The emerging role of immune checkpoint inhibition in malignant lymphoma

Ida Hude,[1] Stephanie Sasse,[2] Andreas Engert[2] and Paul J. Bröckelmann[2]

[1]Department of Internal Medicine, Division of Hematology, University Hospital Center Zagreb, Croatia and [2]Department I of Internal Medicine and German Hodgkin Study Group (GHSG), University Hospital of Cologne, Germany

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ABSTRACT

To evade elimination by the host immune system, tumor cells commonly exploit physiological immune checkpoint pathways, restraining efficient anti-tumor immune cell function. Growing understanding of the complex dialog between tumor cells and their microenvironment contributed to the development of immune checkpoint inhibitors. This innovative strategy has demonstrated paradigm-shifting clinical activity in various malignancies. Antibodies targeting programmed death 1 and cytotoxic T-lymphocyte-associated protein-4 are also being investigated in lymphoid malignancies with varying levels of activity and a favorable toxicity profile. To date, evaluated only in the setting of relapsed or refractory disease, anti-programmed death 1 antibodies such as nivolumab and pembrolizumab show encouraging response rates particularly in classical Hodgkin lymphoma but also in follicular lymphoma and diffuse-large B-cell lymphoma. As the first immune checkpoint inhibitor in lymphoma, nivolumab was approved for the treatment of relapsed or refractory classical Hodgkin lymphoma by the Food and Drug Administration in May 2016. In this review, we assess the role of the pathways involved and potential rationale of checkpoint inhibition in various lymphoid malignancies. In addition to data from current clinical trials, immune-related side effects, potential limitations and future perspectives including promising combinatory approaches with immune checkpoint inhibition are discussed.

Correspondence:
paul.broeckelmann@uk-koeln.de

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Introduction

Even though malignant lymphomas are still considered rare diseases, their incidence has increased over time, so that there are now more than 250.000 new cases per year worldwide, accounting for about 3% of all cancer-related deaths.[1] Lymphoma represents a diverse group of malignancies with distinct clinical, histopathological, and molecular features, as well as heterogeneous outcomes after standard therapy. About 90% of adult lymphomas derive from mature B cells, with the rest being derived from T and natural killer cells.[2] Up until the end of the 20th century, treatment for malignant lymphoma relied mainly on combination cytotoxic chemotherapies, with or without additional radiotherapy. Treatment outcomes were often not satisfactory and associated with significant short- and long-term morbidity and mortality.

The introduction of targeted therapy changed the therapeutic landscape of malignant lymphoma with the advent of monoclonal antibodies targeting surface antigens on malignant cells. In particular, the anti-CD20 antibody rituximab, targeting CD20 in B-cell non-Hodgkin lymphoma (NHL), but also the anti-CD30 antibody-drug-conjugate brentuximab-vedotin (BV) in classical Hodgkin lymphoma (cHL) and T-cell lymphoma, led to higher response rates and prolonged survival in first-line or relapsed/refractory (r/r) disease, while showing acceptable safety profiles.[3-6] Nevertheless, a significant number of patients still undergo multiple lines of treatment, including high-dose chemotherapy and stem cell transplantation (SCT)

with limited outcome due to r/r disease or therapy-associated toxicities. On the other hand, growing insights into the molecular biology of lymphoma have contributed to the development of innovative therapies in recent years: drugs such as kinase inhibitors blocking the aberrant B-cell receptor pathways, or immunomodulators such as lenalidomide obtained regulatory approval for treatment of certain NHL entities after promising activity had been shown in pivotal clinical trials.[7]

More recently, an improved understanding of the interplay between malignant cells and the tumor microenvironment, as well as evasion of the host immune response, has led to identification of new targets in cancer therapy. The idea of harnessing the host immune system to combat cancer effectively has led to the development of agents that target immune checkpoint signaling pathways, enhance T-cell cytotoxic activity and subsequently induce tumor cell lysis. This groundbreaking immunotherapeutic approach has produced exciting results in different malignancies and many clinical trials are currently ongoing or underway to explore immune checkpoint inhibition (ICI) further. The aim of this review is to elaborate on the biology of clinically relevant immune checkpoints, discuss early clinical results with ICI in different lymphoma subtypes, as well as to address potential limitations, current challenges and the future role of ICI in clinical practice.

Immune checkpoints

The biology of immune checkpoints has been thoroughly reviewed elsewhere.[8,9] In brief, naïve T cells become activated after recognizing a unique peptide presented by antigen-presenting cells, via interaction of major histocompatibility complex molecules on antigen-presenting cells with the T-cell receptor, and a co-stimulatory signal. Activating signals are finely modulated by a complex network of inhibitory receptors, referred to as checkpoint molecules.[10] The main function of these molecules is to prevent destructive immune responses, particularly in the presence of chronic infections and inflammation, as well as to maintain peripheral self-tolerance. Tumor cells are capable of evading immunosurveillance by over-expressing the ligands of checkpoint receptors, bringing T cells to a state of non-responsiveness or exhaustion.[11,12] Therapeutic manipulation of these pathways by ICI reverses T-cell anergy, facilitating an effective T-cell-mediated antitumor response. Recently, the cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and programmed death-1 (PD-1) pathways have been the major focus, with several other pathways also described.[10]

CTLA-4 is expressed on activated T cells and plays a crucial role in the priming phase of an immune response thereby representing a prototype for ICI. As depicted in Figure 1, inhibition of this pathway allows co-stimulatory signaling and generates antitumor T-cell responses by inhibiting the interaction between CTLA-4 and B7 (the CTLA-4 ligand on, for example, antigen-presenting cells).[8] One such inhibitor is ipilimumab (Bristol-Myers Squibb), a fully human monoclonal IgG1κ antibody. Its efficacy and the resulting survival benefit led to international approval for its use as first-line treatment of advanced

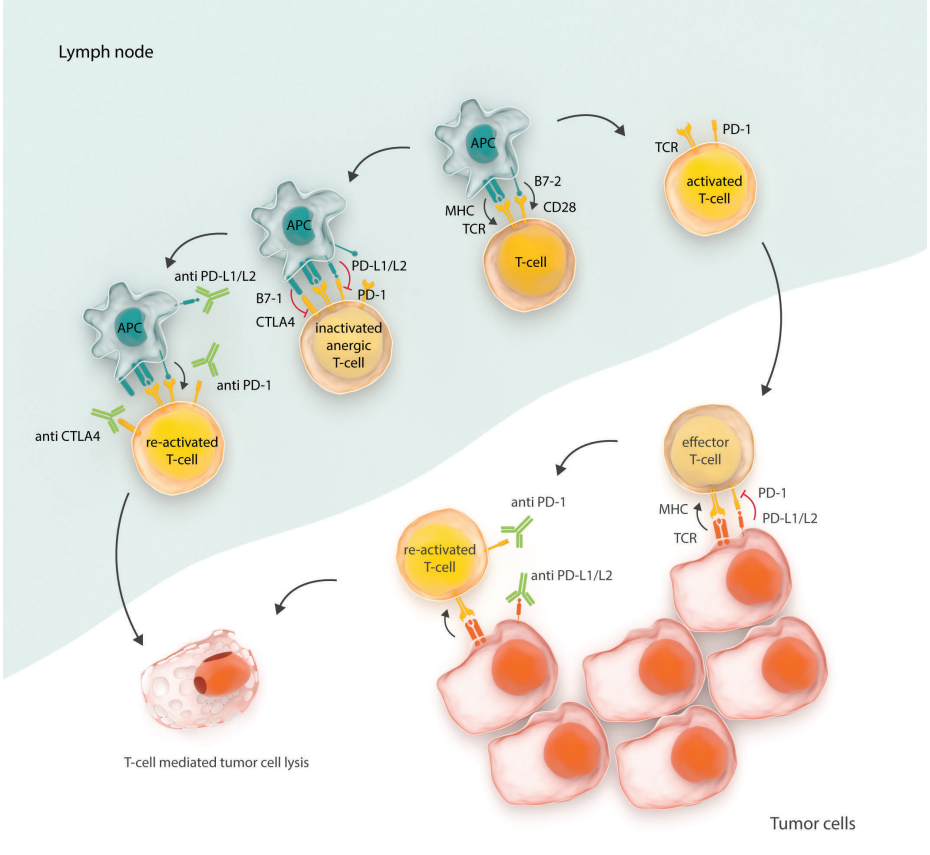

Figure 1. Inhibition of the immune checkpoints PD-1 and CTLA4 to restore T-cell activation. Antigen-presenting cells (APC) present an antigen (e.g., tumor-associated antigen – TAA) to naïve T cells via interaction of T-cell receptor (TCR) and major histocompatibility 1 (MHC-I) molecule, followed by a co-stimulatory signal by CD28/B7-2 interaction, which leads to T-cell activation. The activation is followed by expression of inhibitory checkpoint molecules such as PD-1 and CTLA-4 on T cells. In an immunosuppressive lymph node microenvironment, APC express corresponding inhibitory ligands, bringing T cells to an inactivated or anergic state (via the CTLA4/B7-1 and/or PD-1/PD-L1/L2 interaction). If co-stimulatory signals overpower the co-inhibitory ones, activated effector T cells are released into the blood stream, where they encounter TAA presented on MHC-I molecules on tumor cells. Co-expression of PD-L1 on tumor cells induces inactivation of tumor-specific effector T cells, disabling adequate T-cell-mediated immune responses. Treatment with immune checkpoint inhibitors (ICI) affects both the priming phase of T-cell activation in lymph nodes and the effector phase in the tumor microenvironment (TME), by blocking the inhibitory checkpoint interaction between activated T cells and APC and/or tumor cells, restoring T-cell activity and leading to T-cell-mediated tumor cell lysis. TCR: T-cell receptor; MHC-I: major histocompatibility complex; PD-1: programmed cell death protein 1; PD-L1: programmed death-ligand 1; PD-L2: programmed death-ligand 2; CTLA-4: cytotoxic T-lymphocyte-associated protein 4.

melanoma.[13,14] Clinical results of ipilimumab in other malignancies,[15] as well as results of a second anti-CTLA-4 antibody (tremelimumab; Medimmune/Astra Zeneca) have so far been modest and these drugs are undergoing further clinical investigation.

PD-1 is another inhibitory receptor expressed on activated T cells. It plays a central role in regulating the effector phase of the immune response via its interaction with two ligands, PD-L1 and PD-L2. PD-L1 is expressed on many malignant cells as well as hematopoietic cells and peripheral tissues, while the expression of PD-L2 is mostly restricted to hematopoietic cells as shown in Figure 1.[16] Currently, various antibodies against PD1 and PD-L1 are under clinical evaluation in different malignancies. The anti-PD-1 antibodies nivolumab (a human IgG4 antibody; Bristol-Meyers Squibb/Ono) and pembrolizumab (a humanized IgG4 antibody; Merck) obtained approval from the Food and Drug Administration (and partially from the European Medicines Agency) for use in advanced melanoma, non-small-cell lung carcinoma, and renal-cell cancer. In addition, nivolumab has recently also been approved in the USA for r/r cHL.[17,18] Novel checkpoint targets such as OX-40, LAG-3 and KIR (a natural killer-cell inhibitory receptor) are also currently under investigation (Figure 2).

Inhibition of the PD1/PD1-L and the CTLA-4/B7 pathways in malignant lymphoma has been evaluated in early phase clinical trials. Hereafter, the preclinical rationale and results of recent trials (Table 1) are discussed by lymphoma type.

Hodgkin lymphoma

Hodgkin lymphoma (HL), consisting of a small number of Hodgkin and Reed-Sternberg (HRS) tumor cells surrounded by an abundant, yet ineffective inflammatory immune-cell infiltrate, is considered a typical example of an ineffective anti-tumor immune response.[2,19] Preclinical data indicate that the PD-1/PD-L pathway contributes significantly to the immunosuppressive microenvironment of cHL. PD-1 is expressed on tumor-infiltrating and peripheral T cells in patients with cHL,[20,21] whereas PD-ligands are frequently expressed by HRS cells[22,23] and tumor-infiltrating macrophages.[24] PD-L genes have been shown to be key targets of structural amplification of chromo-

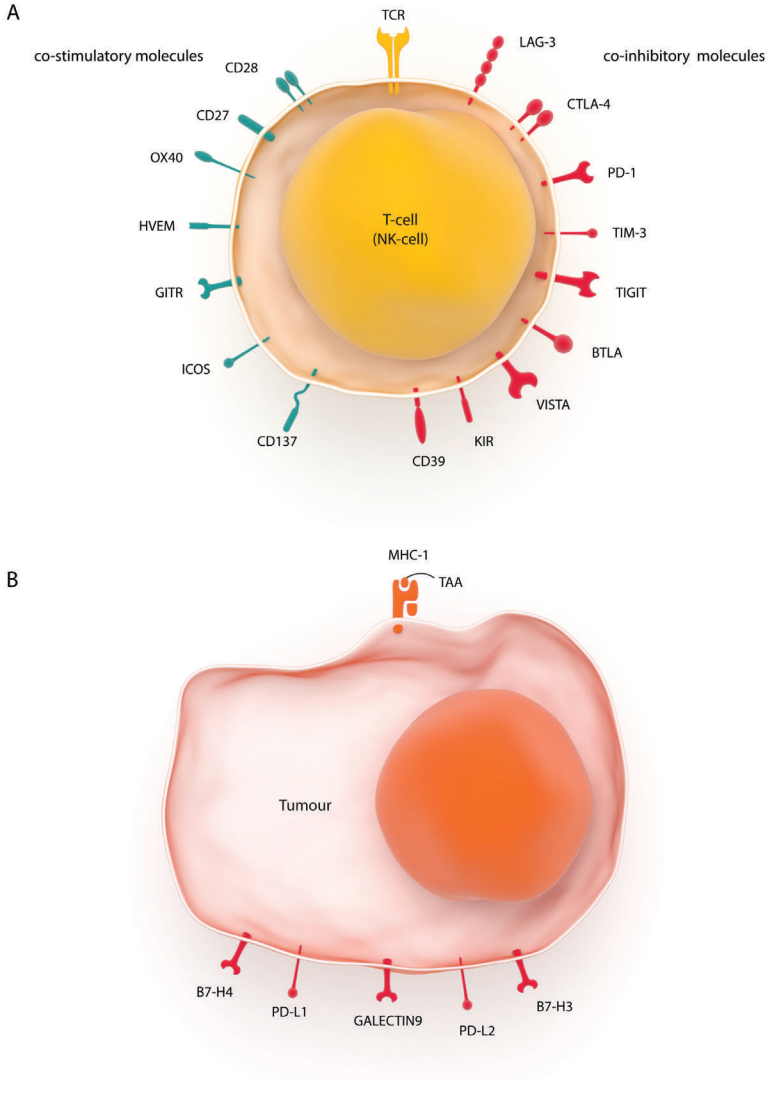

Figure 2. Potential targets of ICI on lymphocytes and tumor cells. (A) Activated T cells (and natural killer cells to a certain extent) express multiple co-stimulatory and co-inhibitory checkpoint molecules on their surface, all of which are potential targets for immunomodulation by checkpoint agonists (co-stimulatory molecules) or inhibitors (co-inhibitory molecules). (B) Tumor cells evade the host immune system by expressing ligands for co-inhibitory checkpoint molecules on T cells, hence targeting these ligands leads to inactivation of inhibitory pathways and reactivation of tumor-specific T cells. TCR: T-cell receptor; MHC-I: major histocompatibility complex I; TAA: tumor-associated antigen; LAG-3: lymphocyte-activation gene 3; CTLA-4: cytotoxic T-lymphocyte-associated protein 4; PD-1: programmed cell death protein 1; TIM-3: T-cell immunoglobulin and mucin-domain containing-3; TIGIT: T-cell immunoreceptor with Ig and ITIM domains; BTLA: B- and T-lymphocyte attenuator; VISTA: V-domain immunoglobulin suppressor of T-cell activation; KIR: killer cell immunoglobulin-like receptor; ICOS: inducible T-cell co-stimulator; GITR: glucocorticoid-induced TNFR-related protein; HVEM: Herpesvirus entry mediator, PD-L1: programmed death-ligand 1; PD-L2: programmed death-ligand 2.

Table 1. Early phase clinical trial data for ICI according to lymphoma type.

Lymphoma	Agent	Ref.	Phase	Disease setting	No of Pts	Treatment plan	Outcome	Safety	Most common AE
HL	ipilimumab	(30)	I	r/r HL after allogeneic SCT	14	dose esc. trial: 0.1 mg/kg - 0.33 mg/kg - 0.66 mg/kg - 1.0 mg/kg - 3.0 mg/kg	ORR: 14% CR: 14%	no Gr 3/4 GvHD 1 pt Gr 4 pneumonitis no TRD	fatigue chills/ fever abdominal pain
	ipilimumab + BV	(31)	I/II	r/r HL	23	dose esc. trial: IPI 1 mg/kg - 3 mg/kg Q21d x 4 doses BV: 1.8 mg/kg Q21d x 16 doses	ORR: 72% CR: 50% median PFS: 1.02y	100% any AE 1 pt Gr 4 AE (thrombocytopenia) no TRD	neuropathy nausea/ vomiting fatigue pruritus/rash
	nivolumab	(18, 32)	I	r/r HL	23	3 mg/kg NIVO at week 1 and 4, Q2w thereafter until 2y	ORR: 87% CR: 22% median OS and PFS not reached after 101m FU 1.5y OS 83%	1 pt Gr 3 pneumonitis 1 pt Gr 3 colitis	rash/pruritus hypothyroidism diarrhea
	nivolumab	(33)	II	r/r HL	80[1]	3 mg/kg Q2w	ORR: 66% CR: 8,8% PR: 57,5% ORR (no BV response)*: 72%	90% any AE 1 TRD 25% Gr 3/4 AE	hypothyroidism/ thyreoiditis rash hypersensitivity
	pembrolizumab	(36)	Ib	r/r HL (failing BV)	31	10 mg/kg Q2w/2y	ORR: 65% CR: 16% PR: 48% 24w PFS 69%	no Gr 4 AE 5 pt Gr 3 AE	hypothyroidism diarrhea nausea/vomiting pneumonitis
	pembrolizumab	(37)	II	r/r HL	90[2]	200 mg Q3w	cohort 1: ORR 73%, CR 27%, PR 47% cohort 2: ORR 83%, CR 30%, PR 53% cohort 3: ORR 73%, CR 30%, PR 43%	4% Gr 4 AE no TRD 7 pt Gr AE	pyrexia diarrhea
DLBCL + PMBCL	ipilimumab	(46)	I	r/r B-NHL	3 DLBCL	dose level 1: 3 mg/kg once + 1 mg/kg Q1m x3 dose level 2: 3 mg/kg Q1m x4	1 CR DOR > 31 months	5 pt Gr 3 AE (diarrhea) no Gr 4 or TRD	fatigue diarrhea abdominal pain thrombocytopenia
	nivolumab	(47)	I	r/r lymphoid malignancies	11 DLBCL + 2 PMBCL	dose level 1: 1 mg/kg at w 1 and 4, thereafter Q2w/2y dose level 2: 3 mg/kg at w 1 and 4, thereafter Q2w/2y	DLBCL: CR 18%, PR 18%, SD 27%, Median PFS 7w PMBCL: SD 100%	all AE 71%[3] Gr 3-5 AE: pneumonitis, ARDS, dermatitis, diplopia, enteritis, eosinophilia, mucosal inflammation, pyrexia, vomiting	fatigue pneumonitis pruritus/rash
	pembrolizumab	(48)	Ib	PMBCL	16	10 mg/kg Q2W or 200 mg Q3W/2y	ORR: 37,5% CR: 6,25% PR: 31,25%	62% TR AE 1 pt Gr 3 AE (neutropenia) no Gr 4 AE, no TRD	decreased apetite, nausea fatigue diarrhea hypothyroidism
FL	nivolumab	(47)	I	r/r lymphoid malignancies	10 FL	dose level 1: 1 mg/kg Q2w/2y dose level 2: 3 mg/kg Q2w/2y	ORR: 40% CR: 10% PR: 30% Median PFS not reached	any AE 72% of pt[3] Gr 3-5 AE: pneumonitis, ARDS, dermatitis, diplopia, enteritis, eosinophilia, mucosal inflammation, pyrexia, vomiting	fatigue pneumonitis, pruritus/rash
CLL	pembrolizumab	(67)	II	r/r CLL (including RS)	16, 7 evaluable	200 mg Q3w	ORR: 57% CR: 14% PR: 14% 2 responses before PD	2 pt Gr 3 AE no Gr 4, no TRD	dyspnea anemia

Gr: grade; ARDS: acute respiratory distress syndrome; IP: ipilimumab; NIVO: nivolumabBV; brentuximab-vedotin; R: rituximab; r/r: relapsed/refractory; autoSCT: autologous stem cell transplantation; RS: Richter syndrome; PMBCL: primary mediastinal B-cell lymphoma; esc.: escalation; pt: patient(s); TR: treatment-related; TRD: treatment-related death; AE: adverse event; ORR: overall response rate; CR: complete remission; PR: partial remission; PD: progressive disease; m: month(s); w: week(s); DOR: duration of response; PFS: progression-free survival; OS: overall survival; QXd: every X days; QXw: every X weeks; QXm: every X months. [1]Results from cohort B: r/r cHL pts who received BV after failing prior autoSCT; [2]30 pts in cohort 1 (r/r cHL after autologous SCT and subsequent BV therapy), 30 in cohort 2 (r/r cHL ineligible for autologous SCT due to chemo-resistance, no response to salvage chemotherapy and prior BV therapy) and 30 in cohort 3 (r/r cHL after autologous SCT without subsequent BV therapy). [3]Adverse events reported for all B-NHL patients enrolled (31 pts).

some 9p24.1, a recurrent genetic abnormality in cHL. Extended amplification of the 9p24.1 region also induces expression of the Janus kinase 2 (JAK2) protein whose activity further induces PDL expression via JAK2/STAT signaling.[22] In addition, Epstein-Barr virus infection has been demonstrated as an alternative, mutually exclusive, mechanism of PD-L1 induction,[23] consistent with the ability of the virus to usurp the PD-1 pathway.[16] A recent retrospective analysis in first-line cHL biopsies suggests a correlation between advanced stage disease and a negative prognostic impact of 9p24.1 amplification.[25] In another immunohistochemical study, PD-1 expression on tumor-infiltrating lymphocytes was suggested as a stage-independent negative prognostic factor for overall survival (OS) in cHL,[26] while others found only rare PD-1-positive tumor-infiltrating lymphocytes.[27] According to two phase I trials investigating the safety and activity of anti-PD1 antibodies in r/r HL, over 90% of the examined patients' samples showed strong expression of PD-L1 on HRS cells, with rather low PD-1 expression on tumor-infiltrating lymphocytes.[18,28] Taking these discrepant data into account, relevant predictive factors for treatment outcome with anti-PD1 antibodies are still unknown. Other potential immune escape mechanisms including the CTLA-4 pathway have been described in cHL[29] and it is thought that these mechanisms contribute to the rather low graft-*versus*-lymphoma effect in cHL after allogeneic SCT.

The first checkpoint inhibitor tested in HL was ipilimumab administered as a single dose in a phase I trial to 14 r/r HL patients after allogeneic SCT. The drug was well tolerated, with no cases of relevant graft-*versus*-host disease (GvHD) and two patients achieved a complete remission (CR).[30] Preliminary results of a NCI-sponsored phase I/II trial testing 3 mg/kg ipilimumab in combination with 1.8 mg/kg BV in r/r cHL showed an overall response rate (ORR) of 72% with a 50% CR rate in 18 evaluable patients.[31]

More recently, two early phase trials with anti-PD1 antibodies in r/r HL patients reported encouraging results: the dose-escalation phase I trial of nivolumab included 23 intensively pretreated r/r cHL patients and the ORR was 87%.[18] In the updated report with a median follow-up of 101 weeks, the median progression-free survival (PFS) was not reached with a 1.5-year overall survival rate of 83%.[32] CR was observed in 22% of the patients, but partial remissions (PR) seem to be durable with 13 patients remaining in stable remission without further treatment. Treatment-related adverse events were observed in 78% of patients, with 22% having grade 3 or 4 events. Preliminary results of a phase II trial in r/r cHL patients who had relapsed after autologous SCT and BV, presented at EHA and ASCO 2016, depicted an ORR of 66% based on central review and of 72% based on investigator evaluation after a median follow-up of 8.9 months, with 51 out of 80 patients still receiving treatment at the time of the data cut-off.[33] Interestingly, a high ORR of 72% was observed among 43 patients without prior response to BV. Drug-related adverse events occurred in 90% of patients, with 25% grade 3–4 adverse events and one non-treatment-related grade 5 multi-organ failure. Of note, correlative questionnaires suggested substantial improvement in quality of life after initiation of treatment.

As far as allogeneic SCT is concerned, preclinical data suggested that anti-PD-1 antibodies might contribute to significant GvHD.[34] Severe GvHD was documented in patients undergoing allogeneic SCT after treatment with nivolumab in the phase I trial. In contrast, recent results of a cohort receiving nivolumab for r/r cHL after allogeneic SCT suggested a more acceptable safety profile: acute GvHD was recorded in three patients, all of whom already had a history of acute GvHD after allogeneic SCT.[35] Among 14 patients evaluated at the time of reporting, the ORR was 92.7% with six patients achieving a CR.

Pembrolizumab is being evaluated in an ongoing phase Ib trial in patients with different r/r hematologic malignancies who had failed prior treatment, were refractory to or refused autologous SCT. The ORR among the 31 r/r cHL patients in whom prior BV treatment had failed was 65% and included five patients who achieved a CR (16%). The 24- and 52-week PFS rates were 69% and 46%, respectively. Similarly to nivolumab, treatment was well tolerated, with grade 3 drug-related adverse events reported in five patients and no grade 4 adverse events or treatment-related deaths.[36] Preliminary results of a multicohort phase II trial in r/r HL patients were presented at EHA and ASCO 2016. The results showed promising activity: in cohort 1 (r/r HL after autologous SCT and BV), cohort 2 (ineligible for autologous SCT after BV) and cohort 3 (r/r HL after autologous SCT without BV) investigator-based ORR of 73%, 83% and 73%, respectively, were reported.[37]

Large B-cell lymphoma

Unlike on HRS cells, PD-L1 overexpression is not commonly seen on B NHL cells. PD-L1 overexpression has been described in the more aggressive, non-germinal center B-cell-like type of diffuse large B-cell lymphoma (DLBCL),[38] in which it was recently also found to be a predictor of poor OS.[39] The ratio of CD4*CD8 to (CD163:CD68 [M2])*PD-L1 in histopathological samples of DLBCL patients treated with R-CHOP also indicated differences in OS.[40] Interestingly, soluble plasma PD-L1 (sPD-L1), measured prior to treatment in newly diagnosed DLBCL patients, has also been found to correlate with poorer 3-year OS in multivariate analysis.[41] Of note, serum-levels of sPD-L1 decreased significantly in patients achieving a CR and were attributed to an immunological effect of treatment, suggesting that sPD-L1 levels mirror the host anti-immune response, rather than the specific presence of malignant cells. This hypothesis is supported by a poor correlation of sPD-L1 and tumor PD-L1 expression in this cohort of patients. The aforementioned 9p24.1 alterations responsible for PD-L1/L2 upregulation in cHL have also been observed in specific subsets of large B-cell lymphoma such as primary mediastinal but also primary testicular lymphoma and primary central nervous system DLBCL.[42-44] Furthermore, the PD-L1/PD-L2 locus was identified as a recurrent translocation partner for immunoglobulin heavy chain locus, a hallmark of DLBCL, by whole genome sequencing analysis. Interestingly, these cytogenetic alterations were more frequently observed in the non-germinal center B-cell-like type of DLBCL.[45]

Even though the role of the CTLA-4 pathway in DLBCL remains unclear, ipilimumab was the first checkpoint inhibitor investigated in this malignancy. The dose-escalation phase I trial of ipilimumab in 18 patients with r/r B-cell NHL included three cases with DLBCL. Two out of the 18 patients had clinical responses and one with DLBCL achieved a durable CR lasting more than 31 months. Analysis of post-treatment samples showed T-cell prolifer-

ation in response to recall antigens after ipilimumab treatment in five of the 16 evaluated cases (31%).[46] A phase I trial of nivolumab monotherapy recruited patients with heavily pretreated r/r lymphoid malignancies including 11 patients with DLBCL.[47] Four patients (36%) responded (2 CR and 2 PR) and three (27%) had stable disease (SD) with a median PFS of 7 weeks. A comparable ORR of 37.5% with a tolerable safety profile was recently reported in heavily pretreated patients with r/r primary mediastinal large B-cell lymphoma receiving pembrolizumab.[48]

Mantle cell lymphoma

Preclinical data suggest that mantle cell lymphoma (MCL) cells evade the host immune response by inducing several microenvironmental changes. In a study investigating B-NHL biopsy tissues including two MCL cases, intratumoral T regulatory cells were shown to inhibit proliferation and cytokine production of $CD4^+CD25^-$ T cells by the PD-1/PD-L1 interaction.[49] PD-L1 expressed by MCL cell lines results in impaired T-cell proliferation after tumor exposure, impaired T-cell-mediated tumor cytotoxicity and inhibited specific anti-tumor T-cell responses.[50]

So far, data available on ICI in MCL are limited: in the aforementioned ipilimumab phase I study,[46] the only MCL patient included did not respond to treatment. In contrast, a PR was observed in a single MCL patient treated with ipilimumab for relapse after allogeneic SCT.[30] Four MCL patients treated with nivolumab did not respond.[47] Results of currently ongoing combination trials with nivolumab and ipilimumab or anti-KIR therapy are pending (Table 2). In addition to the clinical efficacy of single-agent bruton-kinase inhibition in MCL,[50,51] combinations of ibrutinib and ICI look appealing, in light of the immunomodulatory effect targeting interleukin-2-inducible T-cell kinase.[52]

Follicular lymphoma

Preclinical studies described an immunosuppressive microenvironment as the key component of disease sustainability and progression in follicular lymphoma (FL).[49,53] Moreover, the gene expression signature of non-malignant stromal cells is prognostically more relevant than the neoplastic B cells themselves. While a tumor-infiltrating lymphocyte gene expression signature seems to be associated with a favorable outcome, a signature enriched for genes expressed by macrophages and dendritic cells implies poor survival, suggesting that the complex dialog within the tumor microenvironment also plays a crucial role in FL.[54] Despite several attempts at translating these findings into immunohistochemical studies and clinical practice, results are still inconclusive.[55,56] Similarily, attempts to distinguish the prognostic impact of PD-1 expression in the FL tumor microenvironement on survival have resulted in controversial findings, possibly due to technical issues and different prior treatment regimens including different rituximab utilization within the tested cohorts.[57-59]

Ten FL patients were included in a phase I study of nivolumab in a variety of r/r hematologic malignancies;[47] the ORR was 40% and three responses were ongoing after a median follow-up of 91.4 weeks, which encouraged further clinical trials.

Chronic lymphocytic leukemia

Immune dysfunction is common among patients with chronic lymphocytic leukemia (CLL), who may have profound defects in the function of T cells, which eventually develop an exhausted phenotype, resulting in both failure of anti-tumor effectiveness and increased susceptibility to infections. T cells isolated from CLL patients have higher expression of checkpoint molecules such as CTLA-4 and PD-1.[60,61] The cells' cytotoxic and proliferating capacities are reduced, but they maintain the ability to produce cytokines.[62] Unlike the situation in most hematologic malignancies, PD-1 is expressed on both T and CLL cells, while PD-L1 is also highly expressed in the different compartments of the tumor microenvironment, including CLL cells.[61,63] Preclinical data on anti-PD-1 effects in CLL demonstrated restored CD8 T-cell cytotoxicity, immune synapse formation and prevention of CLL development in TCL-1 mouse models.[64,65] These observations and other preclinical data suggesting the importance of additional immune checkpoint pathways[66] provide a strong rationale for investigating immunomodulating therapies in CLL.

A phase I trial of ipilimumab did not show that the drug had efficacy as monotherapy in CLL patients.[30] On the other hand, preliminary results of an ongoing phase II trial of pembrolizumab in r/r CLL patients, including those with Richter syndrome, showed an ORR of 21% in 20 evaluable patients. Responses, including one CR, were documented in three patients with Richter syndrome and also in patients in whom prior ibrutinib therapy had failed. Treatment seemed to be well tolerated, with two patients developing grade 3 adverse events. Correlative studies indicate that sPD-L1 might be a biomarker for response to treatment.[67] After the combination of ibrutinib and an anti-PD-L1 antibody showed synergistic effects in a mouse model resistant to either agent given alone,[52] several combination clinical trials in CLL are underway.

Other lymphoma

There are limited data on the efficacy of ICI in other B-cell malignancies. Due to the rapid clinical course of disease, it is questionable whether monotherapy with ICI is adequate in more aggressive lymphoma subtypes such as Burkitt lymphoma. However, preclinical evidence indicates that some patients with virus-associated aggressive lymphomas might benefit from such treatment: retroviral infection is known to upregulate immune checkpoint pathways[68] and recent evidence shows that PD-1 blockade might be efficient in controlling human immunodeficiency virus infection.[69] This renders anti-PD-1 antibodies interesting agents in human immunodeficiency virus-associated lymphomas, e.g. as part of combinatory therapies to induce host immune restitution, anti-retroviral and anti-tumor effects. Other virus-related lymphomas (i.e. Epstein-Barr virus- or hepatitis C virus-related)[24,70] might be susceptible to a similar approach.

As far as T-cell lymphomas (TCL) are concerned, a phase I trial with nivolumab included five patients with peripheral TCL and 18 with other TCL and obtained an ORR of 17% (2 patients with peripheral TCL and 2 with mycosis fungoides achieved a PR).[47] Encouraged by these results and preclinical data confirming PD-1 and PD-L1 expression in peripheral TCL,[71,72] further studies are currently underway. It is feasible to anticipate that these patients, like those with MCL and indolent lymphoma, might benefit more from combination treatments with other agents. Table 2 provides an overview of the numerous currently ongoing phase I and phase II trials investigating ICI as monotherapy or in combinatory approaches in lymphoid malignancies.

Table 2. Selection of currently ongoing clinical trials with ICI in lymphoma (clinicaltrials.gov as of 1st of June, 2016).

Trial N. (Name)	Malignancies	Agent/Procedure	Immunological Target	Phase
NCT02254772	r/r low grade NHL	ipilimumab + SD-101 + RTx	CTLA-4, TLR9a	I/II
NCT01729806	r/r B-NHL	ipilimumab + R	CTLA-4, CD20	I
NCT00586391	r/r B-NHL, CLL, ALL	ipilimumab + CD19-CAR-T-cells	CTLA-4, CAR-T-cells	I/II
NCT01919619	leukemia and lymphoma, after SCT	ipilimumab + lenalidomide	CTLA-4	I
NCT02581631 (CheckMate 436)	r/r NHL, CD30 positive	nivolumab + BV	PD-1, CD30	I/II
NCT02681631 (CPIT001)	high risk and or r/r lymphoma/myeloma	ipilimumab + nivolumab	CTLA-4, PD-1	I/II
NCT02518958 (PRIMETIME)	solid tumors + lymphoma	nivolumab + RRX-001	PD-1	I
NCT01896999	r/r HL	ipilimumab + nivolumab + BV ipilimumab + BV nivolumab + BV	CTLA-4, PD1, CD30	I
NCT02631746	adult HTLV-assoc. T-cell lymphoma/leukemia	nivolumab	PD-1	II
NCT02758717	HL (first line)	nivolumab + BV	PD-1, CD30	II
NCT02038946 (Checkmate 140)	r/r FL	nivolumab	PD-1	II
NCT02572167	r/r HL (second line)	nivolumab + BV	PD-1, CD30	I/II
NCT02038933 (CheckMate 139)	r/r DLBCL	nivolumab	PD-1	I
NCT02181738 (CheckMate 205)	cHL (r/r cohorts A,B,C, first line cohort D)	nivolumab nivolumab + AVD	PD-1	II
NCT02253992	solid tumors + r/r B-NHL	nivolumab + urelumab	PD-1, CD 137	I/II
NCT01592370	NHL, HL, multiple myeloma	nivolumab nivolumab + ipilimumab nivolumab + lirilumab	PD-1, CTLA-4, KIR	I
NCT01822509	relapsed hematologic malignancies after allogeneic SCT	ipilimumab or nivolumab	CTLA-4, PD-1	I
NCT02327078	multiple	nivolumab + epacadostat	PD-1, IDO	I/II
NCT02329847	CLL, FL, DLBCL	nivolumab + ibrutinib	PD-1	I/II
NCT02362997	r/r HL, r/r DLBCL	pembrolizumab (consolidation after autoSCT)	PD-1	II
NCT02446457	relapsed FL	pembrolizumab + R	PD-1, CD20	II
NCT02541565	DLBCL (first line)	pembrolizumab + R-CHOP	PD-1, CD20	II
NCT02332980	r/r CLL or low-grade NHL	pembrolizumab pembrolizumab + ibrutinib pembrolizumab + idelalisib	PD-1	II
NCT02677155 (Lyuvac-2)	FL (first line or relapse)	pembrolizumab + R + Rtx + dendritic-cell autotransplantation (intra-tumoral) + GM-CSF	PD-1, CD20	II
NCT01953692 (Keynote 13)	multiple hematologic malignancies	pembrolizumab pembrolizumab + lenalidomide (DLBCL)	PD-1, CD20	I
NCT02576990 (Keynote 170)	r/r PMBCL	pembrolizumab	PD-1	II
NCT02501473	low grade NHL	pembrolizumab + G100	PD-1, TLR-4	I/II
NCT02650990	r/r DLBCL, MCL	pembrolizumab (after antiCD19 failure)	PD-1	I/II
NCT02684292 (Keynote 204)	r/r HL	pembrolizumab vs. BV	PD-1, CD30	III
NCT02453594 (Keynote 87)	r/r HL	pembrolizumab	PD-1	II
NCT0266560	r/r HL	pembrolizumab + AFM13	PD-1, CD30/CD16A	I
NCT02779101	recurrent /progressive PCNSL	pembrolizumab	PD-1	II
NCT02595866	r/r or disseminated HIV-related malignancies	pembrolizumab	PD-1	I
NCT02362035 (Keynote 145)	multiple hematologic malignancies	pembrolizumab + ACP-196	PD-1	I/II
NCT02178722 (Keynote 155)	DLBCL, solid tumors	pembrolizumab, epacadostat	PD-1, IDO	I/II
NCT02684617 (Keynote 155)	r/r CLL, DLBCL, multiple myeloma	pembrolizumab + dinaciclib	PD-1	I
NCT02243578	r/r mycosis fungoides and Sezary syndrome	pembrolizumab	PD-1	II
NCT02220842	r/r FL and DLBCL	atezolizumab + obinutuzumab	PD-L1, CD20	I/II
NCT02779896	r/r FL and DLBCL	atezolizumab + obinutuzumab + polatuzumab-vedotin	PD-L1, CD20, CD79	I/II
NCT02631577	r/r FL	atezolizumab + obinutuzumab + lenalidomide	PD-L1, CD20	I/II

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NCT02596971	FL and DLBCL - first line or r/r	atezolizumab + obinutuzumab + bendamustine atezolizumab + obinutuzumab + CHOP maintenance: atezolizumab maintenance: atezolizumab + obinutuzumab	PD-L1, CD20	Ib
NCT02603419	r/r HL	avelumab	PD-L1	Ib
NCT02643303	advanced, biopsy accessible tumors (including cutaneus T-cell NHL)	durvalumab(i.v.) tremelimumab (i.v. or intra-tumoral) poly ICLC (intra-tumoral/i.m.)	PD-L1, CTLA-4, TME modulator	I/II
NCT02733042 (Fusion NHL)	r/r B-NHL or high-risk CLL	durvalumab monotherapy, durvalumab + Ibrutinib durvalumab + R + lenalidomide durvalumab + R + bendamustine	PD-L1, CD20	I/II
NCT02549651	r/r DLBCL	durvalumab durvalumab + tremelimumab durvalumab + AZD9150	PD-L1, CTLA-4	I
NCT02706405	r/r B-NHL	durvalumab + JCAR014 cyclophosphamide + fludarabine	PD-L1, CAR-T-cells	Ib
NCT02401048	r/r FL, DLBCL	durvalumab + ibrutinib	PD-L1	I/II
NCT02205333	advanced solid tumors, DLBCL	MEDI6469 MEDI6469 + tremelimumab MEDI6469 + R	OX40, CTLA4, CD20	Ib/II
NCT02061761	r/r CLL, HL, NHL	anti-LAG-3 anti-LAG-3 + nivolumab	LAG-3, PD-1	I/II
NCT01775631	r/r B-NHL	urelumab + R	CD137, CD20	I
NCT01471210	r/r B-NHL, advanced solid tumors	urelumab	CD137	I
NCT02481297	r/r or high-risk CLL	lirilumab + R	KIR, CD20	II
NCT02271945	r/r agressive B-NHL	AMP-514 + MEDI-551	PD-1, CD19	Ib/II

PMBCL: primary mediastinal large B-cell lymphoma; PCNSL: primary central nervous system lymphoma; RTx: radiotherapy; R: rituximab; CAR: chimeric antigen receptor; AVD: doxorubicin, vinblastine, dacarbazine; GM-CSF: granulocyte-macrophage colony-stimulating factor.

Toxicity of immune checkpoint inhibition

Engaging the host immune system by ICI is associated with specific immune-related adverse events that had not been typical for traditional anti-cancer therapy so far. As a result of generalized immune activation, immune-related adverse events affecting practically every tissue have been described.[73] These side effects commonly involve the skin (vitiligo, rash, pruritus), gastrointestinal tract (diarrhea, colitis), liver (hepatitis) and endocrine glands (hypophysitis, thyroiditis, adrenal insufficiency).

Reports from several phase III trials evaluating anti-CTLA-4 antibodies in different malignancies demonstrated a 61-90% incidence of immune-related adverse events, with 15-43% being grade 3 or higher. Skin toxicities, especially vitiligo and diffuse rash, are most common and develop 3-4 weeks after the initiation of treatment. Most adverse events are manageable with topical corticosteroids and oral antipruritic agents, but sporadic life-threatening cases of Steven-Johnson syndrome and toxic epidermal necrolysis have been reported.[74] Gastrointestinal toxicities such as diarrhea and colitis develop after 6-7 weeks and are of major clinical concern with anti-CTLA-4 therapy. They share features with Crohn disease, seem to be dose-related, and have been reported as causes of treatment-related deaths.[13,75,76] Endocrinopathies mostly occur about 9 weeks after starting treatment and their reported incidence is up to 18%. Hypophysitis has been quite frequently associated with ipilimumab.[77] Thyroid dysfunction has also been commonly reported, with hypothyroidism occurring more often than hyperthyroidism[77] and consequent hormonal deficiencies often require long-term hormone supplementation. Usually asymptomatic, pancreatic and hepatic enzyme elevations have been described, in rare cases manifesting as hepatitis with fever, malaise and abdominal pain. Rarer toxicities of anti-CTLA-4 treatment include neurological, renal and pulmonary side effects.[74]

Comparing monotherapy with anti-PD-1 or anti-CTLA-4 antibodies, anti-PD-1 treatment seems to cause fewer high-grade events. A meta-analysis of immune-related adverse events presented at ASCO 2016 found significantly higher toxicity rates among patients receiving anti-CTLA-4 than among those receiving anti-PD-1 or anti-PD-L1 antibodies ($P<0.0001$). Furthermore, the rates of high-grade (3-5) adverse events was significantly higher with anti-CTLA-4 therapy than with other ICI.[78] The spectrum of reported toxicities is rather similar. Even though maculopapular rash is a common dermatologic adverse effect of inhibiting both pathways, vitiligo seems to occur more frequently with anti-PD-1 treatment.[79] On the other hand, diarrhea, colitis and hepatic toxicities, as well as severe endocrinopathies are less frequently reported.[73] Immune-related pneumonitis occurs in <5% of patients with anti-PD-1 monotherapy, but severe clinical presentations and cases of treatment-related death make this complication an utmost concern of many clinicians.[80] Combinations of

anti-CTLA-4 and anti-PD-1 antibodies are associated with higher rates of treatment-related toxicities, as well as increased rates of high-grade toxicities, including pneumonitis.[81]

In respect to checkpoint inhibition in lymphoma, severe immune-related adverse events have so far been rare. Diarrhea has been reported frequently (56%) among patients receiving ipilimumab,[46] with 28% of these patients developing grade 3-4 adverse events. Among patients with relapsed NHL receiving nivolumab within a phase Ib trial, 4% developed grade 3-5 pneumonitis[47] and newly developed myelodysplastic syndrome was noted in one heavily-pretreated r/r cHL patient.[32] The occurrence of low-grade pancytopenia has been substantial in several studies,[32,46] with rare or no grade 3-4 events. Another adverse event is fatigue, which has been reported to occur in 13-56% of patients, mostly at grade 1-2.[32,46]

Another immune-related adverse event of particular interest is the development or worsening of GvHD after allogeneic SCT in a subset of patients. After favorable results from preclinical studies,[82] the idea of applying ICI to enhance graft-*versus*-tumor effects after allogeneic SCT led to ICI usage in trials and practice. A single dose of ipilimumab in patients who relapsed after allogeneic SCT appeared to be safe with no case of severe GvHD reported among 29 patients.[30] A French study of nivolumab in r/r cHL after allogeneic SCT reported limited toxicity and no cases of significant GvHD,[35] which is in contrast to preliminary results of an ongoing trial of ipilimumab in relapsed malignancies after allogeneic SCT reported at ASH 2015.[83] This trial included 28 patients, five of whom had drug-related toxicities leading to treatment discontinuation, including three cases of grade 3 chronic liver GvHD and one case of acute intestinal GvHD. Additionally, the application of a consolidating allogeneic SCT after re-induction treatment with nivolumab is still a matter of discussion since the occurrence of severe GvHD in r/r cHL patients was observed in the nivolumab phase I trial.[32]

Although severe immune-related adverse events are relatively rare, early recognition and timely management are crucial to prevent irreversibility. Treatment mostly relies on temporary dose delay and immunosuppression by topical, oral or intravenous corticosteroids, with addition of mycophenolate mofetil and other immunosuppressants for refractory cases. Whether and how immunosuppression, including prophylactic measures for infusion-related reactions, affects treatment efficacy is currently unknown. A recent case-presentation showed the feasibility of rituximab therapy for B-cell mediated autoimmune thrombocytopenia during nivolumab treatment, with no added toxicity and the possibility of continuing effective anti-PD-1 treatment.[84]

Future perspectives

Despite the promising responses with ICI in hematologic malignancies, the limited amount of data still calls for some caution. On the other hand, impressive response rates among selected heavily pretreated patients and acceptable treatment tolerability make ICI a valuable therapeutic option. Regarding treatment response evaluation in lymphoma, it is important to keep in mind that all former and current trials used response assessment criteria which were developed on principles of standard antineoplastic treatment[85] and are mainly based on the findings of positron emission tomography (PET) and computed tomography. Response kinetics with ICI are different, with some patients even achieving responses after disease progression by conventional imaging studies.[86] Also, due to anti-tumor immune responses, ICI might result in metabolic activity at previous tumor sites reflected by potentially (false-)positive PET signals. Furthermore, at least in the r/r setting, a revised definition of favorable response is required: despite a rather low complete response rate, a substantial proportion of patients with otherwise desperate prognosis achieve durable disease control, without further treatment necessity and improved quality of life. Similar observations have already led to the development of novel immune-related response criteria proposed in solid tumors.[86]

Two major preconditions are required for effective ICI: a capacitated host immune system to act against the tumor and effective tumor antigen presentation and recognition, enabling a specific immune response. Bearing in mind that all currently available ICI trials in lymphoma only included r/r patients after multiple lines of chemotherapy, it is possible that modest response rates were conditioned by a weakened host immune system. Implementation of ICI earlier in the course of disease, with a potentially more competent immune system, is under investigation. On the other hand, mutational load and mismatch-repair deficiency have been identified as possible biomarkers for ICI response and so r/r disease might be associated with a more obvious benefit from treatment. The optimal treatment duration is unknown; although the majority of responses are being observed within the first 6 months, some responses occur rather late when compared to those following conventional therapy. Most trials investigated ICI until disease progression or intolerable toxicity; other trials allowed treatment for up to 2 years or longer. Some studies were amended to allow cessation of therapy in case of a prolonged PET-negative CR. Treatment duration should ideally be based on biomarkers and minimal residual disease diagnostics in future studies, taking into account both clinical and economic factors.

Evaluation of PD-L1 expression on tumor-cells as a predictive marker has been inconclusive so far, both in solid tumors and hematologic malignancies. This might be due to complex dynamics of expression depending on the tumor microenvironment and the lack of standardized immunohistochemistry.[87] Mutational load, leading to higher neo-antigen presentation, might be a potential biomarker in solid tumors,[88] but frequent mutations of MHC molecules in lymphomas suggest that neo-antigen presentation might still be inefficient, leading to a gradual loss of ICI efficacy.[89] Recently, emerging data that gut microbiota might interact with and have some impact on ICI response[90,91] suggest that probiotics or microbial transplantation could theoretically enhance the efficacy of ICI.

A more detailed understanding of the principle of action of PD-1/PD-L pathway blockade is indispensable in order to apply ICI efficiently and to develop combination treatments. One modality to improve ICI would be to combine this new approach with other immunological agents or conventional therapeutics. Studies combining anti-CTLA-4 and anti-PD-1 antibodies in melanoma and multiple myeloma showed promising results[81,92] and similar trials in lymphoma are underway. It has long been recognized that

chemotherapy has an immunomodulatory effect, e.g. by enhancing antigen availability and presentation by antigen-presenting cells.[93] Many agents efficient in lymphoma treatment such as cyclophosphamide or anthracyclines have known immunomodulatory effects and might be promising partners for ICI. In addition, combination strategies with other, non-cytotoxic targeted immunomodulatory agents could work synergistically and are currently under investigation. Most of these combination strategies to date include ibrutinib or idelalisib and have a strong translational rationale. On the other hand, clinical observation may also identify promising combinations: in a small study of eight r/r cHL patients, the high CR rate of 87.5% with nivolumab might in part have been due to prior exposure to azacitidine.[94] Exposure to hypomethylating agents seems to prime ICI, complementing preclinical data on its immunogenicity.[95] Pidilizumab, a humanized IgG1 antibody thought to target PD-1, showed interesting results in DLBCL and FL.[96,97] However, it has become clear that its mechanism of action is not checkpoint inhibition, but innate immune system activation, which needs further elucidation. A phase II clinical trial testing the efficacy of pidilizumab as consolidation treatment in stage III-IV DLBCL in first CR is underway, and it will be interesting to see – once its mechanism of action is clarified – whether this antibody represents another platform for possible combinations with ICI.

Immunogenic effects of radiotherapy, one of the most effective monotherapies in lymphoma treatment, are also well recognized.[98] Local effects of direct DNA damage and cellular stress can translate into a systemic boost of effica-

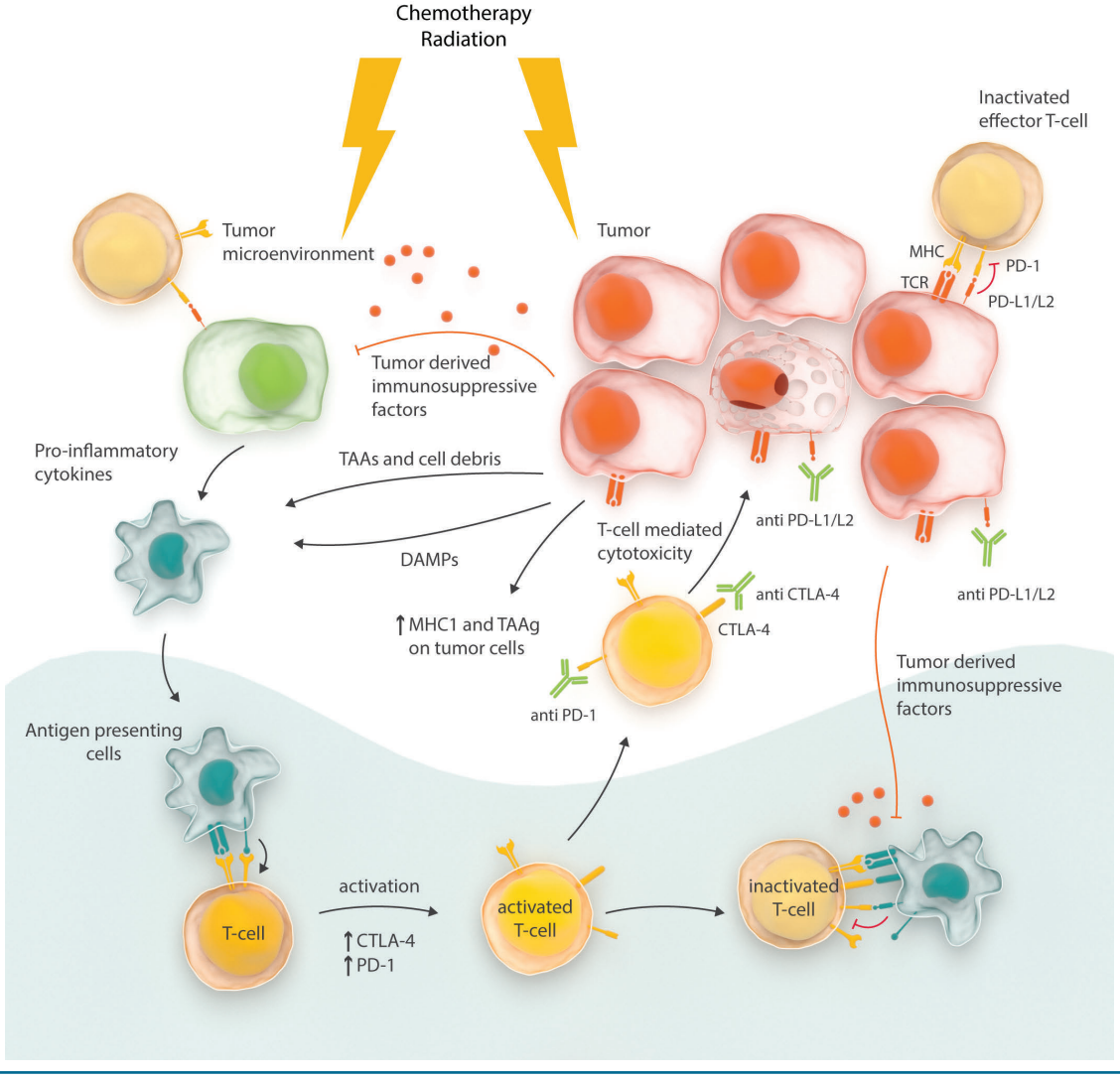

Figure 3. Schematic depiction of synergistic effects of ICI and radiotherapy or chemotherapy. Tumors are able to model the tumor microenvironment (TME) as well as the systemic immune system by production of immunosuppressive factors, thus evading the host immune response and assuring their survival. Chemotherapy and ionizing radiation induce immunogenic tumor-cell death by multiple mechanisms. Expression of major histocompatibility complex I (MHC-I) molecules, presenting tumor-associated antigens (TAA), is up-regulated in tumor cells. The release of TAA and danger-associated molecular patterns (DAMP) in TME stimulates dendritic cell (DC) activation. At the same time, DC activation is additionally enhanced by a newly established pro-inflammatory milieu in TME caused by direct effects of chemotherapy and/or radiotherapy. Activated and mature DC provide co-stimulatory signals to naïve T cells in draining lymph nodes, enabling priming of tumor-specific T cells. Addition of immune checkpoint inhibitors synergistically facilitates activation of T cells and T-cell-mediated anti-tumor cytotoxicity, overcoming inhibitory effects caused by tumor-derived immunosuppressive factors.

cy. The response to immune-activating chemokines and cytokines caused by radiation initiates further innate and adoptive immune responses. This systemic immune response even induces regression of non-radiated lesions, a phenomenon which is often termed the "abscopal effect", presenting a potential platform for combination with ICI. Radiation dose, fractioning and timing as well as safety and efficacy of such combinations are yet to be determined in future clinical trials. In addition to these effects, low-dose total-body irradiation causes transient lymphopenia, with subsequent lymphoid reconstitution and stimulation of tumor-reactive effector T cells[99] – another possible setting in which to exploit T-cell activity enhancement by ICI. The schematic mechanism of how addition of chemotherapy or radiotherapy to ICI may bypass tumor-induced immunosuppression is depicted in Figure 3.

Ongoing preclinical and translational research including correlative analyses of ICI-based therapies will likely create the rationale for evaluation of further combination strategies potentially including adoptive T-cell therapy, oncolytic viruses, metabolic checkpoint blockade or BET inhibitors as well as foster more individualized treatment approaches e.g. with personalized vaccines. Carefully investigating potentially synergistic combinations by evaluating optimal timing, dosage and sequencing is crucial in order to achieve optimal effects and to avoid unprecedented increased toxicity.

Summary

ICI has shown promising activity in r/r cHL, DLBCL and FL, and to some extent also in other lymphoid malignancies. Evidence from preclinical data and clinical trials investigating ICI is emerging, but major issues such as timing and sequencing, treatment duration and synergistic combinatory approaches remain to be resolved. Furthermore, long-term efficacy outcomes and potential development of late toxicities in lymphoma patients are still poorly defined. With its recent approval from the Food and Drug Administration for use in r/r cHL, nivolumab, a first antibody directed against PD-1 has already made its way into standard treatment. Other promising antibodies and ICI-based combination strategies are under investigation to develop efficient and well-tolerated treatments in various disease settings. This new treatment modality is set to reduce late effects of conventional chemotherapy and radiotherapy, potentially leading to less early and late toxicities and an improved quality of life. The emerging role of immunotherapy in lymphoma requires an "out of the box" way of thinking about antineoplastic therapy, redefining treatment outcomes and response assessment, but also raises financial issues regarding prolonged therapies. Results of ongoing trials and collaborative translational research in the field of lymphoma are, therefore, eagerly awaited.

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