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Novel immune checkpoints beyond PD-1 in advanced melanoma

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Summary In malignant diseases, targeting of immune checkpoints successfully changed the therapeutic landscape and helped to unleash anti-tumor T cell responses, resulting in durable clinical outcomes, but only in up to 50% of patients. The success of these therapies and the need to overcome intrinsic and acquired therapy resistance stimulated research to identify new pathways and targets. Numerous clinical trials are currently evaluating novel checkpoint inhibitors or recently developed strategies like modulating the tumor microenvironment, mostly in combination with approved therapies. This short review briefly discusses promising therapeutic targets, currently still under investigation, with the chance to realize clinical application in the foreseeable future.

Keywords Immunotherapy · Skin cancer · Immune checkpoint inhibitors · Inhibitory receptor · Co-stimulatory receptor

Introduction

Over the past decade, immune checkpoint inhibitors (ICIs) successfully shaped the therapeutic landscape of malignant tumors. The most broadly studied and first immune checkpoint targets were cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), programmed cell death protein-1 (PD-1) and its ligand (PD-L1). The binding of CTLA-4 (CD152) to the ligands CD80 (B7-1) and CD86 (B7-2) delivers a negative signal to T cell activation [1], whereas the binding of PD-1 (CD279) to its ligands PD-L1 and PD-L2 (CD273, B7-DC) suppresses the activation and function of T cells, thereby downregulating adaptive immune response [2, 3]. *Ipilimumab*, a monoclonal antibody (mAb) against CTLA-4, was the first ICI approved by the United States Food and Drug Administration (FDA) in 2011 after demonstrating a survival benefit for patients with advanced melanoma over the chemotherapeutic dacarbazine. *Pembrolizumab*, the first humanized mAb against PD-1, gained initial global approval for patients with unresectable or metastatic melanoma by the FDA in 2014 [4]. Since then, the indication of those mAbs to several other tumor entities, and the list of approved ICIs against PD-1/PD-L1 or CTLA-4 have expanded [5]. The current benchmark for efficacy in melanoma therapy is the combinational therapy of anti-CTLA-4 and anti-PD-1 agents [6]. However, roughly half of all patients will not benefit from ICIs, and therefore identification of predictive markers allowing patient stratification regarding first- and second-line treatment strategies and avoiding toxicity of ineffective therapy is of immense clinical interest [7, 8]. The search for new potential targets and pathways has already resulted in a new portfolio of targets for novel treatment options, mostly tested in combination with PD-1 inhibitors. Molecules targeting inhibitory pathways such as the type I transmembrane glycoproteins lymphocyte activation gene 3 protein (LAG-3), T cell immunoglobulin mucin receptor 3 (TIM-3), T cell immunoglobulin mucin receptor 3 (TIGIT) or B7 homolog 3 (B7-H3) are being investigated, as well as agonists of stimulatory checkpoint pathways, such as OX-40, the inducible T cell co-stimulator (ICOS), the glucocorticoid-induced TNFR-related protein (GITR), 4-1BB and CD40 (Fig. 1, Table 1).

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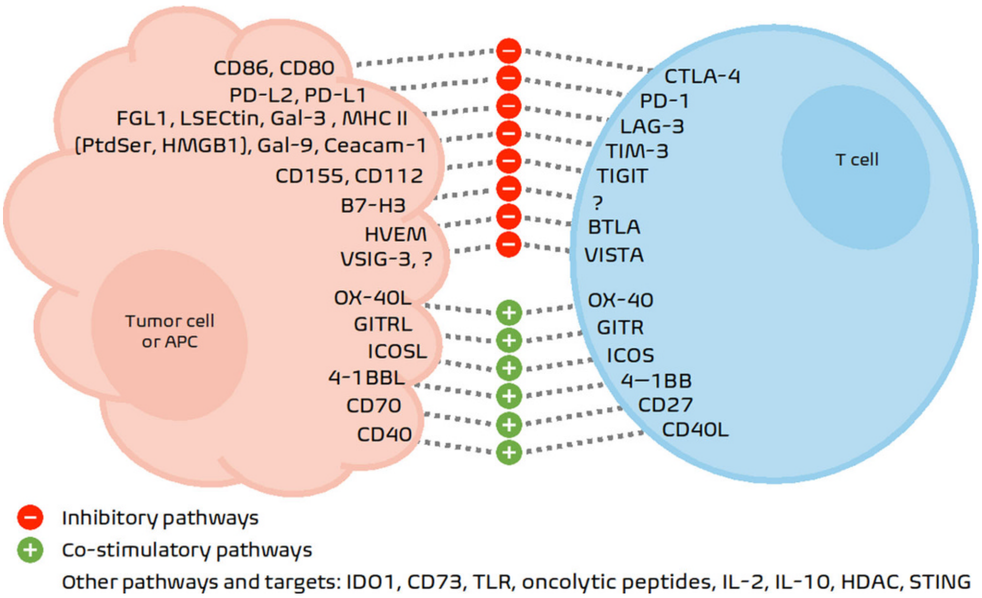

Fig. 1 Overview of discussed immune checkpoints and their respective ligands on a tumor cell and/or antigen presenting cell (*APC*)

Targeting inhibitory pathways

After CTLA-4 and PD-1, lymphocyte activation gene 3 protein (LAG-3) was the third inhibitory receptor targeted with mAbs in clinical trials. LAG-3 is a T cell-associated inhibitory checkpoint protein and member of the immunoglobin (Ig) superfamily, co-expressed with PD-1 and usually present on T cells, B cells, dendritic cells (DCs) and natural killer cells (NK cells) [9]. It is responsible for regulating immune tolerance and T cell homeostasis by its inhibitory effect on effector T cell proliferation and enhancing regulatory T cell function [9, 10]. Pre-clinical studies have shown that dual PD-1 and LAG-3 blockade synergistically stimulate T cell responses and decrease tumor burden more than either agent alone [11]. In addition, the efficiency of the LAG-3 antibody *relatlimab* seems to be increased in tumors with higher LAG-3 expression, indicating that it might be used as a biomarker [6, 12].

The T cell immunoglobulin mucin receptor 3 (TIM-3, CD366) is a type I transmembrane protein that can be found on a variety of immune cells and its expression was also demonstrated on melanoma tumor-infiltrating lymphocytes (TILs) [13–15]. Animal models of advanced melanoma demonstrated that blocking TIM-3 reverses T cell exhaustion and dysfunction [10, 14–16]. Anti-TIM-3 antagonist antibodies, like *cobolimab*, are currently under investigation in phase II clinical studies in combination with other checkpoint inhibitors or as a bispecific antibody (anti-PD-1 and anti-TIM-3) in a phase I multiple-ascending dose study.

Another inhibitory receptor is the T-cell immunoreceptor with Ig and ITIM domains (TIGIT). This transmembrane glycoprotein receptor is expressed not only on T cells, regulatory T cells (Tregs) and NK cells, but also highly expressed on melanoma cells, DCs and monocytes within the melanoma tumor microenvironment (TME) [15, 17, 18]. TIGITs immunosuppressive effects are mediated through a decreased release of pro-inflammatory cytokines and an increased release of IL-10 [19]. *Vibostolimab*, an anti-TIGIT antibody, is currently under investigation in a number of sub-studies to an umbrella study, testing experimental treatments for melanoma.

The B7 homolog 3 (B7-H3, CD276) protein is a type I transmembrane protein commonly expressed on antigen presenting cells (APCs), NK cells, tumor cells and tumor endothelial cells, belonging to the B7-CD28 pathway family. Its overexpression is frequent in multiple malignancies including melanoma, correlating with poor prognosis [20, 21]. Little is known about the molecular mechanisms underlying B7-H3 functions and its receptor(s) have not yet been identified. Research demonstrates that the B7-H3 pathway promotes cancer aggressiveness, while exerting inhibitory function on T cell activation, proliferation and cytokine production [20, 21]. This indicates that besides enhancing innate immunological responses against malignancies, B7-H3 blockade might also directly affect tumor behavior. The safety of the mAb *enoblituzumab* in combination with *pembrolizumab* on B7-H3 expressing melanomas and other cancers is currently evaluated. When combined with chemotherapy or other ICIs, it appears to have a synergistic effect [20, 21].

The B and T cell lymphocyte attenuator (BTLA, CD272) expressed by the majority of lymphocytes is an inhibitory receptor, structurally and functionally related to CTLA-4 and PD-1. Binding of BTLA to its ligand herpes virus entry mediator (HVEM) leads to an inhibition of T and B cell activation, proliferation and cytokine production [20, 22]. By expressing HVEM, melanoma cells have been shown to exploit this pathway and high levels of BTLA/HVEM correlate with progression and poor prognosis, making this

Table 1 Selection of clinical trials on novel immune checkpoints and other targets in advanced melanoma (as indexed on ClinicalTrials.gov, accessed Jan 10th, 2021) B7 homolog 3 (B7-H3); B and T cell lymphocyte attenuator (BTLA); Glucocorticoid-induced TNFR-related protein (GITR); Histone deacetylase (HDAC); Inducible T cell co-stimulator (ICOS); Indoleamine 2,3-dioxygenase (IDO); Lymphocyte activation gene 3 protein (LAG-3); Stimulator of interferon genes (STING); T-cell immunoreceptor with Ig and ITIM domains (TIGIT); T cell immunoglobulin mucin receptor 3 (TIM-3); Toll-like receptors (TLR); V-domain Ig suppressor of T cell activation (VISTA)

Target	Expression	Mechanism	Drug	Type of drug	Phase	Identifier
Inhibitory pathways						
LAG-3	Activated T cells, NK cells, B cells, plasmacytoid DCs	Regulates proliferation, activation and homeostasis of T cells	Relatlimab (BMS-986016)	mAb	II	NCT03743766
			XmAb22841	Bispecific Ab (anti-CTLA-4 and anti-LAG-3)	I	NCT03849469
TIM-3	Th17 cells, Tregs and innate immune cells (DCs, NK cells, monocytes), TILs	Mediates CD8+ T cell exhaustion, regulates macrophage activation	Cobolimab (TSR-022)	mAb	II	NCT04139902
			INCAGN02390	mAb	I/II	NCT04370704
			RO7121661	Bispecific Ab (anti-PD-1 and anti-TIM-3)	I	NCT03708328
TIGIT	T cells, Tregs, NK cells, TILs	Regulates T cell activity, increases IL-10 secretion	Vibostolimab (MK-7684)	mAb	I/II	NCT04305054 NCT04305041 NCT04303169
B7-H3	APCs, NK cells, tumor and endothelial cells	Inhibits T cell activation, proliferation and cytokine production	Enoblituzumab (MGA271)	mAb	I	NCT02475213
BTLA	Majority of lymphocytes	Inhibits T and B cell activation, proliferation and cytokine production	TAB004/JS004	mAb	I	NCT04137900
VISTA	Neutrophils, monocytes, macrophages, DCs, CD4+ and CD8+ T cells, TILs	Regulates TLR signaling in myeloid cells, controls myeloid cell-mediated inflammation and immunosuppression	JNJ-61610588	mAb	I	NCT02671955
			CA-170	Small molecule antagonist of PD-1 and VISTA	I	NCT02812875
Stimulatory pathways						
OX-40	Activated T cells, APCs	Promotes T effector proliferation, inhibits Treg function	PF-04518600	mAb	II	NCT02554812
GITR	Tregs (high), naïve and memory T cells (low)	Down-regulates Tregs, up-regulates CD8+ effector cells and extends their survival	NCAGN01876	mAb	I/II	NCT03126110
ICOS	Activated cytotoxic T cells, Tregs, NK cells	Enhances T cell functions to foreign antigen	GSK3359609	mAb	II	NCT03693612
4-1BB	Innate and adaptive immune cells	Upregulates anti-apoptotic molecules, cytokine secretion, and enhanced effector function	Utomilumab (PF-05082566)	mAb	II	NCT02554812
CD27	T cells, NK cells, Tregs	Enhances CD8+ T cell activation, survival and effector function	Varlilumab (CDX-1127)	mAb	I/II	NCT03617328
CD40	APCs, DCs, B cells, non-immune cells and tumors	Regulates initiation and progression of cellular and humoral adaptive immunity	APX005M	mAb	II	NCT04337931
Other pathways and targets						
IDO	Overexpressed in several malignancies, including melanoma	Inhibits immune cell effector functions and/or facilitates T cell death	Linrodostat (BMS-986205)	Small molecule inhibitor	III	NCT03329846
CD73	Overexpressed by many cancer cells	Inhibits immunosurveillance against tumor cells by upregulating adenosine signaling	LY3475070	Small molecule inhibitor	I	NCT04148937
TLR	Expressed on a variety of cell types	Play a key role in controlling innate immune responses to a wide variety of pathogen-associated molecules	Poly-ICLC (Hiltonol)	Viral mimic immunostimulant	I/II	NCT03617328 NCT04364230
IL-2R	Lymphocytes	Plays vital roles in key functions of the immune system, tolerance and immunity	Bempegaldesleukin (NKTR-214)	PEGylated IL-2	III	NCT04410445 NCT03635983
IL-10	Produced by almost all cell types within the innate and adaptive immune system	Multiple, pleiotropic effects in immunoregulation and inflammation	Pegilodecakin (LY3500518, AM0010)	PEGylated IL-10	I	NCT02009449

Table 1 (Continued)

Target	Expression	Mechanism	Drug	Type of drug	Phase	Identifier
Oncolytic peptides	–	Has the ability to kill human cancer cells and induce specific anticancer immune response when injected locally	Ruxotemitide (LTX-315)	Lytic peptide	I	NCT01986426
HDAC	Primarily found in the nucleus (depending on class)	Regulates DNA expression by acetylation and de-acetylation	Entinostat (MS-275, SNDX-275)	Small molecule inhibitor	II	NCT03765229
STING	T cells, NK cells, myeloid cells	Plays important role in innate immunity	E7766	Agonist compound	I	NCT04144140
			MIW815 (ADU-S100)	Agonist compound	I	NCT03172936

pathway a promising target for checkpoint blockade [23]. The first anti-BTLA mAb approved for clinical trials is currently being assessed regarding its safety and tolerability as monotherapy in advanced malignancies.

The V-domain Ig suppressor of T cell activation (VISTA) is a type I transmembrane protein and B7 family member. It is constitutively expressed on multiple immune cell types, mainly on myeloid cells including neutrophils, monocytes, macrophages and DCs, and can also be found on TILs [24, 25]. Structural analysis suggests that VISTA has the potential to function as a receptor and a ligand [26]. Furthermore, evidence indicates that VISTA could exert a dual role, stimulatory for APCs on the one side and inhibitory for T cells on the other [20]. Recently, VSIG-3 was reported as a binding partner of VISTA [27]. In preclinical studies, VISTA blockade has demonstrated improved infiltration, proliferation and effector function of TILs within the TME, thereby altering the suppressive properties of the TME [28, 29].

Activating co-stimulatory pathways

For optimal cancer control it may not be sufficient to target negative regulatory pathways alone, but may require the activation of co-stimulatory pathways either alone or in combination with checkpoint blockade to enhance the immune response.

OX-40 (CD134, TNFRSF4) is a T cell co-stimulatory protein and member of the tumor necrosis factor receptor superfamily (TNFRSF). It is primarily expressed on activated T cells and APCs, but it is also expressed at high levels on tumor resident Tregs [30]. OX-40 agonism leads to an increase in the number of activated T cells and those cells gaining effector function, while the induction of Tregs in the periphery is suppressed [31].

Interactions of OX-40 and its ligand on activated T cells increase proliferation, effector and cytotoxic function and cytokine production of those T cells, among other features [32]. Several OX-40 agonist antibodies are investigated in clinical phase I and II trials. Increased OX-40 expression on TILs in cutaneous melanoma is associated with improved prognosis [33].

Another promising immunotherapy target is the glucocorticoid-induced TNFR-related protein (GITR), stimulating the acquired and innate immunity. It is highly expressed on Tregs and activated upon binding to its ligand GITRL, mainly expressed on APCs and endothelial cells. This exerts dual effects, down-regulation of Tregs and up-regulation of CD8+ effector cells while extending their survival [15, 34, 35]. Antitumor activity of agonistic anti-GITR antibodies has already been demonstrated in mouse models and is currently evaluated in phase I and II clinical trials in melanoma and other tumor types [36].

The inducible T cell co-stimulator, short ICOS (CD278), is an immune checkpoint protein structurally and functionally related to CD28, a T cell specific cell-surface receptor like CTLA-4, and an important regulator of the immune system. ICOS is expressed on activated cytotoxic T cells, Tregs, NK cells and other types of T cells. It enhances all basic T cell functions to a foreign antigen like proliferation, secretion of cytokines and mediators up-regulating cell–cell interaction and supporting antibody secretion by B cells [37]. Up-regulation of ICOS can be detected in activated T cells, especially after anti-CTLA-4 therapy, where it can serve as a biomarker indicating the binding of anti-CTLA-4 antibodies to their targets. Increased expression on circulating T cells after *ipilimumab* therapy has been associated with improved clinical outcomes [38, 39]. Due to the upregulation of ICOS after anti-CTLA-4 therapy, the combination of those targets can achieve potent synergistic effects [20, 37].

Another important regulator of immune response and member of the TNFRSF is 4-1BB (CD137, TNFRSF9). This co-stimulatory molecule is expressed on innate and adaptive immune cells and triggers proliferation and prolonged survival of CD8+ effector T cells and NK cells upon binding to its ligand 4-1BBL [20, 40]. Anti-4-1BB antibody blockade has been shown to induce potent anti-tumor T cell responses by promoting CD8+ T cell proliferation, enhancing T cell receptor (TCR) signaling and inducing immunologic memory [41, 42]. Several pre-clinical agonistic antibodies are currently under investigation, like *utomilumab*, in a phase II study in melanoma [41].

An additional member of the TNF family and co-stimulatory immune checkpoint receptor is the glycoprotein CD27 expressed on T cells, NK cells and

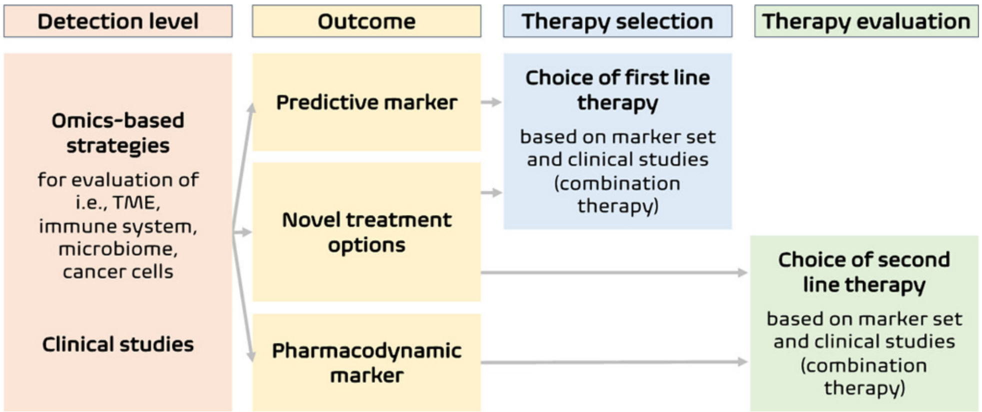

Fig. 2 General algorithm for the optimal treatment choice

Tregs. Its ligand is CD70, which is expressed on DCs, activated B and T cells [43]. When CD27 is bound by CD70, CD8+ T cell activation, survival and effector function are enhanced. To prevent unwanted stimulation of T cells, CD70 is usually not available. In this case, *varlilumab* or other mAbs can substitute and activate T cells receiving TCR stimulation [44].

Immune co-stimulatory receptor CD40 (TNFRSF5) is also part of the TNFRSF and expressed on APCs, including DCs, B cells, macrophages and monocytes. It plays a key role in the activation of the immune system, while binding to its ligand CD40L (CD154) on T cells [20]. Binding of CD40 leads to increased priming and activation of CD8+ T cells, mediated through increased major histocompatibility complex (MHC) surface expression on DCs, production of pro-inflammatory cytokines and B cell proliferation [15, 45]. Healthy tissue exhibits comparatively low to no CD40 expression, indicating strong potential as a cancer-specific immunological target [46]. Several clinical trials are studying the effects of CD40 as monotherapy or in combination.

Other pathways and novel agents

Besides targeting immune checkpoints and thereby inducing inhibitory or co-stimulatory immune responses, further research interest is aimed at other promising pathways and mechanisms.

Modulating the TME through *indoleamine 2,3-dioxygenase 1* (IDO1) is one of those approaches. IDO1 is a tryptophan catabolizing and IFN-inducible enzyme, promoting tumor-mediated immunosuppression. It thereby inhibits effector T cells and NK cells and activates Tregs and myeloid-derived suppressor cells [15, 47]. IDO1 is overexpressed in several malignancies including melanoma, while inhibition shifts the TME from a tumor-promoting inflammatory to an immune stimulating state [48]. Especially in melanoma, previous research established a relationship between CTLA-4, PD-1 and IDO1 associated with poor prognosis, independent of disease stage, making IDO1 a potential target for further investigation [15, 49]. However, a phase III study showed no survival benefit from enhancing anti-PD-1 therapy by IDO1 inhibition [50].

Another major factor in the immunosuppressive TME is the adenosine pathway, which is mediated by ectonucleotidases, like CD39 and CD73, and adenosine receptors, like A2AR. In melanoma, increased CD73 (*ecto-5′-nucleotidase*) expression correlates with a more aggressive, invasive phenotype and can be detected in over 50% of the metastases [51], while CD39 is overexpressed earlier in tumor development, potentially influencing the differentiation of melanocytes to melanoma cells [15]. In pre-clinical models, CD73 blockade showed inhibition of metastasis formation and improved anti-tumor immunity [52]. A first-in-human study is currently evaluating the safety of a small molecule inhibitor targeting CD73 as monotherapy or in combination with *pembrolizumab* in patients with melanoma and other advanced solid malignancies.

Targeting TLRs aims at a family of specialized receptors, stimulating immune responses to pathogen-associated molecular patterns (PAMPs). Among those, TLR9 has been shown to induce potent anti-tumor responses by stimulating innate and adaptive immune responses [53], ultimately leading to strong CD4+ and CD8+ T cell responses that may intensify the efficacy of ICIs [54]. Clinical activity of TLR9 has been shown in advanced melanoma patients unresponsive to PD-(L)1 inhibition.

Oncolytic peptides are cytotoxic chemotherapeutic peptides, injected intratumorally and thereby limiting systemic toxicities as well as their application to disseminated malignancies [20]. Injection of the lactoferrin-derived lytic peptide *ruxotemitide* leads to tumor antigen release followed by increased TIL activity and CTLA-4 expression, suggesting administration in conjunction with anti-CTLA-4 agents [55].

IL-2 is nothing new and is considered the first effective immunotherapy in cancer [56]. Due to severe toxicities, IL-2R agonists have been developed to potentiate and prolong IL-2 anti-tumor effects, thereby allowing lower doses. *Bempegaldesleukin*, an engineered cytokine specifically stimulating through IL-2Rβ (CD122), is currently being investigated in combination with *nivolumab* in a phase III clinical trial in melanoma. *Pegilodecakin*, the PEGylated form of IL-10, is under investigation in regard to safety and tolerability in a phase I study. Also for IL-10, a combination with PD-1 seems reasonable due to both receptors being upregulated on TILs [20].

Promising results have also been shown with histone deacetylase (HDAC) inhibitors and *pembrolizumab* in patients with unresectable or metastatic melanoma that progressed during or after anti-PD-1 therapy. Histone acetylation and deacetylation play a key role in regulating gene transcription, and inhibition of this process has emerged as a potential anticancer therapeutic in various malignancies [15].

Another emerging field of research focusses on intra-tumoral agents, like the stimulator of interferon genes (STING). STING is a transmembrane protein that is activated by cyclic dinucleotides and plays an important role in innate immunity by stimulating type 1 IFN-1 and DC activation [15]. In mouse models, intra-tumoral injection of the dinucleotide caused regression of the injected and untreated lesions. Preclinical data demonstrates that tumor antigen recognition in melanoma can be enhanced by restoring MHC class I surface expression through agonist-induced activation of STING signaling [57]. This suggests that synthetic cyclic dinucleotides activating the STING pathway should be considered as a therapeutic intervention and further investigated. Phase I trials are currently testing stimulators of STING.

Discussion

Understanding the immunobiology of tumors and therapeutic resistance will remain a major challenge for the future. The aim is to develop effective immunotherapies tailored to individual subgroups of patients, especially those not achieving long-term clinical benefit. Omics-based strategies utilizing the respective bioinformatics could deliver a potential breakthrough in this ongoing (re)search in precision medicine (Fig. 2). To understand the complexity of the tumor-immune microenvironment consisting of interactions between multiple cell types, omics-guided approaches can help in assessing for instance the TME, immune system and cancer cells. Microbiome strategies, representing the next wave of treatment approaches in immuno-oncology, will likely enrich this dynamic therapeutic landscape even further. The results from pre-clinical research and subsequently clinical studies will provide potential predictive biomarkers, novel treatment options and pharmacodynamic markers to guide treatment decisions for each individual patient. Ultimately, a combination of immunotherapies or integration of immunotherapy with non-immunotherapies will likely succeed over single agent approaches, given the complexity of the immune response.

Take home message

At present, it seems to be a difficult challenge to surpass the combination of *ipilimumab* and *nivolumab* regarding efficacy. Nevertheless, novel immune checkpoints have the potential to improve clinical outcome for patients with advanced melanoma and other malignancies, by broadening the therapeutic spectrum and increasing the number of therapy options.

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