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PLASMA BIOMARKERS: A POSSIBLE WAY TO PREDICT EFFECTIVITY OF PD-1 BLOCKADE THERAPY IN HODGKIN LYMPHOMA

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Content

Abstract

Hodgkin lymphoma is one of the most frequent lymphomas in the Western world and is characterized by an extremely low number of tumor cells within a large microenvironment. Treatment of Hodgkin lymphoma by radiotherapy and chemotherapy is very effective, but is highly toxic, causing second malignancies to be the leading cause of death among Hodgkin lymphoma survivors. Partly because of the large microenvironment surrounding the tumor cells, immune checkpoint blockade therapy targeting the PD-1/PD-L1 axis has shown great efficacy in relapsed and refractory Hodgkin lymphoma patients. Selection for potential responders to immunotherapy is very important and can ben realized by determination of predictive soluble biomarkers. The aim of this thesis is to identify the potential prognostic and predictive value of sPD-L1, sPD-L2, sPD-1, Indoleamine 2,3-dioxygenase (IDO), sCD83, and VEGF for PD-1 blockade therapy, and the possible impact of these soluble biomarkers on the PD-1/PL-L1 axis in Hodgkin lymphoma. We found that sPD-L1 and sPD-1 are promising independent predictive biomarkers and combination PD-1 blockade therapy with anti-IDO or anti-VEGF antibodies might provide a solution for resistance to immune checkpoint inhibition.

Introduction

Over the past years, immune checkpoint therapy has become an important part of cancer treatment in both solid tumors and hematologic malignancies. Immune checkpoint therapy makes use of the intrinsic antitumor activity of the immune system through induction of an immune response to the tumor. Immune 'checkpoints', including cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) and the inhibitory receptor programmed cell death-1 (PD-1), normally function as inhibitory feedback loops to diminish immune activation. These checkpoints protect the host from autoimmunity or collateral tissue damage as a result of inflammation. This thesis mainly focuses on PD-1.

Programmed death-ligand 1 (PD-L1) is a membrane-bound protein primarily expressed on dendritic cells (DC) and monocytes (1,2). This ligand binds to the PD-1, expressed on activated T cells and B cells, DC, and monocytes (2). Research has shown that membrane-bound PD-1 and PD-L1 also have soluble forms, which will be discussed later (3). Upregulation of PD-L1 on antigen presenting cells can be accomplished when T cells recognize an antigen presented by human leukocyte antigen (HLA) molecules, which leads to the production of inflammatory cytokines (e.g. IFN-γ).

Cancer cells are not automatically recognized as 'foreign' by the immune system. Several mechanisms, such as regulatory immune cells, immunosuppressive cytokines and chemokines, and so-called 'immune-check-point' pathways down-modulate immune functions, resulting in a tumor-tolerant environment (4). This is frequently observed in a variety of cancers (5–8). Tumors subvert the immune response by increasing inhibitory immune checkpoint ligands like PD-L1 in the tumor microenvironment (TME), and therefore preventing an antitumor immune response through host T cell exhaustion (9). The PD-1-PD-L1 receptor-ligand pair serves as a dominant immune checkpoint pathway operative in the TME and regulates the strength of the immune response (10). PD-L1 expression is associated with worse clinical outcomes in several cancer types (1,11,12).

Increasing knowledge about the self-protecting mechanisms of tumor cells has led to the development of anti-PD-1 drugs like nivolumab and pembrolizumab. Monoclonal antibodies (mAbs) block the immune checkpoint pathways and therefore induce antitumor immune responses. Blockade of the PD-1 signaling pathway prevents inhibition of the immune response, allowing for the exhausted T cells to be reinvigorated

and induce an effective immune response, despite elevated levels of PD-L1 in the TME (10). This has appeared to be very effective in Hodgkin lymphoma, advanced melanoma, renal cell carcinoma, and non-small-cell lung cancer (NSCLC) (4,13,14). However, clinical success of this treatment greatly varies between different cancer types and different patients. Objective responses of 65-87% were achieved in Hodgkin lymphoma, whereas responses of 30% were reached in other malignancies (13).

Differences in response rates to immune checkpoint blockade might be explained by resistance. This phenomenon can be divided into two groups: 1) tumors that do not respond to therapy (primary resistance) and 2) tumors that initially respond, but eventually become resistant (acquired resistance) (13). Current knowledge about the mechanisms of resistance is limited, but Veldman, Visser, van den Berg, & Diepstra (13) described six potential mechanisms of PD-1 blockade resistance in Hodgkin lymphoma. 1) Shaping of a TME with downregulation of CD8+ T cells and upregulation of Treg cells (primary resistance); 2) Insufficient attraction and activation of T cells as a result of poor antigen presentation. Tumors with a high tumor mutation burden (TMB) present more neoantigens and as a result, are more sensitive to immune checkpoint blockade (15–17). Solid tumors are often characterized by a great number of genetic and epigenetic alterations, which leads to the presentation of a diverse set of neoantigens (18,19)**.** (primary resistance); 3) elevated IDO levels, resulting in inhibited activation and function of effector T cells and leading to attraction and activation of preexisting Tregs and differentiation of naïve T cells into Tregs (primary resistance); 4) attraction of tumor associated macrophages (TAMs), which, in combination with PD-L1, can inhibit PD-1 + T cells and natural killer (NK) cells (primary resistance); 5) upregulation of PD-1, LAG-3 and TIM-3 by PD-1 blockade therapy (acquired resistance) and 6) deregulated purinergic signaling as a result of increased levels of adenosine. Adenosine is an immunosuppressive molecule that suppresses effector T cells and upregulates Treg in the TME (13).

As mentioned before, like numerous costimulatory molecules in immune regulation pathways, PD-1 and PD-L1 have membrane-bound forms and soluble forms. This increases the complexity and diversity of the PD-1-PD-L1 signaling pathway (3). However, they are a topic of interest in view of PD-L1 as a predictive biomarker because detection of biomarkers in plasma is easier and less invasive for the patient compared to a biopsy. The soluble form of PD-1 and PD-L1 is usually generated by proteolytic cleavage of the membrane-bound form of the molecule, or by translation of alternative spliced mRNA. There is growing evidence that sPD-1/PD-L1 might be as valuable as a predictive biomarker for clinical outcome as membrane-bound PD-1/PD-L1 (3). Tumor-related membrane-bound PD-1/PD-L1 contribute to immunosuppression. However, binding of sPD-1 to membrane-bound PD-L1/PD-L2 may counteract the inhibitory effects of membrane-bound PD-1 on T cells by preventing the T cell inhibition as a result of membrane-bound PD-1-PD-L1/PD-L2 interaction (3,20). Whether higher levels of sPD-1 are associated with a better prognosis remains rather unclear (3,21–25).

Biomarkers including IDO, CD83, Vascular Endothelial Growth Factor (VEGF), IFN-γ, and oncogene pathways such as IFN- γ/JAK2/IFN and PI3K might be involved in the generation of sPD-1 or sPD-L1 in patients, but the exact mechanisms by which this happens remain poorly understood (1,3). sPD-L1 has shown to be a meaningful and practical dynamic biomarker in several solid tumors such as melanoma (1), bladder cancer and NSCLC (4,26,27). IDO is an important immunosuppressive enzyme and expression can be induced by IFN-γ (13). IDO is an important enzyme and the rate-limiting step in the kynurenine pathway. Through this pathway, T cell apoptosis is induced by degradation of tryptophan and increase of products from the kynurenine pathway (13,28), which leads to the conversion of naïve CD4+ T cells into Tregs (29). IDO has

also been described as a predictive biomarker of anti-tumor response by immune checkpoint inhibitors. Expression of recombinant soluble CD83 (sCD83) downregulates DC maturation as well as DC dependent T cell responses. Thus, CD83 has an immunosuppressive function (30). The production of sCD83 is mostly mediated by activated B-cells and DC (31). VEGF is a proangiogenic signal protein but is also closely involved with induction of a immunosuppressive TME in cancer. In the TME, VEGF inhibits DC maturation, and causes induction of Treg cells. VEGF increases the expression levels of several immune checkpoints including PD-1, CTLA-4, TIM-3 and LAG3 on CD8+ T cells in the microenvironment, which results in T cell exhaustion (13,32,33).

Biomarkers can be used to select for patients with a higher likelihood to respond to immunotherapy. However, the role of several biomarkers in resistance to immune checkpoint therapy and the PD-1/PD-L1 axis in Hodgkin lymphoma is not yet clear. The aim of this study is to assess the influence of sPD-L1, sPD-L2, sPD-1, IDO, sCD83 and VEGF as biomarkers for the prediction of durable efficacy to immunotherapeutic agents and the PD-1-PD-L1 axis in Hodgkin lymphoma. To this end, we will summarize the current knowledge about the role of the TME in Hodgkin lymphoma, the mechanisms by which HRS cells evade the immune system and the impact of soluble markers including sPD-L1, sPD-L2, sPD-1, IDO, sCD83, and VEGF, on PD-1/PD-L1 expression in solid tumors as well as Hodgkin lymphoma.

Hodgkin lymphoma

Cause of Hodgkin lymphoma

According to the American Cancer Society, approximately 8,480 new patients will be affected by Hodgkin lymphoma in the United States in 2020, making this disease one of the most frequent lymphomas in the Western world (34,35). The disease has an increased incidence in young adults (age 20-34) and patients 55 years and older. Risk factors for the development as well as the cause of the disease remain poorly understood. However, research suggests that an abnormal immune response to infection as a result of expression of a variety of cytokines and chemokines might play a role in the development of Hodgkin lymphoma (36). Epidemiologic and serologic studies suggest Epstein-Barr virus (EBV) in the cause of Hodgkin lymphoma and viral EBV DNA was actually found in tumor specimens from patients with Hodgkin lymphoma (37). EBV positivity also influences the expression of certain cytokines including CXCL10, CXCL9 and CCL5, resulting in amplification of the intense inflammatory reaction to infection (36,38). However, other childhood infectious diseases including chickenpox, measles and rubella are associated with a decreased risk of Hodgkin lymphoma (39,40).

Hodgkin lymphoma biology

Hodgkin lymphoma is a B cell-lymphoid malignancy that can be subdivided into two distinct entities; classical Hodgkin lymphoma (around 95% of cases) and nodular lymphocyte predominant Hodgkin lymphoma (NLPHL; 5% of cases), based on morphological and clinical differences (13). Classical Hodgkin lymphoma is characterized by the presence of Hodgkin Reed-Sternberg (HRS) cells, while NLPHL is accompanied by the presence of lymphocyte-predominant (LP) cells (36,41). HRS cells are derived from germinal center B cells, but differ because they lack most B cell markers.

CD30 and CD15 are cell membrane proteins of the tumor necrosis factor (TNF)-receptor family. Under normal conditions, CD30 is not expressed in human tissue. However, co-expression of these membrane

proteins is typically associated with classical Hodgkin lymphoma (13,42–44). The presence of CD15 and CD30 on HRS cells promote tumor growth and survival. Therefore, these factors are interesting targets for cancer treatment (45).

Hodgkin lymphoma is a unique type of cancer because the amount of malignant cells is very low compared to the amount of reactive cells in a TME that includes lymphocytes, macrophages, eosinophils, mast cells, plasma cells, stromal cells, fibroblasts and other cells (36,46). In fact, the Hodgkin lymphoma tumor cell mass consists of only about 1% HRS cells (13). Therefore, the microenvironment is an important factor in the progression of the disease.

Microenvironment

Infiltrating CD4+ T cells, directly surrounding the HRS cells, are the most abundant cells found in the microenvironment of the tumor (36,47). Research shows that these cells primarily consist of T helper 2 (TH2) and T regulatory (Treg) cells (48,49). HRS cells express characteristic surface molecules and secrete a variety of cytokines and chemokines, resulting in interactions that shape the reactive infiltrate and signaling for the HRS. An overview of the interacting cells in the TME of Hodgkin lymphoma is shown in Fig 1. In Hodgkin lymphoma, several chemokines including CCL17 and CCL22 promote the attraction of CCR4-expressing TH2 and Treg cells (50–54). CCL5 is a chemokine, commonly found in Hodgkin lymphoma, that has been shown to attract mast cells to the TME (55). Several other mechanisms, like the production of interferon gamma by HRS cells together with T helper 1 (TH1) cells (56,57), and the production of granulocyte colony stimulating factor (CSF1) and fractalkine (CX3CL1), chemokines and differentiation factors of monocytes by HRS cells, enhance the attraction and infiltration of macrophages (58–60). This is important for the progression of several cancers, including Hodgkin lymphoma, since macrophages promote cell migration and suppress antitumor immunity. Moreover, the secretion of macrophage migration inhibitory factor (MIF) by M2 macrophages may contribute to the proliferation of HRS cells (35,36). Activation and proliferation of fibroblasts in Hodgkin lymphoma is mediated by the expression of IL-13, tumor necrosis factor (TNF) α, and fibroblast growth factors by HRS cells (61). This, in turn, contributes to eosinophil and TH2 cell infiltration by secretion of CCL11 (35,62). Altogether, many cytokines and chemokines are present in the TME of Hodgkin lymphoma, which results in the attraction of cells that help the tumor survive. The complex interference of HRS cells with its microenvironment may provide novel strategies for cancer therapy.

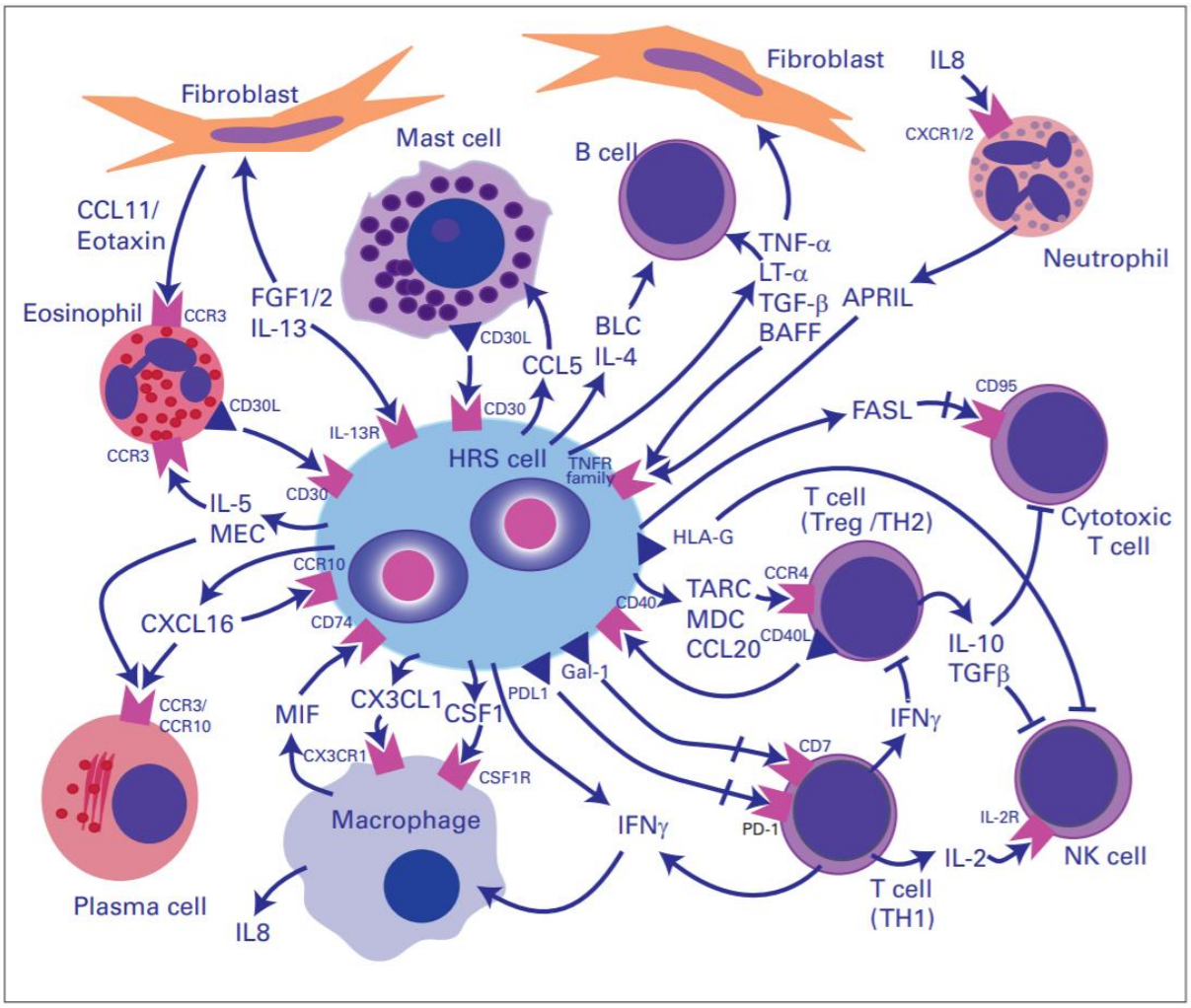

Fig 1. The tumor microenvironment in classical Hodgkin lymphoma. A malignant Hodgkin Reed-Sternberg (HRS) cell expressing characteristic surface molecules as well as secreted cytokines and chemokines. These molecules attract the surrounding non-neoplastic cells. The arrows indicate the main activating and inhibitory functions of predominantly membrane-bound (purple triangles) and secreted molecules (pink), which are mediated by surface receptors. Not all existing interactions are shown. IL, interleukin; FGF, fibroblast growth factor; TNF-α, tumor necrosis factor α; LT-α, lymphotoxin α; TGF-β, transforming growth factor β; BAFF, B-cell activating factor; APRIL, a proliferation-inducing ligand; BLC, B lymphocyte chemoattractant; TNFR, tumor necrosis factor receptor; FASL, Fas ligand; MEC, mucosae-associated epithelial chemokine; Treg, T regulatory cell; TH, T helper cell; TARC, thymus and activation-related chemokine; MDC, Macrophage-derived chemokine; Gal-1, galectin-1; PDL, programmed cell death 1 ligand; IFN-γ, interferon gamma; NK, natural killer (Steidl, Connors, & Gascoyne, 2011; (36)).

Current treatment

Over the last 60 years, there have been enormous improvements in the outlook for patients with Hodgkin lymphoma. Today, it is considered one of the most curable types of cancer because of the use of chemotherapy, sometimes in combination with radiotherapy. However, since the patient group is relatively young, this success has exposed the problem of long-term treatment-related toxicity. In fact, second malignancies are the leading cause of death among Hodgkin lymphoma survivors (63,64). Chemotherapy and radiotherapy are highly toxic, increasing the risk of several cancers, including breast cancer, lung cancer and acute myeloid leukemia (64). Furthermore, there is increased morbidity and mortality from ischaemic heart disease, valvular heart disease, anthracycline-related cardiomyopathy and conduction abnormalities among patients previously treated with radiotherapy (65,66). Altogether, new, less toxic therapy is highly

needed for Hodgkin lymphoma patients. Two essentials of new therapy are the minimization of long-term treatment-related toxicities and the improvement in therapy for patients with relapsed and refractory disease (19).

Immunotherapy

Immune checkpoints can be utilized to modulate the duration and amplitude of the physiological immune response (6,19). In solid tumors, including advanced melanoma and NSCLC, immune checkpoint inhibition has already shown great clinical success (4,13). Hodgkin lymphoma has a low mutational burden, resulting in a lower level of neoantigens. This could be an implication for immunotherapy in Hodgkin lymphoma patients (19,67). However, the amount of T cells, cytokines and chemokines present in the Hodgkin lymphoma microenvironment does make immune checkpoints an interesting target of therapy and Hodgkin lymphoma stands out among all lymphomas because of its high responsiveness to PD-1 blockade (>70% ORR) therapy and significant clinical benefit in patients with relapsed and refractory Hodgkin lymphoma (19,68–70).

Research has shown that Hodgkin patients often show deficiencies of host immune function, and patients with HIV infection or autoimmune disease have an increased risk of both Hodgkin lymphoma and non-Hodgkin lymphoma (19,71). Additionally, both PD-1 and its ligand PD-L1 are overexpressed in the TME of Hodgkin lymphoma patients, which contributes to a potent inhibitory signal in the maintenance of the immunosuppressive TME (19,72). Immune checkpoints are thus misused in Hodgkin lymphoma patients, via a process called “immunoediting” (73), causing T cell exhaustion. This leads to an immunosuppressive microenvironment and contributes to the survival of the tumor. Immunoediting involves three phases: 1) an initial elimination phase, which originally represents the concept of cancer immunosurveillance, eliminating the tumor by making use of both the innate and adaptive immune system. 2) The longest of three phases: an equilibrium phase between the host immune system and tumor cells that survived the elimination. The end result of this phase can be envisioned as the product of Darwinian selection: a population of tumor cells with reduced immunogenicity. 3) An escape phase in which tumor cell variants, that were selected out of the equilibrium phase, evade immune surveillance through a variety of immunoinhibitory mechanisms.

On a molecular level, overexpression of PD-L1 is partly caused by selective amplification of the 9p24.1 region in HRS cells, containing the PD-L1 gene. Moreover, the Jak2 locus is amplified causing Jak2 overexpression, which leads to subsequent transcriptional activation of PD-L1 (13,19,74). EBV infection leads to increased expression of PD-L1 gene and protein (19,75). Hodgkin lymphoma cell lines highly express a chromosomal fusion gene involving a CIITA MHC-II transcriptional activator, which leads to downregulation of surface HLA class II expression and overexpression of PD-L1 (19,76). Antigen presentation by MHC molecules is an important factor in the initiation of an immune response. HRS cells often lack HLA class I molecules, and therefore CD8+ T cells are not likely to be of much importance in immune checkpoint inhibition therapy in Hodgkin lymphoma. No association was found between MHC class I expression on HRS cells and PFS. However, MHC class II expression was found in 92% of complete responders to PD-1 blockade therapy. Furthermore, positive MHC class II expression on HRS cells in Hodgkin lymphoma patients was associated with elongated PFS when the anti-PD-1 antibody was received >12 months after autologous stem-cell transplantation (ASCT) (77). Altogether, HRS cells are able to evade the immune system by shutting down the immune response.

Results of anti-PD-1 antibodies pembrolizumab and nivolumab have been very promising for relapsed and refractory Hodgkin lymphoma patients. An 87% objective response rate (ORR) with a 20% complete response (CR) was achieved in refractory heavily pretreated Hodgkin lymphoma patients (19,78).

PD-1-PD-L1 expression in solid tumors

The PD-1 blocking antibodies pembrolizumab and nivolumab have been approved by the US Food and Drug Administration (FDA) for patients with solid tumors including advanced unresectable melanoma and NSCLC (80). Predictive and progressive biomarkers are needed for the optimization of therapeutic benefit, minimization of risk of the autoimmune toxicities, and decrease of treatment costs (81). Predictive value can be determined by dividing the number of correctly predicted responders by the total number of patients with a positive biomarker result. Direct assessment of PD-L1 expression on tumor cells would be a logical biomarker for the prediction of durable efficacy to PD-1 blockade therapy in patients with solid tumors, like NSCLC and melanoma. However, soluble PD-1 or PD-L1 might be a better and less invasive manner to predict and/or determine the efficacy of immune checkpoint inhibitors on cancer therapy. Moreover, several other soluble factors, including IDO, CD83, and VEGF, have been described influencing the PD-1/PD-L1 pathway and might be of relevance as well.

sPD-L1

As mentioned before, sPD-L1 is often the product of proteolytic cleavage of the membrane-bound form of the molecule, or translation of alternative spliced mRNA. Cytokine treatment with IFN-α, IFN-γ, or TNF- α is also positively associated with sPD-L1 secretion as well as membrane-bound PD-L1 expression in melanoma cells (1). Elevated sPD-L1 is associated with worse clinical outcome in many types of cancer (21–23,82). Studies about higher pretreatment levels of sPD-L1 also support this claim (1,83), however, rise in sPD-L1 after 5 months of treatment resulted in greater likelihood of partial responses after PD-1 inhibition (1). Upregulation of sPD-L1 after immune checkpoint inhibition may be caused by the changes in the TME as a result of the therapy-induced anti-tumor response, which causes enhanced production of cytokines including IFN-α, IFN-γ, and TNF- α (1,84).

sPD-L2

In addition to sPD-L1, sPD-L2 is an important ligand for PD-1. Studies have suggested that sPD-L2 may play a role in the regulation of inflammation and autoimmunity. Costantini, et al. (85) described the predictive role of plasmatic biomarkers such as sPD-L2 in advanced NSCLC when patients were treated with nivolumab. sPD-L2 concentrations and variations showed no impact on clinical benefit, ORR, progression free survival (PFS) or overall survival (OS) in this study. However, it was found that lower concentrations of sPD-L2 at initial diagnosis and at nivolumab initiation were associated with patients presenting grade 3-4 toxicity. This means that sPD-L2 may be an effective biomarker in the prediction of immune-related toxicities rather than in the prediction of tumor response and clinical benefit (85)

sPD-1

In the development of several cancers, there is a strong interaction between sPD-1 and its ligand sPD-L1. Furthermore, these factors are present in elevated plasma levels in patients with a variety of cancers, including advanced pancreatic cancer (86), NSCLC (87), and pancreatic ductal adenocarcinoma (PDAC)

(82). Assessment of the kinetics of sPD-1 before and after treatment with nivolumab shows the impact of treatment with anti-PD-1 antibodies on the production or destruction of sPD-1 and gives insight into clinical outcome. Treatment with nivolumab caused a decrease in sPD-1 levels of NSCLC patients after two cycles of treatment, which was also associated with a worse prognosis. This is in line with previous results of NSCLC patients with epidermal growth factor receptor (EGFR) mutations from Sorensen, Demuth, Weber, Sorensen, & Meldgaard (88).

IDO

In metastatic melanoma patients who were treated with anti-PD-1 agents, it was found that patients with the highest PD-1-PD-L1 interaction score and IDO-1-HLA-DR co-expression appeared to be more likely to respond to therapy and experienced significantly improved progression-free survival. This describes a connection between these factors (89). Furthermore, it was found that patients with advanced melanoma, treated with ipilimumab, an antibody that blocks CTLA-4, showed significant associations between baseline FoxP3 expression together with baseline IDO expression in tumor tissue, and clinical activity and increase in tumor-infiltrating lymphocytes (TILs) before and 3 weeks after start of treatment ($p = 0.005$). FoxP3 is a transcription factor, mainly expressed on CD4+ T cells, and has an immunosuppressive character because expression of Fox3P contributes to the suppression of many genes including IL-2 and effector T cell cytokines (90). The association between Fox3P together with baseline IDO expression and treatment with ipilimumab may be positive for clinical outcome (84,91). IDO expression in tumor tissue was assessed by IHC in tumor tissue, but in another study, in which IDO activity was assessed by serum kynurenine/tryptophan ratio, high IDO expression was associated with worse clinical outcome in NSCLC patients treated with nivolumab (92). This indicates that IDO activity may vary dependent on the assessment. (84). Previous studies have described co-expression of IDO and PD-L1 in several solid malignancies, including colon cancer and breast cancer (93–95). In a study on the relation between IDO and PD-L1 expression in cervical and vulvar squamous carcinomas (SCC), PD-L1 and IDO co-expression was observed in a subset of patients, which suggests a role for these factors in combination immunotherapy in cancer. The presence of invasion is also not expected to be of much benefit for combination treatment with anti-PD-L1 and anti-IDO agents (93).

CD83

In healthy human sera, sCD83 is present at low levels. However, patients with autoimmune disease or hematopoietic malignancies have elevated levels of sCD83. Recombinant soluble extracellular CD83 (rsCD83) mimics the natural sCD83, and binding of this molecule to the TLR4/MD-2 complex on monocytes induces production of anti-inflammatory mediators, such as IDO, leading to an immunosuppressive environment (96,97). Several solid tumors, including small cell lung cancer and other lung adenocarcinomas, have been shown to express CD83 (98). Moreover, polymorphisms of CD83, which were correlated with prognosis of cervical cancer, have been found in several cancers. Little is known about the relation between levels of sCD83 and prognosis in solid cancers (96,99).

VEGF

In a mouse model of colorectal cancer, it was found that the VEGF-A concentration in the TME in vivo was ~10 times higher compared to plasma levels of VEGF-A. After treatment targeting the VEGFA-VEGF receptor (VEGFR) axis with anti-VEGF-A antibodies, a significant reduction of PD-1 expression on

intratumoral CD8+ T cells was found and IFN-γ production in intratumoral CD8+ T cells was restored. Moreover, a combination of antiangiogenic agents such as anti-VEGF-A and immunotherapeutic approaches such as the use of anti-PD-1 induced a much stronger antitumor effect compared with therapy anti-VEGF-A alone or anti-PD-1 alone. Colorectal cancer patients showed overexpression of VEGF mRNA, which was associated with poorer survival than patients without overexpression. In a small cell lung cancer (SCLC) mouse model, mice were treated with a combination therapy of anti-PD-L1 and anti-VEGF agents, which restored the exhausted T cell phenotype of the mice and significantly improved PFS and OS. Thus, VEGF may serve as a predictive biomarker for the prognosis of several solid malignancies and anti-VEGF/anti-PD-L1 as well as anti-VEGFA/anti-PD-1 combination therapy synergistically improves clinical outcome in a SCLC mouse model and colorectal cancer patients, respectively (33,100,101).

PD-1-PD-L1 expression in Hodgkin lymphoma

To our knowledge, no reports have been published about the potential role of sPD-L2 as a biomarker in Hodgkin lymphoma. However, sPD-L1, sPD-1, IDO, CD83, and VEGF have been described as important factors in the PD-1/PD-L1 pathway in Hodgkin lymphoma patients.

sPD-L1

Elevated mPD-L1 levels in classical Hodgkin lymphoma are associated with 9p24.1 amplification, which also leads to significantly shorter PFS for patients (102,103). Elevated sPD-L1 levels are often found in patients with several types of cancer compared to healthy individuals. This is also the case for Hodgkin lymphoma patients. Higher sPD-L1 levels in Hodgkin lymphoma patients are positively correlated with advanced stage and significantly shorter PFS, but not with OS. This suggests a potential role of sPD-L1 as an independent prognostic biomarker in classical Hodgkin lymphoma. Conflicting results have been obtained about the relation between EBV positivity and plasma sPD-L1 levels (103–105).

sPD-1

It is known that sPD-1 can prevent interaction between PD-1 and PD-L1 in vivo (106). In one study, Hodgkin lymphoma patients show high plasma levels of sPD-1 at diagnosis and before treatment with chemotherapy and radiotherapy, and these levels decrease as treatment proceeds. No correlation was found between sPD-1 and sPD-L1 expression. (107). To our knowledge, this is the only study to the role of sPD-1 as a potential biomarker in Hodgkin lymphoma.

IDO

In Hodgkin lymphoma patients, IDO is not expressed by HRS cells or lymphocytes. However, the molecule is active in the TME given its expression by macrophages, dendritic cells and vascular endothelial cells. Research supports that high expression of IDO is a significant prognostic predictor of bad clinical outcome in Hodgkin lymphoma. EBV and HIV-positive Hodgkin lymphoma patients show higher expression of IDO. High IDO expression is also associated with decreased CD4+ T cells and increased CD8+ T cells (108). Since CD8+ T cells recognize tumor cells through (neo)antigens presented by HLA class I molecules, this could be positive for clinical outcome. However, considering the fact that HLA class I molecules are often absent on HRS cells, CD8+ T cells are not likely to be of much importance in immune checkpoint inhibition therapy in

Hodgkin lymphoma. CD4+ T cells are more involved in the antitumor immune response in classical Hodgkin lymphoma, and interaction between PD-1 and CD4+ T cells is also enriched in the TME.

CD83

CD83 is expressed on HRS cells in lymph node biopsies of Hodgkin lymphoma patients. Recently, it was found that the membrane cleaved soluble form of CD83 is released into serum by Hodgkin lymphoma tumor cells, where the protein has an immune-inhibitory function by inhibiting T-cell proliferation. Decreases in circulating sCD83 were observed in patients who showed a CR after treatment with chemotherapy. Furthermore, research shows that CD4+ T cells that express CD83, expressed a higher level of PD-1 than $CD83^-$ T cells. This indicates that combination therapy of CD83 blockade and PD-1 blockade may enhance clinical response (30).

VEGF

VEGF can be produced by HRS cells, leading to upregulation of PD-L1 and PD-L2 expression levels (13,109). A combination of doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD) therapy in addition to anti-VEGF therapy may in fact be effective for Hodgkin lymphoma patients (109). However, elevated levels of VEGF, PD-L1, and PD-L2 are not significantly associated with event-free survival (EFS) as well as OS (109,110). Other studies suggest that high VEGF-A gene expression in classical Hodgkin lymphoma is associated with worse OS (111,112).

Discussion

The aim of this study was to investigate the potential prognostic and predictive value of sPD-L1, sPD-L2, sPD-1, IDO, sCD83, and VEGF for PD-1 blockade therapy, and their impact on the PD-1/PL-L1 axis in Hodgkin lymphoma. Immune checkpoint therapy is shown to be very effective in Hodgkin lymphoma because the disease is characterized by a large TME, which forms an immunosuppressive, tumor tolerant environment with overexpression of PD-1 and PD-L1. Overexpressed PD-1 can be targeted by immunosuppressive agents such as nivolumab and pembrolizumab. Broader knowledge about the impact of these soluble factors on immune checkpoint inhibitors would make a significant contribution to treatment optimization. Soluble factors are preferred over the membrane-bound biomarkers because detection of biomarkers in plasma is easier and less invasive for the patient compared to a biopsy. The identification of potential responders and non-responders to immune checkpoint blockade therapy is very useful because like this, patients lose no time on ineffective therapy and the financial burden of unsuccessful treatment is limited (87,113). To this day, research has mainly been on the effect of the expression of soluble markers in solid malignancies and on tumor PD-L1 expression and current knowledge about the possible role of soluble biomarkers in the prediction and progression of Hodgkin lymphoma is limited (87).

In solid tumors, high pretreatment sPD-L1 levels are often associated with worse clinical outcome, but since rise in sPD-L1 levels during treatment have shown to contribute to better response to PD-1 blockade therapy. In Hodgkin lymphoma, elevated levels of sPD-L1 are associated with advanced stage and significantly shorter PFS. Therefore, we find sPD-L1 a valuable predictive biomarker for Hodgkin lymphoma. However, further investigation to pretreatment sPD-L1 levels and the kinetics of sPD-L1 in Hodgkin lymphoma during treatment is needed and might give insight into the exact role in the progression of the disease. In solid tumors, sPD-L2 has shown little predictive value for efficacy of immunotherapy, and no reports have been published about the potential role of sPD-L2 as a biomarker in Hodgkin lymphoma. sPD-1 expression is decreased in solid tumors after treatment with PD-1 inhibitors, which is also associated with a worse prognosis. Hodgkin lymphoma treatment with radiotherapy and chemotherapy decreases the elevated levels of sPD-1, but little is known about the predictive value for efficacy of PD-1 blockade therapy. However, current knowledge about sPD-1 in solid tumors is promising for sPD-1 as a predictive biomarker in Hodgkin lymphoma. The predictive value of IDO expression has been dichotomous in solid malignancies. However, in Hodgkin lymphoma, IDO expression is described as a significant prognostic predictor of bad clinical outcome. IDO is not expressed by HRS cells, but still has a large role in the TME. sCD83 expression in patients with solid malignancies had not been described in a predictive or progressive relation. However, in Hodgkin lymphoma, CR was associated with decreases in sCD83 levels after chemotherapy. VEGF overexpression in colorectal cancer patients was associated with poorer survival. In contrast, in Hodgkin lymphoma patients, no significant relation to VEGF expression and clinical outcome was found.

It is often observed that despite few impressive clinical successes, immunotherapy with a single-agent immune checkpoint inhibitor can not serve as a sufficiently effective therapy for many cancers (93,114,115). This might be the result of the complex composition of the TME, and the fact that other immunomodulatory molecules compensate for the loss of one immune checkpoint. The presence of the other factors is enough to continue the evasion of the immune surveillance by the tumor, even during PD-1/PD-L1 blockade therapy. Therefore, researchers have recently started to investigate the effects of

targeted combination immunotherapy for the treatment of solid malignancies (93,114). Promising effects have been observed in combination therapy blocking IDO or VEGF in combination with PD-1 or PD-L1 in solid malignancies. Therefore, these markers might also be of importance for combination immunotherapy in Hodgkin lymphoma. Little is known about sPD-L1, sPD-L2, sPD-1 and sCD83 in the field of combination therapy.

High expression of IDO in patients with solid malignancies has been associated with primary resistance to PD-1 blockade therapy. Therefore, combined IDO inhibition and PD-1 blockade may be very effective. IDO was also associated with decreased CD4+ cells and increased CD8+ cells in the TME of Hodgkin lymphoma (108), which might be an interesting aspect for combination immunotherapy in Hodgkin lymphoma. In solid tumors, a possible connection between IDO-1-HLA-DR co-expression and high PD-1-PD-L1 interaction was found and therefore this could be an interesting topic for future studies on the possible role of IDO in combination immunotherapy in Hodgkin lymphoma. Furthermore, baseline FoxP3 expression in combination with IDO expression might have a positive effect on clinical outcome in melanoma patients (84,91). Since Fox3P expression is also elevated in classical Hodgkin lymphoma patients (116), a combination therapy with anti-Fox3P agents and anti-IDO agents or anti-PD-L1 agents might have a positive effect on clinical outcome in Hodgkin lymphoma.

Combination immunotherapy with a combination anti-VEGF-A and anti-PD-1 has been very effective in solid malignancies (33,100,101). The effects of high VEGF expression in Hodgkin lymphoma patients are not yet clear but could be of importance for clinical outcome and as a target immunotherapy. Given the finding that CD4+ T cells that express CD83, express a higher level of PD-1 than $CD83^-$ T cells, a combination of CD83 blockade and PD-1 blockade therapy might be also of clinical value in Hodgkin lymphoma patients.

After assessment with immunohistochemistry, the PD-1 or PD-L1 axis is not always accurately reflected, which could be partly caused by the poor uniformity in the PD-L1 immunohistochemistry anti bodies (14,80). A variety of tumors, including melanoma, renal cell carcinoma, NSCLC, and bladder cancer are associated with good clinical responses to immune checkpoint inhibition with anti-PD-1 agents. However, PD-L1 as a predictive biomarker for these cancers results in a wide range of PD-L1 IHC expression (14-100%), which highlights the issues with PD-L1 as a valuable, predictive biomarker for anti-PD-1 therapy (14,117–119).

Further studies aiming to identify predictive and progressive biomarkers for treatment of Hodgkin lymphoma with immune checkpoint inhibitors should include the kinetics of sPD-L1 in Hodgkin lymphoma during treatment, the effects of EBV and HIV infection on the TME and the role of sPD-L2 in the progression of Hodgkin lymphoma.

Based on our findings, we believe that sPD-L1 and sPD-1 are promising independent predictive biomarkers for Hodgkin lymphoma. Furthermore, combination therapy of anti-IDO with anti-PD-1 agents as well as anti-VEGF with anti-PD-1 antibodies might provide positive clinical outcome for Hodgkin lymphoma patients.

Conflict of interest

There was no conflict of interest.

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