REVIEW ARTICLE

# Catching up with solid tumor oncology: what is the evidence for a prognostic role of programmed cell death-ligand 1/programmed cell death-1 expression in B-cell lymphomas?

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## ABSTRACT

Therapeutic strategies targeting the programmed cell death-ligand 1/programmed cell death-1 pathway have shown significant responses and good tolerability in solid malignancies. Although preclinical studies suggest that inhibiting programmed cell death-ligand 1/programmed cell death-1 interactions might also be highly effective in hematological malignancies, remarkably few clinical trials have been published. Determining patients who will benefit most from programmed cell death-ligand 1/programmed cell death-1-directed immunotherapy and whether programmed cell death-ligand 1/programmed cell death-1 are adequate prognostic markers becomes an increasingly important clinical question, especially as aberrant programmed cell death-ligand 1/programmed cell death-1 expression are key mediators of impaired anti-tumor immune responses in a range of B-cell lymphomas. Herein, we systematically review the published literature on the expression and prognostic value of programmed cell death-ligand 1/programmed cell death-1 in these patients and identify considerable differences in expression patterns, distribution and numbers of programmed cell death-ligand 1$^+$/programmed cell death-1$^+$cells, both between and within lymphoma subtypes, which is reflected in conflicting findings regarding the prognostic value of programmed cell death-ligand 1$^+$/programmed cell death-1$^+$ cells. This can be partly explained by differences in methodologies (techniques, protocols, cutoff values) and definitions of positivity. Moreover, lymphomagenesis, disease progression, and prognosis appear to be determined not only by the presence, numbers and distribution of specific subtypes of T cells, but also by other cells and additional immune checkpoints. Collectively, our findings indicate that programmed cell death-ligand 1/programmed cell death-1 interactions play an essential role in B-cell lymphoma biology and are of clinical importance, but that the overall outcome is determined by additional components. To categorize the exact prognostic value of programmed cell death-ligand 1/programmed cell death-1 expressing cells and cell types, efforts should be made to harmonize their assessment and interpretation, optimally within ongoing clinical immune checkpoint inhibitor trials, and to identify and validate novel high-throughput platforms.

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## Introduction

The immune checkpoint programmed cell death protein 1 (PD-1, CD279) and its ligand PD-L1 (B7-H1, CD274) have rapidly taken center stage in tumor immunology. This is because antibodies targeting this pathway have shown significant responses and good tolerability across a variety of solid malignancies, both in initial phase 1/2 studies and in recently published randomized trials or in combination

with other substances.[1-12] Although a plethora of preclinical studies suggest that inhibiting PD-L1/PD-1 interactions might also be highly effective in hematological malignancies,[13,14] only few PD-L1/PD-1 antibody based clinical trials have been published to date. An initial phase I trial demonstrated a clinical benefit of the PD-1 antibody pidilizumab in several advanced hematological malignancies.[15] Encouraging results were also observed in recently published phase II trials in relapsed follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL),[16,17] as well as in relapsed/refractory Hodgkin lymphoma (HL) patients treated with nivolumab.[18]

Determining which patients benefit most from PD-L1/PD-1-directed immunotherapy is an important clinical question. Yet again, the solid oncology field appears to be one step ahead. Several retrospective and correlative studies examining the prognostic significance of tumor PD-L1 expression and PD-1 expression on tumor-infiltrating lymphocytes (TILs) have already been published, although the exact associations are somewhat controversial and appear to be dependent on tumor entity, treatment setting and the presence of other predictive factors or biomarkers.[19-23]

Similar studies have not been reported in hematological malignancies, even though most of these tumor types, and especially lymphomas, are increasingly understood to closely interact with their surrounding microenvironment.[24] Importantly, we and others have shown that aberrant PD-L1 expression by lymphoma cells and increased expression of PD-1 on T cells are key mediators of impaired anti-tumor immune responses in a range of B-cell lymphomas, including DLBCL, FL and chronic lymphocytic leukemia (CLL),[25-27] and that inhibiting their interaction restores immune function in preclinical models.[28] However, PD-L1 is also expressed on other cell types and in peripheral tissues and is up-regulated during inflammation and in the tumor microenvironment.[29-31] Similarly, PD-1 can be expressed on a variety of physiological immune cells, for example on $CD4^+$ germinal center (GC) follicular helper T cells ($T_{FH}$), which are required for GC development and high-affinity antibody production.[32] As $T_{FH}$ cells also act as negative regulators of immune responses, their numbers and tissue distribution may shape the microenvironment in GC-type lymphomas.[33] Indeed, across multiple solid cancer types, it was recently demonstrated that clinical responses were not only observed in patients with high tumor PD-L1 levels, but also when PD-L1 was expressed by tumor-infiltrating immune cells and when T helper type 1 (TH1) gene signatures and CTLA-4 expression were detected in baseline specimens.[23]

Herein, we aimed to collate and review data from the literature on the prognostic value of PD-L1 or PD-1 expression in patients with the most frequent types of B-cell lymphomas. We hypothesized that increased PD-L1/PD-1 expression confers an adverse prognosis, but that differences exist between lymphoma subtypes and between lymphoma and tumor infiltrating lymphocytes (TIL) expression. Such a systematic comparison has several clinical implications. First, it allows the identification of entity- and cell-type-specific expression patterns and their association with prognosis and survival. Second, it elucidates the clinical importance of this pathway in specific lymphomas, contributing to identifying patient groups that might benefit most from blocking PD-L1/PD-1 interactions. Ultimately, these findings provide direct translational guidance in the implementation and interpretation of assays and techniques assessing PD-L1 or PD-1 as biomarkers in future clinical trials of immune checkpoint inhibitors.

## Methods and Materials

Full-text publications were included if they met prospectively defined criteria: i) investigated DLBCL, FL, CLL/small lymphocytic leukemia (SLL), Hodgkin lymphoma (HL) or primary mediastinal large B-cell lymphoma (PMBCL), ii) quantified PD-1/PD-L1 expression on tumor and/or microenvironmental components by immunohistochemistry (IHC) or flow cytometry, iii) described techniques and quantification methods, and iv) were written in English. Abstracts from conference proceedings were not reviewed, and less frequent B-cell lymphomas such as mantle cell, marginal zone and Burkitt lymphoma were not included. Suitable publications were retrieved from two independent MEDLINE database queries and information on study characteristics, methods/materials (examined tissues, techniques, quantification of PD-L1/PD-1 expression, antigens/antibodies, controls, statistical analyses), patients and treatment characteristics and findings on PD-L1/PD-1 expression and prognostic significance were extracted. The majority of retrieved results were excluded because studies examined T-cell or cutaneous lymphomas. An overview of key information on included studies can be found in Table 1. Expression patterns on lymphoma and lymphoma-associated immune and/or surrounding cells are summarized according to lymphoma type in Table 2 (DLBCL), Table 3 (FL), Table 4 (CLL/SLL) and Table 5 (HL). The prognostic value of PD-L1/PD-1 in all examined lymphoma types is depicted in Table 6.

## Results

### DLBCL

#### PD-L1/PD-1 expression on DLBCL cells

One of the first studies to characterize PD-L1/PD-1 expression in a series of 161 B-cell non-Hodgkin lymphoma (NHL) tissues contained only 25 DLBCL specimens, of which 4 out of 14 examined samples were PD-$L1^+$ on 1-75% of tumor cells[34] (Table 2). In a cohort comprising (Epstein-Barr virus) $EBV^+$ and $EBV^-$ patients, the proportion of PD-$L1^+$ malignant cells ranged from 10-90%.[35] All $EBV^+$ DLBCLs showed strong PD-L1 expression, in contrast to 11% of $EBV^-$ DLBCL patients. Another study found at least 5% of PD-$L1^+$ tumor cells in 55 out of 73 interpretable tissue microarrays (TMAs), which did however not correlate with plasma PD-L1 levels.[36] Slight differences were observed in frozen *versus* paraffin specimens, where heterogeneous PD-L1 tumor expression was observed in 27% of frozen and 20% of paraffin samples.[37] A more recent study detected tumor PD-L1 expression in 61% of DLBCL TMAs, with variable intensities and proportions.[38] Using a threshold of ≥30% of PD-$L1^+$ malignant cells among all malignant cells, another recent study of a total of 1,253 DLBCL TMAs reported a tumor PD-$L1^+$ prevalence rate of 11%.[39] This was significantly associated with non-germinal center B-cell (GCB) type and EBV positivity, and with chromosome 9 gain but not structural abnormalities in chromosome 9p. PD-1 expression was

**Table 1.** Key information on included studies. Information on aim of study, patient/ sample numbers, techniques and examined tissues and PD-L1/ PD-1 scoring methods was extracted and is summarized according to B-NHL subtype.

| Included studies examining several lymphoma types | | | | |
|---|---|---|---|---|
| **Reference** | **Aim of study** | **Patient/sample numbers** | **Techniques and examined tissues** | **PD-L1/PD-1 scoring methods** |
| Amé-Thomas 2012[43] | Functional characterization of intratumoral CD4+ T cells | DLBCL, FL<br>numbers not specified | IHC on paraffin-embedded tissue sections<br>Flow cytometry | Percent positive among CD4+ cells |
| Andorsky 2011[37] | PD-L1 expression in cell lines and lymphoma specimens | Frozen specimens:9 HL, 33 DLBCL (11 GCB, 19 non-GCB), 3 PMBCL<br>SSS: 16 FL, 2 SLL/CLL, 3 MZL, 1 MCL,1 BL<br>paraffin specimens: 5 ALCL, 7 FL, 30 DLBCL | IHC on different sets of frozen or paraffin-embedded DLBCL, HL, PMBCL, FL<br>Fow cytometry on CLL/SLL, MZL, MCL, BL | Not specified |
| Chen 2013[35] | Examination of 237 primary tumors for expression of PD-L1 protein | 25 NSCHL, 8 MCCHL, 5 CHL-NOS, 15 NLPHL, 21 PMBCL, 11 TCHRBCL, 9 EBV+ DLBCL of the elderly, 7 EBV+ immunodeficiency-related DLBCL, 10 EBV+ PTLD, 7 EBV- PTLD, 66 DLBCL-NOS; 9 PMBCL, 4 PEL, 6 ENKTCL, 7 EBV+ BL, 18 NPC, 9 KS | IHC on paraffin-embedded tissue biopsies | Staining intensity:<br>no staining: 0<br>weak: 1+<br>moderate: 2+<br>strong: 3+<br><br>Tumor PD-L1+ if ≥5% of tumor cells 2+/3+ membrane staining<br><br>Microenvironment PD-L1+ if ≥20% of total tissue 2+/3+ membrane or cytoplasmic staining |
| Dorfman 2006[33] | PD-1 expression in B and T-cell lymphoproliferative disorders | 42 B-LPD (25 HL, 4 CLL, 4 MCL, 6 FL, 6 DLBCL, 3 MZL, 3 HCL, 7 BL, 3 LPL, 3 MM, 3 B-ALL), 23 T-LPD | IHC on paraffin-embedded tissues | PD-1+ if ≥20% of neoplastic cells positive staining<br>Staining specificity: comparison to isotype control |
| Muenst 2010[40] | Diagnostic potential and prognostic importance of PD-1 in B-cell lymphomas | 8 BL, 184 DLBCL, 5 T-cell rich large BCL, 7 DLBCL ex SLL/LPL/MZL, 11 DLBCL ex FL, 7 FL grade 3, 42 FL grade 1/2, 33 extranodal MZL, 19 extranodal DLBCL ex MZL, 10 MCL, 20 PMBCL, 58 SLL/CLL | IHC on total or paraffin-embedded sections | Total number of PD-1+ TILs counted in one medium power field (1.33 $mm^2$) at 200x magnification.<br><br>% PD-1+TILs in relation to all cells<br><br>Only absolute count of positive cells and not staining intensity were considered |
| Ramsay 2012[27] | Role of immune checkpoints in immune evasion mechanisms in lymphomas | 68 CLL, 18 CLL median survival 38 mo, 17 CLL median survival >10 yrs, 6 untreated FL, 6 transformed FL, 34 diagnostic FL survival <5 yrs, 25 diagnostic FL survival > 15 yrs | IHC on TMAs<br><br>Flow cytometry | Staining on CD20+ cancer or reactive LN B cells and on CD3+ T cells evaluated for mean intensity expression using automated serial section overlay analysis<br>Percent positive cells and median fluorescence intensity |
| Tonino 2012[63] | Changes in T cell compartment in different B cell malignancies | 29 CLL, 8 FL, 2 HCL, 3 MZL, 2 low-grade lymphoma NOS, 13 aggressive lymphomas, 10 MM | Flow cytometry of PB mononuclear cells | % cells positive |
| Xerri 2008[34] | Expression profile of PD-1, PD-L1 and PD-L2 in B-NHLs | 35 HL (5 LPHL, 22 NSCHL, 8 MCCHL), 11 MCL, 12 MZL, 3 BL,25 DLBCL, 43 FL, 11 T-NHL, 11 CLL | IHC on total or paraffin-embedded sections<br><br>Flow cytometry on CLL blood samples | 0:<1% of cells positive<br>+: 1-50% of cells positive<br>++: 50-75% of cells positive<br>+++: >75% of cells positive |

| Included studies examining DLBCL only or focus on DLBCL | | | | |
|---|---|---|---|---|
| **Reference** | **Aim of study** | **Patient/sample numbers** | **Techniques and examined tissues** | **PD-L1/PD-1 scoring methods** |
| Ahearne 2014[42] | Expression of PD-1 in combination with FoxP3 in DLBCL | 70 | IHC on paraffin-embedded LN<br>Flow cytometry to quantify T-cell subsets | Intensity threshold for definition of PD-1high cells by comparison to PD-1 expression within tonsil sections from normal subjects |

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| Armand 2013[17] | Correlative studies of lymphocyte subsets in phase II trial of pidilizumab in patients with DLBCL undergoing AHSCT | 35 available patients | Flow cytometry on PB mononuclear cells from patients treated at least once with pidilizumab | 41 prospectively specified leukocyte subsets evaluated for absolute (per μL) and relative numbers and median fluorescence intensity |
| Kiyasu 2015[39] | Clinicopathological impact of PD-L1+ in newly diagnosed DLBCL | 1,253<br>Among 273 pts with available clinical information: quantitative analysis of PD-1+ TILs | IHC on formalin-fixed paraffin-embedded tissues | PD-L1+ DLBCL: ≥30% of lymphoma cells distinct membranous and/or cytoplasmic staining and nuclear staining of PAX5, regardless of PD-L1 positivity of nonmalignant stromal cells<br><br>Microenvironmental PD-L1+ DLBCL: PD-L1– DLBCL cases in which PD-L1+ nonmalignant stromal cells represented ≥20% of total tissue<br><br>Number of PD-1+ TILs |
| Ko 2011[41] | Correlation between PD-1+ TILs and clinicopathologic prognostic factors in DLBCL | 65 | IHC on paraffin-embedded tumors | Number of PD-1+ TILs, recorded as average value<br>Positive if >20/hpf<br>Negative if ≤20/hpf |
| Kwon 2015[38] | Expression patterns, clinicopathological features and prognostic implications of PD-1 and PD-L1 in DLBCL tissues | 126 | IHC on formalin-fixed paraffin-embedded tumor blocks | PD-L1 intensity and proportion of cells with membranous and/or cytoplasmic staining:<br>0: negative (no or any staining in<10% of cells)<br>1: weak<br>2: moderate<br>3: strong (>10% of cells)<br><br>Numbers of PD-1+ cells:<br>0: no positive cells/hpf<br>1: <10 positive cells/hpf<br>2: 10–30 positive cells/hpf<br>3: >30 positive cells/hpf |
| Rossille 2014[36] | Clinical impact of soluble PD-L1 at diagnosis in DLBCL | 73 interpretable TMAs | IHC on paraffin-embedded blocks | Protein expression recorded in 5% increments as percentage of positive tumor cells |

**Included studies examining FL only or focus on FL**

| Reference | Aim of study | Patient/sample numbers | Techniques and examined tissues | PD-L1/PD-1 scoring methods |
|---|---|---|---|---|
| Carreras 2009[50] | Role of PD-1 in FL progression and outcome | 100 diagnostic samples, 15 sequential biopsies at relapse, 17 relapse samples only | IHC on paraffin-embedded whole tissue sections<br>Flow cytometry in a subset of samples | Quantification using an automated scanning microscope and computerized image analysis system (under pathologist visual supervision) |
| Koch 2012[52] | Prognostic significance of $T_{reg}$ and $T_{FH}$ in advanced-stage FL | 139 advanced stage, 125 early stage | IHC on paraffin-embedded tissue samples | Number of positive cells among 100 cells/hpf (×400 magnification) |
| Richendollar 2011[53] | Prognostic relevance of numbers of PD-1+ T cells within the tumor microenvironment | 91 | IHC on paraffin-embedded tissue samples | Mean number of follicular PD-1+ cells/hpf (1000×, 3 follicles with 3 fields per follicle) |
| Smeltzer 2014[54] | Cell subtypes associated with transformation in FL | 58 | IHC on paraffin embedded tissues | Patterns of expression and 0–3 scale assessing quantity and intensity<br>Follicular pattern: majority of cells in follicle/perifollicular area |

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| | | | | Diffuse pattern: majority of positive cells not confined to follicle |
| Takahashi 2013[51] | Prognostic implications of PD-1 in patients treated with R-CHOP | 82 | IHC on biopsy specimen<br>10 follicular areas quantified | Nucleated and PD-1+ cells of<br>using an automated scanning microscope and image analysis system |
| Wahlin 2010[59] | Prognostic significance of immune cell subsets | 31 good and 33 bad prognosis patients | IHC on paraffin-embedded TMAs<br>Flow cytometry | Computerized image analysis, separating cells inside and outside the follicles |
| Westin 2014[16] | Correlative studies on available blood samples at baseline from FL patients treated with pidilizumab and rituximab | 25: 18 responders, 7 non-responders | Flow cytometry | Mean fluorescence intensity |
| Yang 2015[55] | Biological and clinical relevance of PD-1 in FL | 32 | IHC on paraffin embedded tissue<br>Flow cytometry on SSS | Bright *vs.* dim<br>Percent cells positive |

**Included studies examining CLL/ SLL only or focus on CLL/ SLL**

| Reference | Aim of study | Patient/sample numbers | Techniques and examined tissues | PD-L1/PD-1 scoring methods |
|---|---|---|---|---|
| Brusa 2013[60] | Expression and functional significance of PD-1/ PD-L1 | 117 | Flow cytometry PB in all samples<br>IHC on paraffin-embedded sections of LNs infiltrated by CLL cells (n=20) | Percent cells positive<br>Percent positive area and patterns of expression in proliferation centers compared to other parts of same slide |
| Grzywnowicz 2012[61] | Characterization of PD-1 and PD-L1 expression | 45 | Flow cytometry (n=45)<br>PD-1 mRNA expression by qRT-PCR (n=43) | Percent cells positive<br>Splicing variants of PD-1 gene |
| Riches 2013[62] | Exhaustion in CD8+ T cells from CLL patients | 39 | Flow cytometry PB, in comparison to CMV-status matched controls | Percent cells positive |

**Included studies examining HL/ PMBCL only or focus on HL/ PMBCL**

| Reference | Aim of study | Patient/sample numbers | Techniques and examined tissues | PD-L1/PD-1 scoring methods |
|---|---|---|---|---|
| Ansell 2015[18] | Correlative studies phase 1 trial assessing PD-L1/PD-L2 loci and protein expression | Pretreatment tumor specimens available from 10 patients | IHC by automated staining system<br>FISH to assess chromosome 9p24.1 | Staining intensities and double-staining techniques |
| Greaves 2013[73] | Characterization of $CD4^+$ cells in the microenvironment of HL | 18 cHL SSS, 122 cHL | Flow cytometry SSS<br>IHC on TMAs (n=122) | Percentage cells positive, median expression levels<br>Median cell count/$mm^2$ and expression levels based on automated image analysis |
| Koh 2015[69] | Prognostic significance of and correlations between PD-1 and PD-L1 and PD-L2 expression in uniformly treated cHL | Diagnostic tissues from 109 cHL pts treated with ABVD | IHC on formalin-fixed, paraffin-embedded tumor samples | ≥10 CD30+ HRS cells were read. PD-L1- or PD-L2-positive if expression was detected in ≥20 % of HRS cells. PD-1-positive if PD-1 expression was detected in ≥20 % of the peritumoral microenvironment |
| Muenst 2009[72] | Distribution of PD-1+ lymphocytes in the HL microenvironment | 280 cHL (156 NSCHL, 93 MCCHL, 11 LRCHL, 7 LDCHL, 13 cHL-NOS), 3 nodular lymphocyte-predominant HL | IHC on TMAs (n=189 evaluable cases) | Absolute number of PD-1+ lymphocytes in relation to other lymphocyte populations |
| Nam-Cha 2008[71] | PD-1 expression on $T_{FH}$ cells in NLPHL and the entities involved in its differential diagnosis | 43 NSCHL, 14 MCCHL, 13 LRCHL, 58 NLPHL, 7 NLPHL with diffuse areas, 12 T-cell rich BCL | IHC on paraffin-embedded tissues | Cells positive and forming rosettes around tumor cells |
| Paydas 2015[68] | Clinical and prognostic importance of PD-1 and/or PD-L1 and association between EBV-encoded RNA | 87 cases with newly diagnosed HL | IHC on formalin-fixed, paraffin-embedded tissue samples | Staining intensity:<br>no staining: 0<br>weak/ equivocal: 1+<br>moderate: 2+ |

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| | (EBER) and PD-1/PD-L1 | | | strong: 3+<br>Tumor PD-L1+ if ≥5% of tumor cells membrane staining<br>Microenvironment positive if ≥20% of total tissue membrane or cytoplasmic staining<br>HRS cells evaluated as positive or negative regardless of intensity |
| Yamamoto 2008[70] | Characterization of PD-L1 and PD-L2 expression | 19 HL, 12 B-NHL | IHC (n=4)<br>Flow cytometry LN SSS (n=3) and PB (n=10) | Cells positive |

*ABVD: doxorubicin, bleomycin, vinblastine, and dacarbazine; AHSCT: autologous hematopoietic stem cell transplantation; ALCL: anaplastic large cell lymphoma; ALL: acute lymphoblastic leukemia; BCL: B-cell lymphoma; BL: Burkitt lymphoma; CHL: classical Hodgkin lymphoma; CLL: chronic lymphocytic leukemia; DLBCL: diffuse large B-cell lymphoma; EBV: Epstein–Barr virus; ENKTCL: extranodal NK/T cell lymphoma; FISH: fluorescence* in situ *hybridization; FL: follicular lymphoma; GCB: germinal center B cell; HCL: hairy cell leukemia; HL: Hodgkin lymphoma; hpf: high-power field; IHC: immunohistochemistry; KS: Kaposi sarcoma; LDCHL: lymphocyte-depleted classical Hodgkin lymphoma; LN: lymph node(s); LPD - lymphoproliferative disorder; LPL: lymphoplasmacytic lymphoma; LRCHL: lymphocyte-rich classical Hodgkin lymphoma; MCCHL: mixed cellularity classical Hodgkin lymphoma; MCL: mantle cell lymphoma; MM: multiple myeloma; mo: months; MZL: marginal zone lymphoma; NHL: Non-Hodgkin lymphoma; NLPHL: nodular lymphocyte-predominant Hodgkin lymphoma; NOS: not otherwise specified; NPC: nasopharyngeal carcinoma; NSCHL: nodular sclerosis CHL; PB: peripheral blood; PEL: primary effusion lymphoma; PMBCL: primary mediastinal large B-cell lymphoma; PTLD: post-transplant lymphoproliferative disorder; SLL: small lymphocytic lymphoma; SSS: single cell suspension(s); TCHRBCL: T-cell/histiocyte-rich large B-cell lymphoma; TIL: tumor infiltrating lymphocytes; TMA: tissue microarray; yrs: years. CHL: classical Hodgkin lymphoma; B-LPD: B cell lymphoproliferative disorder; $T_{FH}$: T follicular helper; R-CHOP: Rituximab, cyclophosphamide, hydroxydaunorubicin, vincristine, and prednisolone; RNA: ribodeoxynucleic acid; NHL: non-Hodgkin lymphoma; LPHL: lymphocyte predominant Hodgkin lymphoma; qRT-PCR; quantitative real-time polymerase chain reaction; HRS: Hodgkin Reed-Sternberg cell; CMV: cytomegalovirus; mRNA: messenger RNA.*

initally not detected on DLBCL cells,[33] but heterogenous expression in a small number of patients was subsequently described.[34,40]

### *PD-L1/PD-1 expression on DLBCL-associated immune cells*

Initial studies described numerous PD-L1/PD-L2$^+$ and variable, non-quantified amounts of PD-1$^+$ reactive lymphocytes[34] (Table 2). More recently, most DLBCL-infiltrating immune cells were characterized as PD-L1 expressing macrophages, with 30% of patients showing PD-L1 expression mainly in macrophages with little expression in tumor cells.[38] Using a threshold of ≥20% PD-L1$^+$ nonmalignant cells among the total tissue cellularity in PD-L1$^-$ patients, the study by Kiyasu *et al.* reported a microenvironment PD-L1$^+$ prevalence rate of 15%.[39] This was significantly associated with non-GCB type and EBV positivity, but not with gain of chromosome 9 nor structural abnormalities in chromosome 9p.

Increased PD-1$^+$ TILs were detected in 11% of 184 DLBCL, but numbers and percentages were lower compared with FL and PMBCL.[40] Similarly variable and low numbers of PD-1$^+$ TILs were described in a Korean cohort.[41] More than half of the included patients were classified PD-1$^+$, with no differences between GCB subtypes. PD-1$^+$ cases had significantly higher clinical stage ($P$=0.025) and higher International Prognostic Index (IPI) ($P$=0.026) than PD-1$^-$ patients. Subsequent studies classified PD1$^+$CD4$^+$ TILs in DLBCL as $T_{FH}$ cells, and noted reduced $T_{FH}$ numbers in DLBCL and reactive lymph nodes (LNs) compared to tonsils.[42,43] CD4$^+$ T-cell numbers correlated with both PD-1$^+$ and FoxP3$^+$ numbers.[42] More recently, PD-1 was detected on TILs in all but two cases, and their quantity correlated positively with the level of PD-L1 expression in tumor cells ($P$=0.042) or in tumor cells/macrophages ($P$=0.03).[38] In the study by Kiyasu *et al.*, the number of PD-1$^+$ TILs was significantly lower in PD-L1$^+$ patients and in those with B symptoms ($P$=0.024), extranodal sites ($P$=0.042) and bulky disease ($P$=0.041), but higher in GCB-type DLBCL ($P$=0.034).[39]

### *Prognostic relevance of PD-1 expression in DLBCL*

Distinct molecular subtypes determine biology and outcome in DLBCL,[44,45] and molecular- and IHC-based algorithms have confirmed additional tumor-promoting roles of the microenvironment.[46] However, findings regarding the prognostic relevance of TILs and tumor-associated macrophages (TAMs) are conflicting. Whereas infiltration with activated CD4$^+$ cells generally correlates with better prognosis, the role of specific subtypes, such as FoxP3$^+$ cells, has been largely contradictory.[47-49] The same appears to be true for PD-1$^+$ TILs in GC lymphomas (Table 6). While actual median values were not reported, the numbers of PD1$^+$$T_{FH}$ ($P$=0.0007), FoxP3$^+$ ($P$=0.0069), and total CD4$^+$ cells ($P$=0.04) above the median were associated with improved overall survival (OS), and had independent prognostic significance in multivariate analyses.[42] This was confirmed in more recent studies; although the quantity of PD-1$^+$ TILs showed no significant association with clinicopathological variables, the presence of PD-1$^+$ TILs (score 1–3) significantly prolonged OS ($P$=0.026) and progression-free survival (PFS) ($P$=0.005), and was an independent favorable prognostic factor in multivariate analyses.[38] In contrast, in another study, patients with PD-1 expression >20/hpf had a trend to poorer OS ($P$=0.120).[41] A similar trend was seen when groups were further refined to 1-10, 11-50, 51-100 and >100 PD-1$^+$ cells/hpf, but numbers were too small to allow valid conclusions.

### *Prognostic relevance of PD-L1 expression in DLBCL*

The prognostic relevance of cellular PD-L1 has only recently been explored (Table 6). Strong tumor and tumor/macrophage PD-L1 expression were significantly associated with B symptoms ($P$=0.005 tumor only, $P$=0.011 tumor and/or macrophages) and EBV infection ($P$=0.015 tumor only, $P$=0.020 tumor and/or macrophages), and tended to be higher in activated B-cell (ABC) than GCB DLBCL.[38] This however did not correlate with survival, which is somewhat inconsistent with another report showing that increased plasma PD-L1 lev-

**Table 2.** Expression of PD-1 and PD-L1 on tumor infiltrating lymphocytes (TILs) and tumor cells in DLBCL.

| DLBCL | Method of quantification | PD-1 expression on | | PD-L1 expression on | |
|---|---|---|---|---|---|
| | | TILs | Tumor cells | TILs | Tumor cells |
| Dorfman 2006[33] | Positive cases/ all cases | nd | 0/6 | nd | nd |
| Xerri 2008[34] | Proportion of positive cells[#] | Variable, not quantified | 2/25 pts: + | Numerous, not quantified | 4/14 pts: + to +++ |
| Muenst 2010[40] | Mean number of positive cells/mm$^2$<br>Mean % of positive cells/ all cells<br>Pts with positive cells >mean | 27±93 (SD)<br>1.1<br>20/184 (11%) | 20/184 pts<br>nd<br>nd | nd<br>nd<br>nd | nd<br>nd<br>nd |
| Andorsky 2011[37] | % positive cells frozen specimen<br>% positive cells paraffin specimen | nd<br>nd | nd<br>nd | nd<br>nd | 9/33 pts: 27%<br>6/30 pts: 20% |
| Ko 2011[41] | Mean number of PD-1$^+$ TILs/hpf[$]<br>Pts with positive cells >mean | 21 (range 0-201)<br>33 (52.4%) | nd<br>nd | nd<br>nd | nd<br>nd |
| Amé Thomas 2012[43] | % $T_{FH}$ cells/ all cells | Median 0.2% (0-20) | nd | nd | nd |
| Chen 2013[35] | % of positive cells | nd | nd | nd | EBV: in 7/66 pts on 10-90% of cells<br>EBV$^+$: present in all pts |
| Rosille 2014[36] | % positive cells | nd | nd | nd | 55/73 pts: ≥5% |
| Ahearne 2014[42] | % positive cells/ all cells | 0.1 - 1.5 % | nd | nd | nd |
| Kiyasu 2015[39] | Prevalence rates of PD-L1+ DLBCL and microenvironment PD-L1$^+$ DLBCL<br>Median TILs/ mm$^2$ | nd<br>Reported according to various clinical features | nd<br>nd | 15.3% (172 of 1121)<br>nd | 10.5% (132 of 1253)<br>nd |
| Kwon 2015[38] | N (%) pts positive<br><br>Staining intensities among positive cells<br><br><br>Quantity of PD-1+ TIL/hpf | <br><br><br><br>0: 38 (31%)<br><10: 30 (25%)<br>10-30: 23 (19%)<br>>30: 30 (25%) | <br><br><br><br> | In tumor cells and/ or macrophages: 115 (91%)<br>weak 55 (44%)<br>moderate 46 (37%)<br>strong 14 (11%) | 77 (61%)<br><br>weak 37 (29%)<br>moderate 27(21%)<br>strong 13 (10%) |

*hpf: high power field; nd: not done; pts: patients; SD: standard deviation. EBV: Epstein-Barr virus; DLBCL: diffuse large B-cell lymphoma. #+ 1-50%, ++ 50-75%, +++ >75% of cells positive; $classified as positive for >20/hpf, negative for ≤20/hpf.*

els were associated with poorer prognosis in DLBCL patients.[36] Inferior OS was also reported in patients with PD-L1$^+$ DLBCL ($P$=0.0009), and the expression of PD-L1 maintained prognostic value for OS in multivariate analysis.[39] Combining the median number of TILs with positive or negative PD-L1 expression patterns, the PD-L1$^+$/TIL$^{low}$ group was significantly associated with poor prognosis compared to the PD-L1$^-$/TIL$^{low}$ group, whereas no prognostic impact was observed in the other two groups (PD-L1$^+$/TIL$^{high}$ and PD-L1$^-$/TIL$^{high}$).

## FL

### PD-L1/PD-1 expression on lymphoma cells

The majority of published studies reported virtually PD-L1 negative FL cells[34,37,50] (Table 3). We found significantly increased PD-L1 on FL compared to healthy B cells, and on tumor cells from patients with <5-year (n=34) *versus* >15-year (n=25) survival.[27] PD-1 was heterogeneously expressed on 1-50% of tumor cells in a minority of FL specimens,[34] whereas others excluded PD-1 expression on B cells.[33]

### PD-L1/PD-1 expression on FL-associated immune cells

PD-L1 expression was detected in some CD3$^+$ cells in both reactive LN and FL samples[50] (Table 3). A number of studies have characterized PD-1$^+$ $T_{FH}$ cells, with similarly high proportions of $T_{FH}$ in tonsils and FL LNs (median 30% and 32%, respectively).[43] At diagnosis (n=100), PD-1$^+$ cells were mainly observed in follicular areas, but numbers were highly variable (mean 21.8%, range 0.12-73.6%) and similar to reactive tonsils.[50] PD-1$^+$ cells decreased with increasing histological grade (P=0.003), but correlated with the number of $T_{Regs}$. PD-1$^+$ cell numbers were also significantly lower in patients with poor performance status ($P$=0.014) and high serum lactate dehydrogenase (LDH, $P$=0.001). At relapse (n=32), the number of PD-1$^+$ cells was similar to diagnosis for all grades. In transformed FL (n=10), PD-1$^+$ numbers were significantly lower than either at diagnosis or relapse. Decreasing but numerous PD-1$^+$ TILs with increasing grade (n=49) and transformation to DLBCL (n=11) were described by others.[40] There might be an association between male gender and increased PD-1$^+$ cells,[51] but further confirmation is lacking.

Several studies have focused on localization patterns of TFH cells. While PD-1 expression generally correlated with T-cell content in both interfollicular and follicular zones, it was mainly expressed within[52] or restricted to follicles.[53] FoxP3$^+$ cells were predominantly found interfollicularly, but a high follicular content of FoxP3$^+$ and PD-1$^+$

**Table 3.** Expression of PD-1 and PD-L1 on tumor infiltrating lymphocytes (TILs) and tumor cells in FL.

| FL | Method of quantification | PD-1 expression on | | PD-L1 expression on | |
|---|---|---|---|---|---|
| | | TILs | Tumor cells | TILs | Tumor cells |
| Dorfman 2006[33] | Positive cases/ all cases | nd | 0/6 | nd | nd |
| Xerri 2008[34] | Proportion of positive cells[#] | nd | 3/43 pts: + | nd | 0/8 pts |
| Carreras 2009[50] | Proportion of positive cells | Diagnosis *vs.* relapse (mean±SD): Gr1/2: 24.3±20% *vs.* 19.8±20%, Gr3: 13.2±17% *vs.* 20.6±18% | nd | Median 9% (2.4-29%) | Median 2.4% (0-4%) |
| Muenst 2010[40] | Mean number of positive cells/mm$^2$ ±SD | Gr1/2: 287±228<br>Gr3: 128±105<br>tFL: 75±107 | nd | nd | nd |
| | Mean % of positive cells/ all cells | Gr1/2: 6.5, Gr3: 4.5, tFL: 2.3 | nd | nd | nd |
| | Pts with positive cells >mean | Gr1/2: 7/42 (17%)<br>Gr3: 2/7 (29%)<br>tFL: 3/11 (27%) | nd | nd | nd |
| Wahlin 2010[59] | Nmber of positive cells/ total area good *vs.* poor outcome pts | Total: 2.7 *vs.* 2.5<br>Follicular: 3.7 *vs.* 2.8<br>Interfoll.: 2.2 *vs.* 2.5 | nd | nd | nd |
| Andorsky 2011[37] | % positive cells flow cytometry | nd | nd | nd | 0/16 pts |
| | % positive cells paraffin specimen | nd | nd | nd | 0/7 |
| Richendollar 2011[53] | Median number of positive cells/hpf | 35.6 cells/hpf (range 4.4-91.2) | nd | nd | nd |
| | Pts> median | 45/91 (49%) | nd | nd | nd |
| Amé Thomas 2012[48] | Median % $T_{FH}$ cells/ all cells | Tonsils: 30% (5--57)<br>FL LN: 32% (10- 57) | nd | nd | nd |
| Koch 2012[52] | Median % positive cells/ 100 cells/hpf | Follicular: 12.7% Interfollicular: 3.3% | nd | nd | nd |
| Ramsay 2012[27] | Mean intensity healthy *vs.* FL[$] | CD3$^+$ cells: ~105 *vs.* 150 | nd | nd | CD20$^+$ cells: ~90 *vs.* 150 |
| | Mean intensity long *vs.* short survival[$] | CD3$^+$ cells: ~140 *vs.* 175 | nd | nd | CD20$^+$ cells: ~135 *vs.* 175 |
| Yang 2015[55] | % positive cells | CD4$^+$: PD-1$^{high}$ 26%, PD-1$^{low}$ 26.4%<br>CD8$^+$: PD-1$^{high}$ 4.8%, PD-1$^{low}$ 42.1% | nd | nd | nd |

*Gr: grade; nd: not done; pts: patients; SD: standard deviation. FL: follicular lymphoma; $T_{FH}$: T follicular helper; LN: lymph node. tFL: transformed follicular lymphoma; hpf: high-power field. [#]+ 1-50%, ++ 50-75%, +++ >75% of cells positive; [$]actual values not given, mean numbers estimated from graphs in figures.*

cells was associated with high interfollicular content of the same cell type.[52] Regardless of region, PD-1 content decreased with stage, and the interfollicular PD-1 content decreased in patients with a high Follicular Lymphoma International Prognostic Index (FLIPI) score. More recent evidence suggests that PD-1$^+$CD4$^+$ cells consist of several sub-populations and include conventional TFH cells and PD-1$^+$TIM-3$^+$ exhausted T cells, which primarily reside in the interfollicular space.[54] Functionally exhausted TIM-3$^+$ cells were PD-1low in another study, while the majority of CD4$^+$PD-1$^{high}$ T cells were conventional $T_{FH}$ cells.[55] Others identified distinct functional T-cell populations displaying specific gene expression profiles on the basis of CD25, namely CD25$^+$ follicular regulatory T cells and CD25- $T_{FH}$.[43] Changes in PD-1, PD-L1 and PD-L2 expression were analyzed with pidilizumab and rituximab treatment in relapsed FL patients.[16] PD-L1 but not PD-1 or PD-L2 was significantly higher in blood T cells and monocytes of responders (n=18) than non-responders (n=7). Additional gene expression signature studies conducted in this trial suggested that T-effector cells had anti-tumor and $T_{FH}$ cells had pro-tumor effects, predicting tumor shrinkage and PFS. As this was not recapitulated in an external dataset of 191 patients largely treated with chemotherapy, the predictive power of the identified gene signature might only be relevant with PD-L1/PD-1 blockade.

### *Prognostic relevance of PD-1 expression in FL*

Gene expression profiling studies demonstrated that the cellular microenvironment plays an essential role in lymphomagenesis and outcome in FL, with enrichment in T-cell and monocyte-restricted genes conferring a favorable prognosis, and with activated macrophages/dendritic genes conferring a poor prognosis.[56] However, it appears that both survival and transformation into DLBCL are influenced by the presence and perifollicular *versus* follicular localization of specific T-cell subtypes, including FOXP3$^+$ $T_{Reg}$s[57,58] (Table 6). Several studies have assessed the prognostic relevance of PD-1$^+$ T cells, but findings are contradictory; increased levels of PD-1$^+$ TILs were associated with improved 5-year OS ($P$=0.004) and PFS in one study ($P$=0.038), but patients were treated independently of the number of PD-1$^+$ cells, and there was no correlation to the type of therapy and therapeutic response.[50] In contrast, increased levels were associated with reduced survival in another study.[53] PD-1 was an independent risk factor (RF) in a scoring system predicting 10-year survival rates of 80%, 60%, and 15% in the low (0 RFs, n=14), intermediate (1/2 RFs, n=64) and high-risk group (3/4 RFs, n=13). Using an extremes of survival approach, we detected increased PD-1 expression on follicular T cells in poor outcome *versus* long-term surviving patients, as well as on CD3$^+$ T cells from patients compared to healthy controls.[27]

Two studies found no impact on time to treatment failure or OS.[51,52] The numbers of CD4+ cells were associated with poor outcome, and CD8+ and PD-1+ cells with improved outcome, independently of FLIPI.[59] In another study, increased numbers of CD4+PD-1high $T_{FH}$ cells had no impact on survival ($P$=0.411), while that of exhausted CD4+PD-1low ($P$=0.007) and of CD8+PD-1+ (most likely also exhausted cytotoxic T cells) reduced survival ($P$=0.026).[55]

A potential prognostic role has also been attributed to patterns of PD-1+ TILs. The prognostic values of CD4+ and PD-1+ cells were accentuated when they were follicular, and that of CD8+ cells when they were interfollicular.[59] Patients with PD-1+ in follicular patterns (i.e. $T_{FH}$, n=38) also had prolonged time to transformation (TTT) and OS compared to patients with diffuse patterns (n=19), and transformation within one year occurred exclusively in patients with diffuse patterns.[54] Multivariate analyses demonstrated that PD-1+ cells with diffuse patterns were associated with shorter TTT (HR 1.9, $P$=0.045) and inferior OS (HR 2.5, $P$=0.012), but that inferior outcome was also independently influenced by follicular dendritic cells (HR 3.0, $P$=0.004). In another study, transformation risk was significantly higher in patients (n=25) with less than 5% PD-1+ TILs compared to other patients.[50]

## CLL/ SLL

### PD-L1/PD-1 expression on tumor cells

In initial IHC studies, neither PD-L1 nor PD-L2 were expressed on LN SLL/CLL cells[34] (Table 4). Larger IHC studies later found significantly higher PD-L1 expression on CLL cells compared to control LN samples.[27,60] Small vessels in CLL LNs also appear to express PD-L1 weakly, whereas this was confined to endothelial cells lining vessels in reactive LNs.[60] Higher PD-L1 expression on CLL cells was also detected in blood in some[27,60] but not all studies.[61] PD-1 was strongly expressed on ≥50% of tumor cells in the majority of SLL LN specimens and on peripheral blood (PB) neoplastic cells from almost all CLL patients.[34] Similar expression patterns were described in flow-cytometry-based studies.[60,61] In contrast, the majority of examined SLL/CLL full tissue sections collected from three different institutions (n=58) were PD-1- in another study,[40] similar to earlier findings of a lack of PD-1 expression on CLL cells.[33]

### PD-L1/PD-1 expression on CLL/SLL-associated immune cells

PD-1+ TILs are generally exceptionally low in CLL/SLL compared to other lymphomas[40] (Table 4). We found significantly increased PD-1 expression on T cells from CLL patients compared to reactive LNs, and on PB CLL T cells compared to age-matched healthy donor T cells (both $P$<0.01).[27] While percentages and numbers of CD4+ and CD8+ T cells are significantly increased in CLL patients,[60,62,63] marked differences exist in the composition of both CD4+ and CD8+ T-cell subsets. This includes decreased naïve and relatively increased effector cells, with differential PD-1 expression compared to age-matched controls, and in specific subpopulations such as BLIMP1HI CD4+ and CD8+ T cells and effector cells.[60,62,63]

### Prognostic relevance of PD-L1/ PD-1 expression in CLL

Studies assessing the prognostic value of PD-L1/ PD-1 in CLL are lacking, and correlations between PD-L1/PD-1 and other conventional prognostic markers have not been identified.[34,60,61] Using an extremes of survival approach and a limited number of patient samples, we found significant-

**Table 4.** Expression of PD-1 and PD-L1 on tumor infiltrating lymphocytes (TILs) and tumor cells in CLL/SLL.

| CLL/SLL | Method of quantification | PD-1 expression on | | PD-L1 expression on | |
|---|---|---|---|---|---|
| | | TILs | Tumor cells | TILs | Tumor cells |
| Dorfman 2006[33] | Positive cases/ all cases | nd | 0/4 | nd | nd |
| Xerri 2008[34] | Proportion of positive cells (IHC)* | nd | SLL: 12/13 pts ++ to +++ | nd | SLL: 0/7 pts |
| | Pts with positive cells (flow cytometry) | nd | CLL: 10/11 | nd | CLL: 0/11 |
| Muenst 2010[40] | Mean number of positive cells/mm² ±SD | 13±37 | nd | nd | nd |
| | Mean % of positive cells/ all cells | 0.2 | Unequivocal in 8/66 pts (5%) | nd | nd |
| | Pts with positive cells >mean | 15/58 (26%) | nd | nd | nd |
| Grzywnowicz 2012[61] | Median % positive | nd | CLL vs. healthy B cells: 47.2 *vs.* 14.81 | nd | CLL cells 52.52% (10.8–97.3) |
| | MFI CLL *vs.* healthy B cells | nd | nd | nd | 9.96 *vs.* 7.93 |
| Ramsay 2012[27] | Mean intensity healthy *vs.* CLL LN (IHC)§ | ~120 *vs.* 150 | nd | nd | ~80 *vs.* 150 |
| | Mean intensity long *vs.* short survival LN (IHC)§ | nd | nd | nd | ~120 *vs.* 150 |
| | MFI healthy *vs.* CLL PB (flow cytometry)§ | ~10 *vs.* 25 | nd | nd | ~12 *vs.* 20 |
| Tonino 2012[63] | % positive effector cells CLL. *vs.* healthy controls§ | CD4: ~20 *vs.*40<br>CD8: ~12.5 *vs.* 25 | nd | nd | nd |
| Brusa 2013[60] | % positive cells pts *vs.* healthy controls (flow cytometry)§ | CD4: ~50 *vs.* 35<br>CD8: ~30 *vs.* 10 | ~18 *vs.* <5 | nd | ~35 *vs.* 20 |
| | % positive areas in proliferation centers *vs.* other parts of same slide (IHC) | ~12 *vs.* 7 | nd | nd | ~10 *vs.* 5 |
| | Pattern of expression (IHC) | nd | nd | nd | Diffuse: 9/20 pts patchy: 10/20 pts |
| Riches 201362 | % positive CLL *vs.* healthy controls§ | Median ~25 *vs.* 18 | nd | nd | nd |
| | AN positive cells/µl CLL *vs.* healthy controls§ | CD8: median ~400 *vs.* 90 | nd | nd | nd |

*IHC: immunohistochemistry; LN: lymph node(s); MFI: median fluorescence intensity; nd: not done; pts: patients; SD: standard deviation. SLL: small lymphocytic lymphoma; CLL: chronic lymphocytic leukemia; PB: peripheral blood. *+ 1-50%, ++ 50-75%, +++ >75% of cells positive; §actual values not given, mean numbers estimated from graphs in figures.*

ly increased expression of PD-L1 on CLL cells and of PD-1 on $CD3^+$ T cells in poor prognosis patients (median survival 38 months, n=18) compared with good prognosis patients (median survival >10 years, n=17)[27] (Table 6). This, however, was based on a relatively small sample size and requires confirmation in independent patient cohorts. Others described an association between stage, need of therapy and molecular markers and levels of $CD4^+$ and $CD8^+$ subsets, but the exact role of PD-1 has not been established.[60]

### HL/ PMBCL

#### PD-L1/ PD-1 expression on HL and PMBCL cells

An underlying molecular mechanism leading to elevated *PD-L1/PD-L2* transcription is present in most patients with HL and PMBCL, as frequent cytogenetic alterations involve chromosome 9p, the coding region for *PD-L1/PD-L2*.[64-67] PD-L1 expression on malignant cells has been described by several studies for the majority of PMBCL patients and on Reed–Sternberg (RS) cells in patients with HL, mostly in conjunction with PD-L2[33-35,37,68-70] (Table 5). Expression seems to differ with histological subtype, with strong tumor PD-L1 expression in the majority of patients with nodular sclerosis classical HL (cHL), mixed cellularity cHL and cHL-not otherwise specified (NOS), but only in a small fraction of nodular lymphocyte-predominant HL patients.[35] Although tumor infiltration varied widely in this cohort, tumor PD-L1

**Table 5. Expression of PD-1 and PD-L1 on tumor infiltrating lymphocytes (TILs) and tumor cells in HL/PMBCL.**

| HL/PMBCL | Method of quantification | PD-1 expression on | | PD-L1 expression on | |
|---|---|---|---|---|---|
| | | TILs | Tumor cells | TILs | Tumor cells |
| Dorfman 2006[33] | Positive cases/ all cases | 14/14 | 0/25 | nd | RS positive but not quantified |
| Nam-Cha 2008[71] | Positive cases/ all cases (rosette formation) | NSCHL 0/43,MCCHL 0/14, LRCHL 10/13, NLPHL 57/58 | nd | nd | nd |
| Yamamoto 2008[70] | %positive cells SSS LN[$] | $CD4^+$: 54.3-76.8% $CD8^+$: 53-66.6% | nd | nd | Increased, but not quantified |
| | %positive cells PB healthy *vs.* HL[$] | ~5-15 *vs.* 5-53 | nd | nd | nd |
| Xerri 2008[34] | Proportion of positive cells[#] | Not quantified | cHL: 0/30 pts LPHL: 0/5 pts | nd | cHL: 8/13 pts + to ++ LPHL: 4/4 pts + to ++ |
| Muenst 2009[72] | Mean number of positive cells/$mm^2$ | NSCHL 275 ± 493, MCCHL 129 ± 175, LRCHL 1044 ± 1116, LDCHL 202 ± 109, cHL-NOS 544 ± 794, NLPHL 296 ± 95 | nd | nd | nd |
| | Median number of positive cells/$mm^2$ | NSCHL 16, MCCHL 37, LRCHL 203, LDCHL 49, cHL-NOS 30, NLPHL 297 | nd | nd | nd |
| Andorsky 2011[37] | % positive cells frozen specimens | nd | nd | nd | HL 8/9 pts 89% of cells, PMBCL 3/3 pts 100% |
| Chen 2013[35] | Median and range percent of malignant cells | nd | nd | nd | NSCHL 5% (2-20), MCCHL 2% (2-10), CHL-NOS 50% (2-90), NLPHL 2% (2-5) |
| | N (%) cases with ≥5% malignant cells positive | nd | nd | nd | NSCHL 21/25 (84%), MCCHL 7/8 (>2+ membranous staining)* (88%), CHL-NOS 5/5 (100%), NLPHL 2/15 (13%), PMBCL 15/21 (71%) |
| | N (%) cases with ≥20% total cellularity positive (>2+ membranous and/or cytoplasmic staining)* | nd | nd | nd | NSCHL 19/25 (76%), MCCHL 7/8 (88%), CHL-NOS 5/5 (100%), NLPHL 1/15 (10%), PMBCL 19/21 (90%) |
| Greaves 2013[73] | Pts with % positive cells (IHC) | Not detectable in 42%, <0.5% of all nucleated cells in another 40% | nd | nd | nd |
| Ansell 2015[18] | %positive cells | Positive cells noted in all examined cases | nd | nd | Range 34-99% with staining intensity ++ to +++ |
| Koh 2015[69] | N (%) pts with ≥20% malignant cells PD-L1 or PD-L2-positive N (%) pts with ≥20 % of microenvironment cells PD-1-positive | 13 pts (11%) membranous positivity | nd | nd | 82 pts (75%) cytoplasmic and/or membranous positivity |
| Paydas 2015[68] | N (%) cases with ≥5% malignant cells or ≥20% microenvironment cells positive staining intensity | 18 cases (20%) n=3: ++ n=15: + | nd | 18 cases (20%) Staining in HRS cells and in microenvironment | |

*LDCHL: lymphocyte-depleted classical HL; LRCHL: lymphocyte-rich classical HL; MCCHL: mixed cellularity classical HL; nd: not done; NLPHL: nodular lymphocyte-predominant HL; NSCHL: nodular sclerosis CHL; PB: peripheral blood; pts: patients; RS: Reed-Sternberg; SSS: single cell suspension. LN: lymph node; HL: Hodgkin lymphoma; NOS: not otherwise stated; PMBCL: primary mediastinal B-cell lymphoma; HRS: Hodgkin Reed-Sternberg cell. °+ 1-50%, ++ 50-75%, +++ >75% of cells positive; #actual values not given, mean numbers estimated from graphs in figures; *0 no staining, + weak or equivocal staining, ++ moderate staining, +++ strong staining.*

expression correlated with expression of PD-L1 on tumor-infiltrating macrophages. A more recent study reported PD-L1 positivity in only 20% of examined HL patients, with staining intensities and patterns not further specified.[68] In contrast with findings in DLBCL, PD-L1 expression is not increased in EBV$^+$ patients.[35,68,70] RS cells and variants appear to lack PD-1 expression, suggesting a potentially mutually exclusive expression pattern with PD-L1.[33,35]

### *PD-L1/ PD-1 on HL/PMBCL-associated immune cells*

Several early studies identified increased numbers of PD-1$^+$ subsets, which frequently form rosettes around tumor cells, especially in lymphocyte-predominant Hodgkin lymphoma subtypes[33,70-72] (Table 5). Elevated levels of PD-1$^+$ TILs were also noted in blood T cells of HL patients (n=10) compared to healthy controls, and appeared to be higher in patients with active disease.[70] In contrast, using both cHL-derived single-cell suspensions (n=18) and TMAs (n=122), our group found only little expression of PD-1 in TILs, with 40% of patients having less than 0.5% PD-1$^+$ cells.[73] In the phase I study on nivolumab, CD3$^+$ TILs in available biopsy specimens largely expressed PD-1, albeit at similarly low levels.[18] Recently published studies assessing diagnostic cHL TMAs reported PD-1 positivity on microenvironment cells in 11%[69] and 20%[68] of patients. Interestingly, there were no clear correlations between PD-L1 and PD-1 expression in either study.[68,69] PD-1$^+$ cell numbers were lower in both cHL patients with 9p24 gains and with higher amounts of FOXP3$^+$ cells, but correlated with Granzyme-B and T-cell restricted intracellular antigen (TIA-1) expression in another study.[72]

### *Prognostic relevance of PD-1 expression in HL*

Associations between microenvironment PD-1 expression and PD-1$^+$ cell numbers and clinical variables or other known phenotypic parameters have not yet been identified.[69,72] Regardless, an increased amount of PD-1$^+$ TILs above the prognostic cutoff score (23 cells/mm$^2$) was a stage-independent negative prognostic factor of OS ($P$=0.005)[72] (Table 6). In a prognostic score incorporating numbers of PD-1, Granzyme-B, and FOXP3 expressing cells, different age- and stage-independent outcomes were found between risk groups (FOXP3$^-$PD-1$^+$GrB$^+$ median survival 91 months *vs.* FOXP3$^+$PD-1-Gr-B- not reached, $P$<0.0001). Similar associations were noted by our group: albeit expressed at low levels; patients with

**Table 6. Prognostic significance of PD-L1 and PD-1 in different types of B-NHL and HL. Orange color signifies reduced survival, green color improved survival, gray color no association between PD-L1/PD-1 and survival.**

| Reference | Cell type analyzed | Cutoff value(s) | Treatment | outcome measurement | Prognostic significance |
|---|---|---|---|---|---|
| **DLBCL** | | | | | |
| Ahearne 2014[42] | PD1$^+$T$_{FH}$ | >median | R-CHOP | OS | Improved survival<br>Independent prognostic significance in MV analysis |
| Kwon 2014[38] | PD1$^+$ TIL | No positive cells/hpf *vs.* presence of positive cells | R-CHOP | OS | Improved survival with increasing numbers of PD1$^+$ TILs/ hpf<br>Independent prognostic significance in MV analysis |
| Ko 2011[41] | PD1$^+$T$_{FH}$ | >20/hpf | Not reported | OS | Decreased survival but not significant |
| Kiyasu 2015[39] | PD1$^+$ TIL in combination with PD-L1 expression patterns | Median number of PD-1+ TILs | Newly diagnosed and untreated | OS | In combination with PD-L1 expression patterns: improved prognosis in PD-L1$^-$/ TIL$^{low}$ group (n=92) *vs.* PD-L1$^+$/ TIL$^{low}$ group (n=25), $P$=0.0086 |
| | | | | | No prognostic difference between PD-L1$^+$/ TIL$^{high}$ group (n=3) and PD-L1– /TIL$^{high}$h group (n=116). |
| Kwon 2014[38] | PD-L1$^+$ tumor cells and/or macrophages | No staining *vs.* staining | R-CHOP | OS | No impact |
| Kiyasu 2015[39] | PD-L1$^+$ tumor cells | ≥30% of lymphoma cells positive | Newly diagnosed and untreated | OS | Decreased survival compared to PD-L1- DLBCL ($P$=0.009) |
| | PD-L1$^+$ microenvironment in patients without tumor PD-L1 expression | ≥20% of total tissue positive | | OS | Decreased survival compared to microenvironmental PD-L1$^-$ DLBCL but not significant |
| **FL** | | | | | |
| Carreras 2009[50] | PD1$^+$T$_{FH}$ | <5% *vs.* 6-33% *vs.* >33% | n=80 fludarabine-based regimes, n=6 alkylating monotherapy, n=3 RT, n=11 w&w | 5-year PFS<br>5-year OS | Increased survival with increasing PD-1+ TILs: 20% (95%CI 2-38), 46% (30-64), 48% (26-70) 50% (30-70), 77% (64-90), 95% (85-100) independent prognostic factor in MV analysis |
| | | <5% | | 5-year risk of transformation | Increased risk of transformation: 29%, 95% CI 7-51% *vs.* 7%, 95% CI 1-13%, $P$<0.05 |
| Richendollar 2011[53] | PD1$^+$T$_{FH}$ | >35.6 cells/hpf | n=23 w&w, n=8 RT, n=12 rituximab, n=48 immunochemotherapy | OS | Decreased survival in MV analysis: HR 1.98, 95% CI 1.09-3.60, $P$=0.03<br>PD-1 independent risk factor in scoring system |
| Ramsay 2012[27] | CD3$^+$ | Extremes of survival | Untreated | Median OS | Increased PD-1 expression in poor survival group<br>Increased PD-L1 expression in poor survival group |

*Continued on the next page*

PD-1 expression in >15 cells/hpf had poorer 5-year disease specific survival, while OS was not affected.[73] Multivariate analyses demonstrated that high PD-1 ($P$=0.007) and low FOXP3 expression ($P$=0.029) were predictors of adverse OS. Significantly reduced OS among PD-1$^+$ patients was also reported in a recently published study, and multivariate analysis identified PD-1 expression as an independent prognostic marker for OS ($P$=0.019) along with high-risk IPS ≥3.[69] This was, however, dependent on Ann Arbor clinical stage; in limited-stage cHL, PD-1-positive patients had a worse OS compared with PD-1-negative patients ($P$=0.048), whereas in advanced stage cHL PD-1-positive status was not associated with OS ($P$=0.13). Another study found median OS and disease-free survival (DFS) to be shorter in patients with PD-1 compared to those without PD-1 expression, as well as in patients with PD-L1 expression compared to those without, but none of these differences were statistically significant.[68] Interestingly, co-expression of PD-1 and PD-L1 emerged as an independent risk factor for prognosis (OR 6.9, 95 % CI 1.9–24.3), and both OS and DFS were significantly reduced among patients with PD-1/PD-L1 coexpression compared to both PD-1 and PD-L1 negative patients.

### *Prognostic relevance of PD-L1 expression in HL*

Koh *et al.* reported that patients with tumor PD-L1 expression were more likely to have a low level of lactate dehydrogenase ($P$=0.024) than PD-L1-negative patients, but neither PD-L1 nor PD-L2 expression were significantly associated with OS (P=0.477 and $P$=0.676)[69] (Table 6).

## Discussion

Preclinical studies suggest that PD-L1/PD-1 are key mediators of impaired anti-tumor immune responses in lymphomas.[13,14] It is therefore reasonable to hypothesize that increased PD-L1/PD-1 expression confers an adverse prognosis, and that such patients might be prime candidates for therapeutic strategies targeting this axis. As prospective studies are currently lacking, we systematically reviewed published data on PD-L1/PD-1 expression and association with prognosis on B-cell lymphoma and lymphoma-associated cells.

We found that PD-L1 expression on DLBCL cells is very heterogeneous and present in only a small number of examined samples, while being affected by EBV status and potentially molecular subtype. On FL cells, PD-L1 is absent

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|---|---|---|---|---|---|
| Koch 2012[52] | PD1$^+$T$_{FH}$ | Continuous variable | CHOP, MCP | OS, TTF | No impact |
| Takahashi 2013[51] | PD1$^+$T$_{FH}$ | <7.5% *vs.* 7.5-24.4%vs. >24.4% | R-CHOP | OS | No impact |
| Yang 2015[55] | CD4$^+$PD-1$^{high}$<br>CD4$^+$PD-1$^{low}$<br>CD8$^+$PD-1$^{low}$ | >25%<br>>26%<br>>45% | Untreated | OS | No impact<br>Poorer survival<br>Poorer survival |
| Wahlin 2010[59] | CD4$^+$<br>CD8$^+$<br>PD1$^+$ | Extremes of survival | Elaborate criteria for good *versus* bad risk pts but treatments not specified | OS | Poorer survival, especially when follicular<br>Increased survival, especially when interfollicular<br>Increased survival, especially when follicular |
| Smeltzer 2014[54] | PD1$^+$T$_{FH}$ | Follicular *vs.* diffuse pattern | n=42 w&w, n=9 CHOP, n= 5 anthracycline-combination | Median TTT<br>Median OS | Follicular pattern prognostically favorable<br>TTT 6.1 *vs.* 3.6yrs, $P$=0.033<br>OS 9.7 *vs.* 4.6yrs, $P$=0.009 |
| **CLL** | | | | | |
| Ramsay 2012[27] | CD3$^+$<br>CD20$^+$ | Extremes of survival | Untreated | Median OS | Increased PD-1 expression in decreased survival group<br>Increased PD-L1 expression in decreased survival group |
| **HL** | | | | | |
| Muenst 2009[72] | PD-1$^+$ TILs | >23 cells/mm$^2$ | Not specified | Mean OS | Increased PD-1$^+$ TILs reduce survival:<br>198 (range 164-234) *vs.* 283 mo (247-318), $P$=0.005<br>PD-1$^+$ counts risk factor in prognostic score |
| Greaves 2013[73] | PD-1$^+$ TILs | >15 cells/hpf | n=56 anthracyclines, n=52 alkylator-based, n=14 RT, n=48 combined modality | 5-year DSS<br>5-year OS | Increased PD-1$^+$ TILs reduce DSS but not OS<br>DSS 63% *vs.* 86%, $P$ =0.012<br>OS 63% *vs.* 84%, $P$=0.18<br>Predictor of adverse OS in MV analysis |
| Koh 2015[69] | PD-1$^+$ microenvironment | ≥20% of cells positive | ABVD | Cumulative OS | OS significantly worse in PD-1$^+$ pts<br>Adverse predictor of OS in MV analysis in limited-stage cHL ($P$=0.048). |
| Paydas 2015[68] | PD-L1$^+$ tumor cells<br>PD-1$^+$ microenvironment | ≥5% of tumor cells positive *vs.* negative<br>≥20% of total tissue positive *vs.* negative | First-line ABVD<br>Second-line DHAP | OS<br>DFS | OS and DFS significantly worse in PD-1$^+$ and PD-L1$^+$ pts:<br>OS 24 *vs.*135mo, $P$=0.002<br>DFS 20 *vs.*,107mo, $P$=0.003 |

*ABVD: doxorubicin, bleomycin, vinblastine, dacarbazine; CI: confidence interval; DFS: disease free survival; DHAP: dexamethasone, cytarabine, cisplatin; DSS: disease specific survival; HR: hazard ratio; MCP: melphalan, chlorambucil, prednisone; mo: months; MV: multivariate; OS: overall survival; pts: patients; R-CHOP: rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone; RT: radiotherapy; TTT: time to transformation; w&w: watch and wait; yrs: years. CHOP: cyclophosphamide, hydroxydaunorubicin, vincristine, and prednisolone; TTF: time to treatment failure; T$_{FH}$: T follicular helper; TIL: tumor infiltrating lymphoma; hpf: high power field; DLBCL: diffuse large B-cell lymphoma; PFS: progression-free survival.*

except in an extremes of survival approach. PD-L1 expression on CLL/SLL cells is increased on both LN and PB cells and in patients experiencing short-term survival. Malignant PMBCL and RS cells strongly express PD-L1 and PD-L2, especially in cHL subtypes, while being less affected by EBV serostatus. PD-1 expression was scarce on DLBCL and FL cells and absent on RS cells and variants, whereas highly conflicting findings exist in CLL.

PD-1$^+$ TILs in DLBCL are predominantly $T_{FH}$ cells, and numbers are reduced compared to tonsils and other lymphomas. This appears unaffected by molecular subtype, but numbers increase with advanced disease. In FL, PD-1+ cells mainly reside in follicles. Their numbers are comparable to tonsils, but decrease with increasing histological grade, advanced stage and transformation. Several sub-populations of PD-1+CD4+ cells with distinct localization preferences and functions have been identified, including conventional TFH, exhausted and follicular regulatory T cells. Compared to other lymphomas, PD-1+ TILs numbers appears to be low in SLL/CLL LNs, but increased relative and absolute T cell numbers and functionally distinct subsets are present in blood. In HL, conflicting findings exist regarding the architectural structure of PD-1+ T-cell subsets and levels of PD-1+ TILs, potentially due to differences in examined histological subtypes and disease activity.

This heterogeneity within and across lymphoma entities is reflected by contradictory findings on the prognostic role of PD-1$^+$ TILs, especially in DLBCL. On first sight, the same seems to be true for FL. However, both prognosis and transformation appear to be determined by follicular *versus* interfollicular localizations of exhausted *versus* functional or regulatory CD4$^+$ and CD8$^+$ cells. A more defined role exists in HL, where despite low and/ or variable overall numbers, elevated numbers of PD-1$^+$ TILs confer a poor prognosis. PD-L1 expression was generally found to be an adverse prognostic marker across examined lymphoma types.

Such heterogeneous findings can partly be explained by differences in the nature and composition of the examined cohorts (sample sizes, patient characteristics, treatment, etc.). Another explanation are differing methodologies, including the choice of reagents, analysis systems and definition of positivity and cutoff values. A validation study from a lymphoma consortium on the FL microenvironment reported considerable differences between manual scoring and automated microscopy systems and flow cytometry, which was also dependent on the investigating laboratory.[74] Within semi-automated image analysis systems, a high concordance seems to exist.[49] Among the included studies, expression was predominantly assessed by IHC. However, methods of quantifying positive cells and the definition of staining intensity and positivity varied widely. In selected studies, different counting methods were compared or verified with flow-cytometry results. Several studies have also accounted for intra- and inter-observer bias, showing good reproducibility especially in areas with fewer PD-1$^+$ cells. Similar issues have been observed in solid malignancies, where the use of PD-L1 as a biomarker is confounded by detection antibodies, differing cutoffs and differences in tissue preparation and processing variability.[75]

It is also likely that biological behavior and prognosis are determined not only by overall PD-1$^+$ TILs and tumor cells, but by functionally distinct subsets. PD-1$^+$ numbers correlated with CD4$^+$ T-cell and FoxP3$^+$ numbers and GrB and TIA-1$^+$ cells in several studies,[42,52,72] and similar associations were found between distribution patterns of FoxP3$^+$ and PD-1$^+$ cells. Modulating effects might also be exerted by other microenvironment components such as TAMs,[35] tumor-associated histiocytes,[37] and small vessels.[60] Studies in CLL, for example, suggest that monocyte-derived suppressor cells with high PD-L1 expression and/ or skewed monocyte subpopulations are increased in patients and preclinical models and modulate T-cell responses.[76,77] In multiple solid cancer types, clinical responses were observed in patients with high PD-L1 expression on tumor-infiltrating immune cells and in those with TH1 gene signatures and T-cell CTLA-4 expression at baseline.[23] Immune dysfunction might also be mediated by other (potentially inducible) immune checkpoint receptor-ligand interactions, for example, by the binding of PD-1 to PD-L2[78] or by signaling *via* CD200, CD270 and CD276,[27] or by additional tumor-associated and/ or genetic determinants.[22] Upregulation of TIM-3 was recently reported in preclinical models of lung adenocarcinoma, where tumors progressed following response to anti-PD-1 therapy.[79] Optimally, the importance of these components should be assessed within one analysis and in conjunction with established clinic-pathological features.

Regardless of the expression and functions of PD-L1/PD-1 expressing cell subsets, blocking PD-L1/PD-1 interactions is safe and effective in patients with relapsed/refractory FL, DLBCL and HL.[16-18] This indicates that PD-L1/PD-1 expression on tumor cells or TILs cannot be used in isolation to predict outcome of treatment for individual patients. This is further supported by observations that the numbers of PD-L1$^+$ $T_{reg}$s, CD4$^+$ and CD8$^+$ central memory cells, and PD-L1$^+$ monocytes increased during treatment.[17] PD-1/PD-L1/PD-L2 expression changes could also be noted in responding *versus* non-responding patients.[16] Altogether, this work highlights that PD-L1/PD-1 expression on tumor cells and the microenvironment is only one aspect, albeit an essential one, determining the biology of lymphomas, and that the inclusion of additional components will be required to form prognostic models.

Therefore, attempts should be made to harmonize quantification methods and reporting of PD-L1/PD-1, optimally in the context of clinical studies on immune checkpoint inhibitors. Clinical study strategies should also include the identification of additional potential biomarkers using high-throughput technologies such as whole-exome sequencing, gene expression signatures/ patterns, epigenetic modifications, protein microarrays and flow and mass cytometry. To address the challenges of assay comparability, performance standardization, interpretation of test results and safe translation into patient care, the US Food and Drug Administration (FDA), the American Association for Cancer Research (AACR) and the American Society of Clinical Oncology (ASCO) recently convened a workshop entitled "Complexities in Personalized Medicine: Harmonizing Companion Diagnostics Across a Class of Targeted Therapies". As a collaboration between several companies, a blueprint proposal was developed with the goal to agree on and deliver a package of information /data upon which analytic comparison of various diagnostic assays may be conducted in non-small cell lung cancer treated with PD1/PD-L1 inhibitors.[80] It is anticipated that the proposed study will build the pre-clinical evidence for PD-L1/PD-1 diagnostic characterization and lead to post-approval studies that will help inform personalized treatment decisions, and ultimately be applied to other tumor entities as well.

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