**A R T I C L E   I N F O**

**Keywords:**
- Esophageal adenocarcinoma
- CD8
- PD-1
- PD-L1
- Radiochemotherapy

**A B S T R A C T**

**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 peritumoral 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 peritumoral 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 peritumoral 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 intratumoral area (LoCD8/HiPD1 + vs. HiCD8/LoPD1: HR 0.25 [0.09-0.69], p = 0.007; LoCD8/HiPD1 + vs. HiCD8/HiPD1 + HR 0.32 [0.12-0.89], p = 0.028) and were associated with negative outcome when seen in peritumoral area (HiCD8/HiPD1 + vs. LoCD8/HiPD1 : HR 0.29 [0.11-0.74], p = 0.010; HiCD8/HiPD1 + vs. LoCD8/HiPD1 : 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.

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**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%)              | n.s. 2    |
| Male                   | 60 (79%)     | 47 (81%)              | 13 (72%)             |           |
| **Age**                |              |                       |                      |           |
| Mean (±SD) - years     | 66.4         | 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 | | | | |
| Median (IQR) - months  | 24 (32%)     | 20 (34%)              | 4 (22%)              | n.s. 2    |
| **Reection quality**   |              |                       |                      |           |
| R0                     | 51 (88%)     | 43 (77%)              | 8 (47%)              |           |
| R1                     | 6 (10%)      | 6 (10%)               | 0 (0%)               |           |
| R2                     | 1 (2%)       | 1 (2%)                | 0 (0%)               |           |
| **TRG (Mandard)**      |              |                       |                      |           |
| 1                      | 20 (34%)     | 16 (27%)              | 4 (22%)              |           |
| 2                      |               | 22 (38%)              | 10 (56%)             |           |
| 3                      | 5 (9%)       | 5 (9%)                | 0 (0%)               |           |
| 4                      | 9 (16%)      | 7 (12%)               | 2 (11%)              |           |
| 5                      | 2 (3%)       | 1 (2%)                | 1 (6%)               |           |

w/o without, n.s. not significant, SD standard deviation, IQR interquartile range, Tx therapy, UICC International Union against Cancer, TRG tumour regression grade

1 Last verification: 2019/04/01
2 p-value for the difference between patients with and without surgery

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 PD-L2 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 past 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.
A

![PD-1 pos Tu (n=52)](image1)

![PD-L1 pos Tu (n=52)](image2)

![PD-1 pos pTu (n=44)](image3)

![PD-L1 pos pTu (n=44)](image4)

B

![unfavourable TRG](image5)

![favourable TRG](image6)

![RR (95% CI)](image7)

![RD (95% CI)](image8)

![Fisher exact test](image9)

![Wilcoxon test](image10)

![Mann-Whitney-U test](image11)

![Spearman rank-order correlation](image12)

![Spearman’s chi-squared test](image13)

![Pearson’s chi-squared test](image14)

![Fisher’s exact test](image15)

![Wilcoxon test](image16)

![Mann-Whitney-U test](image17)

![Spearman’s rank-order correlation](image18)

![Spearman’s chi-squared test](image19)

![Fisher’s exact test](image20)

![Wilcoxon test](image21)

![Mann-Whitney-U test](image22)

![Spearman’s rank-order correlation](image23)

![Spearman’s chi-squared test](image24)

![Fisher’s exact test](image25)

![Wilcoxon test](image26)

![Mann-Whitney-U test](image27)

![Spearman’s rank-order correlation](image28)

![Spearman’s chi-squared test](image29)

![Fisher’s exact test](image30)

![Wilcoxon test](image31)

![Mann-Whitney-U test](image32)

![Spearman’s rank-order correlation](image33)

![Spearman’s chi-squared test](image34)

![Fisher’s exact test](image35)

![Wilcoxon test](image36)

![Mann-Whitney-U test](image37)

![Spearman’s rank-order correlation](image38)

![Spearman’s chi-squared test](image39)

![Fisher’s exact test](image40)

![Wilcoxon test](image41)

![Mann-Whitney-U test](image42)

![Spearman’s rank-order correlation](image43)

![Spearman’s chi-squared test](image44)

![Fisher’s exact test](image45)

![Wilcoxon test](image46)

![Mann-Whitney-U test](image47)

![Spearman’s rank-order correlation](image48)

![Spearman’s chi-squared test](image49)

![Fisher’s exact test](image50)

![Wilcoxon test](image51)

![Mann-Whitney-U test](image52)

![Spearman’s rank-order correlation](image53)

![Spearman’s chi-squared test](image54)

![Fisher’s exact test](image55)

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![Spearman’s rank-order correlation](image58)

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![Wilcoxon test](image61)

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![Spearman’s rank-order correlation](image63)

![Spearman’s chi-squared test](image64)

![Fisher’s exact test](image65)

![Wilcoxon test](image66)

![Mann-Whitney-U test](image67)

![Spearman’s rank-order correlation](image68)

![Spearman’s chi-squared test](image69)

![Fisher’s exact test](image70)

![Wilcoxon test](image71)

![Mann-Whitney-U test](image72)

![Spearman’s rank-order correlation](image73)

![Spearman’s chi-squared test](image74)

![Fisher’s exact test](image75)

![Wilcoxon test](image76)

![Mann-Whitney-U test](image77)

![Spearman’s rank-order correlation](image78)

![Spearman’s chi-squared test](image79)

![Fisher’s exact test](image80)

![Wilcoxon test](image81)

![Mann-Whitney-U test](image82)

![Spearman’s rank-order correlation](image83)

![Spearman’s chi-squared test](image84)

![Fisher’s exact test](image85)

![Wilcoxon test](image86)

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![Spearman’s rank-order correlation](image88)

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![Spearman’s chi-squared test](image104)

![Fisher’s exact test](image105)

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![Spearman’s rank-order correlation](image108)

![Spearman’s chi-squared test](image109)

![Fisher’s exact test](image110)

![Wilcoxon test](image111)

![Mann-Whitney-U test](image112)

![Spearman’s rank-order correlation](image113)

![Spearman’s chi-squared test](image114)

![Fisher’s exact test](image115)

![Wilcoxon test](image116)

![Mann-Whitney-U test](image117)

![Spearman’s rank-order correlation](image118)

![Spearman’s chi-squared test](image119)

![Fisher’s exact test](image120)

![Wilcoxon test](image121)

![Mann-Whitney-U test](image122)

![Spearman’s rank-order correlation](image123)

![Spearman’s chi-squared test](image124)

![Fisher’s exact test](image125)

![Wilcoxon test](image126)

![Mann-Whitney-U test](image127)

![Spearman’s rank-order correlation](image128)

![Spearman’s chi-squared test](image129)

![Fisher’s exact test](image130)

![Wilcoxon test](image131)

![Mann-Whitney-U test](image132)

![Spearman’s rank-order correlation](image133)

![Spearman’s chi-squared test](image134)

![Fisher’s exact test](image135)

![Wilcoxon test](image136)

![Mann-Whitney-U test](image137)

![Spearman’s rank-order correlation](image138)
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. 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 test LoCD8/HiCD8 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%).
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 (\( p = 0.028 \)). 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 (\( r = 0.50 \), CI 0.25–0.99). A: PD-1 expression combined with CD8+ density, HiCD8/PD1+ compared to LoCD8/PD1−: HR 0.29 (0.11–0.74). B: PD-1 expression combined with CD8+ density, HiCD8/PD1− compared to LoCD8/PD1+: HR 0.50 (0.25–0.99). C: PD-L1 expression, HR 0.71 (95% CI 0.34–1.45). D: PD-L1 expression combined with CD8+ density, HiCD8/PD1+ compared to LoCD8/PD1−: HR 0.33 (0.12–0.90). Results of logrank test. HiCD8/LoCD8 high/low CD8+ density (median 132.2/mm²), PD1−/PD1+ negative/positive PD-1 expression, PDL1−/PDL1+ negative/positive PD-L1 expression (threshold 1%).

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 =
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...
PD-1 expression showed that PD-L1 or PD-1 expression attenuated the combined evaluation of intratumoural CD8 effect of CD8 prognosis for high peritumoural density. However, our results of the prognosis for high intratumoural CD8 we also analysed the interaction of these variables and could confirm the better survival than low/low infiltration/expression [27]. In our cohort, that high/high infiltration/expression was associated with significantly demonstrable 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 chemoradiotherapy 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-L1 and PD-1 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 preferably be evaluated in the context of underlying immunomolecular 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 intratumoral and peritumoural PD-1 expression seem to be at high risk for disease progression. Moreover, PD-1 and PD-1 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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