PERSPECTIVE

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Is autoimmunity the Achilles' heel of cancer immunotherapy?

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The emergence of immuno-oncology as the first broadly successful strategy for metastatic cancer will require clinicians to integrate this new pillar of medicine with chemotherapy, radiation, and targeted small-molecule compounds. Of equal importance is gaining an understanding of the limitations and toxicities of immunotherapy. Immunotherapy was initially perceived to be a relatively less toxic approach to cancer treatment than other available therapies—and surely it is, when compared to those. However, as the use of immunotherapy becomes more common, especially as first- and second-line treatments, immunotoxicity and autoimmunity are emerging as the Achilles' heel of immunotherapy. In this Perspective, we discuss evidence that the occurrence of immunotoxicity bodes well for the patient, and describe mechanisms that might be related to the induction of autoimmunity. We then explore approaches to limit immunotoxicity, and discuss the future directions of research and reporting that are needed to diminish it.

Immuno-oncology drug development presently encompasses a broad range of agents, including antibodies, peptides, proteins, small molecules, adjuvants, cytokines, oncolytic viruses, bispecific molecules, and cellular therapies[1]. A survey of recent literature indicates that adverse events affecting nearly every organ system have been reported in association with cancer immunotherapy (**Fig. 1**). However, one baseline assumption is that, at present, the frequency of autoimmune complications following cancer immunotherapy is probably underestimated, in part because most cancer trials follow patients for only a brief time after enrollment (typically 6 months), and some symptoms, such as lethargy, might have an unclear etiology. The true incidence is probably further underestimated still, because the numerator does not include patients who died from their cancer. Related to this, the incidence of certain immune-related adverse events (irAEs) is correlated with an increased probability of prolonged survival[2]. However, these associations might be related to lead-time bias, because patients whose tumors progress succumb to their disease, whereas those who respond to immunotherapies have longer treatment duration and more time to develop autoimmune toxicities. In addition, immunotoxicity, as presently defined[3], can have a delayed onset. For instance, some cases of graft-versus-host disease after hematopoietic stem cell transplantation (HSCT) can take more than a year to manifest[4]. Onset of thyroiditis has been reported as late as 3 years after the initiation of therapy with the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) antagonist ipilimumab[5]. Adding more complexity, the natural history of certain autoimmune diseases, such as type 1 diabetes (T1D), is unpredictable; the onset of clinical disease manifestations can vary from weeks to decades after the appearance of islet autoantibodies[6].

The existence of paraneoplastic syndromes in oncology has long been known to clinicians, antedating immune-checkpoint blockade, and it provides the clearest example of naturally occurring tumor immunity and autoimmunity in humans. Patients with occult or advanced tumors may develop a wide variety of syndromes, varying from myasthenia gravis to cerebellar degeneration, owing to acquired cellular and/or humoral immunity to antigens expressed by the tumor[7]. In small-cell lung cancer, the occurrence of paraneoplastic syndromes can portend prolonged survival[8]. These observations raise the question of whether deliberate attempts to provoke paraneoplastic syndrome should be attempted, and, on a related note, whether the occurrence of these syndromes will increase with increasing numbers of cancer patients exposed to immune-checkpoint-blockade administration.

Previous studies have described the evolving spectrum of immunotherapy-associated irAEs[3,9,10]. Here we provide further perspective on the emerging clinical syndromes of immunotoxicity and autoimmunity in cancer therapy.

Barriers to immune recognition of tumors

Immune tolerance is crucial for preventing autoimmunity, and now, we suspect that it might also be highly relevant to cancer immunity. Immune tolerance is defined as a lack of lymphocyte reactivity to self-antigens or foreign-tissue antigens in an organ graft, achieved without the need for long-term immunosuppression and while retaining immune competence and reactivity to all other foreign antigens[11]. Immune tolerance starts in the thymus, where the diverse T cell receptor (TCR) repertoire is created through random somatic recombination events. Autoreactive T cell progenitors expressing TCRs that bind with high affinity to self-peptide–major histocompatibility complex (MHC) complexes are deleted through a process called negative selection. The transcription factor autoimmune regulator (AIRE), which is selectively expressed by a subset of CD80-expressing medullary thymic epithelial cells (TECs), drives the expression of tissue-specific self-antigens to ensure selective removal of thymocytes bearing TCRs that recognize these antigens[12]. Thymocytes that have low affinity for self-peptide-MHC complexes are positively selected to progress to the periphery. In addition, some thymocytes expressing TCRs that bind with high affinity to self-antigen peptide–MHC complexes differentiate into Forkhead box protein 3 (FOXP3)-expressing regulatory T (T_{reg}) cells[13].

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As T cells exit the thymus, additional peripheral tolerance mechanisms prevent autoreactive T cells that escaped negative selection from reacting to or attacking self-antigen-expressing healthy cells. These include T cell–intrinsic mechanisms, such as immunological ignorance, anergy, exhaustion, phenotypic skewing, and apoptosis, as well as extrinsic-cell-based mechanisms[14]. Among the latter, T_{reg} cells stand out as unique because they are comprised both of 'central' T_{reg} cells—formed from high-affinity interactions with self-peptide–MHC complexes in the thymus, as mentioned above—and 'peripheral' T_{reg} cells, formed from T cells that engage in prolonged interactions with low-affinity self-antigens and non-self-antigens, such as allergens, food and commensal microbiota. Together, these pathways help to maintain peripheral tolerance to self-antigens and certain foreign antigens[13,15]. Therefore, although tumors express tumor-specific neoantigens and overexpress self-antigens that can potentially initiate a potent anti-tumor immune response[16], the immune system has developed a complex, redundant, and robust combination of cells and molecules all designed to keep the immune system in check and avoid unwanted inflammation and tissue damage[17].

Several specific cases exemplify the degree to which tumors can take advantage of immune-tolerance mechanisms to disrupt anti-tumor immunity. Some tumor-associated antigens, such as tyrosinase-related protein 1 (TYRP1, also known as TRP1), are expressed in medullary thymic epithelial cells[18]. In addition, tumors can escape anti-tumor T cell responses by decreasing their expression of tumor-associated antigens and/or MHC molecules; by secreting immunosuppressive soluble factors (vascular endothelial growth factor, stromal-cell-derived factor, interleukin 10, interleukin 6, transforming growth factor-β, adenosine, and prostaglandins); and/or by engaging immune checkpoints (CTLA-4, PD-1, Tim-3, and LAG-3) that suppress anti-tumor activity. Immunosuppressive cells, including myeloid-derived suppressor cells (MDSCs), T_{reg} cells, tumor-associated macrophages, regulatory B cells, and regulatory dendritic cells[19,20], present within the tumor microenvironment can also suppress anti-tumor responses[21].

Therapeutic induction of anti-tumor responses

Clinical development and approval of immunomodulators, also known as immune-checkpoint inhibitors, have transformed the treatment of certain tumors, such as melanoma, non-small-cell lung cancer, and bladder cancer. Checkpoint inhibitors act by blocking interactions that normally suppress T cell responses. The binding of CTLA-4 on naive T cells in the lymph nodes to B7 on antigen-presenting cells (APCs) produces an inhibitory signal during the primary phase of T cell activation (**Fig. 2**). CTLA-4 can also strip B7 molecules—which are ligands for CD28 costimulatory molecules on T cells—from the APCs through a process of transendocytosis, which further impairs T cell activation[22]. Thus, the blockade of CTLA-4 leads to a more robust costimulatory signal, and this boosted signal may enable otherwise naive T cells with weak affinity to respond to overexpressed and mutant tumor antigens. CTLA-4 is also expressed on $CD4^+CD25^{hi}FOXP3^+$ T_{reg} cells, and its engagement leads to enhanced IL-35, IL-10, TGF-β, and indoleamine 2,3-dioxygenase (IDO) production, T_{reg} cell proliferation, and decreased effector T cell activation and proliferation[23].

By contrast, PD-1 binding to PD-L1 and PD-L2 regulates those already activated T cells later in the immune response in the peripheral tissues. PD-1 engagement inhibits T cell proliferation, IFN-γ, TNF-α, and IL-2 production[24], although some data suggest that it may also be involved in early T cell activation[25], central tolerance,

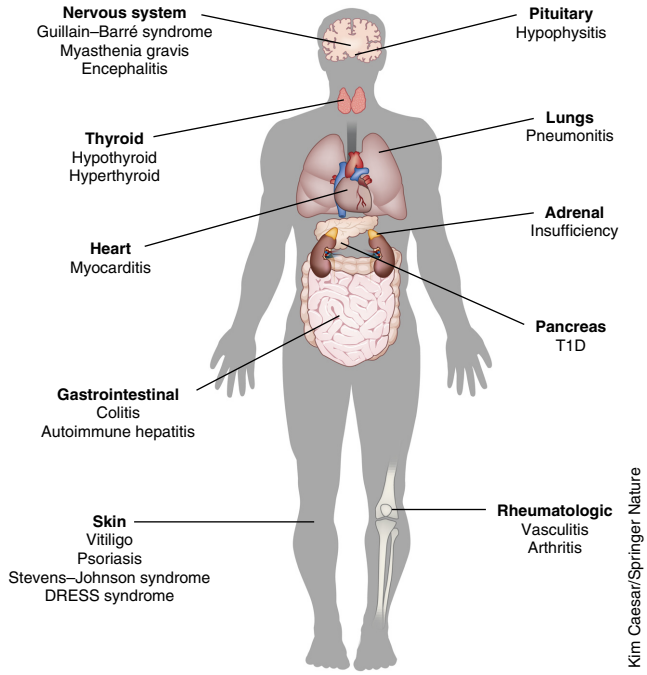

Figure 1 Examples of autoimmune and other immune-related adverse effects associated with cancer immunotherapy. See **Supplementary Note** for references describing each of these autoimmune and immune-related adverse events. T1D, type 1 diabetes.

and regulation of negative selection[26]. In addition to the direct effect of checkpoints on effector T cells, blockade of the molecular interactions can also affect the tumor microenvironment (TME). For example, altering IDO expression and CTLA-4 engagement on APCs, and decreasing T_{reg} cells in the TME through mechanisms such as STING/IFN-αβ signaling and myeloid-derived suppressor cells improves cancer immunity[27].

Autoimmune consequences

Considering their diverse mechanisms of action, it is perhaps not surprising that these immunomodulators induce multiple immune-mediated adverse events that lead to antigen-specific autoimmune manifestations. In humans, autoimmune manifestations caused by drugs targeting the CTLA-4 and PD-1 pathways seem to be dependent on the pathway(s) targeted[5,10,28,29]. For example, the most commonly reported endocrine irAE following therapy with the CTLA-4-blocking antibody ipilimumab is hypophysitis, an event that is rarely observed after PD-1-antibody therapy. Ectopic expression of CTLA-4 in the pituitary gland may be responsible for this effect[30], and antibody-dependent, cell-mediated cytotoxicity (ADCC) with activation of complement might be involved in the destruction of the hypophysis. By contrast, the most commonly reported endocrine-related toxicity after PD-1-antibody therapy is hypothyroidism, a syndrome that is rarely observed in patients treated with ipilimumab. These autoimmune syndromes could be a consequence of revealing pre-existing conditions in these patients. However, with one exception[31], analysis of pre-existing autoantibodies and single-nucleotide polymorphisms (SNPs) associated with autoimmune disease has not proved useful in the identification of patients at risk for irAE.

Given that the function of CTLA-4 is to primarily affect $CD4^+$ T cells at an early stage in lymphatic tissue, its blockade might be expected to have broader and more nonspecific consequences than

the blockade of PD-1, which interacts with its ligand primarily within the peripheral tissue and tumor microenvironment[32]. Moreover, CTLA-4 inhibition lowers the threshold required for T cell activation, which results in increased expansion and diversification of circulating, low-avidity T cells[33]; it also causes an Fc-γR-mediated depletion of T_{reg} cells[34]. In agreement with this, treatment with ipilimumab was found to broaden the TCR repertoire more robustly, within 2 weeks, in those experiencing irAEs than in those without irAEs, and treatment response improved along with the increase in TCR diversity. This further underscores cancer immunotherapy as a double-edged sword in which patients and clinicians must weigh the risk of immunotoxicity against the benefit of tumor destruction[35]. By comparison, PD-1 blockade is likely to reinvigorate a previously overactive immune system[32]; studies of T cell exhaustion in chronic infection show that PD-1 functions to limit effector T cell–mediated inflammatory injury[36]. Studies of individuals with cancer have not yet compared T cell exhaustion profiles from patients treated with PD-1 with those from patients receiving CTLA-4 blockade as treatment. Some effects, however, might be common to CTLA-4 and PD-1 blockade. For example, local T_{reg} cells in the TME of human tumors also show upregulated expression of PD-L1 and PD-L2 (ref. 37), and therefore, might be affected by PD-1 blockade; if this is the case, then alterations in T_{reg} cell activity might contribute to both PD-1- and CTLA-4-induced autoimmunity.

An anti-tumor immune response can kill tumor cells, and host APCs can then pick up the antigens, which, in a form of antigen presentation referred to as cross-presentation, leads to the priming of secondary immune responses. There is increasing evidence that cross-presentation of neoantigens or shared antigens might induce a loss of tolerance and subsequent autoimmunity in patients treated with checkpoint blockade. In two patients with fulminant myocarditis resulting from a combination treatment of ipilimumab and nivolumab, an analysis of T cells infiltrating the skeletal muscle, myocardium, and tumor revealed an increase in the most abundant TCR type in one of the patients; tumors in both patients expressed abundant muscle-specific antigens, including desmin and troponin[38]. This led to the suggestion that an epitope shared by tumor and healthy tissue contributed to the myocarditis, but it is also possible that this finding was coincidental, because no predominant TCR clonotype was detected in the other patient[38].

Indeed, autoimmune manifestations may be indirect and due to epitope spreading (ES) caused by immunotherapy-induced inflammation and tumor lysis. ES refers to the recruitment of additional T cells and the development of an immune response to epitopes distinct from and non-cross-reactive with the primary epitope recognized by the original effector T cells[39]. Recognition of multiple epitopes might enhance anti-tumor responses, for example, by promoting additional help from CD4-mediated T cells; through linked recognition; or through direct tumor destruction, if it is a CD8 T cell–activating epitope. Linked recognition is a process of CTL priming whereby CD4 helper cells recognize antigens on the same APC that cross-presents the CTL epitope[40]. ES might result through unknown mechanisms during the initiation of T cell responses that target self-antigens (**Fig. 3**) shared by normal tissue, which thereby leads to loss of tolerance and autoimmunity. ES has been documented in patients receiving tumor vaccines[41] and in patients who have undergone adoptive transfer (**Box 1**) of CTLs and CAR T cells[42,43]. Checkpoint therapy with ipilimumab can induce ES[44], and it is likely that neoantigen-directed immune responses against tumors, when followed by ES, can trigger autoimmunity against nonmalignant tissues in which the neoantigens

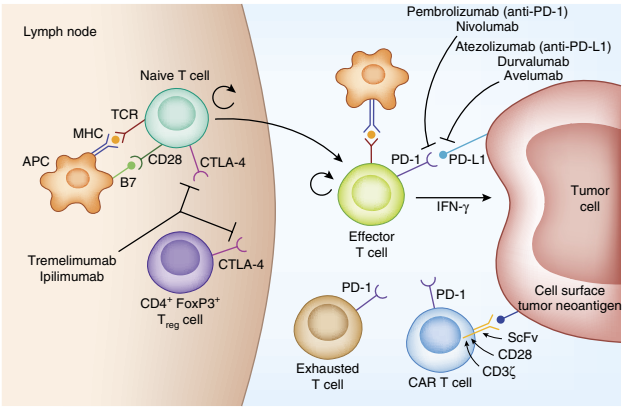

Figure 2 CTLA-4 and PD-1 checkpoint blockade affects T cells at different stages of differentiation and at different anatomical locations. In lymphoid tissues, CTLA-4 expression is induced in naive T cells. After the TCR is triggered by an antigen–MHC encounter, CTLA-4 is expressed on the cell surface. CTLA-4-blocking therapies suppress negative signals delivered by CTLA-4, which permits sustained T cell activation and proliferation. The major role of the PD-1 pathway is at a later stage of T cell activation. In peripheral tissues (including tumors), activated T cells upregulate PD-1 expression. Inflammatory signals in the tissues induce the expression of PD-1 ligands, which downregulate the activity of T cells through binding to PD-1 and CD80, a feedback mechanism to limit collateral tissue damage. T cell exhaustion, a state of terminal T differentiation, is induced by prolonged exposure to high levels of antigen. Anti-PD-1 or PD-L1 therapies prevent this negative regulation and may reinvigorate exhausted T cells or delay T cell exhaustion in response to chronic antigen exposure. Alternative new therapies with CAR T cells that are highly specific for tumor antigens cause destruction directly within the tumor microenvironment.

are absent. Although many efforts are under way to exploit ES using several immune and nonimmune (for example, radiation and chemotherapy) treatment modalities, in certain circumstances, the consequences of ES might need to be curtailed through the development and administration of selective inhibitors of cross-presentation[45], perhaps by manipulating proteasome activity or regulatory T cell function (**Fig. 3**).

Another mechanism that might influence autoimmune side effects is T cell functional flexibility and plasticity. Changes in epigenetic-control mechanisms may allow for switching between exhausted and activated T cell states. In addition, T_{reg} cells can be converted into T_H17 cells in the presence of IL-6 and TGF-β[46,47], which might underlie certain forms of autoimmunity, such as autoimmune hepatitis and psoriasis[48,49], and CXCL11 promotes T_{reg} cell differentiation into $CXCR3^+CD4^+$ effector T cells, suggesting that it might be involved in the development of autoimmune encephalitis[50]. Control of T_{reg} cell plasticity is further regulated by EZH2, a target for multiple small-molecule inhibitors in cancer trials[51]. EZH2 is a histone modifier that functions as the catalytic component of the polycomb repressive complex 2 (PRC2). As a lysine methyltransferase, EZH2 promotes the addition of the repressive mark histone H3K27me3 to target chromatin, which thereby induces chromatin compaction and transcriptional repression by restricting access to transcriptional regulators such as RNA polymerase II and other transcription-associated factors. Hyperactivation of or mutations in EZH2 are found in a variety of malignancies[52]. However, EZH2 activity in tumor cells can shape the immune microenvironment of tumors by controlling the expression of chemokines[53]. Thus, in cancer, small molecules targeting proteins that are mutated in tumor cells could have dual effects: they

might directly suppress proliferation or survival of cancer cells, and at the same time, they might modulate the anti-tumor immune response at the level of T_{reg} cells. Understanding the complex network of transcription factors involved in the regulation of T cell plasticity will be crucial to the development of targeted therapies that can effectively treat cancer without also introducing autoimmune risk.

Efforts to predict and understand toxicity

Understanding and manipulating the mechanisms and factors that determine a patient's risk of developing immune toxicity during or after checkpoint blockade will require basic and preclinical research, as well as changes in current clinical-reporting practice. With regard to the latter, although existing observations of immune-related toxicity are helpful, most literature reporting autoimmune-associated disease consists of case reports[54,55], in which, for example, autoantibodies are often not described. Moreover, frequently, autoantibodies are negative when reported and autoimmune disease presents rapidly, as seen with diabetic ketoacidosis and T1D; because subsequent longitudinal data are often not reported, scientists have generally been unable to assess whether autoantibodies, such as GAD-65, ICA-512, and ZnT8, become detectable over time. At present, it remains unclear whether the autoimmune manifestations seen after immunotherapy are clearly connected to the 'classical' autoimmune diseases that they symptomatically represent, because simple correlations with factors such as MHC haplotypes, autoantibodies, and antigen-specific T cell identification are mostly absent from current analyses. Moreover, organ involvement in an autoimmune response is unpredictable from patient to patient, perhaps owing to different genetics, epigenetics, or microbiota environment, and/or because of polymorphisms in the checkpoints themselves[56]. For example, in terms of genetic associations, *CTLA4* polymorphisms have been linked to an increased risk of autoimmune diseases such as T1D, and preclinical models have shown that anti-CTLA-4 can increase the risk of autoimmune diabetes[57]. In summary, large patient databases containing more metadata will be required to determine whether similar genetic predispositions and biologic pathways are shared between classic autoimmune diseases and the autoimmune syndromes induced by various cancer immunotherapies.

Patients with a history of or ongoing autoimmune disease are currently excluded from clinical trials, but whether this is justified remains unclear, given that the underlying mechanisms of immunotoxicity from checkpoint inhibition may be distinct and have no substantial impact on a patient's existing autoimmune disease. Crucial to clinicians' understanding of and ability to predict immunotoxicity from these drugs will be enhanced pharmacovigilance. The establishment of a national database to track long-term outcome would enable a deeper understanding of irAEs. One effort in this area is an initiative by the US Food and Drug Administration (FDA) Center for Biologics Evaluation and Research (CBER) that used the High-Performance Integrated Virtual Environment (HIVE) to establish a database on the safety of engineered T cells. Other efforts, such as the Cancer Moonshot launched as part of the 21st Century Cures Act, also highlight the need for a more complete understanding of the consequences of these new therapies as well as the importance of collating large data sets and maintaining robust clinical and basic research efforts. Together, these academic, industry, philanthropic, and government efforts will help us to shape and take advantage of these exciting new therapeutic opportunities.

Some insights into the mechanisms by which cancer immunotherapy promulgates autoimmunity might be gained by analysis of T cell responses in other settings. For instance, $CD8^+$ T cell exhaustion

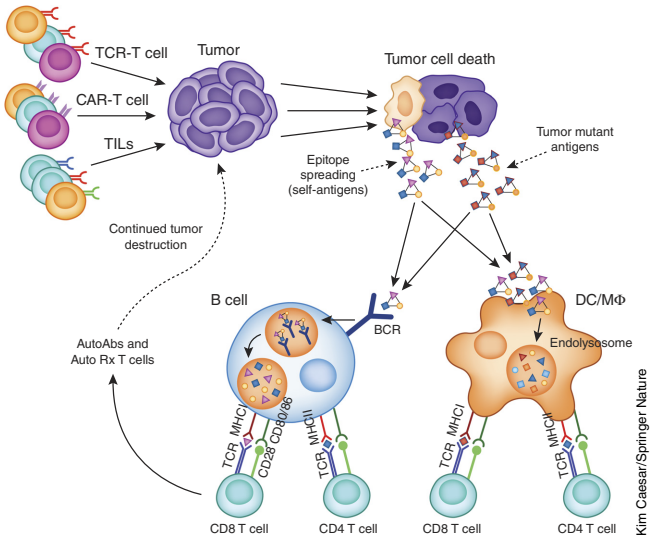

Figure 3 Potential mechanism of epitope spreading leading to autoimmunity. Top, T cells recognizing antigens (for example, neoantigens or antigens overexpressed on tumor cells) on tumor cells induce cytokine secretion and/or cytotoxic killing of tumor cells. This can also result in the death of nontransformed bystander cells. The antigens released by all cells in the environment are ingested by APCs (for example, dendritic cells, macrophages, and B cells), which migrate to the lymph nodes. Bottom, in the lymph nodes, these activated APCs can present tumor antigens as well as antigens from bystander cells, thereby priming a second wave of T cells that can re-enter the tissue and cause additional tumor destruction and off-target destruction of normal tissue. This, in turn, leads to autoimmunity. AutoAbs, autoantibodies. Auto RX, autoreactive.

is frequently observed in chronic viral infections[58]. Moreover, PD-1 blockade seems to reactivate effector T cells through the targeting of certain transcriptional factors (i.e., nuclear factor kappa-light-chain-enhancer of activated B cells, interferon regulatory factors 1 and 2, orphan nuclear receptor NR4A1, and B-lymphocyte-induced maturation protein 1) that cause a reengagement of the effector mechanisms in the epigenome of exhausted T cells[59]. CTLA-4 has also been found to have a clear role in multiple chronic infections, including HBV, HCV, and HIV, and its inhibition can increase the function of pathogen-specific T cells[60,61]. Epigenetic and transcriptional mechanisms determine the functional plasticity of T cells to switch between their exhausted and effector states[62], and so altering the transcriptional landscape—for example, by manipulating transcription-factor and gene-enhancer expression—and epigenetic landscape—for example, by manipulating the activity of histone-modifying methylation or demethylation enzymes, histone acetylases, and DNA demethylases—of adoptively transferred T cells may yield a more specific anti-tumor response than generalized PD-1 and CTLA-4 blockade. Genomic editing of chimeric antigen receptor (CAR) T cells to render them resistant to exhaustion has been proposed[63,64], and Sen and colleagues suggested that mapping state-specific enhancers in exhausted T cells could enable more precise genome editing for adoptive T cell therapy[62].

Improved clinical reporting and new basic and preclinical research is especially important because more and more patients are receiving checkpoint-blockade treatment. Indeed, the emerging standard of care for patients with many forms of disseminated cancer is therapy with a checkpoint antagonist. Moreover, for patients in clinical trials, the trend is to combine one or several of the increasing 'toolbox of therapies,' including vaccines, CAR T cells, oncolytic viruses, radiation,

Box 1 Adoptive T cell therapy

Adoptive transfer of T cells engineered with defined specificity is another ongoing approach to limit autoimmunity while preserving anti-tumor efficacy. The TCR must be chosen to target tumor-specific antigens, such as cancer-testis antigens or neoantigens, because infusions of T cells with transgenic TCRs that recognize antigens shared by normal tissue induce an unacceptable degree of toxicity[92]. Experiments with a panel of TCRs with varied affinity and targeting self-antigens show that the induction of anti-tumor activity and autoimmunity are closely coupled[93]. Furthermore, autoimmune syndromes might occur following the adoptive transfer of transgenic T cells that retain the endogenous TCR[94], presumably owing to the formation of heterodimers that create self-reactive specificities.

The adoptive transfer of TCR transgenic T cells and CAR T cells can cause severe immunotoxicity owing to cytokine release upon target recognition[95,96]. Cytokine-release syndrome can be effectively managed with cytokine blockade[29]. The propensity of adoptive transfer to cause autoimmunity seems to be low, although there is not as much experience with adoptive transfer as with checkpoint therapy. Consistent with a reduced propensity for autoimmunity, infusions of allogeneic CAR T cells might cause diminished GVHD[97–99].

The potential for autoimmunity with adoptive transfer can be abrogated by reducing or eliminating the endogenous TCR. Genome editing to create CAR T cells or TCR transgenic T cells devoid of the endogenous TCR in order to prevent GVHD and enhance the function of the transgenic TCR has been reported[64,100,101].

An increasing number of efforts are taking place to combine therapeutic T cells with checkpoint inhibitors, either as combination therapies or directly, by altering the checkpoint targets (such as PD-1) within the cell through genome-editing technologies. These attempts to create checkpoint-resistant T cells are likely to increase both their general efficacy and autoantigen-mediated effects[63,64,102]. Thus, controlling potential off-target effects of CAR and TCR-transduced T cells will depend on choosing an appropriate tumor-specific antigen, eliminating the endogenous TCR, engineering CAR T cells reliant on multiple tumor-associated antigens for activation[103], and inserting suicide genes, such as iCaspase-9 or other regulated receptors[75], that allow for quick inactivation in the event of off-target effects.

Finally, future approaches combining synthetic biology with adoptive transfer may solve many problems with immunotoxicity and autoimmunity. CAR T cells can be engineered with Boolean logic gates—for example, ON-switch CARs, so that activity of the CAR T cell can be rapidly titrated and reversed, allowing, in principle, for a high level of physician-enabled remote control[104]. AND-gate engineered T cells can express two or more receptors of desired specificity to increase tumor killing and discrimination from normal tissue. For example, AND-gated chimeric receptors can recognize a target surface antigen but, upon engagement, activate transcription and the expression of a second receptor (i.e., CAR or TCR) that mediates cell killing[105]. Only when both antigens are present does sustained T cell priming and activation occur.

chemotherapy, and targeted therapies, with a checkpoint blocker[65]. We expect the incidence of inflammatory and autoimmune toxicity to increase with the complexity and duration of combination therapies, as has been observed with ipilimumab and PD-1 antagonist combinations[66]. The toxicity that emerges after combination therapy can be unexpected, as illustrated when ipilimumab was combined with vemurafenib[67]; in this case, neither agent, when given as a single agent, resulted in unacceptable liver toxicity; the combination, however, elicited severe liver toxicity that resulted in termination of the trial.

Biomarker studies of tumor biopsies have identified tumor and immune markers predictive of beneficial anti-tumor responses to checkpoint therapy[68], including PD-L1 expression on tumor cells and $CD8^+$ T cell infiltration of the TME. The occurrence of autoimmune skin depigmentation (vitiligo) after immunotherapy with a variety of modalities is associated with improved long-term survival[69]. In addition, the typical kinetics of the onset of immunotoxicity have been described for some therapies, including ipilimumab, in which skin conditions are typically seen as the first manifestation, followed by colitis, and later, hepatitis and polyendocrinopathies[70]. As was mentioned above, the occurrence of certain immune-mediated paraneoplastic syndromes might portend prolonged survival. By contrast, biomarker studies are just beginning to identify patients who are at increased risk for immunotoxicity[71]. A recent SNP analysis of high-risk loci for T1D in a human case of fulminant T1D treated with combination ipilimumab and nivolumab failed to reveal a high-risk genetic profile, and so the role of genetic predisposition is unclear with regard to identifying those who might develop autoimmunity when treated with checkpoint blockade[54]. More accurate predictions of irAEs require the development of better insights into the genetics, epigenetics, and environmental elements that control immunity in both animal models and humans[72]. For example, it is not yet clear whether reductions in T cell exhaustion as a result of checkpoint inhibition may explain the mechanisms of autoimmunity observed in mice and patients, because epigenetic transcriptional-control factors and enhancers that determine the plasticity between exhausted and effector T cells have not as yet been compared in different immune settings.

Emerging strategies to limit toxicity

One main rationale for immunotherapy is that it is a 'living drug;' the adaptive immune response might persist for years, as opposed to targeted therapies and chemotherapy, in which the active agent is rapidly metabolized. A fundamental question to address is whether patients are 'cured' after cancer immunotherapy, and if so, is the remission a 'sterile cure' or the induction of some form of tumor dormancy? In mice, tumor dormancy is maintained by adaptive immunity as immune equilibrium[73]. Clinical data suggest that subclinical melanoma may persist for more than a decade after therapy in patients who remain in remission[74], which points to the existence of prolonged tumor dormancy, likely related to cancer stem cells and adaptive immunity. Given the development of sensitive assays that can determine the absence of residual dormant tumor, it is conceivable that methods could be implemented to terminate cancer immunotherapy and thereby limit the incidence and severity of autoimmunity. In the case of adoptive cell transfer, various suicide systems have been deployed successfully to rapidly and specifically terminate immunotherapy[75]. As discussed above, checkpoint blockade may spontaneously abate by exhaustion, but methods to terminate 'at will' by physician control are not presently available, other than through the administration of systemic immunosuppression.

The manipulation of immune-cell cytokine effects and/or systemic host metabolism also has the potential to modulate immunotoxicity. For example, cancer immunotherapy with IL-2 and agonistic CD40

antibody is more toxic in aged mice and mice with obesity[76]. Treatment with etanercept to block TNF in young, obese mice receiving the same immunotherapy prevented the toxic effects of cytokine storm[77].

Many epidemiologic studies indicate that vitamin D deficiency is associated with an increased risk of cancer incidence and mortality[78]. There is an increased incidence of GVHD in patients who have vitamin D deficiency before undergoing allogeneic HSCT[79]. Both macrophages and T_{reg} cells are implicated in the above studies. PD-L1 expression on dendritic cells (DCs) is required for the induction of T_{reg} cells by vitamin D3, perhaps through reverse signaling by PD-L1 to DCs[80]. It is possible that the regulation of PD-1–PD-L1 signaling at discrete stages during the immune response could diminish immunotoxicity in individuals with cancer. Thus, vitamin D3 supplementation might reduce the risk of several autoimmune disorders in those who are deficient[81], but this has yet to be documented in patients because the human data available are primarily observational[82].

It is possible that manipulation of the microbiome could enhance or diminish immunotoxicity. In mice, Vétizou *et al.*[83] found that optimal responses to CTLA-4 blockade required the presence of specific *Bacteroides* spp. Similarly, it was reported that *Bifidobacterium* spp. enhanced the efficacy of anti-PD-L1 therapy[84]. It is possible that interindividual differences in the microbiome account for substantial heterogeneity in therapeutic efficacy and the immunopathology that is invoked after checkpoint blockade[85]. Fecal stool transplants can ameliorate colitis associated with GVHD[86], and it is likely that this approach may reduce toxicity from checkpoint-blockade therapy.

Targeting tumor-specific antigens, such as cancer-testis antigens, and mutation-specific neoantigens are attractive strategies for mitigating the risk of autoimmunity. Several approaches are currently testing this idea, including vaccines that target neoantigens derived from nonsynonymous mutations that occur during cellular transformation; adoptive transfer with TCR transgenic or CAR T cells; and targeted radiotherapy to induce abscopal effects[87]. The rationale for vaccines targeting neoantigens is that the functional avidity of TCRs is improved as compared to that of T cells that target shared antigens, because neoantigen-specific T cells are not subjected to thymic-tolerance mechanisms, and so off-tumor toxicity should not occur in the absence of epitope spreading[88]. But if epitope spreading were to occur, these strategies would also risk inducing autoimmunity.

CONCLUSIONS

Given the recent success of immunotherapy, the incidence of immunotoxicity will likely continue to rise as these therapies become more widely used. For instance, lung cancer, classically thought to be non-immunogenic, has now shown substantial response to checkpoint inhibitors; this suggests that we might only be at the tip of the iceberg with regard to their oncologic treatment potential[89,90]. Furthermore, CAR T cells are being tested in a variety of solid-tumor settings, either alone or in combination with drugs that alter the TME and inherent immune response. Moreover, current use has been limited primarily to patients with advanced or unresectable disease, but use in less advanced disease has begun to show promise, and many clinical trials are pending. In these settings, the immune system is likely to be more intact, given that it has not been exposed to immune-suppressive chemotherapies and radiation. It will be important to monitor the field for an increasing incidence of immunotoxicity as patients with more vigorous and diverse immune systems are exposed to immunomodulation. Finally, the incidence of autoimmunity in the general population, particularly in developed nations, has been rising—and this begs the question of whether autoimmunity with cancer immunotherapy will also increase[91].

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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The authors declare competing financial interests: details are available in the online version of the paper.

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Corrigendum: Is autoimmunity the Achilles' heel of cancer immunotherapy?

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In the version of this article published in print, Jeffrey A Bluestone was missing an affiliation. His affiliation information has been changed to include the Parker Institute for Cancer Immunotherapy in San Francisco, California. Accordingly, the affiliation information for Carl H June has been updated to distinguish the Parker Institute from the University of Pennsylvania; these are now two separate affiliations. All affiliation numbers have been revised. The error did not appear in the HTML and PDF versions of the article.