Targeting Tumor-Associated Macrophages

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Immune checkpoint inhibitors (ICIs) represent a promising therapeutic intervention for a variety of advanced/metastatic solid tumors, including melanoma, but in a large number of cases, patients fail to establish a sustained anti-tumor immunity and to achieve a long-lasting clinical benefit. Cells of the tumor micro-environment such as tumor-associated M2 macrophages (M2-TAMs) have been reported to limit the efficacy of immunotherapy, promoting tumor immune evasion and progression.

macrophages | immune escape | immune checkpoint inhibitors | clinical trials | melanoma

1. Introduction

“Immune checkpoints” refer to a family of proteins expressed on the surface of T-cells, interacting with specific receptors/ligands located on antigen-presenting cells (APCs) or cancer cells, and inhibiting T-cell receptor (TCR)-mediated immune functions. Up-regulated during T-cell activation, the immune checkpoint molecules, such as programmed cell death 1 (PD-1), programmed cell death protein ligand 1 (PD-L1) and cytotoxic T-lymphocyte associated protein 4 (CTLA-4), prevent an excessive immune response, potentially leading to tissue damage or to the establishment of an autoimmune disease. Immune checkpoint inhibitors (ICIs) allow the adaptive immune system to overcome this “turn-off” signal and to maintain an effective immune surveillance against cancer cells.

In the last decade, different monoclonal antibodies (mAbs) targeting immune checkpoints have been developed, i.e., pembrolizumab, nivolumab and cemiplimab, directed against PD-1; atezolizumab, durvalumab and avelumab, which target PD-L1; ipilimumab and tremelimumab, specifically recognizing CTLA-4. Indications of ICIs currently approved by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) are reported in Table 1.

Unfortunately, data accumulated in recent years suggest that the clinical efficacy of ICIs is confined to a limited percentage of cancer patients. Furthermore, certain tumor types, including pancreatic, colorectal, ovarian cancer, show little benefits or are completely refractory to therapies based on immune checkpoint blockade [1]. Therefore, ICIs are not always able of efficiently reactivate exhausted tumor-specific T-cells and to restore a proper cancer immune surveillance [2], due to intrinsic or acquired mechanisms of resistance still not fully understood.

Little information is presently available concerning the potential interactions between ICIs and components of the tumor micro-environment (TME). Among the cell populations extensively recruited in the tumor mass, tumor-associated macrophages (TAMs) are known to hamper cancer patient’s response to traditional chemotherapy, and

a growing literature shows their involvement in the failure of the anti-tumor immune surveillance, as well as of immunotherapy with ICIs.

Aim of this review is to recapitulate the pro-tumor functions of TAMs, in particular the molecular mechanisms by which TAMs polarized toward the M2 phenotype promote cancer progression and immune escape. A special focus is provided on the preclinical evidence suggesting TAMs involvement in melanoma immune evasion, and on promising clinical investigations combining TAMs targeting molecules with ICIs for metastatic melanoma treatment.

Table 1. Approved ICIs by FDA and EMA.

ICI	Molecular Target	FDA-Approved Indication (Year of Approval) [a]	EMA-Approved Indication (Year of Approval) [a]
Ipilimumab	CTLA-4	Melanoma: - adults, metastatic (2011); - BRAF V600 wild-type unresectable/metastatic, in combination with nivolumab (2015); - adjuvant treatment, stage III (2015); - unresectable/metastatic regardless of BRAF mutational status, in combination with nivolumab (2016); - pediatric patients ≥12 years, unresectable/metastatic (2017). Renal cell carcinoma: - first-line, intermediate/poor-risk, advanced, in combination with	Melanoma: - adults, unresectable or metastatic (2011); - pediatric patients ≥12 years, unresectable/metastatic (2018); - advanced, in combination with nivolumab (2016). Renal cell carcinoma:

	nivolumab (2018).	- first-line, intermediate/poor-risk, advanced, in combination with nivolumab (2018).
	Colorectal cancer: - microsatellite instability high (MSI-H) or mismatch repair deficient (dMMR), metastatic, previously treated with a fluoropyrimidine, oxaliplatin and irinotecan, in combination with nivolumab (2018).	
	Hepatocellular carcinoma: - previously treated with sorafenib, in combination with nivolumab (2020).	
	Non-small cell lung cancer (NSCLC) (squamous and non-squamous): - first-line, metastatic, ≥1% PD-L1, without EGFR or ALK mutations, in combination with nivolumab (2020);	
	- first-line, metastatic or recurrent, without EGFR or ALK mutations, in combination with nivolumab and two cycles of platinum-doublet chemotherapy (2020).	NSCLC (squamous and non-squamous): - first-line, metastatic, without EGFR or ALK mutations, in combination with nivolumab and two cycles of platinum-doublet chemotherapy (2020).

		Mesothelioma: - previously untreated unresectable, in combination with nivolumab (2020).	
Nivolumab	PD-1	Melanoma: - unresectable/metastatic and resistant to other agents (2014); - unresectable/metastatic, BRAF V600 wild-type, in combination with ipilimumab (2015); - unresectable/metastatic, regardless of BRAF mutational status, in combination with ipilimumab (2016); - adjuvant, lymph node involvement or metastatic, after completely resection of the tumor (2017). NSCLC (squamous or non-squamous): - metastatic, in progression during or after platinum-based chemotherapy (2015); - first-line, metastatic, ≥1% PD-L1, without EGFR or ALK mutations, in combination with ipilimumab (2020);	Melanoma: - unresectable or metastatic, regardless of BRAF mutational status, as single agent (2015) or in combination with ipilimumab (2016); - adjuvant, lymph node involvement or metastatic, after completely resection of the tumor (2018). NSCLC: - locally advanced or metastatic forms, following prior chemotherapy (2016); - first-line, metastatic or recurrent, without EGFR or ALK mutations, in combination with ipilimumab and 2 cycles of platinum-doublet chemotherapy (2020).

- first-line, metastatic or recurrent, without EGFR or ALK mutations, in combination with ipilimumab and 2 cycles of platinum-doublet chemotherapy (2020).	
Small cell lung cancer (SCLC):	
- metastatic, progressed after platinum-based chemotherapy and at least one other line of therapy (2018).	
Mesothelioma:	
- first-line, unresectable, in combination with ipilimumab (2020).	
Renal cell carcinoma:	
- advanced/metastatic, previously treated with antiangiogenic therapy (2015);	
- first-line, advanced, intermediate/poor-risk, in combination with ipilimumab (2018).	Renal cell carcinoma:
	- advanced, after prior therapy (2016);
Classical Hodgkin lymphoma:	- first-line, advanced, intermediate/poor-risk, in

	- relapsed/progressed after autologous hematopoietic stem cell transplantation and brentuximab vedotin and/or ≥3 lines of prior systemic therapy (2016).	combination with ipilimumab (2018).
	Head and neck squamous cell carcinoma: - recurrent or metastatic with disease progression during or after platinum-based chemotherapy (2016).	Classical Hodgkin lymphoma: - relapsed/progressed after autologous hematopoietic stem cell transplantation and brentuximab vedotin (2016).
	Urothelial carcinoma: - locally advanced or metastatic, in progression during or after platinum-containing chemotherapy or within 12 months from platinum-containing adjuvant or neoadjuvant chemotherapy (2017).	Head and neck squamous cell carcinoma: - recurrent or metastatic, with disease progression during or after platinum-based chemotherapy (2017). Urothelial carcinoma: - locally advanced, unresectable or metastatic, as second-line treatment, after failure of prior platinum-based chemotherapy (2017).
	Colorectal cancer: - adult and pediatric patients, metastatic with MSI-H or dMMR metastatic, progressed after treatment with a fluoropyrimidine, oxaliplatin, and irinotecan, as a single agent (2017) or in combination with ipilimumab (2018).	

		Hepatocellular carcinoma: - previously treated with sorafenib, as single agent (2017) or in combination with ipilimumab (2020).	
		Esophageal squamous cell carcinoma: - unresectable, advanced, recurrent or metastatic, after prior fluoropyrimidine and platinum-based chemotherapy (2020).	
			Esophageal squamous cell carcinoma: - unresectable advanced, recurrent or metastatic, after prior fluoropyrimidine- and platinum-based chemotherapy (2020).
Pembrolizumab	PD-1	Melanoma: - unresectable or metastatic non-responding to previous treatment	Melanoma: - first-line, unresectable or metastatic (2015);

	(2014) and as first-line regardless of BRAF mutational status (2015); - adjuvant, completely resected, with lymph node involvement (2019).	- adjuvant, completely resected, with lymph node involvement (2018).
	NSCLC: - advanced/metastatic, progressed after other treatments and expressing PD-L1 (2015); - first-line, metastatic, high (≥50%) PD-L1 (2016); - first-line, metastatic, non-squamous, in combination with pemetrexed and carboplatin (2017) and without EGFR or ALK mutations (2018), irrespective of PD-L1 expression; - first-line, metastatic, squamous, in combination with carboplatin and either paclitaxel or nab-paclitaxel (2018); - first-line, metastatic or stage III not candidate for surgical resection or definitive chemo-radiotherapy, ≥1% PD-L1 (2019). SCLC: - metastatic, progressing on or after platinum-based	NSCLC: - locally advanced or metastatic, after at least one prior chemotherapy regimen, high (≥50%) PD-L1 (2016); - first-line, metastatic, with high PD-L1 expression, without EGFR or ALK mutations (2017); - first-line, metastatic non-squamous, without EGFR or ALK mutations in combination with pemetrexed and a platinum compound (2017); - first-line, metastatic, squamous, in combination with carboplatin and either paclitaxel or nab-paclitaxel (2019).

	chemotherapy (2019).	
	Head and neck squamous cell carcinoma: - recurrent or metastatic, progressing on or after platinum-based chemotherapy (2016); - first-line, metastatic or unresectable, recurrent, as monotherapy in tumors expressing ≥1% PD-L1 or in combination with platinum and 5-fluorouracil (2019).	Head and neck squamous cell carcinoma: - recurrent or metastatic, progressing on or after platinum-based chemotherapy, with high PD-L1 (2018); - metastatic or unresectable, recurrent, as monotherapy in tumors expressing ≥1% PD-L1 or with platinum and 5-fluorouracil (2019).
	Classical Hodgkin lymphoma: - adult and pediatric patients, refractory or relapsed after ≥3 prior lines (2017) or ≥2 prior lines of therapy (2020).	Classical Hodgkin lymphoma: - refractory or relapsed after autologous hematopoietic stem cell transplantation and brentuximab vedotin or who are transplant-ineligible and have failed brentuximab vedotin (2017).
	Urothelial carcinoma: - locally advanced or metastatic, not eligible for cisplatin-containing chemotherapy (as first-line, 2017),	Urothelial carcinoma: - locally advanced or metastatic, not eligible for cisplatin-containing chemotherapy (2017), ≥10% PD-L1

	≥10% PD-L1 (2018) or progressing during or following platinum-containing chemotherapy (2017); - high-risk, non-muscle invasive bladder cancer, with carcinoma in situ, with or without papillary tumors, not eligible for cystectomy and unresponsive to Bacillus Calmette-Guérin (BCG) (2020).	(2018) or after platinum-containing chemotherapy (2017).
	Renal cell carcinoma: - first-line, advanced, in combination with axitinib (2019).	Renal cell carcinoma: - first-line, advanced, in combination with axitinib (2019).
	Gastric or gastroesophageal junction cancer: - recurrent, locally advanced or metastatic, ≥1% PD-L1, progressing on or after ≥2 prior lines of therapy with a fluoropyrimidine, platinum-containing and anti-HER2 therapy (2017).	
	Cervical cancer: - recurrent or metastatic, ≥1% PD-L1, progressing on or after chemotherapy (2018).	

Primary mediastinal large B-cell lymphoma:

- adult and pediatric patients, refractory or relapsed after ≥2 lines of therapy (2018).

Hepatocellular carcinoma:

- previously treated with sorafenib (2018).

Merkel cell carcinoma:

- adult and pediatricpatients, recurrent, locally advanced or metastatic (2018).

Esophageal squamous cell carcinoma: - recurrent locally advanced or metastatic, ≥10% PD-L1, progressing after ≥1 line of therapy (2019).

Endometrial carcinoma:

- advanced, not MSI-H or dMMR, not candidate for curative surgery or radiotherapy, in combination with lenvatinib (2019).

		Cutaneous squamous cell carcinoma: - recurrent or metastatic, not curable by surgery or radiotherapy (2020). Colorectal cancer: - unresectable or metastatic, progressing after treatment with a fluoropyrimidine, oxaliplatin and irinotecan (2017); - first-line, unresectable or metastatic, MSI-H or dMMR (2020). Solid tumors: - adult and pediatric patients, unresectable or metastatic, MSI-H or dMMR (2017) or high tumor mutational burden (2020) progressing after prior treatment and without satisfactory alternative therapeutic options.	
Cemiplimab	PD-1	Cutaneous squamous cell carcinoma: - metastatic or locally advanced not eligible for curative surgery or radiotherapy (2018).	Cutaneous squamous cell carcinoma: - metastatic or locally advanced not eligible for curative surgery or radiotherapy (2019).
Atezolizumab	PD-L1	Urothelial carcinoma:	Urothelial carcinoma:

	- locally advanced or metastatic, worsened during or following platinum-containing chemotherapy or within 12 months from platinum-containing adjuvant or neoadjuvant chemotherapy (2016); - locally advanced or metastatic, not eligible for any platinum-containing chemotherapy regardless of PD-L1 expression level (2017) or not eligible for cisplatin-containing chemotherapy, ≥5% PD-L1 (2018). NSCLC: - metastatic, progressing during or after platinum-containing chemotherapy or, in case of tumors with EGFR or ALK mutation, after prior targeted agents (2016); - first-line, metastatic, non-squamous, without EGFR or ALK mutations, in combination with bevacizumab, paclitaxel and carboplatin (2018); - first-line, metastatic, non-squamous, without EGFR or ALK mutations, in combination with nab-paclitaxel and carboplatin (2019);	- locally advanced or metastatic, after prior platinum-containing chemotherapy, or cisplatin-ineligible (2017) and ≥10% PD-L1 (2018). NSCLC: - locally advanced or metastatic, non-squamous, after prior chemotherapy or, in case of tumors with EGFR or ALK mutation, after prior targeted agents (2017); - first-line, metastatic, non-squamous, without EGFR or ALK mutations, in combination with bevacizumab, paclitaxel and carboplatin; if EGFR or ALK mutation are present, the combination with bevacizumab,

	- first-line, metastatic, high PD-L1 (i.e., 50% of tumor cells or PD-L1 positive tumor-infiltrating immune cells covering ≥ 10% of the tumor area) (2020).	paclitaxel and carboplatin is administered only after failure of targeted agents (2019); - first-line, metastatic, non-squamous, without EGFR or ALK mutations, in combination with nab-paclitaxel and carboplatin (2019).
	SCLC: - first-line, extensive-stage, in combination with carboplatin and etoposide (2019).	SCLC: - first-line, extensive-stage, in combination with carboplatin and etoposide (2019).
	Triple-negative breast cancer: - unresectable locally advanced or metastatic, ≥1% PD-L1, in combination with nab-placlitaxel (2019).	Triple-negative breast cancer: - unresectable locally advanced or metastatic, ≥1% PD-L1, not receiving prior chemotherapy (2019).
	Hepatocellular carcinoma: - unresectable or metastatic disease, not receiving prior systemic therapy, in combination with bevacizumab (2020).	Hepatocellular carcinoma: - advanced or unresectable carcinoma, not receiving prior systemic therapy, in combination with bevacizumab (2020).
	Melanoma: - BRAF V600 mutation-positive, advanced, in combination with	

		vemurafenib and cobimetinib (2020).	
		Urothelial carcinoma: - locally advanced or metastatic, progressing during or following platinum-containing chemotherapy or within 12 months from platinum-containing adjuvant or neoadjuvant chemotherapy (2017).	
Durvalumab	PD-L1	NSCLC: - unresectable stage III, not progressed after platinum-based chemotherapy and radiotherapy (2018). SCLC: - first-line, extensive-stage, in combination with platinum-etoposide (2020).	NSCLC: - locally advanced, unresectable tumor, ≥1% PD-L1, not progressed after platinum-based chemotherapy and radiotherapy (2018). SCLC: - first-line, extensive-stage, in combination with platinum-etoposide (2020).
Avelumab	PD-L1	Merkel cell carcinoma: - adult and pediatric patients, metastatic, not receiving prior chemotherapy (2017). Urothelial carcinoma:	Merkel cell carcinoma: - metastatic (2017).

	- locally advanced or metastatic disease, progressing during or following platinum-containing chemotherapy or within 12 months from platinum-containing adjuvant or neoadjuvant chemotherapy (2017); - first-line maintenance treatment, locally advanced or metastatic, not progressed following first-line platinum-based chemotherapy (2020).	
	Renal cell carcinoma: - first-line, advanced, in combination with axitinib (2019).	
		Renal cell carcinoma: - first-line, advanced, in combination with axitinib (2019).

[a] Data updated to October 2020.

2. Tumor-Associated Macrophages and Anti-tumor Immune Surveillance Evasion

Several mechanisms have been identified through which TAMs suppress anti-tumor immunity and may hamper ICIs activity, thus promoting cancer progression and resistance to immunotherapy [3]. In particular, it has been suggested that M2-TAMs inhibit cytotoxic T-cell function by producing anti-inflammatory cytokines, depleting

essential metabolites for T-cell proliferation, and turning off T-cell activation through interaction with inhibitory immune checkpoints (Figure 1).

IL-10, prostaglandin E2 (PGE2), and TGF-β are examples of signaling molecules, produced by M2-TAMs under the influence of tumor-derived factors, which inhibit T-cell-mediated immune responses and contribute to the establishment of a self-propagating immunosuppressive TME [3].

IL-10 plays a crucial role in dampening anti-tumor immunity by suppressing the activity of different immune cells, eventually leading to the inactivation of effector T-cells [4]. In detail, TAMs-derived IL-10 inhibits APCs function [5], suppresses intratumoral dendritic cells (DCs) maturation, and reduces IL-12 production by DCs, thereby limiting cytotoxic T-cell activity [6]. Furthermore, IL-10 can directly down-regulate the activation of CD8+ T-cells, by increasing the expression of a glycosyltransferase that promotes N-glycan branching of surface glycoproteins. This event physically prevents CD8 protein and TCR co-localization and reduces the antigen sensitivity of CD8+ T-cells [7].

PGE2, a COX-2 product acting as a molecular mediator of inflammation and known to be involved in macrophage M2 polarization [8], contributes to suppress the cytotoxicity of natural killer (NK) cells and CD8+ cytotoxic T lymphocytes (CTLs). Moreover, PGE2 induces the expression of Foxp3, a transcription factor that stimulates the differentiation of immunosuppressive regulatory T-cells (Tregs) from naïve T-cells [9]. Another important immunosuppressive effect of PGE2 is the inhibition of the production by monocytes and DCs of CCL-19, a key chemokine that recruits naïve T-cells and activates effector T-cells [10]. Finally, through inhibition of IL-2 signaling, PGE2 promotes a switch from Th1 to Th2 immune responses [11], the first favoring cellular immunity by stimulating IFN-γ and TNF-α production, and, consequently, the cytotoxic activities of macrophages and CTLs.

M2-TAMs-derived TGF-β contributes to immune evasion by affecting both the adaptive and the innate immune responses, as assessed in many tumor types [12][13]. In metastatic urothelial cancer, TGF-β expression was associated with the exclusion of CD8+ T-cells from the tumor parenchyma, and with their delocalization in the fibroblast- and collagen-rich peritumoral stroma [14]. In colorectal cancer, increased TGF-β levels in the TME not only promoted T-cell exclusion but also blocked the acquisition of the Th1 effector phenotype [15].

Among chemokines, macrophages produce CCL-2, CCL-3, CCL-4, CCL-5, CCL-20, and CCL-22 that recruit Tregs to the TME and sustain their survival [16], with consequent inhibition of effector T-cell function.

By secreting arginase 1 (ARG-1) in the TME, M2-TAMs are also able to deplete arginine reservoir, a metabolite with a crucial role in T-cell proliferation and activation [17][18]. Lactic acid produced by tumor cells, known to exert a critical role in inducing M2-like polarization of TAMs, is a key player in promoting ARG-1 expression by macrophages [19]. ARG-1 metabolizes L-arginine to L-ornithine and other anti-inflammatory products, such as urea. L-ornithine, in addition to promote tissue re-modeling and wound healing [20], stimulates cancer cell proliferation, while L-arginine depletion reduces the expression of CD3 ζ-chain in the TCR complex, impairing effector T-cell-mediated responses to tumor antigens [21][22]. Furthermore, by up-regulating ARG-1, M2-TAMs also deplete the

arginine pool for inducible nitric oxide synthase (iNOS), another enzyme that uses arginine to produce nitric oxide (NO), an important mediator of the immune responses against parasites and cancer [23].

Modulation of tryptophan metabolism is another way to affect the immune functions: both human and murine M2-TAMs overexpress indolamine 2,3 dioxygenase (IDO), an enzyme which converts tryptophan to formylkynurenine, and significantly decreases tryptophan availability for T-cells [24][25]. Furthermore, tryptophan depletion induces the stress kinase general control nonderepressible 2 (GCN2), which in turn down-regulates the expression of the CD3 ζ-chain in the TCR complex of CD8+ cytotoxic T-cells, and inhibits the differentiation of Th17 cells (IL-17 producing T-cells, generally considered to be positive regulators of the immune responses) [26][27]. In addition, kynurenine itself is a potent suppressor of T-cell function, since it can induce T-cell death or interfere with TCR signaling.

TAMs-induced immune suppression can be also mediated by the expression of PD-L1/PD-L2 and CD80/CD86, the ligands of the immune checkpoint inhibitory receptors PD-1 and CTLA-4, respectively [28][29]. Moreover, TAMs can sequester anti-immune checkpoint mAbs through the Fcγ receptor present on their cell surface, preventing the interaction of the antibody Fab regions with the target [30]. Indeed, in vivo imaging studies in different murine cancer models demonstrated that after intraperitoneal administration, an anti-PD-1 mAb co-localized with tumor-infiltrating T-cells at early time points, being then captured by TAMs [30]. Other immune checkpoint ligands expressed by TAMs, with a potential direct suppressive effect on tumor-infiltrating T-cells, are B7-H4 (also known as B7x, B7S1 or VTCN1) and V-domain Ig-containing suppressor of T-cell activation (VISTA, also known as PD-1H, B7-H5, DD1α) [31][32][33]. Cells expressing B7-H4 may negatively modulate the immune response by inhibiting T-cell proliferation and production of cytokines [34]. Remarkably, B7-H4 expression on TAMs correlated with the clinical stage in cancer patients [35]. VISTA, instead, is an immunosuppressive molecule expressed either on cells of the myeloid and lymphoid lineages (it seems to acts both as a ligand on APCs and as an inhibitory receptor on T-cells) that reduces T-cell proliferation and cytokine production, while sustaining Tregs function [36]. Consistently, VISTA has been proposed as an independent negative prognostic factor for multiple cancers, among which primary cutaneous melanoma. In fact, a recent study demonstrated a strong correlation between VISTA expression and tumor infiltration by myeloid cells and PD-1+ inflammatory cells. Interestingly, VISTA levels negatively correlated with patients' survival [37]. Unlike the other better characterized immune checkpoints (PD-1, CTLA-4), induced at different stages after immune cells activation, VISTA is constitutively expressed. This property suggests an important homeostatic role of VISTA in regulating the immune system and qualifies VISTA as a promising target of cancer immunotherapy [38]. Modulation of both innate and adaptive immunity, obtained through an antibody targeting VISTA, slowed tumor growth in murine cancer models [39] by promoting a pro-inflammatory TME that favored T-cell infiltration. Furthermore, a recent study showed that VISTA-deficient myeloid cells presented a reduced chemotactic ability and that tumors grown in VISTA-deficient mice were markedly devoid of macrophages [40].

Still unknown is the mechanism through which M2-TAMs hamper anti-tumor immunity by physically preventing CD8+ T-cells from being properly recruited in the TME [41][42]. Fibrosis could represent a possible condition allowing TAMs to inhibit T-cell accumulation within the tumor mass: through interaction with fibroblasts, macrophages are

known to actively participate in tissue re-modeling, inducing collagen synthesis and secretion [43]; furthermore, by producing granulin, M2-TAMs were shown to remodel the ECM [44] and induce fibrosis in the tumor stroma [45][46].

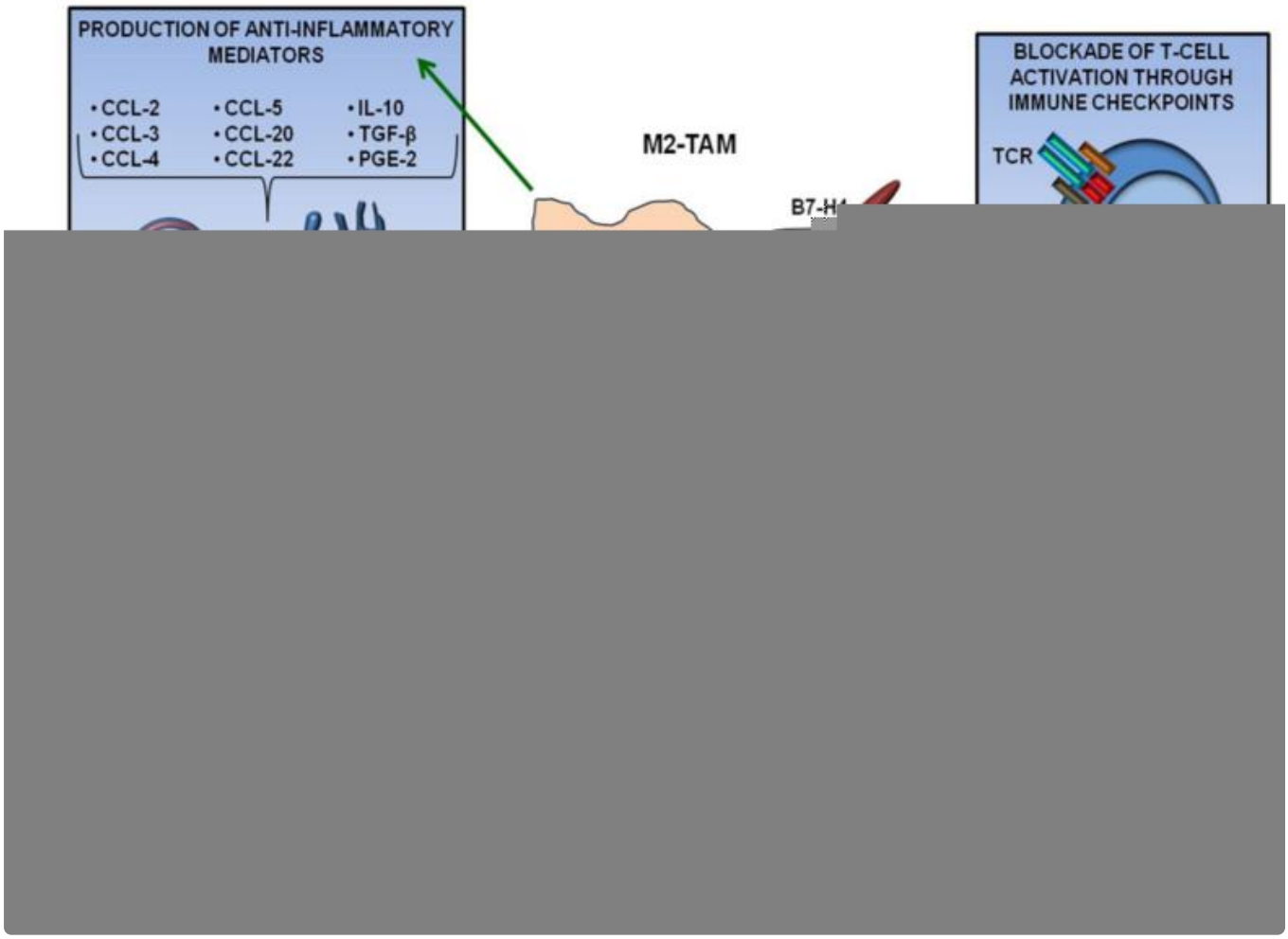

Figure 1. Mechanisms involved in the suppression of anti-tumor immunity mediated by TAMs. Immunosuppressive mechanisms supported by TAMs include: production of anti-inflammatory cytokines and chemokines and other inflammatory mediators that sustain Treg differentiation and hamper dendritic cell function; blockade of T-cell activation through the interaction with inhibitory immune checkpoints; depletion of essential metabolites for T-cell proliferation, such as arginine and tryptophan, due to the expression of specific metabolic enzymes (arginase-1, ARG-1, and indoleamine 2,3-dioxygenase, IDO, respectively); physical hindrance of T-cell recruitment in the TME. See text for further details.

3. Clinical Trials Combining Immune Checkpoint Inhibitors and Tumor-Associated Macrophages Targeting Agents

Data obtained from preclinical studies provided a strong rationale for clinical trials testing removal/re-polarization of immunosuppressive macrophages to overcome resistance to ICIs and/or enhance their anti-tumor activity. Several

studies combining ICIs with immunomodulatory molecules [47][48], resulting in inhibition of M2-TAMs activity, have been carried out or are currently ongoing in melanoma patients (figure 2).

Increase of GM-CSF and decrease of M-CSF (CSF-1) levels are examples of practicable and interesting approaches to re-polarize M2-TAMs into M1-TAMs, currently under investigation in combination with ICIs.
In regard to GM-CSF, phase 2 studies are evaluating the safety and efficacy of the recombinant human analogue (sargramostim) combined with ipilimumab, in patients with unresectable stage III or IV metastatic melanoma (NCT01363206; NCT01134614). Interestingly, in the NCT01363206 trial, the median overall survival evaluated from 22 patients was double, compared to that reported for second-line ipilimumab monotherapy (21.1 months vs. 10.1 months) [49]. Similarly, in the NCT01134614 study carried on a total of 245 patients, during a median follow-up of 13.3 months, the reported values of overall survival were 17.5 months (95% CI; 14.9, not reached) and 12.7 months (95% CI; 10.0, not reached) for the combined treatment and ipilimumab, respectively. Moreover, the 1-year survival rate for the ipilimumab plus sargramostim combination was significantly higher than that of ipilimumab alone (68.9% vs. 52.9%); although no difference in progression-free survival was revealed [50], it is undoubting the promising impact of these results. A currently recruiting phase 2/3 clinical trial, with no data available, is testing the side effects of nivolumab and ipilimumab when given together, with or without sargramostim, in patients with stage III–IV unresectable melanoma (NCT02339571). Used as a vaccine adjuvant, sargramostim is also one of the agents used in a still recruiting phase 2 study (NCT04382664) investigating the efficacy and safety of the cancer vaccine UV1, in combination with nivolumab and ipilimumab, as first-line treatment of adult patients with histologically confirmed unresectable or metastatic melanoma. Another recruiting phase 2 clinical trial (NCT02965716), with no reported results, aims at testing the combination of talimogene laherparepvec (T-VEC) plus pembrolizumab in stage III–IV melanoma patients. T-VEC is an oncolytic, recombinant herpes simplex type-1 virus (HSV) encoding human GM-CSF, which selectively infects and replicates in tumor cells, thereby inducing tumor cell lysis. In addition, the encoded GM-CSF may stimulate a cytotoxic T-cell response against tumor cells, resulting in immune-mediated tumor cell death. Thus, T-VEC would convert the TME from an exhausted to a "hot" immune compartment, and might increase melanoma susceptibility to ICIs. Another recent, not yet recruiting, phase 2 study (NCT04330430) will evaluate T-VEC plus nivolumab in the neoadjuvant setting for resectable early metastatic (stage IIIB/C/D-IV M1a) melanoma. Furthermore, an active phase 1 pilot study (NCT03003676) is testing the safety of ONCOS-102, an engineered oncolytic adenovirus expressing GM-CSF, followed by pembrolizumab, in patients with advanced or unresectable melanoma progressing after PD-1 blockade. On June 2019 the sponsor biotechnology company, announced in a press release that clinical responses were observed in 3 out of 9 patients, corresponding to an overall response rate of 33%, in part 1 of this ONCOS-102 trial.

On the other hand, targeting the M-CSF cytokine is expected to result in M2-TAMs depletion and potential increase of ICI activity. This approach has been investigated in a phase 1b/2 study (NCT02807844) assessing the safety, tolerability, pharmacokinetics, pharmacodynamics, and anti-tumor activity of the anti-M-CSF mAb MCS110 (lacnotuzumab), administered in combination with the experimental anti-PD-1 mAb PDR001 (spartalizumab), to adult patients with solid tumors, including melanoma. As reported, the combination was well tolerated overall and anti-tumor activity was observed, in particular in the pancreatic cancer cohort. The most common (10%) grade ≥3 adverse events were increased aspartate transaminase (12%), asthenia (10%), and hyponatremia (10%), and the

most frequent suspected drug-related adverse events were periorbital edema (30%), increased aspartate transaminase (24%) and blood creatine phosphokinase (24%) [51]. The M-CSF receptor (CSF1R) represents another promising target to reduce the immunosuppressive behavior of TAMs and several ongoing or completed clinical trials were designed in order to evaluate the therapeutic potential of combined CSF1R inhibition and ICIs in patients with solid tumors, such as NCT02829723 (CSF1R inhibitor BLZ945 and anti-PD-1 mAb PDR001, recruiting with no data available), NCT02718911 (CSF1R inhibitor LY3022855 and durvalumab or tremelimumab, completed without published results) and NCT02323191 (anti-CSF1R mAb emactuzumab and atezolizumab, completed but no data are available). A currently still recruiting phase 1/1b clinical trial (NCT03502330) is studying the triple combination of nivolumab, cabiralizumab (a humanized mAb directed against CSF1R) and APX005M (a humanized agonistic mAb that binds to CD40 and acts as an immuno-activating agent by triggering the release of IFN), in advanced melanoma, NSCLC and renal cell carcinoma. APX005M is also under evaluation, in combination with nivolumab, in a phase 1/2 study (NCT03123783) aimed at assessing the safety and efficacy of the co-administered treatment in adult subjects with metastatic melanoma (and NSCLC). Interestingly, published results demonstrated that the combination was associated with a good safety profile and a promising anti-tumor activity in melanoma patients with disease progression during previous anti-PD-1 therapy (anti-CTLA-4 therapy was allowed more than 3 months prior to study entry), and the overall toxicity profile was consistent with the profiles of each individual agent [52].

Due to its involvement in T-cell exhaustion, IDO is another interesting target of therapies aimed at avoiding TAMs-mediated immune evasion and resistance to ICIs. A completed phase 1/2 study (NCT02073123) tested the IDO inhibitor indoximod with ICIs (ipilimumab, pembrolizumab and nivolumab) in adult patients with metastatic stage III/IV melanoma. The combination was well tolerated, most common adverse effects being fatigue, nausea, and pruritus. In terms of efficacy, the indoximod plus pembrolizumab regimen demonstrated an overall response rate of 55.7%, favorably comparable with the reported overall response rate for pembrolizumab alone (33%) [53]. A completed phase 1/2 study (NCT02327078) evaluated the safety, tolerability, and efficacy of another IDO inhibitor, i.e., epacadostat, when administered in combination with nivolumab, in various advanced cancer types, including melanoma. As reported, overall response rate was 62% across all patients, while in treatment-naïve patients it was 65%, including both PD-L1-positive and PD-L1-negative patients. The rate of grade 3 treatment-related adverse events was 48% with epacadostat higher dose (300 mg, twice a day) and 13% with the lower dose (100 mg, twice a day), allowing to conclude that the combination showed promising anti-tumor activity in patients with advanced melanoma and that the lower dosage was well tolerated [54]. Nevertheless, a completed phase 3 study (NCT02752074) assessing the efficacy and safety of epacadostat plus pembrolizumab, used to treat almost one thousand patients with unresectable or metastatic melanoma, posed some doubts about the usefulness of IDO inhibition as a strategy to enhance the efficacy of an anti-PD-1 approach. In fact, the administration of epacadostat plus pembrolizumab twice daily did not significantly improve the progression-free survival and overall survival, if compared with placebo plus pembrolizumab [55]. In another active non-recruiting trial with no shared results (NCT03347123), epacadostat was given in combination with nivolumab and other immunotherapies (ipilimumab or lirilumab), in subjects with advanced or metastatic malignancies, comprising melanoma. Lirilumab is a fully human

mAb that binds to the inhibitory receptors KIRDL1/L2/L3 (specifically expressed by NK cells) and avoid their interaction with HLA-C, lowering the threshold for NK cell activation.

Particularly important is the potential role of combined approaches on controlling brain metastases, a very common event that drastically reduces patient's survival. A phase 2 multicenter clinical trial indicated a promising activity for the combination of ipilimumab and nivolumab also in the central nervous system [56]. An intracranial response rate up to 46% was reported, with higher benefit in patients with asymptomatic untreated brain metastases. However, not all patients obtained substantial benefit from ICI treatment. Importantly, a recent study suggested that IDO enzyme might represent a suitable target in this particular clinical context to enhance the efficacy of ICIs in the brain, being a major product of macrophage/microglia populations infiltrating the TME of melanoma metastases in the central nervous system [57].

As with IDO, ARG-1 is another metabolic enzyme whose inhibition could restore T-cell function, by replenishing arginine storage. A phase 1/2 clinical trial (NCT02903914) is currently testing the efficacy of the ARG-1 inhibitor INCB001158 (or CB-1158), as monotherapy and in combination with pembrolizumab, in patients with advanced/metastatic solid tumors, including melanoma. Results of the ongoing phase 1 study demonstrated that CB-1158 was well tolerated, with no drug-related grade 3 adverse events, and achieved a substantial target inhibition, resulting in increased arginine plasma levels [58].

Given the potential of PI3K inhibition in re-polarizing pro-tumor M2-TAMs into pro-inflammatory M1-TAMs, a phase 1/1b dose-escalation study (NCT02637531) is testing the safety, tolerability, pharmacokinetics and pharmacodynamics of the small-molecule PI3K-inhibitor IPI-549, as monotherapy and in combination with nivolumab, for advanced melanoma and other solid tumors. Interestingly, according to first published results, the IPI-549 plus nivolumab combination demonstrated favorable tolerability, early signs of clinical activity, and immune modulation: patients' blood samples showed evidence of immune activation and reduced immune suppression, in terms of up-regulation of IFN-γ-responsive factors, and dose-dependent proliferation of exhausted PD1+ CD8+ T-cells [59]. A phase 1/2 study (NCT03131908) is also testing the selective PI3K-inhibitor GSK2636771, in combination with pembrolizumab, in patients with refractory metastatic melanoma characterized by the loss of the tumor suppressor PTEN gene. Safety results are available, suggesting that renal toxicity precludes the higher tested doses; although no objective responses have been observed among the 13 treated patients, two patients experienced a prolonged clinical benefit, and in one case a 27% decrease in tumor burden was obtained [60]. A single completed dose-escalation phase 1 clinical trial (NCT02812875) is testing CA-170, an orally available small molecule designed to target VISTA along with PD-L1 and PD-L2, in patients with advanced solid tumors, comprising also melanoma. The rationale for this study, whose data are unpublished, is that compared to mAbs, small-molecule immune checkpoint inhibitors may offer advantages, in terms of oral bioavailability and lower immunogenicity [61].

Figure 2. Recent strategies aimed at targeting TAMs in combination with ICIs for melanoma treatment. The schematic drawing illustrates agents, evaluated in preclinical studies (brown) or clinical trials (blue) for melanoma treatment, acting through agonistic (green arrows or bracket) or antagonistic (red blunted arrows or brackets) mechanisms, in combination with anti-PD-1/PDL-1 or anti-CTLA-4 mAbs. GM-CSF agonists, CSF-1 antagonists and CSF1R inhibitors hamper a signaling pathway involved in M2-TAMs recruitment and polarization. IDO and ARG-1 inhibitors counteract depletion of tryptophan and arginine reservoir, respectively, both required for T-cell activity. The adenyl cyclase is a feasible target of anti-TAMs approaches since it inhibits TLR dependent pro-inflammatory NF-kB signaling, by increasing cAMP levels and promoting ICER expression. The same signaling pathway is negatively regulated by PI3K, thus justifying the experimental use of molecules targeting PI3K- . Consistently, another TAMs reprogramming pharmacological approach is represented by TLR agonists. Finally, the D16F7 mAb, directed against VEGFR-1, counteracts a signaling pathway involved in M2-TAMs chemotaxis and recruitment to the TME.

References

1. Quaranta, V.; Schmid, M.C. Macrophage-Mediated Subversion of Anti-Tumour Immunity. Cells 2019, 8, 747.

2. Li, X.; Shao, C.; Shi, Y.; Han, W. Lessons learned from the blockade of immune checkpoints in cancerimmunotherapy. J. Hematol. Oncol. 2018, 11, 1–26.
3. De Palma, M.; Lewis, C.E. Macrophage Regulation of Tumor Responses to Anticancer Therapies. Cancer Cell2013, 23, 277–286.
4. Shalapour, S.; Karin, M. Pas de Deux: Control of Anti-tumor Immunity by Cancer-Associated Inflammation.Immunity 2019, 51, 15–26.
5. Ouyang, W.; O'Garra, A. IL-10 Family Cytokines IL-10 and IL-22: From Basic Science to Clinical Translation.Immunity 2019, 50, 871–891.
6. Russell, B.; Chang-Strachan, D.; Chan, V.; Rosenbusch, A.; Ho, C.M.; Pryer, N.; Daniel, D.; Hwang, E.S.;Rugo, H.S.; Coussens, L.M. Macrophage IL-10 Blocks CD8+ T Cell-Dependent Responses to Chemotherapyby Suppressing IL-12 Expression in Intratumoral Dendritic Cells. Cancer Cell 2014, 26, 623–637.
7. Smith, L.K.; Boukhaled, G.M.; Condotta, S.A.; Mazouz, S.; Guthmiller, J.J.; Vijay, R.; Butler, N.S.; Bruneau, J.;Shoukry, N.H.; Krawczyk, C.M.; et al. Interleukin-10 Directly Inhibits CD8+ T Cell Function by EnhancingN-Glycan Branching to Decrease Antigen Sensitivity. Immunity 2018, 48, 299–312.e5.
8. Liu, L.; Ge, D.; Ma, L.; Mei, J.; Liu, S.; Zhang, Q.; Ren, F.; Liao, H.; Pu, Q.; Wang, T.; et al. Interleukin-17 andProstaglandin E2 Are Involved in Formation of an M2 Macrophage-Dominant Microenvironment in LungCancer. J. Thorac. Oncol. 2012, 7, 1091–1100.
9. Nakanishi, M.; Rosenberg, D.W. Multifaceted roles of PGE2 in inflammation and cancer. Semin. Immunopathol.2013, 35, 123–137.
10. Muthuswamy, R.; Mueller-Berghaus, J.; Haberkorn, U.; Reinhart, T.A.; Schadendorf, D.; Kalinski, P. PGE2transiently enhances DC expression of CCR7 but inhibits the ability of DCs to produce CCL19 and attractnaive T cells. Blood 2010, 116, 1454–1459.
11. Betz, M.; Fox, B.S. Prostaglandin E2 inhibits production of Th1 lymphokines but not of Th2 lymphokines.J. Immunol. 1991, 146, 108–113.
12. Ghiringhelli, F.; Puig, P.E.; Roux, S.; Parcellier, A.; Schmitt, E.; Solary, E.; Kroemer, G.; Martin, F.; Chauert, B.;Zitvogel, L. Tumor cells convert immature myeloid dendritic cells into TGF-–secreting cells inducingCD4+CD25+ regulatory T cell proliferation. J. Exp. Med. 2005, 202, 919–929.
13. Viel, S.; Marçais, A.; Guimaraes, F.S.-F.; Loftus, R.; Rabilloud, J.; Grau, M.; Degouve, S.; Djebali, S.;Sanlaville, A.; Charrier, E.; et al. TGF- inhibits the activation and functions of NK cells by repressing themTOR pathway. Sci. Signal. 2016, 9, ra19.
14. Mariathasan, S.; Turley, S.J.; Nickles, D.; Castiglioni, A.; Yuen, K.; Wang, Y.; Iii, E.E.K.; Koeppen, H.;Astarita, J.L.; Cubas, R.; et al. TGF attenuates tumour response to PD-L1 blockade by

contributing toexclusion of T cells. Nature 2018, 554, 544–548.

15. Tauriello, D.V.F.; Palomo-Ponce, S.; Stork, D.; Berenguer-Llergo, A.; Badia-Ramentol, J.; Iglesias, M.;Sevillano, M.; Ibiza, S.; Cañellas, A.; Hernando-Momblona, X.; et al. TGF drives immune evasion ingenetically reconstituted colon cancer metastasis. Nat. Cell Biol. 2018, 554, 538–543.
16. DeNardo, D.G.; Ruell, B. Macrophages as regulators of tumour immunity and immunotherapy.Nat. Rev. Immunol. 2019, 19, 369–382.
17. Mazzone, M.; Menga, A.; Castegna, A. Metabolism and TAM functions-it takes two to tango. FEBS J. 2017,285, 700–716.
18. Grzywa, T.M.; Sosnowska, A.; Matryba, P.; Rydzynska, Z.; Jasinski, M.; Nowis, D.; Golab, J. MyeloidCell-Derived Arginase in Cancer Immune Response. Front. Immunol. 2020, 11, 938.
19. Colegio, O.R.; Chu, N.-Q.; Szabo, A.L.; Chu, T.; Rhebergen, A.M.; Jairam, V.; Cyrus, N.; Brokowski, C.E.;Eisenbarth, S.C.; Phillips, G.M.; et al. Functional polarization of tumour-associated macrophages bytumour-derived lactic acid. Nat. Cell Biol. 2014, 513, 559–563.
20. Rath, M.; Müller, I.; Kropf, P.; Closs, E.I.; Munder, M. Metabolism via Arginase or Nitric Oxide Synthase:Two Competing Arginine Pathways in Macrophages. Front. Immunol. 2014, 5, 532.
21. Geiger, R.; Rieckmann, J.C.;Wolf, T.; Basso, C.; Feng, Y.; Fuhrer, T.; Kogadeeva, M.; Picotti, P.; Meissner, F.;Mann, M.; et al. L-Arginine Modulates T Cell Metabolism and Enhances Survival and Anti-tumor Activity.Cell 2016, 167, 829–842.e13.
22. Szefel, J.; Danielak, A.; Kruszewski, W.J. Metabolic pathways of L-arginine and therapeutic consequences intumors. Adv. Med. Sci. 2019, 64, 104–110.
23. Bronte, V.; Zanovello, P. Regulation of immune responses by L-arginine metabolism. Nat. Rev. Immunol.2005, 5, 641–654.
24. Wang, X.; Wang, H.-S.; Wang, H.; Zhang, F.; Wang, K.-F.; Guo, Q.; Zhang, G.; Cai, S.-H.; Du, J. The role ofindoleamine 2,3-dioxygenase (IDO) in immune tolerance: Focus on macrophage polarization of THP-1 cells.Cell. Immunol. 2014, 289, 42–48.
25. Platten, M.; Doeberitz, N.E.K.; Eoezen, I.; Ewick, W.; Eochs, K. Cancer Immunotherapy by TargetingIDO1/TDO and Their Downstream Eectors. Front. Immunol. 2015, 5, 673.
26. Munn, D.H.; Sharma, M.D.; Baban, B.; Harding, H.P.; Zhang, Y.; Ron, D.; Mellor, A.L. GCN2 Kinase in T CellsMediates Proliferative Arrest and Anergy Induction in Response to Indoleamine 2,3-Dioxygenase. Immunity2005, 22, 633–642.
27. Fallarino, F.; Grohmann, U.; You, S.; McGrath, B.C.; Cavener, D.R.; Vacca, C.; Orabona, C.; Bianchi, R.;Belladonna, M.L.; Volpi, C.; et al. The Combined Eects of Tryptophan Starvation and Tryptophan CatabolitesDown-Regulate T Cell Receptor -Chain and Induce a Regulatory Phenotype in Naive T Cells. J. Immunol.2006, 176, 6752–6761.

28. Mantovani, A.; Marchesi, F.; Malesci, A.; Laghi, L.; Allavena, P. Tumour-associated macrophages as treatmenttargets in oncology. Nat. Rev. Clin. Oncol. 2017, 14, 399–416.
29. Guerriero, J.L. Macrophages: The Road Less Traveled, Changing Anticancer Therapy. Trends Mol. Med. 2018,24, 472–489.
30. Arlauckas, S.P.; Garris, C.S.; Kohler, R.H.; Kitaoka, M.; Cuccarese, M.F.; Yang, K.S.; Miller, M.A.; Carlson, J.C.;Freeman, G.J.; Anthony, R.M.; et al. In vivo imaging reveals a tumor-associated macrophage-mediatedresistance pathway in anti-PD-1 therapy. Sci. Transl. Med. 2017, 9, eaal3604.
31. Gao, J.; Ward, J.F.; Pettaway, C.A.; Shi, L.Z.; Subudhi, S.K.; Vence, L.M.; Zhao, H.; Chen, J.; Chen, H.;Efstathiou, E.; et al. VISTA is an inhibitory immune checkpoint that is increased after ipilimumab therapy inpatients with prostate cancer. Nat. Med. 2017, 23, 551–555.
32. Kryczek, I.; Zou, L.; Rodriguez, P.; Zhu, G.; Wei, S.; Mottram, P.; Brumlik, M.; Cheng, P.; Curiel, T.;Myers, L.; et al. B7-H4 expression identifies a novel suppressive macrophage population in human ovariancarcinoma. J. Exp. Med. 2006, 203, 871–881.
33. Ni, L.; Dong, C. New checkpoints in cancer immunotherapy. Immunol. Rev. 2017, 276, 52–65.
34. Li, J.; Lee, Y.; Li, Y.; Jiang, Y.; Lu, H.; Zang, W.; Zhao, X.; Liu, L.; Chen, Y.; Tan, H.; et al. Co-inhibitoryMolecule B7 Superfamily Member 1 Expressed by Tumor-Infiltrating Myeloid Cells Induces Dysfunction ofAnti-tumor CD8+ T Cells. Immunity 2018, 48, 773–786.e5.
35. Ceeraz, S.; Nowak, E.C.; Noelle, R.J. B7 family checkpoint regulators in immune regulation and disease.Trends Immunol. 2013, 34, 556–563.
36. Nowak, E.C.; Lines, J.L.; Varn, F.S.; Deng, J.; Sarde, A.; Mabaera, R.; Kuta, A.; Le Mercier, I.; Cheng, C.;Noelle, R.J. Immunoregulatory functions of VISTA. Immunol. Rev. 2017, 276, 66–79.
37. Kuklinski, L.F.; Yan, S.; Li, Z.; Fisher, J.L.; Cheng, C.; Noelle, R.J.; Angeles, C.V.; Turk, M.J.; Ernsto, M.S.VISTA expression on tumor-infiltrating inflammatory cells in primary cutaneous melanoma correlates withpoor disease-specific survival. Cancer Immunol. Immunother. 2018, 67, 1113–1121.
38. Eltanbouly, M.; Schaafsma, E.; Noelle, R.J.; Lines, J.L. VISTA: Coming of age as a multi-lineage immunecheckpoint. Clin. Exp. Immunol. 2020, 200, 120–130.
39. Le Mercier, I.; Chen, W.; Lines, J.L.; Day, M.; Li, J.; Sergent, P.; Noelle, R.J.; Wang, L. VISTA Regulates theDevelopment of Protective Antitumor Immunity. Cancer Res. 2014, 74, 1933–1944.
40. Broughton, T.W.K.; Eltanbouly, M.A.; Schaafsma, E.; Deng, J.; Sarde, A.; Croteau, W.; Li, J.; Nowak, E.C.;Mabaera, R.; Smits, N.C.; et al. Defining the Signature of VISTA on Myeloid Cell Chemokine Responsiveness.Front. Immunol. 2019, 10, 2641.

41. Rashidian, M.; LaFleur, M.W.; Verschoor, V.L.; Dongre, A.; Zhang, Y.; Nguyen, T.H.; Kolifrath, S.; Aref, A.R.;Lau, C.J.; Paweletz, C.P.; et al. Immuno-PET identifies the myeloid compartment as a key contributor to theoutcome of the antitumor response under PD-1 blockade. Proc. Natl. Acad. Sci. USA 2019, 116, 16971–16980.
42. Peranzoni, E.; Lemoine, J.; Vimeux, L.; Feuillet, V.; Barrin, S.; Kantari-Mimoun, C.; Bercovici, N.; Guérin, M.;Biton, J.; Ouakrim, H.; et al. Macrophages impede CD8 T cells from reaching tumor cells and limit theecacy of anti-PD-1 treatment. Proc. Natl. Acad. Sci. USA 2018, 115, E4041–E4050.
43. Nicolás-Boluda, A.; Donnadieu, E. Obstacles to T cell migration in the tumor microenvironment.Comp. Immunol. Microbiol. Infect. Dis. 2018, 63, 22–30.
44. Afik, R.; Zigmond, E.; Vugman, M.; Klepfish, M.; Shimshoni, E.; Pasmanik-Chor, M.; Shenoy, A.; Bassat, E.;Halpern, Z.; Geiger, T.; et al. Tumor macrophages are pivotal constructors of tumor collagenous matrix.J. Exp. Med. 2016, 213, 2315–2331.
45. Nielsen, S.R.; Quaranta, V.; Linford, A.; Emeagi, P.; Rainer, C.; Santos, A.; Ireland, L.; Sakai, T.; Sakai, K.;Kim, Y.-S.; et al. Macrophage-secreted granulin supports pancreatic cancer metastasis by inducing liverfibrosis. Nat. Cell Biol. 2016, 18, 549–560.
46. Quaranta, V.; Rainer, C.; Nielsen, S.R.; Raymant, M.L.; Ahmed, M.S.; Engle, D.D.; Taylor, A.; Murray, T.;Campbell, F.; Palmer, D.H.; et al. Macrophage-Derived Granulin Drives Resistance to Immune CheckpointInhibition in Metastatic Pancreatic Cancer. Cancer Res. 2018, 78, 4253–4269.
47. Anfray, C.; Ummarino, A.; Andón, F.T.; Allavena, P. Current Strategies to Target Tumor-Associated-Macrophages to Improve Anti-Tumor Immune Responses. Cells 2019, 9, 46.
48. Aris, M.; Mordoh, J.; Barrio, M.M. Immunomodulatory Monoclonal Antibodies in Combined ImmunotherapyTrials for Cutaneous Melanoma. Front. Immunol. 2017, 8, 1024.
49. Kwek, S.S.; Kahn, J.; Greaney, S.K.; Lewis, J.; Cha, E.; Zhang, L.;Weber, R.W.; Leonard, L.; Markovic, S.N.;Fong, L.; et al. GM-CSF and ipilimumab therapy in metastatic melanoma: Clinical outcomes and immunologicresponses. Oncoimmunology 2015, 5, e1101204.
50. Hodi, F.S.; Lee, S.; McDermott, D.F.; Rao, U.N.; Butterfield, L.H.; Tarhini, A.A.; Leming, P.D.; Puzanov, I.;Shin, D.; Kirkwood, J.M. Ipilimumab Plus Sargramostim vs Ipilimumab Alone for Treatment of MetastaticMelanoma: A randomized clinical trial. JAMA 2014, 312, 1744–1753.
51. Calvo, A.; Joensuu, H.; Sebastian, M.; Naing, A.; Bang, Y.-J.; Martin, M.; Roda, D.; Hodi, F.S.; Veloso, A.;Mataraza, J.; et al. Phase Ib/II study of lacnotuzumab (MCS110) combined with spartalizumab (PDR001) inpatients (pts) with advanced tumors. J. Clin. Oncol. 2018, 36, 3014.
52. Kluger, H.; Weiss, S.A.; Olszanski, A.J.; Schuchter, L.; Linette, G.P.; Garland, L.; Iannotti, N.O.;Johnson, M.; Avsar, E.; Srivastava, M.K.; et al. Phase Ib/II of CD40 agonistic antibody

APX005M incombination with nivolumab (nivo) in subjects with metastatic melanoma (M) or non-small cell lung cancer(NSCLC). In Proceedings of the American Association for Cancer Research Annual Meeting, Atlanta, GA, USA,29 March–3 April 2019; American Association Cancer Research: Philadelphia, PA, USA, 2019.

53. Zakharia, Y.; Rixe, O.; Ward, J.H.; Drabick, J.J.; Shaheen, M.F.; Milhem, M.; Munn, D.; Kennedy, E.P.;Vahanian, N.N.; Link, C.J.; et al. Phase 2 trial of the IDO pathway inhibitor indoximod plus checkpointinhibition for the treatment of patients with advanced melanoma. J. Clin. Oncol. 2018, 36, 9512.
54. Daud, A.; Saleh, M.N.; Hu, J.; Bleeker, J.S.; Riese, M.J.; Meier, R.; Zhou, L.; Serbest, G.; Lewis, K.D. Epacadostatplus nivolumab for advanced melanoma: Updated phase 2 results of the ECHO-204 study. J. Clin. Oncol.2018, 36, 9511.
55. Long, G.V.; Dummer, R.; Hamid, O.; Gajewski, T.F.; Caglevic, C.; Dalle, S.; Arance, A.; Carlino, M.S.; Grob, J.-J.;Kim, T.M.; et al. Epacadostat plus pembrolizumab versus placebo plus pembrolizumab in patients withunresectable or metastatic melanoma (ECHO-301/KEYNOTE-252): A phase 3, randomised, double-blindstudy. Lancet Oncol. 2019, 20, 1083–1097.
56. Long, G.V.; Atkinson, V.; Lo, S.; Sandhu, S.; Guminski, A.D.; Brown, M.P.; Wilmott, J.S.; Edwards, J.;Gonzalez, M.A.; Scolyer, R.; et al. Combination nivolumab and ipilimumab or nivolumab alone in melanomabrain metastases: A multicentre randomised phase 2 study. Lancet Oncol. 2018, 19, 672–681.
57. Herrera-Rios, D.; Mughal, S.S.; Teuber-Hanselmann, S.; Pierscianek, D.; Sucker, A.; Jansen, P.; Schimming, T.;Klode, J.; Reifenberger, J.; Felsberg, J.; et al. Macrophages/Microglia Represent the Major Source of Indolamine2,3-Dioxygenase Expression in Melanoma Metastases of the Brain. Front. Immunol. 2020, 11, 120.
58. Papadopoulos, K.P.; Tsai, F.Y.-C.; Bauer, T.M.; Muigai, L.; Liang, Y.; Bennett, M.K.; Orford, K.W.;Fu, S. CX-1158-101: A first-in-human phase 1 study of CB-1158, a small molecule inhibitor of arginase,as monotherapy and in combination with an anti-PD-1 checkpoint inhibitor in patients (pts) with solidtumors. J. Clin. Oncol. 2017, 35, 3005.
59. Sullivan, R.; Hong, D.S.; Tolcher, A.W.; Patnaik, A.; Shapiro, G.; Chmielowski, B.; Ribas, A.; Brail, L.H.;Roberts, J.; Lee, L.; et al. Initial results from first-in-human study of IPI-549, a tumor macrophage-targetingagent, combined with nivolumab in advanced solid tumors. J. Clin. Oncol. 2018, 36, 3013.
60. Tawbi, H.A.-H.; Peng,W.; Phillips, S.; Milton, D.R.; Amaria, R.N.; Diab, A.; Glitza, I.C.; Patel, S.P.;Wong, M.K.;Yee, C.; et al. Safety results from phase I/II study of the PI3K inhibitor GSK2636771 (G) in combinationwith pembrolizumab (P) in patients (pts) with PD-1 refractory metastatic melanoma (MM) and PTEN loss.J. Clin. Oncol. 2020, 38, e22000.

61. Li, K.; Tian, H. Development of small-molecule immune checkpoint inhibitors of PD-1/PD-L1 as a newtherapeutic strategy for tumour immunotherapy. J. Drug Target. 2019, 27, 244–256.