Blocking tumor escape in hematologic malignancies: The anti-PD-1 strategy

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A R T I C L E   I N F O

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A B S T R A C T

Immunotherapy remains an important tool for treatment of hematologic malignancies. The Programmed Death-1 (PD-1) immune checkpoint pathway has emerged as a mechanism of tumor evasion from the anti-tumor immune response. The recent development of anti-PD-1 monoclonal antibodies has offered a targeted approach to cancer therapy. Several agents are in various stages of development and have shown clinical responses across a broad spectrum of both solid and hematologic malignancies. The use of anti-PD-1 therapy in hematologic malignancies is limited but has demonstrated clinical responses in relapsed/refractory disease following multiple lines of therapy. PD-1 blockade may reduce relapse rates for patients who fail to obtain a complete remission prior to autologous hematopoietic cell transplant. The role of the PD-1 pathway for tumor escape is reviewed. We explore the use of anti-PD-1 therapy in hematologic malignancies. The proposed mechanism of PD-1 blockade as a modulator of the innate and acquired immune response is considered. Finally, the challenges of anti-PD-1 therapy and the future direction of investigation in this area are reviewed.

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1. Introduction

The immune system plays an important role in the development of cancer [1,2]. The ability of the immune response to target malignant cells is best illustrated by treatments like hematopoietic cell transplantation and donor lymphocyte infusion where a graft versus leukemia/lymphoma effect has been well demonstrated [3,4]. Why immune targeting may fail can be explained by the concept of “tumor escape”, which results from direct inhibition of cytotoxic T-lymphocytes, thereby allowing malignant cells to evade the immune response [5–7]. The Programmed Death-1 (PD-1) pathway has emerged as an important mechanism for tumor escape. Exploration into the tumor microenvironment has uncovered key mechanisms that regulate the unchecked nature of tumor cell growth. Treatment strategies that block the PD-1 pathway are currently under development and recent clinical trials have shown clinical responses in a variety of malignancies including both solid and hematologic cancers. In this review, we will discuss the role of newly developed agents for PD-1 blockade in hematologic malignancies.

2. PD-1 pathway

Programmed Death-1 (PD-1) is a member of the B7 receptor family, which plays an important role in the regulation of the immune response. The PD-1 receptor is a 288 amino acid type I transmembrane protein that is part of the immunoglobulin superfamily [8,9]. The PD-1 receptor, in conjunction with receptor ligands PD-L1 and PD-L2, functions to regulate the immune response primarily by down regulating signals of the T-cell receptor (Fig. 1). The interaction of PD-1 and the receptor ligands induces processes resulting in apoptosis of activated T lymphocytes [10–13]. PD-1 is expressed on progenitor T-cells, activated T and B lymphocytes, natural killer cells, and myeloid cells. While PD-1 has broad expression across multiple immune cell types, the primary function of PD-1 is on effector/memory T lymphocytes resulting in regulation of T-cell activation and apoptotic pathways [8,14]. The mechanism of PD-1 regulation is related to its close proximity to the T-cell receptor (TCR) in activated T-cells. An increase in SHP-2, a cytoplasmic SH2 domain containing protein tyrosine phosphatase, is recruited to the cytoplasmic tail and interferes with the TCR signaling complex, thus blocking activation of the PI3K pathway and downstream activation of Akt. Blockade of PI3K results in a decrease in survival proteins, including Bcl-xL, a transmembrane mitochondrial molecule key to the intrinsic apoptotic pathway [8]. The end result of the PD-1 pathway is to function as a regulator of immune tolerance [15].

The role of PD-1 and its receptor ligands has been described in the regulation of immune defense mechanisms against microbes related to both acute and chronic infections. The PD-1 pathway is instrumental in controlling the balance of effective antimicrobial immune defenses against immune-mediated damage to host tissues. Chronic viral infections including HIV, hepatitis B, and hepatitis C have been studied with established alterations in the PD-1 pathway [16–19]. Chronic
infections with *Helicobacter pylori* have also been shown to utilize the PD-1 pathway in promoting T-cell suppression through regulation of effector/memory T-cells [20]. Additionally, parasitic worms utilize the PD-1 pathway to induce macrophages to produce immune suppression [21]. In inflammatory states like chronic infections, sustained expression of PD-1 and the receptor ligands results in T-cell exhaustion and immune escape. The resulting changes protect the host from an excessive immune response. There is ongoing investigation into PD-1 blockade for the treatment of chronic infectious diseases.

Similarly, tumors have adopted this mechanism in order to escape the anti-tumor activity of tumor-infiltrating lymphocytes that are present in the microenvironment [22]. The pathway has been demonstrated in a broad spectrum of solid malignancies including breast, colon, esophageal, lung, pancreatic, renal cell, and skin cancers. Furthermore, hematologic malignancies including lymphomas and leukemia have adopted the PD-1 pathway to mitigate anti-tumor immune response. The aberrant expression of PD-L1 has been demonstrated on tumor-infiltrating lymphocytes of lymphomas [23]. The result is T-cell exhaustion, resulting in inhibition of the anti-tumor response. The mechanism likely affects both the innate and the adaptive immune response. Innate immunity is demonstrated by upregulation of PD-L1 expression via amplified gene expression. Chromosomal abnormalities of 9p24.1 in Hodgkin lymphoma have been correlated with increased PD-L1 expression [24]. Adaptive immunity is demonstrated by upregulation of PD-L1 expression in response to inflammatory cytokines related to tumor development [25]. Together, tumors are protected from the destructive processes of a targeted immune response. Using a mouse animal model, researchers were able to show that by increasing expression of PD-L1 on tumor cells, one could induce resistance to previously established effective immunotherapies [26]. For tumors, the chronic antigen exposure creates persistently elevated levels of PD-1 expression resulting in exhaustion or anergy of antigen-specific T-cells.

### 3. Targeting the PD-1 pathway in hematologic malignancies

Expression of the PD-1 pathway markers has been demonstrated in multiple hematologic malignancies (Table 1). Plasmacytoma myeloma cells, but not normal plasma cells express PD-L1 [27]. PD-L1 is expressed on primary T-cell lymphomas including high expression particularly in anaplastic large T-cell lymphomas [28]. Nodular lymphocyte-predominant Hodgkin lymphomas have tumor-infiltrating T-cells that express PD-1 [29]. Interestingly, the PD-1 expressing cells form a rosette surrounding the tumor nodules within involved lymph nodes [28]. Both PD-1 and PD-L1 expression is found on T-cells of HTLV-1 mediated adult T-cell lymphoma and leukemia [30]. Marker expression related to the PD-1 pathway has been demonstrated in acute myeloid leukemia [31]. PD-L2 expression has been identified in primary mediastinal B-cell lymphoma, a specific subtype of diffuse large B-cell lymphoma [32]. Other B-cell non-Hodgkin lymphomas expressing PD-1 include small lymphocytic lymphoma, follicular lymphoma (FL), and diffuse large B-cell lymphoma (DLBCL) [33–35]. For T-cell non-Hodgkin lymphomas, PD-1 is restricted to angioimmunoblastic T-cell lymphoma [35]. PD-1 is expressed by tumor-infiltrating lymphocytes of the tumor microenvironment in several hematologic malignancies including follicular lymphoma, diffuse large B-cell lymphoma, and classical Hodgkin lymphoma [33,34,36].

Expression of PD-1 and the receptor ligands has proven to be a difficult marker for predicting prognosis. PD ligand expression including both PD-L1 and PD-L2 on malignant cells has been negatively correlated with prognosis for solid tumors [37]. For hematologic malignancies, particularly lymphomas, PD-1 expression as a prognostic marker is variable across lymphoma subtypes. The expression of PD-1 on tumor-infiltrating lymphocytes is associated with improved disease specific survival, progression free survival, and importantly overall survival in follicular lymphoma [33,38]. In follicular lymphoma, the type of tumor-infiltrating PD-1 positive T-cell subset, exhausted T-cells versus T follicular helper cells, may have differing influences on patient outcomes accounting for the discrepancies in previous clinical observations [39]. Early clinical data may support the use of soluble PD-L1 protein expression in the peripheral blood as a predictive biomarker in diffuse large B-cell lymphoma [40].

The expression of PD-1 and the receptor ligand, PD-L1, has drawn interest as potential therapeutic targets. Preclinical models for PD-1 pathway blockade have shown promising results in tumor responses across a broad spectrum of malignancies [41]. The proposed mechanism of PD-1 blockade as a therapeutic intervention is likely through either of two processes. The first proposed process is direct anti-tumor effect related to binding to PD-1 or PD-L1 receptors expressed on tumor cells. The varied receptor expression and inconsistent clinical responses

### Table 1

<table>
<thead>
<tr>
<th>Disease</th>
<th>Receptor(s)</th>
<th>Location(s)</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATL</td>
<td>PD-1</td>
<td>Tumor cells</td>
<td>[35]</td>
</tr>
<tr>
<td>ALC</td>
<td>PD-L1</td>
<td>Tumor cells</td>
<td>[28]</td>
</tr>
<tr>
<td>AML</td>
<td>PD-1</td>
<td>Tumor cells</td>
<td>[31]</td>
</tr>
<tr>
<td>ATL, HTLV-1 mediated</td>
<td>PD-1, PD-L1</td>
<td>Tumor cells</td>
<td>[30]</td>
</tr>
<tr>
<td>CLL/SLL</td>
<td>PD-1</td>
<td>Tumor cells</td>
<td>[35]</td>
</tr>
<tr>
<td>DLBCL</td>
<td>PD-1</td>
<td>Tumor cells</td>
<td>[34,35]</td>
</tr>
<tr>
<td>FL</td>
<td>PD-1</td>
<td>Tumor cells</td>
<td>[32,33]</td>
</tr>
<tr>
<td>Classical</td>
<td>PD-1</td>
<td>TMI</td>
<td>[36]</td>
</tr>
<tr>
<td>NLPbL</td>
<td>PD-1</td>
<td>TMI</td>
<td>[29]</td>
</tr>
<tr>
<td>PCM</td>
<td>PD-L1</td>
<td>Tumor cells</td>
<td>[27]</td>
</tr>
<tr>
<td>PMBCL</td>
<td>PD-L2</td>
<td>Tumor cells</td>
<td>[32]</td>
</tr>
</tbody>
</table>

**Legend:**
- **ATL:** angioimmunoblastic T-cell lymphoma
- **ALC:** anaplastic large T-cell lymphoma
- **AML:** acute myeloid leukemia
- **ATL:** adult T-cell leukemia/lymphoma
- **DLBCL:** diffuse large B-cell lymphoma
- **FL:** follicular lymphoma
- **HDL:** Hodgkin lymphoma
- **HTLV-1:** human T-lymphotropic virus-1
- **NLPbL:** nodular lymphocyte predominant Hodgkin lymphoma
- **PCM:** plasma cell myeloma
- **PMBCL:** primary mediastinal B-cell lymphoma
- **SLL:** small lymphocytic lymphoma
- **TMI:** tumor microenvironment

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related to receptor expression make a direct anti-tumor effect unlikely. The second hypothesis is anti-PD-1 therapy functioning as an immune checkpoint inhibitor thus enhancing anti-tumor effect. PD-1 blockade has been proposed to function as an enhancer of T-cell activity, to restore NK cell activity, and to induce PD-1 + B-cell antibody production. PD-1 blockade ultimately results in the reestablishment of the immune anti-tumor response. Correlative studies from recent clinical trials in FL and DLBCL have shown immune subset alterations with an increase in the absolute number of T-cells and PD-1 ligand bearing lymphocytes. The immune subset alterations support the proposed immune checkpoint inhibition as the therapeutic mechanism.

Several agents targeting the PD-1 pathway have been studied in the treatment of hematologic malignancies (Table 2). The agents are in various stages of investigation (Table 3). Each agent is discussed separately in the following sections with a review of the active clinical trials or available supporting clinical evidence.

3.1. Pidilizumab (CT-011)

Pidilizumab is a humanized IgG-1 kappa recombinant monoclonal antibody developed by CureTech Ltd. The development of pidilizumab was based on a murine antibody, mCT-011 or BAT, which was generated through the immunization of Balb/c mice with membranes of a human B-cell lymphoma cell line (Daudi). The PD-1 receptor is the target of pidilizumab with high specific activity to an epitope on several immune cells including activated T-cells, B-cells, NK-cells, and myeloid cells (CD14+ cells). In ex vivo studies, pidilizumab was found to block PD-1 activity with effects demonstrated on the CD4+ CD45RO+ effector/memory T lymphocytes. The binding of pidilizumab to the PD-1 receptor results in the induction of the PI3K signaling pathway. There is similar action demonstrated on NK cells via the PI3K pathway. In NK cells, the PI3K pathway is central to activation signaling and the effect on the NK cells augments the anti-tumor activity. The modulation of effector/memory T cells and NK cells results in the generation of tumor-specific memory cells and the enhancement of the anti-tumor immune response.

3.2. Clinical trials with pidilizumab

Three clinical trials have investigated the role of pidilizumab in hematologic malignancies. Each resulted in positive results with minimal side effects and no serious adverse events related to therapy. Pidilizumab was given as a single administration in escalating doses for the Phase I trial and two differing schedules for the subsequent Phase II trials. Therefore, no standard regimen has been established. The second Phase II trial used pidilizumab in combination with rituximab. Each trial will be discussed separately. Overall, pidilizumab is well tolerated with a limited side effect profile consistent with anti-PD-1 therapy in the treatment of solid malignancies (Table 4).

3.2.1. Phase I results with pidilizumab

A Phase I, open-labeled, dose-escalation trial was conducted in seventeen patients with a variety of hematologic malignancies. Enrolled patients included acute myeloid leukemia (8), non-Hodgkin lymphoma (4), chronic lymphocytic leukemia (3), Hodgkin lymphoma (10), and plasma cell myeloma (1). The non-Hodgkin lymphoma patients included acute lymphocytic cell lymphoma (ALCL), follicular lymphoma (FL), and two with diffuse large B-cell lymphoma (DLBCL). All patients had relapsed disease following several lines of conventional therapy and nine had previously undergone hematopoietic cell transplant. Pidilizumab was administered in escalating doses ranging from 0.2 to 6.0 mg/kg. The primary endpoint was safety, dose-limiting toxicity, and maximal tolerated dose.

No dose-limiting toxicity was reached nor was a maximum tolerated dose determined. Diarrhea was reported as the most common side effect as seen in two patients though symptoms were not felt related to pidilizumab. Four patients suffered severe adverse effects with death related to progression of AML. No clear toxicity profile emerged during the trial. Despite only a single administration of pidilizumab, clinical responses were observed in six patients. Clinical responses included a FL with complete remission, AML with minimal response, and stable disease in patients with CLL, HL, and MM. Correlative studies showed an increase in CD4+ lymphocytes in 15 of the 17 treated patients. The elevation of CD4+ lymphocytes persisted for up to 3 weeks following treatment with pidilizumab. The authors concluded that pidilizumab is a safe and well tolerated agent. The clinical responses were encouraging results and have prompted further investigation.

3.2.2. Phase II trial with pidilizumab in DLBCL following AHCT

A Phase II, international, open-labeled trial was conducted in patients with DLBCL, primary mediastinal B-cell lymphoma (PMBCL), and transformed indolent B-cell NHL following autologous hematopoietic cell transplant (AHCT). The rationale for the protocol was to utilize the reconstituted immune landscape following transplantation in hopes to break immune tolerance with PD-1 blockade and prevent disease relapse. The median age of patients was 57 with a majority, 74%, having DLBCL. Patients with chemotherapy sensitive disease obtaining at least partial remission proceeded to AHCT. A total of 72 patients met enrollment criteria and received at least one dose of pidilizumab. Only 66/72 patients completed all three cycles and were included in the treatment analysis. Treatment with pidilizumab was initiated within 30 to 90 days after AHCT. Pidilizumab 1.5 mg/kg was administered every 42 days for 3 cycles. Patients were followed with CT scans obtained at screening, prior to cycles two and three, and then at 30, 44, and 69 weeks from the initial pidilizumab dose. Additionally, patients had FDG-PET/CT imaging at the treating investigator’s discretion. The primary endpoint was progression-free survival at 16 months from the initial pidilizumab dose. The secondary objectives included safety and toxicity, progression free survival, and overall survival.

Based on FDG-PET/CT imaging, 24 patients (36%) had a positive scan prior to AHCT consistent with pre-transplant residual disease. This did not include 11 patients (17%) that did not undergo pre-transplant FDG-PET/CT imaging. Following AHCT, 35 patients (53%) had residual disease by CT imaging and 9 patients (18%) had a positive FDG-PET/CT scan. A total of 12 patients (14%) did not undergo FDG-PET/CT evaluations following AHCT. Outcomes were compared to historical 18-month PFS of 0.60 to 0.65 with a calculated 16-month PFS of 0.69 to be considered a positive study. The discrepancy between the planned 16-month follow-up accounts for the median 2-month period following AHCT prior to initiation of pidilizumab. The observed 16 month PFS was 0.72 (90% CI, 0.6 to 0.82) and met the primary endpoint. The OS was 0.85 (90% CI, 0.74 to 0.92). The authors reported a PFS of 0.52 for similar AHCT patients within their institution. For the 35 patients with residual disease following AHCT as defined by CT imaging, the overall response rate (ORR) was 51% including 12 (34%) with a complete response (CR)

Table 2

<table>
<thead>
<tr>
<th>Agent</th>
<th>Target</th>
<th>Isotype</th>
<th>Manufacturer(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pidilizumab (CT-011)</td>
<td>PD-1</td>
<td>IgG1</td>
<td>CureTech</td>
</tr>
<tr>
<td>Pembrolizumab (MK-4375)</td>
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<td>IgG4</td>
<td>Merck</td>
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<tr>
<td>Nivolumab</td>
<td>PD-1</td>
<td>IgG4</td>
<td>Bristol-Meyers Squibb</td>
</tr>
<tr>
<td>AMP-224</td>
<td>PD-1</td>
<td>IgG1</td>
<td>Amgen</td>
</tr>
<tr>
<td>AMP-514 (MEDI0680)</td>
<td>PD-1</td>
<td>NA</td>
<td>Amgen</td>
</tr>
<tr>
<td>MEDI3280A (RG7446)</td>
<td>PD-L1</td>
<td>IgG1*</td>
<td>Genentech &amp; Roche</td>
</tr>
<tr>
<td>MEDI4736</td>
<td>PD-L1</td>
<td>IgG*</td>
<td>MedImmune</td>
</tr>
</tbody>
</table>

NA: not available.

* Fusion protein with 87-D/C PD-L2.

* Optimized Fc region.
on-target effect. T-cell subsets is consistent with pre-clinical animal models. It is felt sustained throughout the 16 weeks following treatment. A significant increase in circulating CD8 + peripheral and central memory T-cells as of therapy. A statistically significant increase in the absolute number of PD-LI bearing activated T-cells was observed. The increase was measured when starting potential immune subset changes through therapy. The absolute increase in the associated genes by T-cells and natural killer cells in both the peripheral and central memory T-cells as well as CD4 + central memory cells was identified. The increase in the T-cell subsets is consistent with pre-clinical animal models. It is felt that the immune subset changes were consistent with a pidilizumab on-target effect.

**Table 3**

<table>
<thead>
<tr>
<th>Agent</th>
<th>ClinicalTrial.gov ID</th>
<th>Phase</th>
<th>Additional Therapy</th>
<th>Disease(s)</th>
<th>Trial status</th>
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<tr>
<td>Pidilizumab (CT-011)</td>
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<td>I</td>
<td>None</td>
<td>Heme malignancies</td>
<td>Complete</td>
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<td></td>
<td>NCT005312259</td>
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<td>None</td>
<td>DLBCL post AHCT</td>
<td>Complete</td>
</tr>
<tr>
<td></td>
<td>NCT00904722</td>
<td>II</td>
<td>+ rituximab</td>
<td>FL</td>
<td>Complete</td>
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<tr>
<td></td>
<td>NCT01067287</td>
<td>II</td>
<td>+/- DC myeloma vaccine</td>
<td>PCM</td>
<td>Recruiting</td>
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<tr>
<td></td>
<td>NCT01096602</td>
<td>II</td>
<td>+/- DC AML vaccine</td>
<td>AML</td>
<td>Recruiting</td>
</tr>
<tr>
<td></td>
<td>NCT02077959</td>
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<td>+ lenalidomide</td>
<td>PCM</td>
<td>Recruiting</td>
</tr>
<tr>
<td>Pembrolizumab (MK-4375)</td>
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<td>Heme malignancies</td>
<td>Recruiting</td>
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<tr>
<td></td>
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<td>+ lenalidomide</td>
<td>PCM</td>
<td>Recruiting</td>
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<tr>
<td>Nivolumab (BMS936558)</td>
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<td>+/- ipilimumab</td>
<td>PCM, CML, HL, NHL</td>
<td>Recruiting</td>
</tr>
<tr>
<td></td>
<td>NCT02011945</td>
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<td>+ dasatinib</td>
<td>CML</td>
<td>Recruiting</td>
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<tr>
<td></td>
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<td>None</td>
<td>DLBCL</td>
<td>Recruiting</td>
</tr>
<tr>
<td></td>
<td>NCT02038946</td>
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<td>FL</td>
<td>Recruiting</td>
</tr>
<tr>
<td>AMP-224</td>
<td>NCT01352884</td>
<td>I</td>
<td>None</td>
<td>CTCL</td>
<td>Active, not recruiting</td>
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<tr>
<td>AMP-514 (MEDI0680)</td>
<td>NCT02118337</td>
<td>I</td>
<td>None</td>
<td>Solid/Heme malignancies</td>
<td>Not yet recruiting</td>
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<tr>
<td></td>
<td>NCT01735842</td>
<td>I</td>
<td>None</td>
<td>Solid/Heme malignancies</td>
<td>Recruiting</td>
</tr>
<tr>
<td></td>
<td>NCT04736</td>
<td>I</td>
<td>None</td>
<td>MDS</td>
<td>Recruiting</td>
</tr>
</tbody>
</table>


and 4 (11%) with a partial response (PR). Patients with a positive FDG-PET/CT prior to AHCT have a poorer outcome and a single institution experience in 46 similar patients had an 18-month PFS of 0.52 (90% CI, 0.39 to 0.63) [48]. For the patient cohort with a positive FDG-PET/CT, the 16-month PFS was 0.72 (90% CI, 0.42 to 0.88). The improvement in PFS supports the hypothesis that PD-1 blockade may overcome the poorer prognostic implications of a positive FDG-PET/CT prior to AHCT; however, the study was not powered for this analysis.

A total of 613 adverse events were reported in the 72 enrolled patients. There were 135 adverse events attributed to pidilizumab. Neutropenia (19%) and thrombocytopenia (8%) were the most common adverse events. The most common adverse event was grade 1 anemia in 14 patients treated with pidilizumab and 24 h following the initial dose. Patients with stable disease could receive up to 8 additional cycles of pidilizumab on the same 28 day cycle for a total of 12 doses. Responses were defined by CT imaging and bone marrow biopsies. Assessments were performed at enrollment, following the second and fourth cycles of pidilizumab, and every 12 weeks through 2 yrs. FDG-PET/CT was used at the discretion of the treating investigator. Correlative studies included immune phenotyping on peripheral blood by flow cytometry and gene expression profiling on lymph node biopsies. Patients had both peripheral blood draws and lymph node biopsies prior to therapy and 14 days following the initial pidilizumab dose. The primary endpoint was the objective response rate defined as patients that received either complete or partial responses. The secondary endpoints included safety and toxicity, proportion of complete responses, proportion of partial responses, PFS, and correlative analysis to determine immunologic effects of therapy. The median number of pidilizumab treatments on protocol was 10 with a range of 1–12 and 29 patients (97%) completed the four infusions of rituximab as planned. The median follow-up was 15.4 months. Of the 29 patients evaluated, 19 (66%) achieved an objective response with 15 (52%) having a CR and 4 (14%) having a PR. The median time to response was 88 days with 6 patients (21%) having long duration response greater than 4 months following initiation of pidilizumab. The observed median PFS was 18.8 months though not reached for 19 patients with response to therapy. The PFS appears improved in comparison to the estimated median time to progression of 17.8 in patients treated with rituximab monotherapy. The observed PFS was 19.6 months for 25 patients that had tumor regression through therapy. Treatment was well tolerated with no reported grade 3 or 4 treatment related adverse events. The most common adverse event was grade 1 anemia in 14 patients and grade 1 fatigue in 13 patients. The most common grade 2 adverse event was respiratory infections seen in 5 patients. No deaths occurred during the trial period. Combination therapy with pidilizumab and rituximab was well tolerated and appears active in FL.

**Table 4**

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>Grade 1/2</th>
<th>Grade 3/4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatigue</td>
<td>16 (28%)</td>
<td>0</td>
</tr>
<tr>
<td>URI</td>
<td>20 (17%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>20 (13%)</td>
<td>1 (11%)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>16 (14%)</td>
<td>1 (13%)</td>
</tr>
<tr>
<td>Cough</td>
<td>16 (14%)</td>
<td>1 (13%)</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>15 (13%)</td>
<td>1 (13%)</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>14 (13%)</td>
<td>3 (3%)</td>
</tr>
</tbody>
</table>

URI: upper respiratory infection.

**3.2.3. Phase II trial with pidilizumab in FL**

A Phase II, open-label, non-randomized single institution trial was conducted in patients with FL [44]. Patients all had grades 1 to 2 relapsed FL following one to four lines of therapy. A total of 30 patients were enrolled and treated with a median age of 61. Patients were treated with combination pidilizumab and rituximab. Pidilizumab was administered at 3 mg/kg every 4 weeks for 4 cycles. Rituximab 375 mg/m² was given weekly for 4 doses starting 17 days following the initial pidilizumab dose. Patients with stable disease could receive up to 8 additional cycles of pidilizumab on the same 28 day cycle for a total of 12 doses. Responses were defined by CT imaging and bone marrow biopsies. Assessments were performed at enrollment, following the second and fourth cycles of pidilizumab, and every 12 weeks through 2 yrs. FDG-PET/CT was used at the discretion of the treating investigator. Correlative studies included immunophenotyping on peripheral blood by flow cytometry and gene expression profiling on lymph node biopsies. Patients had both peripheral blood draws and lymph node biopsies prior to therapy and 14 days following the initial pidilizumab dose. The primary endpoint was the objective response rate defined as patients that received either complete or partial responses. The secondary endpoints included safety and toxicity, proportion of complete responses, proportion of partial responses, PFS, and correlative analysis to determine immunologic effects of therapy. The median number of pidilizumab treatments on protocol was 10 with a range of 1–12 and 29 patients (97%) completed the four infusions of rituximab as planned. The median follow-up was 15.4 months. Of the 29 patients evaluated, 19 (66%) achieved an objective response with 15 (52%) having a CR and 4 (14%) having a PR. The median time to response was 88 days with 6 patients (21%) having long duration response greater than 4 months following initiation of pidilizumab. The observed median PFS was 18.8 months though not reached for 19 patients with response to therapy. The PFS appears improved in comparison to the estimated median time to progression of 17.8 in patients treated with rituximab monotherapy. The observed PFS was 19.6 months for 25 patients that had tumor regression through therapy. Treatment was well tolerated with no reported grade 3 or 4 treatment related adverse events. The most common adverse event was grade 1 anemia in 14 patients and grade 1 fatigue in 13 patients. The most common grade 2 adverse event was respiratory infections seen in 5 patients. No deaths occurred during the trial period. Combination therapy with pidilizumab and rituximab was well tolerated and appears active in FL.

Correlative studies revealed an increase in expression of activation-associated genes by T-cells and natural killer cells in both the peripheral blood and tumor microenvironment. An increase was also observed following pidilizumab in the absolute number of CD4 + effector and...
memory T-cells consistent with an anti-tumor immune response. The authors found that expression of PD-L1 in the peripheral blood was significantly higher for responders than non-responders; however, higher expression was not associated with PFS.

3.3. Clinical investigations with pidilizumab

CureTech is sponsoring three additional clinical trials investigating pidilizumab in hematologic malignancies. Two single institution trials will utilize a tumor specific dendritic cell fusion vaccine in combination with pidilizumab. The first is a Phase II, dual staged, non-randomized, open-label clinical trial for relapsed/refractory PCM (NCT10677287). The primary endpoints are assessment of immunological response post transplant and toxicity assessment. Secondary endpoints include assessment of cellular immunity with pidilizumab and further defining the anti-tumor effect. Patients are treated at six week intervals for three doses starting one to three months following AHCT. The dendritic cell fusion vaccine will be administered one week following each pidilizumab infusion. A second Phase II clinical trial will be conducted using pidilizumab in combination with a tumor specific dendritic cell fusion vaccine to AML (NCT01096602). The trial has a similar design and endpoints; however, pidilizumab will be given at four week intervals for three doses. The dendritic cell fusion vaccine will be administered two weeks after each pidilizumab dose. The final trial is a Phase I/II, open-label, multi-center, safety and efficacy study in relapsed/refractory PCM (NCT20779599). Pidilizumab will be used in combination with lenalidomide and dexamethasone for patients that have failed two prior therapies. Primary endpoints include maximal tolerated dose and overall response rates. Secondary endpoints include time to progression, overall survival, and correlative studies. Patients will receive lenalidomide daily on days 1–21 and pidilizumab on day 3 every 28 days until disease progression or unacceptable toxicity. All three trials are active and currently recruiting patients.

3.4. Pembrolizumab (formerly lambrolizumab; MK-3475)

Pembrolizumab is a humanized IgG-4 kappa isotype monoclonal antibody [49]. The drug was developed by Merck. The target for pembrolizumab is PD-1 receptor on human T-cells. The variable region sequences of a very-high-affinity mouse antihuman PD-1 antibody were grafted into a human IgG-4 immunoglobulin with a stabilizing S228P Fc alteration. The IgG-4 immunoglobulin subtype does not engage Fc receptors or activate complement, thus avoiding the cytotoxic effects of the antibody when binding to T-cells. The therapeutic effect on the PD-1 pathway is similar to the mechanism as described in the section on pidilizumab (3.1).

3.5. Clinical investigations with pembrolizumab

Merck is currently pursuing clinical trials with pembrolizumab in hematologic malignancies. Pembrolizumab has been studied in solid malignancies including melanoma, renal cell cancer, and lung cancer with promising results. Pembrolizumab is an emerging therapy for treatment of advanced melanoma with noted durable response rates of 38% in patients with prior exposure to immunotherapy including ipilimumab [49]. Common adverse effects were low grade and included rash, fatigue, pruritus, and diarrhea. There are no published data for the use in hematologic malignancies. An international Phase Ib trial is currently registered on clinicaltrials.gov for study of pembrolizumab in blood cancers (NCT01953692). Eligible patients are those with relapsed/refractory malignancies including myelodysplastic syndrome (MDS), plasma cell myeloma (PCM), nodular sclerosis or mixed cellularity Hodgkin lymphoma, primary mediastinal large B-cell lymphoma, or other non-Hodgkin lymphomas. For the non Hodgkin lymphoma patients, they must be ineligible for hematopoietic cell transplantation and demonstrate PD-L1 positivity on a lymph node biopsy. Pembrolizumab 10 mg/kg will be administered every 2 weeks until disease progression for up to 2 years. We await the results of this investigation. A second clinical trial recently opened to investigate combination pembrolizumab with lenalidomide and dexamethasone in patients with relapsed/refractory plasma cell myeloma (NCT02036502). This Phase I open-labeled, international trial is a safety and tolerability investigation with a primary endpoint of dose limiting toxicities. Pembrolizumab will be administered on days 1 and 15 of a 28 day cycle on an escalating dose schedule. The secondary endpoints will explore the anti-tumor effect with overall response rate, time to progression, duration of response, and other survival parameters. The trial is currently active and accruing patients.

3.6. Nivolumab (BMS936558)

Nivolumab is a fully humanized IgG-4 blocking monoclonal antibody. The drug was developed by Bristol Myers Squibb. The target for nivolumab is the PD-1 receptor on activated human T-cells and in vitro studies have demonstrated high-affinity binding [50]. Nivolumab binds specifically to PD-1 and not to related members of the CD28 family such as CD28, inducible co-stimulator (ICOS), cytotoxic T lymphocyte associated antigen-4 (CTLA-4), and B7 and T lymphocyte attenuator (BTLA). Blockade of the PD-1 pathway by nivolumab results in a reproducible enhancement of both proliferation and IFN-γ release in the mixed lymphocyte reaction (MLR). In vivo blockade of PD-1 by a murine analog of nivolumab enhances the anti-tumor immune response and results in tumor rejections and tumor growth delays in MC38 and SA1/N immuno-competent mouse tumor models [51].

3.7. Clinical investigations with nivolumab

The use of nivolumab has been previously studied in patients with renal cell carcinoma, lung cancer, and melanoma with objective responses in ~20% of patients [52]. The toxicity profile included grade 3 or 4 adverse effects in 14% of patients though overall the drug was well tolerated. Clinical responses were demonstrated in melanoma with a median overall survival of 16.8 months and 2 year survival rates of 43% that both compare favorably with available treatments for this patient population [53]. Bristol Myers Squibb is currently pursuing several clinical trials for hematologic malignancies registered on the clinicaltrials.gov including two Phase I trials. An initial Phase I, multi-institutional, safety and tolerability trial is underway (NCT01592370). The trial is a non-randomized, open-label investigation comparing nivolumab vs. combination therapy with nivolumab plus ipilimumab. Eligible patients include relapsed/refractory plasma cell myeloma, CML, Hodgkin lymphoma, and non-Hodgkin lymphomas. The trial has an initial dose escalated phase with nivolumab monotherapy at a starting dose of 1 mg/kg up to 3 mg/kg administered every 2 to 3 weeks for up to 48 to 96 weeks. The second arm will open with nivolumab 3 mg/kg and ipilimumab 1 or 3 mg/kg every 2 to 3 weeks for up to 48 to 96 weeks. The primary endpoint is safety and tolerability with multiple endpoints evaluating pharmacokinetics and anti-tumor activity. The other Phase IB international trial is investigating safety and efficacy for the combination of nivolumab with dasatinib in patients with CML (NCT02011945). Patients will have relapsed/refractory CML that has failed ≥ 2 tyrosine kinase inhibitors. The trial will include a dose-escalation phase for nivolumab starting at 0.3 mg/kg up to 3 mg/kg every 2 weeks for up to 2 years. The primary endpoints include safety and tolerability with secondary endpoints evaluating anti-tumor effect. Two Phase II trials are currently registered on clinicaltrials.gov for nivolumab in hematologic malignancies. The first is a single-arm, open-labeled, Phase II study of patients with relapsed/refractory DLBCL that failed AHCT or two prior standard regimens in patients that are transplant ineligible (NCT02038933). The primary endpoint is overall response rate (ORR) and secondary endpoints include further anti-tumor effects. Nivolumab will be administered at 3 mg/kg every
2 weeks until progression of disease. The second is a single-arm, open-labeled, Phase II study of patients with relapsed/refractory follicular lymphoma of all grades that have failed ≥2 prior standard regimens that included rituximab (NCT02038946). The primary endpoint is overall response rate (ORR) and secondary endpoints include duration of response and other anti-tumor effects. Nivolumab will be administered on the same schedule. Both international trials are currently attracting patients. We await the results of these investigations.

3.8. AMP-224

AMP-224 is a recombinant Fc-fusion protein fusing the extracellular domain of human B7-DC/PDL-2 to IgG1 currently under development [54]. The drug is a co-sponsored agent by GSK and Amplimmune. AMP-224 blocks the interaction between PD-1 and the receptor ligands, B7-DC (PD-L2) and B7-H1 (PD-L1 or CD274). AMP-224 functions to inhibit the activation of the PD-1 pathway. The pre-clinical models show a potential to block the inhibitory B7-DC/PD-1 interaction while engaging NK cells. The drug effect has been demonstrated as both single agent activity and further enhancement when given in combination with cyclophosphamide [55]. A similar effect was demonstrated when comparing AMP-224 with cyclophosphamide plus vaccine therapy [56].

3.9. Clinical investigations with AMP-224

AMP-224 is an early investigational agent that was recently evaluated in a closed Phase I trial of advanced cancers (NCT01352884). Enrolled patients included melanoma, solid tumors, and cutaneous T-cell lymphomas. Eligible patients had relapsed/refractory disease following prior standard treatment regimens. The trial was an open-labeled, multi-center, first in human evaluation of AMP-224 with an initial experimental arm evaluating an escalating dose schedule. Stage 2 further evaluated the safety, pharmacokinetics, and preliminary anti-tumor effects based on a recommended stage 1 maximal tolerated dose. Phase I results are pending. Of note, Amplimmune has a second anti-PD-1 agent, AMP-514, that will enter a Phase I, open-label safety and tolerability evaluation of patients with advanced malignancies (NCT02118337). Details of this agent are limited.

3.10. MPDL3280A (RG7446)

MPDL3280A is a humanized, Fc optimized, monoclonal antibody targeted against PD-L1 that has demonstrated immunomodulating and anti-tumor activity in pre-clinical models. The drug is co-sponsored by Genentech and Roche. The Fc region is modified to avoid induction of antibody-dependent cytotoxicity and complement-dependent cytotoxicity. MPDL3280A binds to PD-L1 thus blocking the PD-L1 receptor ligand binding to PD-1 resulting in T-cell mediated enhancement. Additionally, MPDL3280A prevents the binding of PD-L1 to the B7.1 receptor (CD80).

3.11. Clinical investigations with MPDL3280A

A Phase I, multicenter, first in human, open-labeled study is investigating MPDL3280A in patients with locally advanced or metastatic solid tumors (NCT01375842). Eligible patients include an unrestricted mix of both solid and hematologic malignancies but exclude those with CNS involvement. The primary endpoint is dose limiting toxicities with secondary endpoints further evaluating safety and tolerability. The dose and treatment schedule are not defined. The trial is currently active and recruiting patients.

3.12. MEDI4736

MEDI4736 is a humanized immunoglobulin G1 kappa (IgG1κ) monoclonal antibody targeted against PD-L1. The drug was developed by MedImmune LLC, a biologics research and development arm of AstraZeneca. The mechanism of action for MEDI4736 is PD-L1 binding resulting in T-cell mediated enhancement. MEDI4736 also blocks binding to the B7.1 receptor (CD80). The fragment crystallizable (Fc) domain contains a triple mutation in the constant domain of the IgG1 heavy chain that reduces binding to the complement component C1q and the Fcγ receptors responsible for mediating antibody dependent cell-mediated cytotoxicity (ADCC).

3.13. Clinical investigations with MEDI4736

Phase I results for MEDI4736 remain pending from the clinical trial NCT01693562. Patients with advanced solid tumors including non-small cell lung cancer, melanoma, hepatocellular carcinoma, squamous cell carcinoma of the head and neck, gastroesophageal, triple negative breast cancer, and pancreatic adenocarcinoma were treated with escalating doses. The maximal tolerated dose was established at 10 mg/kg IV given every two weeks. Preliminary reports include a low toxicity profile similar to other anti-PD-1 therapy. An actively recruiting Phase I, multicenter, open-label trial is underway investigating the safety, tolerability, pharmacokinetics, and anti-tumor activity in patients with relapsed/refractory myelodysplastic syndrome (NCT02117219).

4. Conclusions and future directions

The PD-1 pathway is a promising target for the treatment of hematologic malignancies. The concept of modulating an immune checkpoint pathway to re-establish the anti-tumor response has been well demonstrated in pre-clinical models. Results from clinical trials have demonstrated a clear therapeutic effect in solid tumors. More recent results have shown benefit in several lymphoma subtypes. The application of anti-PD-1 blockade is currently being expanded across a broad range of hematologic malignancies. The rapidly growing number of clinical trials using numerous anti-PD-1 agents will likely show clinical responses. The duration of response and influence on overall survival are yet to be determined.

The adaptation of anti-PD-1 therapies into standard treatment needs to be defined. The timing of initiating these therapies will be important once response to anti-PD-1 agents is established in relapsed/refractory disease. The ability to alter the host’s immune response to eradicate malignant cells is an attractive strategy as it provides a targeted effect with likely minimal side effects. Important questions, however, need to be explored: 1. Can PD-1 blockade be used prior to standard chemotherapy to improve response? 2. Can PD-1 blockade be used concurrently with standard chemotherapy to maintain response? 3. Can PD-1 blockade be used following standard chemotherapy to prolong remission or result in cure? 4. What is the role of PD-1 blockade in the setting of hematopoietic cell transplantation? Clearly, the adaption of these agents into treatment regimens is rich with opportunities.

Prior pre-clinical and available clinical research has investigated the proposed mechanism of PD-1 blockade. No clear biomarker has emerged to fully assess the effects of anti-PD-1 therapy. An important step in developing these therapies will be defining the mechanism of immune modulation. Given the broad range of tumor types, it is possible that the immune checkpoint modulation may exist through differing immune subsets. Important questions regarding immune subset changes with treatment are required to better clarify both the mechanism but also the potential to predict response to PD-1 blockade. The status of receptors present on both tumor cells or in the tumor microenvironment is worth continued exploration to better select disease specific application of the anti-PD-1 agents. A blanket application of these agents across a multitude of malignancies will only exhaust resources. Careful clinical trial design in the appropriate tumor types is crucial to proving the applicability of PD-1 blockade in the treatment of hematologic malignancies.
The strategy of PD-1 blockade has recently developed as a treatment for hematologic malignancies. The reviewed clinical trials, many Phase I, will provide important insight into the clinical benefit. The side effect profiles have been minimal and the anticipated safety and tolerability are expected to be acceptable. Though likely underpowered to confirm clinical effect, many of these trials will provide a first look into a treatment response for a broader range of tumor subtypes. More Phase II trials can be anticipated as PD-1 blockade continues to demonstrate anti-tumor effect. PD-1 blockade represents continued advancement in the future of cancer treatment.

Conflict of interest statement

None.

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Practice points:

- Several anti-PD-1 therapies are in various stages of development for the treatment of hematologic malignancies.
- PD-1 blockade is investigational for hematologic malignancies and the incorporation into standard therapy remains undetermined.
- Anti-PD-1 therapy should be considered for patients with relapsed/refractory hematologic malignancies.
- Patients should be referred for clinical trial enrollment using clinicaltrials.gov as a resource to initially screen patients for eligibility.

Research agenda:

- Active phase I safety and tolerability trials with anti-PD-1 agents will provide initial assessment of disease specific anti-tumor response.
- Active phase II trials with anti-PD-1 agents in relapsed/refractory hematologic malignancies.
- Active phase I and phase II trials combining anti-PD-1 agents with standard therapies to assess anti-tumor response.
- When determine to incorporate anti-PD-1 therapy with standard therapy to improve response rates and prolong remission.

References


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