Immune checkpoint inhibition in lymphoid disease

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Summary

It has long been understood that the immune system has intrinsic anti-tumour activity in humans, and that a key mechanism of tumour progression is the ability of a tumour to escape this immune surveillance. A number of attempts have been made to harness this anti-tumour immunity in both solid tumour oncology and haematological malignancies with variable success. Examples include the use of allogeneic stem cell transplantation and donor lymphocyte infusion in haematological cancer and vaccine studies in solid tumours. Enhanced signalling of the Programmed cell death-1 (PDCD1, PD-1)/cytotoxic T-lymphocyte-associated protein 4 (CTLA4) ‘immune checkpoint’ pathway has emerged recently as a critical mechanism by which tumours can escape the natural anti-tumour immune response. As such, novel therapies have been developed to help enhance this natural immunity by switching off the PDCD1/CTLA4 immune checkpoint pathway. The following review will discuss the pathobiology of these pathways and the exciting new data now available in lymphoid malignancies.

Keywords: Hodgkin lymphoma, pembrolizumab, nivolumab, PDCD1 (PD-1), CD274 (PD-L1).

Immune checkpoints

Normal T-cell physiology is highly complex, involving activation and proliferation as a result of antigen stimulation, trafficking to sites of damage or infection and the execution of a variety of effector functions, such as providing help, cellular cytotoxicity or suppression of the immune response. It is necessary that this process is highly regulated with balancing stimulatory and inhibitory signals mediated largely through membrane receptors engaging with cell surface bound or soluble receptor ligands (Zou & Chen, 2008). The inhibitory signals, also known as immune checkpoints, are crucial in the protection against autoimmunity. This checkpoint therefore enables the maintenance of self-tolerance. When a T-cell is activated by engagement of the T-cell receptor (TCR) with antigen in the context of the major histocompatibility complex (MHC), T cells expand and proliferate. It is important, however, that T cell function is highly regulated, so that the subsequent immune response minimizes collateral damage to the host (Keir et al, 2008). A plethora of regulatory molecules have been identified (see review by Pardoll (2012) for a useful summary), although two have been studied most intensively: CTLA4 binding to CD80 or CD86 and PDCD1 binding to CD274 (also known as PDCD1 ligand 1, PD-L1) or PDCD1 ligand 2 (PDCD1LG2).

Cytotoxic T-lymphocyte-associated antigen-4

It has been appreciated for nearly two decades that Cytotoxic T-lymphocyte-associated antigen-4 (CTLA4) is crucial for the regulation of the immune response. Studies involving knockout mice confirm the importance of this system whereby deletion of the Cita gene results in a lethal systemic immune hyperactivation phenotype characterized by profound lymphoproliferation (Tivol et al, 1995; Waterhouse et al, 1995). The effect of CTLA4 activation is the reduction in the amplitude of the early T-cell response to antigen and it is therefore active at the very first stage of immune response. In a resting T-cell, CTLA4 is not expressed on the cell surface but is instead sequestered within cytoplasmic vesicles. Upon activation, translocation to the cell surface occurs. The extent of this translocation is dependent on the strength of antigen binding to the TCR (Egen & Allison, 2002). Cytotoxic T-lymphocyte-associated antigen-4 therefore serves to regulate and attenuate the T cell immune response following the antigen-TCR interaction.

Although the exact mechanism underlying the suppression of T-cell activation is a matter of debate, it involves the interaction with two ligands: CD80 and CD86 (also known as B7.1 and B7.2) (Linsley et al, 1991, 1994). These molecules were already recognized as being important for the augmentation of the T-cell response to antigen after the TCR binds to antigen in the context of MHC via binding to CD28 on T-cells (Lenschow et al, 1996). It therefore follows that CTLA4 may function by outcompeting CD28 for binding to ligand, thus dampening down the immune activation resulting from this interaction. In keeping with this hypothesis, the affinity of CTLA4 for CD80 and CD86 is greater than...
that of CD28 for these ligands (Linsley et al, 1994). Other mechanisms are clearly at play however. Cytotoxic T-lymphocyte-associated antigen-4 binding to CD80/86 appears to result in a removal of these proteins from the cell surface (Qureshi et al, 2011) and CTLA4 binding activates intracellular phosphatases [such as PTPN11 (SHP2) and PP2A], which are thought to interfere with the kinase signalling cascade induced by T-cell activation (Rudd et al, 2009).

Perhaps not surprisingly, at a cellular level the activation of CTLA4 results in complex changes in the activity of different subsets of T-cells. Although CTLA4 was first recognized as being expressed on activated CD8+ T-cells (hence its name), its major physiological effect is mediated through profound changes in CD4+ T-cells, both with a helper and regulatory function (Lenschow et al, 1996). Within T regulatory cells (Tregs), FOXP3 is a vital transcription factor mediating the regulatory phenotype (Gavin et al, 2007) and CTLA4 has been shown to be a target gene resulting in high levels of expression of the protein on the surface of these cells (Hori et al, 2003; Peggs et al, 2009). Transgenic models have demonstrated that engagement of CTLA4 enhances the regulatory function of these cells (Wing et al, 2008) as well as independently suppressing the activity of effector T-cells, such as CD4+ T helper cells (Peggs et al, 2009).

Not surprisingly, early suggestions of clinical CTLA4 blockade met with concerns due to the lethal lymphoproliferation seen in knockout mice. However, animal studies using blocking antibodies showed that these agents could be given safely. Indeed these studies provided proof of concept of an anti-cancer effect as CTLA4 inhibition resulted in potent anti-tumour effects in animal models (Leach et al, 1996; Van Elsas et al, 1999). Poorly immunogenic tumours were not sensitive to CTLA4 inhibition alone, unless animals were additionally treated with a granulocyte-macrophage colony-stimulating factor (GM-CSF)-transduced cellular vaccine (Van Elsas et al, 1999). Interestingly, following successful application of anti-CTLA4, re-challenge with tumour was rejected by the animals, suggesting the establishment of a memory component to the immune response (Leach et al, 1996).

**Programmed cell death protein 1**

Programmed cell death protein 1 (PDCD1) is involved at a different stage of the lymphocyte response to infection or inflammation. Initial stimulation of a T-cell results in expression of PDCD1, which then persists as the T-cell infiltrates peripheral tissues. Within these tissues, inflammatory cytokines induce the expression of ligands to PDCD1, which therefore serve to modulate the T-cell activity at the site of infection or damage to limit tissue damage and autoimmunity (Nishimura et al, 1999; Keir et al, 2006, 2008). Two ligands have been identified for PDCD1: CD274 (PD-L1) and PDCD1 ligand 2 (PDCD1LG2; PD-L2) (Latchman et al, 2001; Dong et al, 2002). The downstream mechanism resulting in T-cell suppression is, in some ways, thought similar to CTLA4, with activation of phosphatases, such as PTPN11 leading to inhibition of kinases mediating T-cell activation (Freeman et al, 2000). In addition, PDCD1 knockout experiments reveal the presence of an additional receptor for CD274 and PDCD1LG2 on T-cells, which leads to stimulation. This raises the possibility of a competition mechanism for PDCD1 action, similar to the interaction between CTLA4 and CD28 (Shin et al, 2005). Similar to CTLA4, the effect of PDCD1 action on different T-cell subsets is complex and again, the role of Tregs is crucial. PDCD1 is highly expressed on Tregs and PDCD1 interaction with ligand can enhance the proliferation of these cells (Francisco et al, 2009). Furthermore, PDCD1 is expressed by a number of other immune cells, such as B-cells and Natural Killer cells and therapeutic manipulation of PDCD1 may well influence the behaviour of these components (Fanoni et al, 2011). It has been demonstrated in a chronic viral infection model, that long term exposure of T-cells to antigen can result in high levels of sustained PDCD1 expression, leading to exhaustion or anergy of responding T-cells – a state which can be at least partly overcome by inhibition of PDCD1 (Barber et al, 2006). This process is probably also at play during the immune response to tumours.

The PDCD1 – CD274/PDCD1LG2 pathway represents an important immune resistance mechanism for tumour cells (Dong et al, 2002; Blank, 2004). Programmed cell death protein 1 is highly expressed by tumour infiltrating lymphocytes (TILs) in a wide variety of tumour types (Ahmadzadeh et al, 2009; Sfanos et al, 2009). The PDCD1 ligands (especially CD274) are frequently expressed on tumour cells and forced expression of CD274 within tumour cells of animal models effectively inhibits local T-cell mediated anti-tumour responses (Dong et al, 2002; Iwai et al, 2002; Konishi et al, 2004). Some studies have also demonstrated upregulation of PDCD1LG2, especially in B-cell lymphomas such as Hodgkin lymphoma (HL), follicular lymphoma (FL) and primary mediastinal B-cell lymphoma (PMBCL) (Rosenwald et al, 2003; Ansell et al, 2014). The mechanism of upregulation in HL is probably gain or amplification of the genomic locus within which these genes lie. The genes encoding the two proteins are thought to be the result of gene duplication and lie within 100 kb of each other within the genome (Tseng et al, 2001; Ansell et al, 2014). Clinical data has recently shown that increase in CD274 and PDCD1LG2 protein expression is associated with underlying chromosome 9p copy gain, amplification or polysomy (Ansell et al, 2014). Other mechanisms for ligand upregulation have also been postulated: amplification or upregulation of the class II MHC transactivator locus CIITA (Steidl et al, 2011); activation by constitutively active oncogenic signalling pathways via STAT3 (Marzec et al, 2008); an adaptive response to endogenous anti-tumour immunity (Taube et al, 2012).
An obvious translational question was to test whether inhibition of PDCD1 or its ligands would lead to tumour regression. This was a particularly attractive target due to the relatively mild phenotype of Pdcd1, Cd274 and Pdcd1lg2 knockout mice, suggesting that blockade maybe well tolerated. Numerous mouse models have validated this approach (Dong et al, 2002; Iwai et al, 2002; Blank, 2004) and led to clinical trials in humans (see below).

Clinical trials in checkpoint inhibition

As detailed above, there has been a dramatic evolution in understanding of the ‘immune checkpoint’ pathway over recent years. Several novel agents have therefore been developed in an attempt to harness the anti-tumour activity of the immune system. Although the immune system has long been believed to be crucial in the control of tumour growth, certain immunotherapy approaches, such as some vaccination strategies, have been fundamentally disappointing. Over recent years, clinicians have pioneered a novel targeted approach following the development of humanized IgG monoclonal antibodies with the ability to primarily target the PDCD1 - CD274/PDCD1LG2 and CTLA4 – CD80/86 interactions. The five most well developed agents are the anti-CTLA4 IgG humanized antibodies, ipilimumab and tremelimumab, and the anti-PDCD1 IgG monoclonal antibodies pidilizumab, pembrolizumab and nivolumab. Their respective sites of activity and the mechanism of such activity are displayed in Fig 1. Within medical oncology, the best example of the rapid progress in immunotherapy is within the field of metastatic melanoma. Ipilimumab has been shown to improve the median overall survival (OS) when compared with a glycoprotein placebo (10/11 vs. 6/4 months hazard ratio (HR) for death, 0.66; P = 0.003) in a seminal trial in relapsed, refractory melanoma (Hodi et al, 2010) and in a subsequent trial comparing dacarbazine alone (Robert et al, 2011). A recent randomized study has shown similar benefit for the anti-PDCD1 monoclonal antibody nivolumab when compared with dacarbazine, demonstrating a significantly improved OS at 12 months [72.9%; 95% confidence interval (CI): 65.5–78.9] in the nivolumab group compared to 42.1% (95% CI: 33.0–50.9) in the dacarbazine group (HR for death, 0.42; 95% CI: 0.25–0.73; P < 0.001) (Robert et al, 2015). This led to the US Food and Drug Administration licensing ipilimumab for unresectable or metastatic melanoma in 2011, and in 2014 both nivolumab and pembrolizumab for the same indication in those who had received ipilimumab.

The large data sets in solid tumour oncology have preceded trials in haemato-oncology. However, recent developments in lymphoid malignancies have provided compelling evidence of the efficacy and tolerability of targeting the immune checkpoint pathways in these disorders. Table I summarizes the clinical trial data published to date using immune checkpoint inhibition in lymphoid disease.

Phase I studies in advanced haematological cancer

Ipilimumab was initially investigated in a dose escalation study in relapsed B cell lymphoma (Ansell et al, 2009) and pidilizumab (initially known as CT-011) in a separate dose escalation study in advanced haematological malignancies (Berger et al, 2008).

In relapsed B cell lymphoma, ipilimumab was initiated at 3 mg/kg and then given at 1 mg/kg once per month for 3 months. The second dose level escalated to a flat dose of 3 mg/kg for 4 months. Eighteen patients received treatment on this study, the majority of whom had relapsed, refractory FL. Two patients had clinical responses and the overall response rate (ORR) was 11%. Of note, a single patient with diffuse large B cell lymphoma (DLBCL) achieved a durable

Fig 1. The PDCD1-CD274/PDCD1LG2 CTLA4-CD80/CD86 pathway: action of anti-PDCD1 and anti-CTLA4 monoclonal antibodies.
Table I. A summary of the current clinical responses to checkpoint inhibition in lymphoid disease.

<table>
<thead>
<tr>
<th>Lymphoid disease</th>
<th>Agent and dosing</th>
<th>Mechanism</th>
<th>Phase</th>
<th>Patients (n)</th>
<th>Patient characteristics</th>
<th>Response rates</th>
<th>PFS</th>
<th>OS</th>
<th>Activity related to CD274/PDCD1LG2 expression</th>
<th>Toxicity</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>Relapsed B-NHL</td>
<td>Ipilimumab 3 mg/kg and then monthly at 1 mg/kg for 3/12; escalation to 3 mg/kg monthly for 4/12</td>
<td>Anti-CTLA4 fully humanized IgG1 mAb</td>
<td>I</td>
<td>18</td>
<td>14 FL, 3 DLBCL, 1 MCL, Median prior therapies 2 (1–4)</td>
<td>11% ORR</td>
<td>Not stated</td>
<td>Not stated</td>
<td>Nil stated</td>
<td>Grade 3 fatigue 6%</td>
<td>Grade 3 diarrhoea 28%</td>
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<tr>
<td>Multiple haematological cancers</td>
<td>CT-011 (Pidilizumab) escalating from 0-2 to 6 mg/kg</td>
<td>Anti-PDCD1 fully humanized IgG1 mAb</td>
<td>I</td>
<td>18</td>
<td>Advanced disease: 8 AML, 1 MDS, 3 CLL, 3 NHL, 1 HL, 1 MM</td>
<td>Clinical benefit was observed in 35% with 1 CR</td>
<td>Not stated</td>
<td>21 days 76%, Mean OS 25 weeks</td>
<td>Nil stated</td>
<td>Safe and well tolerated. No single MTD. No DLT reached.</td>
<td>Berger et al (2008)</td>
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<td>DLBCL post-ASCT</td>
<td>Pidilizumab 1.5 mg/kg every 42 days beginning 1 to 3/12 after AHSCRT.</td>
<td>Anti-PDCD1 fully human mAb IgG1 recombinant mAb</td>
<td>II</td>
<td>66</td>
<td>Median age 57 (19-80) years.</td>
<td>51% ORR</td>
<td>54% CR</td>
<td>17% PR</td>
<td>37% SD</td>
<td>Insufficient tissue to assess CD274 expression</td>
<td>Grade 3–4 neutropenia (19%) and thrombocytopenia (8%)</td>
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<td>Relapsed FL</td>
<td>Rituximab 375 mg/m² in 4 weekly doses with Pidilizumab 3 mg/kg iv every 4 weeks for 4 infusions, plus 8 optional infusions every 4 weeks for 5D or better</td>
<td>Anti-PDCD1 fully human mAb IgG1 recombinant mAb</td>
<td>II</td>
<td>32</td>
<td>Median age 61 (35–79) years. All prior rituximab. Median prior treatments 1 (1–4). FLIPI2: 7 (54%) Low, 15 (50%) Intermediate, 8 (27%) High</td>
<td>66% ORR</td>
<td>CR 52%</td>
<td>PR 14%</td>
<td>Median PFS 18.8 months (95% CI 14–7–NR)</td>
<td>Responders expressed higher CD274 on peripheral blood T cells and monocytes at baseline vs. non-responders. GEP (tumour and peripheral blood) of T-cell activation or T effector cells associated with PFS</td>
<td>No grade 3–4 toxicity</td>
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<td>Lymphoid disease</td>
<td>Agent and dosing</td>
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<td>Patients (n)</td>
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<td>Relapsed or Refractory HL</td>
<td>Nivolumab 3 mg/kg at weeks 1 and 4, and then every 2 weeks until PD or CR or for a maximum of 2 years.</td>
<td>Anti-PDCD1 fully human mAb IgG4</td>
<td>I</td>
<td>23</td>
<td>Median age 35 (20–54) years. 78% post-ASCT and BV</td>
<td>87% (95% CI, 66–97) CR 17% PR 70% SD 13%</td>
<td>24 weeks 86% (95% CI, 62–95).</td>
<td>Median OS not reached (21–75)</td>
<td>10 available tumours: 3–15 copies of CD274 and PDCD1LG2 (amplification, relative copy gain or polysomy) of chromosome 9p by FISH and high expression of CD274 and PDCD1LG2 by IHC.</td>
<td>Grade 1–2 Rash 22% Decreased platelet count 17%</td>
<td>Ansell et al (2014)</td>
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<td>Relapsed or Refractory HL</td>
<td>Pembrolizumab single-agent 10 mg/kg iv every 2 weeks until confirmed tumour progression, excessive toxicity or completion of 2 years therapy.</td>
<td>Anti-PDCD1 fully human mAb IgG4</td>
<td>Ib</td>
<td>15</td>
<td>Median age 28 (21–67) years. Median prior therapies = 4. Relapse or failure to respond to BV. 67% failed prior ASCT.</td>
<td>Best ORR 53% at 12 weeks 20% CR 33% PR</td>
<td>Not stated</td>
<td>Not stated</td>
<td>Nil stated</td>
<td>No SAEs, 1 grade 3 pain and grade 3 joint swelling: unrelated to IMP. Grade 1–2 respiratory events (20%) and thyroid disorders (20%). One discontinued: grade 2 pneumonitis</td>
<td>Moskowitz et al (2014)</td>
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<td>Lymphoid disease</td>
<td>Agent and dosing</td>
<td>Mechanism</td>
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<tr>
<td>Relapsed or Refractory lymphoid malignancies</td>
<td>Dose escalation design (1 mg/kg and 3 mg/kg) of nivolumab administered every two weeks for up to two years.</td>
<td>Anti-PDCD1 fully human mAb IgG4</td>
<td>1 11 DLBCL, 10 FL</td>
<td>11 DLBCL, 10 FL: ORR 36%, (CR 9%, PR 17%), PFS at 24 weeks: DLBCL 24%</td>
<td>Not stated</td>
<td>Most cases were not positive for CD274 and did not have 9p alterations</td>
<td>SAEs in B-NHL: pneumonitis (7%), ARDS, dermatitis, diplopia, enteritis, eosinophilia, mucositis, pyrexia and vomiting, each in 3%. SAEs in T-NHL: pneumonitis, rash, and sepsis, each in 4%. SAEs in MM: pneumonitis, myositis, each 4%</td>
<td>Lesokhin et al (2014)</td>
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<td>8 other B-NHL, 2 FL: ORR 40%, Other B-NHL 58%</td>
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<td>PMBCL, 13 (CR 10%, PR 30%) MF</td>
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<td>5 PTCL, 5 MF: ORR 15%, PTCL 10%</td>
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<td>other T-NHL (all PR) MF</td>
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<td>27 MM PTCL ORR</td>
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<td>1 CML</td>
<td>CML 100%</td>
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<td>All ≥ 3 prior treatments No ORR in MM, other T-NHL, other B-NHL, CML and PMBCL</td>
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ORR, overall response rate; CR, complete remission; PR, partial response; PFS, progression-free survival; SD, stable disease; PD, progressive disease; OS, overall survival; SAE, serious adverse event; AE, adverse event; DLBCL, diffuse large B cell lymphoma; FL, follicular lymphoma; B-NHL, B cell Non-Hodgkin lymphoma; HL, Hodgkin lymphoma; cHL, classical Hodgkin lymphoma; PMBCL, primary mediastinal B cell lymphoma; PTCL, peripheral T cell lymphoma; MDS, myelodysplastic syndrome; AML, acute myeloid leukaemia; CML, chronic myeloid leukaemia; CLL, chronic myeloid leukaemia; NMC, mantle cell lymphoma; MM, multiple myeloma; T-NHL, T cell Non-Hodgkin lymphoma; MF, Mycosis Fungoides; ARDS, acute respiratory distress syndrome; MTD, maximum tolerated dose; DLT, dose limiting toxicity; ACST, autologous stem cell transplantation; mAb, monoclonal antibody; BV, brentuximab vedotin; FISH, fluorescence in situ hybridization; GEP, gene expression profiling; IMP, investigational medicinal product.
complete response (CR) of over 31 months and one patient with FL achieved a partial response (PR) lasting 19 months. Ipilimumab was shown to increase T cell proliferation greater than two-fold, in the third [5 of 16 cases tested (31%)] of patients tested with Keyhole limpet haemocyanin/odor tetalus antigen stimuli. Three dose-limiting toxicities (DLT) were noted in the first 12-patient cohort (all grade 3 diarrhoea) and no DLT was noted in the second cohort of six patients.

Pidelizumab was first tested in multiple types of haematological cancers in a phase I, non-randomized, open-label dose escalation trial (Berger et al, 2008). Eighteen patients were dosed with a single intravenous (iv) infusion. Three patients were dosed at each of the following doses 0, 0.5, 1, 1.5, 3 and 6 mg/kg. No major adverse events were noted and the maximum tolerated dose (MTD) was not reached. Over the 21 days of the study, a single patient with acute myeloid leukaemia (AML) reduced their peripheral blood blast count from 50% to 5%. The other seven patients with AML exhibited no change in blast count. At 21 days, all patients with lymphoproliferative disease and lymphoma had stable disease (SD). The cumulative OS at 21 days was 76% with a mean OS of 25 weeks. There was a notable case of a patient with FL who entered CR for 68 weeks when the data was censored and published.

**Diffuse large B-cell lymphoma**

Following the initial interest of the durable CR of a patient with Diffuse large B-cell lymphoma (DLBCL) in the phase I ipilimumab study (Ansell et al, 2009), pidelizumab was investigated in relapsed or refractory DLBCL post-autologous stem cell transplantation (ASCT) in a phase II open label trial of 66 patients (Armand et al, 2013). CD274 is known to be expressed by at least a subgroup of patients with DLBCL and PMBCL (Rosenwald et al, 2003; Green et al, 2010; Andersky et al, 2011; Li et al, 2012). It was hypothesized that this expression and activity within the tumour micro-environment may result in immune evasion. The authors of the study considered the setting of low volume, minimal residual disease (MRD) in the context of remodelling immunity post-ASCT to be ideal for further study of enhanced PDCD1 blockade with the aim to further eradicate MRD.

Sixty-six patients with DLBCL were treated with 1.5 mg/kg of pidelizumab from one to 3 months post-ASCT every 6 weeks for a maximum of three cycles (Armand et al, 2013). Of the 66 patients within the study, 35 had measurable disease at screening post-ASCT by standard CT. Of those 35 patients, 34% achieved a CR following pidelizumab, 17% a PR and 37% had SD. The overall progression-free survival (PFS) for all patients was 68% (90% CI: 59–77%) at 16 months with an OS of 84% (90% CI: 77–91%). The most frequently reported grade 3 to 4 toxicities were neutropenia (19%) and thrombocytopenia (8%). In the discussion, the authors recognized that the design of the study did allow the possibility that some responses seen could relate to late cytotoxic effects and that residual measurable ‘disease’ could have reflected treated tumour (Armand et al, 2013). These findings have, however, led to a further worldwide ongoing phase II clinical trial investigating PDCD1 inhibition (nivolumab) in patients with relapsed, refractory DLBCL that have either failed or are unfit for ASCT (NCT02038933).

**Relapsed follicular lymphoma**

As described above, immune checkpoint inhibition was initially investigated in relapsed non-Hodgkin lymphoma (NHL) in a dose escalation phase I ipilimumab study that included within it 14 patients with FL (Ansell et al, 2009) and reported a single PR. Notably a patient with FL in the pidelizumab phase I dose escalation study obtained a very durable CR (Berger et al, 2008).

A recently published phase II study investigated the combination of pidelizumab with rituximab in 30 patients with relapsed FL (Westin et al, 2014). Patients had a median age of 61 years and had received between one and four previous lines of treatment. Pidelizumab at 3 mg/kg was given every 4 weeks for four cycles alongside four weekly doses of rituximab 375 mg/m², which started 17 days after the first pidelizumab treatment. Patients assessed as obtaining SD or better were able to receive a further eight cycles of four-weekly pidelizumab. With a median follow-up of 15.4 months and a mean 10 doses of pidelizumab, 19 of 29 patients achieved an overall response (ORR 66%) with 52% having achieved a CR and 14% a PR. The median PFS was 18.4 months. No grade 3–4 toxicity was observed. It is possible that adverse immune-mediated toxicity was suppressed by rituximab within this study. The group also studied a number of interesting genetic and cellular markers in correlative analyses. Gene expression profiling (GEP) was performed on tumour specimens from baseline in 18 of the 30 patients. The group found that gene signatures associated with upregulated T-cell activation and/or T-regulatory cell suppression were significantly predictive of PFS. Moreover, in the eight patients whose paired biopsies post-treatment were analysed, increased expression of T-cell activation signatures by GEP was also associated with improved PFS. Mean fluorescence intensity of PDCD1, CD274 and PDCD1LG2 on peripheral blood CD4-positive T cells, CD8-positive T cells and CD14-positive monocytes was investigated by flow cytometry from peripheral blood mononuclear cells in 18 responders and seven non-responders. This exploratory analysis demonstrated an enhanced expression of absolute numbers of CD4-positive effector and memory T cells post-treatment and that patients with higher expression of CD274 in the peripheral blood had better initial response rates (CD4: P = 0.04, CD8: P = 0.001, CD14: P = 0.03).

**Classical Hodgkin lymphoma**

The most exciting new development in the evolution of immune checkpoint inhibition is the recent data published.
and presented regarding PDCD1 blockade in classical HL (cHL). A recent article published in the New England Journal of Medicine described outstanding results for nivolumab treatment in a phase I study in relapsed, refractory cHL (Ansell et al, 2014). Similar, although less mature, data was also recently presented at the 2014 American Society of Hematology (ASH) conference regarding pembrolizumab in a similar patient cohort (Moskowitz et al, 2014). Twenty-three young patients (median age 35 years, range 20–54 years) with relapsed, refractory cHL received nivolumab in a dose escalation schedule from 1 mg/kg to 3 mg/kg (Ansell et al, 2014). The primary objective was to evaluate the tolerability and adverse event profile of nivolumab within this heavily pre-treated cohort. No DLT was reached in these patients and therefore the dose of 3 mg/kg on weeks one and four, and then fortnightly thereafter, was taken forward to the dose expansion phase of the study. Patients were treated until progression or CR and up to a maximum of 2 years. Seventy-eight percent of patients on the study had previously received an ASCT and subsequently relapsed on or after brentuximab vedotin (BV). Conventional computerized tomography (CT) of the neck, chest, abdomen and pelvis was performed to follow disease response at regular time intervals with PET CT being used to confirm CR. Grade 3–4 adverse events occurred in 12 participants (52%); the most common grade 3–4 toxicities seen were rash (22%) and grade 3–4 thrombocytopenia (17%). Additional grade 3 toxicities that were felt likely to be related to nivolumab included lymphopenia, myelodysplastic syndrome, increased lipase, pancreatitis and stomatitis. Despite these adverse effects, the median number of treatment doses received by the cohort was 16 (range 6–37) with 15 patients receiving >90% of the intended dosing schedule (Ansell et al, 2014). Nivolumab-induced PDCD1 inhibition resulted in an impressive ORR of 87% (Ansell et al, 2014). A 17% CR rate and 70% PR rate were noted in these 20 patients. The PFS for this heavily-pre-treated cohort was 86% at 24 weeks. Interestingly, in the five patients who had not been exposed to BV, three achieved a CR. These numbers are small but raise the possibility that CD30-positive T-cells may be required for full activity of the drug. Ten patients within the cohort had tumour biopsies available for analysis of both CD274 and PDCD1LG2 protein expression by immunohistochemistry in addition to an analysis of CD274 and PDCD1LG2 copy numbers within the Hodgkin cells by fluorescence in-situ hybridization (FISH). All tumour tissue examined displayed an increase in CD274 and PDCD1LG2 protein expression and all tumour cells displayed between 3 and 15 copies of CD274 and PDCD1LG2, in keeping with underlying chromosome 9p copy gain, amplification or polysomy.

These exciting results were presented at ASH alongside similarly impressive, albeit early data from a phase I dose escalation study investigating PDCD1 inhibition in approximately 106 patients evaluating the safety, tolerability, and efficacy of pembrolizumab monotherapy in a range of haematological cancers, including myelodysplastic syndromes, multiple myeloma (MM), cHL, PMBCL and NHL (Moskowitz et al, 2014). The data presented from the KEYNOTE-013 phase 1b study evaluated pembrolizumab monotherapy in 29 patients with relapsed, refractory cHL at 10 mg/kg every 2 weeks in patients who had progressed on or after BV after failure of ASCT, or who were transplant-ineligible (Moskowitz et al, 2014). The median age of participants was 32 years and they had received a median number of four prior treatments. All patients had received prior BV and the majority had failed prior ASCT. This study is ongoing, and at the data cut-off on 17 November 2014, the initial ORR in this sub-cohort was a very promising 66%, with 21% achieving a CR and 45% a PR, albeit at an early 12-week evaluation time point. Results of this study are immature and it is possible that they will improve with further follow-up. Grade 3 adverse events included axillary discomfort, joint swelling and pneumonitis in one patient each. One patient discontinued the treatment due to pneumonitis; an adverse effect well described with immune checkpoint inhibition, particularly with ipilimumab (Hodi et al, 2010). As outlined in Table II, ongoing studies are investigating immune checkpoint inhibition in cHL, alone or in combination with novel agents. For example, a phase 1 study of ipilimumab with BV in relapsed or refractory cHL is currently recruiting (NCT01896999).

Peripheral T cell lymphoma

The final set of data presented at ASH 2014 described the responses to single agent nivolumab in a range of relapsed, refractory lymphoid malignancies in an open label, dose escalation phase I study (Lesokhin et al, 2014). A range of lymphoid tumour types were treated within the study including DLBCL, FL, cHL and MM. Five patients with relapsed, refractory peripheral T cell lymphoma (PTCL) displayed an ORR of 40% (all PR), although with such few patients it is hard to draw conclusions as to the efficacy in this disease. Of note, the ORR for FL and DLBCL was 40% and 36%, respectively.

Future directions

These exciting new developments will undoubtedly lead to further assessment of immune checkpoint inhibition in many other settings within lymphoproliferative disorders. Table II summarizes all of the ongoing clinical trials investigating checkpoint immunotherapy in this disease area. At this stage there are many unanswered questions about the timing of such treatment, the ability to be able to combine immune checkpoint inhibition therapy with standard chemotherapy or other immunotherapy (including immunomodulatory agents and cellular therapies), the relative risk and benefit of enhancing endogenous immunity pre- and post-ASCT or allogeneic stem cell transplantation (SCT), and the correlative benefits
### Table II. Ongoing trials of checkpoint inhibition.

<table>
<thead>
<tr>
<th>ClinicalTrials.gov Identifier</th>
<th>Study title</th>
<th>Phase trial</th>
<th>Checkpoint inhibition</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT01592370</td>
<td>Safety study in Nivolumab alone and in combination with Ipilimumab or Lirilumab in lymphoma and multiple myeloma</td>
<td>I</td>
<td>Anti-PDCD1 +/- anti-CTLA4 or +/- anti-KIR2DL1/2DL3</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT02253992</td>
<td>Study of the safety and tolerability of Urelumab administered in combination with Nivolumab in solid tumours and B-cell Non-Hodgkin Lymphoma</td>
<td>I/II</td>
<td>Anti-PDCD1 +/- Anti-CD137</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT02181738</td>
<td>Non-comparative, Two-cohort, Single arm, Open-label, Phase 2 study of Nivolumab in classical Hodgkin lymphoma (cHL) subjects after failure of autologous stem cell transplant (ASCT)</td>
<td>II</td>
<td>Anti-PDCD1</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT02038946</td>
<td>A single arm, Open-label phase 2 study of Nivolumab (BMS-936558) in subjects with relapsed or refractory follicular lymphoma (FL)</td>
<td>II</td>
<td>Anti-PDCD1</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT02038933</td>
<td>A single-arm, Open-label, Phase 2 study of Nivolumab (BMS-936558) in subjects with relapsed or refractory diffuse large B-cell lymphoma (DLBCL) after failure of autologous stem cell transplant (ASCT) or after failure of at least two prior multi-agent chemotherapy regimens in subjects who are not candidates for ASCT</td>
<td>II</td>
<td>Anti-PDCD1</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT02243579</td>
<td>Pembrolizumab in treating patients with relapsed or refractory stage IIB-IVB mycosis fungoides or Sezary syndrome</td>
<td>II</td>
<td>Anti-PDCD1</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT02289222</td>
<td>Anti-PDCD1 (MK-3475) and IMiD (Pomalidomide) combination immunotherapy in relapsed/refractory multiple myeloma</td>
<td>I/II</td>
<td>IMiD and Anti-PDCD1</td>
<td>Not yet recruiting</td>
</tr>
<tr>
<td>NCT02036502</td>
<td>A study of Pembrolizumab (MK-3475) in Combination with lenalidomide and dexamethasone in participants with multiple myeloma (MK-3475-023/KEYNOTE-023)</td>
<td>I</td>
<td>Anti-PDCD1 with IMID/steroid</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT01953692</td>
<td>A trial of pembrolizumab (MK-3475) in participants with blood cancers (MK-3475-013)(KEYNOTE-013) N.B. MM, NHL, HL, MDS</td>
<td>I</td>
<td>Anti-PDCD1</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT02077959</td>
<td>Lenalidomide and Pidilizumab in treating patients with relapsed or refractory multiple myeloma</td>
<td>I/II</td>
<td>Anti-PDCD1</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT01919619</td>
<td>Lenalidomide and Ipilimumab post allo- or auto-stem cell transplantation (SCT)</td>
<td>Pilot study</td>
<td>Anti CTLA4 with IMiD</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT01750983</td>
<td>A phase I trial of Ipilimumab (Anti CTLA- 4 Antibody) in combination with Lenalidomide (IMiD) in Patients with advanced malignancies</td>
<td>I</td>
<td>Anti CTLA4 with IMiD</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT01822509</td>
<td>Ipilimumab in treating patients with relapsed hematologic malignancies after donor stem cell transplant</td>
<td>I</td>
<td>Anti CTLA4</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT02254772</td>
<td>TLR9 Agonist SD-101, Ipilimumab, and radiation therapy in treating patients with low-grade recurrent B-cell lymphoma</td>
<td>I/II</td>
<td>Anti CTLA4 with Intratumoral Injection of SD-101 (toll-like receptor 9 agonist) and low dose radiation</td>
<td>Recruiting</td>
</tr>
</tbody>
</table>
of treatment relative to CD274/PDCD1LG2 expression. When considering cHL, these questions are particularly relevant and the following section will discuss these questions with reference to cHL.

**A bridge to allogeneic SCT?**

At present, nivolumab is being investigated worldwide in patients who have typically failed ASCT and BV (NCT02181738). Given the demographic of patients here, nivolumab is sometimes being used as a bridge to allogeneic SCT. Given the outstanding ORR described in the phase I trial in a similar setting, this seems a very reasonable approach, although the effects of this immunotherapy on both graft-versus-host disease (GVHD) risk and engraftment rates post-allogeneic SCT is not understood. In the UK, alemtuzumab often forms part of the conditioning regimen for patients with cHL in both sibling and matched-unrelated donor allogeneic SCT (Peggs et al., 2007) and this may abrogate the risk of these complications. The approach of allografting cHL patients may, however, be challenged by the very high response rates and impressive durability of these responses. It remains to be seen however whether immune checkpoint inhibition alone or in combination results in a cure for these patients.

**Use in the front line setting?**

Given the clear efficacy in cHL, it seems highly probable that immunotherapy will be investigated in the first and second line settings. Already there is an ongoing study of ipilimumab with BV in relapsed cHL (NCT01896999). It is unknown at present whether utilizing immunotherapy in the front line setting will be ultimately advantageous for patients. A number of questions arise and are particularly pertinent in cHL where the frontline treatment success rates are excellent. Should immune checkpoint inhibition be used concurrently with chemotherapy, for example AVD (doxorubicin, vinblas-
tine, dacarbazine), in the first line setting? Should it precede or follow standard chemotherapy in an attempt to either avoid chemotherapy or minimize relapse by treating MRD? Does chemotherapy render immune checkpoint inhibition less effective by depleting the microenvironment? Will checkpoint blockade immunotherapy remain as effective in the first line setting if CD274/PDCD1LG2 expression is important for later evasion of the immune system in the relapsed or refractory setting rather than in the initial phase of the disease? And will this differ between different lymphoma subtypes? Much is still to be understood about the correlational importance of protein expression of CD274/PDCD1LG2, PDCD1 and CTLA4 as predictive biomarkers of treatment success, and whether specific underlying genetic aberrations beyond abnormalities in chromosome 9p are important predictive biomarkers.

**Eradicating minimal residual disease/mixed chimerism?**

The benefits of immunotherapy may also be harnessed with the aim to eradicate MRD, with the potential to benefit patients with a range of haematological cancers following both autologous and allogeneic SCT. As discussed, this has been trialled in the relapsed DLBCL setting post-ASCT (Armand et al., 2013), but in the future it may well be that immune checkpoint inhibition is used to enhance the graft-versus-lymphoma/leukaemia effect post-allogeneic SCT and may prove particularly useful in the setting where donor lymphocytes are not available. Early phase I dose escalation trial data suggests that this approach may be feasible (Bashey et al., 2009). Of the 30 patients treated with ipilimumab post-allogeneic SCT in the study, three patients with lymphoid malignancy developed objective disease responses: complete remission in two patients with HL and one partial remission in a patient with refractory mantle cell lymphoma. Interestingly, no GVHD was noted in the study. The same group showed that ipilimumab can induce T-cell activation without an increase in Tregs in this cohort post-allogeneic

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**Table II. (Continued)**

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<tr>
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<th>Status</th>
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<tbody>
<tr>
<td>NCT01896999</td>
<td>Ipilimumab and Brentuximab Vedotin in treating patients with relapsed or refractory Hodgkin lymphoma</td>
<td>I</td>
<td>Anti-CTLA4 with Anti-CD30 immunonjugate</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT01729806</td>
<td>Ipilimumab and Rituximab In treating patients with relapsed or refractory B-Cell lymphoma</td>
<td>I</td>
<td>Anti-CTLA4 with Anti-CD20 mAb</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT00060372</td>
<td>Ipilimumab after allogeneic stem cell transplant in treating patients with persistent or progressive cancer</td>
<td>I</td>
<td>Anti-CTLA4</td>
<td>Completed</td>
</tr>
<tr>
<td>NCT00047164</td>
<td>Monoclonal antibody therapy in treating patients with lymphoma or colon cancer that has not responded to vaccine therapy</td>
<td>II</td>
<td>Anti-CTLA4</td>
<td>Completed</td>
</tr>
</tbody>
</table>

MM, multiple myeloma; HL, Hodgkin lymphoma; NHL, non-Hodgkin lymphoma; MDS, myelodysplastic syndrome; CTLA4, cytotoxic T-lymphocyte-associated protein 4; IMiD, immunomodulatory drug; mAb, monoclonal antibody; PDCD1, programmed cell death-1.
SCT (Zhou et al, 2011). This therapeutic approach will clearly need careful thought, sensible dosing strategies and accurate data collection on the risk and incidence of GVHD.

An option for elderly Hodgkin lymphoma?

Outcomes in elderly patients with newly diagnosed cHL have been poor when compared to young patients. They are typically not fit enough for curative combination chemotherapy and their disease seems more biologically chemo-resistant (Klimm et al, 2007; Evens et al, 2008). BV is currently being investigated as a monotherapy in the front line setting in the elderly or co-morbid, and also in combination with dacarbazine or bendamustine (NCT01716806). It seems highly likely that immune checkpoint blockade will also be investigated in this setting. The toxicity of autoimmune adverse events will require particular attention in this more vulnerable population.

Combination therapy?

Given the success of targeting this pathway, it is unsurprising that further novel agents are in development, targeting CD274 (BMS935559 (MDX-1105), MEDI4736, MSB0010718C) and PDCD1 (BMS935559 (MDX-1105), MPDL3280A, MEDI4736, AMP-224). The utility of combining immunotherapy is now well described in the solid tumour setting, albeit with the ongoing concern regarding enhanced autoimmune-related toxicity (Wolchok et al, 2013). A study of ipilimumab and nivolumab in combination in relapsed, refractory metastatic melanoma showed that in the non-randomized setting, concurrent nivolumab and ipilimumab was associated with ORR that exceeded the previously reported results with either immunotherapy alone. Notably, this came at a cost of toxicity. Grade 3–4 treatment-related toxicities were seen in 53% of patients, compared to previous rates of 20% with ipilimumab monotherapy and 15% nivolumab monotherapy. Pneumonitis, colitis, hepatitis, rash and uveitis were concerning adverse events. It remains to be seen whether this pattern of toxicity and efficacy holds true in patients with lymphoma.

Lenalidomide has shown interesting properties of immunomodulation and enhancement of endogenous cytotoxic T-cells in recent studies. At a cellular level, lenalidomide affects the tumour microenvironment. In vitro studies have demonstrated enhanced T cell activity in chronic lymphocytic leukaemia (Chang et al, 2006) and the ability to repair T cell effector synapses in FL (Ramsay et al, 2009). A recent phase II trial (Fowler et al, 2014) of rituximab and lenalidomide in relapsed indolent NHL noted how lenalidomide treatment increased the numbers of CD8-positive T cells within the tumour alongside an increased number of PDCD1-positive cells, suggesting activation of infiltrating CD8-positive T cells. In pre-clinical studies in myeloma, in vitro exposure to lenalidomide gave rise to a decrease in Tregs and a decrease in T-cell expression of PDCD1. Both these mechanisms promote T-cell proliferation (Luptakova et al, 2013). Ongoing studies are investigating its use in combination with immune checkpoint blockade (NCT02077959, NCT01919619 and NCT01750983).

Vaccination studies?

A major goal of immunotherapy has long been to create tumour vaccines for patients that specifically target tumour surface proteins and enable long-term endogenous tumour immunity. This approach to date has produced mixed results but has largely been unsuccessful (Muraro et al, 2013). The development of immune checkpoint inhibition may well re-invigorate the investigation of combining vaccination with immunotherapy in lymphoma and other cancer types. An example of particular interest may be ALK-positive anaplastic large cell lymphoma (ALCL) where the pathological translocation t(2;5) creates the fusion protein NPM1-ALK. Patients with long term disease free survival following treatment for ALK-positive ALCL have been noted to produce endogenous anti-NPM1-ALK antibody activity (Pulford et al, 1997, 2000; Ait-Tahar et al, 2010). It may be possible in the future to enhance this type of antibody response using immunotherapy and vaccination in a number of lymphoma subtypes after debulking the initial disease with conventional chemotherapy.

Response assessment?

Modern response assessment for many lymphoma subtypes involves fluorodeoxyglucose-postion emission tomography (FDG-PET). Whilst it is well demonstrated that PET is more accurate at staging, for example cHL patients than CT and interim scans after two (or one or three) cycles of conventional combination chemotherapy provide useful prognostic information, their utility for assessing response to immunomodulators is unknown (Terasawa et al, 2009; Cheson et al, 2014). It is possible that the T-cell activation caused by immune checkpoint inhibition would result in a persistently positive PET signal despite excellent clinical efficacy. This may be a reason why in the phase 1 Hodgkin study, only a 17% CR rate was observed despite an excellent 87% ORR rate and impressive PFS (Ansell et al, 2014). This potential phenomenon will need careful and systematic evaluation in the future.

Summary

Immune checkpoint inhibition represents a significant success for translational medicine. Pre-clinical studies identified first CTLA4 and then PDCD1 and its ligands as important molecules regulating the immune response to both exogenous antigen and tumour. Animal testing showed proof of concept that immune checkpoint inhibition represented a
sensible strategy whilst also highlighting possible safety concerns. Initial development in solid tumours has led to some of the most dramatic results seen since the advent of trastuzumab (Herceptin®) for breast cancer. Although investigation of these agents in haematological malignancies has lagged behind, the demonstrable efficacy in cHL will encourage further study in lymphoid disorders. High early response rates have been seen with both these agents as monotherapy, in a relatively non-toxic manner. These agents are likely to represent a new treatment paradigm in cHL, but follow-up data at present is short. This approach very nicely complements the other main translational strategy of genomic investigation resulting in targeting of actionable mutations. Combining these strategies in the future offers real hope of using more effective, better-tolerated therapeutics, hopefully reducing the burden of late effects seen following conventional cytotoxic chemotherapy and radiotherapy.

Acknowledgment

Many thanks to Dr Caroline Watson who provided expert assistance in the production of Fig 1.

Funding source: Nil specific for the paper.

Author contributions

TE and GC wrote the manuscript. TE Produced Tables I and II. TE and GC reviewed and revised the manuscript.

Conflict of interest

Nothing to report.

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Zhou, J., Bashey, A., Zhong, R., Corranging, S., Messer, K., Pu, M., Ma, W., Chut, T., Souillet, R., Mitrovich, R.C., Lowy, I. & Ball, E.D. (2011) CTLA-4 blockade following relapse of malignancy after allogeneic stem cell transplantation is associated with T cell activation but not with increased levels of T regulatory cells. *Biology of Blood and Marrow Transplantation : Journal of the American Society for Blood and Marrow Transplantation*, 17, 682–692.