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Review

# Uncoupling Therapeutic Efficacy from Immune-Related Adverse Events in Immune Checkpoint Blockade

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## SUMMARY

**Immunotherapy with monoclonal antibodies targeting immune checkpoint molecules, including programmed death-1 (PD-1), PD ligand-1 (PD-L1), and cytotoxic T-lymphocyte-associated antigen (CTLA)-4, has become prominent in the treatment of many types of cancer. However, a significant number of patients treated with immune checkpoint inhibitors (ICIs) develop immune-related adverse events (irAEs). irAEs can affect any organ system, and although most are clinically manageable, irAEs can result in mortality or long-term morbidity. Factors that can predict irAEs remain elusive. Understanding the etiology of ICI-induced irAEs and ways to limit these adverse events are needed. In this review, we provide basic science and clinical insights on the mechanisms responsible for ICI efficacy and ICI-induced irAEs. We further provide insights into approaches that may uncouple irAEs from the ability of ICIs to kill tumor cells.**

## INTRODUCTION

Recent breakthroughs in cancer immunotherapy with immune-targeting antibodies, cancer vaccines, modified cytokines, and adoptive cellular therapies, with or without genetic engineering, have yielded remarkable clinical outcomes in the treatment of various cancer types. These approaches have created a paradigm shift in cancer treatment. Among these approaches, immune checkpoint blockade (ICB) therapy has shown the broadest efficacy across cancer subtypes. Immune checkpoint inhibitors (ICIs) elicit anti-tumor immunity by antagonizing the negative immune regulators expressed on immune cells and cancer cells. Several immune checkpoint antibodies targeting cytotoxic T-lymphocyte antigen-4 (CTLA-4) or programmed death 1 (PD-1), or PD-1's principal ligand PD-L1, have been the most widely evaluated in clinical trials and have been approved by the Food and Drug Administration (FDA).

Despite the clinical utility of these molecules, antibodies targeting ICI are also capable of inducing autoimmune effects on healthy organs, which have been termed immune-related adverse effects (irAEs). The pathophysiology underlying irAEs results from the same mechanisms that confer anti-tumor activity, i.e., inhibition of immune system negative regulators. Although irAEs can occur in any organ in the body, the organs most frequently affected are endocrine organs such as the thyroid gland and parenchymal lung tissue. irAEs have also been observed in the kidney, skin, gastrointestinal tract, liver, skeletal muscle, central nervous system, and bone marrow. Although discontinuation of therapy and/or glucocorticoids or other immunosuppressants are usually effective for the clinical management of irAEs, they remain a significant clinical problem and can result in discontinuation of effective therapy, acute and chronic morbidity, and in some cases death (Horvat et al., 2015; Postow et al., 2018; Wang et al., 2018a; Weber et al., 2017).

The precise pathophysiology underlying irAEs is still unclear but is believed to be associated with releasing breaks in the immune system. Among the proposed mechanisms, irAEs could result from activation of cytotoxic $CD8^+$ T cells and resultant host cell lysis or from the generation of autoantibodies (Iwama et al., 2014; Johnson et al., 2016; Oh et al., 2017; Osorio et al., 2017; Puzanov et al., 2017). The predominant organ-specific irAEs also vary with regards to ICI, severity, duration, and management strategies. In some reports, the occurrence of irAEs has been associated with increased anticancer efficacy and prolonged survival, but this correlation has not been fully yet established (Freeman-Keller et al., 2016). A more detailed comprehension of the etiology and pathogenesis of irAEs is needed, as are means of combating or preventing these adverse events. In this review, we will outline the current understanding of ICIs based on the existing

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published literature. We will also provide insights into the relationship between ICI-mediated efficacy and irAEs and offer possible solutions to uncouple antitumor immunity from irAEs.

## MECHANISMS OF ACTION ON IMMUNE CHECKPOINT MOLECULES

Understanding the normal function of CTLA-4 and PD-1/PD-L1 is critical for effective use of ICIs as cancer therapy. Although the two receptors share some functional similarities, CTLA-4 and PD-1 have distinct expression profiles, impacts on signal transduction, and mechanisms of action.

### CTLA-4-Mediated Inhibitory Co-stimulation

CTLA-4, an inhibitory receptor upregulated on nascently activated T cells, is expressed within 2 h following T cell receptor (TCR) engagement in the primary phase of T cell activation, and peaks 2–3 days following activation (Jago et al., 2004; Walunas et al., 1994). CTLA-4 dampens T cell activation by sequestering B7-1 and B7-2 (also known as CD80 and CD86) from binding to the T cell co-stimulatory receptor CD28. Since naive T cells require two signals for activation (binding of an MHC-presented peptide to the TCR [signal one] and activation of CD28 [signal two]), and because B7-1 and B7-2 are highly upregulated in the presence of innate inflammatory signals, CTLA-4 likely functions to prevent aberrant activation of naive T cells from antigen-presenting cells (APCs) expressing self-peptide and low levels of CD28 ligand. CTLA-4 has increased binding affinity for B7-1 and B7-2; thus, these ligands need to be expressed at significantly high levels to saturate upregulated CTLA-4 and productively engage CD28 (Rudd et al., 2009). CTLA-4 up-regulation is biphasic and results from rapid upregulation of CTLA-4 present on intracellular vesicles that translocate to the cell surface membrane after T cell activation (Egen and Allison, 2002) and subsequent transcriptional upregulation (Perkins et al., 1996). Immediately after T cell activation, CTLA-4 function is dependent on physical mobilization to the immune synapse (Teft et al., 2006), a spatiotemporal site on T cells (which also includes the TCR and CD28) that integrates positive and negative regulators to contribute to the amplitude of T cell activation. Once transported to immune synapses on the cell surface, CTLA-4 accumulates on the cell surface through stable binding to B7 ligands (Pentcheva-Hoang et al., 2004). In addition to sequestering B7-1 and B7-2, CTLA-4 is also capable of removing these proteins from the surface of antigen-presenting cells (APCs) through transcytosis, thereby further limiting their ability to promote T cell activation (Qureshi et al., 2011). Through these activities, CTLA-4 exquisitely dampens activation of naive T cells (Wei et al., 2018).

In contrast to its role in regulating naive T cell activation, CTLA-4 is constitutively expressed on $CD4^+CD25^{hi}$ $FoxP3^+$ (Treg) cells. CTLA-4 on Tregs has been postulated to serve as a ligand to stimulate APC-mediated generation of immunosuppressive molecules such as indolamine 2,3-dioxygenase (IDO), which limits tryptophan accessibility to expanding T cell populations (Wing et al., 2008). Its engagement on Tregs also stimulates the production of inhibitory cytokines including IL-10, IL-35, and TGF-β. The suppressive function of CTLA-4 on Tregs was demonstrated via studies of germline deletion in Treg cells, which resulted in inappropriate activation and expansion of effector T cells and lethal autoimmunity (Waterhouse et al., 1995; Wing et al., 2008). In support of a crucial role for CTLA-4 in Tregs, lineage-specific deletion of CTLA-4 in mouse Tregs results in spontaneous lymphoproliferation, hyper-gammaglobulinemia, and autoimmune disorders, albeit with less severity than that observed in germline CTLA-4-deficient mice (Klocke et al., 2016; Paterson et al., 2015). The attenuated phenotype may in part be related to up-regulation of other Treg-expressed inhibitory molecules, including the surface receptor LAG3 and secretion of the cytokine IL-10 (Paterson et al., 2015). Collectively, these studies reveal the complex and significant role of CTLA-4 in T cell biology.

### PD-1/PD-L1-Mediated Inhibition of T Cells

The main biological role of PD-1/PD-L1 is to prevent autoimmunity resulting from persistent antigen exposure, which may occur during aberrant activation of T cells that respond to self-antigens and have evaded other tolerance mechanisms (Virgin and Todd, 2011). Chronic activation of effector T cells triggers expression of PD-1 on T cells resulting in T cell "exhaustion"; a negative feedback loop is formed to alleviate the local T cell response and reduce damage to tissues (Wherry, 2011). PD-1 inhibits T cell activity after binding with its specific ligands PD-L1 (CD274) and PD-L2 (CD273). PD-1 is also transiently expressed on acutely activated immune cells to limit overexuberant immune responses of Th1 $CD4^+$ and $CD8^+$ T cells, B cells, natural killer (NK) cells, and dendritic cells (Keir et al., 2008). As a target for cancer therapy, expression of PD-1 is thought to be most relevant to exhausted T cells where it can re-invigorate responses of

tumor-antigen specific cells $CD8^+$ T cells (Barber et al., 2006), although evidence is accumulating for additional roles for targeting PD-1 in early T cell activation (Jin et al., 2011), central tolerance, and negative selection (Blank et al., 2003). The two ligands for PD-1 demonstrate differential expression. PD-L1 is expressed ubiquitously on cells, especially after exposure to inflammatory cytokines such as IFN-γ, whereas PD-L2 expression is restricted to DCs and macrophages (Keir et al., 2006). The engagement of PD-1 on effector T cells results in attenuation of TCR signaling, at least in part through activation of the tyrosine phosphatase SHP2, which de-phosphorylates and antagonizes proximal signaling mediators of the TCR complex such as Zap-70 and Lck (Yokosuka et al., 2012). Genetic loss of the *Pdcd1* gene (encoding PD-1) results in autoimmune pathologies in mice, such as lupus-like autoimmune pathology in aged C57BL/6 mice or autoimmune dilated cardiomyopathy in BALB/c mice (Nishimura et al., 1999, 2001).

In contrast to its critical role in regulating immune tolerance, PD-1/PD-L plays a deleterious role by impairing effective immune responses in cancer and chronic viral infection (Francisco et al., 2010). PD-L1 is highly expressed in at least 40 cancer types including non-small cell lung cancer (NSCLC) (Konishi et al., 2004), small cell lung cancer (SCLC) (Takada et al., 2016), melanoma (Hino et al., 2010), bladder cancer (Nakanishi et al., 2007), renal cancer (Fay et al., 2015), non-Hodgkin's lymphoma (Andorsky et al., 2011), and hepatocellular carcinoma (HCC) (Calderaro et al., 2016). PD-L1 and PD-L2 are also elevated on the cell surface of tumor-infiltrating macrophages and myeloid cells in response to inflammatory cytokines, including IFN-γ, which further dampen anti-tumor immunity and facilitate T cell exhaustion (Freeman et al., 2000; Ishida et al., 2002; Iwai et al., 2002; Latchman et al., 2001). PD-1 is the best studied marker and effector of exhaustion, whereas other inhibitory proteins are subsequently expressed in T cells chronically exposed to antigens, including TIM3, 2B4, and LAG3, among others (Blackburn et al., 2009). In exhausted T cells, the expression of PD-1 and other inhibitory receptors result in decreased effector function, including decreased proliferation and cytokine production in response to stimulation, and ultimately apoptosis. Expression of PD-L1 on tumors correlates with anti-PD-1/PD-L1 efficacy in cancer treatment (Khunger et al., 2017).

## MECHANISMS OF ICI-MEDIATED TUMOR REJECTION

### CTLA-4 Blockade

Although the mechanisms underlying how CTLA-4 blockade exerts antitumor effects are not fully understood, at least two physiologic mechanisms have been identified (reviewed in Wei et al., 2018). These include enhancing T cell differentiation and expansion of tumor antigen-specific T cells from direct sequestration of B7-1 and B7-2 during acute activation of effector T cells (Chen et al., 2009; Fehlings et al., 2017; Liakou et al., 2008; Ng Tang et al., 2013) and depletion of inhibitory Treg cells through antibody-dependent cellular cytotoxicity (ADCC) resulting from constitutive expression of CTLA-4 on Tregs (Arce Vargas et al., 2018; Romano et al., 2015). Additional studies have identified a potential third role of anti-CTLA-4 in remodeling and enriching the peripheral TCR repertoire through mechanisms that may be related to or be independent of the other two mechanisms (Cha et al., 2014; Kvistborg et al., 2014; Robert et al., 2014b).

### PD-1/PD-L Blockade

PD-1/PD-L1 blockade potentially reinvigorates exhausted $CD8^+$ T cells that have been chronically stimulated by persistent antigens present in chronic viral infection or tumor cells. Molecularly, antibodies targeting this axis act by attenuating proximal TCR signaling, thereby enhancing the functions of exhausted $CD8^+$ T cells (Huang et al., 2017). Apart from acutely increasing activation of downstream transcription factors such as AP-1 and NF-κB, blockade of PD-1/PD-L also may partially reverse impaired metabolic activity of $CD8^+$ T cells, which contribute to exhaustion reversal (Gubin et al., 2014; Patsoukis et al., 2015). Interestingly, some studies of various antibodies used to inhibit PD-1 or PD-L1 have shown that individual antibodies targeting the proteins may not function identically. These differences may result from how effectively individual antibodies bind to cognate epitopes and successfully disrupt antibody/antigen interactions or could be due to changes in binding of the antibodies to FcRs; anti-PD-L1, but not anti-PD-1, is dependent on FcR binding *in vivo* (Dahan et al., 2015). In addition, B7-1 and PD-L1 interaction also increases the variability in the effect of antibodies targeting PD-L1 and PD-1. Briefly, T cell-expressed PD-L1 can interact with APC-expressed B7-1 (*trans*-PD-L1/B7-1 interaction), and T cell-expressed B7-1 engages APC-expressed PD-L1 (*tran*-B7-1/PD-L1 interaction) to transduce inhibitory signals for T cell activation (Butte et al., 2007; Park et al., 2010).

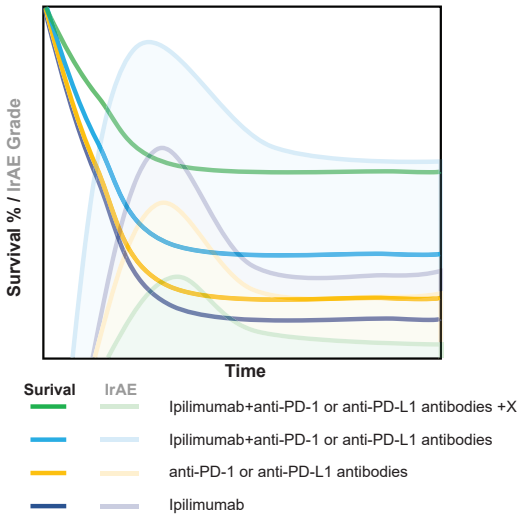


**Figure 1. A Model Illustrating the Potential Impact of Monotherapy and Combination Immunotherapies on Survival and irAEs**

The dark curves represent theoretical survival rates, and the corresponding transparent curves and their shaded areas represent predicted irAEs. An emerging concept is that blockade of CTLA-4 and PD-1/PD-L1 represent initial treatments to combine with other therapies (illustrated as X). Thus, combination immunotherapy regimens (Ipilimumab + anti-PD-1 or anti-PD-L1 antibodies + X) have the potential to generate productive tumor responses with less irAEs.

### Combination Immunotherapies

Compared with monotherapy, the combined targeting of PD-1/PD-L and CTLA-4 improves efficacy (Figure 1). This partially results from compensatory upregulation of other immune checkpoint molecules with the use of monotherapy (Wei et al., 2018). Combined blockade also results in shared biological effects that synergistically enhance T cell activity (Wei et al., 2018). For instance, recent studies have shown that PD-1 inhibits the signal transduction of CD28 in addition to the proximal signal transduction element of the TCR complex (Hui et al., 2017). Thus, in combination with CTLA-4, which inhibits T cell activation primarily by limiting CD28 stimulation, blockade of PD-1 can significantly enhance T cell co-stimulation. Additionally, combination therapies may work together to modulate different T cell subpopulations (Wei et al., 2017). For example, CTLA-4 blockade leads to increased numbers of tumor-infiltrating $ICOS^{+}$Th1-like $CD4^{+}$ effectors, whereas PD-1 blockade acts primarily by targeting $CD8^{+}$ T cell subsets. Thus, at the cellular level, combined anti-CTLA-4 and anti-PD-1 antibodies therapies can differentially activate different components of adaptive immunity. It is worth noting that the number of highly exhausted ($PD1^{hi}TIM3^{hi}LAG3^{hi}$) $CD8^{+}$ T cells present after anti-PD1 monotherapy is greatly reduced with combination therapy, indicating that combination therapy can also alter individual T cell subsets in murine tumor models (Wei et al., 2019).

## FDA-APPROVED ICIS

The FDA has approved the use of seven ICIs as single-agent therapies across multiple tumor subtypes. Detailed clinical evidence supporting FDA approval on ICIs from 2011 to 2020 is summarized in Table 1. Ipilimumab, an anti-CTLA-4 monoclonal antibody, was the first FDA-approved ICI drug for cancer treatment in melanoma. Anti-PD-1 therapies were next approved and include nivolumab, pembrolizumab, and cemiplimab. Anti-PD-L1 antibodies have also been approved and include atezolizumab, avelumab, and durvalumab. Antibodies targeting PD-1/PD-L1 have received approval for multiple indications including the treatment of unresectable or metastatic melanoma; advanced renal cell cancer (RCC); microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) metastatic colorectal cancer; NSCLC; metastatic or unresectable, recurrent head and neck cancer (squamous cell); locally advanced or metastatic urothelial carcinoma; locally advanced or metastatic gastric cancer (including gastroesophageal junction adenocarcinoma); and recurrent or metastatic PD-L1-expressing (combined positive score [CPS] 1 or higher) cervical cancer.

Recently, the efficacy of nivolumab and ipilimumab combination therapy has been compared with ipilimumab monotherapy in previously untreated patients with melanoma (CheckMate 069, NCT01927419). This trial found that, for melanoma with wild-type BRAF, the objective response for combined checkpoint inhibition versus ipilimumab monotherapy was 61% (95% confidence interval [CI]: 49–72) and 11% (95% CI: 3–25), respectively. Furthermore, 22% of subjects receiving combination therapy showed a complete response compared with none among those receiving ipilimumab monotherapy. This led to combination

| Therapeutic Agent | Target | Tumor Type | FDA Approval year | Clinical Trial | Arms | Study Endpoints (95% CI) | Toxicity Outcomes (Grade>=3 IRAEs, %) | References |
|---|---|---|---|---|---|---|---|---|
| Ipilimumab | CTLA4 | Unresectable/ metastatic melanoma | 2011 | NCT00094653 | IPI + gp100 | OS, mo:10.0 (8.5–11.5) | 10.2% | (Hodi et al., 2010) |
| | | | | | IPI | OS, mo:10.1 ( 8.0–13.8) mo | 14.5% | |
| | | | | | gp100 | OS, mo: 6.4 (5.5–8.7) | 3% | |
| Ipilimumab | CTLA4 | Fully resected stage III melanoma | 2015 | NCT00636168 | IPI | RFS, mo: 26.1(19.3–39.3) | 44% | (Eggermont et al., 2015) |
| | | | | | Placebo | RFS, mo:17.1(13.4–21.6) | <3% | |
| Pembrolizumab | PD-1 | Advanced melanoma | 2014 | NCT01295827 | PEM 2 mg/kg | ORR, %: 26 | 4% | (Robert et al., 2014a) |
| | | | | | PEM 10 mg/kg | ORR, %:26 | 3% | |
| Pembrolizumab | PD-1 | PD-L1 Positive NSCLC | 2016 | KEYNOTE-024 (NCT0214273) | PEM | PFS, mo:10.3 (6.7-not reached) ORR, %: 44.8 (36.8–53.0) | 9.7% | (Reck et al., 2016) |
| | | | | | CHEMO | PFS, mo: 6.0 (4.2–6.2) ORR, %: 27.8(20.8–35.7) | 0.7% | |
| Pembrolizumab | PD-1 | Recurrent/ metastatic HNSCC | 2016 | KEYNOTE-012 (NCT0184883) | PEM | ORR, %: 18 (12–26) or 20 (13–28) | <6% | (Chow et al., 2016) |
| Pembrolizumab | PD-1 | Refractory/ relapsed cHL | 2017 | KEYNOTE-087 (NCT02453594) | PEM | ORR, %:69 (62.3–75.2) | NA | (Chen et al., 2017) |
| Pembrolizumab | PD-1 | Locally Advanced and Unresectable or Metastatic Urothelial Cancer | 2017 | KEYNOTE-052 (NCT02335424) | PEM | ORR, %: 24 (20–29) | NA | (Balar et al., 2017) |
| Pembrolizumab | PD-1 | Advanced Urothelial Carcinoma | 2017 | KEYNOTE-045 (NCT0225643) | PEM | OS, mo:10.3 (8.0–11.8) PFS, mo:2.1 (2.0–2.2) ORR, %: 21.1 (16.4–26.5) | 4.5% | (Bellmunt et al., 2017) |
| | | | | | CHEMO | OS, mo:7.4 (6.1–8.3) PFS, mo:3.3 (2.3–3.5) ORR, %: 11.4 (7.9–15.8) | 1.6% | |

**Table 1. Summary of Clinical Trials for Which Immune Checkpoint Blockade Therapies Are FDA Approved (Updated to July 2020)**

*(Continued on next page)*

| Therapeutic Agent | Target | Tumor Type | FDA Approval year | Clinical Trial | Arms | Study Endpoints (95% CI) | Toxicity Outcomes (Grade>=3 IRAEs, %) | References |
|---|---|---|---|---|---|---|---|---|
| Pembrolizumab | PD-1 | MSI-hi or dMMR tumor | 2017 | KEYNOTE-016, -164, −012, −028, and −158 | PEM | ORR, %:39.6 (31.7–47.9) | NA | (Marabelle et al., 2020) www.ascopost.com |
| Pembrolizumab | PD-1 | Stomach and gastroesophageal cancer | 2017 | KEYNOTE-059 (NCT02335411) | PEM | PFS, mo:13.3(8.2–20.0) | NA | www.fda.gov/drugs |
| Pembrolizumab | PD-1 | Cervical cancer | 2018 | KEYNOTE-158 (NCT02628067) | PEM | ORR, %:12.2 (6.5–20.4) | 5.1% | (Chung et al., 2019) |
| Pembrolizumab | PD-1 | rrMLBCL | 2018 | KEYNOTE-013 (NCT01953692) | PEM | ORR, %:48 (26–70) | 5% | (Armand et al., 2019) |
| | | | | KEYNOTE-170 | PEM | ORR, %:45 (32–60) | 2% | |
| Pembrolizumab | PD-1 | Advanced, treatment-resistant HCC | 2018 | KEYNOTE-224 (NCT02702414) | PEM | ORR, %:17 (11–26) | 4% | (Zhu et al., 2018) |
| Pembrolizumab | PD-1 | MCC | 2018 | KEYNOTE-017 (NCT02267603) | PEM | OS, mo: not reached<br>PFS, mo:16.8(4.6-not estimable)<br>ORR, %:56(41.3–70.0) | NA (28%TRAE) | (Nghiem et al., 2019) |
| Pembrolizumab | PD-1 | High-risk, stage III melanoma | 2019 | KEYNOTE-054 (NCT02362594) | PEM | 1-year rate of RFS, %: 75.4 (71.3–78.9) | 7.1% | (Eggermont et al., 2018) |
| | | | | | Placebo | 1-year rate of RFS,%: 61.0 (56.5–65.1) | 0.6% | |
| Pembrolizumab | PD-1 | Stage III NSCLC | 2019 | KEYNOTE-042 (NCT02220894) | PEM | OS, mo:<br>PDL1 TPS ≥50%: 20·0 (15·4–24·9)<br>TPS ≥20%:17·7 (15·3–22·1)<br>TPS≥1%:16·7(13·9–19·7) | 8% | (Mok et al., 2019) |
| | | | | | Placebo | OS,mo:<br>PDL1 TPS≥50%: 12 ·2 (10·4–14·2)<br>TPS ≥20%: 13·0 (11·6–15·3);<br>TPS≥1%:12·1(11·3–13·3) | 1% | |

**Table 1. *Continued***

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| Therapeutic Agent | Target | Tumor Type | FDA Approval year | Clinical Trial | Arms | Study Endpoints (95% CI) | Toxicity Outcomes (Grade>=3 IRAEs, %) | References |
|---|---|---|---|---|---|---|---|---|
| Pembrolizumab | PD-1 | Metastatic or unresectable recurrent HNSCC | 2019 | KEYNOTE-048 (NCT02358031) | PEM | PFS, mo:<br>PD-L1 CPS ≥20: 3.4 (3.2–3.8)<br>CPS ≥1: 3.2 (2.2–3.4) | NA<br>7% (TRAE) | (Burtness et al., 2019) |
| | | | | | PEM with CHEMO | PFS, mo:<br>PD-L1 CPS ≥20: 5·8 (4.7–7.6);<br>CPS ≥1: 5·0 (4.7–6.2) | NA<br>47% (TRAE) | |
| | | | | | Cetuximab with CHEMO | PFS, mo:<br>PD-L1 CPS ≥20:5.2 (4.8–6.2);<br>CPS ≥1: 5·0 (4·8–5·8) | 39% (TRAE) | |
| Pembrolizumab | PD-1 | Advanced ESCC | 2019 | KEYNOTE-180 (NCT02559687) | PEM | ORR, %:<br>PD-L1 CPS ≥10: 0 (8–37) | 5.8% | (Shah et al., 2019) |
| Pembrolizumab | PD-1 | | | KEYNOTE-181 (NCT02564263) | PEM | OS, mo:<br>PD-L1 CPS ≥10: 10.3 (7.0–13.5) | 18% (TRAE) | (Kojima et al., 2019) |
| | | | | | CHEMO | OS, mo:<br>PD-L1 CPS ≥10:6.7 (4.8–8.6) | 41% (TRAE) | |
| Pembrolizumab | PD-1 | High-risk NMIBC | 2020 | KEYNOTE-057 (NCT02625961) | PEM | CRR: 41 (31–51) | NA | https://www.mrknewsroom.com/news-releases/ |
| Nivolumab | PD-1 | Metastatic melanoma | 2014 | CheckMate-037 (NCT01721746) | NIVO | ORR, %:31(23·5–40·8) | 9% (TRAE) | (Weber et al., 2015) |
| | | | | | CHEMO | ORR, %:10·6 (3·5–23·1) | 32% (TRAE) | |
| Nivolumab | PD-1 | Metastatic NSCLC | 2015 | CheckMate-063 (NCT01721759) | NIVO | OS, mo: 8.1(6.1,10.9)<br>ORR, %:15 (9–22) | 17% (TRAE) | (Lena et al., 2016) |
| | | | | CheckMate-017 (NCT01642004) | NIVO | OS, mo: 9.2 (7.33, 12.62);<br>ORR, %: 20 (14, 28) | 8% (TRAE) | |

**Table 1. *Continued***

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| Therapeutic Agent | Target | Tumor Type | FDA Approval year | Clinical Trial | Arms | Study Endpoints (95% CI) | Toxicity Outcomes (Grade>=3 IRAEs, %) | References |
|---|---|---|---|---|---|---|---|---|
| Nivolumab | PD-1 | Non-squamous NSCLC | 2015 | CheckMate-057 (NCT01673867) | NIVO | OS, mo: 12.2 (9.7–15.0)<br>PFS, mo: 2.3 (2.2–3.3)<br>ORR, %: 19 (15–24) | 10% (TRAE) | (Borghaei et al., 2015) |
| | | | | | CHEMO | OS, mo: 9.4 (8.1–10.7);<br>PFS, mo:4.2 (3.5–4.9)<br>ORR, %:12 (9–17) | 54% (TRAE) | |
| Nivolumab | PD-1 | Metastatic RCC | 2015 | CheckMate-025 (NCT01668784) | NIVO | OS, mo: 25.0 (21.8- not estimable)<br>PFS, mo:4.6 (3.7–5.4) | 19% (TRAE) | (Motzer et al., 2015) |
| | | | | | Everolimus | OS, mo:19.6 (17.6–23.1)<br>PFS, mo: 4.4 (3.7–5.5) | 37% (TRAE) | |
| Nivolumab | PD-1 | Previously untreated BRAF wild-type advanced melanoma | 2015 | CheckMate −066 | NIVO | OS, mo: 37.5 (25.5 -not reached)<br>PFS, mo: 5.1 (3.5–12.2)<br>ORR, %:42.9 | 15% (TRAE) | (Ascierto et al., 2019; Robert et al., 2015a) |
| | | | | | Dacarbazine | OS, mo:11.2 (9.6–13.0)<br>PFS, mo: 2.2 (2.1–2.5)<br>ORR, %:14.4 | 17.6% (TRAE) | |
| Nivolumab | PD-1 | cHL | 2016 | CheckMate-205 (NCT02181738) | NIVO | ORR, %: 66.3(54.8–76.4) | 25% (TRAE) | (Younes et al., 2016) |
| | | | | CheckMate-039 (NCT01592370) | NIVO | ORR, %:<br>FL:40<br>DLBCL:36<br>MF:15<br>PTCL: 40 | 20.4%(TRAE) | (Lesokhin et al., 2016) |
| Nivolumab | PD-1 | Recurrent or metastatic HNSCC | 2016 | CheckMate-141 NCT02105636 | NIVO | OS, mo: 7.5 (5.5–9.1)<br>PFS, mo: 2.0 (1.9–2.1)<br>ORR, %:13.3 | 13.1% (TRAE) | (Ferris et al., 2016) |
| | | | | | CHEMO | OS, mo:5.1 (4.0–6.0)<br>PFS, mo:2.3 (1.9–3.1)<br>ORR, %:5.8 | 35.1% (TRAE) | |

**Table 1. *Continued***

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| Therapeutic Agent | Target | Tumor Type | FDA Approval year | Clinical Trial | Arms | Study Endpoints (95% CI) | Toxicity Outcomes (Grade>=3 IRAEs, %) | References |
|---|---|---|---|---|---|---|---|---|
| Nivolumab | PD-1 | Advanced urothelial carcinoma | 2017 | CheckMate-275 (NCT02387996) | NIVO | ORR, %:19·6 (15·0–24·9) | 18% (TRAE) | (Sharma et al., 2017) |
| Nivolumab | PD-1 | Relapsed colorectal cancer with high MSI-hi | 2017 | CheckMate-142 (NCT02060188) | NIVO | ORR, %: 31·1(20·8–42·9) | 20.3% (TRAE) | (Overman et al., 2017) |
| Nivolumab | PD-1 | Liver cancer | 2017 | CheckMate-040 (NCT0165887) | NIVO | ORR, %: dose-expansion phase:20 (15–26) dose-escalation: 15(6–28) | 25% (TRAE) | (El-Khoueiry et al., 2017) |
| Nivolumab | PD-1 | Metastatic SCLC | 2018 | CheckMate-032 (NCT01928394) | NIVO 3 mg/kg | ORR, %:10 | 13% (TRAE) | (Antonia et al., 2016) |
| | | | | | NIVO 1 mg/kg + IPI 1 mg/kg | ORR, %:33 | 0 (TRAE) | |
| | | | | | NIVO 1 mg/kg + IPI 3 mg/kg | ORR, %:23 | 30% (TRAE) | |
| | | | | | NIVO 3 mg/kg + IPI 1 mg/kg | ORR, %:19 | 19% (TRAE) | |
| Avelumab | PD-L1 | MCC | 2017 | NCT02155647 | AVE | ORR, %:62.1(42.3–79.3) | 20.5% (TRAE) | (D'Angelo et al., 2018) |
| Avelumab | PD-L1 | Advanced bladder cancer | 2017 | NCT01772004 | AVE | OS, mo: 6·5 (4·8–9·5) ORR, %:17(11–24) | 8% (TRAE) | (Patel et al., 2018) |
| Durvalumab | PD-L1 | Advanced bladder cancer | 2017 | NCT01693562 | DURV | ORR, %: PD-L1 high:27.6 (19.0–37.5); PD-L1 low or negative:5.1 (1.4–12.5) Total:17.8 (12.7–24.0); | 2.1% | (Powles et al., 2017) |
| Durvalumab | PD-L1 | Unresectable, stage III NSCLC | 2018 | NCT02125461 | DURV | PFS, mo:16.8 (13.0–18.1) ORR, %:28.4 | 3.4% | (Antonia et al., 2017) |
| | | | | | placebo | PFS, mo:5.6 (4.6–7.8) ORR, %:16.0 | 2.6% | |

**Table 1. *Continued***

*(Continued on next page)*

| Therapeutic Agent | Target | Tumor Type | FDA Approval year | Clinical Trial | Arms | Study Endpoints (95% CI) | Toxicity Outcomes (Grade>=3 IRAEs, %) | References |
|---|---|---|---|---|---|---|---|---|
| Cemiplimab | PD-1 | CSCC | 2018 | NCT02383212; NCT02760498 | CEMI | ORR, %: 47 (34–61) | NA | (Migden et al., 2018) |
| Atezolizumab | PD-L1 | Bladder cancer | 2016 | NCT02108652 | ATEZ | OS, mo:<br>IC2/3, 11·4 (9·0 -not estimable)<br>IC1/2/3, 8·8 (7·1 to 10·6)<br>PFS, mo:<br>IC2/3, 4·0 (2·6 - 5·9)<br>IC1/2/3, 2·9 (2·1–4·1)<br>ORR, %:<br>IC2/3, 27% (19–37)<br>IC1/2/3, 18% (13–24) | 16% (TRAE) | (Rosenberg et al., 2016) |
| Atezolizumab | PD-L1 | Metastatic, chemotherapy-resistant NSCLC | 2016 | Phase III OAK (NCT02008227) | ATEZ | OS, mo: 13.8 (11.8–15.7) | NA | (Yu et al., 2019)<br>www.gene.com/media/ |
| | | | | | Docetaxel | OS, mo: 9.6 (8.6–11.2) | NA | |
| | | | | Phase II POPLAR studies (NCT01903993) | ATEZ | OS, mo: 12.6 (9.7–16.0)<br>ORR, %:15(10–22) | NA | |
| | | | | | Docetaxel | OS, mo: 9.7 (8.6, 12.0)<br>ORR, %:15 (9–22) | NA | |
| Atezolizumab | PD-L1 | Metastatic NSCLC with high PD-L1 expression | | IMpower11 (NCT02409342) | ATEZ | OS, mo: 20.2 (16.5-not estimable)<br>PFS, mo:8.1 (6.8–11.0)<br>ORR, %:38 (29–48) | 12.9% (TRAE) | (Spigel et al., 2019) |
| | | | | | CHEMO | OS, mo: 13.1 (7.4, 16.5)<br>PFS, mo:5.0 (4.2–5.7)<br>ORR, %: 29 (20–39) | 44.2% (TRAE) | |

**Table 1. *Continued***

*(Continued on next page)*

| Therapeutic Agent | Target | Tumor Type | FDA Approval year | Clinical Trial | Arms | Study Endpoints (95% CI) | Toxicity Outcomes (Grade>=3 IRAEs, %) | References |
|---|---|---|---|---|---|---|---|---|
| Ipilimumab plus nivolumab | CTLA4+ PD-1 | Advanced melanoma | 2015 | CheckMate −069 (NCT01927419) | IPI + NIVO | PFS, mo:<br>BRAF wild-type, not reached;<br>BRAF mutation–positive, 8.5 (2.8 to not estimable)<br>ORR, %:<br>BRAF wild-type, 61 (49–72);<br>BRAF mutation–positive,52(31–73) | 54% (TRAE) | (Postow et al., 2015) |
| | | | | | IPI + placebo | PFS, mo:<br>BRAF wild-type, 4.4 (2.8–5.7)<br>BRAF mutation–positive, 2.7 (1.0–5.4)<br>ORR, %:<br>BRAF wild-type: 11 (93–25)<br>BRAF mutation–positive:10(0–45) | 24% (TRAE) | |
| Ipilimumab plus nivolumab | CTLA4+ PD-1 | Advanced renal cell carcinoma | 2018 | CheckMate −214 (NCT02231749) | IPI + NIVO | OS, mo: not reached (28.2 - not estimable)<br>PFS, mo: 11.6 (8.7–15.5)<br>ORR, %: 42 (37–47) | 46% (TRAE) | (Motzer et al., 2018) |
| | | | | | Sunitinib | OS, mo: 26.0 (22.1 - not estimable)<br>PFS, mo: 8.4 (7.0–10.8);<br>ORR, %:27 (22–31) | 63%(TRAE) | |
| Ipilimumab plus nivolumab | CTLA4+ PD-1 | Relapsed or refractory CRC with MSI-hi or dMMR | 2018 | CheckMate-142 (NCT02060188) | IPI + NIVO | OS, mo:54.6 (45.2–63.8)<br>ORR, %: 55 (45.2–63.8) | 32% (TRAE) | (Overman et al., 2018) |
| | | Advanced HCC | 2020 | CheckMate −040 (NCT01658878) | IPI + NIVO | ORR, %:31 | 38% (TRAE) | (He et al., 2020)<br>www.targetedonc.com |

**Table 1. *Continued***

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| Therapeutic Agent | Target | Tumor Type | FDA Approval year | Clinical Trial | Arms | Study Endpoints (95% CI) | Toxicity Outcomes (Grade>=3 IRAEs, %) | References |
|---|---|---|---|---|---|---|---|---|
| Ipilimumab plus nivolumab | CTLA4+ PD-1 | Metastatic NSCLC (PD-L1 tumor expression ≥1%) | 2020 | CheckMate-227 (NCT02477826) | IPI + NIVO | OS, mo: 17.1 (15-20.1)<br>PFS, mo: 5.1 (4.1–6.3)<br>ORR, %: 36 (31–41) | 32.8%(TRAE) | (Hellmann et al., 2019) |
| | | | | | CHEMO | OS, mo:14.9 (12.7–16.7)<br>PFS, mo: 5.6 (4.6–5.8)<br>ORR, %: 30 (26–35) | 36%(TRAE) | |

**Table 1. Continued**

Abbreviations: IPI, Ipilimumab; PEM, Pembrolizumab; NIVO, Nivolumab; AVE, Avelumab; DURV, Durvalumab; CEMI, Cemiplimab; ATEZ, Atezolizumab; CHEMO, Chemotherapy; TRAE, treatment-related adverse events; IRAE, immune-related adverse events; CI, confidence interval; MO, month; OS, overall survival; RFS, recurrence-free survival; ORR, objective response rate; PFS, progression-free survival.

nivolumab and ipilimumab in patients with melanoma becoming the first FDA-approved combination for ICI blockade in cancer treatment. Since then, ipilimumab combined with nivolumab has been approved by the FDA for the treatment of melanoma, renal cell cancer, $MSI^{hi}$ or dMMR metastatic colorectal cancer, advanced hepatocellular carcinoma (HCC), and NSCLC.

## ICI-INDUCED IRAES IN PATIENTS WITH CANCER

Since PD-1 and CTLA-4 both participate in immune homeostasis, the use of ICIs in clinical cancer therapy has the potential downside of disrupting immune tolerance in patients. The irAEs triggered by these treatments can be clinically significant especially when they are administered in combination (Martins et al., 2019a). Evidence from large, prospective clinical trials has identified characteristic adverse event profiles of anti-CTLA-4 and anti-PD-1 or PD-L1 antibodies. These toxicities have been reviewed in detail elsewhere (Boutros et al., 2016; Kennedy and Salama, 2020), but we will reiterate the most salient points here (Figure 1).

### Anti-CTLA 4 Antibody

In a phase I/II study of ipilimumab monotherapy in patients with metastatic melanoma, the incidence of any grade of irAEs was 72% and the incidence of grade 3–4 irAEs was 14% (Weber et al., 2008). irAEs after ipilimumab tended to occur during initial treatment or re-initiation of treatment (Hodi et al., 2010). The most common irAEs of any grade occurred in (1) skin, i.e., pruritus and cutaneous rash; (2) gastrointestinal tract, i.e., colitis and diarrhea; (3) liver, i.e., autoimmune hepatitis; and (4) endocrine, i.e., thyroid dysfunction and hypophysitis (Eggermont et al., 2015; Hodi et al., 2010). Immune-related neuropathy, arthritis, uveitis, and myositis occur infrequently (Weber et al., 2012). Toxicity-related fatality occurred in 1.08% of patients receiving anti-CTLA-4 in a clinical trial (Wang et al., 2018a), although improved management of the resulting colitis has decreased mortality significantly (Larkin et al., 2019).

### Anti-PD-1/PD-L Antibody

The irAEs associated with anti-PD-1/PD-L1 antibodies are less common and differ as to the most commonly involved organs (Boutros et al., 2016). irAEs from checkpoint blockade by anti-PD-1/anti-PD-L1 have been reported in approximately 13%–41% patients, with about 1%–14% of patients experiencing a severe (grade 3–4) irAE (Robert et al., 2015a, 2015b; Topalian et al., 2012; Wang et al., 2019) (Table 1). The most frequent irAEs include pruritus, rash, nausea, diarrhea, and thyroid disorders (Boutros et al., 2016). Autoimmune thyroiditis and pneumonitis in patients with cancer occurs more frequently with PD-1 blockade compared with CTLA-4 blockade (Boutros et al., 2016). Fatal irAEs rarely occur with anti-PD-1/PD-L1 therapies, but the most common causes include pneumonitis (35%), hepatitis (22%), and neurotoxicity (15%). irAEs from anti-PD-1/anti-PD-L1 typically occur within the first 6 months of therapy. There appears to be little difference in toxicity among various anti-PD-1 or anti-PD-L1 antibodies. For instance, the toxicity profile of pembrolizumab is similar to that of nivolumab (Wang et al., 2018b). However, no direct comparative trials have been conducted.

### Combination Therapy

A combination regimen of anti-CTLA-4 and anti-PD-1 antibodies results in increased incidence and severity of irAEs, compared with monotherapy (Wang et al., 2018b). A pooled evaluation of data from the Checkmate 037, 067, and 069 trials involving patients receiving nivolumab, ipilimumab, or nivolumab with ipilimumab reported grade 3–4 irAEs in 8%, 19%, and 40% of the patients, respectively (Hassel et al., 2017). Moreover, a meta-analysis studying fatal irAEs in patients treated with anti-PD-1, CTLA-4, or combined therapy identified 0.36%, 1.08%, or 1.23% events, respectively (Wang et al., 2018b). Patients receiving combined treatment experienced earlier-onset irAEs compared with patients receiving monotherapy, with a median onset time of 14.5 days (Kennedy and Salama, 2020; Martins et al., 2019a). The most frequently experienced irAEs of any grade with nivolumab and ipilimumab combination therapy include diarrhea, pruritus, and rash. The most common grade 3 or higher irAEs with combination therapy include hepatitis (as evidenced by increases in ALT or AST) and colitis (Xing et al., 2019). The most common causes of treatment-associated fatalities in patients treated with combined PD-1/PD-L1 and CTLA-4 blockade result from autoimmune colitis (37%) and myocarditis (25%) (Wang et al., 2018b).

## ASSOCIATION BETWEEN IRAES AND CLINICAL OUTCOMES

Several studies have observed that the occurrence of irAEs during ICI treatment is a positive prognostic indicator of treatment response. However, this correlation has not yet been prospectively studied. We will review the current available literature on the linkage between ICI-induced irAEs and therapeutic outcomes.

### Overview of the Association between irAEs and Clinical Outcomes

A meta-analysis assessing the association between disease control rate (DCR) and irAEs in patients with melanoma treated with ipilimumab identified a difference in DCR between the patients who experienced grade 0–1 irAEs versus those with grade 2 or greater irAEs (20%–24% versus 34%, respectively). Additionally, the overall survival (OS) of patients who experienced irAEs was also improved in patients with grade 2 or greater irAEs compared with grade 0 or 1 irAEs (14.8 versus 8.2 months) (Lutzky et al., 2009). In two adjuvant trials involving 75 patients with high-risk stage 3–4 resected melanoma, a marked correlation between development of irAEs and relapse-free survival was also observed (Weber et al., 2009).

The correlation between anti-PD-1 antibody-induced irAEs and clinical response has not been fully explored. In a retrospective study involving 148 patients with melanoma treated with nivolumab, OS was significantly different in patients experiencing irAEs of any grade versus those without irAEs ($p <= 0.001$). Subgroup analysis of the same patient cohort showed a significant improvement in OS for patients who experience rash (hazard ratio [HR], 0.423; 95% CI, 0.243–0.735; $p = 0.001$) and vitiligo (HR, 0.184; 95% CI, 0.036–0.94; $p = 0.012$) (Freeman-Keller et al., 2016). Furthermore, in an observational cohort study of 38 patients with NSCLC treated with nivolumab, patients who experienced irAEs had remarkably higher objective response rates (ORRs) and longer PFS than those without irAEs (63.6% versus 7.4%; $p < 0.01$). Similarly, in a pooled analysis involving 531 patients with metastatic NSCLC from five different retrospective studies, irAEs were positively associated with progression-free survival (HR 0.68 95% CI [0.55–0.85]; $p = 0.001$) as well as OS (HR 0.66 95% CI [0.52–0.82]; $p < 0.001$) (Naqash et al., 2020).

### Temporal Relationship of irAEs to Clinical Efficacy of ICI

One important consideration in understanding the relationship between irAEs and clinical efficacy of ICIs is that patients responding to therapy are likely to be treated longer with ICI and thus be at greater risk of eventually developing an irAE. Adjuvant studies with fixed duration of therapy are useful in minimizing this caveat. A study of adjuvant therapy involving 1,019 patients with resected stage IIIA, IIIB, and IIIC melanoma demonstrated that treated patients who developed irAEs had prolonged recurrence-free survival (RFS) compared with patients without irAEs (HR 0.61; 95% CI 0.39–0.95; $p = .03$). No correlation between the onset of irAEs and RFS was seen in the placebo treatment group (Eggermont et al., 2019). Moreover, a recent extensive systematic review of patients treated with ICI also identified a correlation between irAEs and clinical efficacy, although the data were largely generated from retrospective analyses and primarily focused on patients with melanoma or lung cancer (Cortellini et al., 2019). In addition to these studies, a pooled retrospective analysis of CheckMate 069 and 067 found that even patients who suspended induction therapy of nivolumab (1 mg/kg) plus ipilimumab (3 mg/kg) (4 doses every 3 weeks) because of irAEs still retained some clinical benefit (Schadendorf et al., 2017).

### Relationship of irAE Organ Site and Clinical Efficacy of ICI

It has not been established whether the occurrence of irAEs in particular organs is associated with response rates to ICI treatment. Generally, cutaneous toxicity, one of the most common irAEs, has correlated with improved prognosis. For instance, in a retrospective study involving patients with melanoma treated with pembrolizumab, the occurrence of cutaneous irAEs, including rash, pruritus, hypopigmentation, xerosis, keratosis, and facial erythema, correlated with markedly prolonged progression free survival (PFS) (Sanlorenzo et al., 2015). Similarly, another retrospective analysis of patients with advanced melanoma ($n = 318$) treated with anti-PD-1 monotherapy or anti-PD-1 in combination with ipilimumab identified a correlation between dermatologic irAEs and superior response rate (RR) (Quach et al., 2019).

Some studies have explored the link between endocrine irAEs and RR in ICI treatment. In a comprehensive meta-analysis of patients with metastatic head and neck and lung cancer treated with ICIs, endocrine irAE significantly correlated with OS ($p = .019$) (Gomes-Lima et al., 2019). Furthermore, a single-centered study involving patients with metastatic melanoma treated with ipilimumab revealed that patients who

experienced hypophysitis exhibited prolonged median survival compared with those who did not develop hypophysitis (Faje, 2016; Faje et al., 2014). Furthermore, a study on the correlation of thyroid dysfunction and survival outcomes of patients with advanced NSCLC treated with pembrolizumab therapy as part of KEYNOTE-001 (NCT01295827) revealed that the median OS was markedly longer in patients who developed thyroid dysfunction than in those patients without thyroid dysfunction (Osorio et al., 2017). It has been postulated that the relationship between specific irAE sites and ICI efficacy could result from common antigens between the tumor and the affected organs (Khoja et al., 2017). However, further studies will need to evaluate this hypothesis in greater detail.

### Tumor Type and Relationship between irAE and Clinical Efficacy of ICI

The adverse effects of immunotherapy are inconsistent across tumor types, which likely results from differential effects of histology on the tumor microenvironment. Alterations in tumor microenvironment impact immune cell infiltration, generation of adaptive immune responses, and neoantigen formation (Khoja et al., 2017). For instance, vitiligo, an autoimmune reaction against melanin-containing skin cells, develops in patients with melanoma who receive immunotherapy but not in patients with other malignancies (Burdick and Hawk, 1964). In general, however, the safety profiles of patients with different types of tumors treated with ICI are similar, with irAEs most frequently involving the skin, gastrointestinal tract, and endocrine system (Elias et al., 2019; Grangeon et al., 2019; Weber et al., 2017). These observations indicate that the predominant toxicity of ICI results from non-specific effects on the immune system irrespective of tumor subtype.

### IrAE Severity and ICI Efficacy

irAEs result from activation of autoreactive T cells. Therefore, patients who develop severe irAEs theoretically have T cells more responsive to ICI than those with lower degrees of irAEs (Passat et al., 2018). As previously noted, in a meta-analysis of patients treated with ipilimumab, DCR was elevated in patients with at least grade 2 irAEs than in patients with only grade 1 or no irAEs (34%–43% versus 20%–24%) (Lutzky et al., 2009). In another retrospective study involving patients receiving nivolumab, there was a significant difference in OS between patients with irAEs at any level and those without irAEs, with patients with grade $\geq 3$ irAEs demonstrating the highest OS (Hua et al., 2016). This is supported by a recent retrospective review on ICI-treated patients conducted at the MD Anderson Cancer Center, which found that patients with grade $\geq 3$ irAEs experienced improved overall response rates (ORRs) (25% versus 6%; $p = 0.039$) and an extended median time to progression (30 versus 10 weeks; $p = 0.0040$) than those without grade $\geq 3$ irAEs (Fujii et al., 2018). Moreover, vitiligo, rash, and pruritus are all cutaneous toxic effects that correlate with improved outcomes (Quach et al., 2019). In contrast to data suggesting a correlation between severity of irAE and ICI efficacy, other studies have found that low-grade irAEs better correlate with disease response (Judd et al., 2017). This has been postulated to result from the considerable morbidity resulting from severe irAEs, and the immune suppression intervention used to mitigate these complications (Quach et al., 2019). In summary, it is difficult to generate a strong conclusion between irAE severity and ICI efficacy given the limited available literature and the retrospective nature of the current studies.

## MECHANISMS THAT MAY COUPLE THERAPEUTIC EFFICACY WITH IRAES IN ICI THERAPIES

Considering the multiple mechanisms of immune modulation impacted by ICI, it is predictable that they induce multiple irAEs while activating antitumor immunity. The underlying mechanisms of irAEs are likely to result from components of innate and adaptive immunity, sensitivity of host tissues to direct antibody binding, and the microbiome (Figure 2) (Esfahani et al., 2020; Wei et al., 2018).

### Activation of Self-Reactive T Cells

Although the exact pathophysiology underlying irAEs has not been fully revealed, activation of self-reactive T lymphocytes is considered to be a key event in the immune pathogenesis of most irAEs. The loss of T cell tolerance leads to many self-directed immune processes (Richards et al., 2016). For example, in some diseases, autoreactive T lymphocytes are major effectors, whereas in other cases, the predominant mode of action is to help B lymphocytes produce autoantibodies that mediate the disease (Boehncke and Brembilla, 2019; de Moel et al., 2019). $CD8^+$ T cells are the predominant effector arm of ICI-mediated responses to cancer, and to autoimmunity. Recently, alterations in the T cell repertoire following ICI therapy have been shown to correlate with therapeutic response and the severity of irAEs. For instance, peripheral $CD8^+$ T cell clonal expansion in patients with prostate cancer receiving ipilimumab was found to correlate

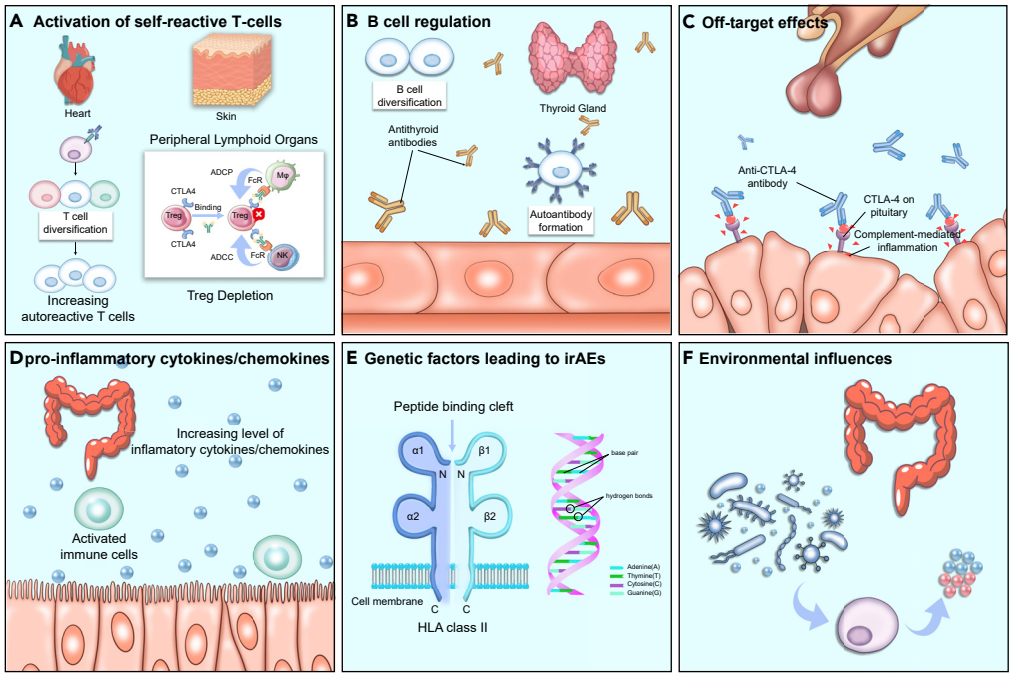


**Figure 2. Mechanisms that Potentially Couple Therapeutic Effect and irAEs**

Several mechanisms regulate systemic inflammation and T cell activation. These include (A) increased T cell responsiveness, which results in the production of pro-inflammatory cytokines by T cells and Treg deletion during ICI therapy; (B) B cell regulation, including pathogenic autoantibody formation; (C) off-target effects on normal tissue expressing the target immune checkpoint ligand; (D) increasing levels of pro-inflammatory cytokines and chemokines from activated immune cells; (E) genetic vulnerability such as HLA haplotypes; and (F) environmental influences including the microbiome.

with the development of severe irAEs (Subudhi et al., 2016). Another study showed that ipilimumab induced greater expansion of the T cell repertoire, including CD4+ as well as CD8+ T cells, within 2 weeks following treatment, in the periphery of patients with irAEs compared with patients without irAEs (Oh et al., 2017). The activation and proliferation of autoreactive T cells are thought to contribute significantly to the onset of irAEs. The shared TCR repertoire found in tumors and other tissues in which irAEs appear may be linked through antigens shared between tumor and healthy tissues (Berner et al., 2019; Johnson et al., 2016). In this situation, T cells activated by ICI that are reactive to the tumor also inflame and kill normal cells. In a postmortem study involving two patients with metastatic melanoma who experienced fulminant myocarditis following combination therapy with nivolumab plus ipilimumab, infiltrating T-cells and macrophages were observed in the myocardium as well as the cardiac conduction system (Johnson et al., 2016). More in-depth exploration of the infiltrating T-cells via TCR sequencing indicated that there were shared high-frequency TCRs in cardiac and skeletal muscle and in tumor cells. Consistent with this study, a recent prospective cohort study involving patients with NSCLC revealed that the skin, lung, and colon demonstrate a high tumor-tissue similarity score with tumors (Berner et al., 2019). Apart from simply appearing antigenically similar to tumor, the authors were able to show that nine common antigens between the skin and the tumor could be used to induce IFN-γ-based T cell responses in activated peripheral blood mononuclear cells from patients with dermatologic irAEs.

Apart from effects on $CD8^+$ T cells, alterations in Tregs resulting from ICI also contribute to irAEs. Alissafi et al. demonstrated that Tregs suppress autoimmune responses by inhibiting autophagy in DCs in a CTLA-4-dependent manner and anti-CTLA-4 disrupts the association linking Tregs and DCs to enhance autoimmunity (Alissafi et al., 2017). Furthermore, CTLA-4 blockade also induces ADCC (antibody-dependent cellular cytotoxicity) or ADCP (antibody-dependent cellular phagocytosis)-mediated depletion of Tregs by Fcγ receptor-expressing NK cells or APCs (Arce Vargas et al., 2018; Selby et al., 2013; Simpson et al., 2013). This systemic depletion of Tregs results in the loss of peripheral tolerance and failed feedback control of acutely activated $CD4^+$ Th1 and $CD8^+$ T cells and the subsequent development of irAEs.

### Impact of ICI on B Cell Regulation

A growing body of research suggests a role for B cells in ICI-mediated irAEs. For instance, histopathologic evaluation of colon tissue in immune-related colitis induced by anti-CTLA-4 demonstrated B cell and T cell enrichment (Beck et al., 2006). Moreover, humans lacking sufficient levels of CTLA-4 also demonstrate B cell alterations (Kuehn et al., 2014). These alterations are characterized by a progressive loss of circulating B cells with an accumulation of autoreactive $CD21^{lo}$ B cells. In patients with melanoma treated with combination ICI therapy, the development of variations in B cells early after treatment initiation increases the likelihood of irAEs (Das et al., 2018). These variations include an overall decrease in B cells along with an associated increase in plasmablasts and $CD21^{lo}$ B cells.

ICI may lead to a loss of B cell self-tolerance, which is associated with the activation of autoreactive B cells and the formation of autoantibodies. Osorio et al. found that thyroid disease occurs in patients treated with pembrolizumab who have pre-existing or nascently generated anti-thyroid antibodies (Osorio et al., 2017). This is likely due to direct effects from high levels of PD-1 expression on B cells (Velu et al., 2009) and indirect effects from the regulation of B by T cells (Thibult et al., 2013) or other immune factors (Kawamoto et al., 2012; Sage et al., 2013). Separate from PD-1 mechanisms, Moel et al. showed that inhibition of CTLA-4 also leads to loss of B cell self-tolerance. They tested 23 common clinical autoantibodies in pre- and post-treatment sera of 133 patients with melanoma treated with ipilimumab and found that autoantibodies developed in 19.2% (19/99) of patients who did not have pre-existing autoantibodies (de Moel et al., 2019). The investigators suggested that this resulted from inappropriate T cell-dependent activation of autoreactive B cells, which in turn resulted in autoantibodies and correlated with expansion of the T cell repertoire, suggesting that autoantibodies may act as biomarkers of ICI-efficient induction of immunogenicity.

### Direct ICI Off-Target Effects on Host Organ Tissues

Immune-related adverse events may also result from off-target effects of ICI on non-hematopoietic cells expressing immune checkpoint molecules. In a cohort of 20 patients with advanced melanoma or prostate cancer, 7 experienced hypophysitis induced by ipilimumab (Iwama et al., 2014). At baseline, pituitary antibodies (primarily targeting thyroid-stimulating hormone [TSH], follicle-stimulating hormone, and adrenocorticotropic hormone) were not present in any patients but developed in all of the 7 patients with hypophysitis; these antibodies were not present in any of the 13 patients without hypophysitis. Some groups have found that CTLA-4 is expressed on normal pituitary cells that secrete human prolactin and TSH (Caturegli et al., 2016; Iwama et al., 2014). Considering ipilimumab is an immunoglobulin G1 (IgG1) antibody, which can activate the classical complement cascade (antibody-dependent complement-mediated cytotoxicity, CDC), ipilimumab-induced hypophysitis may be caused by direct binding of monoclonal antibodies to CTLA-4 on pituitary cells and not by $CD8^+$ T cell-mediated cell killing.

### ICI-Induced Generation of Pro-inflammatory Cytokines/Chemokines

Cytokine and chemokine levels could also be involved in the pathophysiology of irAEs or serve as biomarkers of ICI-mediated immune dysregulation (Esfahani et al., 2019; Lim et al., 2019). For example, Khan et al. found that irAE development was highly correlated with upregulation of cytokines, such as CXCL9, 10, 11, and 13, which are chemotactic for T cells (Khan et al., 2019). Additionally, IL-17 has been observed to be elevated in patients with metastatic melanoma with colitis triggered by ipilimumab (Callahan et al., 2011). Supporting this correlation, a recent study reported that patients with melanoma with a high baseline serum IL-17 level are more likely to develop colitis during neoadjuvant ipilimumab treatment (Tarhini et al., 2015).

### Genetic Factors Leading to irAEs in ICI

Genetic vulnerability is a crucial factor in the predisposition to autoimmunity. Various studies have reported relationships between specific human leukocyte antigen (HLA) haplotypes or polymorphisms in immune checkpoint genes and autoimmune diseases (Gough et al., 2005; Sharpe et al., 2007) and suggest that certain HLA haplotypes may predispose to the occurrence of irAEs. In one example, expression of the HLA allele DR4 was overrepresented in patients who develop autoimmune insulin-dependent diabetes after therapy with anti-PD-1 or anti-PD-L1 (Stamatouli et al., 2018). Despite these preliminary findings, studies involving larger patient cohorts will be required to elucidate a definitive genetic association with the pathogenesis of irAEs.

### Environmental Influences of ICI-Mediated irAEs

It is becoming increasingly clear that the microbiome constitutes a crucial factor in sustaining immune homeostasis and impacting response and toxicity to ICI. Microbial diversity and composition differ between responders and non-responders in patients with melanoma treated with anti-PD-1. Specific microbes responsible for conferring anti-PD-1 specificity have not been uniformly identified, but various studies have found associations with *Akkermansia muciniphila* (Routy et al., 2018), *Bifidobacterium* (Matson et al., 2018), and *Faecalibacterium* (Gopalakrishnan et al., 2018). Intriguingly, fecal transplants from responsive patients confer anti-PD-1 tumor efficacy in mice, whereas anti-PD-1 therapies are ineffective in mice receiving fecal transplants from human nonresponders (Gopalakrishnan et al., 2018; Matson et al., 2018; Routy et al., 2018). Furthermore, a study involving 26 patients with metastatic melanoma receiving ipilimumab revealed that a distinct baseline gut microbiota composition correlates with clinical response as well as ICI-induced colitis. The patients with enriched baseline microbiota consisting of the genus *Faecalibacterium* plus other members of the Firmicutes phylum have extended PFS and OS and increased incidence of colitis when compared with patients with a baseline of *Bacteroides*-enriched microbiota (Chaput et al., 2017). Patients with elevated Firmicutes members had lower levels of Tregs and $\alpha4^+\beta7^+CD4^+$ and $\alpha4^+\beta7^+CD8^+$ T cells. In contrast, an increase in the Bacteroidetes phylum, such as *B. fragilis*, has been associated with resistance to anti-CTLA4-induced colitis (Vétizou et al., 2015). The mechanistic basis of microbiome effects on ICI sensitivity has not been completely defined, but it is currently thought that several factors may play a role including bacterial antigenic similarity to endogenous peptides or tumor-derived neoantigens, bacterial-induced influences on host immune metabolic activity, or alterations in immune cell recruitment and activation (Helmink et al., 2019; Young et al., 2018). Notably, a recent study on fatal encephalitis triggered by anti-PD-1 treatment revealed that the inflamed cortex and meninges possess T cell receptors specific to Epstein-Barr (EB) virus, suggesting that past viral infections may be linked to the occurrence of irAEs in some instances (Johnson et al., 2019). Thus far, it remains unclear whether the microbiome influences ICI in a specific manner (e.g., intestinal flora and the risk of colitis) or whether the observed changes result from differences in systemic inflammation. Additional prospective studies are ongoing and may help to resolve these questions.

## EFFORTS TO UNCOUPLE ANTITUMOR IMMUNITY FROM INFLAMMATION-DRIVEN IRAES

A current major effort in the field of ICI biology is to develop an improved understanding of differences between ICI-mediated anti-tumor responses and irAEs, in order to exploit these differences to improve patient safety. Comprehending and modifying the mechanisms and factors that limit patient risk while maintaining efficacy during or after ICB treatment requires systematic advances in fundamental and preclinical research. The primary approaches used to address these issues involve the development of preclinical animal models, collecting multivariate data on clinical samples, and designing and developing biomarkers based on the preclinical and clinical studies (Figure 3).

### Establishing Preclinical Models to Study irAEs

The development of preclinical tumor models to study immunotherapy provides an invaluable tool for exploring organ-defined tolerance mechanisms, as well as helping to examine the pharmacology, efficacy, and safety of immune-based monotherapy and combination therapy.

Since mice are generally more resistant than humans to irAEs, developing relevant model systems is critical for predicting irAEs and efficacy. Long-term depletion of Tregs in mice is known to induce a lethal autoimmune disease comparable with the most severe irAEs (Liu et al., 2016). Liu et al. used Foxp3-GFP-DTR mice, in which Tregs can be inducibly eliminated after administration of diphtheria toxin (DT), to reduce immune self-tolerance in mice. This model seems to recapitulate many physiologic and cellular aspects of patients after ICI blockade (Liu et al., 2016).

An alternative model was proposed by Du et al. who constructed a model of homozygous mice with the humanized *Ctla4* gene (Du et al., 2018; Liu and Zheng, 2020). In this model, the pathological autoimmune outcomes of patients with cancer receiving ipilimumab or anti-CTLA4 and anti-PD combination therapy were similar when antibodies were administered to 10-day-old mice. However, an important caveat of studying 10-day-old mice is the lack of a mature T cell repertoire.

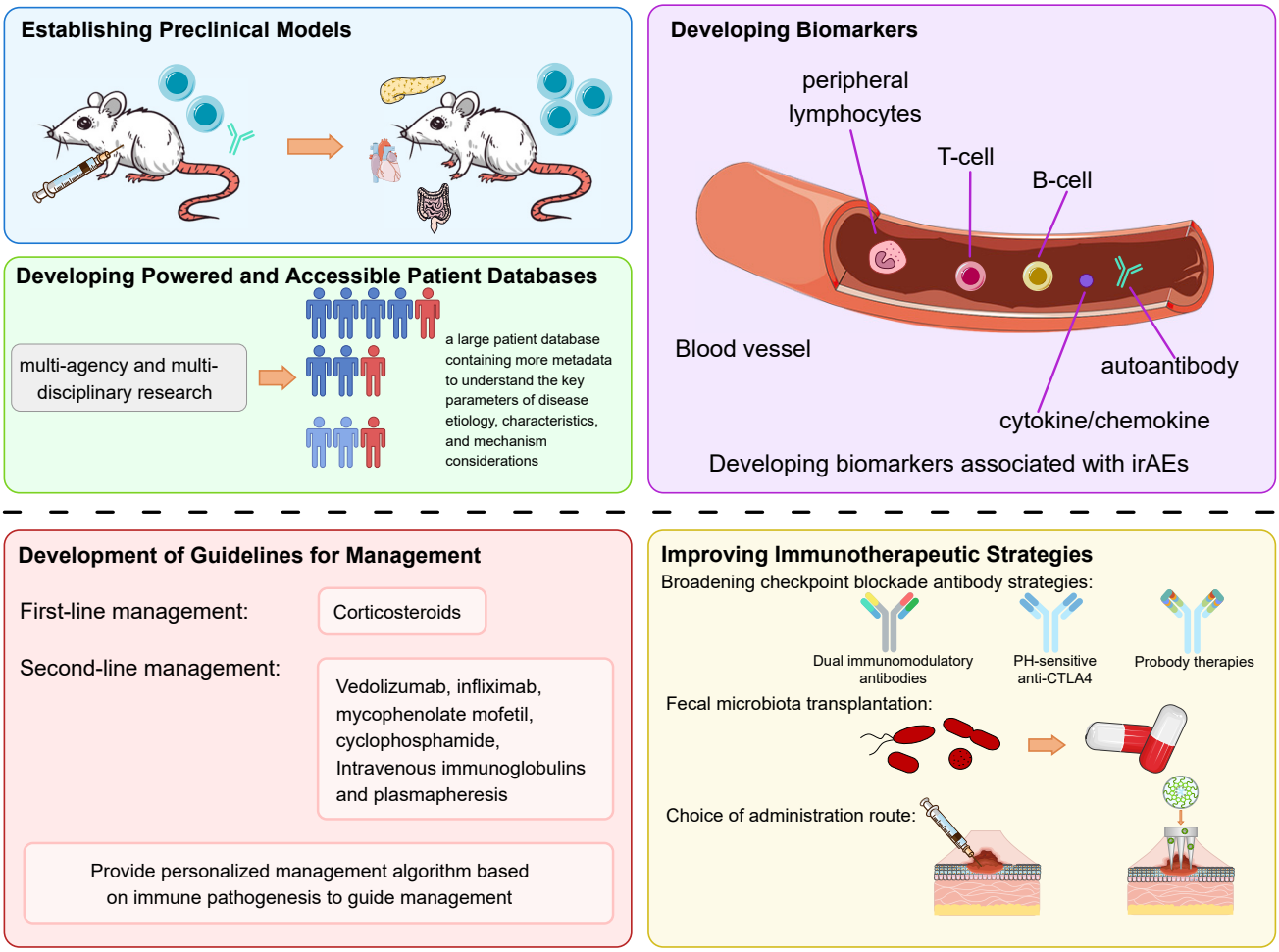


**Figure 3. Efforts to Uncouple Antitumor Immunity from irAEs**

Methods for understanding and predicting irAE toxicity include establishing preclinical models, developing adequately powered and accessible patient databases, and developing biomarkers. For better management of ICI-induced irAEs, developing guidelines is needed. Strategies to limit irAEs without impeding efficacy include broadening checkpoint blockade antibody strategies, altering gut microbiota, and localizing therapies directly within tumor.

Using a dextran sodium sulfate (DSS)-induced colitis model, Perez-Ruiz et al. showed that combination anti-CTLA-4 and anti-PD-1 immunotherapy accelerated colitis, which could be ameliorated by administration of clinically relevant TNF-α inhibitors without concurrent loss of anti-tumor efficacy (Perez-Ruiz et al., 2019). Furthermore, as ICB-induced irAEs share some similarities to the chronic graft-versus-host disease (GVHD) after allogeneic bone marrow transplantation (BMT) (Bakacs et al., 2019), Perez-Ruiz et al. also developed a model in which Rag2−/−Il2rg−/− recipient mice received adoptively transferred human peripheral blood mononuclear cells, resulting in GVHD that could be further aggravated by ipilimumab and nivolumab administration (Perez-Ruiz et al., 2019). Non-murine models are also important for studying irAEs. Notably, Ji et al. reported extensive inflammation in various organs (heart, hepatic, kidney, large intestine, adrenal medulla, and salivary glands) after ipilimumab and nivolumab treatment of monkeys. Interestingly, the myocarditis reported in monkeys demonstrated morphology, cardiac biomarker variations, and immune cell infiltrates comparable with human ICI-associated myocarditis (Ji et al., 2019).

## Developing Adequately Powered and Accessible Patient Databases to Study irAEs

To understand and mitigate the mechanisms and factors responsible for the immunotoxicity of patients during or after ICI blockade, it is necessary to improve current clinical datasets to permit identification of relevant biomarkers. Many factors limit the reporting of irAEs in patients with cancer receiving immunotherapy. Pauken et al. have outlined some of the factors that have prevented adequate reporting of

incidence and associated clinical factors of irAEs, mainly (1) the unclear etiology of certain symptoms such as flu-like symptoms or lethargy; (2) insufficient clinical features, (3) wide variation in time to onset; (4) difficulty in accurately diagnosing autoimmune diseases in clinical practice (Pauken et al., 2019). Additionally, the low frequency of severe or life-threatening irAEs (neurological, heart, and hematological) makes it difficult to adequately power correlation studies. Confounders such as differing underlying malignancies and prior cancer therapies (chemotherapy, radiotherapy, or targeted treatment) that could alter systemic immunity prior to immunotherapy add to challenges related to sample size. Therefore, multi-agency and multi-disciplinary research is required to establish a comprehensive patient database to permit understanding the interplay of disease etiology, prior treatment, and baseline patient co-morbidities. The National Cancer Institute has proposed a "Common Terminology Standard for Adverse Events" (CTCAE) as a tool that can be used to help facilitate development of a shared repository of irAEs from patients treated at different centers.

## Developing Biomarkers

A critical goal of ICI preclinical and clinical research is to identify biomarkers associated with improved clinical prognosis and/or severe irAEs. Screening for biomarkers associated with irAEs could help to identify patients at greatest risk of autoimmune side effects and provide mechanistic insights into irAE pathogenesis, by identifying functionally important pathways in irAE. Possible biomarkers could include signals from patient host and tumor genome/transcriptome or from features in the TME.

The generation of autoantibodies is known to correlate with irAEs (Da Gama Duarte et al., 2018; Tahir et al., 2019). For example, patients with anti-thyroid antibodies (either anti-thyroperoxidase or anti-thyroglobulin antibody) are much more prone to irAEs than those without such antibodies (Maekura et al., 2017; Osorio et al., 2017). One recent study used HuProt, a proteomics database, to characterize baseline serum antibody reactivity in 78 patients with melanoma treated with ICI. An antibody profile with a sensitivity and specificity of over 90% was identified (Gowen et al., 2018). Another study quantified serum proteins by Milliplex MAP assay in 34 patients with advanced NSCLC who had received at least one prior line of chemotherapy followed by nivolumab monotherapy. By multivariate analysis, serum levels of RANTES (CCL5 [Chemokine ligand 5]) were found to correlate with irAEs, and RANTES levels decreased after initiation of corticosteroid use (Oyanagi et al., 2019). High levels of IL-17 (Tarhini et al., 2015) and IL-6 (Valpione et al., 2018) were also found to correlate with high-grade irAEs.

Friedlander et al. developed a whole-blood RNA transcript-based gene signature that correlated with diarrhea in patients with advanced melanoma treated with the anti-CTLA-4 antibody tremelimumab. This 16-gene signature panel distinguished between patients with grade 0–1 and grade 2–4 diarrhea/colitis with a sensitivity of 57.1% and a specificity of 84.4% (Friedlander et al., 2018).

Since T and B lymphocytes are also important mediators of immune tolerance, and serve a crucial role in the occurrence of irAEs, clonal enrichment of T cells in the systemic circulation could serve as a potentially relevant biomarker of irAEs associated with ipilimumab treatment (Oh et al., 2017; Subudhi et al., 2016).

## Development of Guidelines for Management of ICI-Induced irAEs

Several management guidelines for irAEs have been published, including those provided by the European Society for Molecular Oncology (ESMO) (Haanen et al., 2017), the Society for Immunotherapy of Cancer (SITC) Toxicity Management Working Group (Puzanov et al., 2017), and the National Comprehensive Cancer Network (NCCN) (Thompson et al., 2019). These guidelines offer insightful algorithms for dealing with some of the most commonly occurring irAEs. The guidelines also provide suggestions on how to assess the seriousness of an irAE, and potential alternate diagnoses, including infectious complications, tumor progression, pulmonary embolism, cardiac events, and pleural effusion, among others. In general, immunotherapy is suspended or permanently discontinued depending on the severity of the irAE. In highly symptomatic patients, corticosteroids are the first line of management and are effective in relieving symptoms. Immunotherapy can be resumed when there is no evidence of clinical irAE recurrence after discontinuation of steroid therapy. Since corticosteroids act to impair immune activation, especially in T cells, it was initially thought that their use would permanently impact ICI efficacy. However, it is now clear that corticosteroids may not worsen OS or other cancer outcome indicators and that anti-tumor lymphocytes can persist (Horvat et al., 2015; Weber et al., 2017). This comes with the caveat that corticosteroids, in some instances, have demonstrated deleterious effects on ICI therapies. For instance, a study of patients with

NSCLC after treatment with anti-PD-1 and anti-PD-L1 found that patients receiving prednisone >10 mg/day had a poorer prognosis than those taking <10 mg/day (as assessed by decreases in PFS and OS) (Arbor et al., 2018). Additionally, corticosteroids can result in side effects independent of tumor control, including increased risk of infection, impaired glucose control, and GI bleeding (Naidoo et al., 2017). Therefore, the magnitude of effects for high-dose steroids on ICI efficacy is not yet absolutely defined. An adequately powered prospective study of patients treated with ICI who require corticosteroids for treatment of irAEs is required.

Occasionally, discontinuation of ICI and administering corticosteroids are insufficient to ameliorate irAEs. Other agents that have been used to try to suppress irAEs include vedolizumab (anti-integrin $\alpha4\beta7$), infliximab (a chimeric monoclonal anti-TNF-$\alpha$ antibody), tocilizumab (anti-IL6 receptor antibody), mycophenolate mofetil, cyclophosphamide, intravenous immunoglobulins (IVIgs), and plasmapheresis, among others (Martins et al., 2019b). Vedolizumab is a monoclonal antibody against $\alpha4\beta7$ integrin, which is primarily expressed on $CD4^+$ T cell subpopulations in the intestine (Bergqvist et al., 2017). Vedolizumab has been used for the treatment of steroid-refractory colitis. A clinical trial (NCT02723006) in patients with advanced melanoma explored the potential of uncoupling the anti-tumor efficacy and irAE for the combined use of vedolizumab with nivolumab plus ipilimumab. This trial was terminated early and will not be restarted. The results have not yet been posted. Infliximab neutralizes TNF-$\alpha$ and is effective against corticosteroid-refractory colitis. Two small studies have demonstrated that use of corticosteroids plus infliximab does not appear to impact cancer prognosis in patients with colitis differently from corticosteroids alone (Arriola et al., 2015; Wang et al., 2018b). However, the use of infliximab may be beneficial by reducing the duration of corticosteroid therapy and permitting more rapid immune recovery (Young et al., 2018). Preclinical studies have found that prophylactic administration of anti-TNF-$\alpha$ agents decoupled the toxicity from the efficacy of combination therapy with anti-CTLA-4 and anti-PD-1 and also enhanced antitumor response (Perez-Ruiz et al., 2019). Based on these and other results, a phase I clinical trial (NCT03293784) is currently evaluating the use of anti-TNF-$\alpha$ agents (infliximab or certolizumab) in combination with ICIs. Tocilizumab is a humanized monoclonal antibody against the IL-6 receptor (IL-6R); it prevents IL-6 binding to IL-6R, which blocks IL-6 transduction. Tocilizumab is useful for dealing with adverse events secondary to other types of immunotherapy (Kotch et al., 2019). Preclinical data have found that combined blockade of IL-6R and CTLA-4 enhances the antitumor responses in murine pancreatic tumor models, indicating that IL-6R blockade in combination with ICIs in the clinical settings have the potential to augment efficacy and limit irAEs (Ware et al., 2019, 2020). Mycophenolate mofetil is a purine antagonist that inhibits lymphocyte proliferation and is used as a second-line immunosuppressive drug (Brahmer et al., 2018). A case report of heart transplant patients with advanced melanoma showed that mycophenolate mofetil had a significant suppressive effect on T cell responses and ICI treatment could not overcome the potent inhibition (Grant et al., 2018). Obeid et al. suggested that its use should be avoided in patients with immunogenic neoplasm, especially in cases with the intent to cure (Martins et al., 2019b). Cyclophosphamide is a chemotherapeutic drug with strong immunosuppressive properties and is very helpful in the treatment of patients with severe and/or refractory irAEs. However, its carcinogenic risk compromises its clinical use (Martins et al., 2019b). IVIg has been safely employed in the treatment of autoimmune diseases and immunodeficiency. Data from human and animal models have demonstrated that IVIg can inhibit cancer progression as well as prolong survival (Sobieszczańska et al., 2014; Xu et al., 2019). A case report of patients with ICI-induced myocarditis showed that treatment with IVIg and statins resulted in rapid recovery from myocarditis without compromising the effectiveness of ICI therapy (Balanescu et al., 2020). Clinical trials of IVIg in combination with ICI have not yet been conducted. Optimal protocols for the use of second-line therapies after ICI discontinuation and corticosteroids have yet to be defined. Personalized approaches based on the organ involved in the irAE and patient co-morbidities and immunogenetics may help guide the management of severe and/or refractory irAEs. For instance, Martins et al. proposed a personalized management algorithm based on a patient immune pathology model and successfully implemented the use of tocilizumab in patients with nivolumab-induced refractory esophageal stenosis, which resulted in rapid improvement of symptoms and abbreviated duration of corticosteroid use (Horisberger et al., 2018).

## Improving Immunotherapeutic Strategies to Reduce irAEs

A better understanding of the mechanisms that drive irAEs creates avenues for modulating ICIs to improve their anti-tumor specificity. Several broad concepts have recently been implemented to reduce irAEs while maintaining or improving ICI efficacy.

### *Broadening Checkpoint Blockade Antibody Strategies*

The generation of antibodies with the ability to promote local tumor-targeted immunoreactivity is one potential means to prevent irAEs. One way to facilitate this increased localization is with dual immunomodulatory antibodies (bispecific or antibody-based alternative structures) that can bind two different immunomodulatory targets, for instance, PD-1 or PD-L1 in combination with other immune checkpoint receptors such as LAG-3 or TIM-3 (Dahlén et al., 2018). Since tumors often upregulate immunomodulatory molecules (both receptors and ligands), an antibody's dual specificity may promote its retention in the TME, promoting anti-tumor effects, while minimizing the risk of irAEs. ATOR-1015, which is designed to simultaneously target CTLA-4 and OX40, comprises a high-affinity CTLA-4 inhibitory protein combined with an anti-OX40 antibody. This antibody has been shown to suppress CTLA-4/OX40-driven Treg cell proliferation within the TME (Kvarnhammar et al., 2019).

Another approach to decrease irAEs is by modifying anti-CTLA-4 antibodies to permit expression of CTLA-4 on the surface of Tregs. CTLA-4 is required for the inhibitory function of Tregs but undergoes degradation in the lysosome after CTLA-4 binding. pH-sensitive anti-CTLA-4 antibodies (HL12 and HL32) have been developed that do not result in CTLA-4 degradation (Zhang et al., 2019). Upon interaction with CTLA-4 on the Treg surface, the modified antibody is internalized into recycling endosomes, where pH decreases to 6.0–6.5 as the endosomes undergo maturation. The change in pH causes the anti-CTLA-4 antibody to detach from the internalized CTLA-4, where it then binds to LRBA (lipopolysaccharide-responsive vesicle trafficking, beach- and anchor-containing), which in turn recycles CTLA-4 back to the Treg surface. This elegant system results in retention of constitutive CTLA-4 expression on the Treg surface and minimal development of irAEs in murine models (Altman and Kong, 2019; Zhang et al., 2019).

An additional novel approach uses recombinant antibodies that are activated by tumor-associated proteases as a means of preventing irAEs. This antibody therapy approach, termed probody therapy, takes advantage of the aberrant expression of characteristic proteases and is meant to ensure that the "drug" becomes active only inside the TME. Probodies comprise an anti-tumor monoclonal IgG antibody or variable regions fragment, a masking peptide tethered to the N-terminal end of the antibody's light chain, and a protease-cleavable peptide tagged to the antibody (Autio et al., 2020). The probody remains in an inactive form until it encounters the TME where the masking peptide is cleaved off to expose the functional antibody. One of the best characterized probodies is CX-072, which is designed to target PD-L1(Wong et al., 2016). Various studies have shown that low amounts of CX-072 triggers antitumor responses similar to anti-PD-1/PD-L1 antibodies but with a significant decrease in irAEs (Giesen et al., 2020; Wong et al., 2016). One useful feature of probody therapy is the ability to be applied to various therapeutic antibodies. Preliminary studies have demonstrated activities with various approaches including anti-PD-L1 antibody therapy (Wong et al., 2016), antibody-drug conjugates (Singh et al., 2018; Weaver et al., 2015), and T cell-engaging bispecific antibodies (BiTEs) (Boustany et al., 2018).

### *Fecal Microbiota Transplantation*

Fecal microbiota transplantation is the process of altering the gastrointestinal microbiota of non-responders or patients with unacceptable toxicity with fecal microbiota from patients with more favorable responses. Research is ongoing, but at least one Canadian phase I study is actively recruiting patients (NCT04163289). In this study, 20 patients with renal cell carcinoma will be given fecal microbiota transplantation $\geq$7 days before initiation of ipilimumab and nivolumab combined therapy and 1–3 days before the next two treatments. The frequency of high-grade colitis will be calculated (Chan and Bass, 2020).

### *Choice of Administration Route*

It has been reported that the route of therapy administration affects the incidence of irAE (Young et al., 2018). In a mouse model, intratumoral administration of low amounts anti-CTLA-4 antibody in a Montanide ISA 51 emulsion triggers effective anti-tumor CD8+ T cell responses that eradicate the tumor, while maintaining low serum antibody levels (Fransen et al., 2013). Similarly, the intra-tumoral co-administration of the adjuvant CpG and antibodies against CTLA-4 and OX40 at low doses triggered a systemic anti-tumor immune response (Marabelle et al., 2013). Recent studies have shown that advanced biomaterials may facilitate the local sequestration of checkpoint blockade antibodies into tumors. It has been reported that conjugation of checkpoint blockade antibodies to an extracellular matrix (ECM)-super-affinity peptide promotes intra-tumoral antibody retention, resulting in low peripheral antibody levels upon intratumoral delivery (Ishihara et al., 2017). Moreover, a transdermal technique for continuous intra-tumoral delivery

of anti-PD-1 mAb at a reduced dose has been proposed by Gu and co-workers (Wang et al., 2016). This approach utilizes biodegradable microneedle patches bearing pH-sensitive nanoparticles that envelope anti-PD-1 antibody. The microneedles penetrate through the skin into immune cell-rich epidermis, where the antibodies exert their immunotherapeutic function (Shields IV et al., 2020). A study using B16F10 mouse melanoma cells found that a single application of the microneedle patch/antibody triggered a robust immune reaction (Wang et al., 2016). The intra-tumoral application of this therapeutic is currently undergoing evaluation in clinical trials enrolling patients with solid tumors (Ray et al., 2016). It is important to note that, if a systemic anti-tumor effect is not generated with this approach, it may have limited application in the treatment of metastatic disease, where lesions can be numerous.

## Resources Availability

### *Lead Contact*

Further information and requests for resources should be directed to and will be fulfilled by the Lead Contact, Ming You, (myou@mcw.edu).

### *Materials Availability*

Not applicable as this is a review article.

### *Data and Code Availability*

Not applicable as this is a review article.

## CONCLUSION

Immune checkpoint blockade is a critical component of modern cancer treatment. With the widespread use of this therapy, understanding and managing irAEs is essential. Apart from improving treatment, the use of ICIs has also provided a window for better understanding human immune regulation and immune tolerance. Improved understanding of the links between ICI-induced irAEs and efficacy will continue to develop by combining mechanistic basic research and scientifically oriented clinical research in patients. This will eventually enable more patients with cancer receiving ICIs to have optimal outcomes with fewer adverse events.

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## AUTHOR CONTRIBUTIONS

Conceptualization, W.H., G.W., Y.W., M.J.R., and M.Y.; Investigation, W.H., G.W., Y.W., M.J.R., and M.Y.; Writing – Original Draft, W.H. and G.W.; Writing – Review & Editing, Y.W., M.J.R., and M.Y.; Funding Acquisition, M.Y.; Supervision, M.Y. All authors revised and approved the final version of the manuscript.

## DECLARATION OF INTERESTS

M.Y. is a co-founder of OncoImmune, Inc. No potential conflicts of interest were disclosed by other authors.

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