Received: 3 July 2019 | Revised: 31 July 2019 | Accepted: 26 August 2019

DOI: 10.1111/sji.12821

DISCUSSION FORUM

Scandinavian Journal of Immunology WILEY

Exploiting autoimmunity unleashed by low-dose immune checkpoint blockade to treat advanced cancer

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Abstract

As a result of the cancer immunotherapy revolution, more than 2000 immuno-oncology agents are currently being tested or are in use to improve responses. Not unexpectedly, the 2018 Nobel Prize in Physiology or Medicine was awarded to James P. Allison and Tasuku Honjo for their development of cancer therapy by the blockade of co-inhibitory signals. Unfortunately, manipulation of the co-inhibitory receptors has also resulted in a safety issue: widespread iatrogenic immune-related adverse events (irAEs). Autoimmunity is emerging as the nemesis of immunotherapy. Originally, it was assumed that CTLA-4 blockade selectively targets T cells relevant to the antitumour immune response. However, an uncontrolled pan T cell activation was induced compromising tolerance to healthy self-tissues. The irAEs are very similar to that of a chronic graft-versus-host-disease (GVHD) reaction following *allogeneic* bone marrow transplantation (BMT). We hypothesized that ipilimumab induced a graft-versus-malignancy (GVM) effect, which eradicated metastatic melanoma in a minority of patients, but also involved an auto-GVHD reaction that resulted in widespread autoimmunity in the majority. Therefore, we argued for a profound theoretical point against the consensus of experts. The task is not to desperately put the genie back in the bottle by immune-suppressive treatments, but instead to harness the autoimmune forces. In this way, the same goal could be achieved by an antibody as by the adoptive transfer of alloreactive donor lymphocytes, but without severe GVHD. The proof-of-principle of a low-dose-combination immune checkpoint therapy, consisting only of approved drugs and treatments, was demonstrated in 111 stage IV cancer patients.

1 | INTRODUCTION

The immune system is continuously in a state of delicate balance between tolerating normal tissues (self) and attacking foreign substances (non-self). If this balance is perturbed, autoimmune reactions occur. To avoid collateral damage of normal tissues,[1] natural inhibitory feedback loops, generated by co-inhibitory signals, and also referred to as immune checkpoints, reduce inflammation following immune activation.[2]

For a century, it was believed that the immune response can destroy anything containing a foreign substance, such as pathogens or altered host cells (eg cancer). Following the success of vaccines against xenogeneic infectious diseases, the tacit assumption was that host immunity would also be

To the memory of Melvin Cohn, founding fellow and professor emeritus of the Salk Institute for Biological Studies

protective against isogeneic cancer.[3] Cancer immunotherapy trials, however, conducted with the best available science, resulted in only anecdotal responses. As a result, the field of cancer immunotherapy did not fulfil the great hopes of conquering cancer and began to lose credibility.[4]

Studies initiated by J. P. Allison led to a clinical breakthrough via the translation of immune checkpoint inhibitors (ICIs).[4,5] A special issue of Science, *The Cancer Immunotherapy Revolution*, reviewed the newly approved immunotherapies that can manipulate components of the immune system to attack tumours.[6] More than 2000 immuno-oncology agents are currently being tested or are in use to improve responses,[7] and the success stories of terminal cancer patients defying the odds and achieving complete remissions are accumulating.

Unfortunately, this manipulation of the immune system has also resulted in a major safety issue: iatrogenic immune-related adverse events (irAEs). Even the New York Times (NYT) has reported that the immune system unleashed by checkpoint blockades can attack healthy, vital organs. (https://www.nytimes.com/2016/12/03/health/immunotherapy-cancer.html?rref=collection%2Fsectioncollection%2Fhealth&action=click&contentCollection=health&region=rank&module=package&version=highlights&contentPlacement=1&pgtype=sectionfront [last accessed: June 29, 2019]). According to Prof William Murphy (University of California) not enough research has been performed that investigated the risks of the new therapies, which is a massively understudied area. The critical NYT article was quoted by the New England Journal of Medicine (NEJM) Journal Watch (http://www.jwatch.org/fw112314/2016/12/05/cancer-immunotherapies-may-send-immune-system-overdrive?query=etoc_jwonchem&jwd=000020025654&jspc=AI [last accessed: June 29, 2019]).

Highlights

- Checkpoint inhibitors achieved regression of cancer in a minority of patients. However, the majority suffered immune-related adverse events;
- Cancer regression can only be achieved by breaking down the physiologic immune tolerance; autoimmunity is, therefore, emerging as the nemesis of immunotherapy;
- To resolve this very serious safety issue, a therapeutic paradigm shift is required;
- Autoimmune T cells can be harnessed for a graft-versus-tumour (GVT) reaction by an off-label low-dose combined checkpoint blockade, which is complemented with interleukin 2 (IL-2) stimulation and hyperthermia;
- The proof-of-principle was demonstrated in 111 stage IV cancer patients with an overall response rate of 48%, which was associated with irAEs of WHO grade III and IV only in 7% and 2% of patients, respectively.

2 | BLOCKING NEGATIVE FEEDBACK SIGNALS (FROM CO-INHIBITORS) FOR TUMOUR IMMUNOTHERAPY WAS FIRST PROPOSED IN 1971

While popular in the clinical lexicon, regarding scientific understanding, the term 'immune checkpoint' appears to confuse more than it illuminates. It is a vague term, referring broadly to many inhibitory or stimulatory signalling pathways in lymphocytes. The immune checkpoints that have been successfully targeted in cancer have all been well defined, co-inhibitory receptors or their ligands. It is also surprising that the current leaders of co-inhibitory blockade (the mechanism of the new immunotherapy) appear universally unaware of the scientific origin of the concept.

The discovery of co-inhibition is typically not mentioned (for example reference[8]) or has erroneously been described as originating in the early 1990s, with inhibitory receptors in natural killer (NK) cells[9] or with identification of cytotoxic T lymphocyte antigen-4 (CTLA-4) function in T cells.[2,10] However, the first co-inhibitory receptor that was discovered and characterized was FcγRIIb in B cells.[11-14] Although blocking the FcγRIIb receptor is beneficial for some anti-cancer antibody therapies,[15] it is not targeted in current checkpoint blockade approaches, as promoting B cell responses is thought to be rarely effective in cancer. Nevertheless, the concept of co-inhibitory checkpoints originated with these studies and theories about the regulation of B cell responses. A currently popular concept, proposed 23 years ago,[16] is that peripheral tolerance or immunity is determined by the balance of multiple co-stimulatory vs. co-inhibitory signals, or by their temporally distinct expression pattern. The 'co' in both co-stimulation and co-inhibition is the key to the concept that these receptors do not act on their own, but instead they act only when co-engaged with activating receptors such as the T cell receptor (TCR) and B cell receptor (BCR). This limits activation or inhibition of cells that have recently engaged or are currently engaging antigen (ie cells that have recently received an antigen receptor signal). This concept was an extension of earlier proposals that both T and B cell responses are controlled by feedback inhibitory signals.[17-19]

In a 1971 paper remarkably ahead of its time, Sinclair and Chan—based on their data—predicted that B cells have IgG Fc receptors that are inhibitory when co-engaged with

BCR and that blocking such negative feedback signals (from co-inhibitors) would be a beneficial approach in tumour immunotherapy.[11] The concept that peripheral tolerance arises from co-inhibitory signals, rather than signals from the antigen receptor alone (signal 1), was developed by Sinclair based on his unconventional view that the antigen receptor (TCR, BCR) signal alone is activating, not tolerogenic. Tolerance arising from signal 1 *versus* co-inhibition has been debated extensively at the conceptual level.[18,20-22] The two main competing viewpoints are as follows: (a) The proposal that co-inhibitors are key to tolerance during chronic antigen exposure [16,17,21] and (b) The view that the raison d'être of co-inhibitors is not about tolerance but instead about the control of the magnitude and class of the immune response.[20,22] There is now extensive data supporting a role for co-inhibition in tolerance during chronic antigen exposure (a state frequently referred to as exhaustion).[9,23] However, experimentally, it remains unclear whether all cases of peripheral tolerance involve a co-inhibitory signal or if signal 1 alone can be tolerogenic.

3 | CTLA-4 WAS THE FIRST CO-INHIBITOR SHOWN TO BE RELEVANT TO CANCER THERAPY

While the ICIs interrupted T cell pathways responsible for immune down-regulation and mediated regression of established malignant tumours in a minority of patients, the majority suffered irAEs.

In fact, the seminal phase III trial of Hodi et al,[24] which reported improved survival with ipilimumab in patients with metastatic melanoma with a response rate of 10.9% in 676 patients administered ipilimumab, obscured the findings that the complete response rate was only 0.2% with 1 patient out of 403 who received ipilimumab plus a peptide vaccine. Meanwhile, nearly all the patients suffered drug-related toxicity (88.9%). With the benefit of hindsight, Weber correctly predicted that tumour eradication will be associated with tolerance breakdown. 'Abrogation of the function of CTLA-4 would permit CD28 to function unopposed and might swing the balance in favor of immune stimulation, tolerance breakdown and tumor eradication…'.[25]

Bakacs et al critically re-considered the very same published evidence and suggested an alternative interpretation of the widespread irAEs. They did this by comparing the outcomes of the ipilimumab trials and the phase I clinical trial of a humanized 'superagonist', anti-CD28 monoclonal antibody (mAb) (TGN1412), which ended in a catastrophe.[26] It was proposed that the underlying basic mechanism of action of agonistic (anti-CD28) and inhibitory (anti-CTLA-4) immune modulatory therapies are similar such that they cannot be restricted to the targeted T cell population.[27] Anti-CTLA-4 antibodies can block the CTLA-4 receptors not only on tumour-specific T cells, but also on all activated T cells.

4 | ANTI-CTLA-4 ANTIBODY BLOCKADE INDUCES AN UNCONTROLLED T CELL ACTIVATION

We would suggest that the widespread, dose-dependent irAEs of ipilimumab can best be explained by the view that all T cells possess self-reactivity.[27-31] The 'tonic' signal 1 (TCR signal), generated by positively selecting self-peptide/major histocompatibility complex (MHC), promotes activation and homeostatic survival of T cells in the periphery. Furthermore, there is evidence for control of such tonic TCR signals by co-inhibitors.[32] As postulated by Grossman and Paul, tonic signals are a form of chronic signalling that may tune T cells, establishing the threshold for the level of signals needed for activation broadly across the T cell repertoire.[33,34] This is consistent with a critical role for co-inhibitors to establish tolerance in the first T cells—early in life—that seed the periphery.[32,35,36] In addition, the ability of TCRs to interact with tonic self-peptide/MHC ligands opens the possibility that a co-inhibitor blockade causes T cell effector activity to spill over onto nearby healthy cells or tumour cells that have down-regulated tumour antigens. Increased collateral damage is indeed seen during immune responses where a co-inhibitor is lacking.[1] Altogether, the above concepts suggest that all T cells are temporarily activated, expressing co-inhibitors such as CTLA-4 that can then be targeted by anti-CTLA-4 antibodies. This is consistent with aspects of the *immunological homunculus* concept of Irun Cohen, who suggested that the immune system continuously responds to self-molecules.[37-40] In this way, the anti-CTLA-4 mAb blockade induces an uncontrolled T cell activation. It was predicted that the long-lasting objective of cancer regression will be achieved only by paying a price—tolerance to healthy self-tissues will be compromised.

This prediction has been confirmed in many thousands of patients. By now, *Science* has also acknowledged that these patients are 'human experiments' of the autoimmune process.[41] Notwithstanding, we could not find a paper (other than our own) that deduced the widespread irAEs based on the similar outcomes of the TGN1412 and ipilimumab trials, despite the fact that the total number of papers citing ipilimumab has increased over tenfold, from 229 (as of 2011) to 2955 (PubMed search, as of June 2019, using the keywords <ipilimumab>and <TGN1412>).

5 | IN THE FACE OF WIDESPREAD AUTOIMMUNE TOXICITIES, INSISTING THAT THE CTLA-4 BLOCKADE IS TUMOUR SPECIFIC IS IGNORING THE OBVIOUS

As a result of the impaired self-tolerance, irAEs may present with a broad clinical spectrum that mainly involves the gut, skin, endocrine glands, liver and lung, but can potentially affect any tissue; their incidence may reach up to 96% of patients.[42-44] Tawbi et al recently presented an example of this problem, which demonstrated that nivolumab combined with ipilimumab had clinically meaningful intracranial efficacy in patients with melanoma who had untreated brain metastases.[44] However, treatment-related adverse events were reported in 96% of patients, while grade 3 or 4 adverse events occurred in 55% of patients, including events involving the central nervous system, which occurred in 7% of patients. One patient died from immune-related myocarditis. Not unexpectedly, the Nobel committee emphasized that a crucial aspect in the future development of checkpoint inhibitor therapies is to improve the understanding of events leading to adverse events (http://www.nobelprizemedicine.org/ [last accessed: June 29, 2019]) The Puzanov et al meta-analysis reported an overall incidence rate of <75% with anti-CTLA-4 monotherapy (ipilimumab) and ≤30% in Phase 3 trials of anti-PD-1/PD-L1 agents. Up to 43% of patients given ipilimumab and ≤20% of patients given blockers of PD-1/PD-L1 experienced irAEs of ≥grade 3 severity. The incidence of irAEs with ipilimumab and pembrolizumab was shown to be dose-dependent, with greater toxicity at higher dose levels; toxicity also varies between the adjuvant and metastatic disease settings. Death due to irAEs occurred in up to 2% of patients (see in[42]).

The distinct mechanisms of action of anti-CTLA-4 and anti-PD-1/anti-PD-L1 antibodies have led to trials examining combination therapies in a variety of malignancies. Unfortunately, with increased efficacy, the incidence of severe adverse events also increased. The combination of ipilimumab with nivolumab is associated with a high rate (55%) of grade 3/4 adverse events, leading to discontinuation in a third of those treated. Symptoms from ICIs may present as serious and life-threatening events that require timely patient management and adequate therapeutic decisions.[45-50] The Oncology Nursing Society has an immunotherapy wallet card available for patients and providers, which warns that patients have a risk of irAEs that lasts a life-time.[43]

It is therefore very concerning that several centres experienced difficulties in patient compliance with reporting adverse events. Patients often deny their symptoms when they fear their treatment will be stopped due to irAEs.[51]

In the face of widespread autoimmune toxicities, insisting that the CTLA-4 blockade is tumour specific is ignoring the obvious.[52] Clinical remission (partial or complete), or at least cancer stabilization, was noted for 60% of patients who experienced an irAE. Furthermore, a strong correlation was observed between the induction of tumour regression and grade 3/4 autoimmune toxicity, corroborating the idea of coupling autoimmunity and tumour immunity.[53]

Consistent with the CTLA-4 results, a new study revealed an association of irAEs with the efficacy of PD-1 inhibitors in non-small-cell lung cancer (NSCLC), with landmark analysis (which minimized lead time bias potentially associated with time-dependent factors such as irAEs) and multivariable analysis.[54] This finding can be interpreted to mean that the anti-PD-1 antibodies not only modulated the activity of trained killer T lymphocytes that have migrated into tumours,[2] but they also had a systemic effect on T lymphocyte immunity.

6 | IATROGENIC CTLA-4 BLOCKADE TURNS PHYSIOLOGIC AUTOIMMUNITY INTO A PAN-LYMPHOCYTIC ACTIVATION

It was originally assumed that most CTLA-4 expressing T cells are either antitumour effector cells or regulatory T cells inhibiting antitumour response.[52] Consequently, it was believed that CTLA-4 blockade selectively targets T cells relevant to the antitumour immune response. Unfortunately, this assumption cannot be reconciled with the widespread irAEs observed in the vast majority of patients. In support of the viewpoint that CTLA-4 blockade is tumour specific, proponents cite the lack of autoimmunity in mice treated with antagonists of CTLA-4,[52] arguing that releasing the brakes when almost all the T cells are 'in park' cannot promote autoimmunity. This fundamentally misunderstands differences in the activation status of the immune system between mice and humans. Humans have a much more broadly activated state (many fewer cells 'in park') than the 'clean' mice used in laboratory studies.[55] The human experiments that have been performed with ICIs have clearly proven this point.

Since immune cells require regular stimulation for survival,[56] Bakacs et al proposed that self-antigens, from time to time, activate T cells through an internal dialogue via a one-signal mechanism.[28] Temporarily activated T cells express CTLA-4, which is blocked by anti-CTLA-4 antibodies, not only on tumour-specific T cells, but also on all activated T cells.[57] As predicted by Weber,[25] abrogation CTLA-4 function results in immune stimulation, tolerance breakdown and eventually tumour eradication.

Bakacs et al argued, therefore, for a profound theoretical point against the consensus of experts. Since the anti-CTLA-4

immune checkpoint blockade cannot be restricted to the targeted tumour-specific T cell population, such that this blockade induces an uncontrolled pan T cell activation, tolerance to healthy self-tissues will be compromised. They therefore hypothesized that the anti-CTLA-4 therapy may have mechanisms similar to that occurring in inherited human *CTLA4* haplo-insufficiency.[31]

This theoretical proposition is consistent with the finding that inherited human *CTLA4* haploinsufficiency plays a critical quantitative role for CTLA-4 in governing T and B lymphocyte homeostasis.[58] This proposition has since been validated by two new genetic diseases of CTLA-4 checkpoint insufficiency. In 2014, heterozygous, deleterious mutations in the *CTLA4* gene were discovered to be the cause of a dominantly inherited immune dysregulation disorder characterized by lymphocytic infiltration of multiple non-lymphoid organs and termed 'CTLA4 Haploinsufficiency with Autoimmune Infiltration' (CHAI). Another disorder caused by deleterious biallelic mutations in the *LRBA* gene has been termed, 'LRBA deficiency with Autoantibodies, Treg defects, Autoimmune Infiltration, and Enteropathy' (LATAIE), emphasizing the predominant disease features. Overall, the LATAIE resembles CHAI but presents, more often, with an earlier age of onset and a significantly greater disease penetrance.[59,60]

Garcia-Perez et al compared the following three different patient groups with disturbances in the CTLA-4 pathway: CTLA4-haploinsufficiency, LRBA-deficiency and ipilimumab-treated melanoma patients.[61] In these groups, the authors found an inverse correlation between the CTLA4 mRNA expression and degree of CTLA-4 pathway disruption. In fact, CTLA-4 mRNA levels from melanoma patients under therapeutic CTLA-4 blockade (ipilimumab) were increased compared to patients with either CTLA4 or LRBA mutations, which were clinically stable with abatacept treatment. This finding provides experimental confirmation of our theory predicting that anti-CTLA-4 therapy has mechanisms similar to those occurring in inherited human CTLA4 haploinsufficiency.

7 | CLINICAL PHENOTYPES OF *CTLA4* GENE INSUFFICIENCY ARE EERILY SIMILAR TO THAT OF THE CTLA-4 RECEPTOR BLOCKADE

The severe clinical phenotypes of CHAI and LATAIE patients underscore the importance of the negative regulatory molecules in preventing autoimmunity, lymphoproliferation and unnecessary tissue damage in humans. Patients with CHAI and LATAIE present with autoantibody-mediated cytopenias, lymphadenopathy/splenomegaly, hypogammaglobulinemia, organ-specific autoimmunity and lymphocytic infiltration of non-lymphoid organs. Despite varying clinical manifestations, most CHAI and LATAIE patients have lymphocytic overactivation and infiltration of at least one non-lymphoid organ, usually the intestine, lungs or brain. Intestinal involvement that causes enteropathy is most common for both diseases. The lungs are the second most frequently infiltrated organ, while infiltrates in the brain are less common (see Figure 1 in reference[60]).

It is enlightening to compare the symptoms of the *CTLA4* gene insufficiency to that of the iatrogenic CTLA-4 blockade (Table 1). Screening 752 patient files among 19 skin cancer centres, Voskens et al summarized rare and difficult-to-treat ipilimumab-induced side effects.[62] As CTLA-4 is inducible on virtually all T cells, ipilimumab-induced irAEs can virtually affect any organ system or tissues. A total of 88 rare irAEs were observed in 82 patients, affecting the skin, endocrine system, nervous system, liver, respiratory tract, gastrointestinal tract, pancreas, sinuses, renal system, musculoskeletal system, heart, eyes and upper extremities. In addition, a systemic grade IV anaphylactic reaction and a fatal case of tumour mass liquefaction were also reported.[62]

Thus, the theory that anti-CTLA-4 therapy has a similar mechanism to that occurring in inherited human *CTLA4* haploinsufficiency [31] gains credibility due to the overlapping

TABLE 1 Immune-related adverse events (irAEs) of checkpoint inhibitors and clinical phenotypes of *CTLA4* gene insufficiency are very similar

irAEs of checkpoint inhibitors	CHAI and LATAIE diseases
Guillain-Barre syndrome Myasthenia gravis Encephalitis	Brain infiltrates Uveitis
Hypothyroid Hyperthyroid	Autoimmune thyroiditis
Pneumonitis Myocarditis	Lung infiltrates Respiratory infections
Colitis Autoimmune hepatitis	Gut infiltrates Hepatitis Splenomegaly
Hypophysitis Adrenal insufficiency Type I Diabetes	Type I Diabetes
DRESS syndrome	Lymphadenopathy Autoimmune thrombocytopenia Autoimmune anemia Hypogammaglobulinemia Neutropenia
Vasculitis Arthritis	Autoimmune arthritis
Vitiligo Psoriasis Steven-Johnson syndrome	Vitiligo Psoriasis Other skin diseases

clinical phenotypes of the genetic and iatrogenic CTLA-4 insufficiencies. Since the CTLA-4 receptor blockade cannot achieve long-lasting cancer regressions without breaking the physiologic tolerance to healthy self-tissues, a therapeutic paradigm shift is required.

June et al asked whether autoimmunity is the Achilles' heel of cancer immunotherapy.[63] They argued that the true incidence of autoimmune complications is probably underestimated following cancer immunotherapy because most cancer trials follow patients for only a brief time after enrolment and because patients who died from their cancer are not included. Additionally, June et al predicted that the incidence of immunotoxicity will likely continue to rise as these therapies become more widely used. Therefore, they warned that cancer immunotherapy is 'a double-edged sword in which patients and clinicians must weigh the risk of immunotoxicity against the benefit of tumour destruction'.

8 | RATIONALE FOR AN OFF-LABEL, LOW-DOSE ICI THERAPY FOR ADVANCED CANCER

According to the *quantitative paradigm*, T cell activation (not just response magnitude) is the outcome of signals from the TCR, co-stimulatory/co-inhibitory receptors and cytokines added together.[64,65] The rationale for the off-label, low-dose ICI (OL-LD-ICI) combination protocol, and of course the reason for its efficacy, is that the individual (sub-threshold) effect of its components add up such that in combination they are able to achieve the magnitude of T cell stimulation required for tumour eradication.

Based on the quantitative T cell activation paradigm, the OL-LD-ICI therapy administers the lowest doses of ICIs (0.3 mg/kg ipilimumab and 0.5 mg/kg nivolumab), where no patient had an *antitumour* response, but they had Grade 2 or 3 irAEs. (For example, see Table S1 in the Appendix in the paper of Brahmer et al[66] https://ascopubs.org/doi/full/10.1200/JCO.2009.26.7609). This is consistent with the observation that PD-1 occupancy was comparable at 0.3 mg/kg and 10.0 mg/kg (see Figure 4 in reference[66] https://ascopubs.org/doi/full/10.1200/JCO.2009.26.7609). A similar observation was made with ipilimumab by Wolchok et al.[67] These data were interpreted to mean that the appearance of mild to moderate immune-related events suggests a *biological* effect of ICIs, which is the breakdown of physiological immune tolerance.

Based on such observations, Bakacs et al[27] proposed an alternative interpretation of the seminal NEJM paper by Hodi et al.[24] They pointed out that the ipilimumab treatment-induced irAEs by the patients' own lymphocytes were very similar to that of a chronic graft-versus-host-disease (GVHD) reaction following *allogeneic* bone marrow transplantation (BMT). Earlier, auto-graft-versus-host-disease (auto-GVHD) has been reported as an attractive immunotherapeutic strategy to reduce minimal residual disease (MRD) following tumour and immune system cytoreduction.[68] Auto-GVHD occurs either spontaneously or in patients receiving post-transplant immune modulation with cyclosporine A (CsA), IFN-gamma or the combination. According to Kline et al, the development of auto-GVHD depends upon the derangement of self-tolerance. The syndrome appears to be mediated by self-reactive CD8(+) T cells recognizing a self-peptide antigen presented by MHC class II molecules.

Bakacs et al have, therefore, speculated that ipilimumab induced a graft-versus-malignancy (GVM) effect, which eradicated metastatic melanoma in a minority of patients, but also involved an auto-GVHD reaction that resulted in widespread autoimmunity in the majority. The study by Bashey et al [69] supported such speculation, as this study demonstrated that following allogeneic hematopoietic stem cell transplantation (allo-HSCT), ipilimumab increased the GVM effect without exacerbating GVHD. Slavin et al were the first who proposed that a finely tuned, low-dose (0.3 mg/kg) ipilimumab treatment course would induce a prolonged auto-GVHD that would improve the antitumour efficacy of the patients' own lymphocytes.[30] In this way, the same goal could be achieved by an antibody (ipilimumab) as by the adoptive transfer of alloreactive donor lymphocytes, but of course, without severe GVHD.

Our studies in mouse models highlighted the potential risks of combining lymphoablative therapy and HSCT with ICIs.[70] By now, the GVM effect of ICIs has been confirmed by several studies in the allogeneic setting (see in reference[71]). One hundred seven patients received ICIs before allo-HSCT and 176 patients received ICIs after allo-HSCT. The use of ICIs both before and after allo-HSCT was highly effective, but exposure could lead to a significantly increased risk of GVHD related morbidity and mortality in this patient population.

Samstein et al recently found that checkpoint inhibitors were more likely to halt tumour growth in patients with a higher number of mutations than in those with fewer mutations.[72] The authors proposed that the tumour neoantigens generated an immune reaction. In our view, however, this is a transplantation reaction against new antigens which transformed the syngeneic host cells into *semi*-allogeneic ones never seen by the host immune cells. While a limited allogeneic transformation is too weak in itself to provoke an effective T cell attack, the immune checkpoint blockade unleashes T cells against semi-allogeneic tissue,[73] and against tumours resulting in better overall survival (OS). The data of Samstein et al can be interpreted as being in line with our hypothesis that ICI drugs induce an auto-GVHD reaction.

The critical impact of systemic immune responses driving tumour rejection was demonstrated by Spitzer et al in *Cell*.[74]

In fact, the authors stated that the development of new immunotherapies must consider the benefit of systemic immunity. Consistent with this, Poggio et al recently demonstrated that exosomal PD-L1 systemically acts to suppress the antitumour T cell response in draining lymph nodes.[75] While inhibition of exosomal PD-L1 can lead to a long-lasting, systemic antitumour immunity. These findings support our paper calling for a therapeutic paradigm shift in order to exploit systemic autoimmunity to treat advanced cancer.

Clearly, an ICI blockade disrupts the balance between co-stimulatory and co-inhibitory signals, which results in tolerance breakdown. In the context of vast clinical experience with ICI therapy, the immune theory of Sinclair and Anderson, suggesting first that persisting antigens do not generate tolerance by exhaustion but rather a balance between co-stimulatory and co-inhibitory signals, gains more significance.[16]

The problem is that an adjuvant ICI drug alone, when administered at a high dose, created a severe safety issue. A Phase 3 trial compared a high-dose ipilimumab (10 mg/kg; 33.3 times higher dose than that of suggested by Slavin) to placebo in patients who had undergone complete resection of stage III melanoma.[76] In the ipilimumab group, the 5-year recurrence-free survival rate was 41% but only 30% in the placebo group. Also in the ipilimumab group, the 5-year OS rate was 65% compared to 54% in the placebo group. The incidence of a grade 3 or 4 irAE in the ipilimumab group was 41.6%, while only in 2.7% in the placebo group. Importantly, 5 patients (1.1%) died due to irAEs in the ipilimumab group. Notwithstanding, the high-dose (10 mg/kg) adjuvant ipilimumab subsequently gained US Food and Drug Administration (FDA) approval.

9 | PROOF-OF-PRINCIPLE OF LOW-DOSE ICI THERAPY HAS BEEN DEMONSTRATED IN 111 SINGLE CASE STAGE IV CANCER PATIENTS

Fusi and Dalgleish emphasized that there must be a place for back-to-basic, simpler approaches, which just might prime the tumour environment to respond better to checkpoint inhibitors, increase the therapeutic index and convert non-responders into responders.[77] In fact, spectacular therapeutic improvement in childhood cancer was achieved through 'a reassessment of the tools in hand', applying them as combination therapies.[78] A combination therapy consisting of basic low-tech approaches and immune checkpoint drugs in off-label low doses seems to be safer and more efficient in advanced cancer patients than the currently used approved protocols.

We have predicted that ICIs cannot be restricted to the targeted antitumour T cell population because the anti-CTLA-4 mAb blockade induces an uncontrolled T cell activation.[27,29,31,57,79] This prediction has been confirmed in many thousands of patients. By now *Science* has also acknowledged that the patients treated by ICI drugs are 'human experiments' of the autoimmune process.[41] Therefore, in the face of an ipilimumab-induced pan-lymphocytic activation, a therapeutic paradigm shift is required. The task is not desperately trying to put the genie back in the bottle by immune suppressive treatments, but instead harnessing the autoimmune forces for therapeutic purposes.

Our hypothesis was that an off-label low-dose combined anti-CTLA-4 and anti-PD-1 antibody blockade, complemented with hyperthermia and IL-2 treatment, transforms non-responding patients into responding ones.

The low-dose ICI idea was adopted by Kleef et al for stage IV cancer patients. The proof-of-principle was first demonstrated in a heavily pre-treated triple negative breast cancer (TNBC) patient, with far advanced pulmonary metastases and severe shortness of breath, who had exhausted all conventional treatment.[80,81] The patient was treated with a safe, off-label low-dose immune checkpoint blockade, including ipilimumab (0.3 mg/kg) combined with nivolumab (0.5 mg/kg). This was complemented with a moderate-dose IL-2 treatment under taurolidine protection and loco regional- and whole-body hyperthermia, without classical chemotherapy. The patient went into complete remission of her lung metastases and all cancer related symptoms vanished with transient WHO I-II diarrhoea and skin rash. A total gene expression analysis of a metastatic axillary lymph node demonstrated that several checkpoint genes were over-expressed even one year after the initiation of therapy. The patient remained alive for 27 months after the start of treatment, with recurrence of metastases as a sternal mass, and up to 3 cm pleural metastases, which finally classified this patient having a mixed overall response.

Evidently, this TNBC patient with such far advanced lung metastasis had an extremely limited expected survival. Therefore, her response to the low-dose immune checkpoint blockade was exceptional.

'Rare cancer successes should instigate "exceptional" research efforts'.[82] In many clinical trials there were rare patients whose advanced cancer vanished for many months or even for years. The previous US National Cancer Institute director and Nobel laureate, Harold Varmus, stated that we can really learn from such 'exceptional responders' since they may explain why in certain patients a drug sometimes induces unexpected dramatic improvement, which then could be beneficial for more people.

Since the treatment of the first TNBC patient, 111 stage IV cancer patients, with a variety of cancer types, who were treated with the off-label low-dose immune checkpoint blockade, have been evaluated. A retrospective analysis of single cases was presented at the 8th-annual Oncology

Association of Naturopathic Physicians in San Diego, CA, 2019 (https://oncanp.org/event/8th-annual-oncanp-conference/ [last accessed: June 29, 2019]). Staging with iRECIST in stage IV cancer patients (n = 111), the overall response (OR) rate was 48% with an objective response (ORR) of 33% (58 patients had progressive disease, 16 patients had stable disease, while 20 patients achieved major partial remission, and complete remission occurred in 17 patients). The median follow-up period was 22 months (3-47 months). The safety of the low-dose ICI therapy is demonstrated by its excellent adverse events profile: irAEs of WHO grade I were observed in 21% of patients, grade II in 14%, grade III in 7%, while grade IV was only observed in 2% of patients. The safety profile of the low-dose ICI therapy should be compared, for example, to the safety profile of the study by Tawbi et al in which registered doses of ipilimumab (3 mg/kg) and nivolumab (3 mg/kg) were used. Treatment-related adverse events were reported in 96% of patients, while grade 3 or 4 adverse events occurred in 55% of patients, including events involving the central nervous system in 7% of patients, and one patient died from immune-related myocarditis.[44]

Recently, Sen et al, from the University of Texas MD Anderson Cancer Centre confirmed the rationale for our low-dose immune checkpoint blockade protocol.[83] They demonstrated that despite a dose-dependent increase in irAEs, no improvement in progression-free survival (PFS), OS, or disease control rate (DCR) were identified with escalating doses of ICIs. Sen at al. concluded that lower doses may reduce toxicity and cost without compromising disease control or survival. This is consistent with the low-dose protocol first implemented by Ralf Kleef.[80,81]

Furthermore, Sen et al established a 'prognostic scoring system' to help select patients.[84] The system is based on an analysis of 172 patients with advanced metastatic cancer who were enrolled in Phase I clinical trials: 105 of them received anti-CTLA-4 drugs, while the rest received anti-PD-1 agents. The authors found seven factors that predicted the best responding patients. A member of our group (RWM) rearranged these factors by their hazard ratios (HR) indicating the increased risk of death associated with each factor. In those who had zero, one or at most two risk factors, the median survival was over two years, and about half of that group was still alive three years after receiving immune therapy. After two years of starting treatment none of these patients had died.

One can speculate that with better patient selection and lower doses of ICI, this treatment could realistically fulfil the promise of Carl H. June, MD, the 'father' of CAR T cell therapy, that the present moment is only 'the tip of the iceberg' of effective immunotherapy of cancer.[63] In fact, Baik et al reported the limitations, challenges and opportunities in the immuno-oncology clinical trial design. They found that due to the rapidity of development, competition, and race for FDA approval, the optimal dosing and schedule of ICIs are still not fully defined and continue to be under study.[85]

Importantly, Kleef's protocol consists only of approved drugs and treatments. Therefore, our prediction that low-dose ICI-induced autoimmune T cells are powerful therapeutic tools can be confirmed or refuted in prospective, controlled clinical trials. In this context, it is useful to recall the statement of Dr Richard Klausner, former director of the US National Cancer Institute[86]—' As I go around the country, I talk about the tragedy of cancer to remind people that the tragedy is not our inability to prevent the inevitable or to do the impossible; tragedy is when a person, a group or a society fails to achieve the possible.' (text words: 5249).

10 | CONCLUSIONS

The risks of the ICIs are a massively understudied area. Following the discovery of two new genetic diseases of CTLA-4 checkpoint insufficiency, the pan T cell activation theory of anti-CTLA-4 therapy gains credibility. A therapeutic paradigm shift is required. Back-to-basic, simpler approaches (eg, regional hyperthermia[87]) will increase the therapeutic index of ICIs and convert non-responders into responders. Harnessing of the immense forces liberated by the ICI blockade by an off-label, low-dose ipilimumab and nivolumab therapy, supplemented with IL-2 treatment and hyperthermia, could induce a safe and effective GVM. The proof-of-principle of such an approach was demonstrated in 111 advanced metastatic cancer patients whose OR rate was 48% without dangerous autoimmune side effects. Since the low-dose ICI protocol consists only of approved drugs and treatments, our prediction that low-dose ICI-induced autoimmune T cells are powerful therapeutic tools can be confirmed or refuted in controlled clinical trials.

ACKNOWLEDGMENT

For the critical questions and comments which were very helpful in the writing of the manuscript, we would like to thank the late Professor Melvin Cohn, PhD, Salk Institute for Biological Studies 10010 N Torrey Pines Rd, La Jolla, CA 92037.

CONFLICT OF INTEREST

TB is a partner and CSO of PRET Therapeutics Ltd, developing the patented low-dose ICI combination therapy. All other authors have none to declare.

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How to cite this article: Bakacs T, Moss RW, Kleef R, Szasz AM, Anderson CC. Exploiting autoimmunity unleashed by low-dose immune checkpoint blockade to treat advanced cancer. *Scand J Immunol*. 2019;00:e12821. https://doi.org/10.1111/sji.12821