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Cardiotoxicity of immune checkpoint inhibitors

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ABSTRACT

Cardiac toxicity after conventional antineoplastic drugs (eg, anthracyclines) has historically been a relevant issue. In addition, targeted therapies and biological molecules can also induce cardiotoxicity. Immune checkpoint inhibitors are a novel class of anticancer drugs, distinct from targeted or tumour type-specific therapies. Cancer immunotherapy with immune checkpoint blockers (ie, monoclonal antibodies targeting cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), programmed cell death 1 (PD-1) and its ligand (PD-L1)) has revolutionised the management of a wide variety of malignancies endowed with poor prognosis. These inhibitors unleash antitumour immunity, mediate cancer regression and improve the survival in a percentage of patients with different types of malignancies, but can also produce a wide spectrum of immune-related adverse events. Interestingly, PD-1 and PD-L1 are expressed in rodent and human cardiomyocytes, and early animal studies have demonstrated that CTLA-4 and PD-1 deletion can cause autoimmune myocarditis. Cardiac toxicity has largely been underestimated in recent reviews of toxicity of checkpoint inhibitors, but during the last years several cases of myocarditis and fatal heart failure have been reported in patients treated with checkpoint inhibitors alone and in combination. Here we describe the mechanisms of the most prominent checkpoint inhibitors, specifically ipilimumab (anti-CTLA-4, the godfather of checkpoint inhibitors) patient and monoclonal antibodies targeting PD-1 (eg, nivolumab, pembrolizumab) and PD-L1 (eg, atezolizumab). We also discuss what is known and what needs to be done about cardiotoxicity of checkpoint inhibitors in patients with cancer. Severe cardiovascular effects associated with checkpoint blockade introduce important issues for oncologists, cardiologists and immunologists.

INTRODUCTION

Cardiovascular toxicity (left ventricular dysfunction and heart failure (HF), myocardial ischaemia and infarction, hypertension, QT prolongation and arrhythmias, thromboembolism) caused by conventional antineoplastic drugs remains a critical issue.[1 2] Cardiotoxicity may be reversible or irreversible and can occur soon after or after several months/years of treatment.[3] Cardiac dysfunction caused by cytotoxic agents (eg, anthracyclines) has historically been the most relevant problem. However, targeted therapies and biological drugs affecting specific signalling pathways can also induce cardiotoxicity.[1 2 4–6]

The development of immunotherapies in oncology over the past decade has revolutionised the management of an increasing number of advanced-stage malignancies previously endowed with dismal prognosis.[7 8] Monoclonal antibodies (mAbs) targeting immune checkpoint molecules (eg, cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), programmed cell death 1 (PD-1) and its ligand (PD-L1)) have shown unprecedented success in a broad spectrum of solid[9–18] and haematological tumours.[19–23] Several checkpoint inhibition strategies have been developed.[24–26] The first and most widely mAb used is ipilimumab, which targets CTLA-4 and was introduced in 2010 for the treatment of melanoma. More recently several mAbs targeting PD-1 (nivolumab, pembrolizumab) and PD-L1 (atezolizumab, durvalumab, avelumab) have been introduced for the treatment of different types of cancer.[25 27 28] All these drugs have improved the prognosis in melanoma and in other cancer types endowed with poor prognosis.

Unfortunately, these compounds can produce a wide spectrum of immune-related adverse events (IRAEs).[29–32] Interestingly, PD-1 and PD-L1 can be also expressed in rodent and human cardiomyocytes,[33–36] and animal studies have demonstrated that CTLA-4 and PD-1 deletion can cause autoimmune myocarditis.[37–41] During the last years several cases of myocarditis and fatal HF have been reported in patients with cancer treated with immune checkpoint inhibitors (ICIs).[16 30 36 42–44] Severe cardiovascular effects associated with checkpoint blockade introduce important issues for oncologists, cardiologists and immunologists.

IMMUNITY AND CANCER: PATHOPHYSIOLOGY OF CANCER IMMUNOSURVEILLANCE

As cancer arises and progresses, malignant cells accumulate genetic alterations resulting

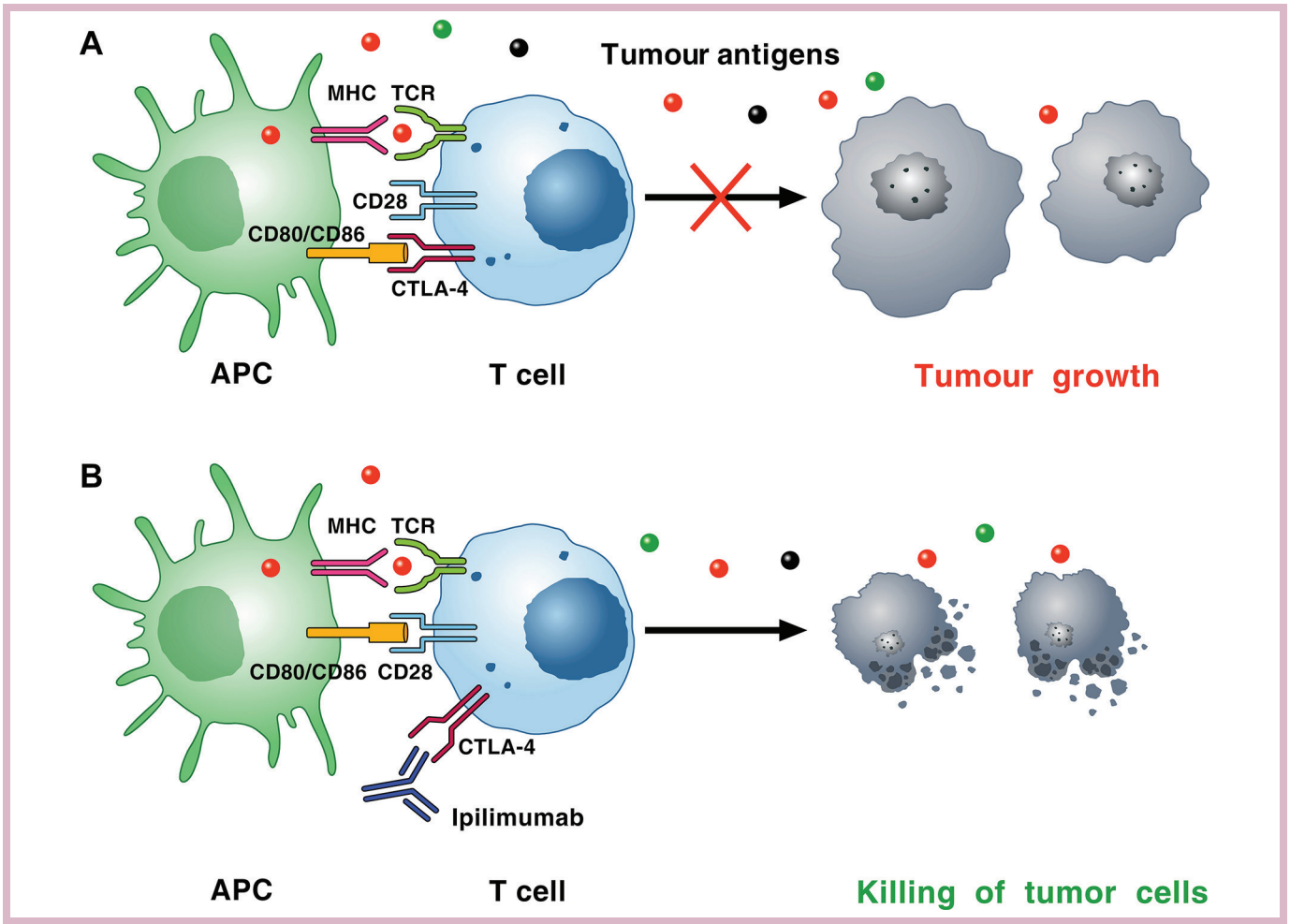

Figure 1 Mechanism of CTLA-4-induced immunosuppression. (A) Cancer cells synthesise and release neoantigens (dots of different colours) that are captured by APCs. These cells present peptides in the context of MHC I molecules/TCRs on the surface of $CD8^+$ cytotoxic T cells within lymph nodes. APCs can also present peptides bound to MHC II molecules to $CD4^+$ T helper cells. T cell activation on TCR signalling requires costimulatory signals transmitted via CD28, which is activated by binding to CD80, and/or CD86, on the surface of APCs. Activated T cells upregulate CTLA-4, which competes with CD28 for binding to CD80 and/or CD86. The interaction of CTLA-4 with CD80 or CD86 results in inhibitory signalling promoting tumour growth. The immunosuppressive activity of CTLA-4 is mediated by downregulation of Th cells and enhancement of Treg cells. (B) CTLA-4 blockade by ipilimumab results in a broad enhancement of immune responses against neoantigen expressing tumour cells, which results in killing of tumour cells. APC, antigen presenting cell; CTLA-4, cytotoxic T lymphocyte-associated antigen 4; TCR, T cell receptor; Th cells, helper $CD4^+$ T cells; Treg, regulatory T cell.

in the expression of several neoantigens.[45] The innate and adaptive immune responses initially prevent tumour outgrowth. However, cancer cells can escape the immune response (immunosurveillance) by selection of non-immunogenic tumour cells (immunoediting) or suppression of immune responses.[46 47] For decades, immunologists and oncologists have attempted to stimulate antitumour immune responses to fight cancer. These initial attempts displayed marginal success for a number of reasons. In particular, several inhibitory pathways, such as CTLA-4, PD-1 and PD-L1, profoundly dampen the antitumour functions of T lymphocytes.[48] These inhibitory pathways play pivotal roles in the maintenance of peripheral tolerance and the prevention of autoimmune diseases.[33 49] Tumours exploit these and many other inhibitory pathways to escape T cell-mediated tumour-specific immunity (figures 1 and 2). The pioneering work of Allison and coworkers led to the discovery that activation of these checkpoint inhibitors is a fundamental tool by which tumour cells evade the immune system.[8 50] Blockade of these immune checkpoints with specific mAbs (eg, anti-CTLA-4, anti-PD-1, anti-PD-L1) has recently revolutionised the entire branch of immunotherapy of a wide spectrum of tumours.[51 52]

Anti-CTLA-4: the first generation of checkpoint inhibitors

The development of blocking mAbs against immune checkpoint molecules is based on the role of these molecules as coinhibitory receptors of T lymphocytes. Indeed, the activation of naïve T lymphocytes requires antigen presentation by dendritic cells (DCs) to T cells through the interaction of major histocompatibility complex

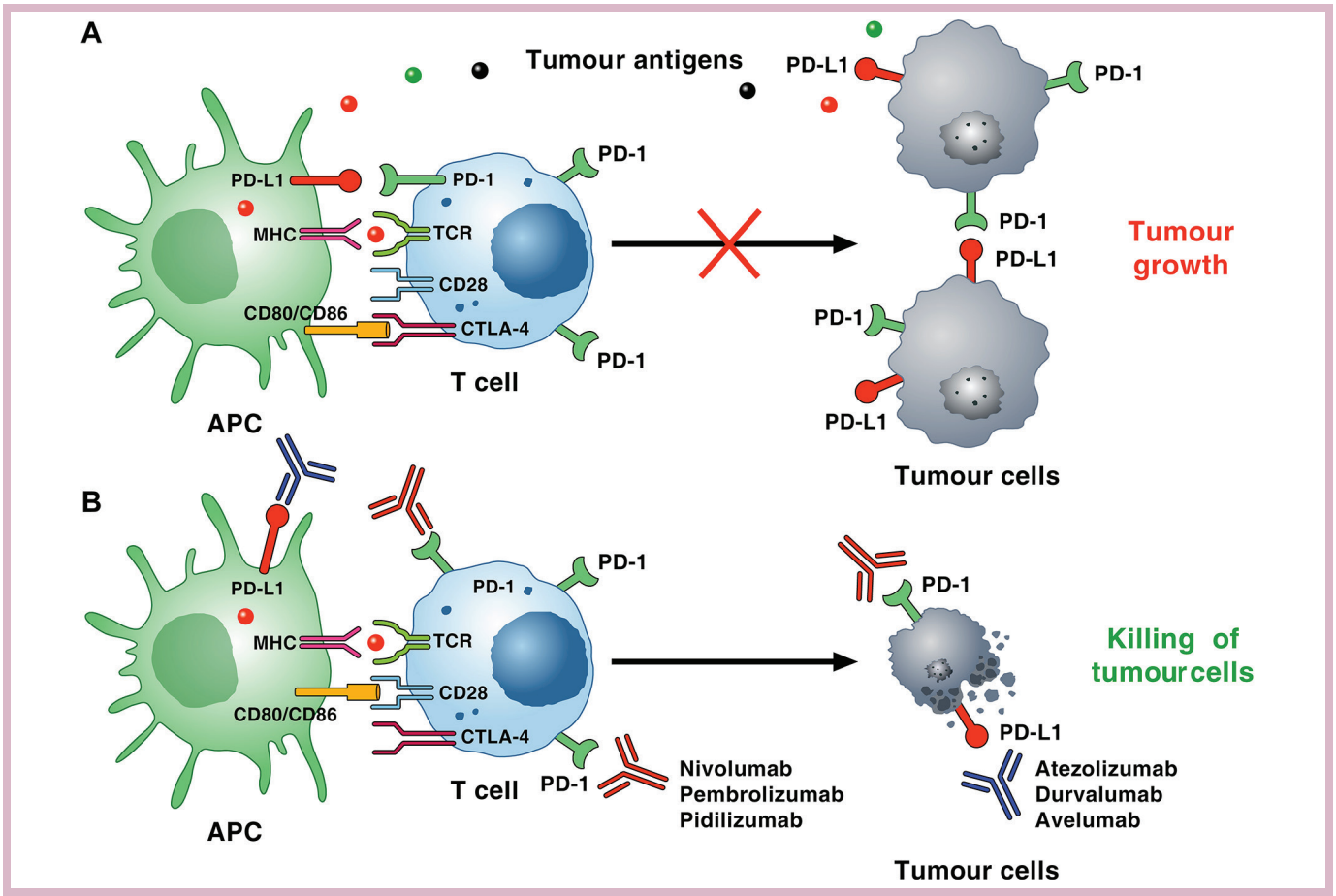

Figure 2 Mechanism of PD-1/PD-L1 pathway-induced immunosuppression within the tumour microenvironment. (A) Tumour neoantigens (dots of different colours) released by cancer cells are captured by APCs. These cells present peptides in the context of MHC molecules/TCRs on the surface of $CD8^+$ cytotoxic T cells. PD-1 is induced on T cells on activation through the TCR and through several cytokines. Tumour cells and other cells in the tumour microenvironment (eg, endothelial cells, mast cells) can express high levels of PD-L1 and/or PD-L2 that binds to PD-1 on T cells, resulting in inhibitory checkpoint signalling that decreases cytotoxicity and leads to T cell exhaustion. Recent evidence suggests that murine and human cancer cell subpopulations can express PD-1 and promote tumour growth. (B) PD-1 blocking antibodies (nivolumab, pembrolizumab, pidilizumab and so on) inhibit the interaction of PD-1 with both PD-L1 and PD-L2, resulting in enhanced T cell cytotoxicity, TAM activity, increased cytokine production, and ultimately killing of tumour cells. $PD\text{-}L1^+$ tumour cells can also induce T cell apoptosis, anergy, functional exhaustion and interleukin-10 production. Anti-PD-L1 antibodies (atezolizumab, durvalumab, avelumab) have similar effects, but only inhibit the interaction between PD-L1 and PD-1. PD-1, programmed cell death 1; PD-L1, programmed cell death ligand 1; TAM, tumour-associated macrophage; TCR, T cell receptor.

(MHC) and T cell receptor (TCR) (signal 1). The process of T cell activation is strengthened by costimulatory signals.[53] Receptors delivering coinhibitory signals (eg, CTLA-4, PD-1) function as immune checkpoints and play a role in maintaining tolerance and in preventing autoimmunity.[49 54 55] The pathways involving either CD80 (also known as B7.1) or CD86 (also known as B7.2), plus either CD28 or CTLA-4, are crucial in T cell activation and tolerance (figure 1).

CTLA-4, expressed almost exclusively on T cells, modulates the amplitude of early stages of T cell activation.[56 57] CTLA-4 competes with CD28 for binding to B7.1 and/or B7.2.[58] CD28 and CTLA-4 share identical ligands, CD80 and/or CD86.[59–62] Overexpression of CTLA-4 on activated T cells dampens their activation competing CD28 in binding CD80 and/or CD86. The crucial role of CTLA-4 was demonstrated by the lethal immune hyperactivation phenotype of *Ctla-4* knockout mice.[54 55] The immunosuppressive activity of CTLA-4 is mediated by downmodulation of helper $CD4^+$ T cell and enhancement of regulatory T cell (Treg) activity.[63 64]

The discovery of these immunoregulating CTLA-4 functions as a negative regulator of immune responses led to a radical shift in cancer immunotherapy: removal of inhibitory signals that block antitumour T cell responses rather than direct activation of the immune system.[50] Indeed, mice bearing immunogenic tumours and treated with an anti-CTLA-4 antibody showed an efficient antitumour response.[65] This seminal observation led to the development of a fully human mAb anti-CTLA-4 (ipilimumab), which was the prototype mAb to demonstrate a survival benefit for patients with metastatic melanoma,[66] and it

Table 1 Immune checkpoint inhibitors under preclinical and clinical development

Target	Agent	Antibody	Manufacturer
CTLA-4	Ipilimumab	Human IgG_1	Bristol-Myers Squibb
PD-1	Nivolumab	Human IgG_4	Bristol-Myers Squibb
	Pembrolizumab	Humanised IgG_4	Merck
	MEDI0680	Humanised	MedImmune
	REGN2810	Human IgG_4	Regeneron/Sanofi
	PDR001	Humanised IgG_4	Novartis
	BGB-A317	Humanised	BeiGene
	Pidilizumab	Humanised IgG_1	Medivation/CureTech
	AMP-224	PD-L2 IgG_{2a} fusion protein	GSK
	AMP-514	PD-L2 fusion protein	GSK
	SHR-1210	Human IgG_4	Incyte/Jiangsu
	JS001	Humanised	Shanghai Junshi Biosciences
	Tsr-042	Humanised	Tesaro
PD-L1	Atezolizumab	Humanised IgG_1	Genentech/Roche
	Durvalumab	Human IgG_1	MedImmune/AstraZeneca
	Avelumab	Human IgG_1	Merck Serono/Pfizer
	BMS-936559	Human IgG_4	Bristol-Myers Squibb
	LY3300054	Not available	Eli Lilly
	MEDI4736	Humanised IgG_1	AstraZeneca
	KNO35	Not available	3D Medicines
PD-L2	rHIgM12B7		Mayo Clinic/NCI
TIM-3	Anti-TIM-3 antibody		
LAG-3	Dual anti-LAG-3/anti-PD-1 antibody		
TIGIT	Anti-TIGIT antibody		
BTLA	Anti-BTLA antibody		
VISTA	Anti-VISTA antibody		

was approved by the Food and Drug Administration in 2010 and the European Medicines Agency in 2011.

Anti-PD-1 pathway: the second generation of checkpoint inhibitors

The immune system has developed several coinhibitory pathways to maintain T cell tolerance and to prevent autoimmunity.[26 67] The pathway consisting of PD-1 (also called CD279) and its ligands, PD-L1 (B7-H1 or CD274) and PD-L2 (B7-DC or CD276), is another important target to stimulate antitumour immune responses. Several mAbs targeting PD-1 and PD-L1 have been developed based on the role of these checkpoint molecules as coinhibitory receptors of T cell activation (figure 2 and table 1). mAbs against PD-1 and/or PD-L1 restore antitumour immune responses and have shown favourable clinical responses across various cancers.[68]

BTLA, B and T lymphocyte attenuator; CTLA-4, cytotoxic T lymphocyte-associated antigen 4; LAG-3, lymphocyte-activated gene-3; PD-1, programmed cell death 1; PD-L1, programmed cell death ligand 1; TIGIT, T cell immunoreceptor with Ig and ITIM domains; TIM-3, T cell immunoglobulin and mucin-containing protein 3; VISTA, V-domain Ig suppressor of T cell activation.

PD-1 is induced on T cells on activation through the TCR and cytokines.[69] PD-1 is expressed at low levels on T cells in the thymus, activated natural killer (NK) cells, B cells, monocytes, tumour-associated macrophage (TAM), immature Langerhans cells and cardiomyocytes.[35 69–71] During T cell activation, PD-1 is translocated to TCR microclusters.[72] Engagement of PD-1 by PD-L1 inhibits the activation of TCR proximal kinases.[73] PD-1 ligation inhibits T cell–APC contacts and thereby contributes to the cessation of T cell effector functions. The role of PD-1 in peripheral tolerance was demonstrated by the development of lupus-like glomerulonephritis and arthritis,[49] as well as in dilated cardiomyopathy in PD-1-deficient mice.[33]

PD-L1 and PD-L2, the ligands of PD-1, display different expression.[69 74] PD-L1 is constitutively expressed at low levels on both professional APCs and non-professional

APCs, as well as on non-haematopoietic cells (ie, endothelial cells, pancreatic islet cells, testes and eye).[75] PD-L1 is also expressed by cardiomyocytes.[36 70] PD-L1 pathway suppresses effector T cells, maintains self-tolerance and promotes the resolution of inflammation.

The expression of PD-L1 and, to a lesser extent, PD-L2 in several tumours[11 75 76] stimulated the exploitation of the PD-1–PD-L1 pathway in cancer immunotherapy. In fact, PD-L1 delivers antiapoptotic signals to cancer cells and prevents immune-mediated cancer cell killing.[77] Cancer cells dampen the host immune response through the upregulation of PD-L1 and PD-L2 in tumour microenvironment and their ligation to PD-1 expressed by tumour-specific $CD8^+$ T cells. PD-L1 and PD-L2 can be upregulated by cancer cells through several cytokines (interferon (IFN), tumour necrosis factor-α (TNF-α) and vascular endothelial growth factor (VEGF)). PD-L1 expression is also modulated by epigenetic mechanisms through microRNAs.[78]

In tumour microenvironment, tumour neoantigens released by dying cancer cells activate T cells that overexpress PD-1. Recent evidence indicates that mouse and human TAM express PD-1.[71] TAM PD-1 expression increases with increasing disease stage in human tumours and dampens macrophage phagocytosis of tumour cells. These events result in the activation of PD-1–PD-L1/PD-L2 inhibitory mechanism(s) leading to selective inhibition of tumour-specific T cells and TAM. Therefore, targeting of the PD-1/PD-L1 checkpoint pathway with mAbs results in the expansion of tumour-infiltrating $CD8^+$ T cells, which recognise tumour antigens.[71 79] $CD8^+$ T cells at the invasive tumour front progressively increase during immunotherapy and correlate with a reduction in tumour size.[12] These findings suggest that anti-PD-1 antibody increases $CD8^+$ memory T cell and TAM functions in the tumour microenvironment.[71 80] In addition, they also suggest that, although anti-PD-1/PD-L1 inhibitors are administered systematically, their mechanism of action is presumably locally active in cancer tissues.

The new generation of checkpoint inhibitors

Immunotherapy targeting CTLA-4 (ipilimumab), PD-1 (nivolumab and pembrolizumab) and PD-L1 (atezolizumab, avelumab, durvalumab) has revolutionised the management of several tumours.[12 81–85] Checkpoint inhibitors provide clinical benefits for a subset of patients with a wide range of solid (melanoma, non-small cell lung cancer (NSCLC), renal carcinoma, ovarian cancer)[9 85 86] and haematological tumours (Hodgkin's lymphoma, primary lymphoma, chronic lymphocytic leukaemia, multiple myeloma).[19–23]

Despite these promising results, dramatic responses are confined to a minority of patients.[28 87 88] This is likely due to the complex network of immunosuppressive pathways in tumour microenvironment, which are unlikely overcome by the blockage of a single signalling checkpoint molecule. In fact, combined anti-CTLA-4 and anti-PD-1 blockade further enhances antitumour activity and patient survival.[66 83 89 90] In addition, combination of four different types of immunotherapies eradicated several experimental tumours viewed as intractable.[91] A new generation of checkpoint inhibitors, beyond CTLA-4, PD-1 and PD-L1, are under preclinical and clinical development for safer and more effective treatment of human cancers (table 1).

The T cell immunoglobulin and mucin-containing protein 3 (TIM-3), expressed on Treg cells, monocytes/macrophages and APCs, regulate their functions.[92 93] Anti-TIM-3 inhibits tumour growth.[92] The lymphocyte-activated gene-3 (LAG-3, CD223) is expressed by $CD4^+$ and $CD8^+$ T cells, Treg and Tr1 cells.[94–96] LAG-3 blockade synergises with PD-1 inhibition to induce tumour regression.[97 98]

The T cell immunoreceptor with Ig and ITIM domains (TIGIT) is a novel member of the immunoglobulin superfamily expressed on activated T cells, Tregs, NK and natural killer t cells (NKT) cells.[99 100] Similar to PD-1, TIM-3 and LAG-3, TIGIT is upregulated on T cells in cancer.[101] TIGIT and PD-1 coblockade inhibits tumour growth in mice.[101] B and T lymphocyte attenuator (BTLA), structurally related to CTLA-4 and PD-1, is expressed on B cells, αβ and γδ T cells and DCs.[102 103] BTLA blockade induces tumour regression in mice.[104]

The V-domain Ig suppressor of T cell activation (VISTA), also known as PD-1 homologue, is expressed on neutrophils, monocytes/macrophages, DCs, Myeloiddendritic cells (MDSDs) and Treg cells, and at lower levels on $CD4^+$ and $CD8^+$ T cells.[105] VISTA blockade, combined with a peptide vaccine, induces tumour regression in a melanoma model.[106]

These novel checkpoint inhibitors, alone or in combination, restore antitumour immunological responses in preclinical models. It is likely that immunotherapeutic approaches of human cancers may enlist the combined use of several checkpoint inhibitors.[91] The assessment of adverse events, including cardiac toxicity, of these novel checkpoint regulators, alone and in combination, will be of fundamental clinical relevance.

IRAES ASSOCIATED WITH CHECKPOINT INHIBITORS

Due to the pivotal role played by immune checkpoints in the maintenance of self-tolerance, their therapeutic blockade can alter immunological tolerance,[87] and give rise to autoimmune or inflammatory side effects, termed 'immune-related adverse events'.[31 107–109] IRAEs associated with the use of ipilimumab were already evident in phase I studies, but now their incidence and severity are well-recognised.[66 110] IRAEs are common, usually reversible and not severe in most patients.[29] However, endocrinopathies (6%–8% of patients)[111] are associated with a high risk of irreversible toxicity. They are caused by the immune infiltration into the thyroid or pituitary glands, resulting in thyroiditis or hypophysitis, respectively.[112]

PD-1 and PD-L1 blocking agents display different adverse effect profiles compared with ipilimumab.[107] The most

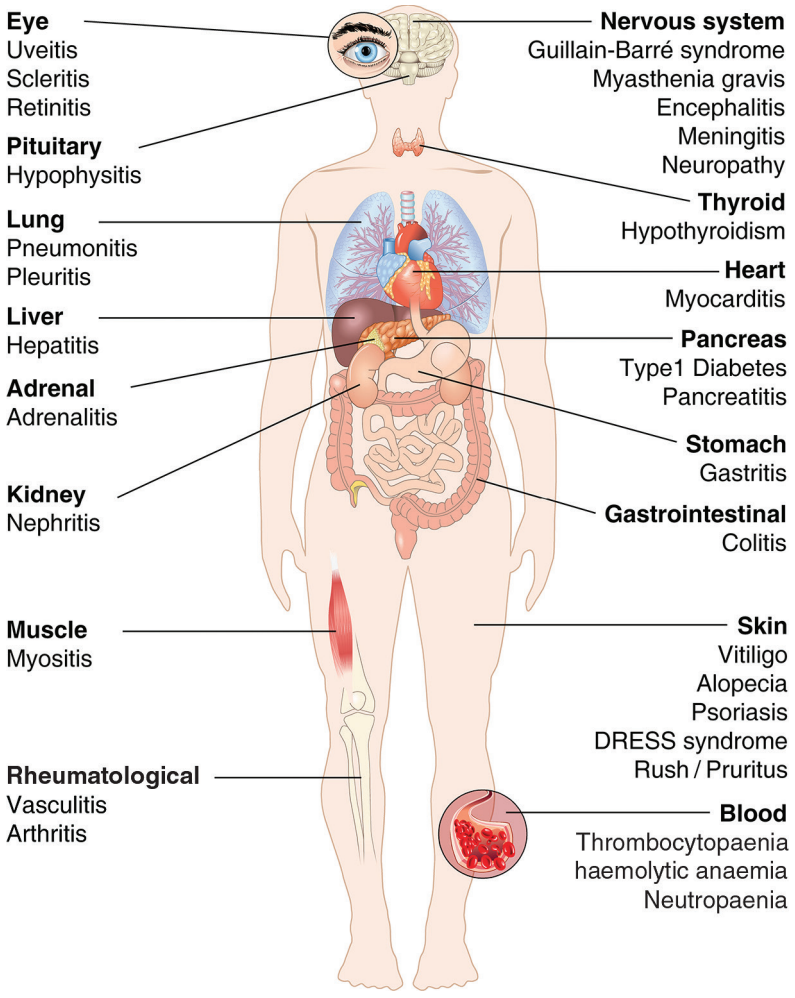

Figure 3 Some of the immune-related adverse effects (IRAEs) associated with checkpoints inhibitors in patients with cancer. DRESS, drug rash with eosinophilia and systemic symptoms.

common adverse events are mild fatigue, rash, pruritus and diarrhoea.[87] The incidence of IRAEs seems to be lower with anti-PD-1 therapy than with ipilimumab.[9 66] In general, IRAEs caused by ICIs resemble the autoimmune manifestations observed in PD-1-deficient mice.[33 49 113] Even though adverse events with combined checkpoint inhibitors are reported as well-tolerated,[114 115] combination of ipilimumab plus nivolumab requires discontinuation of therapy in nearly 40% of patients.[89 90 116] IRAEs can affect nearly every organ in association with checkpoint inhibitors (figure 3). Knowledge of the early-onset and late-onset toxic effects associated with checkpoint inhibitors, as well as effective algorithms for the identification and management of these effects, will be fundamental to optimise the safety and efficacy of these immunotherapies.[117] Finally, the long-term impact of immune checkpoint blockers on quality of life must be evaluated in future studies.

CARDIAC TOXICITY IN PD-1-DEFICIENT AND CTLA-4-DEFICIENT ANIMALS

PD-L1 is expressed in human[34] and murine heart.[35] Freeman and colleagues concluded that PD-L1 may regulate potentially autoreactive lymphocytes at effector sites, thus playing a role in limiting activities of T cells in the heart, where PD-L1 is highly expressed. Nishimura and coworkers demonstrated that disruption of the gene encoding for PD-1 in mice caused dilated cardiomyopathy.[33] They concluded that PD-1 may be an important receptor contributing to autoimmune diseases.

Several mouse models of T cell-dependent myocarditis exist. In a model of $CD8^+$ T cell myocarditis, IFN-γ induced the overexpression of PD-L1 on endothelial cells.[38] Genetic deletion of PD-L1/PD-L2, as well as treatment with anti-PD-L1, transformed transient myocarditis into lethal disease. Deletion of PD-L1 in murphy Roths Large (MRL) mice (genetically predisposed to autoimmunity) resulted in lethal autoimmune myocarditis.[39] Similarly, PD-1 deficiency in MRL mice causes a fatal myocarditis,[40] reminiscent of CTLA-4-deficient mice.[55] In two models

of T cell-dependent myocarditis, PD-1 protected against inflammation and myocyte damage.[41]

Recently, the role of PD-1/PD-L1 has been explored in models of cardiac ischaemia-reperfusion injury and myocardial infarction. Isolated ischaemic-reperfused rat hearts showed increased expression of PD-1 and PD-L1 in cardiomyocytes.[70] Interestingly, PD-1 and PD-L1 were not coexpressed on the same myocytes. Furthermore, myocardial infarction in BALB/c mice increased the percentage of PD-1$^+$ and PD-L1$^+$ cardiac cells. These experimental studies suggest that PD-1/PD-L1 and CTLA-4 play important roles in limiting T cell-mediated autoimmune myocarditis.

CARDIAC TOXICITY OF CHECKPOINT INHIBITORS IN PATIENTS WITH CANCER

With few recent exceptions,[118–121] the vast majority of papers on the toxicities of checkpoint inhibitors have underestimated or even neglected cardiac toxicity.[87 114 115 117] In a multicentre retrospective study on 752 patients with melanoma treated with ipilimumab, one case of myocardial fibrosis was reported.[122] One case report revealed left ventricular dysfunction[123] and one case of Takotsubo cardiomyopathy after treatment with ipilimumab.[44] Interestingly, a late-onset ipilimumab-induced pericarditis was reported in a patient with melanoma.[124] In a multicentre study, six cases of cardiotoxicity after ipilimumab were identified.[30] Two out of six cases were fatal despite intensive treatment. In the same series, one case of myocarditis after ipilimumab plus nivolumab and one case of cardiac arrest after pembrolizumab were described. A case of cardiac arrest was reported in a clinical trial of ipilimumab in melanoma[110] and a fatal case of myocardial infarction in a patient with NSCLC treated with pembrolizumab.[16] Similar case reports have confirmed these findings outside the clinical trial setting.[125 126] Autoimmune myocarditis with variable severity has been described.[127 128] In a multicentre, phase II, non-controlled study on 26 patients with advanced Merkel cell carcinoma treated with pembrolizumab, adverse events occurred in 77% of patients and one case of myocarditis was reported after the first dose of pembrolizumab.[15] Thus, a number of cardiotoxic events (myocarditis, HF, heart block, myocardial fibrosis and cardiomyopathy) were documented in these groups of patients.

Recently, two cases of fulminant myocarditis and myositis associated with combination of ipilimumab plus nivolumab were carefully described.[36] Despite intensive treatment, these two cases were fatal after receiving the first doses of checkpoint inhibitors. Both patients with melanoma had hypertension, but did not display other cardiac risk factors. Histological analysis demonstrated lymphocytes (CD4$^+$ and CD8$^+$ T cells) and macrophages infiltrating the myocardium, the cardiac sinus and the atrioventricular nodes. PD-L1 was highly expressed on injured cardiomyocytes and on infiltrating CD8$^+$ T cells. Figure 4 (reproduced with permission from Johnson *et al*[36]) illustrates the ECGs and the histological findings of the heart of patient 2 described by Johnson and collaborators. Importantly, the overexpression of PD-L1 in the injured myocardium in the two patients described by Johnson and collaborators is consistent with the constitutive expression of PD-L1 in human heart[34 35] and its upregulation in T cell-mediated myocarditis in mice.[38] Recently, PD-1 and PD-L1 were detected on rat cardiomyocytes and overexpressed in the ischaemic-reperfused heart.[70] An analysis of T cells infiltrating the myocardium, skeletal muscle and tumour revealed clonality of TCR. The authors suggested that common antigens present in these tissues could be recognised by clonal lymphocytes. Moreover, overexpression of IFN-γ, granzyme B and TNF-α, presumably produced by activated T cells, might contribute to cardiac damage.

The authors also assessed the frequency of myocarditis in the safety databases of Bristol-Myers Squibb Corporate to verify the occurrence of events in patients treated with nivolumab, ipilimumab or both. Among 20 594 patients treated with these checkpoint inhibitors, 18 drug-related severe adverse events of myocarditis were reported (0.09%). Combination therapy with both drugs was associated with more severe and frequent myocarditis than those who received nivolumab alone (0.27% vs 0.06%).[36] Myocarditis, diagnosed at a median of 17 days after the first treatment, suggests the occurrence of early cardiotoxicity.

In conclusion, although combined immune checkpoint inhibition has produced durable antitumour responses in a percentage of patients with different tumours, IRAEs required discontinuation in nearly 40% of patients.[89 90] Most of these events are manageable with a high dose of glucocorticoids, although severe and even fatal events have occurred in rare instances. Better characterisation of the real incidence of cardiovascular toxicity of checkpoint blockers, alone and in combination, even if uncommon, is a major priority.

It is important to note that extensive cardiac monitoring, including the assessment of troponin, a sensitive and specific marker of cardiotoxicity,[129 130] is not routinely performed in most immunotherapy trials. Therefore, the real incidence of early and late cardiotoxicity associated with immune checkpoint blockade is largely unknown.

MANAGEMENT OF MYOCARDITIS ASSOCIATED WITH ICIS

Oncologists and cardiologists should be aware of early onset of myocarditis, in patients treated with ICIs alone and in combination. Our understanding of the pathophysiology of myocarditis comes largely from animal studies.[131] In the 2000s it was demonstrated that deletion of CTLA-4 and PD-1 axis can cause autoimmune myocarditis.[33 37] Myocarditis is an insidious disease with a wide spectrum of clinical presentation reflecting the different aetiologies and the variability of local or diffuse involvement. In addition, patients with systemic autoimmune disorders (eg, rheumatoid arthritis, lupus erythematosus,

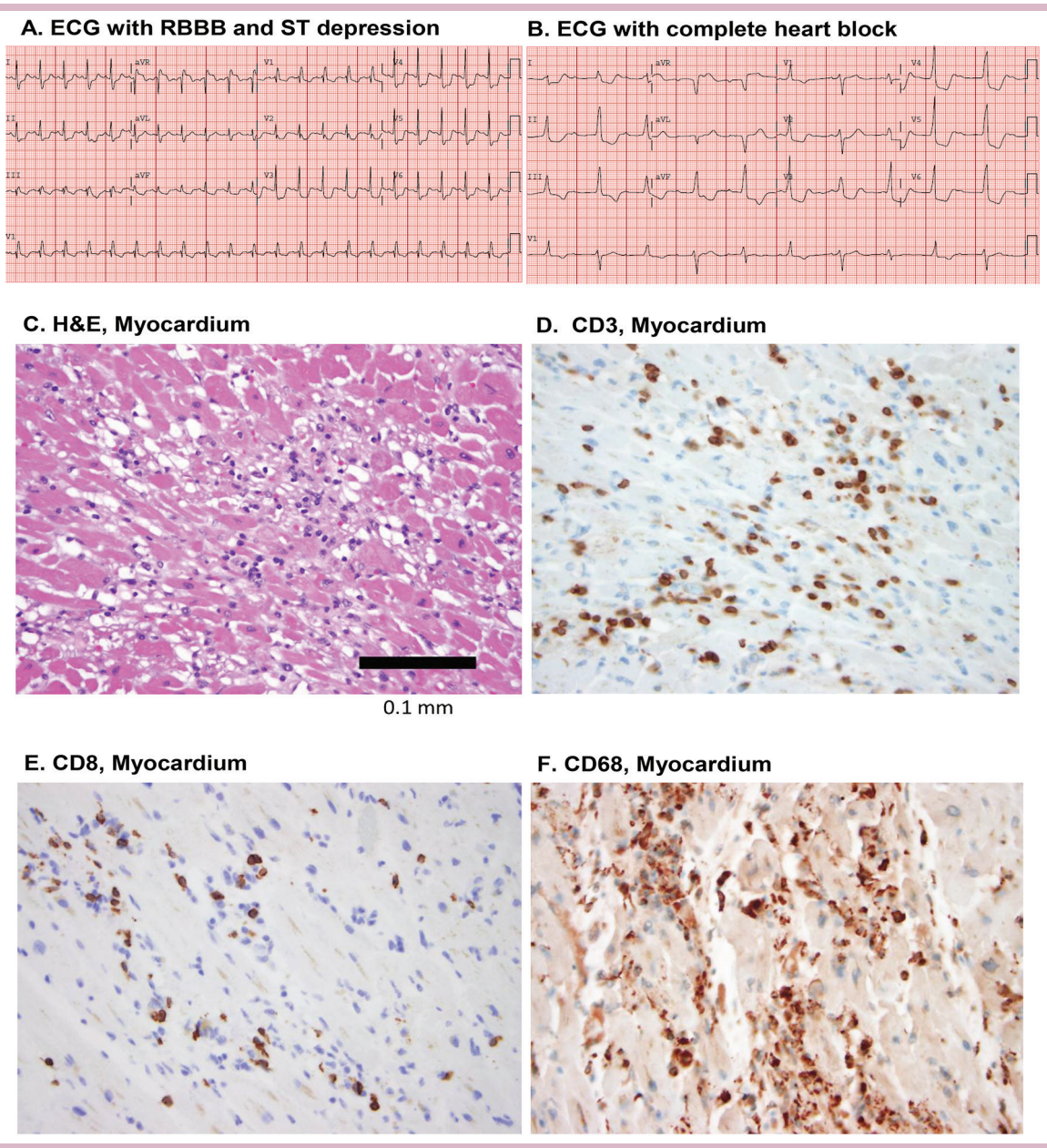

Figure 4 ECG and histological findings of the heart in a 63-year-old man with metastatic melanoma who developed fulminant lymphocytic myocarditis following initial doses of nivolumab and ipilimumab and who developed complete heart block.[36] Despite intense treatment (intravenous methylprednisolone 1 g/kg daily for 4 days plus infliximab 5 mg/kg), fatal complete heart block occurred. Initial right bundle branch block (RBBB) and ST depression (A) progressed rapidly to complete heart block and cardiac arrest (B). Autopsy showed lymphocytic infiltration in myocardium (C) comprised $CD3^+$ T cells (D), many of which were $CD8^+$ lymphocytes (E) and $CD68^+$ macrophages (F) (adapted with permission from Johnson *et al*[36]).

psoriatic arthritis, systemic sclerosis, vasculitis, polymyositis) can have subclinical myocarditis. In the absence of specific studies, clinical experience should guide the use of checkpoint inhibitors in patients with cancer with pre-existing autoimmune diseases.[132]

Similarly, at present there is urgent need of validated guidelines for treatment of myocarditis associated with checkpoint inhibitors. Wang and coworkers[118], based on their extensive personal experience, have proposed an interesting algorithm for management of myocarditis in patients treated with checkpoint inhibitors. Their algorithm represents an excellent basis for an urgently needed consensus guideline for management of different forms of immune-mediated myocarditis.

CONCLUDING REMARKS

Checkpoint blockade has introduced clinical benefits by inducing regression of advanced metastatic tumours, improving patient survival and inducing durable effects in a percentage of patients with a broad spectrum of cancer types. Although IRAEs associated with monoclonal

anti-CTLA-4 and anti-PD-1/PD-L1 antibodies are common and usually reversible, increasing reports of severe cardiac toxicity introduce important questions relevant for future oncology trials and clinical practice.

Patients with autoimmune disorders are usually excluded from clinical trials with checkpoint inhibitors. Patients with a wide spectrum of autoimmune disorders presumably represent 20–50 million people in the USA alone.[119] In addition, approximately 14% of patients with lung cancer have a concurrent diagnosis of autoimmune disease.[133] These findings indicate that clinical and subclinical autoimmune disorders are an important consideration before initiation of checkpoint inhibitor therapy. In 12 patients treated with ipilimumab, worsening or exacerbation of pre-existing autoimmune diseases was observed in 50% of cases.[134 135] Recently, Johnson and collaborators reported their experience with two groups of patients with pre-existing autoimmune disease and melanoma treated with ipilimumab or anti-PD-1.[36 132] Although 20%–30% of these patients experienced an autoimmune flare, the authors concluded that treatment with either ipilimumab or anti-PD-1 is feasible for patients with certain types of pre-existing autoimmunity.[119] Given the heterogeneity of autoimmune disorders and the wide spectrum of their severity, specific guidelines regarding exclusion criteria and treatment are urgently needed.

Cardiac parameters and levels of troponins are not routinely evaluated in most oncology trials. Therefore, the true incidence of cardiac toxicity associated with checkpoint inhibitors may be higher in the real-world population.

Combination therapies such as combined checkpoint inhibitors, or sequential therapies with conventional chemotherapy plus checkpoint inhibitors, or checkpoint inhibitors plus antiangiogenic agents are increasingly being used.[136 137] Cardiovascular monitoring is necessary to assess the occurrence of early and late cardiac toxicity associated with these newer cancer immunotherapies. The use of ICIs is expected to increase within the next years for treatment of new tumour types and presumably also for other immune-mediated disorders such as HIV[138] and infectious diseases.[139] Therefore, prospective cardiovascular evaluation appears necessary to detect potential cardiotoxicity in these disorders.

All cases of cardiotoxicity associated with checkpoint inhibitors reported so far occurred immediately after the infusion or during the first year of therapy.[15 30 36 44] Prospective studies should assess whether late-onset chronic cardiotoxicity can occur after completion of therapy.

Interestingly, interindividual differences in intestinal microbiota are a source of the heterogeneity in immunotherapeutic efficacy and toxicity of ICIs in cancer.[7 140 141] It will be important to investigate whether gut microbiota can also influence cardiac toxicity of ICIs.

Constitutive expression of PD-1 and PD-L1 occurs in human and murine myocytes.[34 35 70] In addition, overexpression of PD-L1 on the surface of injured myocytes has been demonstrated in patients with fulminant myocarditis treated with checkpoint inhibitors.[36] The latter observations open the possibility that, in certain clinical conditions (eg, myocardial ischaemia), cytokines and chemokines produced by infiltrating immune cells can upregulate PD-1/PD-L1 pathway in human myocardium. Additional in vitro and in vivo research is urgently needed to understand the immunological and molecular mechanisms underlying the development of these cardiac toxicities.

Today, oncologists and cardiologists work together mainly to detect and manage cardiotoxicity of antineoplastic treatments. Cardiologists are not always involved in the initial anticancer treatment planning or in the assessment of early cardiac dysfunction. With a growing number of patients treated with different types of ICIs, a collaboration of oncologists, cardiologists and immunologists is now necessary for a better characterisation of the mechanisms of cardiotoxicity of this novel class of anticancer drugs and for a comprehensive identification and management of patients at risk for cardiac adverse events.

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