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Targeting Immune Checkpoints in Hematologic Malignancies

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Abstract—The use of antibodies that target immune checkpoint molecules on the surface of T-lymphocytes and/or tumor cells has revolutionized our approach to cancer therapy. Cytotoxic-T-lymphocyte antigen (CTLA-4) and programmed cell death protein 1 (PD-1) are the two most commonly targeted immune checkpoint molecules. Although the role of antibodies that target CTLA-4 and PD-1 has been established in solid tumor malignancies and Food and Drug Administration approved for melanoma and non-small cell lung cancer, there remains a desperate need to incorporate immune checkpoint inhibition in hematologic malignancies. Unlike solid tumors, a number of considerations must be addressed to appropriately employ immune checkpoint inhibition in hematologic malignancies. For example, hematologic malignancies frequently obliterate the bone marrow and lymph nodes, which are critical immune organs that must be restored

for appropriate response to immune checkpoint inhibition. On the other hand, hematologic malignancies are the quintessential immune responsive tumor type, as proven by the success of allogeneic stem cell transplantation (allo-SCT) in hematologic malignancies. Also, sharing an immune cell lineage, malignant hematologic cells often express immune checkpoint molecules that are absent in solid tumor cells, thereby offering direct targets for immune checkpoint inhibition. A number of clinical trials have demonstrated the potential for immune checkpoint inhibition in hematologic malignancies before and after allo-SCT. The ongoing clinical studies and complimentary immune correlatives are providing a growing body of knowledge regarding the role of immune checkpoint inhibition in hematologic malignancies, which will likely become part of the standard of care for hematologic malignancies.

I. Introduction

Targeting immune checkpoint molecules on the surface of tumor cells or immune cells has proven to be a highly effective approach in cancer immunotherapy. A number of clinical trials in a variety of tumor types have

been conducted using antibodies that target immune checkpoint molecules. Although there are several immune checkpoint pathways that regulate immune cells, to date, the two major approaches to immune checkpoint blockade that have been investigated clinically have targeted cytotoxic-T-lymphocyte antigen (CTLA-4)

ABBREVIATIONS: allo, allogeneic; AML, acute myeloid leukemia; APC, antigen presenting cell; CAR, chimeric antigen receptor; CR, complete response; CTLA-4, cytotoxic-T-lymphocyte antigen; DLBCL, diffuse large B cell lymphoma; FL, follicular lymphoma; GVHD, graft versus host disease; GVL, graft versus leukemia; HL, Hodgkin's lymphoma; MDS, myelodysplastic syndrome; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; NSCLC, non-small cell lung cancer; PD-1, programmed cell death protein 1; PFS, progression-free survival; pHLA, peptide/human leukocyte antigen complex; PR, partial response; SCT, stem cell transplantation; TCR, T cell receptor; TIL, tumor infiltrating lymphocytes; TReg, regulatory T cells.

and the programmed cell death pathway. The programmed cell death pathway includes programmed cell death protein 1 (PD-1) and its ligands programmed death ligands 1 (PD-L1) and 2 (PD-L2). To date, melanoma and non-small cell lung cancer (NSCLC) are the two tumor types for which the use of immune checkpoint inhibition has received Food and Drug Administration approval. However, there is great interest in investigating these agents in hematologic malignancies, which are known to express immune checkpoint molecules and to be susceptible to immune modulation. In addition, there is a desperate need for novel agents to treat a number of hematologic malignancies, because these remain some of the most aggressive tumors to afflict adults and children. This review will provide an update on the current state of immune checkpoint based approaches in the treatment of hematologic malignancies, including stem cell transplantation.

II. T Cell Inhibitory Pathways: Cytotoxic-T-lymphocyte Antigen 4, Programmed Death Protein 1, and Programmed Death Protein Ligand 1

Upon initial encounter with its antigen in a lymphoid organ, there are a number of signaling pathways that must be triggered within the T cell to achieve adequate activation. T cells require binding of their T cell receptor (TCR) to the peptide/human leukocyte antigen complex (pHLA) that is expressed on the target, as well as binding of the T cell costimulatory receptors to their cognate ligands that are expressed by the tumor or antigen presenting cell (APC). CD28 is an important costimulatory molecule expressed on the T cell surface. There are two known ligands for CD28, CD80 (B7.1) and CD86 (B7.2), both expressed on APCs. CD80 and CD86 are also ligands for CTLA-4, an inhibitory molecule expressed on the T cell surface. CTLA-4 binds with a higher affinity to CD80 and CD86 on the APCs, and in effect competes with CD28 for binding to these molecules (Linsley et al., 1994; Leach et al., 1996; Egen and Allison, 2002; Riley et al., 2002; Schneider et al., 2006). In addition, CTLA-4 activates phosphatases such as Src-homology 2 domain-containing phosphatase 2, which counteract the phosphorylation steps that ensue after TCR binding to pHLA and are critical for T cell activation (Rudd et al., 2009). CTLA-4 is expressed by CD8⁺ and CD4⁺ T cells; however, the effects of CTLA-4 are primarily seen in the CD4⁺ T cell population,

including helper T cells and regulatory T cells (T_{Reg}). Engagement of CTLA-4 with its ligands results in the downregulation of helper T cell activities and upregulation of T_{Reg} cell activities. Together, competition for binding with CD80 and CD86, the attenuation of helper T cell functions, and the enhancement of T_{Reg} activities result in a “break” on effector T cell activation that is critical for controlling the immune response and maintaining normal immune homeostasis.

Although CTLA-4 plays a major role in regulating the initial stages of T cell activation, another T cell inhibitory mechanism, PD-1, plays a critical role in abrogating T cell functions during the later stages of the immune response (Nishimura et al., 1999; Freeman et al., 2000; Nishimura et al., 2001). The PD-1 pathway, which involves the T cell inhibitory molecule PD-1 and its ligands PD-L1/PD-L2, modulates the immune response after T cells exit the circulation and home into inflamed and tumor tissues. This mechanism regulates and contains the immune response to prevent tissue damage and autoimmunity that can be deleterious to the host. PD-1 and PD-L1/PD-L2 therefore play an important role in peripheral tolerance. Like CTLA-4, signaling through PD-1 affects phosphatases like Src-homology 2 domain-containing phosphatase 2, which offset the activity of the kinases that mediate T cell activation after TCR/pHLA engagement and CD28 activation (Freeman et al., 2000; Yokosuka et al., 2012). PD-1 signaling also promotes T_{Reg} proliferation and immune suppressive functions (Francisco et al., 2009).

III. Targeting Immune Checkpoint Molecules in Cancer

A number of antibodies that block the interaction between immune checkpoint receptors on T cells and their ligands on tumor cells have been developed and have proven to be efficacious in the setting of solid tumor. Several of these are currently being evaluated in hematologic malignancies (Table 1). The rationale for a therapeutic strategy employing antibodies that target immune checkpoint molecules stems from the concept that impeding the interaction between the immune checkpoint receptor on the T cell and its ligand on the tumor cell releases the inhibitory brakes that abrogate T cells functions and antitumor immune response. There are a number of critical issues to be considered when employing immune checkpoint blockade in cancer immunotherapy. The first is that T cells must be

G.A. is supported by a grant from the Leukemia & Lymphoma Society. N.D. is supported by grants from the Ladies Leukemia League and the Anderson Cancer Center Leukemia SPORE. E.A.M. is an R. Lee Clark Fellow of the University of Texas Anderson Cancer Center supported by the Jeanne F. Shelby Scholarship Fund.

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dx.doi.org/10.1124/pr.116.012682.

TABLE 1
Immune checkpoint antibodies used in hematologic malignancies

	Ipilimumab	Nivolumab	Pembrolizumab	Pidilizumab	Atezolizumab
Target	CTLA4	PD1	PD1	PD1	PD-L1
Class	Humanized IgG1	Human IgG4	Humanized IgG4	Humanized IgG1	Humanized IgG1
FDA approved indication	Unresectable or metastatic melanoma or in the adjuvant setting	Unresectable or metastatic melanoma; metastatic NSCLC; advanced renal cell carcinoma	Unresectable or metastatic melanoma; metastatic NSCLC	None at this time. Promising data in DLBCL and FL	None at this time. Ongoing trial in MM
Pharmaceutical	Bristol-Myers Squibb	Bristol-Myers Squibb	Merck	Cure Tech/Medivation	Genentech/Roche

DLBCL, diffuse B cell lymphoma; FL, follicular lymphoma; MM, multiple myeloma; NSCLC, non-small cell lung cancer.

present within the tumor microenvironment. This is indeed a critical consideration, because it may dictate the timing of administration of the immune checkpoint blockade in relation to other systemic cancer therapies. The majority of systemic cancer therapies is lymphodepleting and can affect the number of lymphocytes within the tumor microenvironment.

The second consideration is that the T cells within the tumor microenvironment need to possess specificity to distinct antigens expressed by tumor cells. The characteristics of the antigens targeted by tumor infiltrating lymphocytes (TIL) have been heavily investigated. Antigens expressed by tumor cells generally fall into two broad categories: 1) mutated antigens that oftentimes account for neoantigens or 2) tumor-associated antigens that are routinely expressed by normal tissues but are differentially expressed by the tumor. In one of the original studies using the anti-CTLA-4 antibody ipilimumab, the response in patients with melanoma directly correlated with a higher number of neoantigens in tumors with a higher mutational load (Snyder et al., 2014). This was confirmed in NSCLC studies where PD-1 blockade with pembrolizumab was used. In that setting, the mutational and neoantigen load, as well as the detection of neoantigen-specific TILs, highly correlated with response to pembrolizumab (Rizvi et al., 2015; McGranahan et al., 2016).

The third consideration is the expression of immune checkpoint receptors on TIL and the presence of their cognate ligands on tumor cells or other immune cells within the tumor microenvironment. A number of studies have demonstrated better efficacy with immune checkpoint blockade in patients who have high levels of CTLA-4 and PD-1 on TIL and high expression of CTLA-4 and PD-L1 ligands on the tumor cells (Taube et al., 2014; Van Allen et al., 2015; McGranahan et al., 2016). However, clinical data have also demonstrated the efficacy of immune checkpoint inhibitors in tumors that have a lower expression of immune checkpoint molecules. For example, a clinical trial testing the efficacy of nivolumab, ipilimumab, and the combination in patients with untreated melanoma, demonstrated clinical response to immune checkpoint blockade even among patients with tumors that expressed a low level of PD-L1, although the response rate was higher in

patients with higher baseline PD-L1 expression (Larkin et al., 2015). Similar results have been observed in a clinical trial in patients with NSCLC (Garon et al., 2015). Although this remains an area of active investigation, the inconsistencies in responses to checkpoint blockade, based on the expression of the immune checkpoint molecules, may be attributable to heterogeneity in the tumor that is not adequately reflected by tumor sampling or to other components of the tumor microenvironment that regulate response to immune checkpoint blockade. These data suggest that the presence of PD-L1 expression may not be an accurate biomarker of response to therapy and many trials no longer use tumor PD-L1 expression as an eligibility criterion.

IV. Immune Checkpoint Inhibition in Hematologic Tumors

There are a number of factors to be considered with the use of immune checkpoint blockade in the treatment of patients with hematologic malignancies, including leukemia, lymphoma, and multiple myeloma (MM). Because they share a common cell lineage, malignant hematologic tumor cells often express markers typically associated with antigen presenting cells, specifically CD80 and CD86, hence making them direct targets for antibodies against CTLA-4. This is different from non-hematologic malignancies wherein CTLA-4 targeting is aimed at removing tolerance to the immune priming events that occur within the lymphoid organs. In addition, because they originate and reside within lymphoid organs, either the bone marrow or lymph nodes, hematologic malignancies could be more susceptible to regulation by targeting CTLA-4 (Fig. 1). On the other hand, the timing of the application of immune checkpoint blockade may be more critical in the setting of hematologic malignancies, especially leukemia, where the tumor itself oftentimes obliterates host immunity. Although the clinical application of immune checkpoint blockade for hematologic malignancies is clearly lagging behind its use in solid tumors, a number of studies have demonstrated encouraging results with immune checkpoint inhibition in hematologic malignancies.

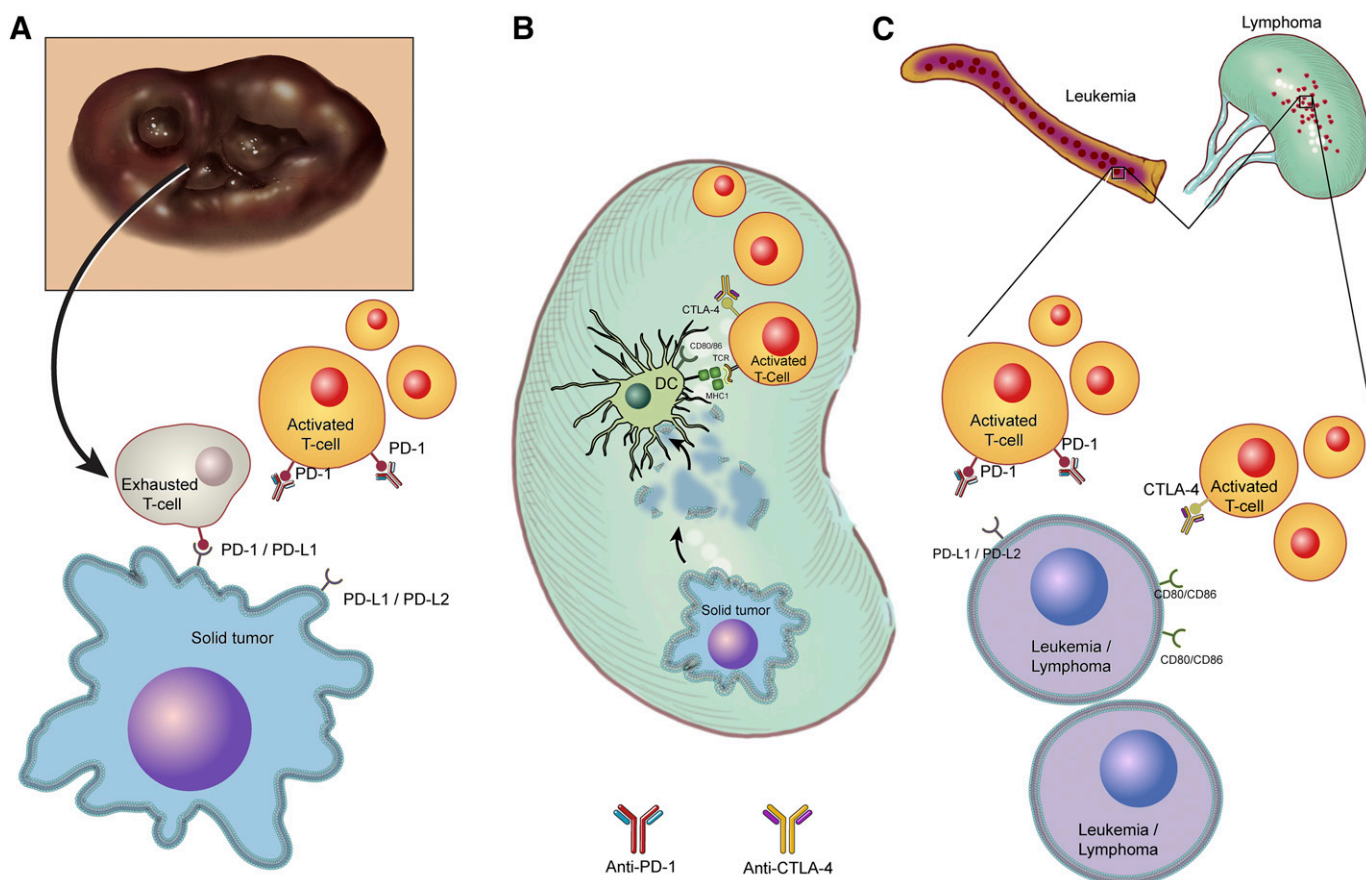


Fig. 1. Malignant hematologic cells express ligand for CTLA-4 and PD-1 and are therefore direct targets for immune checkpoint blockade. (A) Malignant solid tumor cells express PD-L1 and PD-L2 and can attenuate the antitumor immune response through direct interactions with PD-1 on the tumor specific T cells. Immune checkpoint blockade in solid tumor malignancies that interferes with the PD-1/PD-L1 pathway directly removes this inhibition. (B) Anti-CTLA-4 is effective in solid tumor malignancies through its influence on antigen presenting cells (APC) during immune priming. (C) Malignant hematologic cells express PD-L1 and PD-L2, but also express CTLA-4. Because malignant hematologic cells can act as APC, the use of anti-CTLA-4 antibodies in this setting therefore can directly modulate the immune response against the malignant hematologic cell.

A. Lymphoma

The success of anti-PD-1 therapy in Hodgkin's lymphoma (HL) has been the major achievement supporting the use of immune checkpoint blockade in hematologic malignancies. There is a compelling rationale for the use of anti-PD-1 therapy in HL. Firstly, chromosome 9 abnormalities, which contain the PD-L1 and PD-L2 gene loci, are often encountered in HL and lead to overexpression of these ligands (Green et al., 2010). Secondly, there is often a dense immune infiltrate surrounding Reed-Sternberg cells in HL, which if activated could theoretically eliminate the malignant cells. Thirdly, there is a known association between HL and Epstein-Barr virus, which is known to upregulate PD-L1 and PD-L2 (Green et al., 2012). In essence, these observations provided a strong justification for the use of immune checkpoint blockade targeting PD-1 in HL, which was subsequently validated in the clinical setting. In a phase I trial, 23 patients with HL who were heavily pretreated, including 78% who relapsed after autologous (auto) stem cell transplantation (SCT) and 78% who relapsed after therapy with anti-CD30

(brentuximab vedotin), were administered the anti-PD1 antibody nivolumab (Ansell et al., 2015b). Twenty patients (87%) achieved an objective response, including 17% achieving complete response (CR) and 70% partial response (PR). Three patients had stable disease. Tumor samples were available for 10 patients, all of whom demonstrated expression of PD-L1 and PD-L2. Similar results were reported in a phase Ib study using the anti-PD-1 antibody pembrolizumab (Moskowitz et al., 2014). In that study of 15 patients with classic HL, all previously treated with brentuximab vedotin, 3 patients (20%) achieved CR and 5 patients (33%) achieved PR; the overall response rate was 53%. Anti-CTLA-4 therapy with ipilimumab has also been evaluated in patients with HL after allogeneic (allo) SCT. In a study by Bashey et al. (2009), ipilimumab was administered to 29 patients with a variety of relapsed hematologic malignancies after allo-SCT, including 14 patients (48%) with HL. Of the patients with HL, four patients responded to ipilimumab: two achieved CR and two had disease stabilization.

There is also promise for using immune checkpoint blockade in non-Hodgkin lymphoma (NHL). PD-L1 is

TABLE 2
Summary of key studies of immune checkpoint inhibition according to disease type

Disease	Drug	Number of Patients	Results	Study Type	Reference
HL	Nivolumab	23	Confirmed safety and efficacy	Phase 1	Ansell et al., 2015a
HL	Pembrolizumab	15	Confirmed safety and efficacy	Phase 1b	Moskowitz et al., 2014
HL	Ipilimumab	29	Confirmed safety and efficacy	Phase 1	Bashey et al., 2009
NHL	Pidilizumab	17 total patients; DLBCL = 2; FL = 1	Confirmed safety and efficacy	Phase 1	Berger et al., 2008
FL	Pidilizumab	32	Confirmed safety and efficacy	Phase 2	Westin et al., 2014
NHL	Pidilizumab	66 total patients; DLBCL = 49; indolent B cell NHL = 13; mediastinal B cell lymphoma=4	Confirmed safety and efficacy	Phase 2	Armand et al., 2013
NHL	Ipilimumab	18 total patients; FL = 14; DLBCL = 3; MCL = 1	Confirmed safety and efficacy	Phase 1	Ansell et al., 2009
MM	Pidilizumab	17 total patients; MM = 1	Long term stable disease in MM patient	Phase 1	Berger et al., 2008
MM	Nivolumab	82 total patients; MM = 27	No response in the patients with MM	Phase 1	Lesokhin et al., 2014
MM	Pembrolizumab	34	Safety and efficacy	Phase 1	San Miguel et al., 2015
AML/MDS	Pidilizumab	17 total patients; AML = 8; MDS = 1	Minimal response in 1 AML patient	Phase 1	Berger et al., 2008

AML, acute myeloid leukemia; DLBCL, diffuse large B cell lymphoma; FL, follicular lymphoma; HL, Hodgkin's lymphoma; MCL, mantle cell lymphoma; MDS, myelodysplastic syndrome; NHL, non-Hodgkin's lymphoma.

expressed by subtypes of NHL (Green et al., 2010; Andorsky et al., 2011), and immune cell infiltrates in lymphoma tissue have been correlated with clinical outcomes (Lippman et al., 1990; Grogan and Miller, 1993; Ansell et al., 2001). Based on these observations, immune checkpoint blockade has been tested in NHL, with the most encouraging data in the setting of follicular lymphoma (FL) with the use of the anti-PD-1 antibody pidilizumab. In a phase I clinical trial that enrolled 17 patients with lymphoma and leukemia, including 4 patients with NHL [two diffuse large B cell lymphoma (DLBCL), 1 FL and 1 acute lymphocytic cell lymphoma], Berger et al. (2008) showed elimination of tumor masses in the FL patient after pidilizumab treatment. This observation led to a non-randomized, single center phase II clinical trial in 32 patients with relapsed rituximab-sensitive FL (Westin et al., 2014). In that trial, patients were treated with the combination of pidilizumab and rituximab. Results from the study showed safety of the combination of pidilizumab and rituximab and activity in 29 evaluable patients, which included 15 patients (52%) achieving CR and 4 (14%) achieving PR. Furthermore, the investigators identified immune gene signatures that could predict for response to therapy and showed that the frequency of pre-therapy PD-1 expressing effector T cells within the tumor correlated positively with both tumor response and progression-free survival (PFS). These signatures have not yet been validated. Nevertheless, the identified genes extend beyond immune checkpoint molecules, highlighting the complexity of modulating the antitumor immune response with checkpoint antibodies in NHL.

There has also been encouraging data with the use of pidilizumab in the setting of DLBCL after auto-SCT (Armand et al., 2013). In a phase II study, 66 patients

with NHL (49 patients with DLBCL, 4 patients with primary mediastinal B cell lymphoma and 13 patients with transformed indolent B cell NHL) were given pidilizumab within 3 months after auto-SCT. CT and PET scans documented CR in 31 patients (47%) and 45 patients (68%), respectively, before administration of pidilizumab. The overall response rate after pidilizumab treatment in the 35 eligible patients who had measurable disease after auto-SCT was 51% and the 16-month overall survival and PFS were 0.85 (90% CI, 0.74 to 0.92) and 0.72 (90% CI, 0.60 to 0.82), respectively, and were not affected by disease status at the time of administration of pidilizumab. The PFS in the study cohort compared favorably with the PFS of historical controls treated in the same institution, 0.52 (90% CI, 0.39 to 0.63). Unfortunately, the investigators did not have access to tumor tissue and therefore could not provide an analysis of PD-L1 or PD-L2 expression by the tumor cells, which is critical in NHL, because immune checkpoint molecules are not ubiquitously expressed by malignant NHL cells but are often restricted to subgroups of tumors (Green et al., 2010; Andorsky et al., 2011). The investigators did show an increase in the T cell memory subsets in the peripheral blood over the course of treatment and showed an increase in PD-L1 expression in subsets of immune cells in the peripheral blood; however, no clear patterns or correlations were identified.

A number of studies have shown expression of CD80 and CD86 by lymphoma cells, including DLBCL and FL, hence providing the rationale for targeting CTLA-4 in NHL (Dorfman et al., 1997; Tsukada et al., 1997; Chaperot et al., 1999). Promising results in NHL have been seen with the use of the anti-CTLA-4 antibody ipilimumab. In a phase I study of 18 patients with NHL, including 14 patients with FL, 3 with DLBCL and

1 with mantle cell lymphoma, clinical responses were seen in 3 patients, including PR in 1 patient with FL and CR in 1 patient with DLBCL (Ansell et al., 2009). A summary of the aforementioned studies is included in Table 2.

Furthermore, the investigators demonstrated an increase in T cell proliferation to recall antigens after ipilimumab therapy in five patients (31%). Other studies that investigated the use of ipilimumab in the lymphoma setting were conducted after allo-SCT and are discussed in more detail in the following sections. Ongoing clinical trials of immune checkpoint inhibition in lymphoma are listed in Table 3.

B. Multiple Myeloma

The importance of immunotherapy in MM is exemplified by the curative potential of allo-SCT in patients with MM. Despite the potential benefit of allo-SCT, the high risk of toxicity has limited its applicability in these patients (Mehta and Singhal, 1998; Bensinger et al., 2001; Bruno et al., 2007; Blade et al., 2010; Bjorkstrand et al., 2011; Roddie and Peggs, 2011). Antigen specific T cell clones that target MM cells have been identified after allo-SCT, again highlighting the immunogenicity of MM and the potential to target this disease by

immune modulating agents (Atanackovic et al., 2007; Tyler et al., 2013). Furthermore, studies have shown the expression of PD-L1 on MM cells and immune cells and expression of PD-1 on T and natural killer cells within the MM microenvironment (Gorgun et al., 2015; Ray et al., 2015). In addition, T cell exhaustion, primarily in the CD8⁺ T cell compartment, was demonstrated in patients with MM after autologous stem cell transplantation and correlated with disease relapse. Together, these data provide a rationale for targeting immune checkpoint molecules in patients with MM after auto-SCT (Chung et al., 2016).

However, to date, there is limited clinical data of immune checkpoint blockade in MM. In the phase I study of pidilizumab in 17 patients with various hematologic malignancies discussed in the previous section, there was one MM patient enrolled who demonstrated long-term stable disease after treatment (Berger et al., 2008). However, in an interim analysis of a phase I study that tested nivolumab in patients with relapsed or refractory lymphoid malignancies, there were no objective responses in any of the 27 MM patients included (Lesokhin et al., 2014). A number of hypotheses have been postulated to explain the discouraging results of immune checkpoint blockade in

TABLE 3
Select ongoing trials of immune checkpoint inhibition in Hodgkin and non-Hodgkin lymphomas

Data compiled from ClinicalTrials.gov (<https://clinicaltrials.gov>), 7/2016.

Type	Therapy	Primary Outcome	Inclusion	Clinicaltrials.gov Identifier
Phase 1	Nivolumab +/- ipilimumab or lirilumab	Safety	Relapsed/refractory NHL and HL	NCT01592370
Phase 1	Ipilimumab, nivolumab and brentuximab vedotin	Safety	Relapsed/refractory HL	NCT01896999
Phase 1	Ipilimumab or nivolumab post allo-SCT	Safety	Relapsed/refractory NHL and HL	NCT01822509
Phase 1	Ipilimumab post allo-SCT	GVHD and graft rejection	Relapsed/refractory NHL and HL	NCT00060372
Phase 1	Ipilimumab + lenalidomide post allo- or auto-SCT	Safety, including GVHD	NHL and HL	NCT01919619
Phase 1	Pembrolizumab	Safety and objective response rate	Relapsed/refractory NHL and HL	NCT01953692
Phase 1	Pembrolizumab + chemotherapy	Safety and TRM	Untreated DLBCL	NCT02541565
Phase 1	Pembrolizumab + dinaciclib	Safety	Relapsed/refractory NHL	NCT02684617
Phase 1/2	Nivolumab +/- Ipilimumab	Safety and response rate	Relapsed/refractory NHL and HL	NCT02304458
Phase 1/2	Nivolumab + brentuximab	Toxicity and response rate	Relapsed/refractory HL	NCT02572167
Phase 1/2	Nivolumab + urelumab	Toxicity and efficacy	Relapsed/refractory NHL	NCT02253992
Phase 1/2	Nivolumab + epacadostat	Toxicity, ORR and PFS	Relapsed/refractory NHL and HL	NCT02327078
Phase 1/2	Pembrolizumab + epacadostat	Safety and ORR	Relapsed/refractory NHL	NCT02178722
Phase 1/2	Pembrolizumab post CD19 CAR T cell therapy	Safety	Relapsed/refractory NHL	NCT02650999
Phase 2	Nivolumab + brentuximab	Metabolic response rate	Elderly with untreated HL	NCT02758717
Phase 2	Nivolumab post auto-SCT	Objective and overall response rates	Classic HL	NCT02181738
Phase 2	Pembrolizumab	Objective response rate	Relapsed/refractory primary mediastinal lymphoma or Richter syndrome	NCT02576990
Phase 2	Pembrolizumab	Efficacy	Recurrent CNS lymphoma	NCT02779101
Phase 2	Pembrolizumab	PFS	Relapsed/refractory T-cell NHL	NCT02535247
Phase 2	Pembrolizumab + rituximab	ORR	Follicular lymphoma	NCT02446457
Phase 2	Pembrolizumab + idelalisib or ibrutinib	Response rate	Low grade NHL	NCT02332980
Phase 2	Pembrolizumab post auto-SCT	PFS	Relapsed/refractory NHL and HL	NCT02362997
Phase 2	Pidilizumab	Response	Stage III-IV DLBCL in first remission	NCT02530125

allo-SCT, allogeneic stem cell transplantation; auto-SCT, autologous stem cell transplantation; CAR, chimeric antigen receptor; CNS, central nervous system; DLBCL, diffuse large b cell lymphoma; GVHD, graft versus host disease; HL, Hodgkin lymphoma; NHL, non-Hodgkin lymphoma; ORR, overall response rate; PFS, progression free survival; TRM, treatment related mortality.

MM. Clonal T cells have been shown to play an important role in the anti-MM immune response; however, these clonal T cells were shown to have low PD-1 expression (Suen et al., 2015). Another study demonstrated that clonal T cells in MM are not exhausted; rather they exhibit a telomere-independent senescent phenotype or senescence-associated secretory phenotype, which would not be expected to respond to immune checkpoint blockade (Suen et al., 2014). A summary of these studies is included in Table 2.

Nevertheless, despite this somewhat discouraging data, a recent study demonstrated a 76% objective response rate when pembrolizumab was combined with lenalidomide and low-dose dexamethasone for the treatment of patients ($n = 34$) with relapsed/refractory MM (San Miguel et al., 2015). There are currently a number of clinical trials ongoing evaluating checkpoint blockade strategies for MM (Table 4).

C. Leukemia

Even though the majority of clinical studies blocking PD-1 and CTLA-4 using humanized monoclonal antibodies have been conducted in solid tumors and lymphoma, PD-1 and CTLA-4 have also been shown to play a role in leukemia, graft versus leukemia (GVL) and graft versus host disease (GVHD) (Blazar et al., 1994, 1995, 1997; Fevery et al., 2007). Although CD80 and CD86 expression is not expected in solid tumors, both molecules have been detected in acute myeloid leukemia (AML), chronic myeloid leukemia, and myelodysplastic syndrome (MDS), owing to a common lineage shared by leukemia cells and APC, which naturally express CD80 and CD86 (Costello et al., 1998; Re et al., 2002; Vollmer et al., 2003; Whiteway et al., 2003; Graf et al., 2005; Yang et al., 2014). In addition, PD-L1 expression has also been detected in these malignancies and was shown to be associated with aggressive disease

(Mumprecht et al., 2009; Yang et al., 2014). Furthermore, PD-1⁺ T cells are significantly increased in the bone marrow of patients with relapsed AML compared with healthy adult donor bone marrow (Daver et al., 2016).

Previous studies have demonstrated an important role for blocking CTLA-4 in leukemia immunity. Fevery et al. (2007) showed that blocking CTLA-4 augmented the antileukemia immune response in a murine model. Similarly, blocking the PD-1/PD-L1 pathway using anti-PD-L1 antibody enhanced the graft versus leukemia response in murine models (Zhou et al., 2010; Koestner et al., 2011). The aforementioned studies correlating the expression of CTLA-4 and PD-1 ligands with poor outcomes in AML and the preclinical studies showing improved antileukemia activities after blocking CTLA-4 and the PD-1/PD-L1 pathway together support the potential role of immune checkpoint blockade in enhancing the antileukemia immunity.

Another interesting concept that is being explored in checkpoint-based therapies for AML and MDS is the ability of epigenetic therapy to modulate immune checkpoint molecule expression on TIL and tumor cells (Zhang et al., 2011; Wrangle et al., 2013). Azacytidine is an epigenetic drug that is approved by the Food and Drug Administration for the treatment of MDS and approved by the European Medical Agency for the treatment of MDS and elderly AML. Azacytidine upregulates PD-1 and PD-L1 in MDS/AML, and the upregulation of these genes may be associated with emergence of resistance to azacytidine and inferior overall survival (Yang et al., 2014). These data have resulted in clinical trials combining epigenetic therapy with PD-1/PDL-1 blockade to improve response rates and durability of response in AML and MDS (NCT02397720, NCT02530463).

TABLE 4
Ongoing trials of immune checkpoint blockade in multiple myeloma

Data compiled from ClinicalTrials.gov (<https://clinicaltrials.gov>) 7/2016.

Type	Therapy	Primary Outcome	Inclusion	Clinicaltrials.gov Identifier
Phase 1	Nivolumab, nivolumab + ipilumab, nivolumab + lirilumab	Toxicity	Relapsed/refractory lymphoma and MM	NCT01592370
Phase 1	Atezolizumab	Toxicity	Solid tumors, refractory lymphoma and MM	NCT01375842
Phase 1/1b	Ipilimumab or Nivolumab	Toxicity and dose-finding	Hematologic malignancies, including MM, after allo-SCT	NCT01822509
Phase 1/2	Pembrolizumab + pomalidomide + dexamethasone	Toxicity	Relapsed/refractory MM	NCT02289222
Phase 1/2	Pidilizumab + lenalidomide	Pidilizumab MTD ORR	Relapsed/refractory MM	NCT02077959
Phase 2	Pembrolizumab	Clinical response	MM after auto-SCT	NCT02331368
Phase 2	Pidilizumab + DC vaccine	Immunologic response	MM after auto-SCT	NCT01067287
Phase 2	Pembrolizumab	ORR	Residual MM	NCT02636010
Phase 3	Lenalidomide + dexamethasone +/- pembrolizumab	PFS	Newly diagnosed MM	NCT02579863
Phase 3	Pomalidomide + dexamethasone +/- pembrolizumab	PFS and OS	Relapsed/refractory MM	NCT02576977

MM, multiple myeloma; allo-SCT, allogeneic stem cell transplantation; CR, complete response; auto-SCT, autologous stem cell transplantation; MTD, maximal tolerated dose; ORR, overall response rate; PFS, progression free survival; overall survival.

However, the application of immune checkpoint blockade in the setting of leukemia is more challenging in comparison with solid tumors and lymphoma. One significant obstacle in leukemia is that the underlying disease abrogates, and at times may completely obliterate, the immune system. Also, in the case of acute leukemia, the tumor burden and the rate of tumor proliferation suggest that the disease may progress before the checkpoint antibodies have had sufficient time to activate an immune response, especially if these agents are given alone. The timing of checkpoint therapy administration and identification of ideal combinations is critical, and best results may be achieved in the maintenance setting when there is minimal residual disease and a fully competent immune system that can be manipulated with immune checkpoint blockade or when immune checkpoint agents are combined with potentially synergistic standard anti-leukemic therapy. Identification of immune-checkpoint pathways beyond PD-1/PDL-1 and CTLA-4 that dominate in AML may further guide the rational selection of specific antibodies for clinical trials. Clinically targetable checkpoint receptors including PD-1, OX40, and ICOS appear to be overexpressed in the bone marrows of patients with AML (Daver et al., 2016). These findings need to be validated in larger studies.

In a phase I study of pidilizumab in patients with various hematologic malignancies, which included eight patients with AML and one patient with MDS, minimal response was seen in one patient with AML that was manifested by a decrease in the blast percentage from 50% to 5% (Berger et al., 2008). Four deaths were reported in that study, all of which occurred in AML patients and were attributed to leukemia progression. A summary of these studies is included in Table 2. There are a number of clinical trials currently ongoing to test checkpoint antibodies as single agents and in combination with standard antileukemia therapies in newly diagnosed and relapsed leukemia, including AML and MDS, as well as maintenance in AML (Table 5).

D. Immune Checkpoint Inhibition after Stem Cell Transplantation: Timing Is Everything

Clinical trials of immune checkpoint blockade have been conducted after SCT with promising results. Effective immune reconstitution and the low disease burden that are characteristic after SCT provide an ideal setting to enhance the antileukemia/lymphoma immune response by eliminating the direct immunosuppressive effects of the tumor and by providing a microenvironment for the emergence of antigen specific cytotoxic T lymphocytes (CTL) (Guillaume et al., 1998; Molldrem et al., 2000; Atanackovic et al., 2007; Armand et al., 2013; Tyler et al., 2013; Chung et al., 2016). Studies in lymphoma after auto-SCT are discussed in previous sections and appear to be encouraging. However, immune checkpoint blockade in the allo-SCT setting carries the potential risk of flaring GVHD (Saha et al., 2013) and, as a result, there have been fewer clinical studies evaluating immune checkpoint blockade after allo-SCT. The precise timing of T cell reconstitution, including CD8⁺ T cells, CD4⁺ helper T cells, and T_{Reg}, within the first 2 years after allo-SCT has been correlated with promoting GVL activity or inciting GVHD (Dutt et al., 2007; Zheng et al., 2009; Alho et al., 2016). Moreover, although the antigens that drive GVL and GVHD are largely unknown, minor antigens that are common to leukemia and normal tissue have been shown to play a critical role in both processes, and oftentimes patients with GVHD show no evidence of disease. Therefore, it is possible that immune checkpoint blockade could eliminate the underlying leukemia, albeit at the risk of flaring GVHD. Lastly, most patients who receive allo-SCTs are placed on immunosuppressive medications to prevent GVHD for approximately 6 months after the allo-SCT. Together, the particulars of post-SCT immune reconstitution, the target antigens of GVL/GVHD, and the use of immunosuppressive medications after allo-SCT highlight the critical role of the timing in implementing immune checkpoint inhibitors after allo-SCT.

TABLE 5
Ongoing trials of immune checkpoint blockade in acute myeloid leukemia

Data were compiled from ClinicalTrials.gov (<https://clinicaltrials.gov>) 7/2016.

Type	Therapy	Primary Outcome	Inclusion	Clinicaltrials.gov Identifier
Phase 1	Ipilimumab	Acute GVHD graft rejection Autoimmune reaction	Solid tumors, lymphoma and leukemia, including relapsed/refractory AML after allo-SCT	NCT00060372
Phase 1	Ipilimumab	Toxicity and immunologic response	Relapsed/refractory AML or CMML or high-risk MDS	NCT01757639
Phase 1/1b	Ipilimumab or nivolumab	Toxicity and MTD	Relapsed leukemia, including AML, lymphoma and MM after allo-SCT	NCT01822509
Phase 2	Pidilizumab + DC vaccine	Toxicity	AML in CR before cell collection for DC generation	NCT01096602
Phase 2	Azacytidine + nivolumab	Response rate, overall survival	Relapsed AML and frontline elderly (>65 years) AML	NCT02397720
Phase 2	Idarubicin and cytarabine + nivolumab	Event-free survival	Induction in newly diagnosed AML <60 years	NCT02464657
Phase 2	Nivolumab	Recurrence-Free Survival	AML in remission	NCT02532231

allo-SCT, allogeneic stem cell transplantation; AML, acute myeloid leukemia; CMML, chronic myelomonocytic leukemia; CR, complete response; DC, dendritic cell; GVHD, graft versus host disease; MDS, myelodysplastic syndrome; MM, multiple myeloma.

The few reported clinical trials have proven the complexity of immune checkpoint inhibition and the GVL/GVHD balance after allo-SCT. In the study by Bashey et al. (2009), which enrolled 29 patients with lymphoid and myeloid malignancies, ipilimumab given within 125–2368 days (median = 366 days) after allo-SCT did not precipitate GVHD in any of the patients. As discussed in the previous sections, responses were noted in five patients, four of whom had HL and one NHL. There is currently an ongoing study at the Dana Farber Cancer Institute that is testing increasing doses of ipilimumab administered to patients with relapsed malignancy after allo-SCT. Results from this phase I/Ib study of 28 patients with relapsed lymphoid and myeloid malignancies after allo-SCT who received two different dose levels of ipilimumab (3 or 10 mg/kg) showed efficacy of immune checkpoint inhibition in the patients treated at the higher dose level (Davids et al., 2016). Interestingly, patients with extramedullary AML seemed to respond particularly well to the therapy. Of note, acute ($n = 1$) and chronic ($n = 3$) GVHD were observed during treatment at the 10 mg/kg dose level. In addition to blocking CTLA-4 with ipilimumab, there is one report that shows the safety of blocking PD-1 after allo-SCT. In a case report by Angenendt et al. (2016), one patient

with HD received nivolumab 19 months after allo-SCT without inciting GVHD, hence suggesting the possibility of using immune checkpoint blockade in the post-allo-SCT.

In contrast to these encouraging results suggesting the safety of immune checkpoint blockade after allo-SCT, other studies have confirmed the risk of GVHD after immune checkpoint inhibition. In the phase I trial by Berger et al. (2008), previously discussed, 4 of 17 patients who were treated with pidilizumab had received allo-SCT. One of the four patients had received pidilizumab 8 weeks after allo-SCT and subsequently experienced grade 4 GVHD of the gastrointestinal tract and died of persistent AML and GVHD. Because this patient already had evidence of skin GVHD at study entry, it was difficult for the investigators to determine whether the gastrointestinal GVHD was spontaneous or secondary to pidilizumab. Although these studies provide a compelling, nonetheless guarded, rationale to further evaluate immune checkpoint inhibition after allo-SCT, the major advance in this area should be to define the role of immune checkpoint inhibition in patients with evidence of disease after allo-SCT and to delineate the immune mechanisms that can be modulated by immune checkpoint inhibition to favor GVL over GVHD.

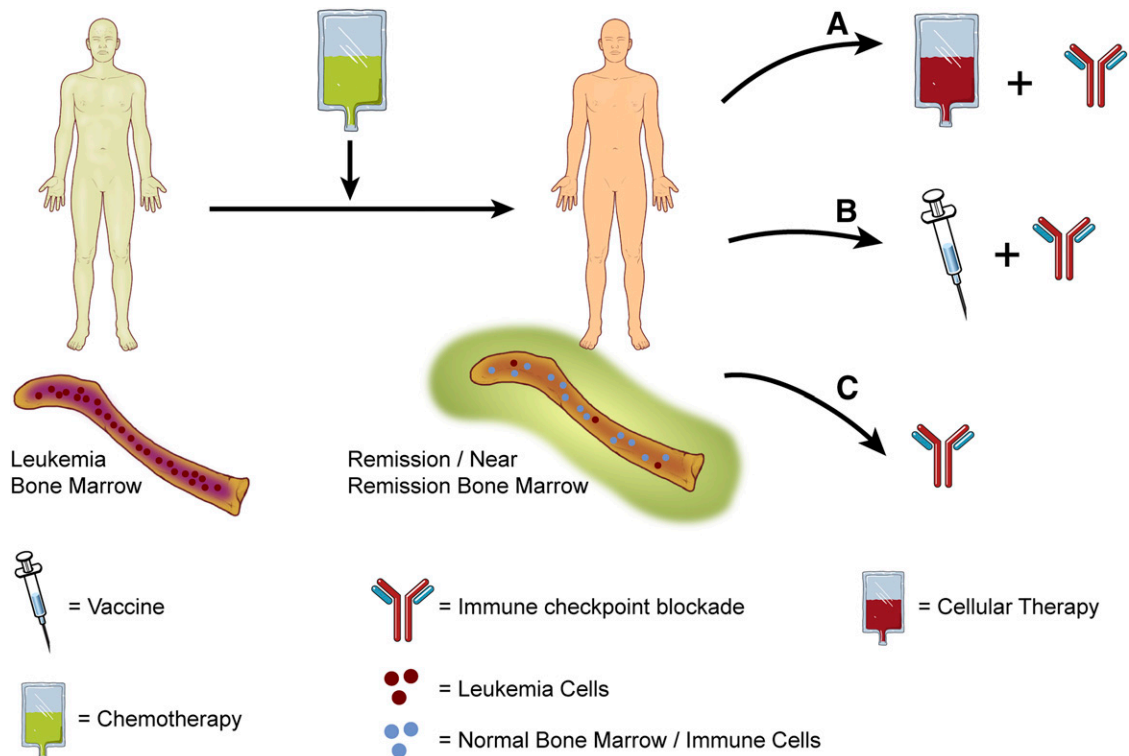


Fig. 2. The timing of the administration of immune checkpoint blockade is critical in determining treatment success in hematologic malignancies. The immune system is often attenuated in patients with active leukemia because of the accumulation of malignant cells in the bone marrow microenvironment. Before, or concomitant with, the administration of immune checkpoint inhibition, the underlying leukemia must be reduced to allow for some degree of immune reconstitution. One such approach includes the administration of immune checkpoint inhibitors to leukemia patients in remission or with a low leukemia burden. At this point, immune checkpoint inhibition can be administered (A) as an adjunct to cellular therapy, including stem cell transplantation, (B) in conjunction with vaccines, or (C) as a single agent.

E. Immune Checkpoint Inhibition in the Setting of Engineered T Cell Therapy

Chimeric antigen receptor (CAR) T cells made their debut clinically in the setting of hematologic malignancies. A CAR combines a single-chain variable fragment antigen-specific extracellular region from a monoclonal antibody fused to intracellular domains providing T cell activation (i.e., CD3- ζ) and costimulation (i.e., CD28, 4-1BB, or OX40). CAR T cells therefore combine the specificity of monoclonal antibodies with the effector functions of T cells. The CD19 CAR T cell is the quintessential example demonstrating the potential of this technology. The efficacy of CD19 CAR T cells was first shown in chronic lymphocytic leukemia (Porter et al., 2011) and recently in acute lymphoblastic leukemia (Grupp et al., 2013; Maude et al., 2015). CAR T cell therapy is rapidly advancing for the treatment of patients with hematologic malignancies (Porter et al., 2011, 2015; Grupp et al., 2013; Maude et al., 2015); however, there remains room for improving the efficacy and safety of CAR T cell therapy.

One approach that could further potentiate the activity of CAR T cells is to combine CAR T cells with immune checkpoint blockade. John et al. (2013) demonstrated the feasibility of this approach in a HER-2 transgenic mouse model. In that study, the combination of anti-HER-2 CAR T cells and anti-PD-1 therapy enhanced the efficacy of the CAR T cells against HER-2-overexpressing tumors. As expected, mice treated with CAR T cells and anti-PD-1 demonstrated higher antitumor activities, but additionally, there was a decrease in myeloid derived suppressor cells in tumors treated with anti-PD-1. The combination of immune checkpoint inhibition and CAR T cell therapy using antibodies or engineered T cells that have modified immune checkpoint receptors (Shin et al., 2012; Ankri et al., 2013) have yet to be tested in preclinical models of hematologic malignancies or in the clinical setting but may provide an essential synergy that could improve the outcomes beyond those seen with each individual therapy. Arguably, hematologic malignancies provide the ideal setting for this approach, because they are cured by immunotherapy, including allo-SCT, CAR T cells, and immune checkpoint inhibition, and the underlying disease itself causes major deficiencies in the immune system, suggesting that an approach that provides both an immune system and an immune modulatory drug may be more effective.

VI. Conclusion and Future Directions

Immune checkpoint inhibition for the treatment of cancer is undoubtedly a great breakthrough in cancer therapy (Couzin-Frankel, 2013; Dizon et al., 2016). The first clinical trial of immune checkpoint inhibition was conducted almost 15 years ago (Tchekmedyian et al.,

2002), and the differences these approaches have made in the therapy of previously untreatable solid tumors and hematologic malignancies have been striking. Through the application of immune checkpoint inhibition, we have learned much about cancer biology and the way tumors shape the immune response. As we gain a better understanding of the intricacies of the tumor microenvironment and the expression of immune checkpoint molecules by the tumor cells and the T cells, beyond CTLA-4 and PD-1 pathways, targeted clinical trials will be designed that take advantage of therapies that target immune checkpoint molecules combined with immune-based therapies, chemotherapies, vaccines, and small molecule targeting therapies (Fig. 2) to induce synergy with an intent to fully eradicate the underlying malignancy and provide long-lasting cures.

Acknowledgments

Figures were designed by David M. Aten, M.A. (MD Anderson Cancer Center).

Author Contributions:

Wrote or contributed to the writing of the manuscript: Alatrash, Mittendorf, and Daver.

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