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REVIEW

Next steps in immuno-oncology: enhancing antitumor effects through appropriate patient selection and rationally designed combination strategies

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Background: Cancers escape immune surveillance via distinct mechanisms that involve central (negative selection within the thymus) or peripheral (lack of costimulation, receipt of death/anergic signals by tumor, immunoregulatory cell populations) immune tolerance. During the 1990s, moderate clinical benefit was seen using several cytokine therapies for a limited number of cancers. Over the past 20 years, extensive research has been performed to understand the role of various components of peripheral immune tolerance, with the co-inhibitory immune checkpoint molecules cytotoxic T-lymphocyte antigen 4 (CTLA-4), programmed death 1 (PD-1), and its ligand (PD-L1) being the most well-characterized at preclinical and clinical levels.

Patients and methods: We used PubMed and Google Scholar searches to identify key articles published reporting preclinical and clinical studies investigating CTLA-4 and PD-1/PD-L1, frequently cited review articles, and clinical studies of CTLA-4 and PD-1/PD-L1 pathway inhibitors, including combination therapy strategies. We also searched recent oncology congress presentations and clinicaltrials.gov to cover the most up-to-date clinical trial data and ongoing clinical trials of immune checkpoint inhibitor (ICI) combinations.

Results: Inhibiting CTLA-4 and PD-1 using monoclonal antibody therapies administered as single agents has been associated with clinical benefit in distinct patient subgroups across several malignancies. Concurrent blockade of CTLA-4 and components of the PD-1/PD-L1 system using various schedules has shown synergy and even higher incidence of durable antitumor responses at the expense of increased rates of immune-mediated adverse events, which can be life-threatening, but are rarely fatal and are reversible in most cases using established treatment guidelines.

Conclusions: Dual immune checkpoint blockade has demonstrated promising clinical benefit in numerous solid tumor types. This example of concurrent modulation of multiple components of the immune system is currently being investigated in other cancers using various immunomodulatory strategies.

Key words: immuno-oncology, cytotoxic T-lymphocyte antigen 4, programmed death 1, peripheral immune tolerance, immune exhaustion, cancer

Introduction

Established cancers develop when they escape immune system regulation [1] and evolve into one of two cancer types. Inflamed cancers are usually immunogenic and rich in innate immune signals, chemokines for recruitment of T cells and other immune cell subsets, as well as tumor infiltration by various immune cell subsets [2]. Conversely, noninflamed cancers are often the end-product of poorly immunogenic transformed cells that have evolved when the host immune system has already eradicated highly immunogenic

transformed cell clones. In noninflamed cancers, there are low or absent chemokine expression, lack of T-cell infiltration, potentially higher numbers of immunoregulatory populations (naturally occurring T-regulatory cells [Treg], myeloid-derived suppressor cells), and denser stroma. Noninflamed cancers comprise the majority of cancers, which, in part, explains the relatively low response rates seen with immunotherapies.

More recently, various mechanisms by which tumors escape immunosurveillance have been identified [3]. These mechanisms are usually induced by tumor cells themselves and/or the microenvironment, although primary or iatrogenic immunosuppression or inefficient activation of effector T cells may have a role (Figure 1 and supplementary Table S1, available at *Annals of Oncology* online). The lack of T cell effector function may be no different from other types of chronic inflammation, such as that seen in infections. More specifically, chronically stimulated effector T cells progressively lose effector function and eventually die. During this progressive decline, typically called exhaustion, immune checkpoint proteins (ICP) play important and dynamic roles. Immune cell death by exhaustion may account for the possibility that some cancers may be immunogenic, although low or absent immune cell infiltration within the tumor is observed [4].

Four issues are critical with respect to T-cell exhaustion in cancer. First, multiple ICPs can be simultaneously expressed [5]. Second, not all ICPs contribute equally to immune cell function and/or dysfunction. Among several co-inhibitory immune checkpoint systems, the CTLA-4/CD80/CD86 and PD-1/PD-L1/PD-L2 pathways have clinically significant roles in peripheral immune tolerance [6]. Third, the net effect on T-cell function is the sum of all co-stimulatory and co-inhibitory molecules simultaneously expressed in T cells. Fourth, T-cell exhaustion often coexists with other immunoregulatory mechanisms within the tumor (Figure 1) [7]. This may explain why single-agent immunotherapies have demonstrated variable efficacy across cancer types and why a combination approach, using agents targeting disease-specific mechanisms of immunosuppression, can be synergistic.

Various immunotherapies targeting distinct aspects of the immune system are either approved for clinical use or in development. This review provides an overview of novel single-agent and combination strategies that target the immune system. We will focus on the combination of CTLA-4 and PD-1 immune checkpoint inhibitors (ICIs), which has recently been approved in the USA for advanced melanoma and is currently being tested in other tumor types. We describe the rationale for this approach, the clinical data to date, and strategies for managing patients receiving combination ICP blockade.

Materials and methods

We used PubMed and Google Scholar searches to identify key articles published since 2004 reporting preclinical and clinical studies investigating CTLA-4 and PD-1/PD-L1, frequently cited review articles about ICPs and the immune system, and clinical studies of CTLA-4 and PD-1/PD-L1 pathway inhibitors, including combination therapy strategies. We also included recent congress presentations from international oncology meetings to cover the most up-to-date clinical trial data and searched the clinicaltrials.gov database to identify ongoing clinical trials of ICI combinations.

Peripheral immune tolerance: focus on the CTLA-4/CD80/CD86 and PD-1/PD-L1/PD-L2 pathways

ICPs are essential for maintaining peripheral self-tolerance during physiologic conditions. Different ICPs operate at various stages, anatomic locations, and impact distinct cell subsets of immune system activation (supplementary Table S2, available at *Annals of Oncology* online) [5]. Most co-inhibitory ICPs, such as CTLA-4 and PD-1, are upregulated in response to T-cell receptor activation as a physiologic response against unnecessary or prolonged immune system activation that may potentially damage normal tissues. CTLA-4 is upregulated early in this process and may induce T-cell inhibition by outcompeting with the costimulatory molecule CD28 for its ligands [8]. CTLA-4 is also required for the suppressive actions of Treg cells in secondary lymphoid organs or other peripheral tissues, including tumor sites [9]. Conversely, PD-1 is highly expressed on activated T cells after prolonged T-cell receptor stimulation [4]. Similar to CTLA-4, PD-1 is also required for their suppressive functions and for development of peripherally induced Treg cells [10, 11]. Therefore, treatment with CTLA-4 inhibitors expands the number of T-cell clones that recognize a broader number of tumor antigens [12], whereas treatment with PD-1 inhibitors preferentially increases the number of preexisting T-cell clones that recognize distinct tumor antigens [13, 14]. The ligands for PD-1, PD-L1 and PD-L2, are physiologically expressed by other immune cells as well as nonimmune cells. However, induction of PD-L1 expression can also be seen in peripheral tissues [8]. In malignancy, the expression of PD-L1 on cancer cells appears to be regulated in a complex set of interactions in part mediated by inflammatory cytokines. Preclinical melanoma models demonstrate an increase in PD-L1 expression in response to IFN-γ and suggest that this is driven by the presence of $CD8^+$ T cells as part of a negative feedback loop [7, 15]. More recent work further highlights the underlying complexity in this system, suggesting specific genetic alterations in the GTPase RAC1 have the ability to modulate PD-L1 expression in melanoma cells [16]. Conversely, it is possible to have induction of PD-L1 that is independent from the presence of tumor-infiltrating lymphocytes (TILs) [17–22]. This observation is clinically relevant because PD-L1-positive, TIL-negative cancers may define a cancer type that may not be responsive to immunotherapies [23].

In summary, CTLA-4 and PD-1/PD-L1 exhibit distinct roles in regulating immune system activation. CTLA-4 limits T-cell activation and clonal expansion, and the PD-1/PD-L1/PD-L2 pathway limits T-cell function in the peripheral tissues, although the extent to which the PD-1 pathway is involved in early T cell priming in addition to modulation of effector function remains to be fully characterized. These spatiotemporal differences in the role of CTLA-4 and PD-1 provide the basis for combined blockade of CTLA-4 and PD-1 to increase effector T-cell response, discussed in further detail below.

Clinical development of inhibitors of CTLA-4 and the PD-1/PD-L1 pathway as single agents in cancers

Table 1 shows key clinical trials testing monoclonal antibodies targeting various ICPs [14, 24–54]. Ipilimumab, a monoclonal

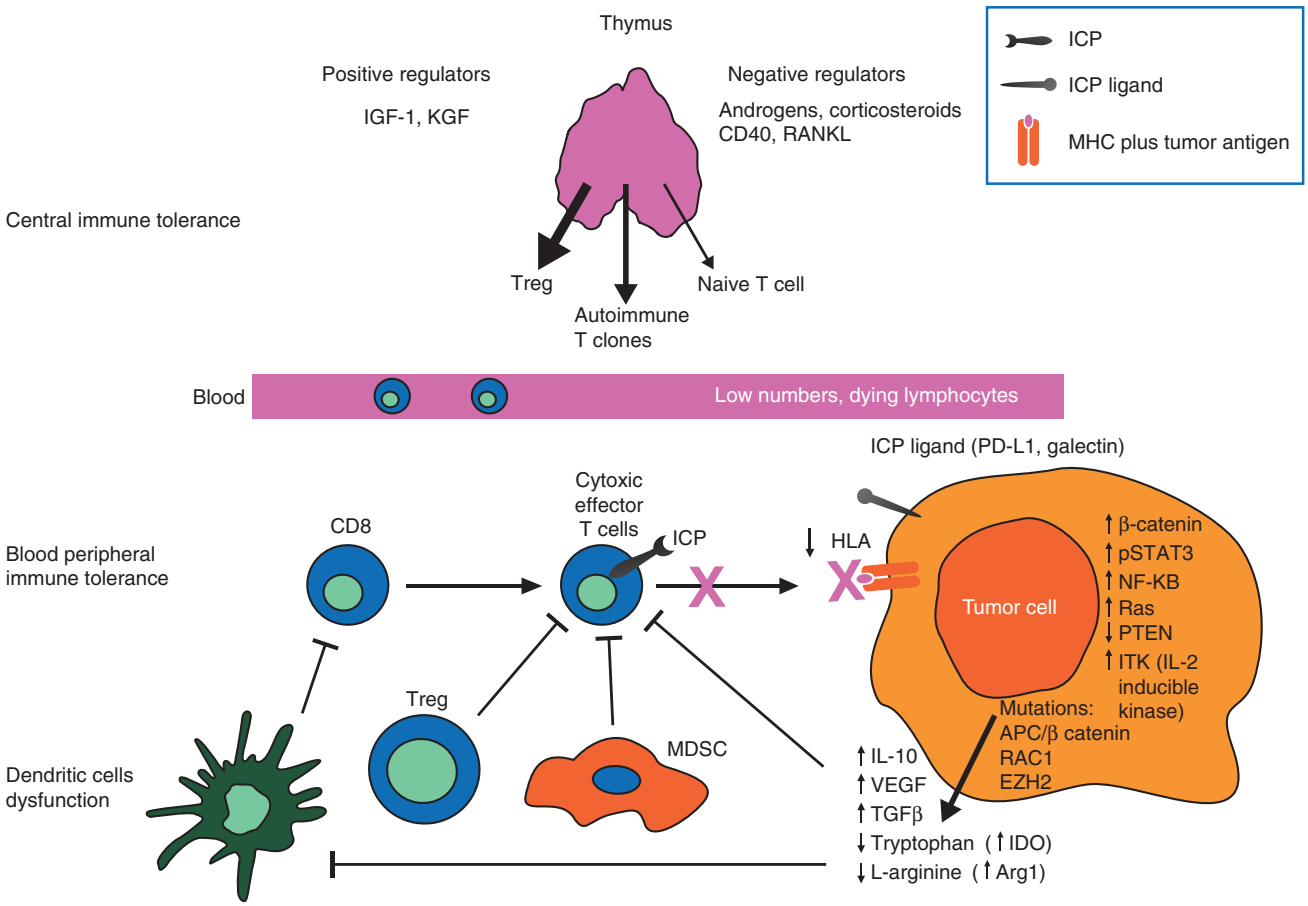

Figure 1. Mechanisms of immune tolerance. Immune tolerance involves a range of overlapping mechanisms that involve not only the periphery (e.g. tumor site), but also central lymphoid organs, especially thymus. They include intrathymic negative regulation (central), decreased costimulation, anergic signals from tumor cells, and immunoregulation (e.g. from Treg and MDSC [peripheral]). Arg1, arginase 1; HLA, human leukocyte antigen; ICP, immune checkpoint protein; IDO, indoleamine 2,3-dioxygenase; IGF-1, insulin-like growth factor; IL-10, interleukin 10; KGF, keratinocyte growth factor; MDSC, myeloid-derived suppressor cell; MHC, major histocompatibility complex; NF-κB, nuclear factor kappa-B; PD-L1, programmed death ligand 1; STAT, signal transducer and activator of transcription; RANKL, receptor activator of nuclear factor kappa-B ligand; TGF, transforming growth factor; Treg, regulatory T cells; VEGF, vascular endothelial growth factor.

antibody against CTLA-4, was approved in the USA in 2011 for the treatment of patients with unresectable or metastatic melanoma on the basis of improved overall survival (OS) in two randomized, controlled phase III trials [24, 55]. In a recent pooled analysis of data from 10 prospective and 2 retrospective studies, including 2 phase III trials, ipilimumab demonstrated long-term OS in ∼20% of patients with advanced melanoma [56]. Although toxicities can be life-threatening, most serious adverse events (AEs) were reversible and treatable in clinical studies using established management algorithms [24, 55]. High-dose ipilimumab (10 mg/kg) has demonstrated increased recurrence-free survival (RFS) of 9 months versus placebo when administered in the adjuvant setting in patients at high risk for relapsing stage III melanoma, although the impact on OS is not yet known [57]. Based on the improvement in RFS, ipilimumab was recently approved by the FDA for this indication. In a randomized phase III trial in metastatic melanoma, tremelimumab, another monoclonal antibody to CTLA-4, was compared against physician's choice chemotherapy, but failed to meet its primary OS endpoint. *Post hoc* analysis suggested that a considerable number of patients who were randomized to the control arm received standard-of-care ipilimumab following progression, potentially confounding the OS difference between these two groups [25].

Nivolumab and pembrolizumab, two monoclonal antibodies against PD-1, were both approved in 2014 for the treatment of patients with unresectable stage III or distant metastatic melanoma and disease progression following ipilimumab and, if harboring a $BRAF^{V600}$ mutation, a BRAF inhibitor [14, 58]. The indications for each agent were subsequently expanded to first-line therapy based on results from two separate studies: nivolumab demonstrated an improvement in OS compared with dacarbazine in patients with previously untreated metastatic melanoma without a $BRAF^{V600}$ mutation [27] and pembrolizumab (at 10 mg/kg every 2 or 3 weeks) showed improved OS when compared to ipilimumab in advanced melanoma patients who had received at most one prior therapy [33]. Based on collective data supporting improved clinical efficacy, as well as reduced rates of toxicity, compared to ipilimumab, PD-1 therapy is established as an option for first-line therapy in patients with advanced melanoma [59]. Additionally, the indications for PD-1/PD-L1 based therapy continue to expand across many tumor types. Patients with advanced, previously treated squamous non-small

Table 1. Selected trials of single-agent immune checkpoint therapies

	Dose	Cancer type	Trial	Primary endpoint	Primary endpoint met (Y/N)	FDA approval status
CTLA-4						
Ipilimumab						
[24]	3 mg/kg Q3W	Previously treated metastatic melanoma	Randomized phase III	OS	Y	Approved
Tremelimumab						
[25]	15 mg/kg Q90D	Treatment-naive, unresectable stage IIIc or IV melanoma	Open-label randomized phase III	OS	N	Not approved
PD-1						
Nivolumab						
[26]	3 mg/kg Q2W	Melanoma. Following ipilimumab, and a BRAF inhibitor, if $BRAF^{V600}$+	Open-label randomized phase III	ORR and OS	Y, ORR (OS pending)	Approved
[27]	3 mg/kg Q2W	Melanoma. *BRAF* WT, treatment-naive	Randomized phase III	OS	Y	Approved
[28]	3 mg/kg Q2W	Squamous-cell NSCLC. Following patient-based chemotherapy	Open-label randomized phase III	OS	Y	Approved
[29]	3 mg/kg Q2W	Advanced RCC	Open-label randomized phase III	PFS	Y	Approved
[30]	3 mg/kg Q2W	Nonsquamous-cell NSCLC. Following patient-based chemotherapy	Open-label randomized phase III	OS	Y	Approved
[31]	3 mg/kg Q2W	Squamous cell carcinoma of the head and neck	Randomized phase III	OS	Y	Breakthrough designation
[32]	3 mg/kg Q2W	Hodgkin's lymphoma	Phase I	Safety	Y	Approved
Pembrolizumab						
KEYNOTE-001 [14]	2 or 10 mg/kg Q3W	Melanoma. Following ipilimumab, and a BRAF inhibitor, if $BRAF^{V600}$+	Phase Ib	ORR	Y	Yes, 2 mg/kg
KEYNOTE-006 [33]	10 mg/kg Q2W or Q3W	Advanced melanoma	Randomized phase III	PFS and OS	Y	FDA approval in first-line setting
KEYNOTE-002 [34]	2 mg/kg or 10 mg/kg Q3W	Melanoma. Following ipilimumab, and a BRAF inhibitor, if $BRAF^{V600}$+	Randomized phase III	PFS and OS	Y	Yes, following ipilimumab and, if $BRAF^{V600}$+, a BRAF inhibitor
KEYNOTE-012 [35]	10 mg/kg Q3W	Squamous cell carcinoma of the head and neck	Open-label phase I	Safety, ORR	Y	Approved
Pidilizumab						
[36]	1.5 mg/kg Q42D	Lymphoma (DLBCL)	Randomized phase II	PFS	Y	Ongoing
PD-L1						
Atezolizumab						
[37]	0.01–20 mg/kg Q3W	Metastatic urothelial bladder cancer	Phase I expansion	N/A	N/A	Approved

Continued

Table 1 *Continued*

	Dose	Cancer type	Trial	Primary endpoint	Primary endpoint met (Y/N)	FDA approval status
BMS-936559						
[38]	0.3–10 mg/kg Q2W	Solid tumors	Phase I	Safety, MTD	N/A	Ongoing
Durvalumab						
NCT02087423 [39]	Q2W	Solid tumors	Phase II	ORR	N/A	Ongoing
NCT01693562 [40]	Dose escalation/expansion Q2W, Q3W or Q4W	Solid tumors	Phase I/II	Safety, ORR	N/A	Ongoing
Avelumab						
NCT02155647 [41]		Merkel cell carcinoma	Phase II	ORR	N/A	Ongoing
PD-L2						
rHIgM12B7; NCT00658892		Melanoma	Phase I	MTD	N/A	Ongoing
CD137						
Urelumab						
[42]	1, 3, and 10 mg/kg Q3W	Advanced cancers	Phase I	Safety, DLT	N/A	Terminated
NCT01471210		Advanced and/or metastatic solid tumors and relapsed/refractory B-cell NHL	Phase I	Safety, MTD, DLT	N/A	Ongoing
KIR						
Lirilumab						
NCT01687387	0.1 mg/kg or 1 mg/kg Q4W	AML	Phase II	Leukemia-free survival	N/A	Ongoing
[43]	0.015, 0.3, 1, 3, 6, and 10 mg/kg Q4W ×4	Hematologic and solid tumors	Phase I	Safety and PK/PD	N/A	Ongoing
LAG-3						
IMP321						
[44]	0.05, 0.25, 1.25, 6.25, and 30 mg Q2W	RCC	Phase I	Safety, MTD, PK, PD	N/A	Completed
BMS-986016	20, 80, 240 mg: 800 mg IV Q2W	CLL, HL, NHL, MM	Phase I	Safety, MTD, PK, PD	N/A	Ongoing
CD200						
Samalizumab						
[45]	50–500 mg/m^2 Q4W	B-CLL, MM	Phase I/II	Safety, MTD, PK, PD	N/A	Completed
CD40						
CP-870,893						
[46]	0.01–0.3 mg/kg	Advanced solid tumors	Phase I	MTD	N/A	Completed
[47]	0.2 mg/kg weekly	Advanced solid tumors	Phase I	Safety, PD	N/A	Completed
Dacetuzumab (SGN-40)						
[48]	Dose escalation	DLBCL	Phase II	ORR	N/A	Completed
[49]	Dose escalation	MM	Phase I	Safety	N/A	Completed
[50]	Dose escalation	NHL	Phase I	Safety, MTD, PK	N/A	Completed

Continued

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Table 1 *Continued*

	Dose	Cancer type	Trial	Primary endpoint	Primary endpoint met (Y/N)	FDA approval status
ChiLob 7/4						
NCT01561911	50–500 mg/m^2 Q4W	Solid tumors, lymphoma, B-cell NHL	Phase I	Safety, MTD	N/A	Completed
OX40 (CD134)						
Anti-OX40						
NCT01644968 [51]	0.1, 0.4, 2 mg/kg Q3W	Advanced solid tumors	Phase I	DLT	N/A	Ongoing
MEDI6383						
[52]	Dose escalation	Advanced solid tumors	Phase I	Safety	N/A	Ongoing
CD70						
ARGX-110						
[53]	0.1, 1, 5, 10 mg/kg Q3W	Advanced solid and hematologic tumors	Phase I	DLT	N/A	Ongoing
SGN-CD70A						
NCT02216890	Nondisclosed	RCC, mantle-cell lymphoma, DLBCL, follicular lymphoma	Phase I	Safety	N/A	Ongoing
CD27						
CDX-1127						
[54]	0.1, 0.3, 1.0, 3.0 or 10 mg/kg every 28 days	Advanced hematologic tumors	Phase I	Safety	N/A	Ongoing

AML, acute myeloid leukemia; B-CLL, B cell chronic lymphocytic leukemia; CLL, chronic lymphocytic leukemia; CTLA-4, cytotoxic T-lymphocyte antigen 4; DLBCL, diffuse large B-cell lymphoma; DLT, dose-limiting toxicity; FDA, US Food and Drug Administration; HL, Hodgkin lymphoma; MM, multiple myeloma; MTD, maximum tolerated dose; N, no; N/A, not available; NHL, non-Hodgkin lymphoma; NSCLC, nonsmall cell lung cancer; ORR, objective response rate; OS, overall survival; PD, pharmacodynamics; PFS, progression-free survival; PK, pharmacokinetics; Q2/3/4W, every 2/3/4 weeks; Q42/90D, every 42/90 days; RCC, renal cell carcinoma; WT, wild type; Y, yes.

cell lung cancer (NSCLC) who received nivolumab had a 41% lower risk of death compared to standard chemotherapy in a randomized phase III trial [28]. Similarly, the hazard ratio for death in patients with metastatic renal cell carcinoma who received nivolumab was 0.73 compared with everolimus in a randomized phase III trial [29] and was 0.70 compared with investigator's choice in patients with squamous cell carcinoma of the head and neck [31]. Early investigation of pembrolizumab in NSCLC and of atezolizumab, a PD-L1 blocking antibody, in advanced urothelial cancer showed high antitumor responses in patients bearing tumors that express high levels of PD-L1 [37, 60]. In addition, pembrolizumab treatment in patients with squamous cell carcinoma of the head and neck resulted in an 18% and 25% ORR in HPV-positive and -negative patients, respectively [35]. Certain aspects of the tumor microenvironment have been associated with favorable immunotherapy responses, such as mutational burden [61–63] and virally driven cancers [64–66], offering insights to the spectrum of activity of co-inhibitory ICPs across more cancers.

Treatment combinations of peripheral ICIs and other strategies

Rationale for combinations other than inhibitors of CTLA-4 and PD-1

There are several barriers that limit responses to immunotherapies and to peripheral immune checkpoint inhibition, in particular. First, constitutive activation of several signaling pathways, such as the Wnt or the PI3K/Akt pathway, prevents influx of TILs [67, 68]. Second, several tumors may have low somatic mutation burden, which has been associated with resistance to immune checkpoint therapies, although this interaction is not completely understood as it is still possible to derive benefit from immunotherapy with a low mutational burden [63]. External beam irradiation has been studied in the context of a combination strategy, and while there are substantial preclinical data to suggest that radiation therapy may synergize with immune checkpoint blockade via various mechanisms, at this point the clinical data are more limited [13, 69]. Third, absolute lymphocyte counts are frequently low in patients with metastatic cancers, which is a result of spontaneous or tumor-cell-induced death [70]. This can occasionally be restored using immunotherapies that promote survival signals for T-cell growth and proliferation, such as high-dose bolus interleukin 2, a T-cell growth factor. Fourth, central (thymic) tolerance, a critical process to prevent autoimmunity, can restrict antitumor responses and limit the generation of tumor antigen-specific effector T cells [71]. Fifth, even within inflamed tumors there are variable degrees of both immunosuppression and peripheral immune tolerance. For example, tumor antigen-specific $CD8^+$ cells that express high levels of two co-inhibitory ICP are more exhausted compared with those that express only one ICP [72], and T cell Ig ad ITIM domain is upregulated on tumor antigen-specific $CD8^+$ cells and $CD8^+$ tumor-infiltrating lymphocytes from patients with melanoma [73]. In addition, tumors may simultaneously contain various immunoregulatory cell types (Treg, myeloid-derived suppressor cells) and/or express high levels of enzymes that breakdown essential amino acids for T-cell growth (indoleamine 2,3-dioxygenase [IDO], arginase), in addition to high levels of ICP. In fact, there is now preclinical and early clinical evidence that targeting the PD-1/PD-L1 pathway in combination with IDO inhibition may be synergistic [74, 75]. Supplementary Table S3 and Table 2, available at *Annals of Oncology* online, show preclinical and clinical evidence, respectively, for combining peripheral ICI with other immunotherapies or treatment modalities [76–82].

Rationale for CTLA-4 and PD-1 combination

CTLA-4 and PD-1/PD-L1 have complementary and synergistic roles in regulating activation via the T-cell receptor [83]. Blockade of CTLA-4 prevents the induction of tolerance and increases the number and repertoire of activated T cells [8, 12, 84]. PD-1 blockade restimulates previously primed T cells that have lost effector and proliferative function during the course of an immune response [4, 5, 12]. Concurrent PD-1 and CTLA-4 blockade restores ability of tumor-infiltrating $CD8^+$ cells to produce IL-2 and therefore stimulates T cell growth, which may inhibit Treg-mediated suppression of antitumor responses [10, 13, 75, 85, 86]. Simultaneous blockade of both CTLA-4 and PD-1 should, therefore, increase the number of T cells participating in an antitumor response and prolong antitumor response by preventing PD-1:PD-L1-mediated downregulation and suppression by Tregs (Figure 2) [17, 87]. A recent study that tested the effects of anti-PD-1 or anti-CTLA-4 alone or in combination in patients' blood and tumor tissue has shown that each treatment induces distinct immunologic effects and no overlapping changes in gene expression [88].

Clinical approaches and efficacy with CTLA-4 and PD-1 blockade

Efficacy of immune-checkpoint combinations

Based on the efficacy seen in preclinical studies, trials using combinations of anti-CTLA-4 and anti-PD-1 have been conducted in patients with melanoma and other cancers.

Phase I trial. A phase I trial evaluated ipilimumab plus nivolumab (I + N) in patients with unresectable stage III or IV melanoma [89]. Patients ($n = 53$) received escalating doses of concurrent nivolumab and ipilimumab for four cycles, followed by nivolumab monotherapy for four cycles. The regimen that consisted of nivolumab 1 mg/kg plus ipilimumab 3 mg/kg was selected for further investigation in phase II and III trials in metastatic melanoma. Across all concurrent cohorts, the objective response rate (ORR) was 40%, including early (i.e. within 12 weeks) and deep (i.e. $\geq$80% tumor shrinkage) responses that were unrelated to $BRAF^{V600}$ mutation status. A recent long-term follow-up demonstrated 1-, 2-, and 3-year OS rates of 85%, 79%, and 68%, respectively [90]. The data from this study are encouraging and represent a dramatic shift from historical OS rates.

Phase II trial. A phase II randomized double-blind study showed significantly improved efficacy with combination I + N versus ipilimumab alone (Table 3) [91, 92]. Treatment-naïve patients with

Table 2. Selected clinical trials testing the combination of peripheral ICI with other therapies

Combination therapy	Study design	Cancer, stage	Study phase and trial number
Vaccines			
gp100, MART-1, and NY-ESO-1+ nivolumab [76]	Vaccines+nivolumab 1, 3, or 10 mg/kg, Q2W×12	Melanoma, IIIC	Phase I, NCT01176461
GM-CSF			
Ipilimumab+sargramostim [77]	Ipilimumab 10 mg/kg (day 1)+sargramostim 250 μg (day 1–14)	Melanoma, III/IV	Phase II, NCT01134614
Interferon			
Ipilimumab+peginterferon alfa-2b [78]	Ipilimumab 3 or 10 mg/kg, Q3W×12+peginterferon alfa-2b	Melanoma, III/IV	Phase II, NCT01496807
VEGF			
Ipilimumab+bevacizumab [79]	Ipilimumab 3 or 10 mg/kg+bevacizumab 7.5 or 15 mg/kg Q3W	Melanoma, III/IV	Phase I, NCT00790010
Small-molecule inhibitors			
Atezolizumab+vemurafenib or Atezolizumab+vemurafenib+ cobimetinib	Atezolizumab 15–20 mg/kg Q3W, 800 mg Q2W or 1200 mg Q3W	Melanoma, III/IV	Phase Ib, NCT01656642
Atezolizumab+cobimetinib	Fixed doses of both or MPD fixed dose+cobimetinib escalating doses	Solid tumors, metastatic	Phase I, NCT01988896
Pembrolizumab+dabrafenib+ trametinib	Pembrolizumab (days 1, 22)+dabrafenib 150 mg/kg BID (day 1)+trametinib 2 mg QD (day 1)	Melanoma, III/IV	Phase I/II, NCT02130466
Dabrafenib+ipilimumab+tra-metinib [80]	Dabrafenib 100 or 150 mg BID+ipilimumab 3 mg/kg Q3W Dabrafenib 100 or 150 mg BID (2 weeks) +trameti-nib 1 or 2 mg QD+ipilimumab 3 mg/kg Q3W	Melanoma, III/IV	Phase I, NCT01767454
Durvalumab+trameti-nib±dabrafenib [81]	Durvalumab 3 or 10 mg/kg Q2W+dabrafenib 150 mg BID+trametinib 2 mg QD or trametinib alone	Melanoma, III/IV	Phase I/II, NCT02027961
IDO inhibitors			
Pembrolizumab+epacadostat (INCB024360)	Pembrolizumab 2 mg/kg Q3W+epacadostat 25 mg BID	Solid tumors, metastatic melanoma	[64]
Ipilimumab+epacadostat	Ipilimumab 3 mg/kg Q3W+epacadostat 25 mg, 50 mg, 75 mg BID	Melanoma, metastatic or unresectable	Phase I/II, NCT01604889
Ipilimumab+indoximod	Ipilimumab 3 mg/kg Q3W+indoximod 600 mg BID	Melanoma, metastatic	Phase I/II, NCT02073123
Oncolytic virus			
Pembrolizumab+T-VEC	Pembrolizumab Q2W (Ib) Q3W (III)+ T-VEC intralesional Q2W (Ib) wk 0, 3, 5, 7 then Q3W	Melanoma, III/IV	Phase Ib/III, NCT02263508
Chemotherapy			
Pembrolizumab+chemotherapy (gemcitabine, docetaxel, nab-paclitaxel, vinorelbine, irinote-can, doxorubicin)	Pembrolizumab 2 mg/kg+gemcitabine 1000 mg/ m^2 (days 1, 8) Q3W Pembrolizumab 2 mg/kg+gemcitabine 900 mg/ m^2+docetaxel 75 mg/m^2 (day 8) Q3W Pembrolizumab 2 mg/kg+gemcitabine 1000 mg/ m^2+nab-paclitaxel 125 mg/m^2 (days 1, 8) Q3W Pembrolizumab 2 mg/kg+gemcitabine 1000 mg/ m^2+vinorelbine 25 mg/m^2 (days 1, 8) Q3W Pembrolizumab 2 mg/kg+irinotecan 300 mg/m^2 (day 1) Q3W Pembrolizumab 2 mg/kg+doxorubicin 30 mg/m^2 (day 1) Q3W	Breast cancer, sarcoma, pancreatic cancer, SCLC, metastatic	Phase I/II, NCT02331251
Pembrolizumab+chemotherapy (paclitaxel, carboplatin, bevaci-zumab, pemetrexed, erlotinib, gefitinib) or immunotherapy (ipilimumab)	Pembrolizumab 2 or 10 mg/kg+paclitaxel 200 mg/ m^2+carboplatin 6 mg/ml/min (day 1) Q3W Pembrolizumab 2 or 10 mg/kg+paclitaxel 200 mg/ m^2+carboplatin 6 mg/ml/min+ bevacizumab 15 mg/kg (day 1) Q3W	NSCLC	Phase I/II, NCT02039674

Continued

Table 2 *Continued*

Combination therapy	Study design	Cancer, stage	Study phase and trial number
	Pembrolizumab 2 or 10 mg/kg+pemetrexed 500 mg/m^2+carboplatin 5 mg/ml/min (day 1) Q3W Pembrolizumab 2 mg/kg+ipilimumab 0.3, 1, or 3 mg/kg (day 1) Q3W Pembrolizumab 2 mg/kg (day 1) Q3W+erlotinib 150 mg QD Pembrolizumab 2 mg/kg (day 1) Q3W+gefitinib 250 mg QD Carboplatin 5 mg/ml/min+pemetrexed 500 mg/m^2±pembrolizumab 200 mg (day 1) Q3W		
Nivolumab±gemcitabine/cisplatin, pemetrexed/cisplatin, carboplatin/paclitaxel, bevacizumab maintenance, erlotinib or ipilimumab	Various doses and schedules	NSCLC	Phase I, NCT01454102
Nivolumab+chemotherapy (temsirolimus, irinotecan, irinotecan+capecitabine)	Nivolumab 3 mg/kg+temsirolimus 25 mg Q2W Nivolumab 3 mg/kg+irinotecan 150 mg/m^2 Q2W Nivolumab 3 mg/kg+irinotecan 175 mg/m^2 (day 1) Q2W+capecitabine 1000 mg BID (days 1–5) QW	Pancreatic cancer, RCC, NSCLC, CRC	Phase I/II, NCT02423954
Atezolizumab+bevacizumab±FOLFOX [82]	Atezolizumab 20 mg/kg Q3W+bevacizumab 15 mg/kg Q3W Atezolizumab 14 mg/kg Q2W+bevacizumab 10 mg/kg Q2W+mFOLFOX6 at standard doses	CRC	Phase I, NCT01633970
Radiation therapy			
Chemoradiation with or without sequential durvalumab		NSCLC	Phase III, NCT02125461

BID, twice daily; CRC, colorectal cancer; GM-CSF, granulocyte-macrophage colony-stimulating factor; ICI, immune checkpoint inhibitors; IDO, indoleamine 2,3-dioxygenase; MPD, myeloproliferative disease; NSCLC, nonsmall cell lung cancer; Q2/3W, every 2/3 weeks; QD, everyday; RCC, renal cell carcinoma; SCLC, small cell lung cancer; T-VEC, talimogene laherparepvec; VEGF, vascular endothelial growth factor.

metastatic melanoma ($n=142$) were randomized 2:1 to receive ipilimumab (3 mg/kg every 3 weeks [Q3W]) concurrently administered with either nivolumab (1 mg/kg Q3W) or placebo for four doses, followed by nivolumab (3 mg/kg) or placebo every 2 weeks (Q2W) until disease progression [91, 92]. Overall, ORR was significantly higher with I + N compared with ipilimumab monotherapy (59% versus 11%) [91]. At a minimum follow-up of 24.5 months, patients who received the combination had prolonged PFS compared with patients who received ipilimumab alone, and the 2-year OS rate for all randomized patients was 64% for the combination and 54% compared with ipilimumab monotherapy; median OS had not been reached in either group (hazard ratio 0.74, 95% CI 0.43–1.26; $P=0.26$) [92]. In the combination group, ORR was independent of tumor PD-L1 status (58% for PD-L1-positive and 55% for PD-L1-negative tumors). In the ipilimumab monotherapy group, a numerically higher ORR was observed among patients with PD-L1-positive compared with PD-L1-negative tumors (18% versus 4%). The results from this trial led to accelerated approval of the combination in the USA based on tumor response rate and durability of response.

Phase III trial. In the first phase III trial to evaluate the role of concurrent versus single-agent immune checkpoint blockade for the treatment of patients with metastatic melanoma (Table 3) [91–100], 945 treatment-naïve patients were randomized 1:1:1 to receive I + N at the phase II schedule or single-agent nivolumab 3 mg/kg Q2W plus placebo, versus single-agent ipilimumab Q3W plus placebo, until disease progression or unacceptable toxicity [93]. At a median follow-up of ∼12 months, both the I + N and nivolumab monotherapy groups demonstrated improved PFS and higher investigator-assessed ORR compared with ipilimumab alone, a benefit that was observed across predefined subgroups [101]. At a median follow-up of 20.7 months, OS data were too immature to analyze [102].

Although PFS for the combination was more prolonged compared with nivolumab alone (11.5 months versus 6.9 months, respectively), the study was not statistically powered to formally assess this difference. In patients whose tumors had at least 5% PD-L1 expression using the PD-L1 IHC 28-8 pharmDx immunohistochemical assay [103], PFS with the I + N combination was numerically higher compared with nivolumab monotherapy (11.2 months versus 5.3 months, respectively). Subset analysis in relation to PD-L1 expression suggests that patients bearing PD-L1-positive tumors who received the combination did not have significantly longer PFS compared with single-agent nivolumab. Accordingly, at this time PD-L1 should not be used for

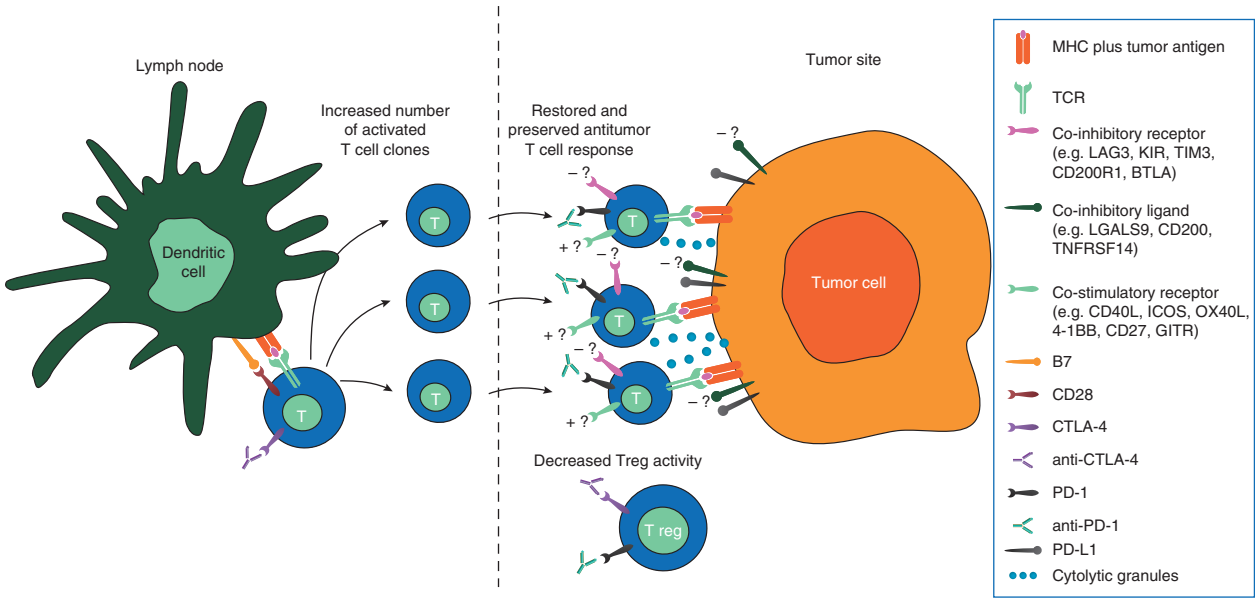

Figure 2. Implications of CTLA-4 and PD-1 dual pathway blockade. Interruption of CTLA-4:B7 binding by T cells in lymph nodes via anti-CTLA-4 increases T-cell proliferation, activation, and survival, potentially leading to an increased number of activated T-cell clones that can respond to tumor antigens. Blockade of PD-1:PD-L1 binding at the tumor site via anti-PD-1 restores the activity of antitumor T cells that have become inactivated. CTLA-4 and PD-1 blockade may also reduce the suppressive effects of Tregs at the tumor site. Please note that T cells may express other (i.e. non-CTLA-4, non-PD-1, PD-L1) co-stimulatory (+?) as well as co-inhibitory immune checkpoint proteins (-?), whereas tumor cells upregulate almost exclusively co-inhibitory ICPs via genetic (gene amplification) [17] or epigenetic mechanisms (upregulation of PI3K) [87]. BTLA, B- and T-lymphocyte attenuator; CTLA-4, cytotoxic T-lymphocyte antigen 4; GITR, glucocorticoid-induced TNFR-related protein; ICOS, inducible costimulator; LAG-3, lymphocyte activation gene-3; LGALS9, lectin, galactoside-binding, soluble-9; KIR, killer-cell immunoglobulin-like receptor; PD-1, programmed death-1; PD-L1, programmed death ligand 1; TCR, T-cell receptor; TNFSRF, tumor necrosis factor receptor superfamily-14; Treg, regulatory T cells.

clinical management and making decisions between combination and single-agent anti-PD-1 therapy, based solely on these results.

Safety profile with dual CTLA-4 and PD-1 inhibition

ICI are associated with immune-related AEs that typically affect skin, gastrointestinal, hepatic, endocrine, pulmonary, and renal organ systems. Table 4 lists grade 3/4 treatment-related AEs reported in trials combining CTLA-4 and PD-1 inhibitors. Although the spectrum of AEs with I + N was similar to monotherapy, the incidence of serious (grade 3 or 4) AEs was higher in the I + N arm compared with monotherapy-treated patients (69% versus 44% and 56%, respectively) [91, 93, 104, 105]. Additionally, there is a suggestion that irAEs may occur early in the course of therapy with combination treatment, potentially after only one cycle [106, 107]. The safety profile across all phases was consistent, and treatment-related AEs were generally well-managed and resolved with established safety guidelines (supplementary Tables S4 and S5, available at *Annals of Oncology* online) [91, 93, 104]. Notably, although four deaths related to combination therapy were reported across the phase I and II studies, no treatment-related deaths were reported in the multicenter phase III trial (109 institutions, 21 countries) among patients receiving the combination regimen [91, 93, 104]. Towards identifying a concurrent I + N regimen with comparable efficacy but a better safety profile, different schedules that decrease frequency and dose of ipilimumab in melanoma and NSCLC may preserve efficacy but definitely reduce life-threatening adverse events [95, 101]. Of note, a recent analysis of 35 patients who discontinued I + N on the phase II study due to toxicity demonstrated a similar response rate (66%) to the overall study population (59%), with the potential for durable benefit [108], suggesting that continued observation may be a reasonable option for this patient cohort.

Clinical insights for managing patients receiving ICIs

Supplementary Table S5, available at *Annals of Oncology* online, provides an overview of immune-related AE management strategies, which emphasizes differential diagnoses, use of steroids, and a multidisciplinary approach. If a patient has a moderate to severe AE that is potentially immune-mediated, treatment should be delayed or discontinued. Steroids are typically used to reduce immune reactions [14, 91, 93]. In some instances, immune checkpoint therapy can be resumed following resolution of an AE; however, patients experiencing a severe AE should permanently discontinue therapy. In patients presenting with acute fatigue, weight loss, diarrhea, nausea, emesis, or arthralgia, a workup for endocrinopathies (in particular, hypophysitis) should be done. Prompt hormone replacement therapy ameliorates symptoms of endocrinopathies and may allow continued therapy with ICI in some cases [109].

As yet, there is insufficient evidence about whether the efficacy of I + N is adversely affected by corticosteroids. To date, pooled data from studies testing single-agent nivolumab or ipilimumab suggest that use of immune modulators to manage immune-related AEs does not significantly alter the efficacy to any of these agents [26, 110, 111].

Granulocyte-macrophage colony-stimulating factor (GM-CSF) has been studied in combination with ipilimumab. In a randomized multicenter study, ipilimumab (10 mg/kg) plus GM-CSF improved

Table 3. Selected completed, ongoing, and planned studies with ICI combinations

Immune checkpoints inhibited	Study design	Treatment	Study population	Primary endpoint(s) and key results (for completed trials)
Melanoma				
CTLA-4+PD-1				
[93]	Phase III, randomized, placebo-controlled *N*=945	Ipilimumab+nivolumab *OR* nivolumab versus ipilimumab (placebo-controlled)	Treatment-naïve, unresectable or metastatic melanoma	OS, PFS Key results: • Prolonged PFS for nivolumab+ipilimumab and nivolumab versus ipilimumab • PFS: nivolumab+ipilimumab, 11.5 mo; nivolumab, 6.9 mo; ipilimumab, 2.9 mo • Grade 3/4 treatment-related AEs: nivolumab+ipilimumab, 55.0%; nivolumab, 16.3%; ipilimumab, 27.3%
[91, 92]	Phase II, randomized, placebo-controlled *N*=142	Ipilimumab+nivolumab versus ipilimumab (placebo-controlled)	Treatment-naïve, unresectable or metastatic melanoma	ORR among patients with *BRAF* WT tumors Key results: • Prolonged ORR for nivolumab+ipilimumab versus ipilimumab • ORR (*BRAF* WT): nivolumab+ipilimumab, 61%; ipilimumab, 11% • Grade 3/4 treatment-related AEs: nivolumab+ipilimumab, 54%; ipilimumab, 24%
NCT02224781	Phase III, randomized *N*=300	Nivolumab+ipilimumab→dabrafenib+trametinib versus dabrafenib+trametinib→nivolumab+ipilimumab	Advanced $BRAF^{V600}$ mutant melanoma	2-year OS rate
NCT02339571	Phase II/III, randomized, open-label *N*=400	Nivolumab+ipilimumab+GM-CSF induction → nivolumab+GM-CSF maintenance versus nivolumab+ipilimumab induction → nivolumab maintenance	Unresectable stage III/IV melanoma of known *BRAF* mutation status	OS
NCT01783938 [94]	Phase II, randomized, open-label *N*=140	Ipilimumab → nivolumab versus nivolumab → ipilimumab (sequential treatment)	Unresectable stage III/IV melanoma	Incidence of treatment-related grade 3/5 AEs during induction
NCT02374242	Phase II, randomized, open-label *N*=75	Nivolumab monotherapy (cohorts 1 and 2, recruited in parallel) versus nivolumab+ipilimumab (cohort 3; randomized allocation versus cohort 1, once 6 patients treated safely in cohort 1)	• Stage IV melanoma • Cohorts 1 and 3: asymptomatic, untreated (with local therapy) brain metastases • Cohort 2: previously treated or symptomatic brain metastases, or with concurrent leptomeningeal disease	Intracranial response rate
NCT02320058	Phase II, open-label *N*=148	Nivolumab+ipilimumab induction → nivolumab monotherapy maintenance	Melanoma with measurable metastases in brain and extracranial compartments	CBR

Continued

doi:10.1093/annonc/mdw534 | 67

Table 3 *Continued*

Immune checkpoints inhibited	Study design	Treatment	Study population	Primary endpoint(s) and key results (for completed trials)
NCT01621490	Phase I, part-randomized, open-label biomarker study *N*=160	• Nivolumab or nivolumab+ipilimumab (nonrandomized) • Nivolumab+ipilimumab versus nivolumab versus ipilimumab (randomized)	Unresectable stage III/IV melanoma	Changes from baseline in activated and memory T cells, IFN, IFN-inducible factors, and T-cell infiltration
NCT02186249	Expanded access	Nivolumab+ipilimumab	Anti-CTLA-4 naïve, unresectable stage III/IV melanoma	Safety and tolerability
NCT02089685 [95]	Phase I/II, randomized, open-label *N*=343	Pembrolizumab+IFN versus pembrolizumab+ipilimumab	• Part 1: advanced melanoma *OR* advanced RCC (nonrandomized) • Part 2: advanced melanoma (randomized)	Safety, MTD and RP2D
Other tumor types				
CTLA-4+PD-1				
NCT02231749	Phase III, randomized, open-label *N*=1,070	Nivolumab+ipilimumab versus sunitinib	Treatment-naïve, advanced or metastatic RCC	PFS, OS
NCT02210117	Pilot, randomized, open-label *N*=45	Nivolumab versus nivolumab+bevacizumab versus nivolumab+ipilimumab	Metastatic RCC eligible for cytoreductive surgery	Safety and tolerability of each combination

NCT01472081 [96]	Phase I, nonrandomized, open-label *N*=175	Nivolumab+ipilimumab induction (3 different dosing arms)→ nivolumab monotherapy maintenance *OR* nivolumab+sunitinib *OR* nivolumab+pazopanib	Advanced or metastatic RCC	Safety and tolerability of each combination
				Key results from expanded cohort (nivolumab 1 mg/kg+ipilimumab 3 mg/kg):
				• Promising antitumor activity
				  • ORR: 43%
				  • Median PFS: 36 months
				  • Median OS: not reached
				• Promising safety findings

NCT02060188	Phase II, dose-escalating, open-label *N*=96	Nivolumab monotherapy *OR* nivolumab+ipilimumab (dose-escalation phase)	Recurrent and metastatic colon cancer	ORR in all MSI-H patients
NCT01454102 [97]	Phase I, randomized, open-label, multi-arm safety study *N*=412	Nivolumab monotherapy *OR* nivolumab+ipilimumab *OR* nivolumab+bevacizumab maintenance *OR* nivolumab+erlotinib *OR* nivolumab+platinum-based doublet chemotherapy	Stage IIIB/IV NSCLC	Safety and tolerability of each combination Key interim results: • Across treatment arms ORR was 11–33% • Activity was seen regardless of PD-L1 status
NCT02039674	Phase I/II *N*=308	Pembrolizumab+chemotherapy *OR* pembrolizumab+ipilimumab *OR* pembrolizumab+targeted therapy	Stage IIIB/IV NSCLC	• Part 1: RP2D of combination • Part 2: PFS, ORR
NCT01592370	Phase I, dose-escalation *N*=315	Nivolumab (cohort 1) *OR* nivolumab+either ipilimumab or lirilumab	Relapsed or refractory lymphoma or multiple myeloma	Safety and tolerability of each combination
NCT02311920	Phase I, randomized, open-label *N*=42	Temozolomide and ipilimumab versus temozolomide and nivolumab versus temozolomide+nivolumab+ipilimumab	Newly diagnosed glioblastoma	Optimal dose of each combination
NCT01928394 [98]		Nivolumab versus nivolumab+ipilimumab		ORR

Continued

Table 3 *Continued*

Immune checkpoints inhibited	Study design	Treatment	Study population	Primary endpoint(s) and key results (for completed trials)
	Phase I/II, randomized, open-label *N*=1100		Advanced or metastatic triple-negative breast cancer, gastric cancer, pancreatic cancer, SCLC, or bladder cancer	Key results for SCLC: • Nivolumab alone or combined with ipilimumab was well-tolerated • ORR was 15% (nivolumab) and 25% (N+I) for evaluable patients • Durable responses were noted
NCT02017717 [99]	Phase I, open label, randomized *N*=100 (cohort 1)	Nivolumab monotherapy versus N+I	Grade IV malignant glioma	Safety and tolerability Key preliminary results for 20 patients: • 80% of N+I patients had grade 3/4 AEs • Drug-related AEs leading to discontinuation occurred in 50% of N+I patients • OS at 6 months was 75%
NCT02039674	See Table 2			
PD-1+LAG-3				
NCT01968109	Phase I, dose-escalation, cohort expanding *N*=198	BMS-986016 versus BMS-986016+nivolumab	Advanced solid tumors	Safety
PD-1+KIR				
NCT01714739	Phase I, dose-escalation, cohort expanding *N*=162	Nivolumab + lirilumab (doses 0.1–3.0 mg/kg)	Advanced solid tumors	Safety and tolerability
CTLA-4+KIR				
NCT01750580	Phase I *N*=22	Lirilumab+ipilimumab	Advanced solid tumors	Safety
PD-1+CD137 (4-1BB)				
NCT02179918 [100]	Phase I *N*=45	Pembrolizumab+PF-05082566	Advanced solid tumors	Dose-limiting toxicities
PD-L1+CTLA-4				
NCT02352948	Phase III, randomized, open-label *N*=900	• Durvalumab versus SOC (Part A) • Durvalumab+tremelimumab versus Durvalumab versus tremelimumab versus SOC (Part B)	• NSCLC • PD-L1 positive (part A) or negative (part B)	OS and PFS
NCT02369874	Phase III, randomized, open-label *N*=720	Durvalumab+tremelimumab OR Durvalumab versus SOC	Recurrent or metastatic head and neck cancer	OS and PFS
NCT02261220	Phase I, open-label *N*=233	Durvalumab+tremelimumab	Advanced solid tumors	Safety
PD-1+PD-L1				
NCT02118337	Phase I, open-label *N*=196	MEDI0680 (AMP-514)+Durvalumab	Advanced malignancies	Safety
PD-L1 or CTLA-4+OX-40				
NCT02205333	Phase Ib/II, open-label *N*=212	MEDI6469 *OR* MEDI6469+tremelimumab *OR* MEDI6469+Durvalumab *OR* MEDI6469+rituximab	Advanced solid tumors or aggressive B-cell lymphomas	MTD and safety

AE, adverse events; CBR, clinical benefit rate; CTLA-4, cytotoxic T-lymphocyte antigen 4; GM-CSF, granulocyte-macrophage colony-stimulating factor; ICI, immune checkpoint inhibitor; IFN, interferon; KIR, killer-cell immunoglobulin-like receptors; LAG-3, lymphocyte activation gene 3; mo, months; MSI-H, microsatellite-high; MTD, maximum tolerated dose; NSCLC, nonsmall cell lung cancer; OR, objective response; ORR, objective response rate; OS, overall survival; PD-1, programmed death-1; PD-L1, programmed death-1 ligand; PFS, progression-free survival; RCC, renal cell carcinoma; RP2D, recommended phase II dose; SCLC, small cell lung cancer; SOC, standard of care; WT, wild type.

doi:10.1093/annonc/mdw534 | 69

Table 4. Rates of grade 3/4 treatment-related AEs reported in trials of concurrent CTLA-4 and PD-1 pathway blockade [91, 93, 104, 105]

	Grade 3/4 AEs (%)
All treatment-related AEs	51–64
Colitis	4–17
Lipase increased	9–15
ALT increased	8–12
AST increased	6–11
Diarrhea	7–11
Rash	5–9
Amylase increased	≤6
Pyrexia	0–3
Fatigue	1–5
Dyspnea	≤3
Hypophysitis	≤2
Pneumonitis	≤2
Headache	≤2

AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CTLA-4, cytotoxic T-lymphocyte antigen 4; PD-1, programmed death-1.

OS, though not PFS, and showed lower rates of serious AEs compared with ipilimumab alone [77]. The implications of reduced toxicity with combination treatment versus monotherapy may be worth exploring once more data are available.

Patient selection

From a safety standpoint, most studies have been conducted in patients with normal hepatic and renal function, although there are no absolute contraindications to therapy with ICI. Additionally, patients with a history of autoimmune disease (AID) have been excluded from clinical trials based on concerns of increased risk of developing immune-related AEs and possible diminished clinical benefit if patients are actively treated with systemic immune modulators [112–114]. It is the authors' practice to consider therapy on an individual basis for patients with AID, after careful discussion of the risks and benefits, as clinical responses can be seen [115].

Development of biomarkers to assist in patient selection for therapy with ICI has trailed that of other therapies, such as small-molecule inhibitors. This may have significant economic implications due to the high cost of these agents over prolonged treatment periods [116]. This is especially likely if they are to be given in combination, either in cancers with low response rates to PD-1/PD-L1 pathway inhibitor studies; or even in cancers with high response rates (e.g. melanoma) but administered during earlier stages of cancer (e.g. adjuvant). Immunohistochemical expression of PD-L1 in tumor tissues seems to be the most promising biomarker so far and is currently used as an FDA-approved companion diagnostic test in patients with NSCLC who are considered for treatment with pembrolizumab [60]. Other tumor tissue-based tests that assess PD-L1 are likely to be FDA-approved as companion diagnostics in combination with other PD-L1 inhibitors [117].

Initial evidence suggested that patients with PD-L1-expressing tumors may have higher response rates and longer PFS to PD-1/PD-L1 pathway inhibitors than patients treated with anti-PD-1 monotherapy with low or negative PD-L1 expression [33, 118–120]. However, PD-L1 was not a predictive biomarker in phase III randomized trials in RCC and squamous cell NSCLC [121]. Moreover, patients with PD-L1 negative tumors still benefit from treatment with these agents when compared with other treatments [120]. This may be attributed to the fact that expression of PD-L1 is heterogeneous with respect to stage (primary versus metastatic), metastatic organs involved, and prior systemic or local treatment effects [122]. Screening for PD-L1 expression by immunohistochemistry, however, may be important in patients who would otherwise be considered for I + N as opposed to single-agent nivolumab, as the margin for PFS benefit to the combination therapy was greatest in patients with PD-L1-negative metastatic melanoma [93]. In the future, PD-L1 expression could factor into the complex decision-making involved with individualized patient treatment, however it has not yet been validated for this purpose at this time.

Analysis of pretreatment tumor tissues from patients who went on to receive pembrolizumab showed that preexisting high numbers of TILs in the vicinity of PD-L1- and PD-1-expressing cells had the greatest tumor response from pembrolizumab in metastatic melanoma [123]. Immunoscore™ is already a commercially available test (HalioDx) that accurately quantifies the density and distribution of TILs using standardized immunohistochemistry and computer imaging algorithms in formalin-fixed, paraffin-embedded tumor tissues with prognostic and predictive implications for therapy [124, 125]. It may likely assist in classifying cancers based on the tumor microenvironment and to facilitate prediction of response to ICI and other immuno-oncology agents [23, 126, 127]. Possibly, a combined tumor tissue biomarker that considers both immunoscore and PD-L1 expression is important [23], especially for patients who are considered for I + N therapy. Other predictive methodologies also continue to be investigated. Recently, multiparameter flow cytometry for PD-1 and CTLA-4 on freshly isolated mononuclear cells from tumor tissues was found to be a predictor of response to PD-1 monotherapy in metastatic melanoma [128]. Functionally, this T-cell subset demonstrated a partially exhausted phenotype. Interestingly, in a separate cohort of 24 patients treated with I + N, increased levels of PD-1 high/CTLA-4 high T cells were not predictive of benefit [129]. While it remains a critical question, the optimal biomarker to guide patient selection has yet to be defined.

Ongoing immune checkpoint combination studies in patients with advanced malignancies

Within melanoma the concurrent I + N regimen is FDA-approved for unresectable stage III or IV disease and is being evaluated in patients with active brain metastases (NCT02374242). Early data on the I + N regimen in other solid tumors suggest that combination treatment may have higher response rates compared with single-agent nivolumab on most occasions (Table 3).

Ongoing studies are investigating other combinations of CTLA-4 and PD-1/PD-L1 pathway inhibitors in other tumor types (Table 3). The anti-PD-L1 agent durvalumab is also being combined with an anti-PD-1 agent (MEDI0680; AMP 514) in the first trial to target both the PD-1 receptor and its key ligand on the basis of preclinical data showing synergy [130]. This combination is being evaluated in patients with advanced malignancies, including melanoma (NCT02118337) (Table 3).

The success and promise of CTLA-4 and PD-1/PD-L1 pathway inhibitors has paved the way to investigate the therapeutic potential of other antibodies that target co-inhibitory or co-stimulatory ICP (Table 3). The list of prospective drug targets is large, and clinical trials testing antibodies against CD137, LAG-3, CD200, and KIR have offered early results of safety and activity. Clinical trials testing drugs against several other ICPs were recently opened to accrual or are ready to enroll patients (e.g. OX-40, CD40, CD27, Tim-3, GITR). Although the number of permutations for simultaneous targeting of these proteins is daunting, the most promising combinations will be ultimately defined by the cancer type-specific biology and *in vivo* testing in appropriate cancer-specific animal models.

Conclusion

The field of immuno-oncology is expanding rapidly, with the potential for broad application across multiple tumor types. ICIs are changing the treatment expectations for cancer patients, offering durable and deep responses for many patients. Combinations of immuno-oncology agents have shown improved response rates compared with single-agent therapy, although the high rate of grade 3/4 AEs remains a potential concern. This emphasizes the need for vigilance in AE identification, prompt management using established guidelines, appropriate risk stratification, and the need for better biomarkers of response that may rely on tumor biology and agent's MOA (PD-L1 negative, immunoscore low/absent). Ongoing studies seek to refine patient selection and identify novel combination approaches, which may lead to safer and more effective treatments.

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