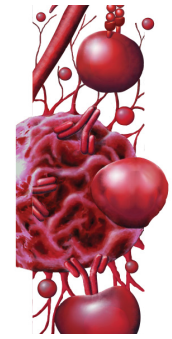
Review

Immunotherapy



Immune checkpoint blockade in human cancer therapy: lung cancer and hematologic malignancies

Tumor immune evasion is one of the hallmarks of cancer, and expression of the B7 family of immune checkpoints (PD-L1, PD-L2, B7-H3, B7x and HHLA2) is one mechanism of immune evasion by tumors to suppress T-cell function. Antibodies blocking these interactions of B7–1/B7–2/CTLA-4 and PD-L1/PD-L2/ PD-1 have had remarkable clinical success in several cancers and are less toxic than traditional chemotherapy. Even though only a small proportion of patients respond to checkpoint blockade, the duration of such responders due to immunological memory is remarkable and is longer than would be expected with any other agent in refractory disease. In this article, we review the therapeutic trials of blocking these pathways in human lung cancer and hematological malignancies.

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Immune checkpoints

Tumor immune evasion is considered one of the hallmarks of malignancy and represents an important step in the evolution of the tumor [1]. The immune system prevents and controls malignancies through immunosurveillance which has been broken down into a three-step process called immunoediting – elimination, equilibrium and escape [2]. Different cancers have different mechanisms to avoid immunosurveillance but one common mechanism is through expression of immune checkpoints. Cancer evolves through genetic and epigenetic instability which leads to failure of conventional chemotherapy and targeted therapy to driver mutations in most patients with metastases. Stimulation of T cells and utilizing T cells to treat cancer is attractive and has advantages: specificity of T cells to a particular antigen; T-cell memory which enables persistent clearance unlike drugs or chemotherapy; and adaptability – the T cell through somatic mutations can generate as many as 10^{15} TCRs (T-cell receptors) and hence can accommodate tumor heterogeneity and tumor evolution [3,4]. Neoantigenic epitopes arise due to somatic mutations in the cancer cells and these antigenic epitopes are normally not expressed in the human genome and play an important role in T-cell immune response. It has been shown that the nonsynonymous mutational burden in non-small-cell lung cancer (NSCLC) correlates with a higher neoantigen burden which in turn is associated with a better response to immune checkpoint blockade [5]. This shows that the genomic landscape of the tumors is important in T-cell response to tumors. In addition to the MHC-peptide/TCR signaling, T-cell-mediated adaptive immune response is regulated by positive costimulation and negative coinhibition from the interaction between the B7 family and their receptor CD28 family. Tumors

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hijack these mechanisms by delivering coinhibitory signals to T cells through expressing coinhibitory ligands on tumor cells such as PD-L1, PD-L2, B7-H3, B7x (B7-H4/B7S1) and HHLA2. The B7–1/B7–2/CTLA-4 and the PD-L1/PD-L2/PD-1 coinhibitory pathways are the furthest along in clinical development, and therapeutic blockade of these pathways has yielded dramatic clinical benefit in melanoma [6–8] and other tumors. Herein, we discuss the clinical trials of immune checkpoint inhibitors in lung cancer and in hematological malignancies.

Immune checkpoint blockade in lung cancer

Blocking antibodies against CTLA-4

Ipilimumab (MDX-010, Bristol-Myers Squibb, Princeton, NJ, USA) is a fully human monoclonal antibody of the IgG1 isotype which binds to CTLA-4. It was first approved based on a Phase III trial, which showed improved overall survival (OS) for the treatment of unresectable or metastatic melanoma in both treatment-naive and previously treated patients [6]. Preclinical models show that taxanes and platinum chemotherapy released tumor antigens and sensitized tumor cells to lymphocyte-mediated killing and hence ipilimumab was tested with this combination in lung cancer [9].

Ipilimumab was tested in a randomized Phase II study in treatment-naive NSCLC patient in combination with carboplatin and paclitaxel in 1:1:1 fashion [10]. Two different modes of administration of ipilimumab were used in the study. In Arm A (concurrent arm) patient received four doses of ipilimumab (10 mg/kg) plus paclitaxel and carboplatin (175 mg/m^2) followed by two doses of placebo plus paclitaxel and carboplatin. Arm B (phased arm) patients received two doses of placebo plus paclitaxel and carboplatin followed by four doses of ipilimumab plus paclitaxel and carboplatin. In the control arm, patient received up to six doses of placebo plus paclitaxel and carboplatin. The eligible patient continued ipilimumab or placebo every 12 weeks as maintenance therapy. This study utilized immune-related progression-free survival (irPFS) as the primary end point [11]. The study met its primary endpoint of irPFS with phased arm (HR: 0.72; p = 0.05), but not with concurrent arm (HR: 0.81; p = 0.13). The phased ipilimumab, concurrent ipilimumab and control arms were associated with a median irPFS of 5.7, 5.5 and 4.6 months, and a median OS of 12.2, 9.7 and 8.3 months, respectively (Table 1) [6]. The rate of grade 3/4 immune-related adverse events was high in the concurrent arm at 20 vs 15% in the phased arm. A nonpreplanned subgroup analysis based on histology showed improved HR of 0.55 for squamous vs 0.82 for nonsquamous (NSCLC) histology. The reason why the phased treatment was superior to concurrent approach was not entirely clear. One hypothesis is that the chemotherapy phased prior to immunotherapy may facilitate immunogenic 'cell death' and lead to improved T-cell priming and better immune responses.

Based on the above study, two Phase III trials are being conducted: NCT01285609, in which patients with squamous NSCLC will be randomized in a 1:1 fashion to carboplatin/paclitaxel/ipilimumab versus carboplatin/paclitaxel alone. The ipilimumab will be given in a phased fashion which will be after two doses of carboplatin and paclitaxel; NCT01450761 being conducted in small-cell lung cancer patients in which patients will be randomized in 1:1 fashion to carboplatin/etoposide/ipilimumab versus carboplatin/etoposide/placebo. Ipilimumab will be infused once every 3 weeks for four doses, then every 12 weeks, until progression of disease or unacceptable toxicity or until the maximum treatment period of 3 years is reached.

Tremelimumab (CP-675,20, AstraZeneca, London, UK) is a human IgG2 monoclonal antibody against CTLA-4 [12]. In a randomized Phase II study of tremelimumab 15 mg/kg every 90 days versus best supportive care in advanced NSCLC patients who had progressed on or after platinum-based chemotherapy, tremelimumab did not improve PFS, but there was a 4.8% relapsed refractory (RR) in the experimental arm [13]. Currently, this drug is being investigated in combination with other anti-PD-1 and anti-PD-L1 antibodies.

Blocking antibodies against PD-1 or PD-L1

To better understand which group of tumors may respond to PD-1/PD-L1 blockade, a simplified model has been proposed to classify tumor microenvironment into four subtypes based on their PD-L1 expression and tumor-infiltrating lymphocyte (TIL) status: type I tumors with PD-L1+TIL+, suggesting adaptive immune resistance and could respond to single agent of PD-1/PD-L1 inhibitors; type II tumors with PD-L1-TIL-, implicating immunological ignorance. This type of tumor may require combination treatment to bring T cells to tumor, such as combining CTLA-4 and PD-1 blockade; type III tumors with PD-L1+TIL-, indicating intrinsic induction of PD-L1 by an oncogenic pathway and the strategy would be to induce T-cell priming and response; type IV tumors with PD-L1-TIL+, suggesting immune tolerance with non-PD-1/PD-L1 suppressors which may need targeting alternative immune checkpoints or signaling pathways [14]. In advanced melanoma, 38%, 41%, 1% and 20% of patients can be categorized to type I, II, III and IV, respectively. In a recent study of tumor lymphocyte infiltration in resected NSCLC, similar to

Table 1. Clinical trials of immune checkpoint inhibitors in non-small-cell lung cancer.

Trial number	Regimen evaluated	Disease/population	Number of patients	Results
NCT00527735	Ipi + carboplatin + paclitaxel (Phase II)	Treatment-naive NSCLC	204	PFS primary end point met in phased ipi arm. Phased Ipi: 5.7 mo (p = 0.05) Concurrent Ipi: 5.5 mo vs Control arm: 4.7 mo irBORR 32% in phased ipi arm Grade 3/4 SAE: 15% in phased Ipi arm
NCT00312975	Tremelimumab vs BSC Phase II	Refractory patients with NSCLC greater than four line of prior treatment	87	ORR: 4.8%
NCT01642004	Nivolumab vs docetaxel Phase III	PD after platinum-based treatment with squamous histology	272	OS: Nivolumab: 9.2 mo (p < 0.001) vs Docetaxel: 6 mo 42% alive at 1 year in nivolumab arm vs 24% in docetaxel arm ORR: 20% in nivolumab (p = 0.008) SAE: pneumonitis 5% in nivolumab arm
NCT01673867	Nivolumab vs docetaxel or pemetrexed (Phase III)	PD after platinum-based treatment with nonsquamous histology	582	OS: Nivolumab:12.2 mos (p = 0.002) vs Docetaxel: 9.4 mo >50% alive at year 1 in nivolumab arm ORR: 19% in nivolumab (p = 0.02) Pneumonitis: 3% Grade 3 or higher SAE: Nivolumab: 10% vs Docetaxel: 54%
NCT01295827	Pembrolizumab (Phase I)	Multiple NSCLC cohorts of treatment-naive as well as previously treated patients	495	OS: 12 mo ORR: 19.4% SD: 21.8% ORR in >50% PD-L1+ : 45.2% Grade 3 or higher SAE: 9.5% Hypothyroidism: 6.9% Pneumonitis: 1.8%
NCT01903993	Atezolizumab vs docetaxel (Phase II)	PD after platinum-based treatment in NSCLC	287	OS: Atezolizumab: 12.6 mo (p = 0.04) vs Docetaxel: 9.7 mo ORR: Atezolizumab: 38% vs Docetaxel: 13%
NCT01633970	Atezolizumab vs platinum-based doublet (Phase Ib)	Treatment-naive NSCLC	37	ORR: 67%
NCT01693562	Durvalumab (Phase I/II)	PD after platinum-based treatment in NSCLC	198	ORR: 14%, Grade 3 or higher SAE: 6%

AEs: Adverse events; BSC: Best supportive care; Ipi: Ipilimumab; irBORR: Immune-related best overall response rate; mo: Months; NSCLC: Non-small-cell lung cancer; ORR: Overall response rate; OS: Overall survival; PD: Progressive disease; PFS: Progression-free survival; SAE: Serious adverse events; SD: Stable disease.

breast cancer, an intense tumor lymphocytic infiltration (>50%) was seen in 11% of patients and was associated with an improved OS (OS: HR: 0.45; 95% CI: 0.23–0.85; p = 0.01) [15]. Hence strategies utilizing the immune profile of the tumor microenvironment can be used to tailor PD-1/PD-L1-based immunotherapy and to design future trials in lung cancer.

Nivolumab (a human IgG4 anti-PD-1 mAb, BMS-936558, MDX-1106, Bristol-Myers Squibb) has been approved by US FDA to treat advanced metastatic NSCLC patients who have progressed on or after platinum therapy based on two trials [16,17]. In Checkmate 017 trial, patients with squamous cell lung cancer were randomized 1:1 to nivolumab, at a dose of 3 mg/kg every 2 weeks, or docetaxel, at a dose of 75 mg/m^2 every 3 weeks [16]. The median OS was 9.2 months with nivolumab versus 6.0 months with docetaxel. The overall response rate (ORR) was 20% with nivolumab versus 9% with docetaxel (p = 0.008). Treatment-related adverse events (AEs) were seen in 7% of patients getting nivolumab versus 55% of patients getting docetaxel. PD-L1 expression was not found to be predictive or prognostic of response or benefit.

Checkmate 057 trial was a global randomized Phase III trial comparing patients with advance stage nonsquamous NSCLC [17], who have progressed on and after platinum-based therapy/tyrosine kinase inhibitors to nivolumab 3 mg/kg every 2 weeks or docetaxel at a dose of 75 mg/m^2 every 3 weeks. Patients randomized to nivolumab had a median OS of 12.2 months (ORR = 19%) versus 9.4 months in the docetaxel arm (ORR = 12%) with 27% reduction in risk of death. 10% of people in the nivolumab arm had grade 3/4 events versus 54% in docetaxel group. In this study, tumor PD-L1 positivity was predictive of benefit to nivolumab. The approval of these drugs represents a major advance, as most lung cancer patients are older and the favorable safety profile of the checkpoint inhibitors makes this very attractive.

Pembrolizumab (anti-PD-1 monoclonal antibody, MK 3475, Merck, Kenilworth, NJ, USA) is a humanized IgG4 anti-PD-1 monoclonal antibody. It was recently granted accelerated approval by FDA based on a large Phase I study KEYNOTE-001, in which 495 patients were treated at doses of 2 or 10 mg/kg every 3 weeks or 10 mg/kg every 2 weeks [18]. The primary endpoint of the study was safety and efficacy of pembrolizumab. Both treatment-naive and patients who have progressed on or after platinum therapy were eligible. The ORR was 19.4% and the median duration of response was 12.5 months. The response rate and duration of response were higher in treatment-naive patients (ORR: 24.8%; 23.3 months) compared with previously treated patients (ORR: 18.0%; 10.4 months). The ORR did not differ by dose, schedule or histology. Current or former smokers had higher response rates (22.5 vs 10.3%) than nonsmokers. Membranous PD-L1 expression in at least 50% of cells was selected as a biomarker cutoff based on the ease of use and receiver operating curve analysis. Tumors with tumor PD-L1 positivity of >50, 1–49 and <1% had ORR of 33, 17 and 3% respectively. 10% of patients had grade 3 or higher side effects; hypothyroidism was the most common immune-related side effect seen in 34 patients (6.9%) followed by pneumonitis in 18 patients (3.6%). Based on these results pembrolizumab is indicated for metastatic NSCLC whose tumors express PD-L1 after progression on platinum-based chemotherapy. Further, Phase II/III confirmatory trials building on these results are ongoing with pembrolizumab in NSCLC.

Atezolizumab MPDL3280A (Genentech/Roche, CA, USA) is an engineered human IgG1 monoclonal antibody (mAb) that targets PD-L1 [19]. This antibody lacks an Fc component, thus avoiding antibody-dependent cytotoxic cellular killing of bystander immune cells. Atezolizumab was studied in multicenter open-label randomized Phase II study (POPLAR; NCT01903993) in which it was compared with docetaxel 75 mg /m^2 in advanced or metastatic NSCLC patients who have progressed on platinum-based therapy [20]. These patients were stratified by histology, PD-L1 status (high, any and no expressors) and prior treatment. The primary outcome was on efficacy, safety and predictive biomarkers. A total of 287 patients were randomized and the ORR was 38% in atezolizumab arm compared with 13% with docetaxel arm. OS was significantly improved in the atezolizumab arm 12.6–9.7 months in the docetaxel (HR: 0.73; p = 0.04). Patients who have the highest expression of PD-L-1 in their tumor derived the most benefit with 41% improvement compared with nonexpressors. Despite longer duration of treatment with atezolizumab (3.7 vs 2.1 months), more patients in docetaxel arm had withdrawals due to treatment-related adverse effect (22 vs 8%) [21]. Atezolizumab has also been studied in the first-line setting in combination with platinum therapy. This Phase Ib study evaluated atezolizumab with a wide variety of platinum doublets [22]. The combination was well-tolerated and the most common atezolizumab-related grade 3-4 serious AEs were anemia, neutropenia and thrombocytopenia. No pneumonitis was seen. There was one atezolizumab-related death due to candidemia after prolonged neutropenia. The ORR across all arms was 67%. These responses were seen in each arm independent of PD-L1 expression.

Durvalumab (MEDI4736, Medimmune, MD, USA) is a fully humanized IgG1 mAb that blocks PD-L1 binding to PD-1 and CD80, leading to enhanced tumor killing by reducing T-cell inhibition [23]. The antibody has a high affinity and specificity to only for PD-L1 and does not bind to PD-L2. In a Phase I dose escalation/expansion study in NSCLC patients, 16% had responded to durvalumab administered 10 mg/kg every 2 weeks. ORR was higher in squamous histology at 21% compared with 10% in nonsquamous NSCLC. The drug was well-tolerated and grade 3–4 AEs were seen in 6% of study population [24]. Currently, both the PD-L1 inhibitors have progressed to Phase III trials in NSCLC (Table 2).

PD-L1 positivity as a biomarker in NSCLC

As noticed from above studies, the response rates of anti-PD-1/PD-L1 antibodies in NSCLC as a single agent are around 20% in unselected patient population. Thus a significant proportion of patients do not derive clinical benefits. It is paramount to identify predictive biomarkers to select appropriate patient population who may or may not benefit from immunotherapy and also to guide future trial designs.

Table 2. Ongoing clinical trials with immune checkpoint inhibitors in non-small-cell lung cancer.

Clinical trial identifier	Phase	Experimental regimen	Population	Primary end points
CTLA-4 antibodies				
NCT01285609	III	Ipilimumab + paclitaxel + carboplatin	Second-line metastatic squamous NSCLC	OS
NCT01450761	III	Ipilimumab + etoposide/platinum	First-line metastatic SCLC	OS
NCT01998126	1B	Ipilimumab + erlotinib/crizotinib	First-line EGFR or ALK (anaplastic lymphoma kinase)-mutated NSCLC	DLT
NCT02477826	III	Nivolumab vs nivolumab + ipilimumab, vs nivolumab + platinum doublet vs platinum doublet chemotherapy	First-line metastatic NSCLC	OS PFS
PD-1 antibodies				
NCT02259621	I	Neoadjuvant nivolumab	Neoadjuvant Stage 1–3 (N1 disease)	DLT
NCT02393625	I	Nivolumab + ceritinib	ALK-positive metastatic lung cancer	DLTw ORR
NCT02343952	II	Pembrolizumab	Unresectable IIIA/IIIB NSCLC after receiving concurrent chemoradiation	Event (death) rate
NCT02564380	II	Pembrolizumab maintenance vs placebo	First-line metastatic setting following platinum-based doublet metastatic squamous NSCLC	PFS
NCT02142738 (Keynote- 024)	III	Pembrolizumab vs carboplatin + paclitaxel	First-line metastatic setting in strong PDL1 expressing NSCLC	PFS
NCT02578680 (KEYNOTE-189)	III	Pembrolizumab + platinum + pemetrexed vs placebo+ platinum + pemetrexed	First-line metastatic setting Nonsquamous NSCLC	PFS
NCT02220894 (Keynote 042)	III	Pembrolizumab vs platinum-based chemotherapy	First-line metastatic setting in strong PDL1 expressing NSCLC	OS
NCT02316002	II	Pembrolizumab	Resected oligometastatic NSCLC	PFS
NCT02504372 (Keynote -091) (PEARLS)	III	Pembrolizumab vs placebo	Resected stage 1b-IIIA NSCLC after standard adjuvant therapy	DFS
PD-L1 antibodies				
NCT02409342 (IMpower110)	III	Atezolizumab + pemetrexed + cisplatin or carboplatin	First-line metastatic nonsquamous NSCLC	PFS
NCT02366143 (IMpower150)	III	Atezolizumab + carboplatin + paclitaxel + bevacizumab	First-line metastatic nonsquamous NSCLC	PFS
NCT02367794	III	Atezolizumab + carboplatin + paclitaxel or nab-paclitaxel	First-line metastatic squamous NSCLC	PFS
NCT02367781	III	Atezolizumab + carboplatin + nab-paclitaxel	First-line metastatic nonsquamous NSCLC	PFS
NCT02125461 (PACIFIC)	III	Durvalumab vs placebo	NSCLC post platinum-based concurrent chemoradiation therapy	OS
NCT02453282 (MYSTIC)	III	Durvalumab + tremelimumab vs durvalumab vs platinum-based therapy	First-line metastatic NSCLC	PFS
NCT02542293	III	Durvalumab + tremelimumab vs platinum-based therapy	First-line metastatic NSCLC	OS
NCT02273375	III	Durvalumab vs placebo	Resected stage 1b-IIIA NSCLC after standard adjuvant therapy	DFS
NCT02352948	III	Durvalumab vs durvalumab + tremelimumab	Third-line metastatic NSCLC	OS

DFS: Disease-free survival; DLT: Dose-limiting toxicity; NSCLC: Non-small-cell lung cancer; ORR: Overall response rate; OS: Overall survival; PFS: Progression-free survival; SCLC: Small-cell-lung cancer.

Expression of PD-L1 in tumor cells has been used and recommended as a biomarker. Indeed, the FDA approval of pembrolizumab comes with a companion diagnostic test, the PD-L1 IHC 22C3 pharmDx assay to select PD-L1 positive lung cancer patients, based on the superior efficacy observed in this subset of patients. On the other hand, nivolumab has been approved without the use of companion diagnostic test and the CHECKMATE-017 study showed no association between PD-L1 expression despite showing high correlation between PDL-1 expression and tumor response in the KEYNOTE-001 study. How to explain these discrepant results? More accurate assessment of the immune environment is important and hence TILs, immune signature showing the degree of activation of the immune system, presence of other immune checkpoint ligands needs to be assessed in conjunction with PD-L1; factors intrinsic to PD-L1 measurement – the use of different antibodies to test the expression of PD-L1, the use of different types of pathology specimen and sites for staining and what constitutes positivity need to be standardized.

Immune checkpoint blockade in hematological malignancies

Hodgkin lymphoma

Hodgkin lymphoma (HL) is a B-cell lymphoma, and relapsed HL has poor survival. In HL, the PD-1/PD-L1 pathway is amplified through three mechanisms. First, 9p24.1 amplification is a recurrent genetic abnormality and this region encodes for the PD-L1 and PD-L2, which are hence amplified. Second, 9p amplification also encodes the JAK/STAT pathway which results in overexpression of PD-L1. Third, HL has a high expression of EBV-related proteins which in turn increases PD-1 expression [25]. Nivolumab was studied in relapsed refractory HL at a dose of 3 mg/kg every 2 weeks. 23 patients were enrolled and 78% had previous autologous stem cell transplant (autologous HSCT; hematopoietic stem cell transplant) and brentuximab vedotin (anti-CD30 mAb) and hence these patients are considered highly refractory [26]. The ORR was 87%, 17% with a complete response (CR), 70% with a partial response and 13% had stable disease and the PFS at 6 months was 86% (Table 3). Tumor tissue showed copy number gains and increased protein expression of PD-L1 and PD-L2. Pembrolizumab was studied in the KEYNOTE-013 trial in relapsed refractory HL and similar to the previous trial most failed autologous HSCT and all failed brentuximab [27]. The ORR was 65% with 16% achieving CR and the median duration of response was not yet reached. A significant increase in the absolute number of circulating total lymphocytes, T cells (CD4 and CD8 subsets) and natural killer (NK) cells was seen post treatment. RNA profiling of pre- and post-treatment blood samples showed that the ten-gene IFN-γ-induced signature, the 18-gene expanded immune signature and the 13-gene TCR signature were all significantly upregulated. These show that enhanced T-cell specific and possibly NK-cell immunity is increased post anti-PD-1 treatment. These studies showed that PD-1 blockade resulted in impressive response rates in HL and significant toxicities were far less when compared with chemotherapy, and immune-related AEs were observed in <20% of those treated. Hence further studies are ongoing in HL as a single agent and in combination with chemotherapy and CTLA-4 inhibitors.

Non-Hodgkin lymphoma

Non-Hodgkin lymphoma (NHL) encompasses a wide variety of lymphomas and based on the cell of origin can be classified as B cell and T cell or based on prognosis as indolent and aggressive. PD-L1 protein is expressed in the tumor cells of various NHL types namely – primary mediastinal large B-cell lymphoma, T-cell/histiocyte-rich B-cell lymphoma, EBV-positive and -negative post-transplant lymphoproliferative disorder, EBV-associated diffuse large B-cell lymphoma, plasmablastic lymphoma, extranodal NK/T-cell lymphoma, nasopharyngeal carcinoma and HHV8-associated primary effusion lymphoma [28,29].

The CTLA-4 inhibitor ipilimumab was tested in relapsed refractory NHL and the ORR was disappointing at 11% (2/18) but the responses in the two patients lasted for >31 and 19 months, which is generally longer than would be expected in relapsed refractory disease [30]. T-cell proliferation to recall antigens was also increased as would be expected. Pidilizumab, a PD-1 blocking antibody was tried in relapsed refractory lymphoma and was given as a single dose with an ORR of 33% and no dose-limiting toxicity was identified, thus providing evidence that PD-1 blockade is safe in hematological malignancies [31]. A sustained increase in $CD4^+$ lymphocytes was seen in the peripheral blood in those treated. Pidilizumab was then combined with rituximab (anti-CD20 Ab) in a Phase II trial for rituximab-sensitive relapsed follicular lymphoma [32]. Pidilizumab also increases NK-cell activity and hence this combination was designed to increase the NK-cell-mediated ADCC activity of rituximab. There were no autoimmune or immune-related AEs of grade 3/4 and the ORR was 66% which suggested activity of this combination, but the population of rituximab sensitive relapse makes it difficult to interpret the true value of this combination [25,33]. Nivolumab was also tested in 29 patients with relapsed refractory NHL at 3 mg/kg

Table 3. Clinical trials of immune checkpoint inhibitors in hematological malignancies.

Trial number	Agent	Disease/ population	Number of patients	Results and notes
NCT01592370	Nivolumab 3 mg/kg every 2 weeks	RR Hodgkin lymphoma	23	ORR: 87%, CR: 17%, PR: 70%, SD: 13% Copy number gains in PD-L1 and PD-L2 was present
NCT01953692 (KEYNOTE 013)	Pembrolizumab 10 mg/kg every 2 weeks	RR Hodgkin lymphoma	15	ORR: 53%, CR: 20%, PR: 33% SAE – respiratory events (20%), thyroid events (20%)
NCT00089076	Ipilimumab 3 mg/kg	RR NHL	18	Two patients with clinical responses lasting 31 and 19 months T-cell proliferation to recall antigens was significantly increased
–	Pidilizumab (CT-011)	Advanced hematological malignancies	17	Cumulative survival at 21 days – 76%, 1 CR (FL). Sustained elevation in peripheral blood $CD4^+$ lymphocytes was present
NCT00904722	Pidilizumab Rituximab	Rituximab sensitive relapsed Follicular lymphoma	29	ORR: 66%, CR: 52%, Median PFS: 18.8 months Expression of activating receptor NKG2D on NK cells was significantly increased
NCT01592370	Nivolumab 1 or 3 mg/kg every 2 weeks for 2 years	RR hematological malignancies	27	ORR by subtypes – DLBCL: 36%, FL: 40%, T-NHL: 17%, PTCL: 40% SAE – pneumonitis: 7%
NCT00532259	Pidilizumab	Post Auto HSCT	66	PFS at 16 week follow-up: 0.72 (met study end point) ORR – 51%. Treatment was associated with an increase in circulating lymphocytes
Ongoing clinical trials with interim results				
MC1485	Pembrolizumab	Relapsed refractory CLL, Richter's	16	4/5 Richter's patients responded 2/2 CLL had stable disease
NCT02289222	Pembrolizumab Pomalidomide Dexamethasone	RR MM	22/16 weeks	ORR: 50%
NCT01953692	Pembrolizumab	RR PMBCL	9/20 weeks	ORR: 49%
NCT02077959	Pidilizumab + lenalidomide	RR MM	12	ORR: 66.6%
NCT01067287	Pidilizumab + dendritic cell vaccine	Post autologous HSCT	22	Three CR's after immunotherapy
NCT02036502	Pembrolizumab Lenalidomide Dexamethasone	RR MM	17/41 weeks	ORR: 76%

CLL: Chronic lymphocytic leukemia; CR: Complete response; DLBCL: Diffuse large B cell lymphoma; FL: Follicular lymphoma; HSCT: Hematopoietic stem cell transplant; MM: Multiple myeloma; NHL: Non-Hodgkin Lymphoma; T-NHL: T-cell non-Hodgkin lymphoma; NK: Natural killer; ORR: Overall response rate; PFS: Progression-free survival; PMBCL: Primary mediastinal B cell lymphoma; PR: Partial response; PTCL: Peripheral T-cell lymphoma; RR: Relapsed refractory; SAE: Serious adverse events; SD: Stable disease.

every 2 weeks. The ORR was 36%, but varied according to the subtype – diffuse large B-cell lymphoma, follicular lymphoma and peripheral T-cell lymphoma having an ORR of 36, 40 and 40%, respectively [34]. Hence these studies show that checkpoint inhibition is feasible in a select subgroup of NHL and this could vary by histology or the molecular pathways involved in B-cell transformation may influence the response.

Autologous HSCT

Autologous HSCT (ASCT) is performed in NHL as a method of consolidation in NHL or for relapsed refractory disease and the time period of immune reconstitution could be a good window to augment T-cell response. Pidilizumab was tested in post autologous HSCT in aggressive non-Hodgkin lymphoma in those who had chemo-sensitive disease [35]. Pidilizumab was

given at 1.5 mg/kg every 42 days, 30–90 days from ASCT during the time of immune reconstitution. The PFS at 16 months was 0.7 (0.51–0.82) while the threshold value was 69% to warrant further study. This study met its primary end point and further randomized studies (NCT02362997) are being done to see whether PD-1 inhibitors can improve disease-free survival after autologous HSCT.

Allogeneic HSCT

Allogeneic HSCT is used as a treatment option for treating relapsed refractory lymphomas. Two concerns remain: is allogeneic HSCT safe after PD-1 inhibitor treatment? Second, exacerbation of acute or chronic graft versus host disease (GVHD) after allogeneic HSCT. Mouse models show that PD-1/PD-L1 interactions dampen acute GVHD while it increases chronic GVHD [36]. Retrospective analyses of allogeneic HSCT after PD-1 inhibitors show that the disease-free survival was higher than would be expected and similarly a higher than expected GVHD and veno-occlusive disease was also seen [37]. Ipilimumab was tested postallogeneic HSCT at dose cohorts between 0.1 and 3 mg/kg [38]. Twenty-nine patients with hematological malignancies which relapsed after allogeneic HSCT were included in three dose cohorts. Dose-limiting toxicity was not encountered and, more importantly, there was no GVHD or graft rejection and immune-related AEs were acceptable. Notably, three patients with Hodgkin disease and one patient with refractory mantle cell lymphoma had a response. This is an important trial to show that checkpoint blockade was feasible in patients with hematological malignancies after allogeneic HSCT without causing GVHD or graft rejection.

Multiple myeloma & leukemia

Nivolumab was also tested in a dose escalation trial in persons with relapsed refractory hematological malignancies and was administered every 2 weeks for 2 years. Interestingly, the stable disease rate in multiple myeloma was 63% with a median duration of follow-up of 62 weeks [39]. Hence there are multiple ongoing trials with PD-1 inhibitor as single agent (NCT01953692) or in combination with immunomodulators (Imids) (NCT02036502, NCT02289222) and dendritic cell vaccines (NCT01067287) in myeloma.

In myeloid malignancies, aberrant expression of PD-L1 was noted in myelodysplastic syndrom (MDS), chronic myelomonocytic leukaemia (CMML) and acute myeloid leukaemia (AML) in 34, 14 and 15% of patient samples tested. Moreover, PD-1 and PD-L1 is highly expressed in MDS blasts after treatment with hypomethylating agents [40], and hence this is being currently tested in a Phase I trial of an anti-PD-1 antibody (MEDI 4736) in combination with azacitidine in this disease. The KEYNOTE-013 trial is exploring pembrolizumab in a multicohort trial (NCT01953692) and patients will be included in different cohorts based on lymphoma subtype and PD-L1 positivity. In CML, PD-L1 is expressed on the leukemic cells and PD-1 is expressed on tumor-specific cytotoxic T cells [41], and hence PD-1 inhibitors are being tested in combination with dasatinib (NCT02011945).

Adverse effects & response criteria

The toxicity of these agents is less and manageable when compared with chemotherapy or even some targeted therapy. Adverse events are more immune related and the common AEs are colitis, pneumonitis, thyroiditis, hypophysitis and dermatitis which could be managed with supportive care and steroids. These AEs are less with the PD-1 and PD-L1 inhibitors than the CTLA-4 inhibitors as PD-L1/PD-1 is more involved in the effector phase of the immune function. Comprehensive reviews of immune-related AEs associated with the checkpoint inhibitors have been published [42–44].

Patients who are treated with checkpoint inhibitors can initially have a disease flare before they have a disease response with a median CR of approximately 30 months in the initial trials [45]. In order to account for the time duration and the pattern of responses, an immune-related RECIST criterion has been developed which requires confirmation of progressive disease by a repeat scan after a certain time interval [11]. As we continue to learn more about the pattern of responses these response criteria will continue to be refined.

Conclusion & future perspective

The CTLA-4 and PD-1/PD-L1 blocking antibodies have shown therapeutic success and many other immune checkpoints are being currently evaluated. Antibodies against other checkpoints inhibitors that are furthest in evaluation are LAG-3 (IMP321, Immuntep; BMS-986016, BMS), TIM-3 and VISTA, which are currently being tested in clinical trials. Other members of the B7 family, namely, B7-H3, B7x and HHLA2 are being tested in preclinical models and are soon to enter drug development. Similarly, agonists for costimulatory T-cell ligands, namely, OX40, 41BB (urelumab, BMS-663513) and ICOS are also in clinical development. Combinatorial therapies with the various checkpoint inhibitors, CAR T cells, radiotherapy, immunogenic chemotherapy like anthracyclines, tyrosine kinase inhibitors, fusion vaccines and immunomodulators are also expected to increase response to these therapies. In particular, combination therapies with neoantigen-based vaccines along with immune checkpoint inhibitors can further drive personalized

medicine toward higher tumor specificity. Biomarker profile of responders including TCR repertoire, CD4/CD8 T-cell profile, cytokine signature, immune check point expression in tumor cells, macrophages or T cells are also being developed to identify markers of early response. Imaging criteria of what constitutes response, and whether the modified response criteria can sufficiently predict overall survival and lead to approval of these drugs are yet to be determined. In conclusion, immune checkpoint inhibitors have shown remarkable clinical success and we are likely to see improved success with these agents in the future.

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Executive summary

Immune checkpoints

- Tumor immune evasion is one of the hallmarks of cancer and expression of immune checkpoints is an important mechanism of immune evasion.
- The two of major coinhibitory pathways currently known are: the B7–1/B7–2/CTLA4 which is involved in early T-cell activation; and the PD-L1/PD-1 pathway which is involved in peripheral tolerance. Inhibition of these pathways has led to a remarkable therapeutic success in some patients.

Blocking antibodies against PD-1 or PD-L1

- The PD-1 inhibitors nivolumab and pembrolizumab are effective in approximately 20% of patients with non-small-cell lung cancer (NSCLC) and have been currently approved as monotherapy for the treatment of metastatic NSCLC after progression on platinum-based therapy.
- The PD-L1 inhibitors atezolizumab and durvalumab have also shown good activity in Phase I trials of NSCLC and are currently being studied in Phase III trials.

PD-L1 positivity as a biomarker in NSCLC

- Factors which influence response to checkpoint inhibitors include smoking status, PD-L1 expression on tumors, TIL infiltrate and mutational burden of tumors.
- Using PD-L1 expression as a predictive biomarker has significant limitations and hence cannot be recommended for nivolumab.

Immune checkpoint blockade in hematological malignancies

- In hematological malignancies, Hodgkin lymphomas has high response rates to PD-1 inhibitors and are likely to be approved for this indication. In non-Hodgkin lymphoma, the checkpoint inhibitors have limited activity which varies depending on the histology and the B-cell pathway activated.
- An important concept is the safety of immune checkpoint inhibitors pre- and postallogeneic transplant, and early studies indicate that the checkpoint inhibitors are safe although their role in acute and chronic graft versus host disease may be different.

Adverse effects & response criteria

- Check point inhibitors have different response patterns and adverse effects when compared with traditional agents and hence it is important to be aware of the immune-related response criteria and immune-related adverse events to checkpoint inhibitors.

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