

## Novel insights into old anticancer drugs

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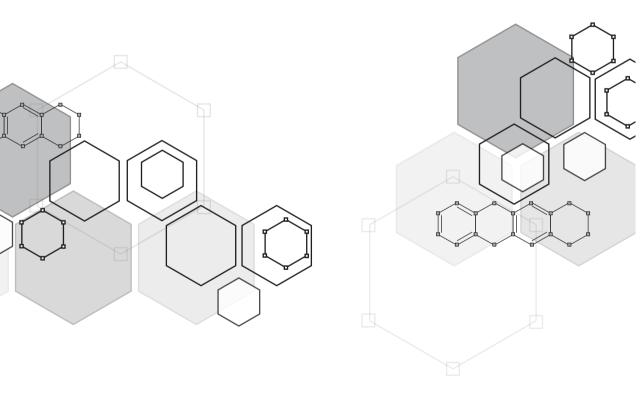
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# Opportunities for small molecules in cancer immunotherapy

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#### **ABSTRACT**

Cancer immunotherapy has proven remarkably successful through instigation of systemic anti-tumor T cell responses. Despite this achievement, further advancements are needed to expand the scope of susceptible cancer types and overcome variation in treatment outcomes between patients. Small-molecule drugs targeting defined pathways and/or cells capable of immune modulation are expected to substantially improve efficacy of cancer immunotherapy. Small-molecule drugs possess unique properties compatible with systemic administration and amenable to both extracellular and intracellular targets. These compounds can modify molecular pathways to overcome immune tolerance and suppression towards effective anti-tumor responses. Here, we provide an overview of how such effects might be achieved by combining immuno-therapy with conventional and/or new small-molecule chemotherapeutics.

#### **ENGAGING IMMUNE PATHWAYS TO TREAT CANCER**

Immunotherapy is rapidly becoming an established cancer treatment next to surgery, chemotherapy, and radiotherapy. In contrast to targeted cancer therapies, immunotherapy relies on tumor-extrinsic mechanisms, which allow it to act on different cancer types in a manner independent of genetic tumor heterogeneity (see Glossary). Its central aim is to activate systemic tumor-specific CD8\* cytotoxic T lymphocyte (CTL) responses against cancer cells. Ideally, a CTL response also eradicates (occult) metastases, even when only the primary tumor has been diagnosed [1]. Immunotherapy strategies include antibody-based 'checkpoint' inhibition, adoptive T cell therapy and therapeutic vaccination [2-5]. Checkpoint blockade using monoclonal antibodies (mAb) against cytotoxic T lymphocyte-associated protein 4 (CTLA-4), programmed death 1 (PD-1), or programmed death ligand 1 (PD-L1) has led to prominent breakthroughs in cancer immunotherapy. Such antibodies are effective boosters of anti-tumor immune responses, but bear the risk of inducing immunerelated adverse events (irAEs) (generally most pronounced for anti-CTLA-4) [6, 7]. Despite its advantages, immunotherapy is successful in only a fraction of patients, and biomarkers broadly predictive of its efficacy remain to be defined. Immune responses to cancer are generally limited by three major bottle-necks: (i) recognition of tumor cells as 'non-self', (ii) peripheral tolerance, and (iii) immunosuppression in the tumor microenvironment (TME). Immunotherapy, on its own or in combination with other strategies, should ideally overcome these bottlenecks. Various combination treatments have been tested to date, with limited success due to lack of synergy or unacceptable toxicity [6]. For instance, combining CTLA-4 and PD-1/ PD-L1 blockades results in stronger anti-tumor responses with unique treatmentlimiting toxicity profiles in melanoma and colorectal cancer patients [8, 9]. Here, use of small-molecule therapies may prove helpful, as such drugs feature a number of advantages over mAbs. Specifically, shorter half-lives of small molecules favor acute and reversible action, as well as reduce the chance of lasting systemic side-effects [10]. In contrast to antibodies, small molecules typically target intracellular proteins and feature distinct toxicity profiles, making them suitable candidates for combination treatments [11, 12]. Moreover, they can be produced at lower costs compared with antibodies and can often be administered orally [11, 12]. Hence, new strategies based on molecular insights of immunological and oncological processes are needed to advance the potential of small molecules in immunotherapy. Here, we provide our perspective on the future of cancer immunotherapy, with emphasis on small molecules expected to improve checkpoint blockade success against cancer (Figure 1; Key Figure, Table 1).

#### IMPROVING TUMOR-SPECIFIC T CELL PRIMING

In order to evoke a T cell response, tumor-derived proteins need to be proteolytically processed into pep-tides, which are subsequently presented by major histocompatibility complex class I and class II molecules (MHCI and MHCII) on the surface of professional antigen-presenting cells, in particular dendritic cells (DCs). T cells in secondary lymphoid organs can then recognize these peptide-MHC complexes via their T cell anti-gen receptors (TCRs). However, to undergo clonal expansion and effector- and memory-differentiation, T cells require additional signals provided by specific costimulatory molecules and cytokines. DCs provide these signals upon pattern recognition receptor (PRR) activation by pathogen-associated molecular pat-terns (PAMPs) or danger-associated molecular patterns (DAMPs), in concert with specific cytokines, such as type I interferons (IFNs), Furthermore, tumor cells must present suitable (neo)antigens (peptides to which no central tolerance has been developed) for recognition by T cells. Tumors with a high mutational load, including melanoma, smoking-induced lung cancers, microsatellite-instable colon cancer, and virus-induced cancers, generally express neoantigens. Hence, these tumors are often immunogenic and raise T cell responses as they develop. Consequently, these cancers can be sensitive to checkpoint blockade [13]. On the other hand, recognition of tumors that are not immunogenic on their own may be facilitated through induction of immunogenic cell death with the help of radiotherapy, certain

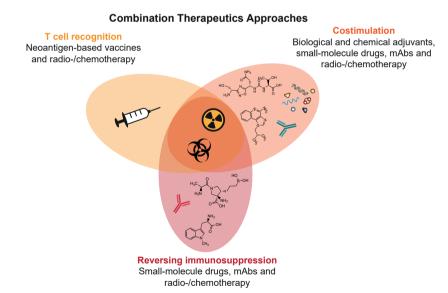


Figure 1, Key Figure. Combination therapeutic approaches in cancer immunotherapy. Neoantigen-based vaccines, conventional chemotherapeutic drugs, radiotherapy, adjuvants, monoclonal antibodies (mAbs), and small-molecule drugs may be designed and targeted to work at different stages of impeded anti-tumor immunity. A combination of strategies can be exploited to ideally boost T cell immunity and overcome tumor-associated immunosuppression.

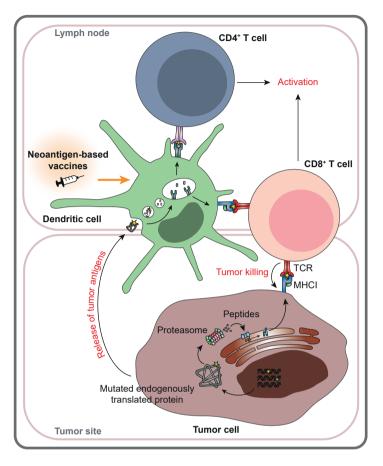
		:	- I - in El - in il O	
largeted pathway	Compound	Function	Clinical Irlais.gov	Cinical trial details
	Cisplatin	DNA crosslinker	NCT02578680 <sup>III</sup>	III Phase 3, randomized, multicenter, double-blind KEYNOTE-189 trial. Combination with anti-PD-1 mAb. First-line treatment of metastatic non-small cell lung cancer (NSCLC).
			NCT02499367 <sup>IV</sup>	IV Phase 2, randomized, single center, non-blinded TONIC trial. Combination with anti-PD-1 mAb. Triple-negative breast cancer (TNBC).
	Doxorubicin	Topoisomerase Ila inhibitor	NCT02499367 <sup>IV</sup>	<sup>IV</sup> Phase 2, randomized, single center, non-blinded TONIC trial. Combination with anti-PD-1 mAb. TNBC.
	Nab-paclitaxel	Microtubule stabilizer	NCT02425891 <sup>V</sup>	<sup>V</sup> Phase 3, randomized, multicenter, double blind IMpassion 130 trial. Combination with anti-PD-L1 mAb. Locally advanced or metastatic TNBC.
	Olaparib	PARP inhibitor	NCT02484404 <sup>VI</sup>	<sup>VI</sup> Phase 1/2, non-randomized trial. Combination with anti-PD-L1 mAb and/or cediranib. Advanced solid tumors and advanced or recurrent ovarian, TNBC, lung, prostate and colorectal cancer.
			NCT02734004 <sup>VII</sup>	VII Phase 1/2 trial, single group assignment. Combination with anti-PD-L1 mAb. Advanced solid tumors.
Standard-of-care drugs / Immunogenic cell death	Tolozonorih	O A DO inhihitor	NCT03964532 <sup>VIII</sup>	VIII Phase 1/2 trial, single group assignment. Combination with anti-PD-L1 mAb. Advanced breast cancer.
	alazopaiio		NCT03330405 <sup>IX</sup>	<sup>IX</sup> Phase 1b/2, non-randomized trial. Combination with anti-PD-L1 mAb. Locally advanced and metastatic solid tumors.
	Rucaparib	PARP inhibitor	NCT03639935 <sup>X</sup>	x Phase 2, multicenter trial, single group assignment. Combination with anti-PD-1 mAb. Advanced or metastatic biliary tract cancer.
	Veliparib	PARP inhibitor		
	Palbociclib	CDK4/6 inhibitor		
	Abemaciclib	CDK4/6 inhibitor		
	Enzalutamide	Androgen receptor antagonist		
	Bicalutamide	Androgen receptor antagonist	NCT03650894 <sup>×1</sup>	X Phase 2 trial, single group assignment. Combination with anti-PD-1 and anti-CTLA-4 mAbs. Advanced breast cancer.
	GTX024	Androgen receptor modulator	NCT02971761 <sup>MI</sup>	<sup>XII</sup> Phase 2 trial, single group assignment. Combination with anti-PD-1 mAb. Androgen receptor positive metastatic TNBC.
	- Inches	to compare a compare	IIX.conoccorto.	MII Phase 1b/2, multicenter, randomized trial. Combination with anti-PD-L1
	- מאסוומון		102200303	IIIAD. LOCAII) auvarioed arid IIIetastaric IIN-positive/IIETX-IIegative Dieast cancer.
	BMS-103	PD-L1 antagonist		
	BMS-142	PD-L1 antagonist		
	BMS-200	PD-L1 antagonist		
	BMS-202	PD-L1 antagonist		
PD-1/PD-L1	BMS-242	PD-L1 antagonist		
	BMS-1001	PD-L1 antagonist		
	BMS-1166	PD-L1 antagonist		
	CA-170	PD-L1, PD-L2, VISTA antagonist NCT02812875 <sup>II</sup>	NCT02812875 <sup>11</sup>	Il Phase 1 trial, single group assignment, dose escalation. Advanced tumors and lymphomas.

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	Imiquimod	TLR7 agonist	NCT03276832 <sup>AV</sup>	<sup>NV</sup> Early phase 1 trial, single group assignment. Combination with anti-PD- 1. Metastatic melanoma.
πRs	Motolimod (VTX-2337)	TLR8 agonist	NCT03906526 <sup>XV</sup>	<sup>30</sup> Phase 1b, multicenter, non-randomized trial. Combination with anti-PD-1 mAb. Head and neck squamous cell carcinoma (HNSCC).
	Resiguimod	Dual TLR7/TLR8 agonist	NCT02126579 <sup>XVI</sup>	<sup>XVI</sup> Phase 1/2, randomized trial. Combination with long peptide vaccination. Melanoma.
			NCT01204684 <sup>XVII</sup>	<sup>XVII</sup> Phase 2, randomized trial. Combination with vaccination. Brain tumors.
	DMXAA/Vadimezan	Murine STING agonist		
	MK-1454	Human STING agonist	NCT03010176 <sup>xxxvII</sup>	xxxvII Phase 1, multicenter, non-randomized trial. Single agent or in combination with anti-PD-1 mAb. Advanced/metastatic solid tumors or lymphomas.
cGAS/STING			NCT02675439 <sup>xxxvIII</sup>	xxxvIII Phase 1, multicenter, non-randomized trial. Single agent and in combination with anti-CTLA-4 mAb. Advanced/metastatic solid tumors and lymphomas.
	ADU-S100	Human STING agonist	NCT03172936 <sup>XXXIX</sup>	XXXX Phase 1b, multicenter, non-randomized trial. Combination with anti-PD. 1 mAb. Advanced/metastatic solid tumors or lymphomas.
			NCT03937141 <sup>xxx</sup>	xxx Phase 2, multicenter trial, single group assignment. Combination with anti-PD-1 mAb. Recurrent or metastatic HNSCC.
	ABZVABZI analogs	Murine/human STING agonist		
	Indoximod	IDO1 inhibitor		
			NCT02178722 <sup>XVIII</sup>	<sup>Will</sup> Phase 1/2, multicenter ECHO-202/KEYNOTE-037 trial. Combination with anti-PD-1 mAb. Multiple advanced solid tumors.
1001	Epacadostat	IDO1 inhibitor	NCT02752074 <sup>XX</sup>	<sup>XX</sup> Phase 3, randomized, double-blind, placebo-controlled ECHO-301- KEYNOTE-252 trial. Combination with anti-PD-1 mAb. Melanoma.
			NCT02318277 <sup>XX</sup>	<sup>37</sup> Phase 1/2 trial, single group assignment. ECHO-203. Combination with anti-PD-L1 mAb. Advanced solid tumors.
	Celecoxib	Dual COX-2/IDO1 inhibitor		
	Melafolone	Dual COX-2/EGFR inhibitor		
	SH-6809	Dual EP <sub>1</sub> /EP <sub>2</sub> antagonist		
	TG4-155	EP <sub>2</sub> antagonist		
:	TG6-129	EP <sub>2</sub> antagonist		
Prostaglandin pathway	PF-04418948	EP <sub>2</sub> antagonist		
	AH6809	EP <sub>1/2</sub> antagonist		
	RQ-07	EP <sub>4</sub> antagonist		
	RQ-15986	EP <sub>4</sub> antagonist		
	AH23848	EP <sub>4</sub> antagonist		
			NCT02903914 <sup>™</sup>	<sup>xxi</sup> Phase 1/2, non-randomized trial. As single agent or in combination with anti-PD-1 mAb. Advanced/metastatic solid tumors.
Arginine metabolism	CB-1158 (INCB001158)	ARG I antagonist	NCT03910530 <sup>xx</sup> 1	XXII Phase 1b, non-randomized trial. As single agent or in combination with a small-molecule PD-1 inhihitor I orally advanced or metastatic solid
<b>)</b>				tumors.
	NCX-4016	Dual ARG-I/iNOS antagonist		
	TA38	Dual ARG-I/iNOS antagonist		

	АТМезя	A2A recentor antegonist	NCT02740985 <sup>XXIII</sup>	XIII Phase 1, multicenter, non-randomized trial. As single agent or in combination with anti-PD-1 mAb. Advanced solid malignancies.
			NCT04089553 <sup>XXV</sup>	XIV Phase 2, non-randomized trial. Combination with anti-PD-1 or anti-CD73 mAbs. Prostate cancer.
	00 444	Cincomplete and an ACA	NCT02655822 <sup>xxv</sup>	<sup>xxv</sup> Phase 1/1b, multicenter, randomized, dose-selection trial. Combination with anti-PD-L1 mAb. Advanced renal cell and prostate cancer.
Adenosine receptor	1	בייסטיט מוימטטורט	NCT03454451 <sup>xxvI</sup>	XXVI Phase 1/1b, multicenter, randomized trial. As single agent and in combination with anti-CD73 and anti-PD-1 mAbs. Advanced tumors.
	PBF-509	A2A receptor antagonist	NCT02403193 <sup>XXVIII</sup>	<sup>xxvII</sup> Phase 1/2b, non-randomized trial. Single agent and in combination with anti-PD-1 mAb. Advanced NSCLC.
	Vipadenant	A2A receptor antagonist		
	Preladenant (SCH-420815, MK-3814	(SCH420815, MK-3814) A2A receptor antagonist	NCT02929862 <sup>xxvIII</sup>	************************************
			NCT03396497 <sup>XXX</sup>	XXX Phase 1b, multicenter trial. Combination with anti-PD-1 mAb. NSCLC.
RORyt transcription factor LYC-55716	r LYC-55716	RORyt agonist		
	Plerixafor (AMD3100)	CXCR4 antagonist		
	AMD070 (AMD11070)	CXCR4 antagonist		
	SX-682	Dual CXCR1/2 inhibitor	NCT03161431 <sup>xxx</sup>	<sup>xxx</sup> Phase 1/2, non-randomized trial. Single agent or in combination with anti-PD-1 mAb. Melanoma.
	AZD5069	CXCR2 antagonist	NCT02583477 <sup>xxx</sup>	<sup>xxx</sup> Phase 1b/2, multicenter, non-randomized trial. Combination with anti- PD-L1 mAb and chemotherapy. Metastatic ductal adenocarcinoma.
	X4P-001	CXCR4 antagonist	NCT02923531 <sup>xxx</sup> II	<sup>xxxI</sup> Phase 1b/2a, single group assignment. Combination with anti-PD-1 mAb. Renal cell carcinoma.
Chemokine receptor	PF-413609	CCR2 antagonist		
	Maraviroc	CCR5 antagonist	NCT03274804 <sup>xxx111</sup>	<sup>xxxIII</sup> Phase 1, single group assignment. Combination with anti-PD-1 mAb. Metastatic colorectal cancer.
	00 00 00 00 00 00 00 00 00 00 00 00 00	to incomplete and a second	NCT03496662 <sup>xxxv</sup>	<sup>xxxv</sup> Phase 1/2, non-randomized trial. Combination with anti-PD-1 mAb and chemotherapy. Locally advanced pancreatic ductal adenocarcinoma.
	001510-0110		NCT03184870 <sup>xxxv</sup>	<sup>xxx</sup> Phase 1/2 trial. Combination with anti-PD-1 mAb or chemotherapy. Metastatic colorectal and pancreatic cancer.
	FLX475	CCR4 inhibitor	NCT03674567 <sup>XXXVI</sup>	**** Phase 1/2, non-randomized dose-escalation trial. As single agent or in combination with anti-PD-1 mAb. Advanced solid tumors.
and was a property of the	GNF351	AhR antagonist		
nypoxic tumor environment	PT2385	HIF-2α antagonist	NCT02293980 <sup>x∞∞</sup>	xxxv Phase 1, non-randomized, dose-escalation trial. Advanced renal cell carcinoma.

Table 1. Small-molecule drugs in cancer immunotherapy. Abbreviations: AhR, Aryl hydrocarbon receptor; ARG I, arginase 1; CCR, C-C chemokine receptor; CXCR, Č-X-C chemokine receptor; CDK, cyclin-dependent kinase; COX-2, cyclo-oxygenase 2; CTLA-4, cytotoxic T IDO1, in-doleamine-2,3-dioxygenase-1; mAb, monoclonal antibody; NSCLC, non-small cell lung cancer; PARP, poly-ADP-ribose polymerase; PD-1, programmed death 1; PD-L1, programmed death ligand 1; RORyt, retinoic acid receptor-related orphan receptor gamma; TLR, toll-like receptor; TNBC, triple-negative breast cancer, VISTA, V-domain Ig containing suppressor of T cell activation. lymphocyte-associated antigen 4; HER2, human epidermal growth factor receptor 2; HNSSC, head and neck squamous cell carcinoma,



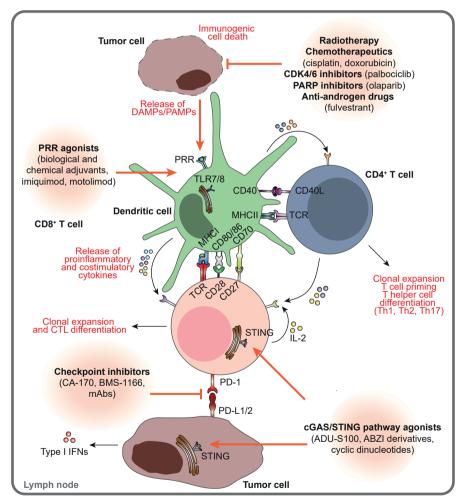
**Figure 2. Promotion of tumor recognition.** In order to be recognized by naïve CD8<sup>+</sup> and CD4<sup>+</sup> T cells, tumor cells must generate (neo)antigens that can be presented by MHCI and MHCII, respectively, on dendritic cells. After activation, T cells clonally expand and differentiate into effector cells that can infiltrate the tumor. Cytotoxic CD8<sup>+</sup> T cells can kill the tumor cells, thus promoting the release of tumor antigens. (Neo)antigen-based vaccines can provide DCs with tumor antigens, and in some cases, boost the tumor-specific CD8<sup>+</sup> T cell response and thereby improve anti-tumor immunity. The yellow arrow illustrates a possible point of interception. Abbreviations: MHC, Major histocompatibility complex; TCR, T cell receptor.

chemotherapeutics, or therapeutic vaccinations (Figures 2 and 3), if not counteracted by suppressive cells in the TME. Tumors often do not supply PAMPs or DAMPs and therefore fail to activate DCs. Immunosuppressive cells or cytokines may further attenuate DC signals. **Thymic regulatory T cells (Tregs)** warrant against autoimmunity by suppressing T cell responses to self-antigens. A key function of thymic Tregs is downregulation of costimulatory ligands CD80 and CD86 on DCs, whereby co-stimulation of conventional T cells by CD28 is attenuated [14]. These mechanisms ordinarily maintain **peripheral tolerance**, a safeguard against autoimmunity, but lack of DC activation constitutes a second major bottleneck in the T cell response against cancer (Figure 3). Biological adjuvants are widely used in this context to promote activation of DCs via PRRs with compounds such as CpG, poly

IC:LC (polyinosinic and polycytidylic acid) or (incomplete) Freund's adjuvant [15]. Here, synthetic approaches could offer ample opportunities for further improvement by boosting therapeutic vaccination (Box 1).

#### Small-molecule drugs targeting PD-1 or PD-L1

The PD-1/PDL-1 axis inhibits TCR and CD28 signaling and can thus limit optimal priming of tumor-specific T cells and their anti-tumor activity [16]. Currently, this axis is targeted by antibodies; however, small-molecule PD-1/PD-L1 antagonists may be useful to reduce toxicity. Some of these appear to act via a novel dimer-locking mechanism (e.g., BMS-103, -142, -200, -202, -242, -1001, and -1166; Table 1) with promising results *in vitro* [17-19]. Another small-molecule antagonist for PD-L1, PD-



**Figure 3. Overcoming peripheral tolerance.** Dendritic cells must receive activating signals in the form of DAMPs and PAMPs, as well as signals from CD4\* T cells, in order to supply the costimulatory signals (via CD27 and CD28) and cytokines (primarily IL-12 or IL-15) needed for clonal expansion and differentiation of newly activated CD8\* T cells. Tumors often do not provide these activating signals, even when their antigens are recognized by T cells.

#### **BOX 1. Combining chemical adjuvants with antigenic vaccines**

Excellent examples of a vaccines aimed at overcoming peripheral tolerance and promoting recognition of tumor cells as 'non-self' are highlighted by recent studies on synthetic long peptides (SLPs) with both CD4<sup>+</sup> and CD8<sup>+</sup> T cell epitopes covalently linked to synthetic ligands that trigger two PRRs: nucleotide-binding oligomerization domain-containing protein 2 (NOD2) and toll-like receptor 2 (TLR2) [103, 104]. The resulting synergy increases proinflammatory cytokine secretion relative to the free TLR and SLP. Investigation of multiple structural combinations of SLPs conjugated to muramyl-dipeptide (MDP), the minimal peptidoglycan component in Freund's adjuvant activating NOD2, and Pam3CSK4, a synthetic lipopeptide activating TLR1/2, revealed enhanced murine DC activation [103]. This in turn led to elevated secretion of vaccine-specific CD8<sup>+</sup> T cells expressing IFNγ and IL-2 *in vitro*, which illustrates the potential of combining chemical adjuvants with antigenic vaccines to boost the anti-tumor response.

L2, and VISTA (CA-170) is currently being evaluated in a Phase I, dose escalation trial (NCT02812875)<sup>I</sup> for patients with advanced tumors and lymphomas (300 participants; primary outcomes measurements were the number of patients with a dose-limiting toxicity in the first treatment cycle, a maximum tolerated dose, and recommended Phase II dose) [20]. However, development of small molecules targeting the PD-1/PD-L1 pathway lags behind that of mAb, due to challenges in designing molecules to occupy the hydrophobic PD-1/PD-L1 interface with high affinity.

#### Therapeutic vaccination

Therapeutic vaccines aim to prime tumor-specific CD8<sup>+</sup> T cells to generate a CTL response. For optimal CTL priming, CD4<sup>+</sup> T cell help is required. Therefore, therapeutic vaccines encompass specific antigens for CD4<sup>+</sup> and CD8<sup>+</sup> T cells, as well as compounds to activate DC [21]. Leading strategies use synthetic long peptides (SLP) (around 20-40 amino acids in length) or antigen-encoding mRNA or DNA, encompassing both MHCI and MHCII epitopes to ensure CD8<sup>+</sup> CTL priming and CD4<sup>+</sup> T cell help for a robust CTL response [5]. These vaccines have shown a degree of therapeutic promise in treating early stage virus-induced cancers [22]. Addi-

Figure 3. Continued. Dendritic cell activation can be induced by biological- and small-molecule adjuvants, or by small-molecule PRR agonists targeted at extracellular or intracellular PRRs. Additionally, treatment of the tumor with selected standard-of-care (chemo)therapeutics or radiation can induce immunogenic cell death and thereby stimulate neoantigen release. STING agonists can induce type I IFN production, promoting DC activation and T cell priming. To evade CD8+ T cell killing, tumor cells can upregulate suppressive molecules such as PD-L1/2. Suboptimally primed CD8+ T cells that have not experience CD4+ T cell help express PD-1. To block the PD-1/PD-L1 interaction, different monoclonal antibodies (mAbs) or small-molecule checkpoint inhibitors have and are being developed. Orange arrows indicate possible points of interception; pointed and flat arrowheads indicate activation and inhibition, respectively. Drugs between brackets are examples of small-molecule drugs or biologicals targeting the indicated proteins/cells. Abbreviations: CTL, cytotoxic T lymphocyte; DAMP, danger-associated molecular pattern; IFN, interferon; MHC, major histocompatibility complex; PAMP, pathogen-associated molecular pattern; PD-1, programmed death 1; PD-L1/2, programmed death ligand 1/2; PRR, pattern recognition receptor; STING, stimulator of IFN genes; TCR, T cell receptor; TLR, toll-like receptor.

tionally, a recent Phase Ib randomized glioblastoma trial (NCT02287428)" indicated that vaccination with a multi-epitope, personalized neoantigen successfully induced intratumoral neoantigen-specific CD4<sup>+</sup> and CD8<sup>+</sup> immune responses, according to single-cell T cell receptor analysis [23]. However, all patients included in the study eventually relapsed, suggesting that tumor-associated immunosuppression and/or other challenges represented a significant and persistent bottleneck.

#### Small molecules targeting toll-like receptors (TLRs)

The first small-molecule immuno-oncology drug approved by the FDA for the treatment of basal cell carcinoma was imiguimod, an imidazoguinoline derivative, commonly used in the treatment of genital warts [24]. Imiguimod targets toll-like receptor 7 (TLR7), a PRR that binds conserved PAMPs, such as double-stranded RNA, lipopolysaccharide, or unmethylated CpG DNA [25]. Most TLRs are expressed on the cell surface, but TLR3, 7, 8, and 9 locate predominantly in endosomes [26]. A small-molecule TLR8 agonist, motolimod (VTX-2337), exhibits anti-tumor activity in recurrent or metastatic head and neck squamous cell carcinomas (HNSCC), by stimulation of natural killer (NK) cells and enhanced antibody-dependent cell-mediated toxicity [27]. Motolimod treatment in combination with cetuximab (an anti-EGFR antibody) or conventional chemo-therapy resulted in a decrease of Tregs in the TME, elevation of circulating EGFR-specific CD8<sup>+</sup> T cells and increase in progression-free and overall survival in a subset of HNSCC patients in, as compared with cetuximab or chemotherapy alone [28, 29]. Imiguimod, motolimod, and resiguimod (relatives of imiquimod targeting TLR7 and TLR8), are currently under investigation in a number of clinical trials (NCT03276832)XIV, (NCT03906526)XV, (NCT02126579)XVI, (NCT01204684)XVIII for treatment of solid tumors, typically as adjuvants to vaccination. Thus, the search for small molecules targeting other (and preferably multiple) TLRs continues, often using high-throughput screening of drug libraries in cell-based assays [30]. Other PRRs, such as NOD-like receptors (NLRs), C-type lectin receptors (CLRs) or RIG-I-like receptors (RLRs) have been less extensively studied, but agonists targeting these families are likely to enhance immune responsiveness and are currently being developed [31].

## Small molecules targeting the cyclic-GMP-AMP synthase (cGAS)/stimulator of IFN genes (STING) pathway

STING is a PRR on the endoplasmatic reticulum membrane that binds cyclic dinucleotides derived from cytosolic DNA converted by cGAS. Activation of the cGAS/STING pathway leads to type I IFN production, which promotes DC activation and T cell priming, as shown in tumor-bearing mice [32], highlighting STING as a putative target for cancer immunotherapy (Box 2). The STING pathway is regulated by ectonucleotide pyrophosphatase/phosthodiesterase-1 (ENPP1) that hydrolyzes cGAMP and thereby controls activation of the signaling cascade. As a consequence, various attempts are made to activate the STING pathway by inhibition of ENPP1 [33-35]. However, other studies report that cGAS/STING signaling can induce indoleamine-2,3-dioxygenase (IDO1; a tryptophan catabolic enzyme found to induce immunosuppression and immunoevasion [36]) and suppress homologous-mediated DNA repair, thus dampening the immune response and promoting tumor growth in a Lewis lung carcinoma mouse model [37, 38]. These studies suggest that more research is needed on the function of the cGAS/STING pathway in cancer immunity before we understand the effects of STING agonist therapies sufficiently.

#### BOX 2. STING as a target for cancer immunotherapy

Intratumoral injection of small-molecule STING agonist DMXAA (5,6-dimethylxantheone-4-acetic acid) in mice showed specificity and efficacy in controlling B16 melanoma tumor outgrowth (of both injected and distant tumors in the same animal) [105]. However, this drug was ineffective in humans because of structural differences with murine STING [106]. Considerable efforts to create derivatives of DMXAA active against human STING are ongoing [107]. For example, among three amidobenzimidazole (ABZI)-based small-molecule STING agonists reported in the same study. the most potent compound was shown to bind several human and one murine isoforms of STING with high affinity, inducing dose dependent activation of STING and secretion of IFNB in human PBMCs, and its intravenous delivery strongly reduced subcutaneous CT26 colon tumor growth in mice [108]. Also, in a high-grade serous carcinoma mouse model, a cyclic dinucleotide STING agonist, combined with anti-PD-1 antibodies and chemotherapy, showed increased survival and decreased tumor burden compared to single treatments [109]. Transcriptomic tumor analysis revealed elevated expression of IFN response and antigen-presenting genes for tumors treated with the STING agonist over control samples. Various STING small agonist are currently tested in early phase clinical trials: MK-1454 (NCT03010176) XXXVII, ADU-S100 (NCT02675439)XXXVIII, and (NCT03172936)XXXIX. However, preliminary results for ADU-S100 presented at the American Society of Clinical Oncology (ASCO) meeting in 2019 showed that only 6 out of 83 patients achieved confirmed responses, with a single complete response (CR), 3 partial responses (PR) among PD-1 naïve TNBC patients, and 2 PRs among previously immunotherapy-treated melanoma patients [110]. A multicenter, Phase II trial combining ADU-S100 and anti-PD-1 antibodies to as-sess safety and efficacy as first-line treatment of PD-L1- positive recurrent or metastatic HNSCC is ongoing (NCT03937141)XXXX.

#### Immunogenic capacity of standard-of-care therapy

Radiotherapy and chemotherapy can directly kill tumor cells, but they may also enhance anti-tumor immunity. The prevailing idea is that these treatments may induce immunogenic cell death, characterized by the release of tumor antigens and danger signals (e.g., cytosolic DNA) capable of activating DCs via PRRs, such as toll like receptors (TLRs) and cGAS/STING [39, 40]. Remarkable effects were reported when standard-of-care therapy was followed by immunotherapy [41], as illustrated by cisplatin treatment and CTLA-4 inhibition in a lung epithelial tumor mouse model [42]. Furthermore, based on the Phase III KEY-NOTE-189 trial (NCT02578680)", the combination of pembrolizumab (anti-PD-1 mAb) with cisplatin and pemetrexed is now FDA approved as first-line treatment for metastatic non-small cell lung cancer (NSCLC) [43]. Moreover, anthracycline drugs such as doxorubicin can induce type I IFN production in a fibrosarcoma mouse model and selectively deplete immunosuppressive myeloid derived suppressor cells (MDSCs) in a murine breast cancer model, which impairs tumor development in vivo [44, 45]. The recent Phase II, single center TONIC trial (NCT02499367)<sup>IV</sup> showed that the combination of either cisplatin (overall response (OR) 23%) or doxorubicin (OR 35%) with nivolumab (anti-PD-1 mAb) improves treatment outcomes of triple-negative breast cancer (TNBC) patients relative to anti-PD-1 alone [46]. Similarly, atezolizumab (anti-PD-L1 mAb) in combination with paclitaxel, a chemotherapeutic that blocks mitosis via stabilization of microtubules, is now FDA approved for treatment of locally advanced or metastatic TNBCs. This was based on the Phase II IMpassion130 trial (NCT02425891)<sup>V</sup>, showing improved progression-free survival for the combination therapy over chemotherapy alone. Other chemotherapeutics that have been reported to boost the effects of checkpoint inhibitors in pre-clinical trials and are under evaluation in clinical trials include: cyclophosphamide; platinum drugs, such as **oxaliplatin**; and **PARP inhibitors** olaparib (NCT02484404)<sup>VI</sup>, (NCT02734004)<sup>VII</sup>, talazoparib (NCT03964532)<sup>VIII</sup>, (NCT03330405)<sup>IX</sup>, rucaparib (NCT03639935)<sup>X</sup> and veliparib. In addition, various cyclin-dependent kinase 4 and 6 inhibitors (CDK4/6i), including palbociclib and abemaciclib, as well as anti-androgen drugs enzalutamide, bicalutamide (NCT03650894) <sup>XI</sup>, GTX-024 (NCT02971761)<sup>XII</sup>, and fulvestrant (NCT03280563)<sup>XIII</sup>, are also being tested in combination approaches with checkpoint blockade for various cancers in pre-clinical and clinical trials [47-52].

#### **ENABLING T CELL ACTIVITY IN THE TUMOR MICROENVIRONMENT**

The TME and the signals it exudes in concert with the T cell response may lead to a state of immunosuppression. The TME might be hypoxic (Box 3) and it may present physical barriers that exclude T cells or express inhibitory molecules, such as IDO1 and PD-L1, that can directly inhibit effector T cell function [53]. Furthermore, it can express cytokines such as transforming growth factor  $\beta$  (TGF- $\beta$ ) and IL-10 that can alter cellular phenotypes (e.g., macrophages) and modulate the function of CD4<sup>+</sup> T cells and promote Treg generation and expansion, thereby inhibiting effector T cell

#### **BOX 3. Targeting the hypoxic environment in many solid tumors**

Hypoxia is often observed in solid tumors and can induce a plethora of effects promotive of tumor growth and metastases. A critical signaling molecule in hypoxia is hypoxia-induced factor (HIF-1α classical helix-loop-helix (HLH) transcription factor. Under oxygen-rich conditions, HIF-1a interacts with VHL in the cytosol, resulting in its ubiquitination and degradation by the proteasome [111]. Under hypoxic conditions, HIF-1α translocates into the nucleus and pairs with the aryl hydrocarbon receptor (AhR) nuclear translocator protein (ARNT) [112]. The HIF1α-ARNT dimer mediates transcription of hypoxia-specific genes that stimulate erythropoiesis, metabolism and angiogenesis, but also induce PD-L1 expression and Treg differentiation [113, 114]. Inhibition of HIF-1α transcription in these hypoxic tumors could prevent tumor outgrowth, as well as improve immune responses, such as in the case of AhR antagonist GNF351, shown to decrease migra-tion and invasion of HNSCC tumor cell lines in vitro [115]. Recently, the first HIF-2α antagonist PT2385, which inhibits its interaction with ARNT, has been tested in a Phase I, nonrandomized, dose-escalation trial (NCT02293980)XXXXI for safety and efficacy in patients with advanced renal cell carcinoma. They show that the drug is well tolerated, with clinical benefit observed in 66% of the patients [116]. Many other HIF-1 inhibitors are currently under development. These drugs have not been tested in combination with immunotherapy, but a synergistic effect on tumor control through modulation of the TME can be expected. One of the adaptive responses of tumor cells to hypoxia involves increased expression of carbonic anhydrase IX (CA IX), an enzyme located at the cell surface of tumors that catalyzes conversion of carbon dioxide to bicarbonate ions and protons. CA IX expression increases adaptation of tumor cells to a hypoxic TME and con-fers an increased ability to migrate and metastasize [117, 118]. The last years, various CA IX mAbs and small-molecule inhibitors have been developed as potential anticancer therapies or for tumor-imaging purposes [119, 120].

responses [54]. Additionally, the TME may attract or create suppressive immune cells, including arbitrarily designated MDSCs, Tregs and certain **tumor-associated macrophages (TAMs)**, which would render T cells dysfunctional and attenuate the efficacy of immunotherapy [55]. Elucidating and targeting immunosuppression and -evasion mechanisms may help improve clinical outcomes. Small-molecule drugs may also be used to spe-cifically target suppressive factors and induce or restore immune reactivity in the TME (Figure 4).

#### **Targeting IDO1**

One such a TME target is IDO1. IDO1 is the most broadly expressed of three enzymes (together with IDO2 and tryptophan 2,3-dioxyenase (TDO)), involved in the first step of the kynurenine pathway. The immuno-suppressive effects of IDO1/kynurenine include Treg cell expansion and recruitment of MDSCs. IDO1 de-prives

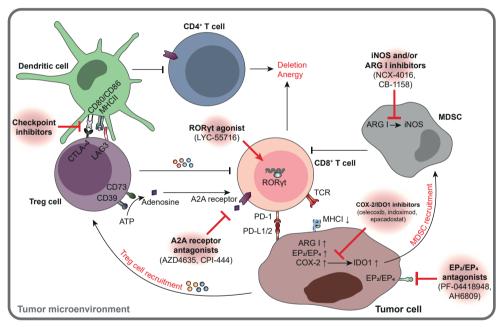


Figure 4. Reversing immunosuppression in the tumor microenvironment. Tumor cells. as well as immune- and stromal cells in the tumor microenvironment, can collaborate to establish an immunosuppressive environment, through upregulation of inhibitory molecules, such as PD-L1 and IDO1, conversion of conventional CD4\* T cells into Tregs, alteration of cytokine profiles, hypoxia, recruitment of suppressive cell types such as myeloid-derived suppressor cells (MDSCs), and the production/upregulation of specific proteins and metabolites. Suppression may be relieved by small-molecule drugs targeted at relevant mechanisms and, in combination with checkpoint blockade this could enhance the anti-tumor response. Red lines indicate possible points of interception; pointed and flat arrowheads indicate activation and inhibition, respectively. Drugs between brackets are examples of small-molecule drugs or biologicals targeting the indicated proteins/cells. Abbreviations: ARG, Arginase; COX-2, cyclooxygenase 2; CTLA-4, cytotoxic T lymphocyte-associated antigen 4; IDO1,indoleamine-2,3-dioxygenase; iNOS, nitric oxide synthase; LAG-3, lymphocyte-activation gene 3; MHC, major histocompatibility complex; PD-1, programmed death 1; PD-L1/2, pro-grammed death ligand 1/2; RORyt, retinoic acid receptor-related orphan receptor gamma; TCR, T cell receptor; Treg, regulatory T cell.

effector T cells of tryptophan, which is required for CTL activation and antagonizes CD8<sup>+</sup> T cell effector function by PD-1 expression [56, 57]. Indoximod was the first IDO1 inhibitor tested in humans, but with confounding results, as this compound might in fact inhibit mTORC1, a downstream effector of IDO1 [58], Epacadostat, a specific and more potent IDO1 inhibitor, in combination with pembrolizumab (anti-PD-1 mAb) showed promising results in the Phase I/II ECHO-202/KEYNOTE-037 trial (NCT02178722)XVIII for patients with multiple advanced solid tumors, but did not increase anti-PD-1 efficacy in a Phase III clinical trial in melanoma patients (NCT02752074)XIX [59]. Likewise, epacadostat was combined safely with anti-PD-L1 in the Phase I/II ECHO-203 trial (NCT02318277)xx for advanced solid tumors, but yielded no combined responses in these patients [60]. Consequently, various pharmaceutical companies have stopped or are downsizing the development of IDO1 inhibitors, which significantly curtails the early clinical development of these types of small molecules [61]. Further robust research is needed to optimize the timing of IDO1 inhibitor administration in combination with checkpoint blockade antibodies. In addition, patient selection and testing of other IDO1 inhibitors will be warranted to ideally find a more successful combination regimen. The kynurenine produced in the IDO1 pathway is an endogenous ligand for the aryl hydrocarbon receptor AhR, a transcription factor that regulates immunological responses [62]. Inhibition of AhR may therefore be an alternative for IDO1 inhibitors. Crosstalk was also observed between IDO1 and the amino acid-sensing kinase general control nonderepressible 2 (GCN2), which is important in inflammation and viability of cancer cells in the TME [63, 64]. Therefore, efforts are made to test the potential of GCN2 antagonists as anticancer drugs [64].

#### Small molecules targeting the prostaglandin pathway

One of the drivers of IDO1 expression is cyclooxygenase 2 (COX-2), an underexplored target in cancer immunotherapy, but a common target of nonsteroidal antiinflammatory drugs (NSAIDs) [65]. COX-2 catalyzes the synthesis of prostaglandins. lipid compounds involved in the response to injury and inflammation. This enzyme is expressed in several cancers and therefore, celecoxib, an NSAID that inhibits COX-2 as well as IDO1, is being explored for cancer therapy [66]. One study developed analogs of celecoxib and showed a potent cytostatic effect on melanoma and colon cancer cell lines in vitro [67]. Concurrent inhibition of COX-2 and EGFR was previously reported to have a synergistic effect on cell proliferation and apoptosis in NSCLC cell lines in vitro [68]. Dual inhibition of COX-2 and EGFR by melafolone (a naturally occurring flavonoid) shows improved effects of PD-1 blockade in a Lewis lung carcinoma and lung carcinoma mouse model through vascular normalization and PD-L1 downregulation [69]. These studies demonstrate the potential of combination therapies targeting multiple tumor-associated molecules simultaneously. Downstream of the COX-2 signaling pathway are the G protein-coupled prostanoid receptors EP2 and EP4, which bind prostaglandin E2 (PGE2). Signaling via the prostaglandin pathway through EP2 and/or EP4 has been implicated in establishment of an immunosuppressive environment by blocking DC activity, redirection of DC differentiation towards suppressive phenotypes and suppression of macrophages [70]. Consequently, interest in small-molecule antagonists targeting these receptors is growing and various EP2 and EP4 an-tagonists are being developed (e.g., AH6809, AH23848, TG6-129, TG4-155, PF-04418948, RQ-07, and RQ-15986; Table 1) [70-72]. Dual inhibition of EP2 and EP4 in combination with checkpoint inhibitors shows increased production of antigen-specific proinflammatory cytokines by tumor-derived CTLs in epithelial ovarian cancer ex vivo [73]. Thus, manipulating the signaling of prostaglandins in the TME may boost anti-tumor immunity.

## Targeting arginine metabolism to overcome the immunosuppressive function of MDSCs and TAMs

Arginase is another potential therapeutic target in the TME. This ubiquitous manganese-containing enzyme catalyzes the hydrolysis of L-arginine to L-ornithine and urea and plays an important role in various aspects of inflammation [74]. Mammals express two isoforms of the enzyme: the cytoplasmic arginase I (ARG I), predominantly in the liver, and arginase II (ARG II) in the mitochondrial matrix. In the TME. MDSCs and TAMs can release high amounts of ARG I into the extracellular space to locally deplete arginine concentrations and thereby impair TCR signaling and proliferation [75]. In T cell cocultures, ARG I inhibitor CB-1158 (INCB001158) blocks the myeloid cell-mediated immunosuppression of T cell proliferation, reducing tumor growth in different mouse models [76]. Furthermore, profiling the TME shows that CB-1158 treatment increases expression of interferon-inducible genes, inflammatory cytokines, and tumor-infiltrating NK and CD8<sup>+</sup> T cells, compared with controls [76]. This drug is currently being tested as single agent and in combination with anti-PD-1 mAb and small-molecule inhibitors in two early stage clinical trials for advanced/ metastatic solid tumors (NCT02903914)<sup>XXI</sup>, (NCT03910530)<sup>XXII</sup>. Another enzyme expressed at high levels in MDSCs and TAM is the nitric oxide synthase (iNOS), iNOS hydrolyzes L-arginine into nitric oxide (NO), which subsequently suppresses T cell function via interference with the JAK3-STAT5 signaling pathway [77]. When ARG-I is inhibited, iNOS has more substrate for NO production, resulting in immunosuppression via the formation of nitrogen species [77]. To overcome this, dual ARG I/ iNOS inhibitors, such as NCX-4016 and TA38, have recently been developed and will be tested in the near future [78, 79].

#### **Targeting Tregs in the TME**

Tregs can express extracellular ectonucleotidases CD39 and CD73; membrane molecules that produce adenosine via dephosphorylation of ATP. Adenosine can subsequently bind to A2A or A2B receptors on the surface of conventional T cells and was found to thereby inhibit CD8<sup>+</sup> T cell infiltration in a melanoma tumor mouse model [80]. Adenosine can also bind to A2A receptors on Tregs, resulting in expansion of the Treg population to strengthen their immunosuppressive effects *in vitro* [81]. To relieve Treg-mediated suppression in the TME, small-molecule A2A antagonists, such as CPI-444, AZD4635, vipadenant, preladenant (SCH-420815, MK3814, MSD), and PBF-509, have been developed [82-84]. These compounds are currently tested in Phase I and II clinical trials either alone or in combination with anti-PD-1 or anti-PD-L1 inhibitors for various solid tumors (NCT02740985)<sup>XXIII</sup>, (NCT04089553) XXIV, (NCT02655822)<sup>XXV</sup>, (NCT03454451)<sup>XXVII</sup> and (NCT02403193)<sup>XXVIII</sup>.

Another Treg target is retinoic acid receptor-related orphan receptor gamma (RORyt), a transcription factor involved in the proinflammatory IL-17 pathway in T cells. RORyt agonists can induce the production of cytokines and chemokines, decrease the proliferation of Tregs, and revoke immunosuppression by tumor cells [85]. Synthetic small-molecule RORyt agonists promote activity, proliferation, and survival of Th17 (CD4+) and Tc17 (CD8+) cells *in vitro* relative to the endogenous agonist desmosterol, and result in enhanced Th17 effector function in an adoptive T cell therapy mouse model [86, 87]. Two Phase II clinical trials have been designed to test the effects of these agonists, one to test safety and tolerability as a single drug (NCT02929862)

xxviii and the other to test safety/tolerability either alone or in combination with anti-PD-1 mAb in NSCLC (NCT03396497)xxix. The outcome of these trials is difficult to predict, since Th17 cells have been associated with poor prognosis in a number of cancer types [88-90]. In these cases, RORγt antagonists might provide therapeutic benefit. However, design of inhibitors is complicated because RORγt has a large and lipophilic ligand-binding domain [91]. Furthermore, stimulating RORγt may promote autoimmune disorders, such as inflammatory bowel disease [92]. Thus, considering that the 'classical' checkpoint inhibitors anti-CTLA-4 and anti-PD-1 antibodies can also induce autoimmunity, it is necessary to caution that this type of combination might induce strong side effects.

#### Small molecules targeting chemokine receptors

Chemokines and their receptors guide both tumor cells to metastatic locations and immune cells to defined tissues. The chemokine receptor CXCR4 is frequently activated in cancer cells and contributes to epithelial-mesenchymal transition, invasion. metastasis, and tumor vascularization [93, 94]. A series of small-molecule antagonists of chemokine receptors have been developed, of which one of the most wellknown, plerixafor (AMD3100), has reported efficacy in acute lymphocytic leukemia and relapsed acute myeloid leukemia [95-97]. Plerixafor reduced primary tumor growth and suppressed metastasis in combination with chemotherapy in a small cell lung cancer xenograft mouse model [98]. CXCR4 inhibition by plerixafor counteracted CXCL12-dependent upregulation of PD-L1 in the TME and recruitment of immunosuppressive Tregs and M2 macrophages [99]. This study in a hepatocellular carcinoma mouse model showed that CXCR4 inhibition in combination with anti-PD-1 mAb and sorafenib inhibits tumor growth, reduces lung metastasis, and im-proves survival [99]. The chemokine receptor CXCR2, overexpressed in various cancers, is correlated with poor prognosis in human pancreatic ductal adenocarcinoma patients [100]. CXCR2 inhibition prevent entry of MDSCs into the TME in pancreatic-, breast- and colorectal cancer mouse models and has therefore been suggested to sensitize tumors to immunotherapy [100-102]. Currently, a Phase I/ II, nonrandomized trial is recruiting melanoma patients to test the safety and efficacy of the dual CXCR1/2 inhibitor SX-682, as single drug or in combination with anti-PD-1 mAb (NCT03161431)<sup>xxx</sup>. Other chemokine receptor-targeting small molecules are under evaluation in pre-clinical and clinical trials as single agents or in combination with checkpoint blockade. These include CXCR2 antagonist AZD5069 (NCT02583477)XXXI, CXCR4 inhibitor X4P-001 (NCT02923531)XXXII, CCR2 inhibitor PF-413609, CCR5 inhibitor maraviroc (NCT03274804)XXXIII, dual CCR2/5 antagonist BMS-813160 (NCT03496662)XXXIV, (NCT03184870)XXXV and CCR4 inhibitor FLX475 (NCT03674567)XXXVI [84].

#### What will the future bring?

The field of cancer immunotherapy is exploding, and a new phase of directed and specific modulation of immune responses by small molecules is taking hold. There are many exciting developments and it is likely that new small molecules will be explored in combination with anti-PD-1/PD-L1 or anti-CTLA-4 blocking mAbs in the near future. The number of potential targets for small molecules has dramatically increased by a novel therapeutic strategy that induces specific protein degradation by proteolysis-targeting chimeras (PROTACs) (Box 4). We anticipate that these PROTACs will greatly expand the options to manipulate im-mune responses. These, along with other novel drug developments are expected to further expand the arse-

## BOX 4. Small-molecule-based proteolysis-targeting chimeras (PROTACS) in cancer immunotherapy

PROTACs are bifunctional hybrid molecules, consisting of two ligands connected by a linker. The first ligand targets a protein of interest, while the second ligand targets an E3 ubiquitin ligase. By bridging a protein of interest to the E3 ubiquitin ligase, PROTACs engage the ubiquitin-proteasome system to degrade the protein of interest [121]. Numerous studies have shown that targeting (onco-)proteins for degradation presents a successful strategy in anti-cancer therapy in vitro [122, 123]. This is illustrated by small-molecule-based PROTACs against FKBP12 and BTK, which have shown rapid (24-72 hours) and global knockdown of their targets in different organs of mice and non-human primates, highlighting their potential for further clinical testing in the context of putative cancer therapies in human patients [124]. Meanwhile, development of small-molecule PROTACS is rapidly increasing, and recently, the first orally bioavailable PROTAC drug (ARV-110) targeting the androgen receptor has been approved for a Phase I, single group assignment, dose escalation clinical trial to evaluate its safety, tolerability, pharmacokinetics, and pharmacodynamics in metastatic castration-resistant prostate cancer (NCT03888612)XXXXIII. which is currently recruiting patients. Although there are numerous issues to solve before broad clinical application of PROTACs is possible, including their cellular permeability and stability, these agents constitute a major focus in drug development. offering a conceptually simple and general approach. Indeed, PROTAC compounds can selectively induce specific peptide presentation by MHCI molecules, indicating that this strategy can promote neoantigen presentation on tumor cells [125]. PRO-TACS could then inhibit tumor growth and sensitize tumor cells for CTL mediated elimination.

nal of approaches heading into the future of cancer treatment.

#### **CONCLUDING REMARKS**

Targeting the PD-1/PD-L1 and CTLA-4 signaling pathways in immunotherapy can induce potent anti-tumor CTL responses in patients with various cancer types. However, only a subset of patients responds and available treatment combinations often coincide with severe adverse events. Therefore, novel treatment op-tions are essential for further improvement of cancer immunotherapy efficacy, all while lowering toxicity. We propose that small-molecule drugs provide opportunities for improving treatment success. Small molecules can easily penetrate into tissues compared with most antibodies and can therefore be directed towards both extracellular and intracellular targets to promote anti-tumor immunity. Additionally, their half-lives are general-ly short, lowering their chance for adverse effects. Because of these features, there is extensive interest in the development of small-molecule-based strategies in the cancer immunotherapy field. The lasting challenge is to rationally select chemoimmunotherapy combinations, that are based on known molecular mechanisms underlying the lack of immune activation against cancer and subvert this state. Also, focus should be on optimizing dose and timing of these combination treatments to maximize their synergistic effect. There are many targets to evaluate in the space of chemo-immunotherapy and only few combinations have been evaluated or are currently tested. Thus, the future of chemo-immunotherapy remains broad and exciting (Figure 1).

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#### **GLOSSARY**

**Central tolerance:** the absence of self-reactive T cells to avoid autoimmunity. T cells that recognize self-antigens are deleted during negative selection in the thymus. **Checkpoint blockade:** inhibition of immune checkpoints PD-1/PD-L1 or CTLA4.

Cisplatin: platinum-based chemotherapeutic, functions by interfering with DNA replication.

**Cytotoxic T lymphocyte (CTL):** (generally CD8<sup>+</sup>) killer T cell that recognizes intracellular alterations in the context of major histocompatibility class I complexes expressed on all tissues.

**Danger-associated molecular patterns (DAMPs):** danger signals released by damaged or dying cells, such as cytosolic or nuclear proteins, or DNA. Binding of DAMPs to pattern recognition receptors induces innate immunity and DC activation. **Doxorubicin:** anthracycline chemotherapeutic that induce cell death by DNA double strand break formation via inhibition of topoisomerase II and the induction of chromatin damage.

**Heterogeneity:** here, phenotypical variations between cells of the same cancer in one patient, often of genetic origin, that affect therapy response and hamper treatment design.

**Immune-related adverse events (irAEs):** inflammatory side effects that may occur during immune therapy. Any organ system can be affected, but irAEs most commonly involve the gastrointestinal tract, endocrine glands, skin, and liver.

**Immunogenic cell death:** form of cell death resulting in the release of immune-stimulating factors.

**Immunosuppression:** here, inhibition of immunity induced by tumor cells and their microenvironment that results in escape from elimination.

**Microsatellite instability:** genetic predisposition to mutation caused by the loss of DNA mismatch repair activity.

**Myeloid-derived suppressor cells (MDSCs):** population of immature myeloid cells that are presumed to have a strong immunosuppressive function in the tumor microenvironment.

**Neoantigen:** tumor antigen arising from somatic DNA mutations, so that no central tolerance has been raised. T cells may be able to recognize these antigens and attack tumor cells expressing them.

**Oxaliplatin:** platinum-based chemotherapeutic, functions by interfering with DNA synthesis.

**PARP inhibitors:** pharmacological inhibitors of poly-ADP-ribose polymerase, which plays a role in DNA repair, genomic stability, and programmed cell death.

**Pathogen-associated molecular patterns (PAMPs):** molecules found on/in microorganisms that trigger innate immunity by binding pattern recognition receptors. Classic PAMPs are double-stranded RNA, endotoxins, or bacterial cell wall constituents.

**Pemetrexed:** antifolate chemotherapeutic that interferes with folate-dependent metabolic processes essential for replication.

Peripheral tolerance: suppression in the periphery of self-reactive immune cells

that have escaped central tolerance.

**Regulatory T cells (Tregs):** subset of CD4<sup>+</sup> T cells that modulate the immune response by suppressing effector cells.

Sorafenib: small-molecule kinase inhibitor for the Raf/Mek/Erk pathway.

**Toll-like receptors:** single-pass membrane-spanning receptors that plays a key role in the innate immune response.

**Tumor-associated macrophages (TAMs):** macrophages present in the tumor microenvironment of solid tumors, usually associated with an unfavorable prognosis due to their immunosuppressive function.

All clinical trials described in this manuscript are registeren with ClinicalTrials.gov, and refences to these trials can be found online: https://www.cell.com/trends/immunology/fulltext/S1471-4906(20)30069-7

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