

EDITORIAL



The pioneers behind immune checkpoint blockers awarded the Nobel Prize in physiology or medicine 2018

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The pioneers who discovered the mechanisms for activation of cytotoxic T-cells and developed antibodies which inactivate the T-cell brakes, professor James P Allison, USA, and professor Tasuku Honjo, Japan, have recently been awarded the Nobel Prize in Physiology or Medicine 2018 by the Nobel Assembly at Karolinska Institutet, Stockholm, Sweden. Immunotherapy, particularly the use of immune checkpoint blockers (ICBs) has been approved for standard clinical use in some settings, but not all tumors respond to the treatment. The aim of the present editorial is, therefore, to present the background, mechanisms of action and the current role of ICBs for clinical anticancer therapy.

Immunotherapy has a more than 100 year long history of preclinical and clinical trials. The general success of vaccines to prevent viral and bacterial diseases has inspired the use of vaccines against malignant diseases [1]. Despite many different approaches, the use of vaccines towards cancer cells has largely been of modest clinical benefit [2]. However, modern sequencing technologies have disclosed that cancer cells have many genetic or epigenetic changes that ultimately produce proteins and peptides classified as unique driver mutations or passenger mutations [3]. Despite the great success of imatinib and newer tyrosine kinase inhibitors against the Bcr/Abl oncoprotein in chronic myelogenous leukemia [4], and the success of antagonists of the Her2/neu tyrosin kinase for breast cancer [5], the clinical results of personalized targeted therapy have long lasting effects in only a limited number of patients with systemic disease, and the responses have generally been short lived.

Cancer development is likely associated with immune suppression: cancer develops in patients with HIV infection or patients on immune suppressive therapy after organ transplantations [6,7]. Newer studies have disclosed that the immune system may recognize cancer cells, but also that cancer can avoid immunologic suppression and control. Some facts necessary to understand the clinical use of ICB drugs which are the recent candidates for immunotherapy will be presented.

Mechanisms for activation and inhibition of cytotoxic T-cells

When cancer cells are exposed to chemotherapy, they generally die by apoptosis [8]. Apoptosis was previously considered not to stimulate an immunological response, in contrast to necrosis which typically elicits an inflammatory response [9]. These two separate mechanisms of dying are in fact interrelated and autophagy seems to be involved in selection between apoptosis and necrosis [10]. Both mechanisms expose tumor-related antigens (neoantigens) which the immune system can identify and direct both the innate immune system and cytotoxic T-lymphocytes (CD8 T-cells) against [11–13]. A clear relation between the number of neoantigens in different tumor entities and their response to immune stimulating therapy by ICBs has been demonstrated [14–16]. Tumors with high number of mutations exhibiting microsatellite instability (MSI) seem especially to be good candidates for ICBs [17,18]. Genetic mutation analyses revealed MSI in 3.8% of tumor and normal tissues assessed from 39 cancer types [19]. However, only a minority of the neoantigens produced by DNA mutations seems to be recognized by CD8 T-cells [20].

To understand the mechanism of action of ICBs, some basic insight into the activation and function of CD8 T-cells are necessary. Cells dying after cancer therapy may undergo a process termed *immunogenic cancer cell death* [21,22]. In addition to neoantigens, tumor cells exposed to stress including therapeutic methods (radiation therapy, chemotherapy, and hyperthermia) also transfer internal molecules to the surface of the tumor cells as *danger or damage associated molecular patterns* (DAMPs) which function as semaphores for the immune system's immature *antigen presenting cells* (APC) [23–25]. Typical DAMPs are calreticulin, high mobility group box 1 (HMGB1), heat shock protein 70 and 90 (Hsp 70, Hsp 90), extracellular adenosine triphosphate (ATP), S100A4, and uric acid [26,27]. The chemokine axis CXCR4–CXCL12 and DAMPs act also as chemo-attractants which activate dendritic cells and attracts macrophages,

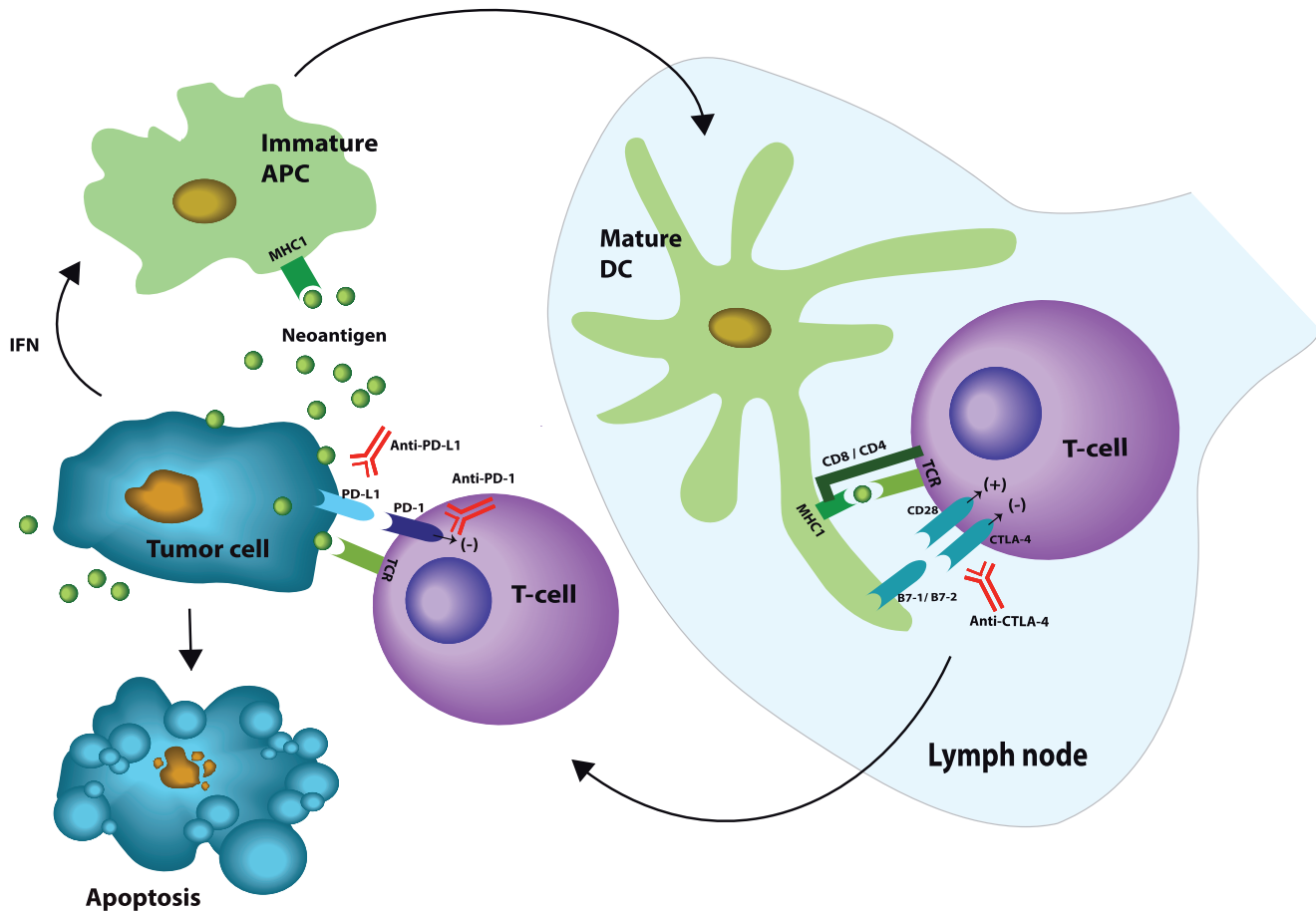


Figure 1. Review of major mechanisms for activation of CD-8 T-cells by neoantigens from a tumor. First immature antigen presenting cells (APC) is activated to mature dendritic cells (DC) by binding of neoantigens to major histocompatibility complex type I (MHC I). Co-stimulation for activation of CD8 T-cells is illustrated by the binding of CD28 receptors on T-cells to either B7-1 or B7-2 ligands on the mature DC. The inhibiting receptor CTLA-4 is also illustrated. The main inhibition of peripheral effect of T-cell is exerted by binding of programmed death receptor 1 (PD-1) to either programmed death ligand 1 or 2 (PD-L1 or PD-L2). Immune check-point blockers (ICBs) are represented with antibodies raised against the blocking molecules (marked in red). Interferons (IFN) together with transforming growth factor β (TGF β) protect tumor cells from immunologic inactivation. Stimulating signals marked (+), inhibition marked (-) (illustration: Kristin Risa).

granulocytes, and eosinophils [28–31]. The innate immune system is also involved in anticancer immunity by stimulation of dendritic cells and by natural killer cells responding to DAMP's [32].

The tumor antigens include different overexpressed antigens, cancer testis antigens, and mutated tumor neoantigens [33]. It seems that chaperoning proteins like Hsp 70, Hsp 90, and Hsp 27 bind to antigen peptides which are then presented for immature APC [34]. The peptide antigens from inside of the tumor cells then bind to the *major histocompatibility complex type I* (MHC I) on APC [35,36]. The human homologs of MHC I and II are termed *human leukocyte antigen* (HLA) complex [35]. When the antigens are bound to MHC I on APCs these cells migrate to regional lymph nodes where they mature to *dendritic cells* which present the antigens bound to MHC I to a specific receptor, *T cell receptor* (TCR) on CD8 T-cells or macrophages [36,37]. The discovery of these mechanisms was pioneered by Allison and his colleagues among others, who disclosed that in order to activate the CD8 T-cell a second signal must be simultaneously sent by binding of the B7 receptor on the APC/dendritic cell to the CD28 ligand on the CD8 T-cell [38]. Later it was shown that a molecular homolog of CD28 termed *cytotoxic T-lymphocyte antigen 4* (CTLA-4) bound even stronger to the

B7 receptors (CD80 and CD86) [39,40]. CTLA-4 is mainly expressed on CD4 and CD8 T-cells [41]. A seminal study in mice then documented that CTLA-4 inhibited initial priming and activation of CD8 T-lymphocytes [42]. Antibodies against CTLA-4 can also deplete immunosuppressive CD4 T-cells (regulatory T-cells) [43,44]. The recent achievements in immunotherapy are largely based on further insight on the molecules and their receptors which normally control immunological responses as needed: activating immune responses or damping the immune effector cells when an infectious agent is controlled or to avoid autoimmune conditions (Figure 1). The level of antigens and the degree of naïve CD8 T-cells in the draining lymph nodes seems to determine whether the immune system responds with tolerance or an active anticancer response, both processes may take place simultaneously changing the immunogenicity of a tumor, a process now termed *cancer immunoediting* [45,46].

When studying programmed cell death in a murine hybridoma and hematopoietic progenitor cell line, Honjo and his group identified a gene they termed *programed death 1* gene (PD-1 gene) coding for a surface receptor of the Ig superfamily [47,48]. They then discovered a ligand (PD-L1) as a member of the B7 family on APCs that dampen the CD8 T-cell response [49]. Later, it was shown that cancer cells

Table 1. Clinical indications approved by U.S. Food and Drug Administration (FDA) and/or European Medicines Agency (EMA) for immune checkpoint blockers. Only the year of first approval for an indication of a drug given in the table.

Target	Drug	Clinical setting	FDA approved	EMA approved
CTLA-4	Ipilimumab (Yervoy)	Advanced malignant melanoma Hormone refractory prostate cancer, NSCLC, SCLC, bladder cancer	2010	2010
CTLA-4	Tremelimumab (CP 675)	Mesothelioma	2018	
PD-1	Nivolumab (Opdivo)	Advanced melanoma Advanced NSCLC Advanced renal cell cancer Hodgkin lymphoma Advanced urothelial cancer Advanced squamous cell head and neck cancer Advanced melanoma Advanced SCLC Advanced squamous cell head and neck cancer, breast cancer Mediastinal large B-cell lymphoma Recurrent or metastatic cervical cancer Any cancer with microsatellite-high/DNA mismatch repair-deficient biomarker	2014	2015
PD-1	Pembrolizumab (Keytruda)	Advanced NSCLC Advanced urothelial cancer, colorectal cancer, breast cancer Renal cell cancer	2014	2015
PD-1	Atezolizumab (Tecentriq)	Advanced NSCLC Advanced urothelial cancer, colorectal cancer, breast cancer Renal cell cancer	2016	2016
PD-L1	Durvalumab (Imfinzi)	Locally advanced NSCLC	2017	2017
PD-L1	Avelumab (Bavencio)	Metastatic Merkel cell carcinoma Advanced urinary system cancers	2017	2017

express *programed death 1 or 2 ligands* (PD-L1, PD-L2) on the surface which bind to *programed death 1* (PD-1) receptors on CD8 T-cells and thereby exploit the natural immune damping system for inactivation of CD8 T-cells, which is expressed on macrophages and bone marrow derived cells [50–53]. By reducing this mechanism antibodies against PD-1 or PD-L1 can re-invigorate exhausted CD8 T-cells to attack tumor cells [54].

Clinical use of immune checkpoint inhibitors

Antibodies inhibiting CTLA-4 (ipilimumab, tremelimumab) and PD-1 (pembrolizumab, nivolumab) and PD-L1 (durvalumab, avelumab, atezolizumab) are approved by U.S. Food and Drug Administration (FDA) or European Medicines Agency (EMA) for clinical use (Table 1). The clinical indications are given in Table 1; therefore, only some typical studies will briefly be presented here.

The initial dose finding studies with ipilimumab in advanced malignant melanoma reported only objective responses in 10% of the patients [55]. For pembrolizumab the response was better (20–30%), and some long duration responses were observed. The two drugs were then combined, based on the rationale that CTLA-4 inhibition with ipilimumab stimulates CD8 T-cells locally, while PD-L1 inhibition

with pembrolizumab or nivolumab works on exhausted CD8 T-cells at any site. The combination yielded a response rate of 50–70% in advanced malignant melanoma and 3–5 year survival of 60% is now reported [56,57]. In a randomized study using ipilimumab versus placebo as adjuvant therapy after resection of malignant melanoma stage III, the survival was 41% and 30%, respectively [58]. In two randomized studies including resected malignant melanoma stage III or IV comparing pembrolizumab versus placebo and nivolumab versus ipilimumab, early results show best durable effect of the of pembrolizumab and nivolumab arms compared to the control groups with placebo and ipilimumab, respectively (difference about 10% at 5 and 2 years) [59,60]. Grades 3–4 toxicity was 14% for nivolumab versus 46% for ipilimumab.

In advanced non-small cell lung cancer (NSCLC), the objective response rate was 19%, after treatment with pembrolizumab [61]. Treatment with nivolumab and pembrolizumab improved median progression free survival in NSCLC with 3–4 months more than docetaxel and platinum-based regimens [62–64]. Combining nivolumab and ipilimumab in different schedules for advanced NSCLC yielded confirmed partial responses in 47% and 38% in the best combinations, and median follow up was not yet achieved after 12 months follow up [65].

Pembrolizumab monotherapy yielded 18% objective responses in recurrent or metastatic head and neck

Table 2. Mechanisms for primary and acquired resistance to immune checkpoint inhibitors.

Primary resistance	Acquired resistance	References
Tumor intrinsic mechanisms		
Constitutively high PD-L1 in tumor		[84,134]
HLA class I loss (Alters antigen presentation)	HLA mutations, Loss β -2-microglobulin	[135]
Low antigen expression	Loss/alterd antigens	[136]
Secreted signals (VEGF, IL8) inhibit T-cell recruitment	Secreted signals (VEGF, IL8) inhibit T- cell recruitment	
Interferon γ	Interferon γ	[137]
Overexpressed antiautophagy genes confer CTLA4 resistance		[138]
Host/environmental factors		
Gut microbiome	latrogen dysbiosis (Use of antibiotics)	[103,139]
latrogen immunosuppression (steroids)	latrogen immunosuppression (steroids)	
Carcinoma associated fibroblasts	Carcinoma associated fibroblasts (FAP positive)	[140]
(FAP positive) activated by CXCL12–CXCR4 axis	Activated CXCL12 – CXCR4 axis influence recruitment of T-cells	
Expression of PD-L1 in microenvironment (HIF-1a induced)	Expression of PD-L1 in microenvironment (HIF-1a induced)	[141]
T regs, myeloid derived suppressor cells, M2 macrophages in microenvironment, indole 2,3-dioxygenase (IDO) activity (kynurenine)	T regs, myeloid derived suppressor cells, M2 macrophages in microenvironment, indole 2,3-dioxygenase (IDO) activity (kynurenine)	[29,136,142]
Interferon γ	Interferon γ	[137]
	Upregulation of alternative immune checkpoints (T-cell Immunoglobulin mucin-3, TIM-3)	[95,143]

squamous cell carcinomas patients [66]. Median overall survival was 13 months. In a randomized trial using nivolumab versus standard therapy for recurrent or metastatic head and neck cancer, 1-year survival was 36% and 17%, respectively [67]. In Merkel cell carcinoma, a rare aggressive skin cancer (related to a polyoma virus in 80%), objective response was observed in 56% of 28 patients treated with pembrolizumab as first line therapy and 33% in 88 chemotherapy refractory patients treated with avelumab [68,69]. Some patients obtained long duration of response.

Renal cell cancer is generally resistant to chemotherapy, but metastatic disease is currently treated by targeted agents like temsirolimus and sorafenib. When nivolumab was compared with everolimus in metastatic renal cell cancer 25% and 5% obtained objective responses, respectively, but few obtained a complete response [70]. In advanced renal cell cancer patients the combination of nivolumab and ipilimumab yielded 75% survival at 18 months in contrast to 60% for sunitinib [71]. Several clinical studies are now ongoing testing ICBs in renal cell cancer patients [72].

In early bladder cancer, immunotherapy with BCG-instillations into the bladder is standard therapy. In a series of trials different ICBs (CTLA-4 blockers ipilimumab, tremelimumab, PD-1blockers nivolumab, and pembrolizumab or PD-L1 blockers atezolizumab, avelumab, durvalumab) have yielded objective responses of 13–30% in advanced urothelial tumors, but generally short lived [73,74]. Only pembrolizumab increased significantly median overall survival from 7.4 months in controls to 10.3 months [75].

Several types of immunotherapy have been tested in gastrointestinal cancers [76]. In therapy resistant PD-L1 positive esophageal cancer an objective response rate of 30% were seen in 23 patients which lasted for 15 months [77]. In a phase II study using nivolumab and ipilimumab in MSI high colorectal cancer, objective response was seen in 55% of 119 patients, and progression free and overall survival were 71% and 85%, respectively [78]. Eight had responses lasting at least 12 months. In unresectable metastatic anal

cancer, treatment with nivolumab resulted in 24% objective responses, and the median progression free survival was 4.1 months [79].

Very promising results were published in Hodgkin's lymphoma patients treated with nivolumab or pembrolizumab where objective response was observed in 65–87%, of these 16–20% were long lasting complete responses [80–82]. Interestingly, Reed Sternberg cells in Hodgkin's lymphoma express PD-L1 to secure immunological protection. In other types of lymphomas objective responses ranging from 20 to 40% have been reported [83,84].

Biomarkers

Tumors may express PD-L1 or PD-L2 on the surface membrane to protect them from cytotoxic CD8 T-cells. Some studies have reported higher response rate in tumors which express PD-L1 or PD-L2 on the membrane [85,86], but the level of expression varies from tumor to tumor, and responses are also seen in tumors lacking these markers. The density of infiltrating CD8 T-cells in tumors and number of neoantigens in tumor cells are also linked with better tumor response [86,87]. Immune gene signatures and markers in serum are under investigation as biomarkers for response to ICB therapy, but we still lack reliable markers to select patients suitable for ICBs. Documented MSI high or mismatch repair deficiency in any tumor is now approved by FDA as an indication for use of ICBs.

Resistance

It should be noted that an increase in tumor volume may occur for some time after initiation of ICB therapy before tumor regression, probably due to infiltration of cytotoxic CD8 T-cells or edema [88]. Classical RECIST v 1.1 criteria for response evaluation may underestimate the benefit of pembrolizumab in approximately 15% of melanoma patients [89];

therefore, the criteria for response has been specified to immune-related RECIST and immune RECIST criteria [90].

Resistance to ICB treatment can be caused by primary properties in the tumor or the host before treatment or as acquired as a response to the treatment, see Table 2. Examples of adaptive tumor responses are reduction of tumor neoantigens or changes in the antigen presentation like mutations in β -2 microglobulin as part of the HLA complex or reduced maturation of the dendritic cells by changes in DAMPs [91–93].

The structure and adaptive changes in the microenvironment like tumor-associated macrophages type 2, cancer associated fibroblasts and presence of proangiogenic growth factors, infiltration of regulatory T-cells (T-reg), myeloid derived cells or cytokines (CXCR4 –CXCL12 axis) all influence the contact between the CD8 T-cells and tumor cells [94,95]. Other mechanisms may be exhaustion of CD8 T-cells or upregulation of other inhibitory immune checkpoints like TIM-3, LAG-3, TIGIT, or VISTA [96–99]. Some of these checkpoints are targets for clinical trials. The host gut microbiome is important for general priming of the immune system to discern between own cells and infectious agents by influencing the microenvironment [100,101]. Commensal bacterial flora is also associated with the effect of ICBs targeting the PD-1 axis [102–106]. Use of antibiotics during ICB treatment has been linked to reduced clinical benefit [107].

ICB treatment may also in some patients cause an accelerated proliferation of tumor cells termed *hyperprogression* [108–110]. The rapid clinical deterioration within two months seems linked to amplifications of MDM2 and MDM4 and aberrations in the EGFR genes [111].

Toxicity

Immunotherapy by ICBs is well tolerated by most patients, with treatment-related grade 3–4 adverse events in 16% after nivolumab and 27% after ipilimumab, but combination of these cause serious side effects in 55% [112]. However, side effects like endocrinological adverse effects (hypophysitis, thyroiditis), cutaneous side effects, gastrointestinal side effects (colitis), cardiac and pulmonary toxicities are largely manageable [113–115]. Autoimmunity may therefore limit the use of ICB treatment in some patients [116]. The effect of antibodies blocking CTLA-4 on CD4 regulatory T-cells may explain the higher rate of autoimmune toxicity related to this class of ICBs [44]. Fortunately, the response rate and duration of response remained high in malignant melanoma patients who had to stop combined treatment with nivolumab and ipilimumab due to side effects [117].

Prospects

It is encouraging to see clinical responses in tumors generally not expected to respond or have a low response rate to available therapy. However, one must acknowledge that many patients does not respond to ICB monotherapy as the objective response rates are generally 20–30%, with substantial higher rates for malignant melanoma and Hodgkin's

lymphoma [118]. Whereas most responses are short lived, the combination of antibodies targeting CTLA-4 and PD-1/PD-L1/PD-L2 seems to yield more durable responses, but the side effects also increase.

Combinations of ICB therapy with targeted drugs [119–121], chemotherapy [122,123] and radiation [124,125] are currently in clinical trials. It is important that CD8 T-cells may attack cancer cells in any site in the body, thus being a systemic anticancer agent. Experience indicates that most anticancer drugs as well as immunotherapy are most effective when the cancer cell burden is limited.

As mentioned above, ICBs are effective as adjuvant therapy after surgery for melanoma patients [58,60,126]. Both experimental studies [127] and recent clinical experience in resectable lung cancer [128], malignant melanoma [129,130], urothelial bladder cancer [131], and MSI high colon cancer [132] show that ICB can totally eradicate a tumor after one to three courses when administered as neoadjuvant therapy before surgery. More than 60 clinical trials are ongoing using neoadjuvant ICB therapy [133].

Immune checkpoint inhibition is now a part of our standard armamentarium to control malignant diseases. As there are many more signal molecules steering the activity of the immune responding cells, of which several are under active investigation, the role of this class of drugs are promising. The expectation of this novel group of anticancer agents is high, but ICBs final role will have to be documented in clinical trials. A major limitation for general use in palliative settings is the high cost of ICBs, especially when combination of several substances is used. However, if the promising neoadjuvant data can be reproduced, a substantially reduced cost will follow opening for a wider use of these drugs.

Disclosure statement

No potential conflict of interest was reported by the authors.

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