Review

Immuno-oncology Combinations: A Review of Clinical Experience and Future Prospects

Scott J. Antonia¹, James Larkin², and Paolo A. Ascierto³

Abstract

Immuno-oncology is an evolving treatment modality that includes immunotherapies designed to harness the patient’s own immune system. This approach is being studied for its potential to improve long-term survival across multiple tumor types. It is now important to determine how immunotherapies may be most effectively used to achieve the best possible patient outcomes. Combining or sequencing immunotherapies that target distinct immune pathways is a logical approach, with the potential to further enhance the magnitude of the antitumor immune response over single agents. Early clinical data in patients with melanoma treated with two immune checkpoint inhibitors, ipilimumab and nivolumab, suggest support for this combination approach. Numerous other combination approaches are being evaluated in early-phase clinical trials; however, their clinical activity remains unknown. Clinical experience to date has shown that when combining an immuno-oncology agent with an existing therapeutic modality, it is important to determine the optimal dose, schedule, and sequence.

Introduction

Tumors avoid immune destruction by a range of complex and often overlapping mechanisms that disrupt key components of the immune system involved in mounting an effective antitumor response (1–4). Tumors can avoid recognition and elimination by the immune system by disrupting antigen presentation mechanisms, either through down-regulation of MHC class I molecules or by disabling antigen-processing machinery. Alternatively, or additionally, tumors may suppress the immune system by disrupting pathways involved in controlling T-cell inhibition (checkpoint) and activation (3, 5), or by recruiting immunosuppressive cell types, such as regulatory T cells (Treg) and myeloid-derived suppressor cells (MDSC). The release of factors, including adenosine and prostaglandin E2, and the enzyme indoleamine 2,3-dioxygenase (IDO) is another mechanism that tumors may use to suppress immune activity (3).

The idea of targeting the immune system as a therapeutic approach in cancer is not new. Cytokines [interleukin-2 (IL2) and interferon-α (IFNα)] have been used for decades, predominantly in patients with renal cell carcinoma (RCC) and melanoma. However, these cytokines are not target specific, and have been associated with significant toxicity and limited efficacy; these factors restrict use to healthy patients and only a select group of these patients will derive benefit (6, 7).

Immuno-oncology is an evolving treatment modality that includes immunotherapies designed to target and harness the patient’s immune system directly to kill tumor cells (8, 9). Numerous strategies for overcoming tumor immune evasion are under evaluation (Table 1). Because these approaches directly target the patient's immune system, they have the potential for activity across multiple types of cancer. Examples of immunotherapeutic approaches under clinical investigation include T-cell checkpoint inhibitors or agonists for T-cell–activating pathways, novel cytokines such as IL12 and IL15, therapeutic vaccines, elimination of immunosuppressive cells, and other agents and approaches designed to enhance immune cell function (Table 1; refs. 10–12).

Since the approval of IL2, sipuleucel-T (a therapeutic vaccine composed of recombinant antigen protein designed to stimulate T-cell responses) and ipilimumab [a cytotoxic T-lymphocyte antigen 4 (CTLA-4) immune checkpoint inhibitor] were the first immunotherapies to be approved for patients with cancer. Sipuleucel-T was approved in 2010 for asymptomatic or minimally symptomatic metastatic castrate–resistant prostate cancer (CRPC) and ipilimumab in 2011 for unresectable or metastatic melanoma. Both agents were shown to significantly improve overall survival (OS) in phase III clinical trials (Fig. 1; refs. 13–16).

Monoclonal antibodies targeting programmed death-1 (PD-1) ligand (PD-L1) interaction, another immune checkpoint pathway, are the most advanced in clinical development after ipilimumab and sipuleucel-T, and various agents are being tested in clinical trials across a range of tumor types.
Table 1. Potential strategies for overcoming tumor immune evasion mechanisms and examples of agents in clinical development (12)

<table>
<thead>
<tr>
<th>Treatment strategy</th>
<th>Examples of agents in clinical development</th>
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<tbody>
<tr>
<td><strong>Reversing the inhibition of adaptive immunity (blocking T-cell checkpoint pathways)</strong></td>
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</tbody>
</table>
| • Inhibiting the CTLA-4 checkpoint molecule\(^a\) | Ipilimumab: approved for melanoma  
Tremelimumab: phase II for malignant mesothelioma, HCC, melanoma |
| • Inhibiting the interaction between PD-1 checkpoint and its ligands\(^a\) | Nivolumab (anti–PD-1): phase III for melanoma, NSCLC, RCC  
Pembrolizumab (MK-3475; anti–PD-1): phase III for NSCLC, melanoma  
MPDL3280A (RG7446; anti–PD-L1): phase III for NSCLC  
Pidilizumab (CT-011; anti–PD-1): phase II for FL, prostate, pancreatic, melanoma  
AMP-514 (MEDI0680; anti–PD-1): phase I for solid tumors  
MEDI4736 (anti–PD-L1): phase I for solid tumors  
AMP-224 (recombinant PD-L-Fc fusion protein): phase I for solid tumors  
rHlgM12B7 (anti–PD-L2): phase I for melanoma |
| • Inhibiting the LAG-3 checkpoint molecule | IMP321: phase I for breast, RCC; phase II for melanoma  
BMS-986016: phase I for solid tumors |
| • Inhibiting the TIM-3 checkpoint | No agent undergoing clinical evaluation |
| • Inhibiting the adenosine A2A receptor | No agent undergoing clinical evaluation |
| **Switching on adaptive immunity (promoting T-cell costimulatory receptor signaling using agonist antibodies)** | |
| • Promoting CD137 signaling | Urelumab: phase I for B-cell NHL, CLL, solid tumors  
PF-05082566: phase I for NHL |
| • Enhancing OX-40 signaling | MEDI6469: phase II for breast, prostate, solid tumors |
| • Promoting GITR signaling | TRX518: phase I for melanoma, solid tumors |
| • Enhancing CD27 signaling | CDX-1127: phase I for CD27–expressing hematologic malignancies and solid tumors  
CP-870,893: phase I for pancreatic, melanoma  
Chi Lob 7/4: phase I for advanced malignancies |
| • Systemic recombinant IL21 administration (range of effects that enhance immune cell function) | Denenicokin: phase II for melanoma, ovarian and phase I for RCC, NHL |
| • Systemic recombinant IL15 administration (range of effects that enhance immune cell function) | rhIL 15: phase I for melanoma, kidney, NSCLC, SChN |
| • Systemic recombinant IL7 administration (range of effects, including on T-cell development) | rhIL 7: phase II for various solid tumors |
| **Improving the function of innate immune cells** | |
| • Manipulating the activation of NK-cell inhibitory receptors (KIR) | Lirilumab: phase II for AML (maintenance); phase I for solid tumors |
| • Stimulating macrophages and DCs | Toll-like receptor agonists:  
Bacillus Calmette-Guérin (TLR 2/4 agonist): approved for bladder carcinoma  
Hiltonol (TLR7 agonist): phase II various solid and hematologic malignancies  
Imiquimod (TLR7 agonist): approved basal cell carcinoma  
Resiquimod (TRL7/8 agonist): phase I/II various solid and hematologic malignancies  
CpG 7909 (TLR 9 agonist): phase II various solid tumors |
| **Activating the immune system (potentiating immune-cell effector function)** | |
| • IDO inhibition | INCBO24360: phase II for melanoma, EOC, PPC, FTC  
Indoximod: phase II for breast, prostate |

(Continued on the following page)
One of the more exciting aspects of immunotherapies is demonstrated with data from clinical trials for ipilimumab, nivolumab, and pembrolizumab that show the potential for long-term survival. In a phase III study of ipilimumab in previously treated patients with metastatic melanoma (study MDX010-020), the survival rate at 2 and 3 years was 25% for each (17). In addition, in a pooled analysis of data from 12 ipilimumab clinical studies with follow-up of up to 10 years in some patients, an OS plateau started at approximately 3 years and the 3-year survival rate was 22% (18). The PD-1 immune checkpoint inhibitors nivolumab and pembrolizumab have also shown durable responses in phase I studies (19–21).

Although targeting the immune system has emerged as an effective treatment approach for patients with CRPC and metastatic melanoma (17, 18), for the development of this treatment modality to progress, it is important to determine how agents should be used to achieve the best possible patient outcomes. Combining immunotherapies with other established and investigational cancer therapies is a field of active investigation, with a multitude of approaches under consideration. This review focuses on (i) combining or sequencing immunotherapies that target distinct immune pathways, particularly T-cell checkpoints, and (ii) combining immunotherapies with existing therapeutic modalities, specifically BRAF-targeted therapies, chemotherapies, and radiotherapy.

### Table 1. Potential strategies for overcoming tumor immune evasion mechanisms and examples of agents in clinical development (12) (Cont’d)

<table>
<thead>
<tr>
<th>Treatment strategy</th>
<th>Examples of agents in clinical development</th>
</tr>
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<tbody>
<tr>
<td>• Inhibition of TGF-β signaling</td>
<td>GC1008; phase I for melanoma, RCC</td>
</tr>
<tr>
<td></td>
<td>LY2157299: phase I/II various solid tumors</td>
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<td></td>
<td>TEW 7197: phase I solid tumors</td>
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<tr>
<td></td>
<td>IMC-TR1 (LY3022859): phase I solid tumors</td>
</tr>
<tr>
<td>• Systemic IL2 or IFNα administrationª</td>
<td>Agents approved</td>
</tr>
<tr>
<td>• Various vaccine-based strategiesª</td>
<td>Various approaches under clinical evaluation</td>
</tr>
</tbody>
</table>

Abbreviations: ADCC, antibody-dependent cell-mediated cytotoxicity; AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia; EOC, epithelial ovarian cancer; FL, follicular lymphoma; FTC, fallopian tube cancer; GITR, glucocorticoid-induced tumor necrosis factor related gene; HCC, hepatocellular carcinoma; NHL, non-Hodgkin lymphoma; PPC, primary peritoneal cancer; SCHN, squamous cell head and neck cancer; TIM, T-cell immunoglobulin mucin; TLR, Toll-like receptor.

ªApproaches in which approved compounds or investigational compounds are being studied in phase III trials.
Combining immunotherapies that target distinct immune pathways

Combining or sequencing immunotherapies that target distinct immune pathways is a rational strategy to determine whether the magnitude of the antitumor immune response may be improved over that generated with a single agent. Potential combination approaches undergoing clinical evaluation include dual T-cell checkpoint inhibition, T-cell checkpoint inhibition combined with immunomodulatory antibodies designed to enhance T-cell activity through agonistic interaction with costimulatory receptors (aiming to switch on adaptive immunity), T-cell checkpoint inhibition combined with approaches to improve the function of innate immune cells, and T-cell checkpoint inhibition combined with other approaches to enhance the immune response (Table 2; refs. 3, 5, 12).

Dual T-cell checkpoint inhibition

Given that T-cell checkpoint inhibitors (e.g., ipilimumab, nivolumab, pembrolizumab) have shown single-agent clinical activity in several tumor types (5, 13), and preclinical data suggest checkpoint molecules may act synergistically to regulate T-cell function and promote tumor immune escape, it is rational to evaluate whether combining checkpoint inhibitors improves activity, achieving an OS benefit in a greater proportion of patients compared with either agent alone (Fig. 2; refs. 16, 22–24). Initial support for dual T-cell checkpoint inhibition has come from a phase I study in which patients with advanced stage III or IV melanoma were treated with both ipilimumab (1 or 3 mg/kg) and nivolumab (0.3 mg/kg, 1 mg/kg, or 3 mg/kg) in a concurrent or sequenced regimen (16, 25). An objective response rate (ORR) rate of 40% was achieved in patients treated with the concurrent regimen (ORR was 53% at the maximum tolerated dose, nivolumab 1 mg/kg, ipilimumab 3 mg/kg). The preliminary 1-year OS rate with the concurrent regimen was 82% [95% confidence interval (CI) 69.0–94.4; Fig. 1; ref. 16]. These promising results prompted the initiation of a phase III study (CheckMate 067) to further evaluate concurrent treatment with ipilimumab and nivolumab (12).

Phase I studies are in progress to evaluate ipilimumab plus nivolumab in patients with a range of solid tumors [including RCC, colon cancer, triple-negative breast cancer, gastric cancer, pancreatic cancer, and small-cell lung cancer (SCLC); ipilimumab plus pembrolizumab (anti–PD-1) in patients with melanoma, RCC, and NSCLC; tremelimumab (an anti–CTLA-4 agent) plus MEDI 4736 (anti–PD-L1 agent) in NSCLC; and an anti–lymphocyte activation gene 3 (LAG-3) monoclonal antibody BMS-986016 plusnivolumab (anti–PD-1) in patients with solid tumors (Table 2; ref. 12)]. The latter combination is supported by preclinical data that showed strong synergistic antitumor activity when both the PD-1 and LAG-3 immune checkpoint pathways were blocked (23). Dual anti–LAG-3/anti–PD-1 antibody treatment cured most mice of established fibrosarcoma and colon adenocarcinoma tumors that were largely resistant to single antibody treatment (22).

T-cell checkpoint inhibition combined with agonistic antibodies against T-cell costimulatory receptors

In theory, if agents designed to release the checkpoint-mediated inhibition of T cells were combined with agonist antibodies designed to enhance costimulatory T-cell signaling, a more effective immune response may be generated (5). To date, no data are available from clinical trials evaluating these combinations, but studies are in progress. CD40 plays a key role in the development of T-cell–dependent antitumor immunity, and is essential in enabling antigen-presenting cells to process and present antigen effectively to T cells (26–28). Combining T-cell checkpoint blockade (using anti–CTLA-4 agent tremelimumab) with an agent that targets the costimulatory molecule CD40 (CP-870,893) is being investigated in a phase I trial in patients with melanoma (12).

Other agonist antibodies designed to target receptors, including OX-40, CD27, GITR, and CD137, are in development. The clinical evaluation of these agents as monotherapy is at an early stage, although the limited data available suggest they can be safety administered to patients. Data from a large phase I trial with urelumab (anti–CD137) in more than 100 patients did show liver toxicity, with 2 deaths reported at higher doses. Clinical evaluation of urelumab is continuing at lower doses in advanced solid tumors and hematologic malignancies (29). Evaluating combinations of these antibodies with checkpoint inhibitors and other immunotherapies is an exciting possibility, but one that should be evaluated with caution.

Another agonistic therapeutic approach that was evaluated with catastrophic effects was the CD28 agonist TGN1412. In a first-in-human phase I trial, TGN1412 administration resulted in a cytokine storm that caused severe adverse events in the six volunteers (30). As explained by Curran and colleagues (31), CD28 is widely expressed on all mature T-cell populations; therefore, an agonistic CD28 antibody may be expected to have a polyclonal “super agonist” effect—this is in contrast to other costimulatory modules, such as CD137 or OX-40, which are only expressed on a proportion of T cells, so agonist antibodies are likely to have a more selective effect.

T-cell checkpoint inhibition combined with approaches to improve the function of innate immune cells

Adaptive immune responses to cancer involve various components of innate immunity. In view of this, combining therapies designed to enhance T-cell function with agents designed to improve innate immune cell function are worthy of evaluation. Natural killer (NK) cells are innate effector cells that maintain tolerance to self-tissue via the expression of killer cell immunoglobulin-like receptors (KIR), which negatively regulate NK-cell activity by binding to the MHC class I molecules expressed on most “normal” cells (32–35). Tumor cells may appear like normal cells by
Table 2. Combination approaches in clinical development (12)

### Selected clinical trials of immunotherapies

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Target</th>
<th>Development phase/tumor type</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dual T-cell checkpoint blockade</strong></td>
<td></td>
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</tr>
<tr>
<td>Ipilimumab + nivolumab</td>
<td>CTLA-4 + PD-1</td>
<td>Phase III: melanoma; phase I/II: RCC, colon, NSCLC, triple-negative breast cancer, gastric cancer pancreatic cancer, and SCLC</td>
</tr>
<tr>
<td>Ipilimumab + pembrolizumab</td>
<td>CTLA-4 + PD-1</td>
<td>Phase I: melanoma, RCC, and NSCLC</td>
</tr>
<tr>
<td>Tremelimumab + MEDI4736</td>
<td>CTLA-4 + PD-L1</td>
<td>Phase I: NSCLC; solid tumors</td>
</tr>
<tr>
<td>Nivolumab + BMS-986016</td>
<td>PD-1 + LAG-3</td>
<td>Phase I: solid tumors</td>
</tr>
<tr>
<td><strong>T-cell blockade + costimulatory receptor agonists</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP-870,893 + tremelimumab</td>
<td>CTLA-4 + CD40</td>
<td>Phase I: metastatic melanoma</td>
</tr>
<tr>
<td><strong>T-cell blockade + improving the function of innate immune cells</strong></td>
<td></td>
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<tr>
<td>Lirilumab + ipilimumab</td>
<td>CTLA-4 + KIR</td>
<td>Phase I: solid tumors</td>
</tr>
<tr>
<td>Lirilumab + nivolumab</td>
<td>PD-1 + KIR</td>
<td>Phase I: solid tumors</td>
</tr>
<tr>
<td><strong>T-cell blockade + other immune system activators</strong></td>
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<tr>
<td>Denenicokin + ipilimumab</td>
<td>CTLA-4 + IL21</td>
<td>Phase I: melanoma</td>
</tr>
<tr>
<td>Denenicokin + nivolumab</td>
<td>PD-1 + IL21</td>
<td>Phase I: solid tumors</td>
</tr>
<tr>
<td>INCB024360 + ipilimumab</td>
<td>CTLA-4 + IDO</td>
<td>Phase I: melanoma</td>
</tr>
<tr>
<td>Indoximod + sipuleucel-T</td>
<td>IDO + vaccine</td>
<td>Phase II: prostate</td>
</tr>
<tr>
<td>Nivolumab + gp100, NY-ESO-1</td>
<td>PD-1 + vaccine</td>
<td>Phase I: melanoma</td>
</tr>
<tr>
<td>Ipilimumab + sipuleucel-T</td>
<td>CTLA-4 + vaccine</td>
<td>Phase I: prostate cancer</td>
</tr>
<tr>
<td>Ipilimumab + TriMix-DC</td>
<td>CTLA-4 + vaccine</td>
<td>Phase I: melanoma</td>
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<tr>
<td>Ipilimumab + NY-ESO-1 vaccine</td>
<td>CTLA-4 + vaccine</td>
<td>Phase I: melanoma</td>
</tr>
<tr>
<td>Ipilimumab + adoptive cell transfer</td>
<td>CTLA-4 + passive immunotherapy</td>
<td>Phase I/II: melanoma</td>
</tr>
</tbody>
</table>

### Selected clinical trials of immunotherapies (excluding ipilimumab) in combination with other treatment modalities

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Target</th>
<th>Combination treatment modality</th>
<th>Development phase/tumor type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tremelimumab</td>
<td>CTLA-4</td>
<td>Gefitinib</td>
<td>Phase I: NSCLC</td>
</tr>
<tr>
<td>Nivolumab</td>
<td>PD-1</td>
<td>Chemotherapy(^a)</td>
<td>Phase I: NSCLC</td>
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<tr>
<td></td>
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<td>Dasatinib</td>
<td>Phase I: CML</td>
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<td></td>
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<td>Bevacizumab</td>
<td>Phase I: NSCLC</td>
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<td></td>
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<td>Erlotinib</td>
<td>Phase I: NSCLC</td>
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<td></td>
<td></td>
<td>Sunitinib or pazopanib</td>
<td>Phase II: RCC</td>
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<tr>
<td>Pembrolizumab</td>
<td>PD-1</td>
<td>Pazopanib</td>
<td>Phase I: RCC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lenalidomide + dexamethasone</td>
<td>Phase I: multiple myeloma</td>
</tr>
<tr>
<td>MPDL3280A</td>
<td>PD-L1</td>
<td>Bevacizumab</td>
<td>Phase II: RCC</td>
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<tr>
<td></td>
<td></td>
<td>Erlotinib</td>
<td>Phase I: NSCLC</td>
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<tr>
<td></td>
<td></td>
<td>Vemurafenib</td>
<td>Phase I: melanoma</td>
</tr>
<tr>
<td>MEDI14736</td>
<td>PD-L1</td>
<td>Trametinib + dabrafenib</td>
<td>Phase I/II: melanoma</td>
</tr>
<tr>
<td>Pidilizumab</td>
<td>PD-1</td>
<td>Rituximab</td>
<td>Phase II: FL</td>
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<td></td>
<td></td>
<td>Gemcitabine</td>
<td>Phase II: pancreatic</td>
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<td></td>
<td></td>
<td>FOLFOX</td>
<td>Phase II: CRC (completed)</td>
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<tr>
<td></td>
<td></td>
<td>Sipuleucil-T + cyclophosphamide</td>
<td>Phase II: prostate cancer</td>
</tr>
<tr>
<td>IMP321</td>
<td>LAG-3</td>
<td>Paclitaxel</td>
<td>Phase I: breast (completed)</td>
</tr>
<tr>
<td>Urelumab</td>
<td>CD137</td>
<td>Rituximab</td>
<td>Phase I: NHL</td>
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<tr>
<td></td>
<td></td>
<td>Chemotherapy</td>
<td>Phase I: solid tumors</td>
</tr>
<tr>
<td>PF-05082586</td>
<td>CD137</td>
<td>Rituximab</td>
<td>Phase I: NHL</td>
</tr>
<tr>
<td>CP-870,893</td>
<td>CD40</td>
<td>Paclitaxel/carboplatin</td>
<td>Phase I: solid tumors (completed)</td>
</tr>
<tr>
<td>Denenicokin</td>
<td>IL21</td>
<td>Sunitinib</td>
<td>Phase I/II: RCC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sorafenib</td>
<td>Phase I/II: RCC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rituximab</td>
<td>Phase I: NHL</td>
</tr>
<tr>
<td>Indoximod</td>
<td>IDO</td>
<td>Docetaxel</td>
<td>Phase I: breast</td>
</tr>
</tbody>
</table>

Abbreviations: CML, chronic myeloid leukemia; CRC, colorectal cancer; FL, follicular lymphoma; NHL, non-Hodgkin lymphoma. \(^a\)Chemotherapy regimens include gemcitabine/cisplatin, pemetrexed/cisplatin, and carboplatin/paclitaxel.
retaining or upregulating MHC class I to escape immuno-surveillance by NK cells (33). Lirilumab is an anti-KIR antibody that blocks the inhibitory KIR signal, thereby potentiating NK-cell killing of tumor cells, despite expression of MHC I. A regimen designed to enhance innate and adaptive immunity, respectively, could theoretically achieve more favorable biologic and clinical activity compared with either agent alone (36, 37). This could be achieved in a variety ways, such as by using an anti-KIR agent (lirilumab) in combination with PD-1 or CTLA-4 immune checkpoint inhibitors. Clinical trials are under way evaluating such combinations (Table 2; ref. 12).

Other immunotherapy combination partners

Cytokine therapy. Cytokines have the capacity to stimulate an immune response, although arguably less specifically compared with other immunotherapeutic approaches (3). IL21 has a role in NK and T-cell activation, and systemic administration of a recombinant IL21 (rIL21) has demonstrated antitumor activity in tumors, including metastatic melanoma (38). On the basis of preclinical studies in mouse tumor models which showed enhanced antitumor activity when rIL21 was combined with either anti–CTLA-4 or anti–PD-1 agents (39), phase I dose-escalation studies are evaluating these combinations in patients with advanced or metastatic melanoma (ipilimumab; ref. 40) or solid tumors (nivolumab; ref. 41).

Other cytokines are under evaluation as monotherapy for advanced or metastatic melanoma (ipilimumab; ref. 40) or solid tumors (nivolumab; ref. 41).

Therapeutic vaccines. Although the various mechanisms of action of therapeutic vaccines are beyond the scope of this review (46, 47), most vaccines are designed to (i) present tumor antigens to the immune system and (ii) provide immune modulation. Because of their differing mechanisms of action, vaccines and other immunotherapies are potential combination partners. Clinical data have shown promising results with some combinations, for example, gp100 peptide vaccine and IL2 in melanoma, and ipilimumab combined with granulocyte macrophage-colony stimulating factor cell-based vaccine in pancreatic cancer (48, 49). However, no survival advantage was seen in patients with melanoma treated with gp100 plus ipilimumab versus those given ipilimumab alone in a phase III trial (13, 17).

Various phase I and II clinical trials combining a vaccine with a checkpoint inhibitor are ongoing in patients with responsive immune cells. Data from preclinical studies suggest that inhibiting IDO can promote the proliferation, survival, and function of various immune cells [e.g., T cells, NK cells, and dendritic cells (DC)], reduce the generation of Tregs, and significantly inhibit tumor growth (42, 43). Furthermore, studies in murine models showed that host-derived IDO can suppress the antitumor activity of an anti–CTLA-4 antibody. However, inhibition or absence of IDO combined with therapies targeting immune checkpoints, such as CTLA-4, PD-1/PD-L1, and GITR, acts synergistically to control tumor growth and improve OS (44). Thus, combining an agent that inhibits IDO with another immunotherapy would appear to be a rational approach and is being evaluated in several clinical trials (Table 2). A phase II trial is evaluating the IDO inhibitor indoximod in combination with the therapeutic vaccine sipuleucel-T in patients with prostate cancer (Table 2; ref. 12).

Adoptive cell transfer and T-cell engineering. Adoptive cell transfer (ACT) involves the collection of tumor-infiltrating lymphocytes (TIL) from patients, the in vitro expansion of autologous lymphocytes with reactivity to tumor antigens, and the subsequent transfer back to the patient, with the expectation that the tumor-specific lymphocytes will attack the tumor (11, 45). ACT has demonstrated durable complete responses in patients with melanoma (45). In a phase II study, 20 of 93 patients with metastatic melanoma (22%) had durable, complete remissions (3–7 years) after treatment with IL2 and ACT of TILs.

In addition to the expansion and transfer of TILs, approaches to modify the patient’s T cells are under evaluation. These include engineering T cells using chimeric antigen receptors (CAR) to redirect them to specific tumor-antigen targets before reinfusion. T-cell receptor (TCR) gene therapy is another strategy in development; the objective is to induce immune reactivity against tumors by introducing genes encoding a tumor-reactive TCR into patients’ T cells, improving immune reactivity. Combining these types of approaches with other immunotherapies may further improve clinical efficacy. Trials of ACT, CARs, and TCR gene therapy in combination with immune checkpoint inhibitors or other approaches are ongoing or under consideration.
melanoma or prostate cancer (Table 2; ref. 12). However, a clear demonstration of the vaccine's ability to induce clinically relevant antitumor responses in patients is required, as historically, the clinical translation of cancer vaccines into efficacious therapies has been challenging (with the exception of sipuleulce-T, the only approved therapeutic cancer vaccine; ref. 47). Data suggest that T cells activated at the vaccine site are "shut down" when they enter the tumor microenvironment, most likely due to tumor-mediated T-cell-suppressive mechanisms (50, 51). With tools such as PD-1 immune checkpoint inhibitors that are designed to block tumor-mediated T-cell suppression in the tumor microenvironment, it is worth evaluating whether vaccines may improve clinical efficacy when combined with a checkpoint inhibitor. However, data from the only published vaccine/PD-1 checkpoint inhibitor study showed the addition of a vaccine did not improve the efficacy of PD-1 inhibition (52).

Integrating immunotherapies with existing therapeutic modalities

Existing treatment modalities, (e.g., chemotherapy, radiotherapy, and molecularly targeted therapies) cause tumor reduction, not only through cytotoxic/cytostatic effects, but also through mechanisms that may potentiate immune activity, including modification of the tumor microenvironment and release of tumor antigens. This activity may be complementary, even synergistic, to the immunotherapies designed to support an antitumor immune response.

The immune effects of chemotherapy and radiotherapy are widely recognized and reviewed elsewhere (53–62). Immune potentiating mechanisms include release of tumor antigens for immune presentation, depletion of immunosuppressive cells (e.g., MDSCs, Tregs), activation of immune effectors (NK cells, DCs, B cells, conventional effector T cells), and sensitization of tumor cells to lysis.

Targeted therapies may also sensitize tumor cells to immune-mediated killing by a variety of mechanisms. These have been reviewed by Vanneman and colleagues (58), and include promoting effective DC maturation, T-cell priming, activation, and differentiation into long-lived memory T cells, increasing expression of death receptors or "distress" ligands, reducing expression of prosurvival signals, abrogating the production of tumorigenic inflammation, and inhibiting immunosuppressive cell types (63). BRAF inhibitors may also increase TILs and enhance antigen presentation (64, 65). Interestingly, while the BRAF inhibitors have a potentiating effect on the immune system, MEK inhibitors have a possible reverse effect, reducing the secretion of cytokines (66) and reducing the activity of T lymphocytes (65) and DCs (67).

Clinical experience and considerations in combining novel immunotherapies with existing treatment modalities

Ipilimumab is the most widely studied combination partner for existing treatment modalities, and data highlight the need for careful consideration in the choice of combination partner and approach to treatment. Preliminary data for ipilimumab in combination with chemotherapy, radiotherapy, and targeted therapy with BRAF inhibitors, are discussed below, alongside data with other immunotherapies. Table 2 provides a summary of ongoing clinical trials with immunotherapies (excluding ipilimumab) in combination studies with chemotherapy, radiotherapy, and targeted therapies (12).

Chemotherapy combinations. Ipilimumab has shown promising results when combined with chemotherapy in patients with melanoma and lung cancer; however, data indicate that careful consideration of the combination approach is going to be important in regard to tolerability and optimizing patient outcomes.

Patients with previously untreated melanoma who received ipilimumab (10 mg/kg) plus chemotherapy (dacarbazine) had significantly improved OS compared with those who received chemotherapy alone (11.2 months vs. 9.1 months; ref. 15). However, the benefit of the combination relative to ipilimumab alone remains unclear, as there was not an ipilimumab-alone arm in the trial. The combination was also less well tolerated compared with dacarbazine alone. Grade 3 or 4 adverse events occurred in 56.3% of patients treated with ipilimumab/dacarbazine compared with 27.5% treated with dacarbazine/placebo (P < 0.001; ref. 15). Similarly, data from a three-arm, phase I study showed that ipilimumab could be safety combined with either dacarbazine or carboplatin/paclitaxel in patients with melanoma (68).

Combining ipilimumab with paclitaxel and carboplatin significantly improved immune-related progression-free survival (irPFS) compared with chemotherapy alone in a phase II study in patients with NSCLC and extensive-disease SCLC (69, 70). However, the improvement in irPFS was only evident when the drugs were given on a phased schedule (e.g., two doses of placebo plus paclitaxel/carboplatin followed by four doses of ipilimumab plus paclitaxel/carboplatin), not when they were given concurrently. Phased ipilimumab, concurrent ipilimumab, and control, respectively, were associated with median irPFS of 5.7, 5.5, and 4.6 months in patients with NSCLC, and 6.4, 5.7, and 5.3 months in patients with SCLC. The overall incidence of treatment-related grade 3/4 adverse events was similar across the arms, and ipilimumab did not appear to exacerbate the adverse events associated with chemotherapy (69, 70). Ongoing trials are further evaluating ipilimumab/chemotherapy combinations in melanoma and lung cancer, as well as in various other solid tumors, and will hopefully provide information about how best to combine these treatment modalities.

Nivolumab is being investigated in combination with a variety of agents in a large phase I trial (CheckMate 012, NCT01454102) in chemotherapy-naive patients with NSCLC. Treatment arms include nivolumab monotherapy and control, respectively, were associated with median irPFS of 5.7, 5.5, and 4.6 months in patients with NSCLC, and 6.4, 5.7, and 5.3 months in patients with SCLC. The overall incidence of treatment-related grade 3/4 adverse events was similar across the arms, and ipilimumab did not appear to exacerbate the adverse events associated with chemotherapy (69, 70). Ongoing trials are further evaluating ipilimumab/chemotherapy combinations in melanoma and lung cancer, as well as in various other solid tumors, and will hopefully provide information about how best to combine these treatment modalities.

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erlotinib (EGFR-mutation positive nonsquamous NSCLC patients). Preliminary data indicate that nivolumab plus platinum-based chemotherapy has a manageable safety profile with no drug-related deaths reported so far. Objective responses have been observed in each arm, and 1-year OS rates ranged from 50% to 87% (71).

**Radiotherapy combinations.** Ipilimumab has been evaluated in combination with radiotherapy in patients with metastatic CRPC and melanoma. Promising activity with manageable tolerability was observed in a phase I/II trial in patients with CRPC who had progressed after anti-androgen therapy (72); however, results from a phase III trial showed no significant improvement in OS with the addition of ipilimumab to radiotherapy in post-docetaxel CRPC. A subgroup analysis did suggest benefit for patients with less advanced disease (73). An analysis of clinical data from 21 patients with advanced melanoma who had received radiotherapy after ipilimumab progression on the Italian Expanded Access Program indicated that radiotherapy after ipilimumab treatment may further potentiate its effect (74). A local response to radiotherapy was detected in 13 patients (62%), while 8 patients (38%) did not show any local regression. The median OS for all 21 patients was 13 months (range 6–26). Eleven (85%) of 13 patients with local response showed an abscopal effect, suggesting that local response to radiotherapy may be predictive for the abscopal response and outcome. The median OS for patients with and without abscopal responses was, respectively, of 22.4 months (range 2.5–50.3) and 8.3 months (range 7.6–9.0). There are now over 15 clinical trials alone in progress to evaluate ipilimumab plus radiotherapy.

Initial data from a phase I trial of MPDL3280A, an anti-PD-L1 monoclonal antibody, in combination with local radiotherapy showed evidence of activity in the five patients treated (75). Overall, case reports and data from several small clinical studies showing successful, sometimes dramatic, outcomes with radiotherapy/immunotherapy combinations in patients with melanoma provide additional support for further evaluation; these are comprehensively discussed by Barker and Postow (76).

**Targeted therapy combinations.** Clinical data are limited on the efficacy of combining ipilimumab with targeted agents, although numerous trials are ongoing, particularly in melanoma, where three targeted therapies are now approved in the United States for patients with melanoma and mutated BRAF (dabrafenib, vemurafenib, and trametinib).

Immunotherapy and BRAF inhibitor combinations are extensively reviewed by Hu-Lieskovan and colleagues (77). Some data indicate that the sequencing of BRAF inhibitors and ipilimumab has a marked effect on the efficacy and tolerability of the combination in patients with BRAF-mutant melanoma, and indicate that the drugs should be sequenced (78–80). Data from a recent retrospective analysis of a cohort of patients treated with immunotherapy and then a BRAF inhibitor (with or without a MEK inhibitor) showed prior immunotherapy did not appear to have an adverse effect on response to a BRAF inhibitor. However, outcomes were poor when ipilimumab was given after BRAF inhibitor discontinuation (81). More data are needed, but there is some rationale to use either agent first in a sequencing approach, depending on the disease kinetics. In more rapid progressors, a BRAF inhibitor may be used first to reduce tumor load followed by ipilimumab to maintain a response; in patients with more indolent disease, ipilimumab may be given first followed by vemurafenib to reduce tumor burden (78).

In a phase I trial, concurrent administration of vemurafenib and ipilimumab at the approved monotherapy doses or with a lower dose of vemurafenib resulted in hepatotoxicity that was greater than expected for either agent alone (80). These safety analyses demonstrate the risk of using vemurafenib and ipilimumab concurrently, and these drugs should not be used in combination outside of a clinical trial. Ongoing studies are evaluating the optimal sequence of these agents in patients with BRAF-mutant metastatic melanoma. Severe cutaneous and neurologic toxicity has also been reported in two patients with melanoma during therapy with vemurafenib after receiving treatment with a PD-1 immune checkpoint inhibitor (nivolumab or pembrolizumab; ref. 82). It is also noteworthy that dose-limiting toxicities have been observed in patients with RCC treated with the targeted agent sunitinib and either rhIL21 (hematologic toxicity) or the anti–CTLA-4 agent tremelimumab (renal failure), further emphasizing the need for caution when evaluating combinations (83, 84).

RCC is a tumor for which combining immunotherapy and targeted therapy is of substantial interest. Preliminary data from a phase II trial of nivolumab in combination with pazopanib or sunitinib in patients with metastatic RCC showed evidence of activity with ORRs of 45% and 52%, respectively, and a manageable safety profile (85). This trial and others evaluating various combinations in RCC continue.

The anti-CD137 agents urelumab and PF-05082566 are both in phase I trials in combination with rituximab in patients with non-Hodgkin lymphoma (Table 2). Clinical study of these agents with rituximab is based on preclinical data that have shown enhanced tumor regression when an anti-CD137 agent was used after a therapeutic monoclonal antibody (86, 87). The anti-CD137 antibody is proposed to enhance rituximab-dependent cytotoxicity through antigen-dependent cell-mediated cytotoxicity (86). Recent preclinical data showing enhanced antilymphoma activity with rituximab combined with KIR blockade (lirilumab) also support clinical investigation of this combination (34).

**Conclusions**

Immu-no-oncology is an evolving treatment modality, with agents being studied for their potential to provide long-term survival across a broad range of tumor types, and for their synergistic activity when combined with other treatment modalities. It is important now to determine how
to advance this field and how to use these new immunotherapies most effectively to achieve the best patient outcomes. Areas of investigation are broad, and include combining or sequencing immunotherapies that target distinct immune pathways, combining or sequencing an immunotherapeutic agent with existing treatment modalities, and determining the optimal schedule of therapies in combination regimens. At present, it is difficult to identify the best combination approaches to pursue given the limited data and the somewhat unexpected occurrence of toxicity with some combinations (e.g., ipilimumab and vemurafenib). Future data from preliminary clinical studies will help to direct research.

Combining immunotherapies has the potential to overcome more than one of the barriers that tumor cells develop to evade the immune system, and may provide an OS benefit in a greater portion of patients compared with either agent alone (Fig. 1). However, the ideal sequence, schedule, and combination of immunotherapies need to be determined. Likewise, it is important to determine optimal dose, schedule, and sequence when combining an immunotherapy with radiotherapy, chemotherapy, or targeted agents, as these therapies all have different mechanisms of action. A final consideration for combining immunotherapies will be to identify the regimens with the best risk–benefit profile. We can expect improvements in overall clinical efficacy as new agents targeting alternative or overlapping tumor-associated immunosuppressive mechanisms are developed and used in combination or sequentially.

Disclosure of Potential Conflicts of Interest
S.J. Antonia is a consultant/advisory board member for Bristol-Myers Squibb and MedImmune/AstraZeneca. J. Larkin reports receiving commercial research grants from Bristol-Myers Squibb, Novartis, and Pfizer, and is a consultant/advisory board member for Bristol-Myers Squibb, GlaxoSmithKline, Merck, Novartis, Pfizer, and Roche/Genentech. [Note: all consultancy/advisory board membership was uncompensated after 2012.] P.A. Ascierto reports receiving commercial research grants from Bristol-Myers Squibb, Merck, Roche/Genentech, and Ventana; speakers bureau honoraria from Bristol-Myers Squibb, GlaxoSmithKline, and Roche/Genentech; and is a consultant/advisory board member for Bristol-Myers Squibb, GlaxoSmithKline, Merck, Novartis, Roche/Genentech, and Ventana. No other potential conflicts of interest were disclosed.

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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): P.A. Ascierto
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