Immune checkpoint inhibitors: new strategies to checkmate cancer

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Summary

Immune checkpoint inhibitors (ICIs) targeting cytotoxic T lymphocyte-associated protein-4 (CTLA-4) or programmed cell death protein 1 (PD-1) receptors have demonstrated remarkable efficacy in subsets of patients with malignant disease. This emerging treatment modality holds great promise for future cancer treatment and has engaged pharmaceutical research interests in tumour immunology. While ICIs can induce rapid and durable responses in some patients, identifying predictive factors for effective clinical responses has proved challenging. This review summarizes the mechanisms of action of ICIs and outlines important preclinical work that contributed to their development. We explore clinical data that has led to disease-specific drug licensing, and highlight key clinical trials that have revealed ICI efficacy across a range of malignancies. We describe how ICIs have been used as part of combination therapies, and explore their future prospects in this area. We conclude by discussing the incorporation of these new immunotherapeutics into precision approaches to cancer therapy.

Keywords: antibodies, cancer, T cell, tumour immunology

Introduction

There are extensive interactions between tumour cells and the components of the immune system. The process of immune surveillance ensures that aberrant cells with tumorigenic potential can undergo immune destruction before they develop into cancers, and from the very earliest stages of tumour development the tumour microenvironment (TME) contains often-substantial populations of leucocytes, including various subsets of T cells [1,2]. Effector T cell responses against tumour antigens are induced early during tumour development. Local dendritic cells (DCs) can acquire and process tumour proteins from lysed tumour cells, including mutated versions of normal proteins, and present peptides from these proteins to naive T cells in secondary lymphoid organs. This has the potential of generating potent tumour-specific effector T cells that could home to the tumour and facilitate selective tumour cell killing. However, tumours evolve diverse mechanisms of immune evasion and immunosuppression to prevent or restrain anti-tumour T cell responses [3,4]. This includes changing their antigen profile (to make them unrecognizable to effector T cells [5,6]), blocking T cell recruitment [7,8] and exploiting immunosuppressive leucocytes, such

as regulatory T cells (T_{regs}) and tumour-associated macrophages [9–11]. In addition, cancer cells and/or the TME can produce molecules that actively inhibit any tumour-specific T cells that manage to enter the tumour [12–14]. All these processes are potential targets for therapeutic interventions that aim to induce, reinstate or enhance anti-tumour T cell responses. There have been several exciting recent successes with cancer immunotherapies: principal among these are antibodies targeting the 'immune check-point' proteins cytotoxic T lymphocyte-associated protein-4 (CTLA-4) or programmed cell death protein 1 (PD-1).

Enhancing anti-tumour T cell responses with anti-CTLA-4 antibodies

Full activation of naive T cells results in clonal expansion and the development of effector functions. This requires positive signals from several membrane receptors. 'Signal one' comes from the T cell receptor (TCR) after it engages cognate antigen displayed on target cell major histocompatibility complex (MHC), but secondary positive signals (co-stimulation) are also essential, while further signals through cytokine receptors shape effector T cell phenotype [15–18]. Co-stimulatory signals are delivered principally

through CD28 receptors after they bind CD80 and CD86 (also known as B7·1 and B7·2, respectively), which are expressed abundantly on mature DCs. CTLA-4 also binds to CD80/86 with higher affinity than CD28, but it regulates co-stimulation negatively [19,20]. CTLA-4 is induced transiently on activated T cells, peaking 2–3 days after initial activation [19], and is expressed strongly by T_{reg}: naturally occurring T_{reg} (nT_{reg}) are the major cell type constitutively expressing this molecule [9]. The receptor CTLA-4 is reported to transduce negative intracellular signals [21,22] and enhance T cell motility [23,24], but perhaps more significant is its ability to outcompete CD28 molecules for CD80/86 binding [19,24,25]; displace CD28 to distal regions of the immunological synapse [26,27]; and strip CD80/86 from the surface of dendritic cells [28,29]. These immunosuppressive activities prevent or restrain T cell activation, and ensure that autoreactive T cells, or those that bind weakly to antigen, fail to become activated and instead enter a state of unresponsiveness (anergy).

The immunosuppressive properties of CTLA-4 are evident in *Ctla4*-deficient mice, which develop fatal lymphoproliferative disease and multi-organ failure due to unopposed T cell activation and loss of immunological tolerance [30,31]. These phenotypes are rescued by blocking or deleting CD80/86 or CD28. Moreover, germline heterozygous mutations in *CTLA4* in humans are linked to severe immune dysregulation [32,33], and a *CTLA4* variant is associated with early-onset Crohn's disease and autoimmunity [34]. Individuals with *CTLA4* mutations have defective T_{reg} function, hyperactivated effector T cells and reduced numbers of circulating B cells [32,33].

It is well established that anti-CTLA-4 monoclonal antibodies can block the immunosuppressive properties of CTLA-4 and enhance anti-tumour T cell responses in animal models [35,36]. Blockade induces a reduction in T_{regs} and an increase in effector T cells within the TME, and simultaneous blockade of CTLA-4 on these cells can synergize to enhance anti-tumour responses in animal models [37–43]. Anti-CTLA-4 antibodies can also bind Fcγ receptors (FcγRs) to mediate antibody-dependent cell-mediated cytotoxicity against CTLA-4$^+$ T_{regs}, leading to their selective depletion [40–43]. This is largely dependent upon the presence of atypical, FcγR-expressing macrophages in the tumour [40–43] and this mechanism of intratumoural T_{reg} depletion has been identified in humans treated with ipilimumab [44].

The functional effects of anti-CTLA-4 antibodies on anti-tumour responses in a patient depend upon the characteristics of the patient's tumour, the underlying TME and the nature of any previous or concurrent therapies. Predicting responses is challenging and, given the profound immunological consequences of *Ctla4* deletion or genetic variation in *CTLA4*, the potential side effects of administering anti-CTLA-4 antibodies may be significant. However, the preclinical research and clinical trial data from CTLA4

blockade has provided a proof-of-principle and laid the groundwork for further targeted disruption of other immune checkpoints, such as the programmed cell death-1/programmed cell death ligand-1 (PD-1/PL-L1) axis.

Dismantling tumour defence by inhibiting PD-1 activity

PD-1 is a surface receptor for the cognate ligands PD-L1 and PD-L2. It is expressed predominantly by activated T cells, but is also found on other leucocyte subsets, including activated B cells, DCs, monocytes and natural killer (NK) cells [45–47]. PD-L1 can be found on activated T and B cells, DCs, macrophages and many tissue cells [47–50], while PD-L2 appears limited to DCs and macrophages and some stromal cells [47]. On effector T cells, PD-1 ligation causes dephosphorylation of key signalling molecules that lie downstream of the TCR, thereby dampening TCR-mediated T cell activation [51,52]. It has also been identified recently that PD-1/PD-L1 interactions trigger dephosphorylation of CD28 preferentially over the TCR, and is the primary mechanism of T cell suppression [53]. PD-1 also enhances T cell motility to limit T cell/DC interactions [54]. PD-L1 has also been reported to bind to CD80 and might, like CTLA-4, compete with CD28 [55]. Deletion of *Pd-1* leads to autoimmune disease [56,57], but this develops later in life than the more severe, early-onset disease in *Ctla4*$^{-/-}$ mice [30,31]. Expression of PD-L2 in lymphoid organs is thought to help maintain tolerance [51], while PD-L1, which is up-regulated in response to interferons, particularly IFN-γ, protects tissues from excessive immune cell activity [48,49]. This occurs during chronic viral infection when persistent viral antigen exposure causes T cell exhaustion, a form of anergy [58,59]. In this context, blockade of PD-L1 by administration of anti-PD-L1 antibodies causes a resurgence of effector T cell activity [60], predominantly through reactivation of the CD28 signalling pathway [61]. Thus, the physiological functions of the PD-1 pathway are to maintain T cell tolerance and suppress effector T cell responses in peripheral tissues.

Cancers can exploit the PD-1 pathway, up-regulating PD-1 ligands to suppress T cell-mediated cytolysis. Up-regulated PD-1 expression by tumour-infiltrating lymphocytes is associated with poor outcomes in many human cancers, and correlates with an exhausted T cell phenotype that impedes tumour immunity [62–67]. Early *in-vivo* experiments demonstrated that myeloma cells were targeted more effectively when *Pd-1* was absent, and that anti-PD-L1 antibodies could suppress myeloma in wild-type mice [68]. In other mouse models, transplantation of *Pd-1*$^{-/-}$ T cells [69] or administration of anti-PD-L1 antibodies [68,69] induced regression of established tumours. PD-L1 expression by infiltrating myeloid cells has also been shown to impair anti-tumour T cell immunity [70,71] but, interestingly, this may be due in part to the induction and
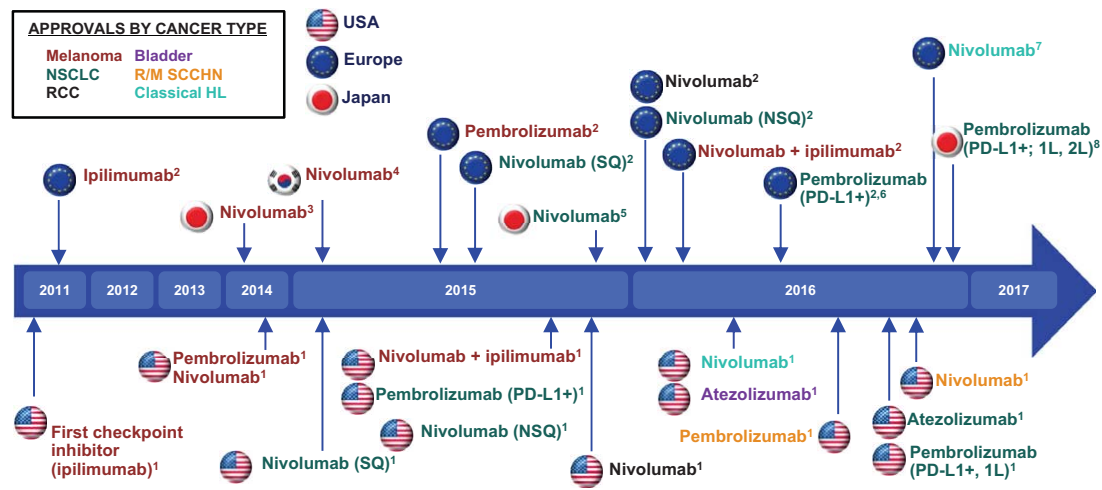
Fig. 1. History of checkpoint inhibitors: key milestones. Timeline showing when checkpoint inhibitors were approved for the treatment of specific cancers in the United States, Europe and Japan. Tumour type is indicated by the colour of each immune checkpoint inhibitor (ICI) depicted in the figure, according to the key in the top left. Abbreviations: 1L = first-line; 2L = second-line; NSCLC = non-small-cell lung cancer; NSQ = non-squamous; PD-L1 = programmed death ligand 1; RCC = renal cell carcinoma; R/M = recurrent/metastatic; SCCHN = squamous cell carcinoma of the head and neck; SQ = squamous. [1] US Food and Drug Administration. http://www.fda.gov, accessed 11 November 2016. [2] European Medicines Agency. http://www.ema.europa.eu, accessed 11 November 2016. [3] ONO Pharmaceutical Co. Ltd [press release], 4 July 2014. [4] ONO Pharmaceutical Co. Ltd [press release]. March 23, 2015. [5] ONO Pharmaceutical Co, Ltd. [press release], 17 December 17, 2015. [6] Merck [press release], 27 June 2016, accessed 8 August 2016. [7] Bristol-Myers Squibb Company [press release], 22 November 2016. [8] Merck [press release], 10 December 2016.

regulation of peripherally induced T_{reg} subsets (iT_{reg}) by the PD-L1-expressing myeloid cells [72]. Disruption of this process may represent an additional mechanism underpinning the enhanced anti-tumour immunity that can be induced by PD-1 blockade.

Translating efficacy from bench to bedside

CTLA-4 and PD-1 provide critical but discrete mechanisms of physiological immunoregulation. A wealth of experimental data shows that antibodies blocking these checkpoints can enhance anti-tumour immune responses in animal models. Despite some concerns over potential toxicities, this drove the development of humanized monoclonal antibodies targeting these molecules. These novel immunotherapies are referred to as 'immune checkpoint inhibitors' (ICIs). These have now been licensed in a number of different tumour types (Fig. 1), and are discussed briefly here.

Anti-CTLA-4: licensing ipilimumab for metastatic melanoma

Ipilimumab (Bristol Myers Squibb, New York, NY, USA), an anti-CTLA-4 antibody, was licensed for malignant melanoma in 2011 [71]. This followed a landmark Phase III clinical trial involving approximately 600 patients with previously treated malignant melanoma [73] (Table 1). There were three treatment arms: ipilimumab plus gp100 vaccine,

ipilimumab alone or gp100 vaccine alone. The gp100 vaccine targets a protein expressed abundantly by melanomas [74,75]. The treatments containing ipilimumab conferred greatest median overall survival (OS): 10·0 months (ipilimumab/gp100) and 10·1 months (ipilimumab monotherapy) *versus* 6·4 months (gp100 vaccine alone). Severe immune-related adverse effects (IAEs) were reported in 10–15% of patients receiving ipilimumab, and were of longer duration in the ipilimumab/gp100 cohort [73]. Established protocols for managing immune-mediated toxicities were adopted from previous trials [76–78]. Subsequent analysis of data from 1861 metastatic melanoma patients in 12 individual clinical trials of ipilimumab showed that OS at 3 years was 20% for previously treated patients and 26% for treatment-naive patients [79]. The survival curve plateaued at approximately 3 years, with evidence in some patients of long-term progression-free survival of up to 10 years.

PD-1 blockade moves into the clinic

The safety and efficacy of pembrolizumab (Merck), a monoclonal antibody targeting PD-1, was demonstrated in patients with ipilimumab-refractory malignant melanoma. In an expansion cohort of a Phase I trial, 173 patients were treated every 3 weeks with pembrolizumab [92]. Equivalent efficacy, as determined by overall response rates (ORR) [93], was observed at both dosing regimens (ORR = 26%). Pembrolizumab was well tolerated: only 12% of patients

Table 1. Key clinical trials of immune checkpoint inhibitor (ICIs) in patients with malignant melanoma and their associated survival data

Trial name and/or Phase [Ref]	Trial drugs (dose)	Patient no.	1-year OS rate (%)	18-month OS rate (%)	2-year OS rate (%)	Median OS (months)
Phase III [73]	Ipi (3 mg/kg) versus gp100 vaccine	676	45·6 versus 25·3	33·2 versus 16·3	23·5 versus 13·7	10·1 versus 6·4
Phase II [76]	Ipi (10 mg/kg) + budesonide versus Ipi (10 mg/kg)	115	55·9 versus 62·4	n.r.	40·5 versus 41·7	17·7 versus 19·3
Phase II [77]	Ipi (10 versus 3 mg/kg)	217	48·6 versus 39·3 versus 39·6	34·5 versus 30·2 versus 23·0	29·8 versus 24·2 versus 18·4	11·4 versus 8·7 versus 8·6
Phase II [78]	Ipi (10mg/kg)	226	47·2	39·4	32·8	10·2
KEYNOTE-006 Phase III [80,81]	Pembro (10mg/kg every 2 weeks) versus Pembro (10mg/kg every 3 weeks) versus Ipi (3mg/kg every 3 weeks)	834	74·1 versus 68·4 versus 58·2	n.r.	55·1 versus 55·3 versus 43·0	n.a. versus n.a. versus 16·0
Phase I [82,83]	Pembro (1–10 mg/kg)	104	62	n.r.	43	16·8
Phase III [84]	Niv (3 mg/kg) versus dacarbazine (1000 mg/m²)	418	72·9 versus 42·1	n.a.	n.a.	n.a. versus 10·8
Phase I [85,86]	Cohort 1: Niv (0·3 mg/kg) +I pi (3 mg/kg)	14	56	n.a.	n.a.	14·8
	Cohort 2: Niv (1 mg/kg) + Ipi (3 mg/kg)	17	94	n.a.	n.a.	N.R.
	Cohort 2a: Niv (3 mg/kg) + Ipi (1 mg/kg)	16	89	n.a.	n.a.	N.R.
	Cohort 3: Niv (3 mg/kg) + Ipi (mg/kg)	6	100	n.a.	n.a.	N.R.
CheckMate-069 Phase II [87,88]	Niv (1 mg/kg) + Ipi (3 mg/kg) versus Ipi (3 mg/kg)	142	73·4 versus 64·8	n.r.	63·8 versus 53·6	N.R.
CheckMate-067	Niv (3 mg/kg)	316	n.a.	n.a.	59	37·6
Phase III [89,90]	Niv (1 mg/kg) + Ipi (3 mg/kg), then Niv (3 mg/kg) maintenance	314	n.a.	n.a.	64	n.a.
	Ipi (3 mg/kg)	315	n.a.	n.a.	45	19·9
CheckMate-064	Niv (3 mg/kg), then Ipi (3 mg/kg)	68	76	n.a.	n.a.	n.a.
Phase II [91]	Ipi (3 mg/kg), then Niv (3 mg/kg)	70	54	n.a.	n.a.	16·9

Ipi = ipilimumab; pembro = pembrolizumab; niv = nivolumab; OS = overall survival; n.a. = not available; n.r. = not reported.

developed drug-related grade 3/4 toxicities. Given the promising clinical responses, good safety profiles and the absence of other effective therapies, pembrolizumab was licensed for ipilimumab-resistant advanced melanoma in 2014 [94].

In 2015, Robert and colleagues demonstrated significant improvements in progression-free survival, OS, response rates and treatment-related adverse events of pembrolizumab monotherapy compared with ipilimumab in advanced melanoma [80] (Table 1). A total of 834 patients were randomized to either pembrolizumab every 2 weeks, pembrolizumab every 3 weeks or four cycles of ipilimumab every 3 weeks. The 12- and 24-month OS was significantly greater in the pembrolizumab groups, and progression-free survival at 6 months was 47·3, 46·4 and 26·5%, respectively, with more durable responses seen in the pembrolizumab groups at 7·9 months [81]. Lower rates of significant immune-mediated toxicities were also seen in the pembrolizumab groups.

A Phase I clinical trial of another anti-PD-1 monoclonal antibody, nivolumab (Bristol Myers Squibb), was conducted in 296 patients with melanoma, non-small-cell lung cancer (NSCLC), renal cell carcinoma (RCC) or other selected treatment-refractory malignancies [82]. Melanoma patients were treated every 2 weeks with increasing doses of 0·1–10 mg/kg and showed overall objective response rates of 28%, with the highest response rate (41%) at a dose of 3 mg/kg. Responses were durable in many responders. Grade 3/4 treatment-related adverse events occurred in 14% of patients, with IAEs in 6%. Longer-term follow-up of melanoma patients showed 1- and 2-year survival rates of 62 and 43%, respectively, with an OS of 16·8 months [83]. Long-term safety evaluation was comparable to the original analysis, with 22% experiencing grade 3/4 treatment-related adverse events and 5% having grade 3/4 IAEs. Toxicities were not cumulative and occurred almost exclusively in the first 6 months of therapy.

In an open-label, randomized, Phase III study involving patients with ipilimumab-refractory melanoma, nivolumab was associated with a higher ORR than chemotherapy (32 versus 11%) [95]. Subsequently, nivolumab was compared with dacarbazine in patients with previously untreated metastatic melanoma without proto-oncogene B-Raf (BRAF) mutation [84]. Nivolumab gave superior 1-year survival (72 versus 42%), progression-free survival (5·1 versus 2·2 months), and ORRs (40 versus 13·9%). The survival benefit with nivolumab was observed across prespecified subgroups, including those defined by PD-L1 status. Grade 3/4 drug-related adverse events occurred in 11·7% of nivolumab-treated patients and 17·6% of those receiving dacarbazine.

Combination regimens of ICIs in patients with advanced melanoma

The distinct mechanisms of actions of anti-PD-1 and anti-CTLA-4 infer that their co-administration could have enhanced efficacy (Fig. 2). The first Phase I study combining two ICIs, nivolumab and ipilimumab, was undertaken in patients with malignant melanoma and used an initial dose-escalation trial design to identify safe doses of these drugs when used in combination regimens [85]. One mg/kg nivolumab and 3 mg/kg ipilimumab were the maximum doses associated with acceptable levels of adverse events. This regimen yielded objective responses in 21 of 52 patients (40%). Durable responses, ranging from 60·1 to 72·1 weeks, were ongoing in 19 patients at the time of publication. Impressively, 16 patients had tumour reduction ≥ 80% at 12 weeks, including five with a complete response, although treatment-related grade 3/4 adverse events were reported in 53% of patients. Updated OS data were 82 and 75% for 1- and 2-year survival, respectively (Table 1) [86]. Thus, this preliminary trial data indicated that simultaneous targeting of PD-1 and CTLA-4 could be tolerated and result in durable responses.

The Checkmate 069 trial compared nivolumab plus ipilimumab, with ipilimumab monotherapy in patients with untreated malignant melanoma (Table 1). Greater objective responses were seen with combination therapy (61 versus 11% with ipilimumab alone), with complete responses in 22% of patients on combination therapy but none with ipilimumab alone [87]. One- and 2-year OS was 73·4 and 63·8%, respectively, in the combination cohort, compared with 64.8% and 53.6% with ipilimumab alone [88]. Importantly, 2-year survival was not affected significantly by BRAF mutation or tumour PD-L1 expression status at diagnosis. A large subsequent Phase III trial (Checkmate 067) compared nivolumab alone, ipilimumab alone and nivolumab plus ipilimumab: median progression-free survival was 6·9, 2·9 and 11·5 months, respectively [89]. Notably, in patients with PD-L1-negative tumours, progression-free survival was greater with combination therapy than either monotherapy. Furthermore, with a minimum follow-up of 36 months, the median overall survival has not been reached in the combination regimen arm and was 37·6 months in the nivolumab arm of the study compared with 19·9 months with ipilimumab monotherapy [hazard ratio (HR) = 0·55, $P < 0·001$ for combination versus ipilimumab; HR= 0·65, $P < 0·001$ for nivolumab versus ipilimumab]. The overall survival at 3 years was 58, 52 and 34% for the combination regimen, nivolumab and ipilimumab monotherapy, respectively. However, treatment-related grade 3/4 toxicities occurred in 59, 21 and 28% of patients treated with the combination regimen, nivolumab and ipilimumab, respectively [90].

A Phase II trial (Checkmate 064) has also revealed intriguing results of switching between nivolumab and ipilimumab [91]. Melanoma patients underwent induction with either nivolumab (3 mg/kg every 2 weeks; six doses) and then ipilimumab (3 mg/kg every 3 weeks; four doses), or the reverse sequence. This was followed by maintenance therapy with nivolumab (3 mg/kg every 2 weeks) in both

cohorts. Greater efficacy was observed in the nivolumab followed by ipilimumab cohort, with significantly greater 1-year OS than the reverse sequence (Table 1). As expected from previous combination trials, the frequency of IAEs was relatively high (43–50%), although this was not affected by the order of drug administration.

Collectively, these trials have allowed ICIs, either alone or in combination, to herald in a new era of immunotherapy for the treatment of malignant melanoma. Impressive

durable responses have led to unprecedented improvements for some patients with metastatic disease. Administering ICIs in combination with other therapeutics will no doubt expand their use further in cancer patients.

New therapeutic approaches for non-small-cell lung cancer

Anti-CTLA-4 therapy has been trialled in patients with NSCLC, but promising outcomes akin to those seen in patients with melanoma have not been forthcoming. One initial trial used tremelimumab, an anti-CTLA-4 antibody, but it showed minimal efficacy and a significant side-effect burden [96] and, when used in combination with chemotherapy, anti-CTLA-4 antibodies have consistently demonstrated only modest improvements in responses in NSCLC patients [97,98]. However, antibodies targeting the PD-1/PD-L1 axis have shown greater efficacy and marked reductions in side effects, so have largely replaced anti-CTLA-4 antibodies as the ICI of choice in NSCLC.

In 2015, nivolumab received Food and Drug Administration (FDA) approval for use in patients with advanced squamous-cell NSCLC who progressed during or after platinum chemotherapy (Table 2) [94]. This followed a Phase III trial (CheckMate-017) of nivolumab compared with docetaxel chemotherapy in patients with progressive disease following first-line chemotherapy [99]. Median OS was 9·2 months in the nivolumab cohort, compared with 6·0 months in those receiving docetaxel. At 12 months, OS was 42% with nivolumab and 24% with docetaxel.
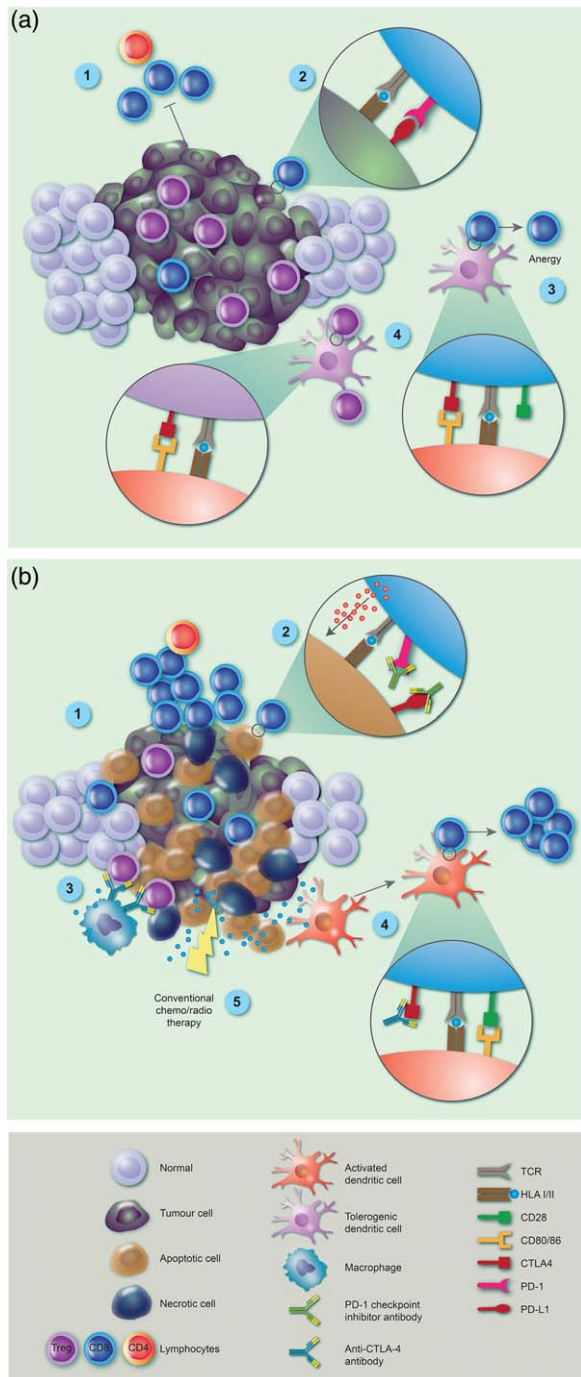

Fig. 2. Immune checkpoint inhibitor (ICI) blockade reverses tumour-mediated immune suppression. (a) Established tumours block immune attack through a variety of mechanisms, including inhibition of tumour-specific cytotoxic T lymphocyte (CTL) and CD4 T cell activation and function (1). This is driven by tumour over-expression of programmed cell death protein 1(PD-L1), interacting with tumour-specific T cell PD-1 receptor (2) and T cell anergy induced by tumour-mediated T cell expression of cytotoxic T lymphocyte-associated protein 4 (CTLA-4) inhibitory receptor (4). In addition, tolerogenic dendritic cells (DC) drive regulatory T cell (T_{reg}) induction and expansion via CTLA-4 (3) and accumulation of T_{regs} then contributes to the immunosuppressive milieu of the tumour microenvironment (TME). (b) After ICI therapy, there is re-activation and proliferation of tumour-specific CTLs via blockade of the PD-1 axis (1), and return of functional cytotoxicity, resulting in perforin release and tumour cell killing (2). As tumour damage increases, the TME is disrupted allowing macrophages to deplete T_{regs} via fragment crystalline receptor (FcR) binding of anti-CTLA-4 antibody (3). Tumour antigen release is driven by immune lysis of tumour cells which are processed by conventional DC and presented to naive T cells in context of checkpoint inhibition of CTLA-4, enhancing CTL proliferation and function (4). Tumour damage and antigen release is also supplemented by concomitant use of conventional chemo/radiotherapy, which can reveal new tumour-associated antigens and contribute to anti-tumour immune responses (5).

Pretreatment tumour PD-L1 expression was not predictive of efficacy. A similar Phase III trial (CheckMate-057) demonstrated considerable improvements in survival for patients with advanced non-squamous NSCLC treated with nivolumab compared to those receiving docetaxel [100]. OS at 12 and 18 months was higher for patients on nivolumab compared with those on docetaxel. An additional Phase II trial (CheckMate-063) supports the survival benefit of nivolumab, reporting a 1-year OS of 40·8% [101]. Similarly, compared with docetaxel, pembrolizumab showed greater efficacy than docetaxel in advanced NSCLC in a Phase II/III trial (KEYNOTE-010) [102,103]. Patients with $\geq$ 1% PD-L1 expression on the tumour received 2 mg/kg pembrolizumab, 10 mg/kg pembrolizumab or docetaxel, resulting in median OS of 10·4, 12·7 or 8·5 months, respectively. Greater efficacy was seen in patients with high tumour PD-L1 expression. These results echo findings from a similar Phase I study (KEYNOTE-001) investigating pembrolizumab in NSCLC [104], while a Phase III trial (KEYNOTE-024) demonstrated survival benefits, durable responses, increased response rates and reduced treatment-related adverse events with pembrolizumab compared with platinum-based chemotherapy in previously untreated patients, with $\geq$ 50% tumoral PD-L1 expression [105]. This has led to pembrolizumab being licensed for treatment of naive metastatic NSCLC with $\geq$ 50% PD-L1 expression [92]. In contrast, nivolumab did not improve progression-free or overall survival compared with chemotherapy in patients ($n = 423$) with previously untreated stage IV or recurrent NSCLC and with a PD-1 expression of 5% or more [106]. This suggests that robust predictive markers are required to select patients optimally for anti-PD-1 therapy, and that the sequence of administration with other standard therapies may also be relevant for optimal treatment strategies.

In the Phase II for patients with atezolizumab *versus* docetaxel for previously treated non-small-cell lung cancer (POPLAR) trial, atezolizumab (Genentech, South San Francisco, CA, USA), an antibody targeting the PD-L1 rather than PD-1, showed improved OS for NSCLC patients who had progressed following platinum chemotherapy [107]. This study included prospective evaluation of tumour PD-L1 expression, and used immune gene expression to define and quantify effector T cell activity within the TME. It was clear that efficacy was related closely to both PD-L1 expression and effector T cell abundance in the tumour. Future studies should consider incorporating this pretreatment analysis of the presence of active tumour-infiltrating effector T cells that may be a predictive marker of ICI efficacy. The clinical outcomes of POPLAR were reiterated in atezolizumab *versus* docetaxel for patients with previously treated non-small-cell lung cancer (OAK), a Phase III clinical trial demonstrating improved median OS with atezolizumab *versus* docetaxel (13·8 *versus* 9·6 months) in NSCLC patients progressing on platinum

chemotherapy, although improvement in OS was seen regardless of tumour PD-L1 expression [108,109]. As a result of these trials, in 2016 the FDA approved atezolizumab for the treatment of metastatic NSCLC that has progressed on platinum chemotherapy [94].

Significantly, PD-L1/PD-1 inhibitors not only improve OS but they are also associated with fewer treatment-related adverse events ($\geq$ grade 3) than chemotherapy [99,100,102,105–108]. Moreover, they may have broad usage: although strongest responses were seen in patients with high tumour PD-L1 expression, they also showed similar, if not greater, efficacy than chemotherapy in patients with very low tumour PD-L1 expression [99–104,107,108]. It has not yet been possible to stratify patient responses accurately based on PD-L1 expression, although this typically involves the analysis of a single pretreatment tumour biopsy, sometimes taken long before starting checkpoint blockade therapy. In the Checkmate-063 trial, for example, there was a median of 1·3 years between biopsy and commencing nivolumab [101]. Thus, more detailed studies are required to explore the prognostic value of tumour PD-L1 expression, and ideally analysis of effector T cell abundance and phenotype should also be included.

ICIs offer new hope for treating NSCLC which, historically, has poor survival outcomes in patients with metastatic disease, and they have become standard second-line therapies in NSCLC patients. They are also being used as first-line treatments, particularly for patients with PD-L1-expressing tumours, although optimal ICI treatment strategies, with or without chemotherapy, still need to be defined more precisely in this context [111]. Trials using a combination of anti-CTLA4 and anti-PD-L1 antibodies have begun in NSCLC patients, with two Phase I studies demonstrating safety profiles comparable to those seen in melanoma patients (Table 2) [110,112]. The results of ongoing Phase II/III trials are eagerly anticipated [113–115]. Other studies have focused upon including ICIs into treatment strategies either in combination or sequentially after other existing therapies in NSCLC. For example, patients ($n = 713$) with locally advanced unresectable NSCLC were assigned randomly (2 : 1) to receive consolidation therapy with either the anti-PDL-1 antibody durvalumab or placebo every 2 weeks for up to 12 months after definitive chemoradiotherapy (chemotherapy with concurrent radiotherapy). Patients treated with durvalumab had a significantly improved progression-free survival (16·8 *versus* 5·6 months; HR = 0·52, $P < 0·001$) and objective response rates 28·4 *versus* 16%; $P < 0·001$) [116].

ICIs show efficacy in other cancers

ICIs have been, or are being, investigated in clinical trials across a broad range of other cancers in the hope that responses seen in patients with melanoma or NSCLC can be reproduced in patients with other tumour types.

Table 2. Key trials of immune checkpoint inhibitor (ICIs) targeting the programmed cell death-1 (PD-1) pathway in patients with non-small-cell lung cancer (NSCLC)

Trial name, Phase [Ref]	Disease	Trial drugs (dose)	Patient no.	1-year OS rate (%)	Median OS (months)	Objective responses rate (%)	Median PFS (months)	Correlation between tumour PD-L1 expression and response?	Treatment-related adverse events (grade ≥ 3) (%)
CheckMate-063 Phase III [101]	Sq NSCLC	Niv (3 mg/kg)	117	40·8	8·2	14·5	1·9	Yes	17
CheckMate-017 Phase III [99]	Sq NSCLC	Niv (3 mg/kg) versus docetaxel	272	42 versus 24	9·2 versus 6·0	20 versus 9	3·5 versus 2·8	n.r.	7 versus 55
CheckMate-057 Phase III [100]	Non-Sq NSCLC	Niv (3 mg/kg) versus docetaxel	582	51 versus 39	12·2 versus 9·4	19 versus 12	2·3 versus 4·2	Yes	10 versus 54
KEYNOTE-001 Phase I [104]	NSCLC	Pembro (2–10 mg/kg)	495	n.r.	12·0	19·4	3·7	Yes	9·5
KEYNOTE-010 Phase II/III [102,103]	>1% PD-L1 + NSCLC	Pembro (2 mg/kg) versus Pembro (10 mg/kg) versus Docetaxel	1034	43·2 versus 52·3 versus 34·6	10·4 versus 12·7 versus 8·5	n.r.	3·9 versus 4·0 versus 4·9	Yes	13 versus 16 versus 35
KEYNOTE-024 Phase II [105]	>50% PD-L1 + NSCLC	Pembro (200 mg) versus docetaxel	305	n.r.	n.r.	44·8 versus 27·8	10·3 versus 6·0	High PD-L1 a prerequisite for inclusion in trial	26·6 versus 53·3
POPLAR Phase II [106]	NSCLC	Atezo (1200 mg) versus docetaxel	277	47·9 versus 37·8	12·6 versus 9·7	15 versus 15	2·7 versus 3·0	Yes	40 versus 53
OAK Phase III [107,108]	NSCLC	Atezo versus docetaxel	1225	55 versus 41	13·8 versus 9·6	14 versus 13	2·8 versus 4·0	Yes	37 versus 54
Phase 1b [109]	NSCLC	Durvalumab + tremelimumab	102	n.r.	n.r.	0–23	n.r.	n.r.	36
CheckMate-012 Phase 1 [110]	NSCLC	Niv (3 mg/kg) + Ipi versus Niv (3 mg/kg) + Ipi	77	Not calculated versus 69	n.r.	47 versus 38	8·1 versus 3·9	Yes	37 versus 33

Sq = squamous; non-sq = non-squamous; niv = nivolumab; pembro = pembrolizumab; atezo = atezolizumab; ipi = ipilumumab; OS = overall survival; PFS = progression-free survival; n.r. = not reported.

PD-L1 expression has long been known to be associated with tumour aggressiveness and poorer outcomes in patients with renal cell carcinoma (RCC) [62,117]. Nivolumab demonstrated efficacy in a Phase II trial of 168 patients with metastatic RCC [118]. They received 0·2, 2 or 10 mg/kg of nivolumab: median OS was 18·2, 25·5 or 24·7 months, respectively. In a subsequent Phase III study, patients that had previously received anti-angiogenic therapy had a median OS of 25·0 months on nivolumab compared with 19·6 months on everolimus [a mechanistic target of rapamycin (mTOR) inhibitor recommended after failed anti-angiogenic therapy] [119]. Nivolumab was subsequently approved for use in patients with advanced RCC after failing anti-angiogenic therapy [94]. Interestingly, high tumour PD-L1 expression was not associated with improved responses to therapy in this study: median OS in patients with PD-L1 expression $\geq$ 1% was 21·6 and 18·8 months (nivolumab and everolimus, respectively), but 27·4 versus 21·2 months in patients with $\leq$ 1% tumour PD-L1 expression. This emphasizes the need to consider carefully the suitability of using PD-L1 expression alone to predict responses.

Following two clinical trials [120,121], atezolizumab, and more recently nivolumab, have been granted FDA approval for bladder cancer patients with disease progression within 12 months of treatment with platinum-containing chemotherapy [94]. The Phase II trial using atezolizumab improved objective response rates compared with a historical control [120]. Approximately 15% of patients responded and in this case, PD-L1 expression on tumour-associated immune cells correlated with objective responses. Ongoing responses in 84% of responders demonstrate excellent treatment durability, and for a small fraction (5%) a complete response was observed. This trial attempted to define features of the TME associated with responses. In addition to PD-L1 expression, it determined CD8$^+$ T cell infiltration, mutational load and molecular subtype. Several 'immune cell' genes were associated with complete and partial responses to atezolizumab, including the IFN-γ-inducible genes CXCL9 and CXCL10, which encode chemokines that direct leucocyte and CD8$^+$ T cell homing. Mutational load was also significantly higher in responders than non-responders (12·4 versus 6·4 mutations per Mb). High mutational load has also been correlated with responses to anti-CTLA-4 in malignant melanoma [122], and may predict better survival in melanoma patients treated with anti-PD1 therapy [123]. This is most probably because increased mutation will increase the generation of neoantigens recognizable by T cells. Thus, in addition to characterizing PD-L1 expression and the immune cell infiltrate, efforts to predict ICI responses should take into account the mutational landscape of the tumour.

Tumours with mismatch-repair (MMR) defects contain a large number of somatic mutations, so may be particularly sensitive to ICIs. Indeed, in a recent Phase II trial pembrolizumab was administered every 2 weeks to patients with MMR-deficient colorectal cancers ($n = 11$), MMR-proficient colorectal cancers ($n = 21$) or MMR-deficient cancers that were not colorectal ($n = 9$) [124]. The objective response rate and progression-free survival rate at 20 weeks were 40 and 78% for MMR-deficient colorectal cancers; 71 and 67% for non-colorectal MMR-deficient cancers; and 0 and 11% for MMR-proficient colorectal cancers. This apparent sensitivity of MMR-deficient tumours supports the rationale of combining DNA repair inhibitors with ICIs in clinical trials, and a Phase I/II study combining the poly ADP ribose polymerase (PARP) inhibitor, olaparib, with durvalumab in patients with selected advanced malignancies is ongoing [125].

PD-1 blockade has shown positive outcomes in classical Hodgkin's lymphoma. Nivolumab and pembrolizumab are now FDA-approved for treatment of patients who have relapsed after other therapies. Two clinical trials have reported ORRs of 65 and 87%, with complete response rates in 16 and 17% of patients [126,127]. Nivolumab and pembrolizumab are also approved for use in patients with recurrent or metastatic squamous cell carcinoma of the head and neck (SCCHN) whose disease has progressed after administration of platinum-based therapeutics [94]. Pembrolizumab treatment gave a clinically significant ORR and durable responses were evident in some patients [128], while nivolumab improved overall survival compared to standard single-agent therapy (methotrexate, docetaxel or cetuximab) [129]. However, in a Phase III study of pembrolizumab versus standard of care in patients with recurrent or metastatic SCCHN, pembrolizumab yielded a 19% reduction in the risk of death compared with standard of care but this did not meet the prespecified efficacy boundary, although it is possible that subsequent immunotherapy use in the standard of care arm may have confounded the overall survival analysis [130]. Nonetheless, greater differences in overall survival, progression-free survival and objective response rates were seen in patients with PD-1-expressing tumours [130].

Based on a Phase II clinical trial involving 88 patients, avelumab (Merck–Pfizer, New York, NY, USA), a PD-L1 blocking antibody, received FDA approval for use in patients with metastatic Merkel cell carcinoma [94,131]. This is the first ever drug approved for this rare skin cancer, in which Merkel cell polyomavirus infection is a major aetiological factor [132]. Nearly a third of patients showed objective responses, with eight complete responses reported: five grade 3 treatment-related adverse events were noted [131]. Pembrolizumab has also been trialled on 26 patients with this disease, with an ORR of 56% being reported, including four complete responses [133].

In a large Phase III trial involving 1132 patients with extensive late-stage small-cell lung cancer (SCLC),

ipilimumab with chemotherapy *versus* placebo with chemotherapy demonstrated no significant difference in OS [134]. A previous Phase I/II trial had also given disappointing results in SCLC, with no survival advantage for patients receiving ipilimumab [135]. However, nivolumab, either alone or in combination with ipilimumab, showed anti-tumour activity with durable responses and manageable safety profiles in previously treated patients with SCLC [136]. Patients were administered nivolumab (3 mg/kg); nivolumab (1 mg/kg) plus ipilimumab (3 mg/kg); or nivolumab (3 mg/kg) plus ipilimumab (1 mg/kg). Objective responses were seen in 10, 23 and 19% patients, respectively. Pembrolizumab is currently under investigation in patients with PD-L1+ ($\geq$ 1% expression) SCLC, who have progressed during or after platinum-based chemotherapy [137]. Initial results indicate good tolerability and, while the data are preliminary, the initial report shows that 25% of SCLC patients developed a partial response.

A Phase III trial compared ipilimumab against placebo, following radiation, in patients with metastatic castration-resistant prostate cancer that progressed on docetaxel chemotherapy [138]. They reported no significant improvement in OS: median OS was 11·2 months for ipilimumab and 10·0 months for placebo. However, subgroup analysis revealed improved OS outcomes for patients with better prognostic factors. This is perhaps unsurprising, as it is now established that immunotherapies often take longer to show measurable efficacy [139]. Final results from a completed Phase III trial comparing placebo with ipilimumab in minimally symptomatic, previously untreated patients with metastatic castration-resistant prostate cancer should reveal whether there is a role for ICIs in this malignancy [140]. Finally, promising responses have also been observed with anti-PD-1 antibody treatment in patients with refractory gastric cancer [141,142] or hepatocellular carcinoma [143]: results of randomized trials are eagerly awaited.

Thus, in addition to melanoma and NSCLC, ICI monotherapies, particularly those targeting the PD-1 pathway, are yielding positive outcomes in a range of metastatic cancers. Toxicities are evident and need to be managed, and the long-term health implications of ICI monotherapy have yet to be determined. This might be significant, given the improvements in patient survival seen with ICIs, although the issue of resistance to ICIs raises the challenge of how to treat cancers which fail multiple therapies.

Future prospects for ICIs in combination therapies

Clinical trial data have justifiably generated considerable excitement concerning the effectiveness of ICIs. In many cases responses are durable, perhaps even curative, possibly because they release systemic personalized memory T cell responses against multiple patient-specific tumour antigens. However, only subsets of patients respond to ICI monotherapy. Combining ICIs can improve outcomes, and

further ICI combination trials, with careful side-effect monitoring, are certainly merited. There is also a large number of ongoing clinical trials exploring ICIs in combination with other treatment modalities, such as radiotherapy, chemotherapy or molecular targeted therapies [144]. The administration of ICIs after radiotherapy attempts to augment the 'abscopal effect', where localized radiation-induced tumour cell death can deliver anti-tumour immune responses against distant tumour deposits (Fig. 1) [145–150]. ICIs even show activity against tumours viewed classically as poorly immunogenic, such as pancreatic adenocarcinoma and glioblastoma, with efforts aimed at administering ICIs while either modifying the TME, up-regulating neoantigen abundance [151] or, in glioblastoma, potentiating immune responses using adenovirus [152]. Modulating the chemokine composition of the TME [153], or blocking chemokine receptors [154], can also enhance ICI efficacy in animal models, and these approaches may translate to humans. Preliminary data in animal tumour models have shown that depletion of T_{regs} via modified anti-CD25 antibody plus anti-PD-1 antibody can induce complete regression of established tumours [155]. In short, combination therapies have the potential to further improve the activity and scope of ICIs, leading to the induction of durable effector T cell responses in more patients, and throughout a greater range of malignancies.

Integrating ICIs into precision oncology

The future of oncology will undoubtedly involve a precision approach to patient care, with treatment tailored to an individual's disease to maximize efficacy and ameliorate side effects as far as possible. Numerous factors determine a patient's response to ICIs. These include, but are not limited to, the distribution of the target immune checkpoint protein; the immune cell context within the tumour; the availability, immunogenicity and frequency of tumour antigens; the pharmacodynamics and pharmacokinetics of the ICI; and the amenability of the TME for effective drug delivery [156]. The analysis of these factors must be incorporated into the design of future clinical trials so that combinations of biomarkers that predict response to ICI immunotherapy can be identified and exploited. It will also be important to clarify further the reciprocal relationship between tumour cells and the TME in the context of ICI therapies, particularly if they are to be implemented across a range of different malignancies. Clinical trials have evolved from when tumours were classified according to histological observations, and comprehensive molecular, cellular and genetic phenotyping is now possible. Genomic and transcriptomic analysis of pretreatment melanoma has identified a transcriptional signature associated with innate resistance to anti-PD1 therapy, and demonstrated that mutations in the DNA repair gene *BRCA2* are enriched in patients that do respond [123]. The analysis of serial

tumour biopsies taken before, during and after ICI therapy will further enhance understanding of how anti-tumour responses are induced by ICIs, and how tumours adapt to evade or limit these responses. Interestingly, one study has identified mutations in melanoma cells in patients whose disease progressed after initially responding to PD-1 blockade [157], and at least one trial is under way that aims to understand the selection pressures and evolutionary changes that ICIs impose upon tumour cells [158].

Concluding remarks

The development and application of ICIs has been a major advance in the treatment of cancer, dramatically rekindling academic, clinical and commercial interest in anti-cancer immunotherapies. As monotherapies, they show considerable promise and remarkable responses have been seen in some patients. However, it is clear that there is improved efficacy when these ICIs are used as part of a concerted therapeutic approach for management of malignant disease. The key issue to be resolved is generation of sufficient evaluable data from combinatorial trials to define effective parameters for treatment. Results from current trials where ICIs are combined with chemotherapy, radiotherapy or with other immunotherapies are starting to demonstrate which malignancies are particularly susceptible to immune reactivation. These studies are also providing clear evidence that, although ICIs have produced dramatic improvements in some patients, they are not necessarily sufficient to mediate long-term remission or cure in most. Identification and validation of suitable prognostic markers in patient cohorts will also help to stratify treatment modalities and target therapy to those patients most likely to respond. The corollary to this is that suitable prognostic criteria could also minimize the IAEs associated with ICI treatment and immune reactivation [159]. The management of side effects and the evolution of resistance are ongoing concerns, but further advances are anticipated as these new medicines are incorporated into combination therapies and integrated into precision oncology. Progress is likely to be swift, given the current explosion in clinical trials of ICIs and the rapidly expanding number of preclinical studies involving these drugs will no doubt stimulate further clinical applications. The range of new ICIs becoming available, and the novel checkpoint targets being identified and characterized in preclinical studies, means that the resistance or relapse seen in some patients could be reversed by using alternative checkpoint inhibitors. There is also a significant new approach to tumour immunotherapy with the recent FDA approval for autologous gene-modified chimeric antigen receptor (CAR) T cell therapy. This cell therapy (Kymriah; Novartis, Basel, Switzerland) targets CD19 in B cell acute lymphocytic leukaemia and has shown significant efficacy [160]. There are several other CAR-T cell therapies in development and combination

therapy with ICIs is already planned, offering the potential for even greater benefits. ICIs are important new weapons in the oncologist's arsenal, and exciting times lie ahead in the field of cancer immunotherapy.

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Disclosure

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