# Manuscript Details

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| **Manuscript number** | INTIMP_2020_254_R1 |
| **Title** | Role of immune checkpoint inhibitors in the revolutionization of advanced melanoma care |
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**Abstract**

Melanoma cancer is an important public health concern owing to its prevalence, high recurrence risk, treatment failures and immunosuppressive abilities. Prolonged immune system activation is the main objective of immune checkpoint inhibitors (ICIs) therapies directed against melanoma cancer. Despite the staggering advancements in approved ICIs therapy effectiveness, immune-related adverse events (imAEs) and therapeutic resistance has limited its wide application. Thus, there is a need to establish biomarkers that predict the response to ICIs and imAEs. In this review article, we provide an in-depth understanding of the role of tolerance, immunity, and immunosuppression in antitumor immune response regulation, together with ongoing clinical therapy and suggested biomarkers. These attainments advise that approved ICIs provide a novel approach to durable and prolonged response in cancer patients and will aid in the reduction of treatment cost and duration and enhance patient recovery.

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| **Keywords** | Malignant melanoma; Immune checkpoint inhibitors; T-cell dysfunction; CTLA-4; PD-1/PD-L1; biomarkers |
| **Taxonomy** | Tumor Immunology, Immunology |
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| **Suggested reviewers** | V.R. Sinha, Bapi Gorain, MOHD CAIRUL IQBAL MOHD AMIN, Manish Chourasia, Virendra Gajbhiye, Chitra Thakur |

## Submission Files Included in this PDF

**File Name [File Type]**

1_Cover letter.docx [Cover Letter]

2_Reviewer Comments reply.docx [Response to Reviewers]

4_Highlights.docx [Highlights]

3_Manuscript.docx [Manuscript File]

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**Manuscript Number:** INTIMP_2020_254
**Title:** Role of immune checkpoint inhibitors in the revolutionization of advanced melanoma care
**Corresponding Author:** Dr. Prashant Kesharwani
**Journal:** International Immunopharmacology

**Subject:** Regarding submission of revised manuscript entitled "*Role of immune checkpoint inhibitors in the revolutionization of advanced melanoma care*"

Dear Dr. Barber,

With reference to your e-mail dated 10th March 2020, we are happy to hear that our manuscript has been reviewed by potential reviewer and they **concluded to accept our paper subjected to minor revision**. As per reviewer suggestions, we have cautiously gone through the comments regarding required changes in the manuscript (please see "Response to reviewers file" for the details). We have thoroughly revised the manuscript by "highlighted text" to address reviewer' concerns. Hope you will find revised manuscript suitable for publication.
Thanking you in anticipation and a favourable response.
Kindly acknowledge
Best Regards,

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# Point wise reply to the reviewer comments

## Reviewer Comments:

**Reviewer 1:**

In this review article, the authors summarize the treatment history of melanoma, the progress of immunotherapy in melanoma, the molecular mechanism of immunotherapy, and the need to establish biomarkers to better predict which patients can be suitable for immunotherapy, in order to minimize the immune-related adverse events. In general, the author summarizes comprehensively. However, before fully acceptance for publication, I suggest the authors check the whole manuscript carefully and make necessary revisions.
**Reply:** We would like to thank the valued reviewer for their time and positive recommendation regarding its acceptance. We appreciate reviewer's suggestion, which had certainly helped us in further improvising the quality of this manuscript.

**Comment 1:** There are repeated descriptions in parts 5 and 6 of the article, such as "only a minority of advanced melanoma patients respond to checkpoint blockade, with a 10–40% objective response rate with monotherapy and up to 58% with combined ipilimumab and nivolumab".
**Reply: Thanks for the minor observations.** Complied; as there is a repetition of sentence, authors have removed in part 6.

**Comment 2:** Figure 2 needs to be further adjusted. At present, it is relatively rough.
**Reply:** Complied; "as per the suggestion authors have incorporated new Figure 2 with good resolution.

**Comment 3:** It is suggested to add a schematic diagram of the molecular mechanism of immunotherapy to facilitate the understanding of the author.
**Reply:** We already published one article on "Immune checkpoint inhibitors: a promising anticancer therapy" in Drug Discovery Today, 2019, 25 (1):223-229. There we give general description of molecular mechanism of immunotherapy.

**Comment 4:** Table 1 should add the specific number of patients included in the treatment, as well as the cure rate, etc.
**Reply:** As per the suggestions, we incorporated the number of patients in Table 1.

**Highlights:**

- ICIs therapies provide survival effects for advanced stage melanoma patients.
- ICIs can improve the activity of T-cells toward tumor cells.
- Approved biomarkers will reduce toxic risk and direct novel treatment therapies.

# Role of immune checkpoint inhibitors in the revolutionization of advanced melanoma care

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**Abstract:**

Melanoma cancer is an important public health concern owing to its prevalence, high recurrence risk, treatment failures and immunosuppressive abilities. Prolonged immune system activation is the main objective of immune checkpoint inhibitors (ICIs) therapies directed against melanoma cancer. Despite the staggering advancements in approved ICIs therapy effectiveness, immune-related adverse events (imAEs) and therapeutic resistance has limited its wide application. Thus, there is a need to establish biomarkers that predict the response to ICIs and imAEs. In this review article, we provide an in-depth understanding of the role of tolerance, immunity, and immunosuppression in antitumor immune response regulation, together with ongoing clinical therapy and suggested biomarkers. These attainments advise that approved ICIs provide a novel approach to durable and prolonged response in cancer patients and will aid in the reduction of treatment cost and duration and enhance patient recovery.

***Keywords:*** Malignant melanoma; Immune checkpoint inhibitors; T-cell dysfunction; CTLA-4; PD-1/PD-L1; biomarkers.

## 1. Introduction

Malignant melanoma is one of the most aggressive and highly resistant melanocyte malignancies that can occur throughout the body. It arises from cutaneous, mucosal, and uveal melanocytes. Amongst these, the most prevalent is the cutaneous form. It has been reported to cause the majority of skin cancer-related deaths with a global incidence of 15–25 people among 100,000 of the population [1]. The number of melanoma incidence is on the rise with approximately 91,270 new cases of melanoma and 9,320 mortalities from melanoma reported, globally. These epidemiological figures are based on the latest melanoma report by the National Cancer Institute: Surveillance, Epidemiology, and End Results Program (NIH SEER) database [2]. There are multiple factors which support malignant melanoma promotion, that are affected by positive genetic backgrounds and other factors such as sunburn susceptibility (fair skin, lighter eyes, and hair color), increased exposure to ultraviolet radiation, and arsenic [3,4].

For a long time, removal surgery has been the approved standard treatment choice for the patients diagnosed with early stage primary skin melanoma. Later, chemotherapy was introduced for the treatment of melanoma. To date, Dacarbazine is the only approved chemotherapeutic drug for the treatment of melanoma by the United States Food and Drug Administration (FDA). However, the overall response rate in phase I and II randomized clinical trial was very low, i.e., 10-20 percent with no definite overall survival (OS) [5]. Unfortunately, the development of resistance through complex mechanisms has hampered the effectiveness of commonly used anticancer therapies in melanoma treatment [6–8]. The specific mechanisms by which resistance in melanoma cancer develops and confers therapeutic resistance needs to be elucidated. Numerous situations lead to drug tolerance and further contribute to resistance. These include upregulation of drug transporters, impaired apoptosis machinery, enhanced therapeutic target expression, activation of alternative survival pathways, high molecular heterogeneity, and overactive pro-survival signaling pathways [9]. Unfortunately, the majority of investigations have recommended that the outcome of these mechanisms lead to the development of abilities that allow cancer cells to survive under extremely unfavorable micro environmental conditions and are capable of overwhelming the deficiency of nutrients and metabolic products. Additionally, such cells can deceive the immune response of the host, withstand hypoxia, induce apoptosis, and eventually establish a remarkable tendency for metastatic spread in melanoma patients [10,11]. For metastatic melanoma, several steps along the immune system fail due to the upregulation of immune checkpoints or their ligands on T-cells by suppressing innate immune sensing.

Failure of immunological control has been established as one of the emerging characteristic features of melanoma cancer [12]. Beginning in 2011, a prolonged and frustrating drought of melanoma treatment therapy ended with the first FDA approval of the immune checkpoint inhibitor (ICI), ipilimumab due to significant safety and good tolerability profile [13]. The introduction of ipilimumab

ignited hope in the medical world and drastically transformed melanoma treatment [14]. With FDA approval and introduction of the first anti-cytotoxic T-lymphocyte antigen-4 (CTLA-4) antibody ipilimumab in the clinical system, the golden period for advanced melanoma treatment begun. A few years later, after approval of ipilimumab, programmed cell death protein 1/or, its ligands (PD-1/PD-L1) were introduced with approval of Pembrolizumab and Nivolumab in melanoma patients. The impressive progress of the ICIs blockade, CTLA-4, and PD-1/PD-L1, with its relatively favorable safety profile, has appeared after its full acceptance in clinical trials [15].

Despite significant vital advancements in the treatment of advanced-stage melanoma, most of the ICIs are currently administered through a systemic route, which results in alteration of the immune system with tolerance breakdown and a range of inflammation-induced toxicity known as immune-mediated adverse events (imAEs) [16]. ICIs-induced toxicities restrict its further treatment applications and contribute to the discontinuation of therapy in approximately half of the patients. Management of imAEs is significantly different from the management of adverse events induced by cytotoxic chemotherapy. Considering these similarities and core variations will promote the addition and implementation of site-specific based targeted treatment strategies for the minimization of imAEs cytotoxicities [17].

In this review, we highlight the current understanding of how inactivated T-cells avoid immune system interruption and the effect of ICIs based treatment towards immunosuppressive cells with a vital focus on pre-clinical studies, progress of clinical trials and approved anti-CTLA-4, anti-PD-1, and anti-PD-L1 mAbs. In light of these, we also discuss the complex crosstalk between the emergence of resistance and therapeutic limitations by exploring biomarker-based approaches to overcome resistance to ICIs. The hindrance of the ICIs will be relieved by an improved understanding of immune regulation pathways to be clinically relevant for the treatment of melanoma. Hence, we summarized historical timeline of melanoma as shown in **Figure 1**.

## 2. Inactivation of T cells by CTLA-4, PD-1 and its ligands

T cells are involved in various immune responses in cancers. In acute disease conditions, naive T cells are immediately initiated, and differentiated into effecting T cells ($T_{eff}$) by antigenic stimulation [18]. In opposition to this, in the case of cancer, due to persistent antigen expression, the role of T cells becomes compromised and termed T cell dysfunction [19]. The immune system can identify the expression of persistent antigens, however in the case of T cell dysfunction; an impaired immune system allows cancer cells to persist undetected. The cycle is regulated by maintaining the balance between co-stimulatory and inhibitory signals. These signals are generally known as immune checkpoints [20]. Immune checkpoints attack when T cells identify and attach to partner proteins on other cells. All such proteins are called immune checkpoint proteins. They produce an "off" signal to the T cells. This prevents the T-cell identification mechanism among cancerous and noncancerous cells and further leads

to the destruction of the immune system. There are mainly two types of receptors that downregulate T-cell function, i.e., CTLA-4 and PD-1 [21,22].

CTLA-4 belongs to the group of co-inhibitory receptor immunoglobin superfamily. CTLA-4 (CD152) is known as a T lymphocyte surface protein, and its interference results in the downregulation of immune response [23]. It is mainly expressed on the surface of T cells between 24 - 48 h of activation. There are two well-defined mechanisms by which CTLA-4 functions to inactivate T cells: direct negative signaling upon TCR activation, and competitive antagonism of CD28:B7-mediated co-stimulation. Both mechanisms of T-cell inactivation are functional via a hierarchical regulation of CTLA-4 oligomerization in lipid rafts at the immunological synapse. These two mechanisms of T cell dysfunction by CTLA-4 may have different practical consequences: quick inhibition of T-cell activation or initiation of T-cell activation [24].

PD-1 (CD279) receptor is a member of the B7-CD28 family and is expressed in activated CD8+ T cells, B cells, monocytes, and natural killer T cells, following activation. PD-1 receptors diminish T cell functions by poorly defined oncogenic signalling pathways or by immunostimulating cytokines such as interferon [25]. The interaction of PD-1 with its ligands also alters the cell cycle by preventing progression through the G1 phase by raising p15 expression levels and suppressing transcription of S phase kinase binding protein 2 (SKP2). The interaction among PD-1 and PD-L1 produces a signal that restricts T-cell proliferation, leading to immune damping and anergy with T-cells [26].

## 3. Application of immune checkpoint inhibitors in patients with malignant melanoma

Based on the current understanding of melanoma pathology, unique therapeutic approaches have been developed, which include CTLA-4, PD-1, and PD-L1/2 inhibitors. The principal purpose was to suppress the molecular interplay among tumor cells and immune effector cells. The findings of continued clinical trials represent a groundbreaking advancement in melanoma patient trials.

Ipilimumab (BMS734016, MDX 101, MDX-010, MDX-CTLA-4, MDX-CTLA4, Yervoy, Bristol-Myers Squibb) is an IgG1 mAb attacking the CTLA-4 receptor. It was first discovered by Berkeley University (CA, USA) and authorized to Medarex, which was further purchased by Bristol – Myers Squibb. In 2011, FDA and European Medicines Agency (EMA) approved Ipilimumab mainly for the treatment of more advanced stage (unresectable or metastatic) melanoma on the basis of overall survival confirmed in a phase III clinical trial study [5].

The FDA authorized ipilimumab 3 mg kg−1 as a single intravenously administered drug every 3 weeks for a sum of four doses. Hodi and collaborators have reported statistically significant overall survival (OS) rates mediated by ipilimumab for patients with earlier treated metastatic melanoma. The

average survival was 10.1 months' patients who were treated with ipilimumab alone or glycoprotein 100 (gp100) peptide in combination with ipilimumab. On the other hand, survival was 6.4 months, with the patients receiving gp100 peptide-vaccine alone. They recommend that ipilimumab will generate synergetic impacts in melanoma patients [27]. Consequently, Ipilimumab was further approved by the EMA at the equivalent dose and monotherapy, but for earlier treated patients. Later in 2011, Robert et al. revealed that Ipilimumab (at a dose of 10 mg per kilogram) in combination with dacarbazine in comparison to Dacarbazine plus placebo had improved overall health in patients without any earlier treatment therapy in metastatic melanoma [5]. Based on the outcome of this research, in 2011 ipilimumab was authorized for metastatic melanoma in the United States and Europe [28]. A randomized, double-blind Phase III research of ipilimumab administered at 3 or 10 mg kg−1 in individual patients with earlier treated or untreated unresectable or metastatic melanoma was open-ended to establish the ideal recommended dose of ipilimumab (ClinicalTrials.gov identifier: NCT01515189) [29]. The mechanism of Ipilimumab worked by interfering with the association of CTLA-4 expressed on a subset of elevated T cells with B7 molecules on antigen-presenting cells (APCs) [30]. These findings in tumor-specific T-cell proliferation and activation are owing to blockage of T-cell activation inhibitory modulation. It is expected to prevent tumor development.

PD-1 is a related inhibitory T cell receptor that, in contrast to CTLA-4, principally controls effector T cell response inside the peripheral tissues [31]. Another antibody-based therapy based on antagonism is an anti-programmed cell death protein 1 (PD-1) or its ligand antibody. Raised levels of PD-L1 have been seen in tumors, including melanoma, where the promotion of T-cell apoptosis is supposed to mediate immunity evasion [32]. A positive relationship among melanoma cell PD-L1 activity and overall survival (OS) was established. PD-1 is abnormally manifested in circulating melanoma antigen-specific T cells and tumor-infiltrating lymphocytes (TILs). Melanoma cells are assumed to be capable of initiating and sustaining long-lasting PD-1 signals, as well as T-cell fatigue and T-lymphocyte dysfunction [33]. Therefore, as tumours and their microenvironment display PD-1 and PD-L1, PD-1 blockade could restore abnormal activity and signaling, recover immune effector cellular activity, and stimulate an adaptive reaction to antitumors. Hamid and colleagues presented the first study of pembrolizumab in melanoma in 2013. Pembrolizumab or lambrolizumab (trade title: Keytruda) is the first PD-1 medication approved by the FDA to treat unresectable or metastatic melanoma that was previously handled [34]. Another monoclonal antibody against human PD-1, Nivolumab (BMS-936558), is FDA-approved for treatment in 2014, for patients with metastatic melanoma and the progression of diseases following ipilimumab therapy. It is a genetically modified IgG4 that binds to raised affinity PD-1 (KD 2.6 nmol / l) and inhibits its ligand relationships. Nivolumab can suppress activated T cells by antibody-dependent cellular cytotoxicity (ADCC) [35]. Nivolumab therapy was generally well-tolerated, with antagonistic results in 14 % of patients in grade 3 to 4. It has been reported that no cumulative toxicity treatment has been given to overcome the antagonistic effects

of Nivolumab. [36].

Simultaneously, after a few years, combinational ICIs therapy using CTLA-4 and PD-1 inhibitors were introduced and regarded suitable for the treatment of melanoma patients. The rationale for combining anti-PD-1 and anti-CTLA-4 antibodies was based on their various modes of intervention and their ability to amplify at different phases during the interaction of cancer cells and the immune system. Anti-CTLA-4 functions principally in the priming stage, while anti-PD-1 prevents the effector stage in local tumor tissue [37]. In advanced BRAF negative melanoma, the combination of nivolumab and ipilimumab has revealed significant effectiveness and is currently approved by the FDA for first-line therapy. A Phase III research showed a combined treatment approach as compared to the standard single immune checkpoint inhibitor treatment [38]. It has been shown that anti-CTLA-4 inhibitors can upregulate the application of PD-1 ligand (PD-L1) [39]. As discussed in the previous section regarding approved ICIs therapies, **Table 1** represents ongoing clinical trial studies for the treatment of Melanoma.

## 4. Mechanisms of resistance to immune checkpoint inhibitors

Contemporary ongoing clinical practice and findings have uncovered different mechanisms of resistance development to ICIs. Precise mechanisms of resistance include; micro environmental tumor changes that restrict T-cell activation, tumor invasion, and tumor cell destruction mediated by the effector [40]. A deficiency of tumor-associated antigens can inhibit the activation of tumor-specific T-cells and enable tumors to avoid ICIs. Failure to perform a tumor antigen may result from a complete failure of antigen or a deficit antigen-processing component and/or performance pathway. Breakdown of tumor antigen performance is an important mechanism by which tumors avoid T-cell-mediated immune recognition [41]. Mutations in β2-microglobulin, a protein needed for the folding and transport of major histocompatibility complex (MHC) Class I to the cell surface [42]. It has also been observed in melanoma patients at the time of anti-PD-1 treatment failure.

Mechanisms that hinder T-cell trafficking to tumor tissue also generate resistance to ICIs. BRAF mutations and suppression of phosphatase and tensin homolog (PTEN) expression both lead to resistance of ICIs in murine models and patients by producing multiple immunosuppressive proteins, including VEGF, to be formed [43]. Additionally, it restricts T-cell trafficking to the sites of tumor and inhibits T-cell effector functions [44]. Mutations in the interferon-gamma (IFN- γ) genes signaling pathway also lead to both primary and acquired resistance to ICIs. Tumor-extrinsic mechanisms of resistance development to ICIs have also been established, including new immune checkpoint receptors, immunosuppressive cytokines, and other factors present in the tumor microenvironment and immunosuppressive immune cell populations. The tumor microenvironment generated by tumor cells has few immunosuppressive factors and infiltrating immune cells can also induce resistance to ICIs by

inhibiting T-cell activity. Transforming growth factor-beta (TGF-β) is an immunosuppressive cytokine formed by many different types of human tumors that can restrict the efficacy of ICIs by stimulating Treg cells and impairing the function of T-cells [45].

## 5. Limitations of ICIs in melanoma

Advanced studies have demonstrated that patients with several malignancies benefit from safe ICIs based therapy. However, mainstream preliminary studies of the immune checkpoint approach to treating tumors are restricted by lowered response rate and insusceptible immune-related adverse events in some cancer patients. Some ICIs are presently accessible in clinical practice for the treatment of melanoma. Unfortunately, the current state of ICIs-treatment remains unclear [46]. One issue is the uncertainty of whether immune or standard medication will be more beneficial to patients. The choice of immune therapy or standard cancer medications has become more difficult because immune medications are associated with a new class of unfavorable modern course of antagonistic impacts. Accumulating proof proposes that only a fraction of cancer patients benefit from ICIs therapy, and in most cases irAEs are only seen in a few patients undergoing ICIs therapy [47]. A prime example is that of ipilimumab, which is taken to enhance T-cell responses. However, after certain dose ipilimumab treatment is associated with mechanism-based, irAEs. An early Phase II dosing study demonstrated a dose-dependent increase in irAEs with increasing ipilimumab dose [48]. irAEs were generally reversible when managed with vigilant monitoring and systemic corticosteroids, as documented in the Risk Evaluation and Mitigation Strategy associated with the FDA approval [49,50]. The most common sites for immune-related adverse events were the gastrointestinal tract and skin, as determined in 5.5–7.6% of ipilimumab-treated patients. Deaths associated with immune-related adverse events were a result of septicemia, bowel perforation, liver or multi-organ failure, or Guillain–Barre syndrome [51].

A similar pattern of mechanism-based, immune-related adverse events are seen with PD-1 blockade as with CTLA-4 blockade with ipilimumab. The most common adverse events seen following PD-1 blockade are fatigue, rash, diarrhea, pruritus, and nausea. The clinical development of CTLA-4 and PD-1-/PD-L1-blocking antibodies has had a profound impact on the treatment of melanoma and several other cancers [52]. However, despite this success, only a minority of advanced melanoma patients respond to checkpoint blockade, with a 10–40% objective response rate with monotherapy and up to 58% with combined ipilimumab and nivolumab. As a result, considerable effort is invested in the identification of predictive bio- markers to identify patients most likely to benefit from checkpoint blockade. Thus, patients at high risk for treatment failure can easily be identified. This would limit unnecessary exposure to immune-related adverse events and would direct treatment toward more aggressive combination strategies. Early clinical experience with immune checkpoint blockade has identified several biomarkers associated with treatment efficacy including; tumor mutational burden, the presence of tumor-infiltrating lymphocytes, PD-L1 expression, and intestinal microbiota.

## 6. Biomarkers for the efficacy of immune checkpoint inhibitors in malignant melanoma

The new era of immunotherapy has drastically changed the treatment landscape of metastatic melanoma. The successful evolution of CTLA-4 and PD-1-/PD-L1 ICIs therapies has had a tremendous impact on melanoma management and several other forms of cancer. However, many patients still do not experience the clinical benefit of ICIs therapies. Notwithstanding this success, only a minority of advanced melanoma patients respond to ICIs therapies. Although potentially important associations between biomarkers and clinical responses to ICIs have been observed, these biomarkers are not yet ready for clinical practice until prospectively validated in clinical studies [53]. Heterogeneity in prior therapies and use of archival tissue for biomarker development further cloud interpretation. Consequently, significant effort has been invested in the identification and detection of predictive biomarkers to identify patients most anticipated to benefit from ICIs and those at high risk for treatment failure that would benefit from more aggressive combination therapies to limit unnecessary exposure to immune-related adverse events. Early clinical experience with ICIs has identified numerous biomarkers linked with treatment efficiency, including tumor mutational burden, the presence of tumor-infiltrating lymphocytes and PD-L1 expression [50].

There is an increasing concern to obtain an in-depth understanding of the effect of chronic inflammation, nutrition, and stress on overall and tumour-specific immunity, but much research is needed to gather actionable elements. Many epigenomic, microenvironmental and immune blocking processes have been recognized that have prompted researchers to develop more multi-drug approaches that target them [54] as shown in **Figure 2.**

## 7. Conclusion

Although, ICIs is relatively a 'new kid on the block', it was recently recognized as a potential fourth pillar in anticancer therapies for the treatment of melanoma by activating the host immune system with improved patient's survival rate. ICIs for cancer has just hit its maturity at the right time and is transforming the field of melanoma treatment, both philosophically and rationally. Treatment of melanomas through ICIs varies from traditional chemotherapeutic agents that act via mechanisms, which boost, activates, or strengthens a functional immune response to tumor cells rather than physically removing or killing cancer cells by inherent radiotherapy or chemotherapies. Despite the popularity of anti-CTLA-4 and anti-PD-1/PD-L1 therapies, ICIs help only a fraction of patients. The compilation of evidence suggests that some patients who receive ICI therapy have serious irAEs. irAEs are induced mainly due to immune checkpoints inhibition, which strengthens standard physiological barriers to autoimmunity, leading to numerous local and systemic autoimmune responses. Many subsequent years of efforts and 'proof of principle' for promoting the immune system have been established; yet minimization of irAEs continues to be a challenge. This has resulted in the need to

establish biomarker sets that can predict the response of ICIs by minimizing irAEs and increasing the patient's compliance. In conclusion, it is time to shift the paradigm toward a biomarker-driven ICIs solution that has the potential to bring about dramatic changes in the ICIs immunotherapy landscape.

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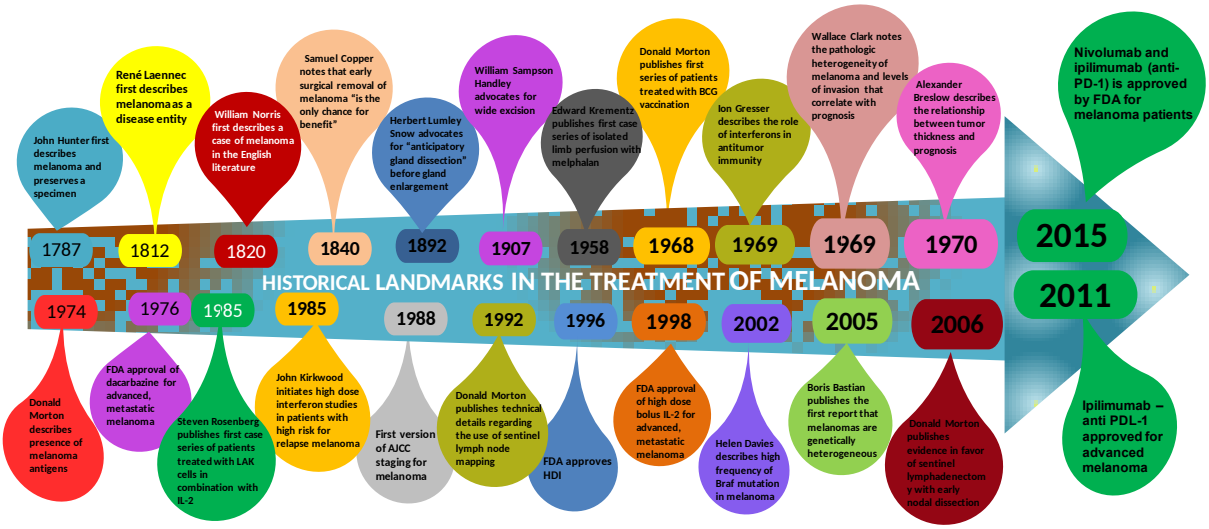


**Figure 1.** Timeline with the key historical landmarks in the treatments for melanoma cancer.

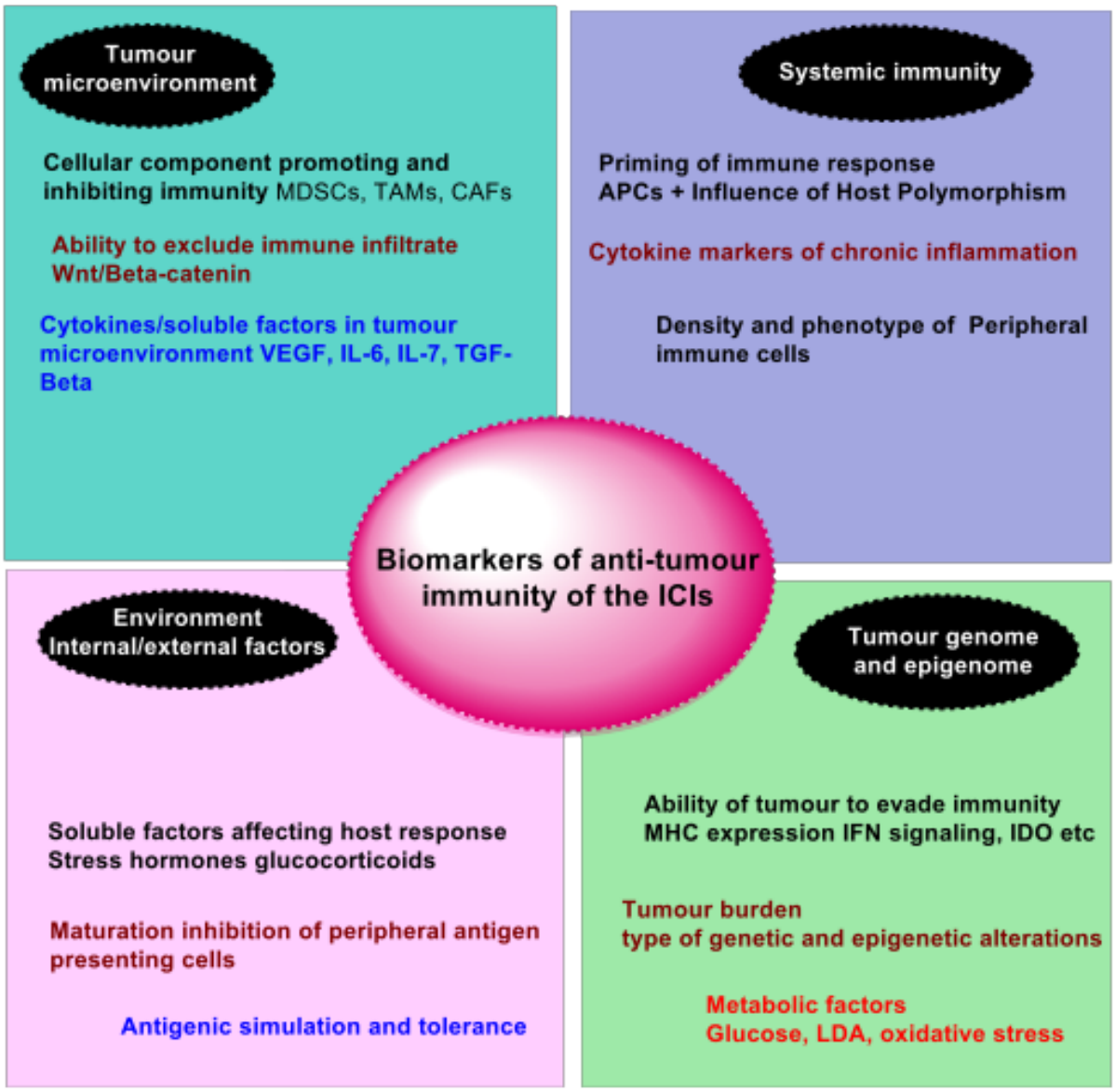


**Figure 2.** The core pillars and biomarkers of anti-tumour immunity to the ICIs.

**Table 1.** Ongoing clinical trial study for the treatment of melanoma

| Trial number | No of Patients | Combinational ICIs therapy | Stage of melanoma | Target | Status |
|---|---|---|---|---|---|
| NCT02905266 | 135 | Nivolumab + ipilimumab | Previously untreated, metastatic melanoma | Anti-PD-1 + anti-CTLA-4 | - |
| NCT03068455 (CheckMate 915) | 1943 | Nivolumab + ipilimumab | Complete resection of stage IIIB/C/D or stage IV melanoma | Anti-PD-1 + anti-CTLA-4 | Active, not recruiting |
| NCT02714218 | 481 | Nivolumab + ipilimumab | Previously untreated, unresectable or metastatic melanoma | Anti-PD-1 + anti-CTLA-4 | Active, not recruiting |
| NCT02599402 (CheckMate 401) | 615 | Nivolumab + ipilimumab | First-line for advanced melanoma | Anti-PD-1 + anti-CTLA-4 | Active, not recruiting |
| NCT03470922 | 700 | Nivolumab + Relatlimab | Unresectable or metastatic melanoma | Anti-PD-1 + LAG-3 inhibitor | Recruiting |