preparation phase 1 - drafting the article: L.G. S.S., U.K., M.K.; Manuscript preparation phase 2- revising it critically for important intellectual content: M.W., M.S., R.W., J.H.; Final approval of the version to be submitted: all Authors.

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