

## Review Article

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# Immune based therapy for melanoma

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**A few years ago therapeutic options in advanced melanoma were very limited and the prognosis was somber. Although recent progresses are far from providing a cure for advanced melanoma, yet these have kindled new hopes and searching for a cure does not seem unreasonable. Seven new medicines have been authorized in various regions of the world in the recent past in the therapy of advanced melanoma, over half of them acting by mechanisms involving the immune system of the host. The anti-CTLA-4 (cytotoxic T lymphocyte associated protein-4) ipilimumab has been followed by anti-PD1 (programmed death1) inhibitors, more effective and safer. Very recently, the first oncolytic immunotherapy, talimogene laherparepvec (T-VEC) has been authorized for placing on the market and a variety of combinations of the new therapies are currently being evaluated or considered. Besides, a plethora of other molecules and approaches, especially monoclonal antibodies, are in the preliminary phases of clinical investigation and are likely to bring new benefits for the treatment of this potentially fatal form of cancer.**

**Key words** Approved drugs - immune checkpoints - immune-therapy - melanoma - oncolytic immunotherapy

## Melanoma and its complex relationship with the host immune system

Melanoma is the most lethal form of skin cancer (rarer forms located elsewhere than skin are also possible), a tumour occurring by the malignant transformation of melanocytes<sup>1</sup>. The number of new cases has been increasing in recent decades<sup>2,3</sup> and an association with higher socio-economic status has been described<sup>3</sup>. Detected early, melanoma may be cured by surgical excision, but the advanced (metastatic) disease is commonly incurable, with 5-year overall survival (OS) under 10 per cent and a median survival under one year<sup>4</sup>. Until recently, the therapeutic options in stage IV melanoma were very limited, mostly centered

on dacarbazine used as monotherapy or in various combinations. Although initially dacarbazine was reported to induce response rates of up to 25 per cent, larger studies conducted later have indicated lower response rates (12% or less), median progression free survival (PFS) under six months and OS of less than 2 per cent at six years<sup>5</sup>. Interleukin-2 (IL-2), never tested in a properly designed pivotal clinical trial, at no time approved in Europe for melanoma treatment, was authorized by FDA (Food and Drug Administration) 18 years ago in the treatment of advanced melanoma<sup>5</sup>. Interleukin-2 had modest efficacy, at best an objective response rate (ORR) of 16 per cent in a retrospective analysis of clinical studies (which included no phase III trials), although some respondents had longer duration

of response but the treatment was accompanied by severe, although mostly reversible adverse effects<sup>5</sup>.

The immune system is physiologically equipped to detect cells with abnormal proliferation and thus destroy them during early neoplasia development<sup>6</sup>. Hence, immune surveillance is the complex pathway responsible for the surveillance and eradication of transformed self, but the theory of immunoediting, developed more than 10 years ago<sup>7</sup>, has gained, in the meantime, valuable experimental and clinical proofs. Immunity is involved in neoplastic cellular transformation but it can also preclude or restrict tumour growth and mould the immunogenicity of tumours. Although these functions seem both pro- and anti-tumoural, specific immune actions get involved in different stages. This is a dynamic progression; the immune system safeguards the host against tumour cell development but it also frames the features of emerging tumours. The process has three stages - Elimination, Equilibrium and Escape<sup>8</sup>.

In the Escape phase the defense cells fail to control the tumour and lead to a clinically visible tumour. Tumour cells lose their specific antigens, express anti-apoptotic molecule Bcl-2 and immunosuppression inducing molecules [indoleamine 2,3-dioxygenase (IDO), tryptophan 2,3-dioxygenase (TDO), programmed death-ligand 1 (PD-L1), galectin-1/3/9, CD39, CD73, adenosine receptors]. Tumour cells become active in secreting vascular endothelial growth factor (VEGF), transforming growth factor beta (TGF- $\beta$ ), interleukin-6 (IL-6), macrophage colony-stimulating factor (M-CSF) thus enhancing the angiogenesis. Myeloid-derived suppressor cells (MDSCs), M2 macrophages and dendritic cells (DCs) are converted to pro-tumoural action, express arginase, inducible nitric oxide synthase (iNOS) and IDO and secrete immunosuppressive regulatory molecules such as IL-10 and TGF- $\beta$ . The effector activity of cytotoxic lymphocytes is inhibited. MDSCs and DCs generate regulatory T cells (Treg) with high immunosuppressive action. T lymphocytes express inhibitory receptor proteins such as PD-1, CTLA-4, Tim-3 and lymphocyte activation gene (LAG-3) suppressing anti-tumour immune response. Immune suppressant cytokines and other signaling proteins like IL-10, TGF- $\beta$ , VEGF, IDO, PD-L1 sustain a pro-tumoural milieu<sup>9</sup> (Figure).

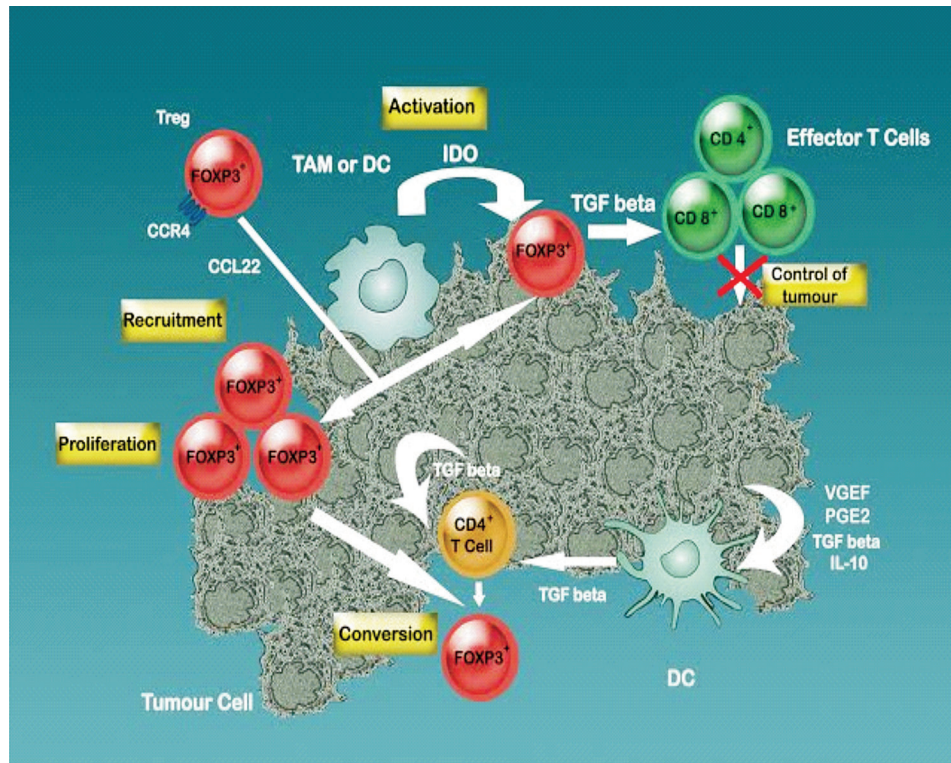
In cutaneous melanoma immune therapy, the three stages have been well studied and the panel of immune therapies includes immune checkpoints inhibitors, monoclonal antibodies, anti-tumour vaccines,

adoptive cell transfer therapies, and a variety of their associations<sup>10</sup>.

Melanoma is notorious for its complex relationship with the immune system, a relationship known for over 25 years. The first convincing proof of the participation of the immunological mechanisms in the response of the host organism against the autologous melanoma was reported in 1987 about the presence of a large number of tumour-infiltrating lymphocytes (TILs) in skin melanoma of a small number of patients, as well as their cytolytic activity against malignant melanocytes when activated with IL-2<sup>11,12</sup>. Numerous therapeutic options have been explored along time in an attempt to improve the immune response of the host against the melanoma cells, but they often resulted in unimpressive successes and even in impressive failures. Recently new hopes have been kindled, as seven new medicines are being or are authorized in various regions of the world in the treatment of late stage melanoma. At least four of these (ipilimumab, nivolumab, pembrolizumab and talimogene laherparepvec) act directly on the immune system, whereas the others interfere at least indirectly with it. The first three belong to the so-called class of "immune checkpoint inhibitors"<sup>13</sup>. Talimogene laherparepvec, an oncolytic virus expressing granulocyte macrophage (GM)-CSF has just been approved in October 2015 both in European Union and United States for the treatment of melanoma<sup>14,15</sup>.

### **Immune checkpoints and co-stimulatory molecules**

Immune checkpoints are molecules (receptors and their corresponding ligands) playing a central part in preserving peripheral immune tolerance, exerting a suppressing role on the immune system and thus preventing the occurrence of autoimmune disturbances. Negation of the negation, their blockade or inhibition is, therefore, equivalent to a boost for the immune system, a boost whose efficacy in controlling tumours has revolutionized cancer therapy in recent years, not only for melanoma, but also for other forms of cancer<sup>16</sup>. Data accumulated over time indicate that tumour cells exploit immune checkpoints as one of their major mechanisms of getting resistant to the immune system, especially to evade the action of T cells that should normally target tumour antigens<sup>17</sup>, and this may explain the efficacy of these products acting through an indirect mechanism (not directly on the tumour cells, but through an action oriented towards the immune system cells).



**Figure.** Immune-suppression mechanisms - therapy targets. Macrophages secrete indoleamine 2,3-dioxygenase (IDO) that induce an inhibition of T cell proliferation due to tryptophan depletion (activation). Moreover, IDO recruits regulatory T cells (FOXP3+) at the tumoural site. Recruiting more TGF beta-secreting Tregs the suppression induced on the effector couple CD4-CD8 increases and therefore, the control of tumour development decreases. Tumoural cells by themselves secrete TGFbeta, IL-10, VEGF, PGE2 that induce DCs to secrete more TGFbeta contributing to the conversion of CD4+ T cells to Tregs phenotype enhancing the cellular immune suppression (conversion). Skin-homing T cells CC-chemokine receptor 4 (CCR4) binds to the CCL22 (macrophage-derived chemokine) of the tumour associated macrophages (TAM) and are recruited to the tumoural site (recruitment). On the whole, an immune suppressive microenvironment is created by the concerted action of several elements, action that induces Tregs proliferation, further hindering CD4+-CD8+ cooperation and therefore, abolishing the effector activity anti-tumoural cytotoxic cells.

Source: Ref. 6; Reproduced with permission.

Over six immune checkpoints have been described. *TIM-3*, belonging to the TIM family (transmembrane immunoglobulin and mucin domain), regulates primarily the Th1 function, its overexpression being accompanied by poor prognosis in different forms of cancer<sup>18</sup>. *BTLA* (B- and T-lymphocyte attenuator, CD272) is a receptor of the CD28 family, that binds to HVEM (herpes virus entry mediator a TNFR family protein), co-inhibiting T-cells together with *CD160*, an immunoglobulin-like molecule<sup>19</sup>. *VISTA* (V-domain immunoglobulin suppressor of T-cell activation) is a programmed death-ligand 1 (PD-L1)-like ligand with a single IgV domain, expressed mostly on myeloid cells and able to suppress strongly T cells, being intensely expressed within the tumour microenvironment<sup>20</sup>. *KIR* (killer immunoglobulin receptor) is expressed on natural killer (NK) cells, involved in the inhibitory regulation of their cytotoxic functions and apparently

also co-opted by tumours to bypass the defense system of the host<sup>21</sup>. *LAG-3*, a type I transmembrane receptor inhibits T lymphocyte proliferation, activity and homeostasis<sup>18</sup>. The two most widely known immune checkpoints exploited for therapeutic purposes in melanoma are cytotoxic T-lymphocyte associated protein-4 (CTLA-4) and PD. CTLA-4 (CD152), the first one to be targeted for therapeutic purposes, is a transmembrane glycoprotein which interacts with its specific ligands CD80 (B7-1) and CD86 (B7-2) and downregulates the activity of T-lymphocytes through several ways, including competition for CD28 ligand binding, tryptophan depletion, production of regulatory cytokines or removal of ligands by a transendocytosis process<sup>22</sup>. The CTLA-4 negatively regulates T-lymphocyte activation, PD-1 (programmed death 1, an immune receptor of the same CTLA-4 family) signaling through a PD-L1 ligand regulates

mainly the effector function of T, B, and NK cells within tissues and tumours<sup>23</sup>. CTLA-4 is considered to intervene in the early immune response, while PD-1 is thought to intervene in the later immune response; the former acts largely in lymph nodes, while the latter occurs predominantly in peripheral tissues<sup>24</sup>.

On the other side, at least four activating co-stimulatory molecules, all belonging to the tumour necrosis factor receptor superfamily (TNFRS), are in the focus of anticancer research groups for their potential of improving the therapy over the current options. *CD40*, a first member of TNFRS, interacts with its specific ligand, CD40L, providing thus a co-stimulatory signal to antigen-presenting cells (APC), resulting in the end in enhanced cytotoxic responses of T cell against tumour cells<sup>25</sup>. *OX40* is a second member of the TNFRS with co-stimulatory effects on T lymphocytes, inducing stimulation of CD4+ and CD8+ T-cells and prolonging their survival, triggering inhibition of the T-regulatory cells subset, and seemingly inducing anti-tumour effects, although the intricacy of the immune signaling pathways related to its activation limits our current understanding of the effects of this ligand-receptor pair<sup>26</sup>. *CD137* (also known as 4-1BB and ILA - because it was initially described as a gene induced by lymphocyte activation) is a third component of the TNFRS, participating to the co-stimulation of T and NK cells, its activation being associated with improved anti-tumour responses in a melanoma non-clinical model<sup>27</sup>. *GITR* (glucocorticoid-induced TNFR-related protein) also belongs to the TNFRS and is expressed on activated T cells, NK cells, B cells, as well as on T regulatory cells; it has not yet been clarified to what extent its activation and consecutive anti-tumour effects are the result of its signaling on typical T lymphocytes or on the CD4+CD25+ and similar subpopulations (Tregs)<sup>28,29</sup>.

In the following two sections we discuss the medicines (monoclonal antibodies) interfering with the CTLA-4 and PD-1 checkpoints that managed to go through the 'Caudine Forks' of the full clinical development and regulatory scrutiny, being approved for melanoma treatment.

### CTLA-4 inhibitors

Generation of an anti-tumour immune response has a complexity that starts with the recognition of tumour antigen with the help of HLA proteins by T cells. The process is strengthened by further cross-talks between T cells and antigen presenting cells (APC). One of the

molecules that adds co-stimulatory events is CD28, an activating checkpoint expressed on T lymphocytes, that interacts with its specific ligands CD80 and CD86 on APC. This co-stimulatory binding leads to the stimulation of the T lymphocytes. CTLA-4 is also present on activated T lymphocytes. T regulatory cells play a critical role in the preservation of immune self-tolerance and equilibrium<sup>30</sup> and these cells harbour a series of immune checkpoints where CTLA-4 is one of the earliest molecular checkpoints that controls T cells response to antigen. CTLA-4 in physiological conditions prevents autoimmune reactions and influences self-tolerance<sup>31</sup>.

One of the first reports showing the clear therapeutical effect of an anti-CTLA-4 antibody in melanoma was presented in 2004<sup>32</sup>. Ribas *et al*<sup>32</sup> have shown that this approach can "break peripheral immunologic tolerance" and further develop an efficient anti-tumoural response. Since then, several positive clinical trials have led to ipilimumab being approved for the first time by FDA in 2011 (followed by other regions, including European Union) to treat late-stage melanoma and then in October 2015 its use extended in the adjuvant setting in the subpopulation with advanced melanoma, to diminish the recurrence hazard<sup>33</sup>. Tremelimumab targets the same receptor and was also initially developed for the the same indication, but after a failed phase III trial it was put on hold at least temporarily for this malignancy<sup>34</sup>; it was later shown that this failure might have been related to the access of a number of patients from the control arm to ipilimumab<sup>35</sup> (leading to misclassification bias) and currently there seems to be a renewed interest in its potential use in melanoma<sup>36</sup>.

In a phase 3 study, ipilimumab was compared with a glycoprotein (gp100) peptide vaccine and a combination of the two therapies in a subpopulation with late stage melanoma (randomization 1:1:3). Median OS in the combination group was 10 months, in the ipilimumab monotherapy group 10.1 months, whereas the gp100 vaccine group had only 6.4 months. OS did not differ significantly between the two ipilimumab groups<sup>37</sup>. In the EORTC 18071 study, a multi-centric, double blind, phase III trial in treatment-naïve subjects with advanced disease (475 on ipilimumab, 476 on placebo), on which the extension of indication to this subpopulation was based, the median recurrence-free survival was 26.1 months (95% CI 19.3-39.3) for the ipilimumab group and 17.1 months (95% CI 13.4-21.6) for the control group<sup>38</sup>. However, the improved efficacy of ipilimumab

came at a safety price, about 85 per cent of patients experienced adverse events of any grade, and although the majority were of minor or reasonable gravity, in a small proportion of patients these were severe and life threatening. Careful monitoring and possibly early use of corticosteroids might be necessary<sup>39</sup>.

### Anti-PD1 inhibitors

PD1 is an immune checkpoint inhibitor expressed on activated T, B and NK lymphocytes, as well as on peripheral myeloid cells, acting through its two ligands to induce T cell tolerance<sup>40</sup>. Its ligand PD-L1 is expressed on a large variety of human tumours including melanoma, being exploited by these to evade the host immune system, for instance by suppressing cytotoxic T lymphocytes<sup>41</sup>. Unlike PD-L1, PD-L2 seems to be expressed in a restricted manner, only on APC cells (dendritic cells, B cells, *etc.*)<sup>23,40</sup>. PD-1 expression or that of its ligand was correlated with poor outcome in several malignancies<sup>42</sup>, and thus, it was to be expected that inhibition of PD-1 signaling, *e.g.* by use of monoclonal antibodies should result in improved outcome. Two such antibodies, nivolumab and pembrolizumab confirmed this hypothesis. Nivolumab has been the earliest anti-PD-1 antibody to be authorized in the European Union, pembrolizumab being the second; in the US, the reverse was true (nivolumab was the second, pembrolizumab the first)<sup>43-46</sup>.

Nivolumab, a fully human antibody blocking the interaction between PD-1 and its ligands, disrupts the T-cell toleration induced by the checkpoint inhibitor. After a promising phase I study in advanced melanoma on pre-treated, nivolumab was tested in a phase III, open label trial against chemotherapy selected by investigator (investigator's choice chemotherapy, ICC - dacarbazine 1000 mg/m<sup>2</sup> Q3W, or carboplatin AUC 6 + paclitaxel 175 mg/m<sup>2</sup> Q3W), in subjects with metastatic melanoma refractory to ipilimumab and to a BRAF inhibitor where relevant. In this open-label study the objective response rate (ORR) for nivolumab was 31.7% (95% CI: 23.5, 40.8), whereas for the ICC group it was only about one third (10.6%; 95% CI: 3.5, 23.1). Nivolumab was also better than ICC in terms of reduction of 50 per cent or more in target lesion burden (82 versus 60%), of median response time (2.1 versus 3.5 months) and in terms of median response duration (not yet attained versus 3.5 months)<sup>47,48</sup>. The pivotal study for market approval was a randomized, double-blind trial comparing nivolumab (3 mg/kg Q2W, plus a dacarbazine-matched placebo Q3W) with

dacarbazine (1000 mg/m<sup>2</sup> Q3W, plus a nivolumab-matched placebo Q2W) in treatment-naïve patients with no BRAF mutations. The one year OS was 72.9 per cent (95% CI 65.5 to 78.9) for nivolumab, whereas for dacarbazine it was almost half, 42.1 per cent (95% CI 33.0 to 50.9); hazard ratio (HR) for death was 0.42 per cent (99.79% CI, 0.25 to 0.73;  $P < 0.001$ ). In terms of PFS (progression free survival) and ORR (objective response rate), nivolumab was also sizably superior to dacarbazine and the benefit was observed irrespective of the PD-L1 status subgroup<sup>49</sup>.

Pembrolizumab (lambrolizumab, MK-3475) is only humanized (such antibodies have in most part human sequences, except for the complementarity-determining regions). It also targets PD-1 and has obtained approval for use in the therapy of late stage melanoma. It showed positive outcomes with respect to both efficacy and safety in the phase I trials<sup>50</sup>, doses of 2 and 10 mg/kg having comparable safety profiles<sup>51</sup>, which led to the use of the latter, higher dose in the pivotal study. This was a randomized, controlled trial with three treatment arms (randomization 1:1:1)<sup>52</sup>: the first two arms were allocated to pembrolizumab (10 mg/kg and every three weeks, respectively) and the third to ipilimumab (3 mg/kg every three weeks). The results were very convincing with regard to both OS and PFS, pembrolizumab being shown to have an efficacy far superior to ipilimumab, but with less adverse reactions. Six-months PFS proportions were 47.3 and 46.4 per cent in the two anti-PD1 arms, versus only 26.5 per cent in the control group (HR with respect to disease progression 0.58,  $P < 0.001$  for each treatment arm versus control). Twelve-months OS rates were 74.1 and 68.4 per cent for the two pembrolizumab regimens, versus 58.2 per cent for ipilimumab. The frequency of responses was about three times higher for pembrolizumab (33.7 and 32.9%) than for ipilimumab (11.9%;  $P < 0.001$ ) for each comparison, but adverse events of grade 3-5 were only about half as frequent in the pembrolizumab arms (13.3 and 10.1 versus 19.9%)<sup>52</sup>. A small, randomized study in subjects non-responding to ipilimumab and, if relevant, to a BRAF or a mitogen-activated protein kinase kinase (MEK) inhibitor or both, compared pembrolizumab (2 and 10 mg/kg) with ICC paclitaxel plus carboplatin, paclitaxel, carboplatin, dacarbazine, or oral temozolomide<sup>53</sup>. Pembrolizumab showed better PFS (38% for 10 mg/kg versus 16% ICC; HR 0.50,  $P < 0.0001$  for 10 mg/kg) and a better safety profile (grade 3-4 adverse

events about half as frequent in those treated with the monoclonal antibody as in the ICC arm, 14 against 26%)<sup>53</sup>. Data obtained in patients with other forms of cancer (colorectal, renal) indicate that the mismatch repair status<sup>54</sup>, the interferon- $\gamma$  signature 10 gene<sup>55</sup> or baseline differential expression  $\geq 1.3$ -fold of a relatively large number of genes (311 genes)<sup>56</sup> may be able to predict treatment response to pembrolizumab, a hypothesis which should be tested in melanoma and also of interest for nivolumab.

### **Oncolytic immunotherapy in melanoma (talimogene laherparepvec)**

Talimogene laherparepvec (T-VEC) is the earliest oncolytic immune therapy to be approved for cancer treatment, a medicinal product using a herpes simplex virus type 1 modified to reproduce in a selective manner inside tumours and to biosynthesize GM-CSF which in turn will induce a systemic anti-tumour immune response<sup>57</sup>. The official prescribing information or assessment reports have not yet been published, but the press releases by the European and American regulatory authorities indicate that the approval is based on the phase III trial recently published<sup>57,58</sup>, which showed that talimogene laherparepvec induced a lasting response in 16.3 per cent of the patients (95% CI, 12.1-20.5) versus 2.1 per cent only (95% CI, 0-4.5) for GM-CSF (OR 8.9,  $P < 0.001$ ). The ORR also favoured talimogene laherparepvec, but the difference in median OS, although favouring the oncolytic therapy marginally failed to reach the conventional significance threshold of 0.05 (23.3 months versus 18.9 months, HR=0.79, 95CI for HR 0.62 to 1.00;  $P = 0.051$ )<sup>57</sup>. In Europe the target population is represented by "adults with unresectable melanoma that is regionally or distantly metastatic (Stage IIIB, IIIC and IVM1a) with no bone, brain, lung or other visceral disease". In this target population the analyses indicated a 25 per cent DRR for the test product versus 1 per cent in the GM-CSF control<sup>58</sup>.

### **Combination therapy**

With a variety of therapeutic approaches available now in melanoma, the idea of combining medicines with different mechanisms of activity is very appealing. Therefore, various combinations have already been assessed, at least tentatively, in clinical trials. Although in this mini-review we have focused especially on the drugs that managed to break through the regulatory wall into clinical practice, there is currently much

excitement about combination therapy in melanoma, with several studies published or underway evaluating molecules not yet approved in combination with some already authorized for use in melanoma, based on the same concept of complementary mechanisms of action. Of the combinations involving at least one mechanism with immunological mechanism are T-VEC (talimogene laherparepvec) + ipilimumab, durvalumab (MEDI4736, another PD-1 inhibitor) + dabrafenib + trametinib<sup>59</sup>, ipilimumab + sargramostim (with improved OS, but not PFS in a relatively large clinical study)<sup>60</sup>, ipilimumab + bevacizumab<sup>61</sup>.

The idea of simultaneously blocking CTLA-4 and PD-1 was supported by non-clinical evidence and confirmed by phase III clinical studies. Nivolumab plus ipilimumab is superior to ipilimumab monotherapy in advanced melanoma, as evidenced in an early trial<sup>62</sup>, followed by a phase III one, the benefit being reached for patients irrespective of their BRAF-mutation status<sup>63</sup>. In treatment naïve patients with metastatic melanoma, nivolumab alone had better outcomes than ipilimumab, but their combination, especially in subjects with PD-L1-negative malignancies, has enhanced efficacy compared to each agent administered as monotherapy<sup>64</sup>. Although various therapeutic vaccines tested so far have not yet fulfilled their promise, a recent small trial explored the efficacy and safety of nivolumab co-administered with a multi-peptide vaccine and reported apparent good results<sup>65</sup>, but given its non-controlled character, the results are difficult to be placed in context. One of the first phase I studies with nivolumab also assessed it as monotherapy or co-administered with a peptide vaccine<sup>66</sup>.

Data accumulated so far indicate that increased toxicity may be a limiting factor of combinations and although with the arrival of newcomers in the field, with novel mechanisms of action, the temptation to use more complex combinations will increase, but safety considerations may impose limits on the number of entities to be combined or will narrow their use. The combination of vemurafenib and ipilimumab, for instance, despite good theoretical reasons supporting it, is currently not recommended because of the increased risk of hepatotoxicity, as evidenced in an early phase trial of the association<sup>67</sup>.

### **Conclusions and perspectives**

A large body of investigations carried out within important research centres and organizations has

led to considerable research advancements in the field, including therapies with new, immunological mechanisms of activity, whose efficacy has been proven in the treatment of late stage of melanoma. The inhibitors of the BRAF/MEK/ERK pathway, anti-CTLA-4 and anti-PD1/PD1-L monoclonal antibodies and the first oncolytic virus have all opened the gates of regulatory approval and are now used in the routine clinical setting<sup>68</sup>. But melanoma as well as other malignancies have not yet been defeated; thus, the exploration of new pathways and new approaches for old(er) targets goes on. For most checkpoint inhibitors and co-stimulatory molecules discussed in this paper, monoclonal antibodies targeting them have already been developed, although not all have been clinically tested in melanoma. IMP-321, a LAG-3Ig fusion protein has been evaluated in an early development trial<sup>69</sup> and an anti-LAG-3 antibody (BMS-986016) is also tested together with nivolumab<sup>70</sup> against melanoma. Lirilumab has been developed against KIR (killer cell immunoglobulin like receptor)<sup>71</sup>, but it has not yet been tested against melanoma. Preliminary data indicate a potential benefit for therapies targeting TIM-3<sup>72</sup> BLTA/CD-160<sup>73</sup> or VISTA<sup>20</sup>, especially in dual blockade combinations. CP-870,893, a human monoclonal antibody<sup>74</sup> and dacetuzumab (SGN-40)<sup>75</sup> are potent CD40 agonists; MEDI6469 is an agonist murine monoclonal antibody (mAb) for the human OX40 receptor tested in phase I in various solid tumours<sup>76</sup>; an early development trial for urelumab (an agonistic anti-hCD137 antibody)<sup>77</sup> was withdrawn before the start of enrollment and TRX518, a monoclonal antibody targeting GITR, is currently evaluated in an early development trial in several advanced malignancies including melanoma<sup>78</sup>. We have left out the whole field of therapeutic vaccines and adaptive cell transfer, which have also to be regarded as forms of immune therapy of considerable interest in melanoma. Due to the well-known and complex relationship of melanoma with the immune system, other forms of therapy with immunological mechanism will continue to be explored against this form of skin cancer.

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