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Original Research Article

PD-1 and PD-L1 expression predict regression and prognosis following neoadjuvant radiochemotherapy of oesophageal adenocarcinoma

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ABSTRACT

Background and purpose: PD-1 and PD-L1 are involved in anticancer immunosurveillance, and their expression may be predictive for therapeutic effectiveness of specific antibodies. Their influence on response to neoadjuvant radiochemotherapy (RCT) and prognosis in patients with oesophageal adenocarcinoma (OAC) remains to be defined.

Materials and methods: Between 10/2004 and 06/2018, complete pre-RCT biopsy-specimens were available from 76 patients with locally advanced, non-metastatic OAC scheduled for trimodality therapy. We evaluated intra- and peritumoural expression of CD8, PD-1 and PD-L1 in pre-treatment specimens to determine their influence on tumour regression grade and survival. PD-1 and PD-L1 expression were considered positive (+) if ≥1% of all cells were stained positive, otherwise negative (-); densities of CD8+ cells were categorized as being high (Hi) or low (Lo) according to the median.

Results: A negative PD-L1 expression in peritumoural cells predicted a poor tumour regression (RD 0.24 [95% CI 0.03–0.44], p = 0.023). A positive PD-1 expression in intra- as well as peritumoural cells was identified as an unfavourable prognostic factor (HR 0.52 [95% CI 0.29–0.93], p = 0.028; HR 0.50 [0.25–0.99], p = 0.047, respectively). With respect to CD8+ infiltration, positive PD-1 and PD-L1 expressions attenuated its favourable prognostic effect in intratumoural area (LoCD8/PD1 + vs. HiCD8/PD1-: HR 0.25 [0.09–0.69], p = 0.007; LoCD8/PDL1+ vs. HiCD8/PDL1-: HR 0.32 [0.12–0.89], p = 0.028) and were associated with negative outcome when seen in peritumoural area (HiCD8/PD1+ vs. LoCD8/PD1-: HR 0.29 [0.11–0.74], p = 0.010); HiCD8/PDL1+ vs. LoCD8/PDL1-: HR 0.33 [0.12–0.90], p = 0.031).

Conclusions: PD-1 and PD-L1 expression were identified to be of predictive and prognostic value in patients with OAC, particularly when considering CD8+ infiltration. Further validation by a large size dataset is required.

Introduction

Trimodality treatment, i.e. neoadjuvant radiochemotherapy (nRCT) followed by surgery, is considered the standard treatment for advanced, non-metastatic adenocarcinoma of the oesophagus and oesophagogastric junction. Specific advantages of including radiotherapy within the combination of treatments are supported by several evidence-based aspects: R0-resection rates, which are the basis for long-term survival, were clearly enhanced by nRCT (92% vs. 69%) as shown by the CROSS trial [1], but not improved by neoadjuvant chemotherapy (69% vs. 66%) within the MAGIC trial [2]. Similar data have been seen when contemplating pCR-rates. In addition, a recent *meta*-analysis provided evidence that nRCT alone resulted in better overall survival than nRCT and surgery in patients with complete response [3]. However, about one fifth of the patients will have only minor or no response to nRCT [1,4]. To preserve this subgroup from unnecessary toxicity it seems of paramount clinical interest to identify biomarkers in biopsies taken before radiochemotherapy (RCT) which can predict the response to RCT and

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Table 1
Patient characteristics.

	All patients	Patients with surgery	Patients w/o surgery	p-value[1]
Number	76 (100%)	58 (100%)	18 (100%)	
Gender				
Female	16 (21%)	11 (19%)	5 (28%)	
Male	60 (79%)	47 (81%)	13 (72%)	n.s.[2]
Age				
Mean (±SD) - years	66.4 (±10.5)	64.2 (±9.5)	73.5 (±10.9)	**p = 0.001**[3]
Range - years	44.3–86.5	44.3–85.4	48.4–86.5	
Staging pre-Tx UICC				
I	0 (0%)	0 (0%)	0 (0%)	
II	4 (5%)	3 (5%)	1 (6%)	
III	70 (92%)	53 (91%)	17 (94%)	
IV	2 (3%)	2 (3%)	0 (0%)	n.s.[2]
cN+	64 (84%)	48 (83%)	16 (89%)	n.s.[2]
Follow up *				
All patients				
Median (IQR) - months	18 (9–43)	22 (8–62)	16 (13–21)	n.s.[4]
Patients being alive, number =	24 (32%)	20 (34%)	4 (22%)	n.s.[2]
Median (IQR) - months	54 (25–97)	68 (34–111)	15 (14–21)	**p = 0.013**[4]
Resection quality				
R0		51 (88%)		
R1		6 (10%)		
R2		1 (2%)		
TRG (Mandard)				
1		20 (34%)		
2		22 (38%)		
3		5 (9%)		
4		9 (16%)		
5		2 (3%)		

w/o without, *n.s.* not significant, *SD* standard deviation, *IQR* interquartile range, *Tx* therapy, *UICC* International Union against Cancer, *TRG* tumour regression grade
* Last verification: 2019/04/01
[1]p-value for the difference between patients with and without surgery
[2] Fisher's exact test, [3] Student's *t*-test, [4] Mann-Whitney-*U* test

may influence the prognosis of the patients.

Tumour infiltrating inflammatory cells (TIC) like tumour infiltrating lymphocytes (TIL) and tumour associated macrophages (TAM) play a key role in anticancer immunosurveillance. Recently, we could identify immunologic parameters, such as CD8+ and FoxP3 + -TIL as well as CD68 + and CD163 + TAM, as being of independent predictive and prognostic value in patients with locally advanced, non-metastatic oesophageal adenocarcinoma (OAC) scheduled for trimodality therapy (RCT followed by surgery) [4]. Here, we present the impact of PD-1 and PD-L1 expression on response to RCT and prognosis of the same cohort of patients.

PD-1 is an inhibitory receptor on T-cells, and its main function is to act as an immune checkpoint receptor to terminate immune response. PD-L1 and PDL-2 are the ligands for PD-1. They are expressed on antigen presenting cells, like dendritic cells, and on a wide variety of non-hematopoietic cell types, like vascular endothelial cells. However, mainly PD-L1 is also expressed in several cancer types which typically escape immune elimination by orchestrating an immunosuppressive microenvironment [5,6]. In the last years, treatment with PD-1 and PD-L1 antibodies led to a substantial progress in anticancer therapy of many tumour entities. Several studies also investigated PD-1 and PD-L1 antibody treatment in gastric carcinoma and adenocarcinoma of the oesophagogastric junction (AEG) [7,8], and based on KEYNOTE-590 [9] and on CheckMate 649 [10], the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) approved Pembrolizumab and Nivolumab as first-line therapy for this indication under certain conditions in 2021.

Outcomes considering the influence of PD-1 and PD-L1 expression on prognosis of patients with OAC are conflicting [11], and data derived from pre-treatment biopsies with possible predictive or prognostic value are rare. Moreover, there is uncertainty about a reasonable threshold to classify patients into PD-1- and PD-L1-positive and negative groups with respect to prognosis. The herein reported study was an explorative analysis based on a consecutive series of patients with locally advanced, non-metastatic OAC that were prospectively treated by nRCT and radical surgery. It was the aim of this study to elucidate the impact of PD-1 and PD-L1 expression in pre-RCT biopsies on response to RCT and prognosis of the patients.

Patients and methods

Definitions and categories are summarized in Supplementary Table 1.

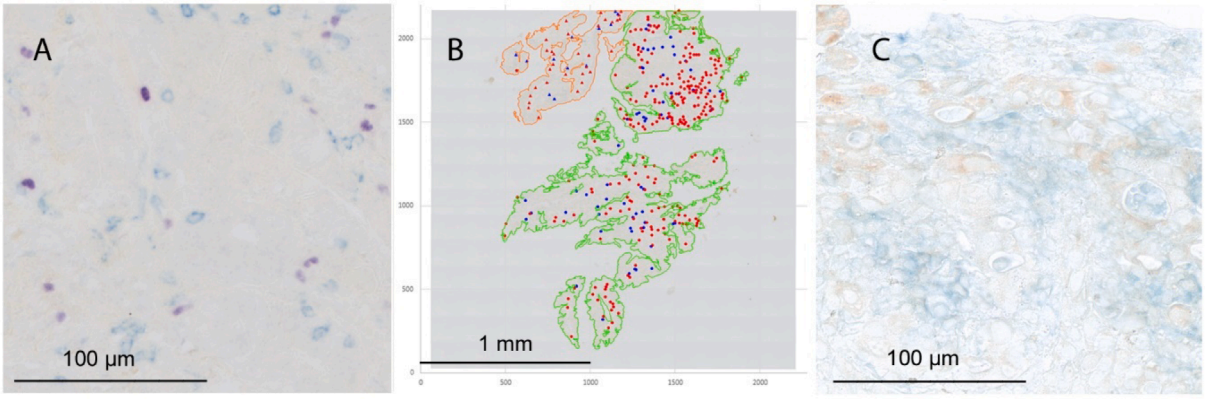


Fig. 1. Evaluation of FoxP3+ and CD8+ tumour infiltrating cells, and PD-1 and PD-L1 expression in adenocarcinoma of the oesophagus and the oesophagogastric junction **A:** Double staining of FoxP3+ (violet) and CD8+ (blue) tumour infiltrating lymphocytes (400x original magnification). **B:** Evaluation of a FoxP3+/CD8+ sample; green lines: surroundings of tumoural compartment; orange lines: surroundings of peritumoural compartment; red markers: FoxP3 + cells; blue markers: CD8+ cells; circles in tumoural compartment and triangles in peritumoural compartment. **C:** Double staining of PD-L1 (blue) and PD-1 (brown) expression.

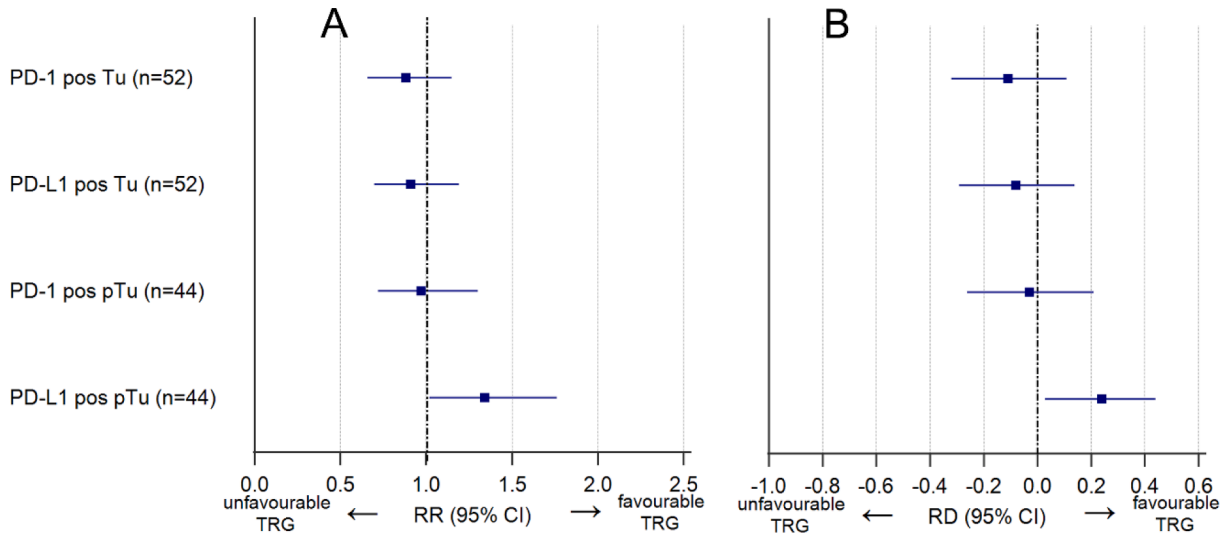


Fig. 2. Pretherapeutic PD-1 and PD-L1expression with possible impact on favourable tumour regression after RCT, forest plots of RR (risk ratio) (**A**) and RD (risk difference) (**B**).RCT radiochemotherapy, TRG tumour regression grade, RR risk ratio, RD risk difference, CI confidence interval, Tu in tumoural area, pTu in peritumoural area Favourable TRG: Mandard 1–3 vs. Mandard 4&5 Results of risk analysis, Pearson's chi-squared test and two tailed z-test.

Patient cohort

Patient characteristics, and details of diagnostic and therapeutic procedures were described elsewhere [4,12]. Briefly, a total of 106 patients with locally advanced OAC or AEG [13] without distant metastases were treated by trimodality therapy. Pretherapeutic biopsies were available from 76 patients (tumoural compartment: 71 specimens, peritumoural: 57), of whom 58 patients underwent radical oesophagectomy by laparatomy and right-sided thoracotomy followed by an immediate intrathoracic gastrooesophageal anastomosis (Ivor-Lewis-procedure [14]). Eighteen patients were not eligible for surgery or refused the procedure (Table 1).

Tumour regression grading (TRG) was categorized according to Mandard (1: complete regression; 2: rare residual cancer; 3: increased number of residual cells, predominantly fibrosis; 4: residual cancer outgrowing fibrosis; 5: no regressive changes) [15].

Informed consent was obtained from all living patients, and the study was approved by the Ethics Committee of the University-Hospitals of Erlangen *(No. 133_17B)*.

Immunohistochemistry and evaluation of TIC

As described in Göbel et al. [4], tissue microarrays (TMA) with a core diameter of 2 mm were constructed from pretherapeutic biopsies and, if available, from resection specimens according to the original HE and immunohistologically stained slides. One of neighbouring histological sections was HE stained, others were double stained using antibodies against CD8/FoxP3 (Dako/Abcam, Fig. 1A) – to determine the CD8+ density in the context of this study –, and PD-1/PD-L1 (Cell Marque/Abcam, Fig. 1C), additionally. Tumoural and peritumoural compartments were marked separately according to the neighbouring HE stained section, their sizes were calculated automatically, TIC were identified semiautomatically (Fig. 1B).

For PD-1 and PD-L1 categorisation, we used a modified score referred to the combined positive score (CPS) which is defined as number of PD-L1 positive tumour cells, lymphocytes, and macrophages divided by the total number of viable tumour cells and multiplied by 100. Because tumour cells are absent in the peritumoural area, we instead evaluated the percentages of positive cells related to the total number of cells for a better comparison. Category 0 to 3 were defined as an estimated percentage of <1%, ≥1% to <10%, ≥10% to <50%, and ≥50%, respectively. According to survival analysis, a percentage of <1% was considered as negative, of ≥1% as positive (Fig. 3). At least in tumoural area, our modified score of PD-L1 should be in good approximation to the widely used CPS of PD-L1.

Statistical analysis

Statistical analysis was performed using MedCalc Statistical Software version 20.014 (MedCalc Software bvba, Ostend, Belgium; 2021) and PAST Paleontological Statistics version 3.25 (Oslo, Norway; 2019) [16]. The Shapiro-Wilk test was used to test for normal distribution of the variables. Subgroups of patients were compared by Student's *t*-test, Fisher's exact test, Wilcoxon test and Mann-Whitney-*U* test. Influence of immunologic markers on TRG was estimated by risk analysis, Pearson's chi-squared test and ROC analysis. Correlation of PD-L1 and PD-1 expression were calculated by Spearman's rank-order correlation. Overall survival (OS), disease free survival (DFS) and no evidence of disease (NED) were analysed by Kaplan-Meier method. The starting point of event analysis was the date of biopsy. NED is defined by time to any event related to the same cancer (recurrence and death) [17]. Logrank test and Cox regression were used to compare survival between subgroups of patients. The proportional hazards assumption was verified by visual examination of the log-minus-log curves. Collinearity was suspected if the correlation coefficient between two independent variables was higher than 0.5. A p-value <0.05 was considered significant, adjusted by the Benjamini-Hochberg procedure to control the false discovery rate (FDR) of multiple hypothesis testing. For the analysis of TRG we tested the influence of PD-1 and PD-L1 expressions on TRG (two hypotheses), and for survival analysis we additionally hypothesized that the impact of PD-1 and PD-L1 expression on prognosis depended on CD8+ density (in summary, four hypotheses). This assumption seemed to be justified as we demonstrated in Göbel et al. [4] that CD8+ density might affect prognosis. The analyses were performed in the tumoural and in the peritumoural compartment separately. A not significant (n.s.) result in the Benjamini-Hochberg procedure was denoted as "FDR n.s."

Results

Tumour regression

Eighty-one percent of the patients experienced major response to RCT (Mandard regression score 1–3 vs. 4&5, Table 1). Albeit intratumoural CD8+ densities were higher in complete responders (Mandard

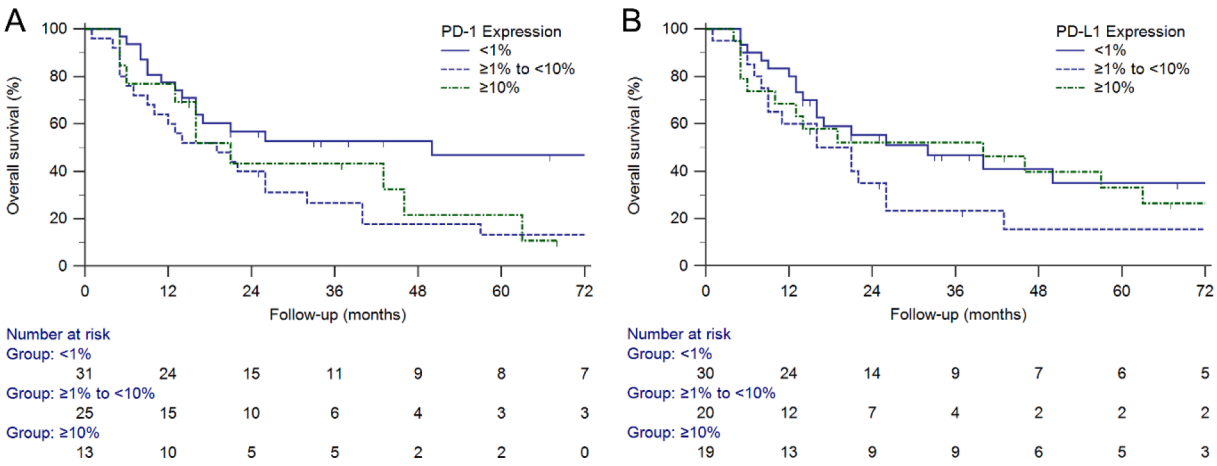


Fig. 3. Dependence of overall survival on PD-1 and PD-L1 expression in tumoural area **A:** Scores of PD-1 expression. **B:** Scores of PD-L1 expression. Expression of PD-1 and PD-L1 was estimated as the number of positive cells divided by the number of all cells in the area.

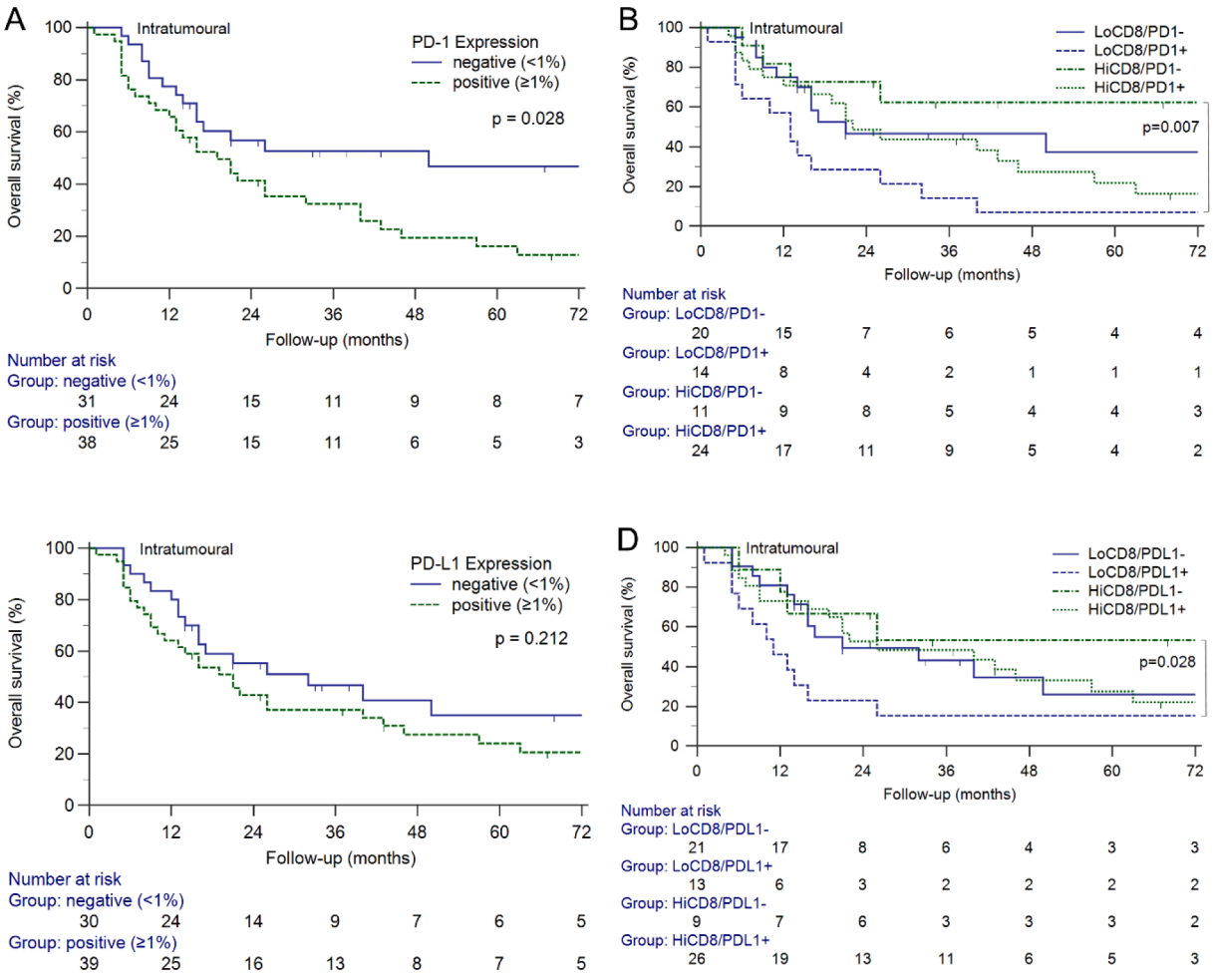


Fig. 4. Influence of PD-1 and PD-L1 expression in tumoural area on overall survival **A:** PD-1 expression, HR 0.52 (95% CI 0.29–0.93). **B:** PD-1 expression combined with CD8+ density, LoCD8/PD1 + compared to HiCD8/PD1-: HR 0.25 (0.09–0.69). **C:** PD-L1 expression, HR 0.69 (95% CI 0.39–1.23). **D:** PD-L1 expression combined with CD8+ density, LoCD8/PDL1 + compared to HiCD8/PDL1-: HR 0.32 (0.12–0.89). Results of logrank testHiCD8/LoCD8 high/low CD8+ density (median 124.3/mm^2), PD1-/PD1 + negative/positive PD-1 expression, PDL1-/PDL1 + negative/positive PD-L1 expression (threshold 1%).

1; median 163 cells/mm^2, 95% CI 60–203) than in non-complete responders (Mandard 2–5; median 110 cells/mm^2, 95% CI 64–138), the difference was not significant (p = 0.223, Mann-Whitney-*U* test). A favourable TRG was found in patients with a positive score of PD-L1 expression in the peritumoural area (RR 1.34 (95% CI 1.02–1.76), p = 0.036, FDR n.s.; RD 0.24 (95% CI 0.03 to 0.44), p = 0.023). PD-L1 expression in the tumoural area and PD-1 expression in both areas had no significant influence on TRG (Fig. 2). As only 11 patients experienced unfavourable TRG, we passed on subgroup analysis.

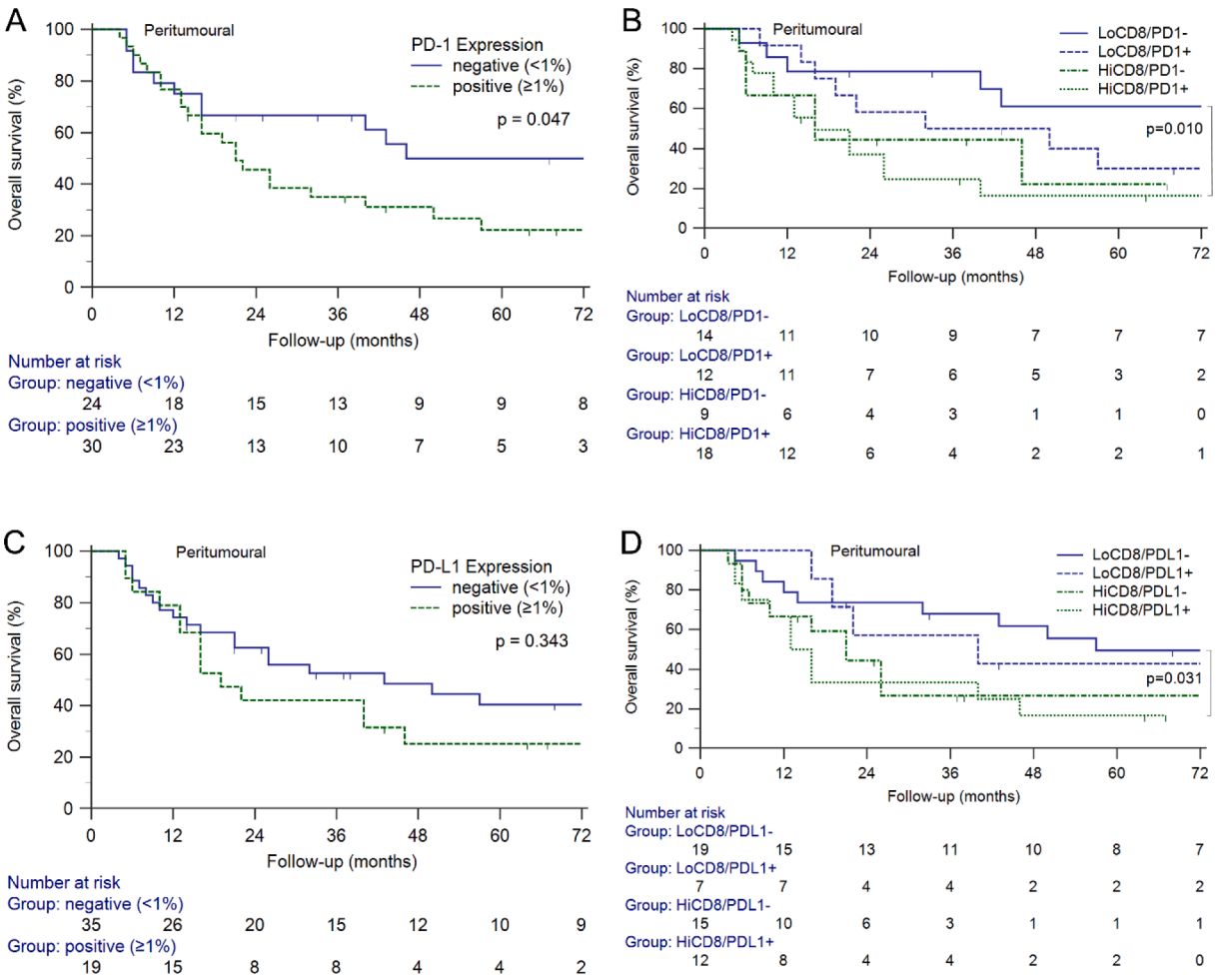


Fig. 5. Influence of PD-1 and PD-L1 expression in peritumoural area on overall survival **A:** PD-1 expression, HR 0.50 (95% CI 0.25–0.99). **B:** PD-1 expression combined with CD8+ density, HiCD8/PD1 + compared to LoCD8/PD1-: HR 0.29 (0.11–0.74). **C:** PD-L1 expression, HR 0.71 (95% CI 0.34–1.45). **D:** PD-L1 expression combined with CD8+ density, HiCD8/PDL1 + compared to LoCD8/PDL1-: HR 0.33 (0.12–0.90). Results of logrank test. HiCD8/LoCD8 high/low CD8+ density (median 132.2/mm^2), PD1-/PD1 + negative/positive PD-1 expression, PDL1-/PDL1 + negative/positive PD-L1 expression (threshold 1%).

Survival analysis

As described in Göbel et al. [4], five-year survival rates with regard to overall survival (OS), disease-free survival (DFS) and no evidence of disease (NED) of the whole cohort were 30%, 24% and 42%, respectively. Survival analysis comparing patients with and without surgery revealed no significant difference, neither in univariate nor in multivariate analysis adjusted for age and cN status (OS: p = 0.314, DFS: p = 0.505, NED: p = 0.208). Intratumoural PD-1 expression was significantly higher in patients with surgery compared to those without (p = 0.01), no significant difference was seen for peritumoural PD-1 expression and PD-L1 expression in both compartments. Overall survival was favourable with high amounts of intratumoural (p = 0.125) and low amounts of peritumoural CD8+ lymphocytes (p = 0.017).

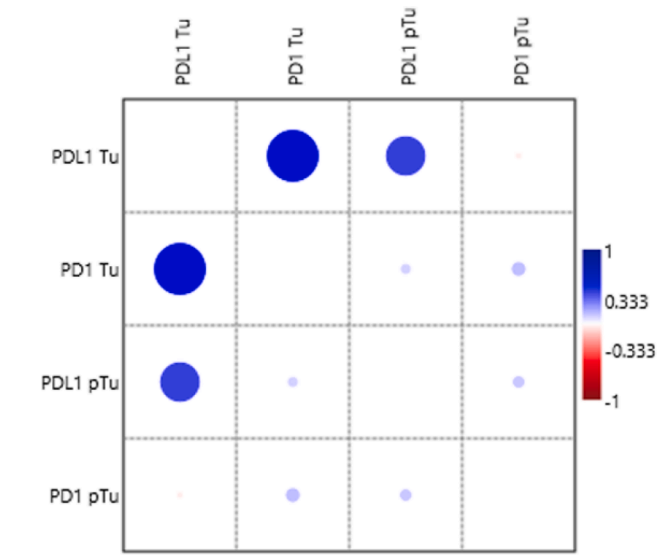
Considering recent clinical studies, as discussed later, and survival analysis of different classes of PD-1/PD-L1 expression in pre-treatment specimens, as shown in Fig. 3, it seemed reasonable to set the threshold of positive expression at 1%.

A negative staining of PD-1 (i.e. <1% of cells) within the tumour was associated with a significantly better prognosis (p = 0.028), whereas PD-L1 expression had no significant influence on outcome (p = 0.212). Taking into account the density of CD8+ TIL, best prognosis was seen in the group with high CD8+ density and negative PD-1 expression, worst prognosis in the group with low CD8+ density and positive PD-1 expression (p = 0.007). Similar effects were seen when combining CD8+ density and PD-L1 expression (p = 0.028). (Fig. 4). Analysis of DFS and NED survival supported the results of OS analysis, albeit not being significant considering multiple hypothesis testing (FDR n.s.) (Supplementary Fig. 1, Supplementary Fig. 4).

In the peritumoural area, again lack of PD-1 expression was linked to a favourable prognosis (p = 0.047, FDR n.s.), and PD-L1 expression had no distinct influence (p = 0.343). Regarding CD8+ density, a negative PD-1 or PD-L1 expression in a low CD8+ density environment was associated with a better prognosis than a positive PD-1 or PD-L1 expression in a high CD8+ density environment (p = 0.010 and p = 0.031, respectively, the latter FDR n.s.) (Fig. 5). Again, analysis of DFS and NED survival showed similar results (Supplementary Fig. 2, Supplementary Fig. 5).

There was a significant correlation between PD-1 and PD-L1 expression in tumoural area (r = 0.50, p = 0.001) and between PD-L1 expression in tumoural and peritumoural area (r = 0.37, p = 0.008) (Fig. 6).

For the patient group with a positive expression of both PD-1 and PD-L1, OS and DFS were significantly higher than in those groups with any of the parameters being negative (Fig. 7, Supplementary Fig. 3), analysis of NED survival confirmed these results as a trend (Supplementary Fig. 6) (p = 0.008, p = 0.017, p = 0.073, respectively). Significance of combined testing was more pronounced than significance of testing each parameter alone. In multivariate analysis adjusted for both parameters, PD-1 expression contributed predominantly to overall prognosis (p =



r|p	PDL1 Tu	PD1 Tu	PDL1 pTu	PD1 pTu
PDL1 Tu		<0.001	0.008	0.809
PD1 Tu	0.496		0.568	0.408
PDL1 pTu	0.373	0.083		0.480
PD1 pTu	-0.035	0.120	0.098	

Fig. 6. Correlation of PD-L1 and PD-1 expression in tumoural and peritumoural area Grade of correlation is represented by different colours according to the colour bar on the right, grade of significance by the diameter of the circles. PD-L1 and PD-1 expressions were scored as 0 = <1%, 1 = ≥1% to <10%, 2 = ≥10% to <50%, 3 = ≥50% number of positive cells compared to the number of all cells in the area Tu tumoural area, pTu peritumoural area, r Spearman's Rho, p p-value Results of Spearman's rank-order correlation.

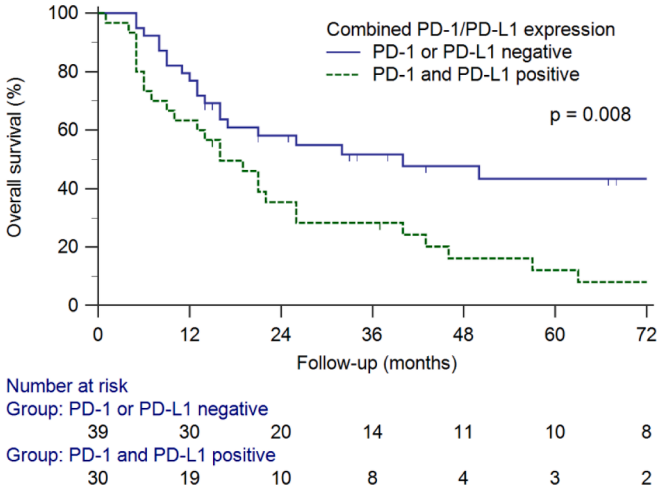


Fig. 7. Influence of combined PD-1 and PD-L1 expression in tumoural area on overall survival PD-1 or PD-L1 negative compared to PD-1 and PD-L1 positive: HR 0.44 (0.24–0.81). Results of logrank test, threshold of positive expression ≥1%.

0.034), whereas the influence of PD-L1 expression was not significant.

Alteration of PD-1 and PD-L1 expression by RCT

As for the intratumoural expression before RCT, 46% of the samples were PD-1 negative and 43% PD-L1 negative, 36% and 29% had a positive score of ≥1% to <10%, 14% and 19% had a score of ≥10% to <50%, 4% and 10% had a score of ≥50%, respectively. The distribution in the pre-RCT peritumoural area was not significantly different from that in the intratumoural area. RCT had no significant influence on the expression in both areas, but the percentage of samples with positive PD-1 expression in intratumoural and peritumoural area tended to be lower after RCT (p = 0.141 and p = 0.109, respectively, Fisher's exact test) (Fig. 8). It has to be considered that the low sample number is limiting statistical power.

Discussion

Neoadjuvant RCT is able to substantially reduce mortality in patients with locally advanced, non-metastatic OAC. However, it is potentially accompanied by serious toxic side effects. To prevent those patients from harm who will not experience any benefit from RCT, predictive parameters are urgently needed. Recently, we identified pretherapeutic immunological biomarkers such as CD8+, FoxP3+, CD68+, and CD163+ TIC with significant influence on TRG and survival [4]. In the current study, we additionally could demonstrate that also PD-1 and PD-L1 expression in the tumoural and peritumoural compartment of pre-treatment specimens may predict TRG and prognosis. As PD-1 and PD-L1 antibodies have been recently proven to be of therapeutic benefit in OAC under certain conditions, our results may also help to identify those patients who should be selected for a combination therapy of RCT and checkpoint inhibition.

Influence of PD-1 and PD-L1 expression on TRG

In oesophageal squamous cell cancer (OSCC), Fassan et al. found that PD-L1 expression was significantly higher in patients who experienced a complete pathological response following neoadjuvant RCT [18]. The authors discuss that a strong immune infiltration within the tumour could be counterbalanced by a high expression of PD-L1 at baseline, but the therapeutic effects could unmask the cancer antigens, allowing a strong immune response and a favourable treatment outcome. In contrast, Chen et al. described a significant correlation of positive PD-L1 staining with poor treatment response following radiotherapy of OSCC [19]. In our cohort, PD-1 and PD-L1 expression inside the tumour area had no influence on TRG, only positive PD-L1 expression in the peritumoural area was significantly associated with a better response following RCT. Interpretation of our results remains difficult. Given that OAC seems to be mostly immune cell excluded [20], the immunological response to tumour spreading may be better characterized in the peritumoural area.

Influence of PD-1 and PD-L1 expression on survival

Since surgery had no significant influence on survival in our cohort and survival curves were very similar, we evaluated PD-1 and PD-L1 expression including patients with and without surgery. However, we cannot exclude a bias as intratumoural PD-1 expression was higher in patients with surgery than in those without.

Most recently published treatment studies investigating the effect of checkpoint inhibitors in gastric cancer and OAC used the CPS to determine PD-L1 expression inside the tumour [8–10]. In our study we evaluated PD-L1 and PD-1 expression simultaneously not only in the tumoural but also in the peritumoural compartment, where tumour cells are absent. For a better comparison of both parameters in both areas we adapted and simplified the CPS and divided the number of positive cells by the total number of all cells. Survival analysis of our cohort revealed that a score of ≥1% was most appropriate to be classified as positive. This threshold may be marginally lower than the CPS of 5 and 10 which was postulated as prerequisite for the recent approval of nivolumab and pembrolizumab for treatment of gastric cancer and OAC, respectively.

In our cohort, a positive PD-1 expression both in tumoural and in peritumoural area was associated with a significantly worse outcome in univariate analysis. Considering multiple hypothesis testing, the effect in peritumoural area lost its significance. In contrast, we could not

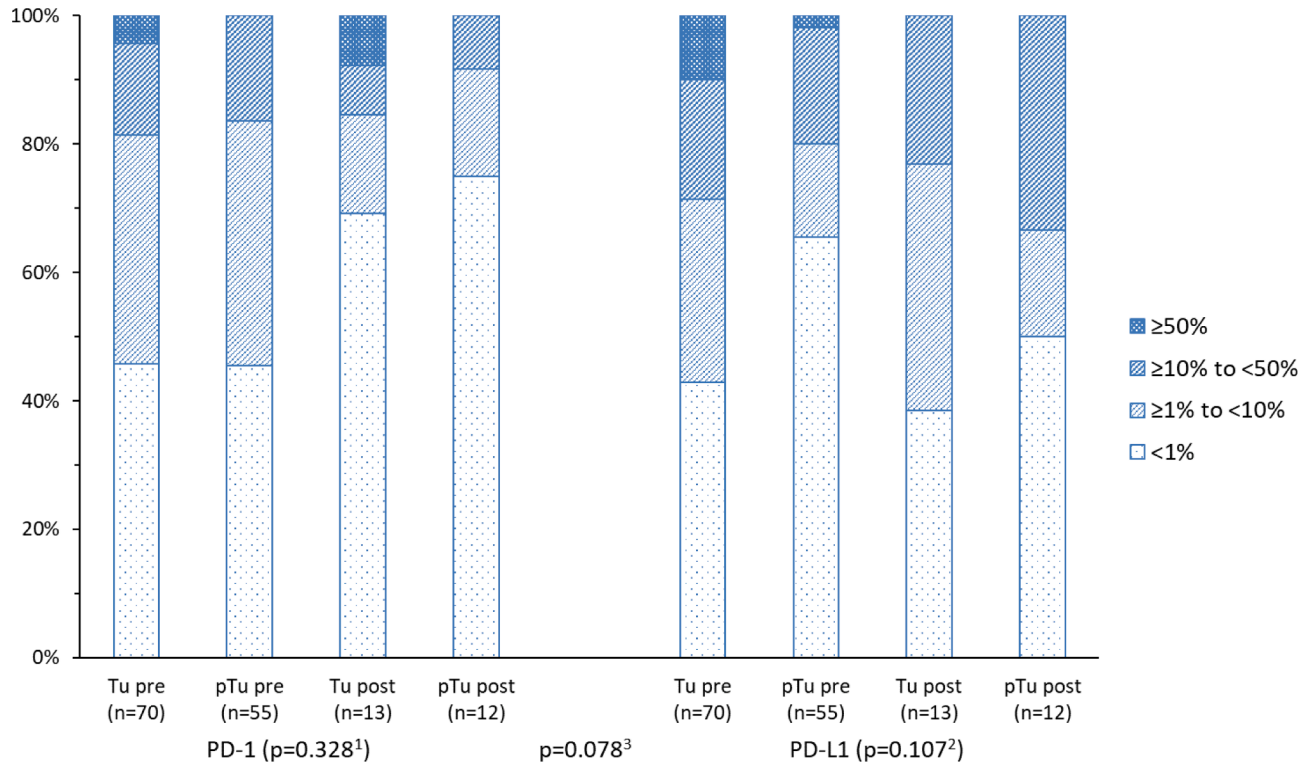


Fig. 8. PD-1 and PD-L1 expression in tumoural and peritumoural area, pre- and post-RCT Expression of PD-1 and PD-L1 was estimated as the number of positive cells divided by the number of all cells in the area. RCT radiochemotherapy, Tu tumoural area, pTu peritumoural area, pre pre-RCT, post post-RCT. Results of Chi-squared test, [1]PD-1 group, [2]PD-L1 group, [3]all groups.

demonstrate any significant influence of PD-L1 expression on survival, neither in tumoural nor in peritumoural area. PD-1 and PD-L1 expressions were highly correlated in tumoural compartment, and a combined evaluation of PD-1 and PD-L1 expression in this area seemed to increase the grade of influence on prognosis. However, multivariate analysis provided that mainly PD-1 expression was responsible for this effect. Our findings may be somewhat surprising, as in gastric cancer, for example, Gao et al. found a significant unfavourable effect of both PD-1 and PD-L1 expression on prognosis [21], and Chang et al. of PD-L1 expression [22]. On the other hand, Wang et al. reported an improved survival of patients with positive tumour PD-L1 expression in gastric cancer [23]. There seems to be some evidence in *meta*-analyses that in patients with digestive system cancer, PD-L1 expression is only a prognostic marker in Asian ethnicity, but not in Non-Asian [11]. The same *meta*-analysis pointed out that the prognostic value in oesophageal cancer may be uncertain. A Swedish study found a prolonged survival for high PD-L1 or PD-1 expression in patients with OAC or gastric cancer; but patients in this study had no neoadjuvant and only 7.5% had adjuvant therapy [24]. Results of a Swiss study support our findings that high PD-1 expression predicts an unfavourable outcome in OAC [25]. As shown for OSCC by Jiang et al., moreover, prediction of PD-L1 expression on survival seems to depend on the tumour stage and lymph node status. In this study, positive tumoural PD-L1 expression was a favourable predictor in UICC stage I-II, but not in III-IV [26].

Däster et al. combined CD8+ and PD-L1/PD-1 evaluation and found that high/high infiltration/expression was associated with significantly better survival than low/low infiltration/expression [27]. In our cohort, we also analysed the interaction of these variables and could confirm the impact of CD8+ infiltration on survival with a trend towards a better prognosis for high intratumoural CD8+ density and a significant worse prognosis for high peritumoural density. However, our results of the combined evaluation of intratumoural CD8+ infiltration and PD-L1 or PD-1 expression showed that PD-L1 or PD-1 expression attenuated the effect of CD8+ infiltration. That means, that in our cohort a high CD8+ infiltration combined with a low PD-L1 or PD-1 expression predicted a favourable prognosis and vice versa. We believe that our results seem to be well plausible in tumoural compartment, as a high immunologic activation should not be hampered by any inhibitory mechanisms, and especially as PD-1 expression is considered to be an indicator of T-cell exhaustion or even hyperexhaustion [6]. In the peritumoural area, we found a similar signature as Däster in tumoural area, but the effect was the opposite: Low/low infiltration/expression was associated with a significantly better outcome than high/high infiltration/expression. As discussed in Göbel et al. [4], we hypothesize that a high peritumoural immunologic activation may support escape mechanisms of tumour cells and therefore impairs prognosis. In turn, activation of CD8+ cells may induce a high expression of PD-L1 and PD-1 via interferon-γ, which consequently has only to be considered as an indicator of immunologic activation.

In our opinion, our results indicate that at least for Non-Asian ethnicities, PD-1 expression could be a more meaningful prognostic factor than PD-L1 expression, and that the expression of PD-L1/PD-1 has to be considered in the context with CD8+ infiltration.

Influence of RCT on PD-1 and PD-L1 expression

As we excluded patients with complete regression from post-therapeutic evaluation and included only samples with clear discrimination of tumoural and peritumoural area, only few post-RCT samples could be evaluated, and results have to be interpreted with caution.

In our cohort, around half of pre-RCT samples was classified as PD-L1 or PD-1 positive both in tumoural and in peritumoural area. Taking into account different scoring systems, this is approximately in line with published results of OAC and gastric cancer [24,28,29]. The high proportion of PD-L1 and PD-1 positive samples from the peritumoural area may reflect the deep involvement of this outside compartment in cancer-related immunologic reactions, and may justify increased interest in further investigation of the peritumoural compartment.

We did not find any significant influence of RCT on PD-L1 or PD-1 expression, at best a weak trend towards a reduced PD-1 expression following RCT. In OAC and gastric cancer, Svensson et al. reported no effect of chemotherapy on PD-L1 [30], whereas Yu et al. found increasing PD-L1 and PD-1 expression after chemotherapy of gastric cancer [31]. In other tumour entities, an up-regulation of PD-L1 expression was reported after RCT of rectal cancer [32], and following chemotherapy of ovarian cancer [33] and of head-neck cancer [34]. It is discussed, that activation of CD8+ cytotoxic T-lymphocytes (CTL), for example by chemotherapy, is accompanied by a shift to a pronounced expression of PD-L1 and PD-1 induced by interferon-γ, which is produced by activated CTL themselves, consequently restoring a relatively balanced environment [23,31]. In our cohort, CD8+ infiltration was not altered significantly by RCT [4], and thus, also the PD-L1 and PD-1 expression could be expected to be unchanged.

Conclusion

We demonstrated substantial influence of PD-1 and PD-L1 expression on TRG and survival of patients with OAC under "real world" conditions. Simultaneous investigation of PD-L1 and PD-1 expression in the tumoural and in the peritumoural compartment may deepen the understanding of immunologic mechanisms responsible for cancer surveillance, and should preferentially be evaluated in the context of underlying immunological environment, mainly of the CD8+ infiltration grade.

In particular, patients with pretherapeutic negative peritumoural PD-L1 expression may not expect a reasonable tumour regression following RCT. Regarding prognosis, patients with positive intratumoural and peritumoural PD-1 expression seem to be at high risk for disease progression. Moreover, PD-1 and PD-L1 expressions influence the prognostic effects of CD8+ CTL infiltration: They attenuate its positive effect in intratumoural area and are indicators for the magnitude of its negative effect in peritumoural area. PD-1 expression seems to be a better prognostic marker than PD-L1 expression.

These results may stimulate prospective trials that evaluate targeted treatment strategies during and after neoadjuvant radiochemotherapy in patients with OAC.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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