Brief Correspondence

PD-L1 Expression and CD8+ T-cell Infiltrate Are Associated with Clinical Progression in Patients with Node-positive Prostate Cancer

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Abstract

Prostate cancer (PCa) patients with lymph node invasion at radical prostatectomy are at higher risk of tumor recurrence and receive immediate androgen deprivation therapy (ADT). While approximately 30% of these patients do not experience recurrence, others experience disease recurrence despite ADT, and currently no biomarkers can accurately identify them. We analyzed tumors from 51 patients with node-positive prostate cancer using immunohistochemistry to investigate whether expression of the immune checkpoint ligand PD-L1 by tumor cells or the density of CD8+ or CD20+ cells are associated with clinical progression. Patients with at least 1% PD-L1+ tumor cells had shorter metastasis-free survival than those with PD-L1+ tumors (p = 0.008, log-rank test). Univariate Cox regression showed that patients with PD-L1+ tumors had almost four times the risk of experiencing distant metastases than those with PD-L1+ tumors (hazard ratio 3.90). In addition, we found that PD-L1 expression was significantly associated with CD8+ T-cell density, but not with CD20+ B-cell density. While these results need to be confirmed in larger studies, they show that PD-L1 and CD8 may be used as biomarkers for node-positive patients at high risk of progression. The study also provides a rationale for selecting patients with node-positive PCa who might benefit the most from adjuvant immunotherapies.

Patient summary: None of the available biomarkers can identify node-positive prostate cancer that will recur after surgery. We found that expression of PD-L1 by tumor cells and a high density of CD8+ T cells in tumor are associated with a higher risk of clinical progression in men with node-positive prostate cancer.

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Overall, up to 15% of contemporary prostate cancer (PCa) patients harbor lymph node metastases detected on radical prostatectomy (RP) and extended pelvic lymph node dissection (ePLND) [1]. However, the optimal postoperative management of PCa patients with lymph node invasion (LNI) is still under debate. While the European Association of Urology (EAU) recommends adjuvant androgen deprivation therapy (ADT) for patients with pN1 disease after ePLND [2], outcomes for men with nodal metastases are heterogeneous according to PCa grading and the extent of nodal invasion [3–5]. Approximately 30% of patients with low-volume LNI do not experience cancer recurrence during long-term follow-up, even without any adjuvant treatment [3]. However, a significant proportion of patients with node-positive PCa may experience recurrence and succumb to their disease [1]. Beyond clinical and pathological characteristics, no current biological markers can accurately stratify node-positive PCa according to the risk of progression.

For solid cancers, several studies have shown that various immune cell subtypes are associated with cancer progression [6]. High CD8+ cytotoxic T- or B-lymphocyte infiltrates correlated with good prognosis and response to immunotherapies [6]. Although data for PCa are discordant, recent reports suggest that high CD8 or B-cell infiltrates are associated with recurrence or progression [7,8]. Furthermore, PD-L1 expression is associated with poor prognosis in most solid tumors [9], including PCa [10]. Interestingly, high PD-L1 expression and CD8+ T-cell infiltrates are also associated with higher susceptibility to immune checkpoint blockade [11]. Since this immune infiltrate has not been specifically investigated in node-positive PCa, we hypothesized that different patterns of immune infiltrates and PD-L1 expression might identify node-positive PCa at higher risk of recurrence. Thus, we analyzed a single-institution series of pN1 patients and correlated immunological characteristics of the primary tumor with oncological outcomes.

After institutional review board approval, a cohort of 51 patients with node-positive PCa treated between 1991 and 2013 with RP and ePLND were randomly selected from our prospectively collected database. No patient had distant metastases at the time of surgery. Patients did not receive either neoadjuvant or adjuvant hormonal therapy. For all patients included in the analysis, complete clinical, pathologic, and follow-up data were available. The baseline characteristics for all the patients are reported in Supplementary Table 1. Patients were stratified according to biochemical recurrence (yes, n = 23 [45%]; no, n = 28 [55%]) and the occurrence of distant metastasis after RP. Median follow-up was 51 mo (interquartile range 30–77). The primary end-point of the study was the onset of clinical recurrence.

![Figure 1](http://dx.doi.org/10.1016/j.euf.2017.05.013)
which was defined as the detection of recurrent or metastatic disease on imaging (bone scintigraphy and/or CT/MRI). Kaplan Meier and univariate Cox proportional hazards regression methods were used to assess the association between predictors (including PD-L1 expression, tumor infiltration by CD8+ cells, CD20+ cells, or CD20+ lymphoid follicles larger than 7000 μm²) and the occurrence of distant metastases over time. All analyses were carried out using the R survival package.

Immunohistochemistry was carried out on 5-μm formalin-fixed, paraffin-embedded prostate tissue sections stained using the antibodies listed in Supplementary Table 2, and were then scanned and analyzed by two independent reviewers in a blinded fashion. The density of CD8+ T cells, CD20+ B cells, and CD20+ B cell follicles (ratio of CD20+ follicular/tumor surface areas) were evaluated in the tumoral zone using Calopix software (Tribvn, France) and the density of positive cells was calculated. The percentage of tumor cells stained positive for PD-L1 was calculated by counting 2000 neoplastic cells.

PD-L1 expression was heterogeneous among tumors (Fig. 1A) and was detected in 1–50% of the tumor cells in seven out of 51 (14%) adenocarcinomas (Fig. 2A). The density of CD8+ T cells was variable (Fig. 1B); the distribution had an asymmetric shape with a long right tail (Fig. 2B). Values above the third quartile (233.1 cell/mm²) were considered as CD8\text{High}. The distribution of non-follicular CD20+ B cells (Fig. 1C) had a similar asymmetric shape; in this case, values above 38.8 cell/mm² (the third quartile) were considered CD20\text{High}. Notably, there was a significant association between PD-L1 expression and CD8+ T-cell density (Spearman’s correlation 0.289, \( p = 0.040 \); Mann-Whitney test comparing CD8+ T-cell values among PD-L1 groups, \( p = 0.049 \); Fig. 2C), but not between PD-L1 expression and CD20+ B-cell density. A significant positive correlation between CD8+ T and CD20+ B cells (Spearman’s correlation 0.432, \( p = 0.002 \)) was also found.

Kaplan Meier analyses were performed to investigate the association between tumor PD-L1 positivity, immune infiltrates, and recurrence. Patients with at least 1% PD-L1+ tumor cells had shorter metastasis-free survival than those with PD-L1- tumors (\( p = 0.008 \), log-rank test; Fig. 2D). Univariate Cox regression showed that patients with PD-L1+ tumors had almost four times the risk of experiencing distant metastases than those with PD-L1- tumors (hazard ratio [HR] 3.90, 95% confidence interval [CI] 1.32–11.47; \( p = 0.014 \); Fig. 2F).

Univariate Cox regression revealed a trend for higher risk of progression for patients with high CD8+ T-cell density (\( p = 0.082 \); Fig. 2F). The density of CD20+ cells (\( p = 0.655 \)) and of CD20+ follicles (\( p = 0.49 \)) in the tumor specimen had no significant impact on clinical recurrence (Fig. 2F).

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**Fig. 2** – Metastasis-free survival analysis for patients according to PD-L1 or CD8+ T-cell infiltration. (A) Distribution of the percentage of PD-L1+ cells among tumors. (B) Distribution of the density of CD8+ T cells among tumors. (C) Density of CD8+ T cells depending on the positivity of PD-L1 expression by tumor cells. Mann-Whitney test, \( p = 0.049 \). (D,E) Kaplan-Meier estimates of metastasis-free survival after surgery according to (D) tumor PD-L1 expression and (E) density of CD8+ T cells. (F) Metastasis-free survival according to univariate Cox proportional hazards analysis, showing the hazards ratios among node-positive patients after radical prostatectomy. The density of follicular CD20+ B cells and total PSA were computed using log_{10} of their original values. Significant poor prognosis values are represented in red, poor prognosis trend (\( p \) value between 0.05 and 0.1) in pink, and non-significant prognostic values in grey.
Among the clinicopathological variables, only total prostate-specific antigen (PSA; HR 6.00, 95% CI 1.47–24.42; \( p = 0.012 \)) and the number of positive lymph nodes (HR 1.15, 95% CI 1.05–1.26; \( p = 0.003 \)) significantly predicted a higher risk of distant metastasis on univariate Cox regression (Fig. 2F). Furthermore, considering the lower rate of adjuvant radiation therapy (aRT) administration among patients who developed clinical recurrence compared to those who did not (35% vs 64%), we tested the association between aRT and risk of distant metastasis. This association was not statistically significant on univariate Cox regression analysis (HR 0.47, 95% CI 0.18–23; \( p = 0.13 \)). The lack of statistical significance might be due to the relatively low number of patients analyzed and the limited follow-up. However, no patient received adjuvant ADT. Thus, it is unlikely that second-line therapies had an impact on our findings.

With the limitations imposed by the relatively small sample size, total PSA \( (p = 0.004) \) and the number of positive lymph nodes \( (p < 0.001) \) were the only variables retained in the multivariable Cox regression model after backward selection. Notably, PD-L1+ and PD-L1+ tumors significantly differed in the number of positive lymph nodes \( (p = 0.004, \text{Mann-Whitney} \text{test}) \).

Taken together, our results identify a subgroup of node-positive PCa patients with PD-L1+ and/or CD8+ tumors with a higher risk of recurrence after RP. Simultaneous high CD8+ T-cell density and PD-L1 expression by tumor cells is evidence of blunted immune surveillance. Indeed, as has been hypothesized for other solid tumors [6,11], activated tumor-infiltrating T cells in these node-positive PCa patients might induce overexpression of PD-L1 on tumor cells via IFN\( \gamma \) release, eventually resulting in T-cell exhaustion. Clinical responses to PD-1/PD-L1 blockade have been limited in PCa patients, probably because treatments were mostly tested in unselected castration-resistant subjects [12]. Our results have identified a subgroup of PCa patients at high risk of disease recurrence after RP, who might benefit the most from adjuvant immunotherapies, and anti-PD-1/anti-PD-L1 antibodies in particular.

Although this study was conducted retrospectively for a relatively small cohort of patients, our results suggest that the expression of PD-L1 and the frequency of CD8+ T cells could be used as biomarkers to predict the risk of clinical progression in LNI PCa. Its replication in larger prospective studies will allow a better evaluation of these markers in multivariate analyses and of their relevance in the choice of therapeutic regimens such as immunotherapies.

**Author contributions:** Matteo Bellone had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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**Supervision:** Sautès-Fridman, Bellone, Doglioni, Briganti.

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**Appendix A. Supplementary data**

Supplementary data associated with this article can be found, in the online version, at [http://dx.doi.org/10.1016/j.euf.2017.05.013](http://dx.doi.org/10.1016/j.euf.2017.05.013).

**References**


