Cancer Treatment Reviews 82 (2020) 101931

Contents lists available at ScienceDirect

Cancer Treatment Reviews

journal homepage: www.elsevier.com/locate/ctrv

Anti-Tumour Treatment

Primary and acquired resistance mechanisms to immune checkpoint inhibition in Hodgkin lymphoma

Johanna Veldman, Lydia Visser, Anke van den Berg, Arjan Diepstra*

Department of Pathology and Medical Biology, University of Groningen, University Medical Center Groningen, 9700RB Groningen, the Netherlands

ARTICLE INFO

Keywords:
Hodgkin lymphoma
Immune checkpoint inhibitors
PD-1 inhibitors
Resistance mechanisms
Tumor immunology
Antigen presentation

ABSTRACT

Hodgkin lymphoma is a B cell derived malignancy characterized by a low number of tumor cells within an environment consisting of inflammatory cells. Recently, immune checkpoint blockade targeting the PD-1-PD-L1 axis has shown to be a great success in relapsed and refractory Hodgkin lymphoma patients. However, complete responses are scarce and median progression-free survival is limited to around 11–15 months. Efficiency of PD-1 blockade in HL might be dependent on CD4+ T cells, but also tumor associated macrophages (TAMs) and NK cells are implicated. The aim of this review is to highlight currently known prominent immune evasion strategies and discuss their possible contribution to primary or acquired resistance to immune checkpoint blockade in Hodgkin lymphoma. These include T cell dependent mechanisms such as shaping of the inflammatory infiltrate, lack of presentation of antigens and neoantigens and production of molecules involved in suppression of T cell functionality such as other immune checkpoints, indoleamine 2,3-dioxygenase and adenosine. Moreover, the role of NK cells and TAMs in efficient PD-1 blockade will be discussed. Targeting these mechanisms in parallel to PD-1 may potentially increase efficiency of PD-1 blockade therapy.

Introduction

Recently, immune checkpoint therapy was shown to be very effective in the treatment of several cancers by reactivating the immune system. This was also shown for hard to treat cancers such as melanoma and lung cancer. At the forefront of immune checkpoint therapy are antibodies targeting cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) and the programmed cell death-1 (PD-1). However, clinical success of immune checkpoint inhibitors greatly varies between different cancer types, ranging from objective responses in 65–87% of Hodgkin lymphoma (HL) patients to around 30% in other malignancies [reviewed by [1–3]].

This review primarily focuses on PD-1, which is an inhibitory receptor mainly present on activated T cells. Under normal physiological conditions, PD-1-PD-L1 signaling regulates the strength of the immune response [reviewed by [4]]. T cells that recognize an antigen presented by human leukocyte antigen (HLA) molecules produce inflammatory cytokines (e.g. IFN-γ) which leads to the upregulation of PD-L1 on the antigen presenting cells. Activated T cells express PD-1 and engagement of PD-1 with PD-L1 causes phosphorylation of the immunoreceptor tyrosine switch motif (ITSM) and the immunoreceptor tyrosine inhibitory motif (ITIM) on PD-1. This leads to the recruitment of the phosphatases SHP1 and SHP2, which in turn results in dephosphorylation of downstream TCR signaling molecules and a reduction of TCR mediated T cell activation. In addition, PD-1-PD-L1 interaction results in dephosphorylation and inhibition of CD28 mediated co-stimulation, whereas PD-L1 can compete with CD80 for binding to CD28 [5,6]. PD-L1 is constitutively overexpressed in multiple cancers. This prevents generation of an antitumor immune response by inhibiting T cell activation. Tumor cells protect themselves from cytotoxic responses mediated by CD8+ T cells by upregulating PD-L1, which can engage with PD-1 on T cells. Indeed, PD-1 blockade in advanced stage non-small cell lung cancer (NSCLC) patients resulted in an increase of Ki67 + PD-1 + CD8 + effector T cells in the circulation [7]. Moreover, in advanced melanoma, responding patients had increased density of CD8+ T cells within tumor tissue serial biopsies after PD-1 blockade therapy, in comparison to patients that progressed [8].

Although responses to immune checkpoint therapy are promising in multiple cancer types, some patients do not respond whilst others initially respond but ultimately relapse. Based on these observations, resistance to immune checkpoint inhibition can be broadly divided into two groups: (1) tumors that do not respond at all (primary resistance) and (2) tumors that initially respond, but become resistant and progress (acquired resistance) [9]. Resistance to immune checkpoint inhibition

* Corresponding author at: Department of Pathology and Medical Biology, University of Groningen, University Medical Center Groningen, Hanzeplein 1, code EA10, P.O. Box 30.001, 9700 RB Groningen, the Netherlands.
E-mail address: a.diepstra@umcg.nl (A. Diepstra).

https://doi.org/10.1016/j.ctrv.2019.101931
Received 9 July 2019; Accepted 3 November 2019
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Table 1
Resistance mechanisms to immune checkpoint blockade described in solid malignancies.

Category	Mechanism	Literature
T cell activation	Lack of neoantigens	[10–12,14,29–31]
	Impaired processing or presentation of neoantigens	[13,15,32]
T cell attraction	Inhibition of T cell influx into the TME	[16,17]
Induction of T cell effector functions	Mutations in immune effector signaling pathways	[13,26]
	Upregulation of immune checkpoint molecules	[19,20,27]
	Production of suppressive cytokines and metabolites	[18,23–25]
	Recruitment of suppressive cell populations	[21,22]
Generation of effector memory T cells	Impaired formation of T effector memory cells	[28]

is mainly studied in solid tumors, especially melanoma and NSCLC. Based on those studies, resistance to immune checkpoint inhibition can occur by intervention at three critical steps of T cell functioning: (1) attraction and activation of T cells; (2) proper induction of T cell effector functions and (3) generation of effector memory T cells.

Table 1 gives an overview of the currently described resistance mechanisms. In summary, tumors with a high tumor mutational burden (TMB) and as a consequence a potential increase in the presentation of neoantigens have an increased sensitivity to immune checkpoint blockade [10–13]. In line with this, clonal selection of tumor cells that lack neoantigen presentation has been described as a way of acquired resistance to immune checkpoint blockade [14]. Moreover, loss of antigen presentation by inactivating mutations in β2 microglobulin (β2M) or HLA class I has been described as a mechanism of primary resistance to CTLA-4 blockade therapy [15] and for acquired resistance after PD-1 blockade therapy [13]. Another mechanism of resistance described in solid cancers is the inhibition of T cell influx to the tumor microenvironment (TME). In general, lymphocyte rich tumors show better outcomes than lymphocyte poor tumors and this is also observed upon PD-1 blockade therapy [16,17]. Inadequate T cell effector functions can be caused by multiple mechanisms related to the interactions with the TME; such as upregulation of additional immune checkpoint molecules, inactivating mutations in signaling pathways making the tumor cells unresponsive to effector T cells (e.g mutations in IFN-γ signaling pathways), attraction of immune suppressive cell populations (e.g. regulatory T cells, Th2, myeloid derived suppressor cells and M2 polarized macrophages) and production of immune suppressive chemokines and metabolites (e.g. adenosine, indoleamine-2,3-dioxygenase, IL-10) [13,18–27]. In addition, generation of sufficient effector memory T cells is a requirement to obtain durable T cell responses. This is supported by the higher frequency of CD8+ effector memory T cells in patients that responded to PD-1 blockade therapy as compared to patients that did not respond [28].

The aim of this review is to combine knowledge on immune evasion mechanisms in solid cancers with current knowledge in HL to propose potential mechanisms of primary and acquired resistance in HL. The focus will mainly be on proper attraction and activation of T cells and induction of T cell effector functions.

Hodgkin lymphoma biology

HL is a B cell malignancy that mainly occurs in young adults (age 20–34). HL can be subdivided into classical HL (cHL), which accounts for around 95% of cases and nodular lymphocyte predominant HL (NLPHL) based on morphological and clinical differences [33]. The tumor cells in cHL, called Hodgkin Reed-Sternberg (HRS) cells, are derived from germinal center B cells, but lack most B cell markers. Two membrane markers commonly expressed on HRS cells are CD15 and CD30 [33–37]. Only about 1% of the HL tumor cell mass consists of HRS cells. These cells are surrounded by an abundant but ineffective inflammatory infiltrate. The inflammatory infiltrate consists of T cells, B cells, eosinophils, macrophages, neutrophils, histiocytes, plasma cells and fibroblasts [33]. The CD4+ T cells directly surrounding the HRS cells have a memory T helper (Th) 2 or regulatory T cell (Treg) phenotype and appear to be anergic [38,39]. These rosetting T cells express several activation markers, such as CD38, CD69 and HLA class II, but not dipeptidyl peptidase IV/CD26 [38–41]. The etiology of HL is largely unknown, but infection with Epstein-Barr Virus (EBV) is common, with an incidence of around 30% in the Western World and > 90% in Central-America [42]. Genome-wide association studies have identified the HLA region on chromosome 6 as the most strongly associated susceptibility locus for EBV positive and EBV negative cases [43,44]. More specifically, within the HLA region different protective and risk loci have been identified for EBV positive and EBV negative cases [44–46]. Several HLA class I alleles are specifically associated with increased or decreased susceptibility to develop EBV positive HL [45,47]. Susceptibility to EBV negative HL is mainly related to HLA class II alleles and therefore implicates a more important role for CD4+ T cells [48]. EBV negative HL have frequently lost expression of HLA class I [49]. In contrast, tumor cells in EBV positive HL usually have retained their HLA class I antigen presentation capacity and are characterized by presence of CD8+ T cells in the TME [50].

Efficiency of immune checkpoint inhibition in Hodgkin lymphoma

Overexpression of PD-L1 on HRS cells has gained a lot of attention as an important mechanism of immune escape. This overexpression is at least partly caused by frequent gain of the 9p24.1 chromosomal region. This gain also often includes the JAK2 gene locus and results in increased JAK/STAT signaling and a further increase in PD-L1 expression [51]. EBV derived latent membrane protein-1 (LMP-1) also leads to activation of the JAK/STAT and AP-1 pathways, which leads to increased PD-L1 expression [52]. Initial phase 1/2 trials exploiting PD-1 blockade were done in relapsed and refractory HL patients as reviewed by [2]. Currently, nivolumab and pembrolizumab are both clinically approved for the treatment of relapsed and refractory cHL by the US Food and Drug Administration. Both drugs target the inhibitory PD-1 receptor. Objective response rates varied between 65 and 87%, whereas complete responses were only observed in 9–22% of HL patients. Moreover, progression-free survival was limited with a median of 11–15 months. To further improve treatment of HL patients it is important to understand the mechanisms underlying efficacy and resistance to PD-1 blockade.

Effector cells of immune checkpoint inhibitors in Hodgkin lymphoma

The mechanism of action of immune checkpoint inhibitors in HL is not completely clear yet. In solid tumors CD8+ cytotoxic T cells seem to be the main effector cells [8]. CD8+ cytotoxic T cells recognize the tumor cells through (neo)antigens presented in the context of HLA class I, which leads to eradication of the tumor cells. However, HLA class I is often absent on HRS cells making a central role for CD8+ T cells in immune checkpoint inhibitor efficacy unlikely [49,53–55]. Several lines of evidence support a significant role of CD4+ T cells in mediating the antitumor immune response in cHL. The inflammatory infiltrate is

dominated by CD4+ T cells, which are more often in direct contact with HRS cells when compared to CD8+ T cells. The CD4 + T cells can recognize the HRS cells via antigens presented in the context of HLA class II molecules. Interestingly PD-1+CD4+ T cells, but not PD-1 +CD8+ T cells, are also enriched in the immediate proximity of PD-L1 + HRS cells [56]. The majority of the complete responders to nivolumab lack membranous HLA class I expression, while being positive for membranous HLA class II expression. To some extent, presence of HLA class II on the tumor cells is predictive for a prolonged progression free survival in patients treated with nivolumab more than 12 months after they have undergone myeloablative autologous stem cell transplantation (ASCT). In contrast, expression of HLA class I did not have a predictive value for survival to nivolumab after ASCT [57]. Altogether this suggests a more profound role for CD4+ T cells in PD-1 blockade efficiency in HL.

Although PD-1 + CD4+ T cells are enriched in close proximity of PD-L1 + HRS cells, they are often not in direct contact with the HRS cells [56]. This suggests that PD-1 blockade therapy is dependent on additional mechanisms. These might involve tumor associated macrophages (TAMs) or natural killer (NK) cells. TAMs can be subdivided in antitumor M1 and tumor promoting M2 macrophages. Macrophages can express both PD-1 and PD-L1 [58]. PD-L1+ TAMs are able to inhibit PD-1+ T cells and NK cells. PD-1 expression on TAM however has been linked to TAM functionality. PD-1+ TAMs have a decreased phagocytotic potential and PD-1 blockade therapy improves phagocytosis, reduced tumor growth and increased survival time in mouse models of cancer [59]. In HL, PD-L1 expression is not limited to HRS cells but can for a large proportion be attributed to TAMs [56]. PD-L1+ TAMs are in closer proximity to HRS cells compared to PD-L1 negative TAMs. In addition, the PD-L1+ TAMs are in close proximity or in direct contact with PD-1+ T cells. These PD-L1+ TAMs might engage with PD-1+ T cells to augment immunosuppression [56].

In mouse models for lymphoma and colon cancer, NK cell responsiveness was increased after PD-1 blockade and tumor growth was reduced even when T cells were absent. PD-1 was specifically upregulated on NK cells that express activation markers (e.g. CD69) and had the highest functional activity when stimulated *ex vivo*. This suggests that by interaction with PD-L1 the most functional and responsive NK cells are inhibited [60]. NK cells in several cancers including HL express PD-1 [61,62]. PD-1 + CD16- NK cells are increased in blood of HL patients compared to healthy controls. In addition, there is an increase of CD163 + PD-L1+ monocytes in HL. *Ex vivo* experiments showed increased activation of CD16- NK cells upon depletion of monocytes from HL patient derived PBMCs compared to healthy control PBMCs. This suggested that monocytes from HL patients suppressed the activity of CD16- NK cells. PD-1-PD-L1 blockade reverted this immunosuppressive effect [61]. In conclusion these studies suggest that besides T cells also other cell types play a prominent role in the high clinical efficacy of PD-1 blockade therapy in HL patients.

T cell related resistance mechanisms to immune checkpoint inhibition in Hodgkin lymphoma

Inadequate T cell attraction and activation

Attraction of specific T cell subsets

One of the steps necessary to elicit an effective antitumor immune response is the attraction of effector T cells towards the tumor cells (Fig. 1A). In solid cancers the lack of CD8+ T cells and the presence of Treg is associated with resistance to PD-1 blockade [8,63]. Melanoma patients that responded to PD-1 blockade had higher pretreatment CD8+ T cell densities in the invasive margins compared to patients that progressed. During treatment the responding group had an increase in CD8+ T cell density in both the invasive margin and the tumor center [8]. In a mouse model of head and neck squamous cell carcinoma, progression on radiotherapy with dual immune checkpoint therapy was

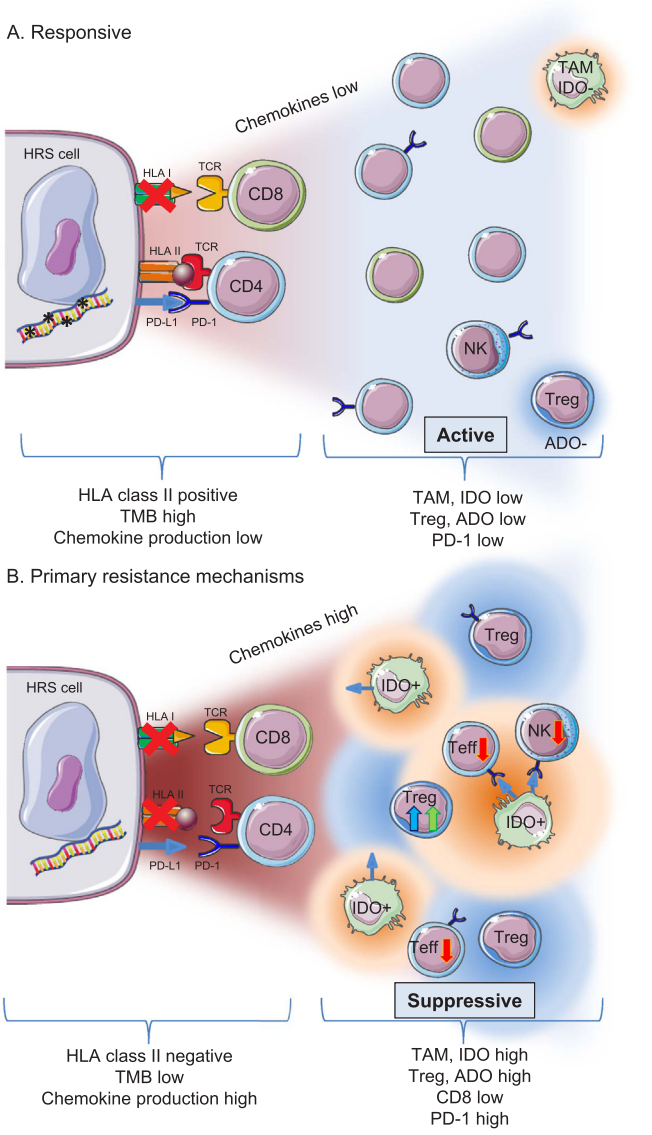

Fig. 1. Mechanisms of primary resistance to immune checkpoint blockade in Hodgkin lymphoma. (A) Patients that are predicted to have a good response are HLA class II positive, with a high TMB that have an active inflammatory infiltrate indicated by less IDO+ TAMs and adenosine generating Treg cells; (B) Primary resistance might be caused by one or a combination of factors including lack of HLA class II positivity, a TME with low CD8, high PD-1, high PD-L1 + IDO+ TAMs and high adenosine generating Tregs, which will result in decreased functionality of effector T cells, NK cells and increased functionality of Tregs. Red arrows indicate a decreased functionality, green arrows an increased functionality and blue arrows increased cell numbers. Blue circles indicate the adenosine (ADO) gradient, orange circles the products from IDO metabolism and red to blue the amount of chemokines produced by HRS cells. This figure was created using Servier Medical Art (http://smart.servier.com/). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

accompanied by an increased proportion of Tregs. Targeted Treg depletion in those mice resulted in sustainable tumor responses. Concordant with the study in mice, a study in two patients that had similar disease presentation but responded differently to radiotherapy in combination with anti-PD-1 showed that the non-responding patient had a higher proportion of Tregs [63]. Together this strongly supports a role for the composition of the TME in primary resistance to PD-1 blockade therapy.

It is clear that the TME in HL contains sufficient T cells, although

CD8+ T cell numbers are very low and they are not in close proximity of the HRS cells. This suggests that these CD8+ T cells do not play a main role in an effective anti-tumor response. Compared to cell suspensions of reactive lymph nodes, HL cell suspensions have significantly higher proportions of Treg [64]. HRS cells create this TME by multiple mechanisms such as the production of CCL17/TARC and CCL22/MDC which cause the attraction of CD4+ Th2 and Treg cells [65,66]. These T cell subsets express CCR4 the receptor for CCL17 and CCL22 [67] and form rosettes around HRS cells [66]. Moreover, *in vitro* studies have shown that HRS cells effectively induce Treg differentiation from naïve CD4+ T cells [68]. In addition, HRS cells can produce vascular endothelial growth factor (VEGF). In a mouse model of colorectal cancer VEGF induced Treg proliferation and increased the expression levels of several inhibitory receptors (e.g. PD-1, CTLA-4, TIM-3, LAG-3) on CD8+ T cells, inducing an exhausted phenotype [69,70]. In HRS cells, VEGF expression shows a significant positive association with PD-L1 and PD-L2 expression levels [71]. In conclusion, HRS cells shape the TME to avoid anti-tumor immune responses by attracting CD4+ Th2 and excluding CD8+ T cells. Moreover, by increasing the numbers of Tregs, activation and effector functions of other T cells can be inhibited. This shaping of the TME and especially the proportion of Tregs present within the TME might influence the efficiency of PD-1 blockade therapy (Fig. 1B).

Presentation of antigens and neoantigens

One of the most plausible mechanisms for resistance against immune checkpoint blockade therapy in HL is the absence or ineffective presentation of antigens by the HLA molecules. The importance of antigen presentation in the pathogenesis of cHL has been indicated by the strong association of the HLA region and specific HLA subtypes with increased cHL susceptibility [44,45].

Neoantigens are antigens derived from genes mutated in tumor cells, which can potentially elicit an effective anti-tumor immune response. Increased TMB has been associated with an improved response to immune checkpoint inhibition in several cancers [12,29]. Recently, TMB was found to be high in 15%, intermediate in 53% and low in 32% of a total of 34 cHL cases [72]. HLA positive cHL patients with high TMB potentially present neoantigens and are likely to respond well to immune checkpoint inhibition (Fig. 1A). Linking PD-1 blockade efficiency to TMB and HLA expression patterns in cHL might shed light on the relevance of these factors.

Presentation of tumor antigens can be compromised by both loss of or aberrant HLA expression (Fig. 1B). Effective presentation of antigenic peptides by the tumor cells is a prerequisite for an effective T cell mediated response to immune checkpoint inhibition. HLA class I cell surface expression is lost in around 70% of cHL cases, more often in EBV negative cases (around 85%) than EBV positive cases (around 30%) [49,53–55,73]. This can be caused by inactivating mutations or loss of the β2M gene region [72,74,75]. β2M is essential for stable cell surface expression of HLA class I. Loss of HLA class I makes HRS cells more susceptible for attack by NK cells, but HRS cells circumvent this by expression of HLA-G a molecule that is highly homologous to HLA class I but that does not present immunogenic peptides [73]. Cell surface expression of HLA class II is lost in around 40% of the HL cases, again more often in EBV negative cases (around 50%) compared to EBV positive cases (around 30%) [49,76]. A major contributor to the loss of HLA class II expression is inactivation of the major histocompatibility complex class II transactivator (CIITA) by genomic rearrangements [77] and somatic mutations [74]. Another factor leading to functional loss of HLA class II antigen presentation is loss of HLA-DM expression. HLA-DM is necessary to displace the class II invariant chain peptide (CLIP) and allow antigen loading into HLA class II [49]. This gives rise to an apparently normal HLA expression, without the ability to present antigenic peptides. Interestingly, the tumor cells in most complete responders to nivolumab are HLA class II positive and progression-free survival was associated with HLA class II positivity in patients who were treated with PD-1 blockade therapy > 12 months after ASCT [57]. In contrast, loss of HLA class I seems to be less relevant as the T cells in close vicinity of the HRS cells are usually not CD8+ [56]. Moreover, response to PD-1 blockade therapy is largely independent of HLA class I expression.

Impaired T cell effector functions

Upregulation of immune checkpoint molecules

Expression of multiple immune checkpoint molecules has been proposed to lead to acquired resistance against immune checkpoint inhibition through PD-1. Most widely described immune checkpoints involved in this in solid malignancies are lymphocyte activation gene-3 (LAG-3) and T cell immunoglobulin and mucin-domain containing-3 (TIM-3). LAG-3 has structural homology to CD4 and competes with CD4 for binding to HLA class II. This results in reduced efficiency of HLA class II mediated antigen presentation. LAG-3 expression did not differ in PD-1 responding versus non-responding melanoma patients (i.e. primary resistance), but was significantly higher in relapsed patients (i.e. acquired resistance). This indicates an important function of LAG-3 in acquired resistance but not in initial treatment response. Combined anti-PD-1 and anti-LAG-3 had a clear antitumor effect in MHC class II positive breast cancer tumors in mice with 6/8 mice achieving complete remission [19]. TIM-3 is an inhibitory receptor expressed on IFN-γ producing CD4 + Th1 and CD8 + T cells. Co-expression of PD-1 and TIM-3 is a feature of severely exhausted T cells based on a prominent decrease in cytokine production and failure to proliferate [78,79]. In head and neck squamous cell carcinoma TIM-3 levels were upregulated on tumor infiltrating lymphocytes (TIL) after *in vitro* PD-1 blockade therapy, whereas CTLA-4 expression was not affected by PD-1 blockade. Moreover, CTLA-4 blockade did not affect TIM-3 expression [27]. TIM-3 expression was also increased on TIL of melanoma and lung cancer patients who progressed on PD-1 blockade therapy [19,20]. However, TIM-3 expression levels were not related to initial PD-1 treatment response implicating its importance in acquired resistance [19]. Treatment with anti-TIM-3 blockade upon PD-1 treatment resistance reduced tumor growth and provided a significant survival advantage in head and neck squamous cell carcinoma or lung cancer mouse tumors [20,27].

T cells in HL frequently co-express PD-1 with other immune checkpoint molecules such as LAG-3 and TIM-3 (Fig. 2) [68,80]. At diagnosis PD-1 is expressed by a proportion of the TILs, with PD-1 expression on average on 25–35% of TILs. Yet, this can reach up to 70% of TILs being positive for PD-1 [80]. Despite the on average low percentage of TILs showing expression of LAG-3 in HL diagnostic biopsies (10–20%), no negative cases were identified [80]. LAG-3 is frequently expressed on T cells located close to HRS cells, especially in EBV+ HL and was associated with decreased EBV-specific CD8+ T cell responses [81]. In contrast, TIM-3 expression is scare on TILs in HL (ranging from 0 to 10% positivity) [80]. In HL, a higher number of PD-1+ T cells has been described in patients that relapse after treatment with PD-1 blockade therapy compared to their pre-immune checkpoint biopsy. It was hypothesized that overexpression of PD-1 on T cells might restore the PD-1-PD-L1 axis and thereby explain the acquired resistance [82]. Also conventional front-line therapies such as chemotherapy and radiotherapy result in higher proportions of PD-1+ T cells and PD-L1+ macrophages in HL patients compared to the diagnostic biopsies [83]. Based on these findings it would be interesting to study LAG-3 and TIM-3 expression in primary and relapse HL tissues of patients treated with PD-1 blockade therapy.

Production of suppressive metabolites

a. Indoleamine 2,3-dioxygenase

Indoleamine 2,3-dioxygenase (IDO) has been described in relation

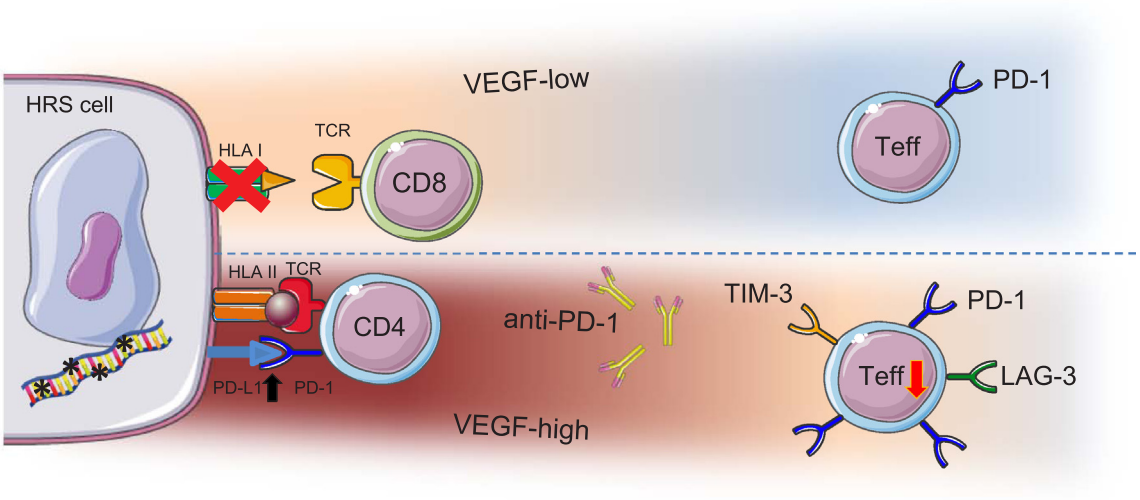

Fig. 2. Mechanisms of acquired resistance to immune checkpoint blockade in Hodgkin lymphoma. Acquired resistance can be caused by an upregulation of either PD-1, LAG-3 and TIM-3 on effector T cells after PD-1 blockade therapy limiting their function. VEGF production by tumor cells can have similar effects, but can also cause upregulation of PD-L1. The red arrow indicates a decreased functionality. The red to blue gradient indicates the amount of VEGF produced by HRS cells. This figure was created using Servier Medical Art (http://smart.servier.com/). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

to resistance to CTLA-4 and PD-1 blockade. Similar to PD-L1, IDO expression can also be induced by IFN-y and is therefore likely to be involved in primary resistance to PD-1 blockade therapy [84]. IDO is the initial and rate-limiting enzyme in the degradation of tryptophan through the kynurenine pathway. Tryptophan depletion or an increase in products from the kynurenine pathway inhibits activation and function of effector T cells and leads to attraction and activation of preexisting Tregs and differentiation of naïve T cells into Tregs [85,86]. In a mouse melanoma model, IDO deficiency synergized with anti-PD-1 and anti-CTLA-4 therapies. Moreover, expression of IDO by tumor cells conferred resistance to anti-CTLA-4, implicating IDO involvement in primary resistance to immune checkpoint blockade [18]. Interestingly, both IDO expressing and non-expressing tumors showed increased tumor responses to combined IDO inhibition and CTLA-4 blockade. The effect in IDO non-expressing tumors was shown to be dependent on IDO expression by the TME [18]. Moreover, non-small cell lung cancer patients with early progression to nivolumab had a significantly higher kynurenine/tryptophan ratio strengthening the hypothesis that IDO might confer primary resistance to immune checkpoint inhibition [24]. In HL, IDO is expressed by histiocytes, macrophages, dendritic cells and some endothelial cells, but not by HRS cells and is present in around 10–20% of the TILs [80,87,88]. A high serum kynurenine/tryptophan ratio in HL patients was associated with worse overall and progression free survival after standard first line treatment compared to patients with a low ratio [88]. These studies underline the importance of IDO expression in patient outcome and implicate IDO expression as an important factor in determining the response to immune checkpoint inhibition, regardless of the cell type expressing IDO (e.g. tumor cells or TME) (Fig. 1B).

b. Deregulated purinergic signaling

Purinergic signaling is important in immune regulation and leads to the production of adenosine. Adenosine is an immunosuppressive molecule, suppressing effector T cells and increasing Treg numbers and immunoregulatory activity via the A2a receptor [89]. Moreover, adenosine signaling also suppresses NK cell maturation and thereby reduces their functionality [90]. Extracellular ATP is metabolized to adenosine by the ectoenzymes CD39 and CD73, which are both highly expressed by Treg [91]. In addition NAD+, which has common structural features with ATP, can also be metabolized to adenosine via an alternative pathway which involves CD38, CD203a and CD73 [92]. Adenosine can be degraded by adenosine deaminase (ADA) when bound to the CD26 cell surface receptor [91]. In a mouse model of NSCLC, CD38 was shown to be upregulated on tumor cells upon resistance to PD-1-PD-L1 blockade therapy. Inhibition of both the A2a receptor or CD38 overcame the resistance [25]. In addition, CD73 expression on tumor cells reduced the effectivity of PD-1 blockade therapy in a mouse model of breast and colon cancer. This could be prevented by dual blockade of PD-1 and the A2a receptor [23]. Moreover, both inhibition of CD73 and A2a increased the effectivity of CTLA-4 blockade in a melanoma mouse model [93]. Blockade of the A2a receptor after viral challenge in mice reduced PD-1, TIM-3 and LAG-3 expression on both effector CD8+ T cells and Treg cells, while the effect on CD4+ T cells was not studied. The authors suggested that decreased expression of PD-1 might lower the threshold for effective PD-1 blockade therapy [94].

In HL, both increased production and a decreased degradation lead to increased adenosine levels (Fig. 3). T cells in cHL have an increased level of CD38 expression compared to tonsil T cells. Moreover, three out of seven cHL patients had increased percentages of CD39-expressing CD4+ T cells [68]. In line with this, expression of CD39 was induced on CD4+ T cells upon co-culture with cHL cells, while no change was seen upon co-culture with diffuse large B-cell lymphoma cells. In addition to CD39, CD73 activity was demonstrated in cHL tissue sections [68]. Together, these results point towards increased adenosine production in cHL. Furthermore, adenosine levels are most likely also increased by a decreased degradation of adenosine, especially in close proximity of HRS cells. Degradation induced by ADA is hampered in HRS cells due to decreased levels compared to germinal center B cells [68,95]. In addition, T cells in the proximity of HRS cells lack CD26 and Treg cells in the cHL TME have lower CD26 expression levels as compared to tonsil Treg cells [39,68]. Lack of CD26 on the T cells prevents binding of ADA and this results in a failure to degrade adenosine. Thus, increased adenosine levels in HL might reduce efficiency of PD-1 blockade and/or induce resistance by counteracting T cell activation through A2a receptor signaling.

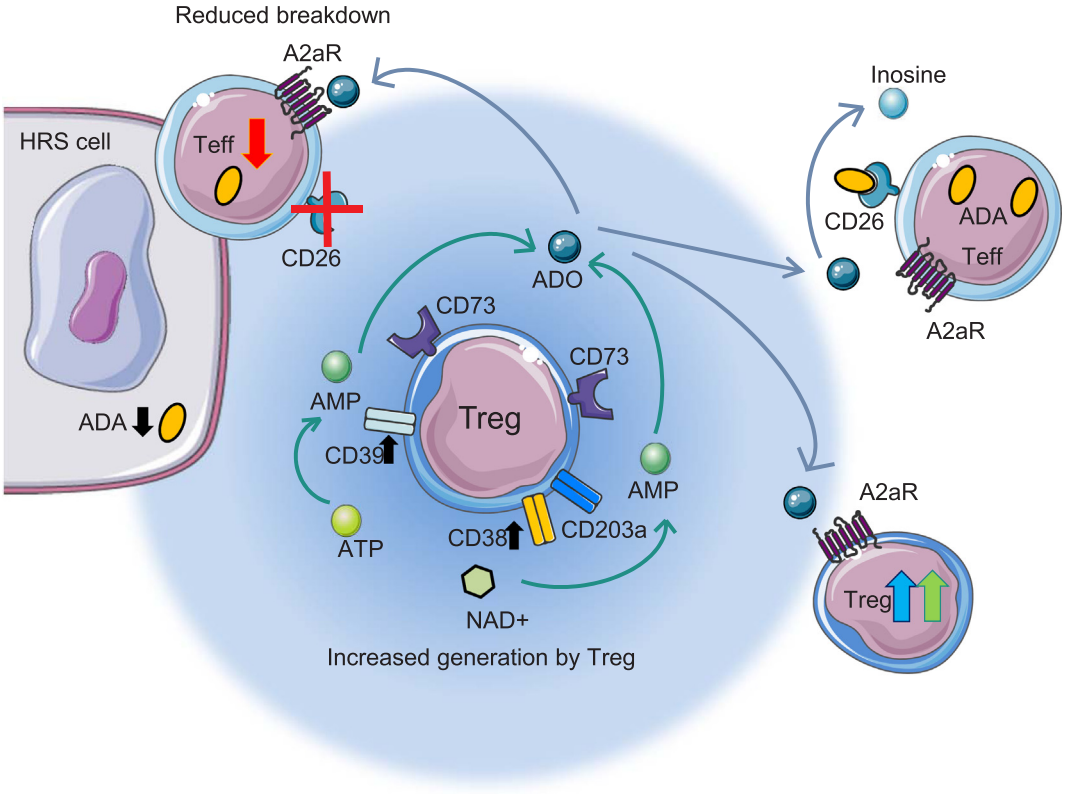

Fig. 3. Deregulated purinergic signaling in Hodgkin lymphoma. In Hodgkin lymphoma purinergic signaling is deregulated especially in close proximity of HRS cells. Due to high numbers of Treg cells in the TME which express high levels of the ectoenzymes there is an increased adenosine production. Moreover, CD38 and CD39 can be increased in T cells of cHL patients. A decreased adenosine breakdown in close proximity of HRS cells is caused by a lack of CD26 and a reduction of ADA. Red arrows indicate a decreased functionality, green arrows an increased functionality and blue arrows increased cell numbers. The blue circle indicates the adenosine gradient created around Treg cells. This figure was created using Servier Medical Art (http://smart.servier.com/). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Tumor associated macrophages and NK cell related resistance mechanisms to immune checkpoint inhibition in Hodgkin lymphoma

Besides T cell related resistance mechanisms, TAMs and NK cells have been implicated in resistance to PD-1 blockade. In a mouse colon cancer model, PD-1 antibodies were bound to CD8+ T cells at early time points, but were captured by PD-1 negative TAMs via the Fc domain of the PD-1 antibody and the FcγR on the macrophages. Blockade of Fc/FcγR inhibited transfer of the PD-1 antibody from CD8+ T cells to TAMs and enhanced anti-PD-1 therapeutic efficiency [22]. Moreover, gastrointestinal stromal tumors and soft tissue sarcomas that were heavily infiltrated by IDO + M2 macrophages were resistant to PD-1 blockade [96]. NSCLC patients responding to PD-1 blockade therapy have enhanced peripheral NK cell reactivity. Moreover, the allelic variant KIR3DS1 present on NK cells is predictive for primary resistance to PD-1 blockade. The authors suggest that continuous stimulation of the activating KIR3DS1 receptor by its ligand HLA-F causes exhausted NK cells [97].

Interestingly, both macrophages and NK cells can be involved in resistance to PD-1 blockade by mechanisms described above for T cells. Macrophages can express IDO and this can create an immunosuppressive environment leading to PD-1 blockade resistance [98]. NK cells can besides PD-1 also express CTLA-4 and LAG-3 [99,100]. In addition, NK cell activity can be inhibited by increased levels of adenosine and products of IDO metabolism [90,101]. These data suggest the importance of TAMs and NK cells in response to PD-1 blockade therapy (Fig. 1B).

Conclusions and future perspectives

Current knowledge on the crucial players defining the effectivity of immune checkpoint blockade therapy and the primary and acquired mechanisms of resistance is limited. In solid malignancies, the focus has mainly been on CD8+ T cells, while in HL a role for CD4+ T cells, TAMs and/or NK cells seems more likely. We discussed six potential mechanisms of resistance to PD-1 blockade in HL: (1) shaping of the TME excluding CD8+ T cells and increasing Treg (primary resistance); (2) inadequate T cell activation by lack of antigen presentation (primary resistance); (3) increased IDO metabolism (primary resistance); [4] attraction of TAMs to augment immunosuppression possibly through NK cells (primary resistance); (5) upregulation of PD-1, LAG-3 and TIM-3 by PD-1 blockade therapy (acquired resistance) and (6) increased adenosine levels especially in the close vicinity of HRS cells (primary and/or acquired resistance) (Figs. 1,2). To date it is unknown which of those mechanisms is most important in defining the efficiency of immune checkpoint inhibition in HL. Currently, studies to investigate PD-1 treatment resistance in HL are hampered by limited biopsy material. Future studies aiming to identify predictive biomarkers for treatment efficiency and/or primary resistance mechanisms should include HLA expression, IDO expression and number of Treg and TAMs in the TME as parameters. In addition, to identify acquired resistance mechanisms, studies on paired pre-immune checkpoint blockade and relapse samples are required. Both adenosine metabolism and multiple immune checkpoint molecules should be studied to identify novel targets for therapy. Ultimately, this might lead to optimized therapeutic regimens with PD-1 therapy combined with other blocking strategies to

prevent resistance. Moreover, it will allow for a selection of patients that are likely to show good and durable responses to PD-1 checkpoint blockade therapy.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgements

JV was funded by the Graduate School of Medical Sciences, University of Groningen, University Medical Center Groningen, the Netherlands.

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