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REVIEW

Improved mouse models to assess tumour immunity and irAEs after combination cancer immunotherapies

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The current excitement surrounding cancer immunotherapy stems particularly from clinical data involving agents mediating immune checkpoint receptor blockade, which have induced unprecedented efficacy against a range of tumours compared with previous immunotherapeutic approaches. However, an important consideration in targeting checkpoint receptors has been the emergence of associated toxicities termed immune-related adverse events (irAEs). In light of the clinical benefits observed after co-blockade of checkpoint receptors and data from preclinical mouse models, there is now a strong rationale to combine different checkpoint receptors together, with other immunotherapies or more conventional therapies to assess if clinical benefits to cancer patients can be further improved. However, one may predict the frequency and severity of irAEs will increase with combinations, which may result in premature therapy cessation, thus limiting the realization of such an approach. In addition, there is a limit to how many different combination therapies that can be tested in a timely manner given the legal, regulatory and budgetary issues associated with conducting clinical trials. Thus, there is a need to develop preclinical mouse models that more accurately inform us as to which immunotherapies might combine best to provide the optimal therapeutic index (maximal anti-tumour efficacy and low level irAEs) in different cancer settings. In this review we will discuss the irAEs observed in patients after checkpoint blockade and discuss which mouse models of cancer can be appropriate to assess the development of tumour immunity and irAEs following combination cancer immunotherapies.
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Cancer immunotherapy harnesses the immune system to eliminate cancer and is poised to be increasingly utilized as a main stream approach for cancer treatment. Notably, in 2013, cancer immunotherapy was awarded the breakthrough award of the year by the journal '*Science*'.[1] The current excitement surrounding cancer immunotherapy stems particularly from clinical data involving agents mediating immune checkpoint receptor blockade, which have induced unprecedented efficacy against a range of tumours compared with previous immunotherapeutic approaches.

Checkpoint receptors pathways are important for maintaining self-tolerance by modulating the duration and amplitude of physiological immune responses and thus limiting collateral damage and preventing autoimmunity. However, we now appreciate that many cancers can hijack these pathways to evade immune attack and generate an immunosuppressive tumour microenvironment. Indeed, the existence of layers of immune suppression in cancer patients was demonstrated by the impressive results obtained following concurrent blockade of two immune checkpoints compared with targeting of one alone.[2] A key issue when targeting checkpoint receptors has been the occurrence of associated toxicities termed immune-related adverse events (irAEs).[3] Although there is clinical benefit observed after co-blockade of checkpoint receptors in mice and humans, one may predict the frequency and severity of irAEs may increase with new combinations. This may result in premature cessation of therapy, hence limiting the efficacy of these new combinations. Furthermore, there is a limit to how many different combination therapies that can be tested in a short time frame given the regulatory and budgetary issues associated with untertaking clinical trials. Therefore, there is a requirement to develop pre-clinical mouse models that more accurately determines which combination immunotherapies have the optimal therapeutic index (maximal anti-tumour efficacy and minimal irAEs) in different cancers. In this review we will highlight the irAEs observed in patients after checkpoint blockade and discuss which mouse models of cancer might be appropriate for predicting the anti-tumour immunity and irAEs following combination cancer immunotherapy in humans.

THE IMMUNE SYSTEM AND CANCER

We now appreciate that the immune system recognizes cancer and that patients with strong natural immune reactions have better survival compared with those with weak responses. This concept was first demonstrated in patients with colorectal cancer (CRC) by Pages *et al.*[4] Here they demonstrated that the immune contexture, defined by number, type and location of tumour immune infiltrates in primary tumours, are key prognostic factors for disease-free and overall survival (OS) in CRC patients. Importantly, the immunoscore,

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an immune-based classification strategy derived from three aspects of the immune contexture, proved superior over the standard tumour–node–metastases classification in predicting outcomes in survival and relapse of CRC patients and their response to therapy.[5–7] Collectively, a good immunoscore, defined as high densities of tumour-infiltrating lymphocytes particularly with a T helper type 1 (Th1) and cytotoxic orientation, was associated with longer disease-free survival and/or improved OS. This correlation was first documented in CRC but has now been extended to a number of other cancers, including melanoma, head and neck, breast, ovarian and lung.[8] Importantly, recognition that cancer development and progression is influenced by the host immune system has led to the development of a conceptual framework called 'cancer immunoediting,' which explains the dual host-protective and tumour-promoting roles of the immune system.[9] Furthermore, the demonstration that cancers actively evade immune destruction has led to its inclusion as an emerging hallmark of cancer in 2011.[10]

Tumours can escape immune detection and destruction by a number of mechanisms that have been previously discussed.[11] These can include: (i) downregulation of antigen and major histocompatibility complex class I (MHC I) expression, (ii) production of immunosuppressive mediators (for example, interleukin-10 (IL-10), transforming growth factor-β, adenosine, vascular endothelial growth factor-A (VEGF-A), indoleamine 2,3-dioxygenase (IDO)) to dampen effector cell activation and (iii) recruitment of immune cell subsets (T regulatory cells (Tregs) and myeloid-derived suppressor cell (MDSC)) to suppress effector immune cell function and facilitate its growth and metastases. In addition to these pathways, we now understand that physiological negative feedback loops that curtail T-cell activation, to prevent autoimmunity, can be exploited by tumours to limit anti-tumour responses. For example, after activation, T cells upregulate checkpoint receptors such as cytotoxic T-lymphocyte antigen 4 (CTLA-4), which bind to B7 ligands with higher affinity than the co-stimulatory receptor CD28, thus attenuating effector T-cell function.[12] Other T-cell checkpoint receptors that limit activated T-cell effector function include programmed cell death-1 (PD-1), B and T lymphocyte attenuator (BTLA), T-cell immunoglobulin mucin 3 (TIM3) and lymphocyte activation gene-3 (LAG-3).[13–15] Recently, it has been demonstrated in melanoma patients that tumours can also counter attack from activated T cells though another mechanism termed adaptive immune resistance.[16] In this study, tumour-infiltrating lymphocytes producing inflammatory cytokines such as interferon-γ in the tumour microenvironment upregulated PD-L1/L2 expression on tumours, which delivers an inhibitory signal to PD-1-expressing T cells. Over the past 5 years, the use of antibodies to target checkpoint receptors (also termed checkpoint blockade) has emerged as one of the most effective ways to overcome tumour-induced immunosuppression and re-activate an endogenous anti-tumour response. In 2011, anti-CTLA-4 (ipilimumab) was the first immunomodulatory monoclonal antibody approved by the Food and Drug Administration for the treatment of patients with advanced melanoma on the basis of improved survival benefits.[17] Recently, second-generation checkpoint inhibitors, anti-PD-1 and anti-PD-L1, have induced better anti-tumour efficacy (31% objective response rate) compared with ipilimumab (6%).[18,19] With the recent observation of further increase in anti-tumour efficacy (53%) through the combination of anti-CTLA-4 and anti-PD-1,[2] the stage is now set for combining checkpoint receptors with other immunotherapeutic approaches. Clinical trials are currently in different stages of accrual and development to assess checkpoint receptor blockade in combination with (1) chemotherapies, (2) radiotherapy, (3) small-molecule inhibitors, (4) vaccines and (5) co-stimulatory receptors (for example, CD40, OX40 (also known as CD134), CD137, GITR (glucocorticoid-induced tumour necrosis factor (TNF) receptor-related protein) and CD27.[17]

TOXICITIES ASSOCIATED WITH TARGETING IMMUNOMODULATORY RECEPTORS

The induction of irAEs is an important factor to consider when attenuating co-stimulatory and co-inhibitory immune receptors for cancer immunotherapy.[3] Thought also has to be given as to how targeting of these receptors in combination or with other therapeutics may induce combination-specific irAEs that may not be predicted or observed by monotherapies. IrAEs are thought to be related to therapy-associated cytokine release and T-cell-mediated organ infiltration[17] and different from *bona fide* autoimmunity as they normally resolve following therapy or with corticosteroids or anti-TNF therapy. IrAEs can generally involve the gastrointestinal, liver, skin, nervous and endocrine systems (Figure 1). In a pooled analysis of 325 patients treated with 10 mg kg^{-1} of anti-CTLA-4, irAEs of any grade were observed in ~72.3%.[20] Similar proportions were also observed in patients treated with anti-PD-1 alone, although with less severity.[19] In most cases, irAEs are mostly mild and generally manageable with appropriate treatment algorithms now having been developed.[20] This includes dose interruption/discontinuation and the use of systemic high-dose corticosteroids.[21] Specifically, grade 3 or 4 irAEs, which developed in each of these patients (14% for anti-PD-1 and 25% for anti-CTLA-4) warranted attention, as in extreme cases these can be severe and life threatening. Patients who resolve their grade 2 irAEs can recommence treatment, although any grade 3 or 4 irAEs (with the exception of grade 3 skin toxicity) is a contraindication to further therapy with ipilimumab. Importantly, almost all patients (93%) developed irAEs following concurrent anti-PD-1 and anti-CTLA-4 monoclonal antibody therapy with an increase in grade 3 or 4 irAEs (50%) being observed compared with those treated with monotherapy alone.[2]

Moving forward, the better safety and clinical efficacy of targeting PD-1 (most likely due to its role in regulating T-cell activation in peripheral tissues) suggest that it will be the checkpoint receptor of choice to combine with other therapies for cancer treatment. Nevertheless, one may predict that the act of combining immunotherapies increases the proportion of patients who may develop severe irAEs. This may limit the number of patients who can fully benefit from combination cancer therapies due to discontinuation, as seen in 21% of patients treated with anti-CTLA-4 and anti-PD-1.[2] IrAEs generally manifest during the induction phase of treatment (initial 9–12 weeks of therapy) and organ-specific irAEs appears to follow kinetics of appearance. In the case of ipilimumab where irAEs of skin and mucosa develop after about 3–6 weeks, this is followed by diarrhoea/colitis around week 5 and liver toxicity and endocrinopathies around week 6.[20] More recently, an update on patients treated with anti-PD-1 reported on 2 years of safety monitoring and found that similar to what has been reported for ipilimumab, most adverse events tended to occur within the first 6 months of therapy.[22] Importantly, cumulative toxicities were also not observed with prolonged drug exposure.[22] Hence, the goal of therapies targeting checkpoint receptors in combination with other approaches should aim to generate a strong therapeutic index over the induction period of therapy. Questions that have to be answered include whether it is possible to release/activate anti-tumour immunity without development of irAEs if the right combination, dosing and

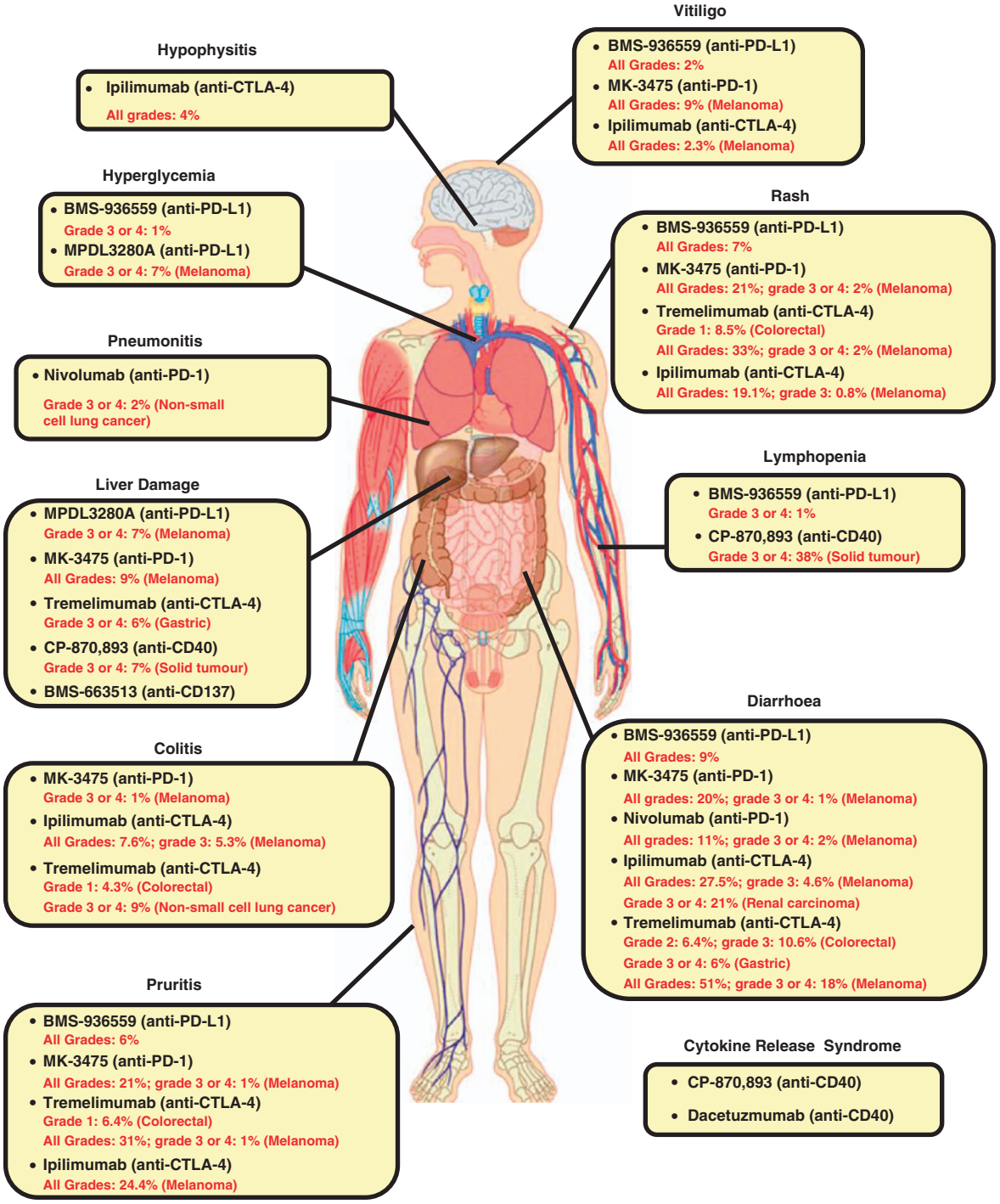

Figure 1 Spectrum of irAEs associated with immunomodulatory antibodies. Although immunomodulatory antibodies such as those that target checkpoint receptors can release endogenous anti-tumour responses, irAEs can be induced through therapy-associated release of cytokines and infiltration of immune effector cells. In cancer patients, treatment with immunomodulatory antibodies particularly with single checkpoint blockade is frequently associated with the development of irAEs. The types of irAEs (proportion and severity) as well and more unique and serious side effects reported in clinical trials with immunomodulatory antibodies such as anti-CTLA-4 (Ipilimumab,[18,49,61] Tremelimumab[62–64]), anti-PD-1 (Nivolumab,[19] MK-3475),[65] anti-PD-L1 (BMS-936559,[66] MPDL3280A[67]), anti-CD40 (CP-870,893)[27,68] and anti-CD137 (BMS-663513)[30] are summarized.

scheduling is utilized. Alternatively, is the development of irAEs a predictor of anti-tumour responses? In one study, it was reported that ipilimumab-treated patients who developed grade 3 or 4 irAEs were more likely to achieve a clinical response compared with those who had no irAEs.[23] Interestingly, in a retrospective analysis of 498 patients pooled from four Phase II clinical trials treated with different doses of ipilimumab, Feng *et al.*[24] reported that higher exposure (dose) was associated with better survival, albeit at a greater risk of developing irAEs in the grade 3 or higher category. A caveat is that this correlation is observed simply because the longer the patients

responding are treated, the greater the possibility of developing irAEs.[20] However, the study by Feng *et al.*[24] also suggested that patients who did not experience irAEs during the induction period were not likely to experience it during maintenance therapy with ipilimumab. It would be interesting to perform retrospective analysis to determine whether these group of patients also did not benefit as much clinically. Potentially these analyses may help answer the question of whether tumour reactive and autoreactive T cells are the same or distinct. It should be noted that, in some studies, there were patients, albeit at a low proportions, who developed durable responses with no associated irAEs.[16] Analysis of these patient cohorts may provide clues as to the requirements necessary to release anti-tumour immunity without inducing irAEs.

For patients who discontinued combination therapy due to irAEs, do they still benefit clinically and what is the minimum period of therapy required to allow immune activation and/or release of immune suppression? A recent report suggests this is likely, where patients with advanced melanoma treated with ipilimumab were found to survive for up to 10 years, confirming the durability of the overall survival (OS) trend observed.[25] These results, the largest analysis of OS for 1861 patients, included 2 Phase III trials, 8 Phase II trials and 2 retrospective trials. Their findings demonstrated that in 10–15% of the total patients who responded, ~22% of patients can achieve 3–10 years OS and this OS reaches a plateau at 3 years. This was observed regardless of dose (3 or 10 mg kg^{-1}), previous treatment history and whether they had been kept on a maintenance therapy with ipilimumab. Analysis of safety data, which were not included in these results, may shed light on the association of irAEs' severity with OS as well as clinical benefits obtained following therapy cessation.

In addition to checkpoint receptors, agonistic antibodies targeting co-stimulatory receptors belonging to the TNF receptor family (CD40, OX40, CD137, GITR and CD27) are also being assessed. These receptors, in general, act through activation of effector cell function and are currently being evaluated for their anti-tumour efficacy in various Phase I/II clinical trials.[26] In particular, the data for agonistic antibodies targeting CD40 and CD137 (4-1BB) are most mature where some objective responses, as well as corresponding irAEs, have been reported. In a number of Phase I clinical trials, agonistic anti-CD40 monoclonal antibodies have been evaluated against solid tumours or B-cell non-Hodgkin's lymphoma.[27] Objective responses were reported in a proportion of these patients, with the most common irAEs being primarily grade 2 cytokine release syndrome, which manifests as transient chills, fever and rigors and mild elevations in liver enzymes and decreases in circulating platelet numbers.[27] Interestingly, irAEs such as colitis or dermatitis, which are normally associated with checkpoint receptor blockade and can result in dose interruption/discontinuation, were not observed in targeting CD40, suggesting the comparative safety of anti-CD40.[27,28] Targeting of another co-stimulatory receptor CD134 (OX40) in one Phase 1 clinical trial was also safe as no acute toxicities were reported at the dose tested.[29] By contrast, grade 3/4 irAEs (severe liver toxicity) were observed in patients treated with anti-CD137 (urelumab) particularly at high doses, resulting in the termination of a number of Phase I trials.[30] Currently, lower doses of anti-CD137 are being evaluated for their safety in a PhaseI/II clinical trial with reports suggesting anti-tumour activity can still be elicited without significant liver toxicity.[26]

The experience with ipilimumab and immunotherapies in general is their kinetics of response is much slower but more durable compared with those seen with cytotoxics.[20,31] Recently, a long-term follow-up study of 177 patients treated with ipilimumab reported a median time to achieve complete response of 30 months, consistent with slow kinetics of tumour regression.[32] Similarly, the kinetics of anti-PD-1 responses is atypical with some patients responding rapidly as early as 8 weeks into therapy, whereas other patients displayed new tumours or growth of existing tumours before responding. In addition, some patients continued to respond despite cessation of anti-PD-1 therapy.[19,22] Strikingly, a different kinetics of response was observed in patients treated with the maximum tolerated doses of anti-PD-1 and anti-CTLA-4, given in combination. Impressively, these patients all had tumour reduction of 80% (classified as deep response) or more at their first scheduled assessment at 12 weeks.[2] This is in contrast to patients who were given anti-CTLA-4 and anti-PD-1 in a sequenced regime. This suggests anti-tumour responses can be rapid if the right combination is given at optimal dosage and timing. The question now is whether (1) maintenance dosage is required for patients treated with combination therapies who responded rapidly so as to minimize their risk of further developing more irAEs or (2) Can patients who developed grade 3 or 4 irAEs and ceased therapy still benefit and can one correlate the number of doses they received before cessation, with clinical benefit to determine what is the minimum period of therapy required? (3) If irAEs are predictors of response, are there biomarkers that can be used to determine which patients will develop them and thus enable monitoring and prophylactic treatment to prevent their escalation into grade 3 or 4 toxicities and cessation of treatment? Given that many patients with irAEs treated with systemic corticosteroids and/or anti-TNF resolve their symptoms rapidly if treated when symptoms first appear, it would suggest serum TNF and other inflammatory cytokines like interleukin-6 could be measured as biomarkers and targeted. There seems to be a consensus that corticosteroids or anti-TNF administration do not impede anti-tumour immune responses following checkpoint blockade.[2,19,20,22,33] Given that most patients will develop some form of irAEs following immunomodulatory therapies, particularly in combination, it would suggest concurrent administration of anti-TNF and/or corticosteroids with combination therapies is an option that can be investigated.

THE BEST PRECLINICAL MOUSE MODELS THAT MORE ACCURATELY MODEL TUMOUR IMMUNITY AND IRAES

The demonstration that targeting checkpoint receptors activates endogenous anti-tumour immunity and leads to significant clinical benefit in different cancer types now spurs the question of how their efficacy can be further improved through rationale combination approaches. In advanced melanoma, concurrent anti-CTLA-4 and anti-PD-1 therapy resulted in clinical benefits in 50% of patients, which to date is the best result obtained with cancer immunotherapies. Nevertheless, 50% of patients did not respond, and in some cancer types such as pancreas and prostate, checkpoint blockade does not appear to provide any significant clinical benefits. Thus, early-phase clinical trials are exploring how checkpoint inhibitors combine with other agents, including (1) conventional therapies (radiotherapy, chemotherapy), (2) alternative immunotherapies (vaccines, cytokine, adoptive cellular therapy), (3) targeted therapies (BRAF or vascular endothelial growth factor inhibitors)[17] or (4) immunomodulatory antibodies.[26] With combination immunotherapies increasingly being tested in clinical trials, irAEs will be an ongoing issue that has to be dealt with (as discussed above) as it may limit their usage. Furthermore, even when both agents have regulatory approval and have distinct mechanisms of action and toxicity profile, unexpected specific irAEs induced by the combination can occur. This was illustrated by a recent clinical trial that reported on the unexpected hepatotoxicity observed with melanoma patients after

concurrent treatment with a Braf inhibitor (Vemurafenib) and ipilimumab, resulting in cessation of the trial.[34] Given the beauracracy, cost and time associated with conducting clinical trials, utilizing preclinical mouse models that can more accurately model tumour immunity and irAEs may allow more informed assessment of which therapies can be combined to induce optimal therapeutic index (Figures 2 and 3).

Tumour-associated autoimmune syndrome is very rare in patients with cancer, such as melanoma-associated retinopathy and vitiligo. However, new data suggest that it is not merely a side effect of robust anti-tumour immunity. The development of vitiligo is considered to be a benefit to the prognosis of the patient with malignant melanoma.[35–38] In a preclinical study, mice with vitiligo generated more $CD8^+$ memory T cells, which protected them from melanoma expressing the same melanocyte antigens.[36] In another study, inflammatory monocytes or dendritic cells accumulated in the skin were shown to be capable of killing melanoma cells as well as normal melanocytes.[39] However, whether the relationship of tumour-associated autoimmune syndrome and tumour immunity is dependent on the innate immune system or adaptive immune system or both requires further investigation. In contrast, irAEs are not true autoimmune diseases, as they typically resolve with cessation of therapy and appropriate immunosuppressive regimes. In addition, the experience with ipilimumab and immunotherapies in general is their kinetics of response is much slower but more durable compared with those seen with cytotoxics.[20,31] Thus utilizing spontaneous mouse tumour models may better mimic the response kinetics that is observed in the clinic and may potentially allow for the development of any therapy-induced toxicity. These models can be divided into those that are carcinogen-induced such as methylcholanthrene (MCA)-induced fibrosarcomas and 7,12-dimethylbenz[α]anthracene (DMBA)/12-O-tetradecanoylphorbol-13-acetate (TPA)-induced skin papillomas or genetically engineered mouse tumour models, which have enforced expression of oncogenes and/or the loss of function of tumour suppressors, often in a tissue-specific and/or temporally controlled manner (Figure 2).[40,41] Examples include the Her2/neu or PyMT transgenic mice to mimic breast cancer, the MT/ret model of spontaneous metastatic melanoma[42] and $Braf^{CA}$Tyr-$creER^{T2}Pten^{fl/fl}$ mice in which 4-hydroxytamoxifen (4-HT) induces *de novo* melanoma[43] as well as the use of adenoviral vectors encoding Cre recombinase to selectively introduce mutations in the oncogene *Kras* and the tumour-suppressor gene *Tp53* in the pulmonary epithelia to induce autochthonous lung tumours.[44] Interestingly, it has been noted that carcinogen-induced models are very immunogenic, whereas germline mutation models are often not, most likely due to the former carrying more passenger mutations, thereby generating more neoantigens that can potentially be recognized by the immune system.[41] This was elegantly illustrated in two studies investigating the role of T cells in selecting for non immunogenic sarcomas using either a mouse model of sarcomagenesis driven by Cre recombinase-triggered activation of the $Kras^{G12D}$ oncogene and inactivation of the *Trp53* tumour-suppressor gene[45] or a MCA-induced mouse model of sarcoma.[46] In the first model, sarcomas that developed did not appear to be recognized by adaptive immunity as these tumours grew equally well when transplanted into wild-type or RAG-2-deficient mice. On the contrary, MCA-induced sarcomas, which had many passenger mutations in addition to *Kras* and *Trp53*, were demonstrated to generate tumour-specific T cells, which could then selectively sculpt tumour immunogenicity.[46] Interestingly, mouse MCA-induced sarcomas were also found to have qualitative and quantitative genomic mutation profiles to carcinogen-induced human cancers, such as smoker's lung cancer. Thus carcinogen-induced mouse models of cancer may better mimic cancers that are immunogenic. In contrast, transplantable tumours, which grow more rapidly and are employed as an initial model to assess the therapeutic potential of combination therapies, may be less useful. As most transplantable tumours are grown subcutaneously on the flanks of mice, an

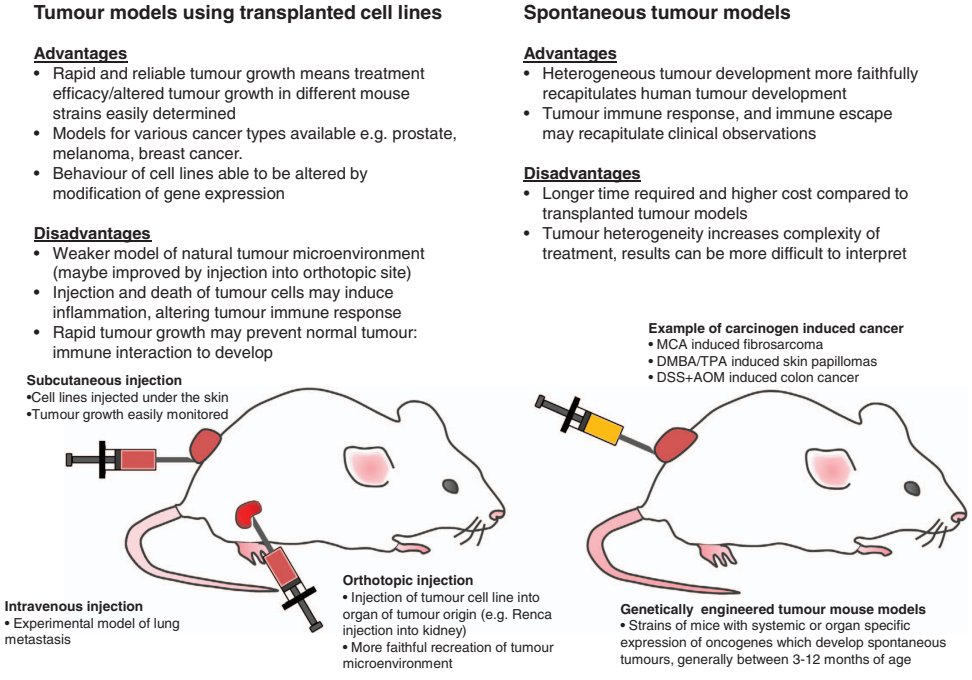

Figure 2 Common preclinical mouse models of cancers to assess the anti-tumour efficacy of cancer immunotherapies. The advantages and disadvantages of using transplantable and spontaneous mouse models of cancer are listed.

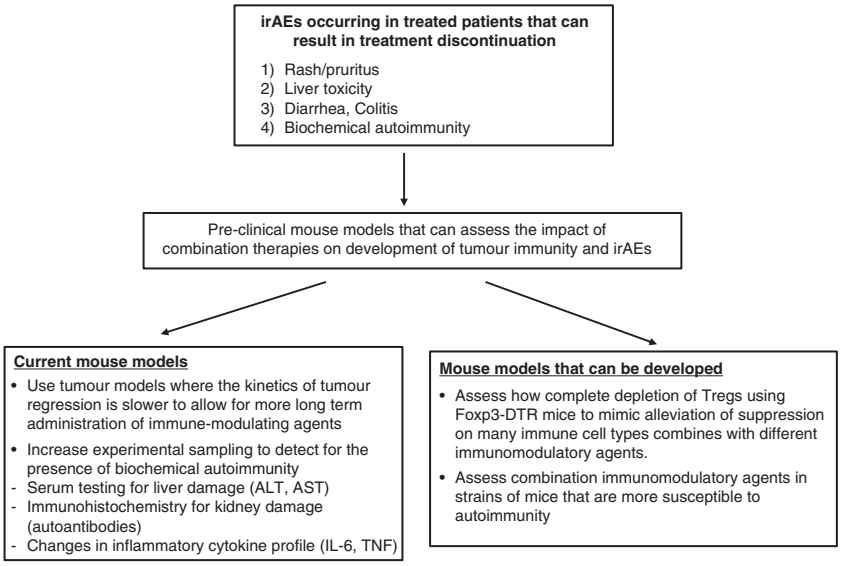

Figure 3 Clinical irAEs that needs to be assessed in preclinical mouse models of cancer following cancer immunotherapies. A number of key irAEs that occurs in patients following treatment with immunomodulatory antibodies and suggestions for how these irAEs could be modelled in preclinical mouse models are listed.

alternative approach is to inoculate them orthotopically to better mimic the tumour environment from which they originated. Indeed, immunotherapies that demonstrate efficacy against a renal tumour grown subcutaneously were not as effective when the same tumour was transplanted into the kidney.[47]

Although checkpoint blockade in patients commonly induces irAEs, such events have rarely been commented upon or observed in preclinical mouse models, even when $\geqslant 3$ different pathways are targeted.[48] It is possible that while treated mice appeared outwardly healthy, closer examination of animals may have revealed the presence of biochemical autoimmunity, which is frequently observed in patients even if they do not display clinical symptoms.[16] In addition, observable irAEs may not have manifested owing to the generally short nature of preclinical mouse models experiments or, perhaps, the strain of mice used may be more resistant to developing irAEs compared with humans. This was elegantly shown in a recent study where repeated dosing of anti-CTLA-4 in SJL/J mice over 5 weeks was able to induce hypophysitis, an irAE that appears to be associated with ipilimumab treatment.[49] Testing checkpoint blockade in combination or with other therapies in mouse strains more prone to development of autoimmune symptoms (for example, NOD, SJL/J) may be one strategy to assess the development of tumour immunity and autoimmunity. Furthermore, it has also been shown that repeated dosing of agonistic antibodies to co-stimulatory receptors such as GITR induced anaphylaxis in tumour-bearing mice that was caused by serum antibodies, and dependent on $CD4^+$ T cells, B cells, basophils, platelet-activating factor and GITR.[50] Alternatively, to observe irAEs in mice, a more dramatic alteration of the immune system such as the complete and systemic depletion of Tregs may be required. Intratumour Treg depletion by antibodies such as anti-CTLA-4 (now a recognized mechanism) does not seem sufficient to induce irAEs in mice, as are observed in humans. In addition, heterogeneity in the kinetics of response is normally seen in patients after immunotherapy, such as those that were treated with anti-PD-1,[19] and thus a tumour model that mimics what is observed in the clinic will be useful. Using the *de novo* model of MCA-induced fibrosarcoma, we observed that complete Treg depletion in DEREG mice bearing established MCA-induced tumours displayed a similar heterogeneous range of tumour responses to that observed in the clinic.[51] In our study, no overt autoimmunity with Treg depletion was observed, but this may be explained by the potential compensatory effect of other nontransgenic regulatory cells in the DEREG mice.[52] Comparative experiments in Foxp3-DTR knock-in mice, where autoimmunity is more easily generated upon Treg depletion,[53] are currently underway in our laboratories.

In clinical trials, concurrent blockade of CTLA-4 and PD-1 checkpoint receptors induced deep and rapid anti-tumour responses in contrast to the slower response kinetics seen in patients given anti-CTLA-4 and anti-PD-1 in a sequenced-regime.[2] This suggests that anti-tumour responses can be rapid if the right combination is given at optimal dosage and timing. It was not made clear if patients who had the rapid deep objective response had high/low grade irAEs. Indeed, we recently reported the use of the poorly immunogenic B16F10 melanoma model to characterize a very heterogeneous anti-tumour effect of the immune response induced by complete Treg depletion in DEREG mice.[54] Strikingly, the duration of the tumour–immune system interaction induced in individual Treg-depleted mice positively correlated with their propensity to develop vitiligo. A rapid complete tumour rejection was not associated with the development of autoimmunity; however, a proportion of mice that suppressed but did not effectively clear B16F10 melanoma did develop vitiligo.[54] We would postulate that approaches that combine with Treg depletion to rapidly reject tumours may also diminish irAEs.

Currently, cancer patients who have chronic autoimmunity are generally precluded from clinical trials with checkpoint blockade.[3] However, patients with certain autoimmune diseases (Pernicious anaemia, Crohn's disease, ulcerative colitis, systemic lupus erythematosis and psoriasis) are generally thought to be at higher risk of cancer due to the underlying dysregulation of their immune system, as well as a consequence of their treatment regime.[55,56] Thus, it is imperative we understand how and if toxicities induced by combination therapies in this cohort of patients differ to cancer patients who do not have underlying autoimmunity, that is, whether the therapies will be more toxic. Given that many tumour-associated

antigens are self-antigens, and that self-reactive T/B cells are increased in patients with autoimmunity, should a case be made that cancer patients with stable underlying autoimmunity be allowed treatment with immunotherapies as they may respond better? Of course, these patients have to be monitored stringently owing to their increased risk of developing irAEs or have their autoimmune symptoms exacerbated. Nevertheless, studies in this cohort of patients may potentially help in answering the question of how much overlap they are between tumour reactive and autoreactive T cells as well as their relative importance in different cancers. The Foxp3-DTR mouse represents one model where we can evaluate how combination immunotherapies attenuate tumour immunity/irAEs. In this setting, tumour-bearing Foxp3-DTR mice can be conditionally depleted of their Tregs to mimic the maximum release of suppression on all immune cells[53] and can then be treated with different immunotherapies/agents. Potentially, this may allow us to assess how different co-inhibitory/co-stimulatory receptors combine with each other or with other therapies to attenuate anti-tumour immunity/irAEs.

Although clinical observations suggest that autoimmune effectors are intricately involved in tumour cell killing, evidence using antigen-specific preclinical mouse models indicates that, in some cases, the immune system can selectively target tumour tissue while sparing self.[57] Indeed how self antigen-specific T cells target tumour versus healthy tissues, and how they are regulated in different microenvironments, is still unclear. To address this issue, Miska *et al.*[57] evaluated the ability of specific T effector cells to kill tumour versus healthy cells expressing the same self antigen in the same animal. Their studies concluded that tumours suppressed autoimmune T effector cells locally without distal impairment, suggesting that higher levels of suppression exist in tumours compared with healthy tissues expressing the same self antigen. Their studies also highlighted an increased potency of regulatory mechanisms such as Treg and MDSCs in tumours, although the molecular mechanisms responsible for this increased suppression remain to be elucidated. Thus antigen-specific $CD4^+$ or $CD8^+$ T cells that are unable to eliminate tumours can still mediate destruction of healthy tissues, suggesting that the threshold of inducing autoimmunity is lower than tumour immunity. This implies that systemic administration of immunotherapies that result in T-cell activation will almost always induce autoimmunity unless a substantial antigenic difference is identified between tumour target and healthy tissue. Thus strategies that can alleviate immune suppression specifically at the tumour site will be the way forward for next-generation combination therapy approaches. Another issue for consideration is that many of the cancer patients may have underlying metabolic syndrome such as obesity[58] and diabetes,[59] which have repeatedly been associated with increased incidence for some common cancers. How would combination therapies impact on this cohort of patients in terms of tumour immunity/irAEs? This question may be assessed by testing immunotherapies with the obese diabetic mouse (ob/ob) model.[60]

CONCLUSION

The durable clinical benefits obtained with immune checkpoint blockade have reinvigorated the field of cancer immunotherapy. Moving forward, checkpoint blockade looks set to be explored in combination with other therapies to further improve their therapeutic efficacy and their impact on more cancer types. However, the proportion of patients developing severe irAEs following combination immunotherapies most likely will increase. Utilizing relevant preclinical mouse models of cancer that better model tumour immunity and irAEs may identify therapies that combine well together without the associating toxicities.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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