



Universiteit
Leiden
The Netherlands

Novel insights into old anticancer drugs

Zanden, S.Y. van der

Citation

Zanden, S. Y. van der. (2021, March 2). *Novel insights into old anticancer drugs*. Retrieved from <https://hdl.handle.net/1887/3135058>

Version: Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/3135058>

Note: To cite this publication please use the final published version (if applicable).

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/3135058> holds various files of this Leiden University dissertation.

Author: Zanden, S.Y. van der

Title: Novel insights into old anticancer drugs

Issue date: 2021-03-02

Opportunities for small molecules in cancer immunotherapy

8



Sabina Y. van der Zanden, Jolien J. Luimstra, Jacques Neeffjes, Jannie Borst and Huib Ovaa

Trends in Immunology (2020)

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 **immune-related 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 treatment-limiting 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 (MHC I and MHC II) 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-liable** 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

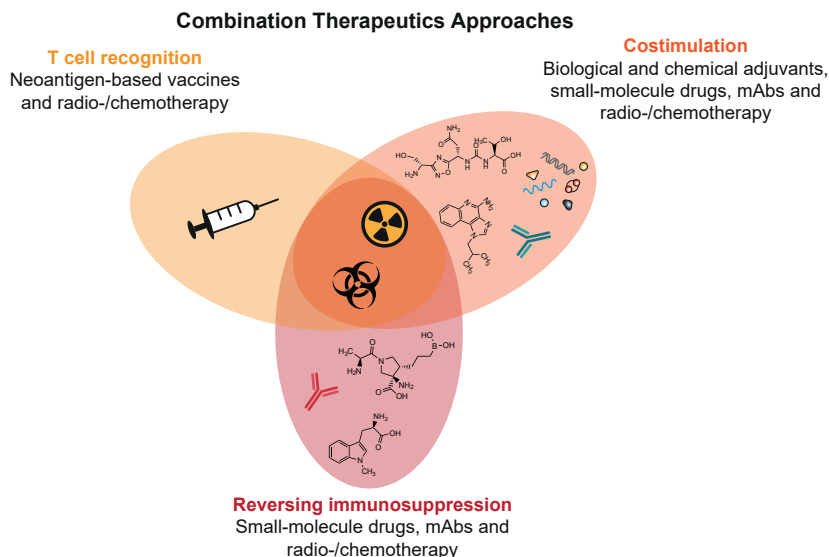


Figure 1, Key Figure. Combination therapeutic approaches in cancer immunotherapy. Neoantigen-based vaccines, conventional chemotherapeutic drugs, radiotherapy, adjuvants, monoclonal antibodies (mAbs), and small-molecule drugs may be designed and targeted to work at different stages of impeded anti-tumor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

Targeted pathway	Compound	Function	ClinicalTrials.gov	Clinical trial details
Standard-of-care drugs / Immunogenic cell death	Cisplatin	DNA crosslinker	NCT02578680 ^{III}	^{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).
	Doxorubicin	Topoisomerase I α inhibitor	NCT02499367 ^{IV}	^{IV} Phase 2, randomized, single center, non-blinded TONIC trial. Combination with anti-PD-1 mAb. Triple-negative breast cancer (TNBC).
			NCT02499367 ^{IV}	^{IV} Phase 2, randomized, single center, non-blinded TONIC trial. Combination with anti-PD-1 mAb. TNBC.
	Nab-paclitaxel	Microtubule stabilizer	NCT02425891 ^V	^V Phase 3, randomized, multicenter, double blind IMpassion130 trial. Combination with anti-PD-L1 mAb. Locally advanced or metastatic TNBC.
	Olaparib	PARP inhibitor	NCT02484404 ^{VI}	^{VI} 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 ^{VII}	^{VII} Phase 1/2 trial, single group assignment. Combination with anti-PD-L1 mAb. Advanced solid tumors.
	Talazoparib	PARP inhibitor	NCT03964532 ^{VIII}	^{VIII} Phase 1/2 trial, single group assignment. Combination with anti-PD-L1 mAb. Advanced breast cancer.
			NCT03330405 ^{IX}	^{IX} Phase 1b/2, non-randomized trial. Combination with anti-PD-L1 mAb. Locally advanced and metastatic solid tumors.
	Rucaparib	PARP inhibitor	NCT03639935 ^X	^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 ^{XI}	^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 ^{XII}	^{XII} Phase 2 trial, single group assignment. Combination with anti-PD-1 mAb. Androgen receptor positive metastatic TNBC.
PD-1/PD-L1	Fulvestrant	Estrogen receptor antagonist	NCT03280563 ^{XIII}	^{XIII} Phase 1b/2, multicenter, randomized trial. Combination with anti-PD-L1 mAb. Locally advanced and metastatic HR-positive/HER2-negative breast cancer.
	BMS-103	PD-L1 antagonist		
	BMS-142	PD-L1 antagonist		
	BMS-200	PD-L1 antagonist		
	BMS-202	PD-L1 antagonist		
	BMS-242	PD-L1 antagonist		
	BMS-1001	PD-L1 antagonist		
	BMS-1166	PD-L1 antagonist		
	CA-170	PD-L1, PD-L2, VISTA antagonist	NCT02812875 ^{II}	^{II} Phase 1 trial, single group assignment, dose escalation. Advanced tumors and lymphomas.

TLRs	Imiquimod	TLR7 agonist	NCT03276832 ^{xv}	^{xv} Early phase 1 trial, single group assignment. Combination with anti-PD-1. Metastatic melanoma.
	Motolimod (VTX-2337)	TLR8 agonist	NCT03906526 ^{xv}	^{xv} Phase 1b, multicenter, non-randomized trial. Combination with anti-PD-1 mAb. Head and neck squamous cell carcinoma (HNSCC).
	Resiquimod	Dual TLR7/TLR8 agonist	NCT02126579 ^{xvi}	^{xvi} Phase 1/2, randomized trial. Combination with long peptide vaccination. Melanoma.
	DMXAA/Vadimezan	Murine STING agonist	NCT01204684 ^{xvii}	^{xvii} Phase 2, randomized trial. Combination with vaccination. Brain tumors.
cGAS/STING	MK-1454	Human STING agonist	NCT03010176 ^{xxxvi}	^{xxxvi} Phase 1, multicenter, non-randomized trial. Single agent or in combination with anti-PD-1 mAb. Advanced/metastatic solid tumors or lymphomas.
	ADU-S100	Human STING agonist	NCT02675439 ^{xxxviii}	^{xxxviii} Phase 1, multicenter, non-randomized trial. Single agent and in combination with anti-CTLA-4 mAb. Advanced/metastatic solid tumors and lymphomas.
			NCT03172936 ^{xxxix}	^{xxxix} Phase 1b, multicenter, non-randomized trial. Combination with anti-PD-1 mAb. Advanced/metastatic solid tumors or lymphomas.
			NCT03937141 ^{xxxx}	^{xxxx} Phase 2, multicenter trial, single group assignment. Combination with anti-PD-1 mAb. Recurrent or metastatic HNSCC.
IDO1	ABZVABZi analogs	Murine/human STING agonist		
	Indoximod	IDO1 inhibitor	NCT02178722 ^{xviii}	^{xviii} Phase 1/2, multicenter ECHO-202/KEYNOTE-037 trial. Combination with anti-PD-1 mAb. Multiple advanced solid tumors.
	Epacadostat	IDO1 inhibitor	NCT02752074 ^{xix}	^{xix} Phase 3, randomized, double-blind, placebo-controlled ECHO-301-KEYNOTE-252 trial. Combination with anti-PD-1 mAb. Melanoma.
			NCT02318277 ^{xx}	^{xx} Phase 1/2 trial, single group assignment. ECHO-203. Combination with anti-PD-L1 mAb. Advanced solid tumors.
Prostaglandin pathway	Celecoxib	Dual COX-2/IDO1 inhibitor		
	Melafolone	Dual COX-2/EGFR inhibitor		
	SH-6809	Dual EP ₁ /EP ₂ antagonist		
	TG4-155	EP ₂ antagonist		
	TG6-129	EP ₂ antagonist		
	PF-04418948	EP ₂ antagonist		
	AH6809	EP _{1/2} antagonist		
Arginine metabolism	RQ-07	EP ₄ antagonist		
	RQ-15986	EP ₄ antagonist		
	AH23848	EP ₄ antagonist		
	CB-1158 (NCB001158)	ARG1 antagonist	NCT02903914 ^{xxi}	^{xxi} Phase 1/2, non-randomized trial. As single agent or in combination with anti-PD-1 mAb. Advanced/metastatic solid tumors.
			NCT03910530 ^{xxii}	^{xxii} Phase 1b, non-randomized trial. As single agent or in combination with a small-molecule PD-1 inhibitor. Locally advanced or metastatic solid tumors.
	NCX-4016	Dual ARG-1/INOS antagonist		
	TA38	Dual ARG-1/INOS antagonist		

Adenosine receptor	AZD4635	A2A receptor antagonist	NCT02740985 ^{xxii} NCT04089553 ^{xxv}	xxiii Phase 1, multicenter, non-randomized trial. As single agent or in combination with anti-PD-1 mAb. Advanced solid malignancies. xxiv Phase 2, non-randomized trial. Combination with anti-PD-1 or anti-CD73 mAbs. Prostate cancer.
	CPI-444	A2A receptor antagonist	NCT02655822 ^{xxv} NCT03454451 ^{xxvi}	xxv Phase 1/1b, multicenter, randomized, dose-selection trial. Combination with anti-PD-1 mAb. Advanced renal cell and prostate cancer. 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 ^{xxvii}	xxvii 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)	A2A receptor antagonist	NCT02929862 ^{xxviii} NCT03396497 ^{xxix}	xxviii Phase 1/2a, multicenter trial. Locally advanced metastatic solid tumors. xxix Phase 1b, multicenter trial. Combination with anti-PD-1 mAb. NSCLC.
	RORγ1 transcription factor			
	LYC-55716	RORγ1 agonist		
	Plerixafor (AMD3100)	CXCR4 antagonist		
	AMD070 (AMD11070)	CXCR4 antagonist		
	SX-682	Dual CXCR1/2 inhibitor	NCT03161431 ^{xxx}	xxx Phase 1/2, non-randomized trial. Single agent or in combination with anti-PD-1 mAb. Melanoma.
Chemokine receptor	AZD5069	CXCR2 antagonist	NCT02583477 ^{xxxi}	xxxi Phase 1b/2, multicenter, non-randomized trial. Combination with anti-PD-1 mAb and chemotherapy. Metastatic ductal adenocarcinoma.
	X4P-001	CXCR4 antagonist	NCT02923531 ^{xxxi}	xxxi Phase 1b/2a, single group assignment. Combination with anti-PD-1 mAb. Renal cell carcinoma.
	PF-413609	CCR2 antagonist		
	Maraviroc	CCR5 antagonist	NCT03274804 ^{xxxiii}	xxxiii Phase 1, single group assignment. Combination with anti-PD-1 mAb. Metastatic colorectal cancer.
	BMS-813160	Dual CCR2/5 antagonist	NCT03496662 ^{xxxiv}	xxxiv Phase 1/2, non-randomized trial. Combination with anti-PD-1 mAb and chemotherapy. Locally advanced pancreatic ductal adenocarcinoma.
			NCT03184870 ^{xxxv}	xxxv Phase 1/2 trial. Combination with anti-PD-1 mAb or chemotherapy. Metastatic colorectal and pancreatic cancer.
	FLX475	CCR4 inhibitor	NCT03674567 ^{xxxvi}	xxxvi Phase 1/2, non-randomized dose-escalation trial. As single agent or in combination with anti-PD-1 mAb. Advanced solid tumors.
	GNF351	AHR antagonist		
	PT2385	HIF-2α antagonist	NCT02293980 ^{xxxix}	xxxix 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 1, arginase 1; CCR, C-C chemokine receptor; CXCR, C-X-C chemokine receptor; CDK, cyclin-dependent kinase; COX-2, cyclo-oxygenase 2; CTLA-4, cytotoxic T lymphocyte-associated antigen 4; HER2, human epidermal growth factor receptor 2; HNSCC, head and neck squamous cell carcinoma, 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; RORγt, 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.

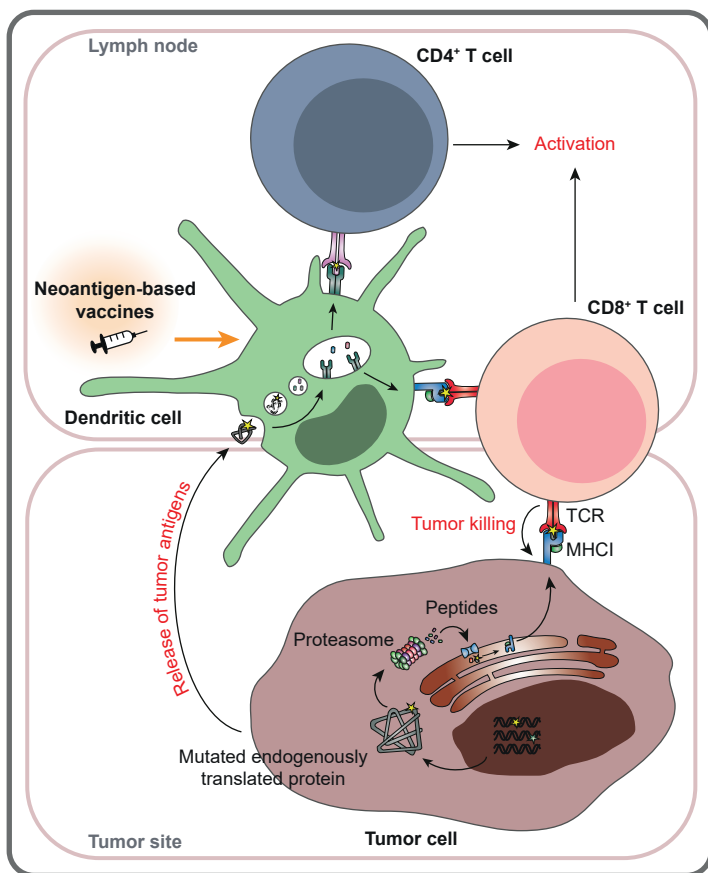


Figure 2. Promotion of tumor recognition. In order to be recognized by naïve CD8⁺ and CD4⁺ T cells, tumor cells must generate (neo)antigens that can be presented by MHC I and MHC II, respectively, on dendritic cells. After activation, T cells clonally expand and differentiate into effector cells that can infiltrate the tumor. Cytotoxic CD8⁺ 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⁺ 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-

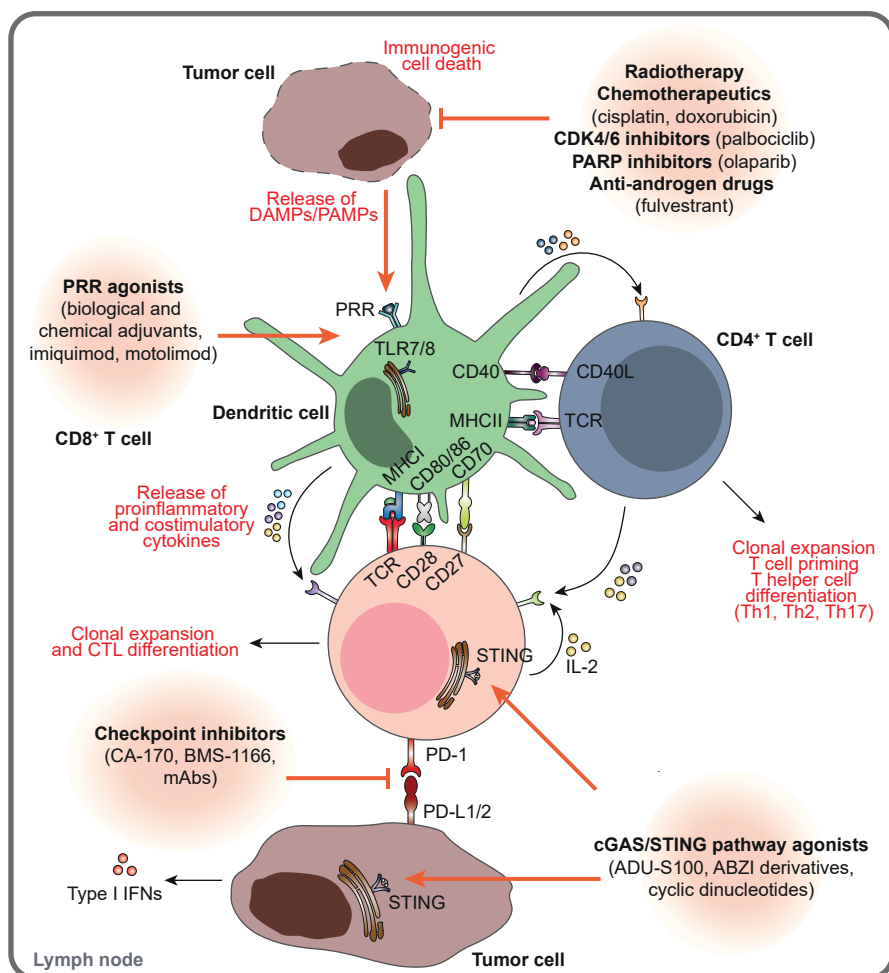


Figure 3. Overcoming peripheral tolerance. Dendritic cells must receive activating signals in the form of DAMPs and PAMPs, as well as signals from CD4⁺ T cells, in order to supply the costimulatory signals (via CD27 and CD28) and cytokines (primarily IL-12 or IL-15) needed for clonal expansion and differentiation 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⁺ and CD8⁺ 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⁺ T cells expressing IFN γ 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)^l 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⁺ T cells to generate a CTL response. For optimal CTL priming, CD4⁺ T cell help is required. Therefore, therapeutic vaccines encompass specific antigens for CD4⁺ and CD8⁺ 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 MHC I and MHC II epitopes to ensure CD8⁺ CTL priming and CD4⁺ 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)ⁱⁱ indicated that vaccination with a multi-epitope, personalized neoantigen successfully induced intratumoral neoantigen-specific CD4⁺ and CD8⁺ 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 imiquimod, an imidazoquinoline derivative, commonly used in the treatment of genital warts [24]. Imiquimod 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⁺ 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]. Imiquimod, motolimod, and resiquimod (relatives of imiquimod targeting TLR7 and TLR8), are currently under investigation in a number of clinical trials (NCT03276832)^{xiv}, (NCT03906526)^{xv}, (NCT02126579)^{xvi}, (NCT01204684)^{xvii} 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/phosphodiesterase-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-dimethylxanthine-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 IFN β 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 assess 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)ⁱⁱⁱ, 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)^{iv} 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)^v, 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)^{vi}, (NCT02734004)^{vii}, talazoparib (NCT03964532)^{viii}, (NCT03330405)^{ix}, rucaparib (NCT03639935)^x 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)^{xi}, GTX-024 (NCT02971761)^{xii}, and fulvestrant (NCT03280563)^{xiii}, 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 β (TGF- β) and IL-10 that can alter cellular phenotypes (e.g., macrophages) and modulate the function of CD4⁺ 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-1 α classical helix-loop-helix (HLH) transcription factor. Under oxygen-rich conditions, HIF-1 α interacts with VHL in the cytosol, resulting in its ubiquitination and degradation by the proteasome [111]. Under hypoxic conditions, HIF-1 α translocates into the nucleus and pairs with the aryl hydrocarbon receptor (AhR) nuclear translocator protein (ARNT) [112]. The HIF1 α -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-1 α 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 migration and invasion of HNSCC tumor cell lines *in vitro* [115]. Recently, the first HIF-2 α 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 confers 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 anti-cancer 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 specifically 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-dioxygenase (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

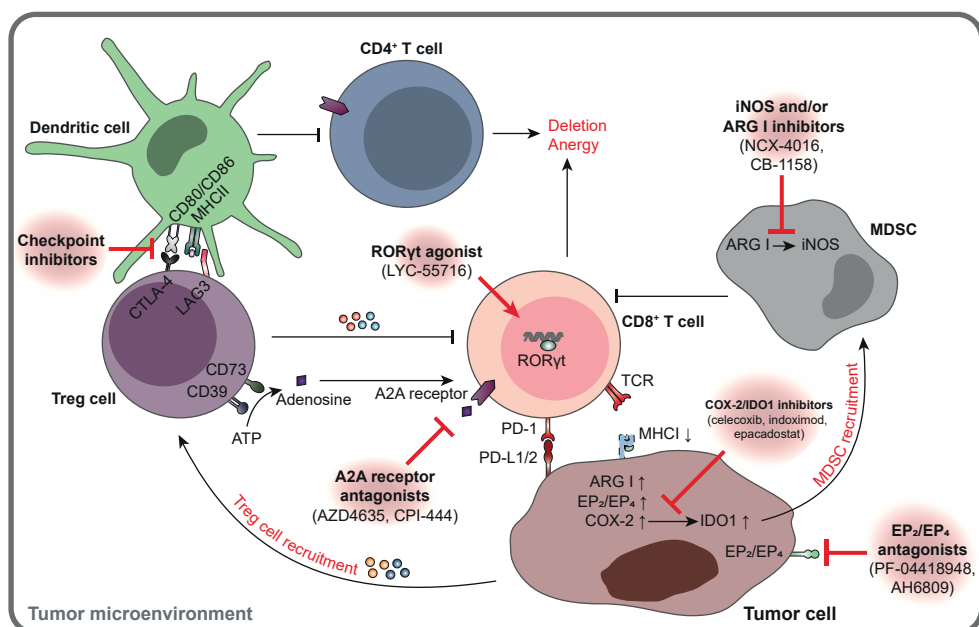


Figure 4. Reversing immunosuppression in the tumor microenvironment. Tumor cells, as well as immune- and stromal cells in the tumor microenvironment, can collaborate to establish an immunosuppressive environment, through upregulation of inhibitory molecules, such as PD-L1 and IDO1, conversion of conventional 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, programmed death ligand 1/2; RORγt, 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⁺ 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 anti-inflammatory 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 antagonists 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⁺ 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)^{xxi}, (NCT03910530)^{xxii}. 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⁺ 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)^{xxiii}, (NCT04089553)^{xxiv}, (NCT02655822)^{xxv}, (NCT03454451)^{xxvi} and (NCT02403193)^{xxvii}.

Another Treg target is retinoic acid receptor-related orphan receptor gamma (RORγt), a transcription factor involved in the proinflammatory IL-17 pathway in T cells. RORγt agonists can induce the production of cytokines and chemokines, decrease the proliferation of Tregs, and revoke immunosuppression by tumor cells [85]. Synthetic small-molecule RORγt 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 well-known, 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 improves 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)^{xxx}. 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 immune 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)^{xxxxii}, 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 MHC I molecules, indicating that this strategy can promote neoantigen presentation on tumor cells [125]. PROTACS 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 options 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 generally 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 chemo-immunotherapy 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).

ACKNOWLEDGMENTS

We thank Ilana Berlin for critical reading and editing of the manuscript. This work was supported by grants from the Institute for Chemical Immunology (ICI, to H. Ovaa, J. Borst and J. Neefjes). This work is part of Oncode Institute, which is partly financed by the Dutch Cancer Society.

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⁺) 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⁺ 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 registered with ClinicalTrials.gov, and references to these trials can be found online: [https://www.cell.com/trends/immunology/fulltext/S1471-4906\(20\)30069-7](https://www.cell.com/trends/immunology/fulltext/S1471-4906(20)30069-7)

REFERENCES

1. Koebel, C. M., Vermi, W., Swann, J. B., Zerafa, N., Rodig, S. J., Old, L. J., Smyth, M. J. & Schreiber, R. D. (2007) Adaptive immunity maintains occult cancer in an equilibrium state, *Nature*. 450, 903-7.
2. Sharma, P. & Allison, J. P. (2015) The future of immune checkpoint therapy, *Science*. 348, 56-61.
3. Ribas, A. & Wolchok, J. D. (2018) Cancer immunotherapy using checkpoint blockade, *Science*. 359, 1350-1355.
4. Hammerl, D., Rieder, D., Martens, J. W. M., Trajanoski, Z. & Debets, R. (2018) Adoptive T Cell Therapy: New Avenues Leading to Safe Targets and Powerful Allies, *Trends Immunol.* 39, 921-936.
5. Melief, C. J., van Hall, T., Arens, R., Ossendorp, F. & van der Burg, S. H. (2015) Therapeutic cancer vaccines, *J Clin Invest.* 125, 3401-12.
6. Ciccarese, C., Alfieri, S., Santoni, M., Santini, D., Brunelli, M., Bergamini, C., Licitra, L., Montironi, R., Tortora, G. & Massari, F. (2016) New toxicity profile for novel immunotherapy agents: focus on immune-checkpoint inhibitors, *Expert Opin Drug Metab Toxicol.* 12, 57-75.
7. Postow, M. A., Sidlow, R. & Hellmann, M. D. (2018) Immune-Related Adverse Events Associated with Immune Checkpoint Blockade, *N Engl J Med.* 378, 158-168.
8. Larkin, J., Chiarion-Sileni, V., Gonzalez, R., Grob, J. J., Cowey, C. L., Lao, C. D., Schadendorf, D., Dummer, R., Smylie, M., Rutkowski, P., Ferrucci, P. F., Hill, A., Wagstaff, J., Carlino, M. S., Haanen, J. B., Maio, M., Marquez-Rodas, I., McArthur, G. A., Ascierto, P. A., Long, G. V., Callahan, M. K., Postow, M. A., Grossmann, K., Sznol, M., Dreno, B., Bastholt, L., Yang, A., Rollin, L. M., Horak, C., Hodi, F. S. & Wolchok, J. D. (2015) Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma, *N Engl J Med.* 373, 23-34.
9. Overman, M. J., Lonardi, S., Wong, K. Y. M., Lenz, H. J., Gelsomino, F., Aglietta, M., Morse, M. A., Van Cutsem, E., McDermott, R., Hill, A., Sawyer, M. B., Hendlish, A., Neyns, B., Svrcek, M., Moss, R. A., Ledeine, J. M., Cao, Z. A., Kamble, S., Kopetz, S. & Andre, T. (2018) Durable Clinical Benefit With Nivolumab Plus Ipilimumab in DNA Mismatch Repair-Deficient/Microsatellite Instability-High Metastatic Colorectal Cancer, *J Clin Oncol.* 36, 773-779.
10. Liston, D. R. & Davis, M. (2017) Clinically Relevant Concentrations of Anticancer Drugs: A Guide for Nonclinical Studies, *Clin Cancer Res.* 23, 3489-3498.
11. Sheng, J., Srivastava, S., Sanghavi, K., Lu, Z., Schmidt, B. J., Bello, A. & Gupta,

- M. (2017) Clinical Pharmacology Considerations for the Development of Immune Checkpoint Inhibitors, *J Clin Pharmacol.* 57 Suppl 10, S26-S42.
12. Khera, N. & Rajput, S. (2017) Therapeutic Potential of Small Molecule Inhibitors, *J Cell Biochem.* 118, 959-961.
13. McGranahan, N., Furness, A. J., Rosenthal, R., Ramskov, S., Lyngaa, R., Saini, S. K., Jamal-Hanjani, M., Wilson, G. A., Birkbak, N. J., Hiley, C. T., Watkins, T. B., Shafi, S., Murugaesu, N., Mitter, R., Akarca, A. U., Linares, J., Marafioti, T., Henry, J. Y., Van Allen, E. M., Miao, D., Schilling, B., Schadendorf, D., Garraway, L. A., Makarov, V., Rizvi, N. A., Snyder, A., Hellmann, M. D., Merghoub, T., Wolchok, J. D., Shukla, S. A., Wu, C. J., Peggs, K. S., Chan, T. A., Hadrup, S. R., Quezada, S. A. & Swanton, C. (2016) Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade, *Science.* 351, 1463-9.
14. Borst, J., Ahrends, T., Babala, N., Melief, C. J. M. & Kastenmuller, W. (2018) CD4(+) T cell help in cancer immunology and immunotherapy, *Nat Rev Immunol.* 18, 635-647.
15. Speiser, D. E., Lienard, D., Rufer, N., Rubio-Godoy, V., Rimoldi, D., Lejeune, F., Krieg, A. M., Cerottini, J. C. & Romero, P. (2005) Rapid and strong human CD8+ T cell responses to vaccination with peptide, IFA, and CpG oligodeoxynucleotide 7909, *J Clin Invest.* 115, 739-46.
16. Hui, E., Cheung, J., Zhu, J., Su, X., Taylor, M. J., Wallweber, H. A., Sasmal, D. K., Huang, J., Kim, J. M., Mellman, I. & Vale, R. D. (2017) T cell costimulatory receptor CD28 is a primary target for PD-1-mediated inhibition, *Science.* 355, 1428-1433.
17. Guzik, K., Zak, K. M., Grudnik, P., Magiera, K., Musielak, B., Torner, R., Skalniak, L., Domling, A., Dubin, G. & Holak, T. A. (2017) Small-Molecule Inhibitors of the Programmed Cell Death-1/Programmed Death-Ligand 1 (PD-1/PD-L1) Interaction via Transiently Induced Protein States and Dimerization of PD-L1, *J Med Chem.* 60, 5857-5867.
18. Zak, K. M., Grudnik, P., Guzik, K., Zieba, B. J., Musielak, B., Domling, A., Dubin, G. & Holak, T. A. (2016) Structural basis for small molecule targeting of the programmed death ligand 1 (PD-L1), *Oncotarget.* 7, 30323-35.
19. Ganesan, J. A., Ahmed, M., Okoye, I., Arutyunova, E., Babu, D., Turnbull, W. L., Kundu, J. K., Shields, J., Agopsowicz, K. C., Xu, L., Tabana, Y., Srivastava, N., Zhang, G., Moon, T. C., Belovodskiy, A., Hena, M., Kandada, A. S., Hosseini, S. N., Hitt, M., Walker, J., Smylie, M., West, F. G., Siraki, A. G., Lemieux, M. J., Elahi, S., Nieman, J. A., Tyrrell, D. L., Houghton, M. & Barakat, K. (2019) Comprehensive in vitro characterization of PD-L1 small molecule inhibitors, *Sci Rep.* 9, 12392.
20. Yang, J. & Hu, L. (2019) Immunomodulators targeting the PD-1/PD-L1 protein-protein interaction: From antibodies to small molecules, *Med Res Rev.* 39, 265-301.
21. Tran, E., Ahmadzadeh, M., Lu, Y. C., Gros, A., Turcotte, S., Robbins, P. F., Gartner, J. J., Zheng, Z., Li, Y. F., Ray, S., Wunderlich, J. R., Somerville, R. P. & Rosenberg, S. A. (2015) Immunogenicity of somatic mutations in human gastrointestinal cancers, *Science.* 350, 1387-90.
22. Sahin, U., Derhovanessian, E., Miller, M., Kloke, B. P., Simon, P., Lower, M., Bukur, V., Tadmor, A. D., Luxemburger, U., Schrors, B., Omokoko, T., Vormehr, M., Albrecht, C., Paruzynski, A., Kuhn, A. N., Buck, J., Heesch, S., Schreeb, K. H., Muller, F., Ortseifer, I., Vogler, I., Godehardt, E., Attig, S., Rae, R., Breitkreuz, A., Tolliver, C., Suchan, M., Martic, G., Hohberger, A., Sorn, P., Diekmann, J., Ciesla, J., Waksman, O., Bruck, A. K., Witt, M., Zillgen, M., Rothermel, A., Kasemann, B., Langer, D., Bolte, S., Diken, M., Kreiter, S., Nemecek, R., Gebhardt, C., Grabbe, S., Holler, C., Utikal, J., Huber, C., Loquai, C. & Tureci, O. (2017) Personalized RNA mutanome

vaccines mobilize poly-specific therapeutic immunity against cancer, *Nature*. 547, 222-226.

23. Keskin, D. B., Anandappa, A. J., Sun, J., Tirosh, I., Mathewson, N. D., Li, S., Oliveira, G., Giobbie-Hurder, A., Felt, K., Gjini, E., Shukla, S. A., Hu, Z., Li, L., Le, P. M., Allesoe, R. L., Richman, A. R., Kowalczyk, M. S., Abdelrahman, S., Geduldig, J. E., Charbonneau, S., Pelton, K., Iorgulescu, J. B., Elagina, L., Zhang, W., Olive, O., McCluskey, C., Olsen, L. R., Stevens, J., Lane, W. J., Salazar, A. M., Daley, H., Wen, P. Y., Chiocca, E. A., Harden, M., Lennon, N. J., Gabriel, S., Getz, G., Lander, E. S., Regev, A., Ritz, J., Neubergh, D., Rodig, S. J., Ligon, K. L., Suva, M. L., Wucherpfenig, K. W., Hacohen, N., Fritsch, E. F., Livak, K. J., Ott, P. A., Wu, C. J. & Reardon, D. A. (2019) Neoantigen vaccine generates intratumoral T cell responses in phase Ib glioblastoma trial, *Nature*. 565, 234-239.

24. Smith, M., Garcia-Martinez, E., Pitter, M. R., Fucikova, J., Spisek, R., Zitvogel, L., Kroemer, G. & Galluzzi, L. (2018) Trial Watch: Toll-like receptor agonists in cancer immunotherapy, *Oncoimmunology*. 7, e1526250.

25. Kaczanowska, S., Joseph, A. M. & Davila, E. (2013) TLR agonists: our best frenemy in cancer immunotherapy, *J Leukoc Biol*. 93, 847-63.

26. Mancini, R. J., Stutts, L., Ryu, K. A., Tom, J. K. & Esser-Kahn, A. P. (2014) Directing the immune system with chemical compounds, *ACS Chem Biol*. 9, 1075-85.

27. Dietsch, G. N., Lu, H., Yang, Y., Morishima, C., Chow, L. Q., Disis, M. L. & Hershberg, R. M. (2016) Coordinated Activation of Toll-Like Receptor8 (TLR8) and NLRP3 by the TLR8 Agonist, VTX-2337, Ignites Tumoricidal Natural Killer Cell Activity, *PLoS One*. 11, e0148764.

28. Shayan, G., Kansy, B. A., Gibson, S. P., Srivastava, R. M., Bryan, J. K., Bauman, J. E., Ohr, J., Kim, S., Duvvuri, U., Clump, D. A., Heron, D. E., Johnson, J. T., Hershberg, R. M. & Ferris, R. L. (2018) Phase Ib Study of Immune Biomarker Modulation with Neoadjuvant Cetuximab and TLR8 Stimulation in Head and Neck Cancer to Overcome Suppressive Myeloid Signals, *Clin Cancer Res*. 24, 62-72.

29. Ferris, R. L., Saba, N. F., Gitlitz, B. J., Haddad, R., Sukari, A., Neupane, P., Morris, J. C., Misiukiewicz, K., Bauman, J. E., Fenton, M., Jimeno, A., Adkins, D. R., Schneider, C. J., Sacco, A. G., Shirai, K., Bowles, D. W., Gibson, M., Nwizu, T., Gotardo, R., Manjarrez, K. L., Dietsch, G. N., Bryan, J. K., Hershberg, R. M. & Cohen, E. E. W. (2018) Effect of Adding Motolimod to Standard Combination Chemotherapy and Cetuximab Treatment of Patients With Squamous Cell Carcinoma of the Head and Neck: The Active8 Randomized Clinical Trial, *JAMA Oncol*. 4, 1583-1588.

30. Zhang, L., Dewan, V. & Yin, H. (2017) Discovery of Small Molecules as Multi-Toll-like Receptor Agonists with Proinflammatory and Anticancer Activities, *J Med Chem*. 60, 5029-5044.

31. Zhu, G., Xu, Y., Cen, X., Nandakumar, K. S., Liu, S. & Cheng, K. (2018) Targeting pattern-recognition receptors to discover new small molecule immune modulators, *Eur J Med Chem*. 144, 82-92.

32. Woo, S. R., Fuertes, M. B., Corrales, L., Spranger, S., Furdyna, M. J., Leung, M. Y., Duggan, R., Wang, Y., Barber, G. N., Fitzgerald, K. A., Alegre, M. L. & Gajewski, T. F. (2014) STING-dependent cytosolic DNA sensing mediates innate immune recognition of immunogenic tumors, *Immunity*. 41, 830-42.

33. Li, L., Yin, Q., Kuss, P., Maliga, Z., Millan, J. L., Wu, H. & Mitchison, T. J. (2014) Hydrolysis of 2'3'-cGAMP by ENPP1 and design of nonhydrolyzable analogs, *Nat Chem Biol*. 10, 1043-8.

34. Chang, L., Lee, S. Y., Leonczak, P., Rozenski, J., De Jonghe, S., Hanck, T., Muller, C. E. & Herdewijn, P. (2014) Imidazopyridine- and purine-thioacetamide deriva-

- tives: potent inhibitors of nucleotide pyrophosphatase/phosphodiesterase 1 (NPP1), *J Med Chem.* 57, 10080-100.
35. Zelikman, V., Pelletier, J., Simhaev, L., Sela, A., Gendron, F. P., Arguin, G., Senderowitz, H., Seigny, J. & Fischer, B. (2018) Highly Selective and Potent Ecto-nucleotide Pyrophosphatase-1 (NPP1) Inhibitors Based on Uridine 5'-Palph α , α -Dithiophosphate Analogues, *J Med Chem.* 61, 3939-3951.
36. Zhai, L., Ladomersky, E., Lenzen, A., Nguyen, B., Patel, R., Lauing, K. L., Wu, M. & Wainwright, D. A. (2018) IDO1 in cancer: a Gemini of immune checkpoints, *Cell Mol Immunol.* 15, 447-457.
37. Liu, H., Zhang, H., Wu, X., Ma, D., Wu, J., Wang, L., Jiang, Y., Fei, Y., Zhu, C., Tan, R., Jungblut, P., Pei, G., Dorhoi, A., Yan, Q., Zhang, F., Zheng, R., Liu, S., Liang, H., Liu, Z., Yang, H., Chen, J., Wang, P., Tang, T., Peng, W., Hu, Z., Xu, Z., Huang, X., Wang, J., Li, H., Zhou, Y., Liu, F., Yan, D., Kaufmann, S. H. E., Chen, C., Mao, Z. & Ge, B. (2018) Nuclear cGAS suppresses DNA repair and promotes tumorigenesis, *Nature.* 563, 131-136.
38. Lemos, H., Mohamed, E., Huang, L., Ou, R., Pacholczyk, G., Arbab, A. S., Munn, D. & Mellor, A. L. (2016) STING Promotes the Growth of Tumors Characterized by Low Antigenicity via IDO Activation, *Cancer Res.* 76, 2076-81.
39. Krombach, J., Hennel, R., Brix, N., Orth, M., Schoetz, U., Ernst, A., Schuster, J., Zuchtriegel, G., Reichel, C. A., Bierschenk, S., Sperandio, M., Vogl, T., Unkel, S., Belka, C. & Lauber, K. (2019) Priming anti-tumor immunity by radiotherapy: Dying tumor cell-derived DAMPs trigger endothelial cell activation and recruitment of myeloid cells, *Oncoimmunology.* 8, e1523097.
40. Galluzzi, L., Buque, A., Kepp, O., Zitvogel, L. & Kroemer, G. (2015) Immunological Effects of Conventional Chemotherapy and Targeted Anticancer Agents, *Cancer Cell.* 28, 690-714.
41. Heinhuis, K. M., Ros, W., Kok, M., Steeghs, N., Beijnen, J. H. & Schellens, J. H. M. (2019) Enhancing antitumor response by combining immune checkpoint inhibitors with chemotherapy in solid tumors, *Ann Oncol.* 30, 219-235.
42. Beyranvand Nejad, E., van der Sluis, T. C., van Duikeren, S., Yagita, H., Jansen, G. M., van Veelen, P. A., Melief, C. J., van der Burg, S. H. & Arens, R. (2016) Tumor Eradication by Cisplatin Is Sustained by CD80/86-Mediated Costimulation of CD8⁺ T Cells, *Cancer Res.* 76, 6017-6029.
43. Gandhi, L. & Garassino, M. C. (2018) Pembrolizumab plus Chemotherapy in Lung Cancer, *N Engl J Med.* 379, e18.
44. Sistigu, A., Yamazaki, T., Vacchelli, E., Chaba, K., Enot, D. P., Adam, J., Vitale, I., Goubar, A., Baracco, E. E., Remedios, C., Fend, L., Hannani, D., Aymeric, L., Ma, Y., Niso-Santano, M., Kepp, O., Schultze, J. L., Tuting, T., Belardelli, F., Bracci, L., La Sorsa, V., Ziccheddu, G., Sestili, P., Urbani, F., Delorenzi, M., Lacroix-Triki, M., Quidville, V., Conforti, R., Spano, J. P., Pusztai, L., Poirier-Colame, V., Delaloge, S., Penault-Llorca, F., Ladoire, S., Arnould, L., Cyrta, J., Dessoliers, M. C., Eggermont, A., Bianchi, M. E., Pittet, M., Engblom, C., Pfirschke, C., Preville, X., Uze, G., Schreiber, R. D., Chow, M. T., Smyth, M. J., Proietti, E., Andre, F., Kroemer, G. & Zitvogel, L. (2014) Cancer cell-autonomous contribution of type I interferon signaling to the efficacy of chemotherapy, *Nat Med.* 20, 1301-9.
45. Alizadeh, D., Trad, M., Hanke, N. T., Larmonier, C. B., Janikashvili, N., Bonnotte, B., Katsanis, E. & Larmonier, N. (2014) Doxorubicin eliminates myeloid-derived suppressor cells and enhances the efficacy of adoptive T-cell transfer in breast cancer, *Cancer Res.* 74, 104-18.
46. Voorwerk, L., Slagter, M., Horlings, H. M., Sikorska, K., van de Vijver, K. K., de

- Maaker, M., Nederlof, I., Kluin, R. J. C., Warren, S., Ong, S., Wiersma, T. G., Russell, N. S., Lalezari, F., Schouten, P. C., Bakker, N. A. M., Ketelaars, S. L. C., Peters, D., Lange, C. A. H., van Werkhoven, E., van Tinteren, H., Mandjes, I. A. M., Kemper, I., Onderwater, S., Chalabi, M., Wilgenhof, S., Haanen, J., Salgado, R., de Visser, K. E., Sonke, G. S., Wessels, L. F. A., Linn, S. C., Schumacher, T. N., Blank, C. U. & Kok, M. (2019) Immune induction strategies in metastatic triple-negative breast cancer to enhance the sensitivity to PD-1 blockade: the TONIC trial, *Nat Med.* 25, 920-928.
47. Orecchioni, S., Talarico, G., Labanca, V., Calleri, A., Mancuso, P. & Bertolini, F. (2018) Vinorelbine, cyclophosphamide and 5-FU effects on the circulating and intratumoural landscape of immune cells improve anti-PD-L1 efficacy in preclinical models of breast cancer and lymphoma, *Br J Cancer.* 118, 1329-1336.
48. Zhou, J., Yang, T., Liu, L. & Lu, B. (2017) Chemotherapy oxaliplatin sensitizes prostate cancer to immune checkpoint blockade therapies via stimulating tumor immunogenicity, *Mol Med Rep.* 16, 2868-2874.
49. Paz-Ares, L., Luft, A., Vicente, D., Tafreshi, A., Gumus, M., Mazieres, J., Hermes, B., Cay Senler, F., Csoszi, T., Fulop, A., Rodriguez-Cid, J., Wilson, J., Sugawara, S., Kato, T., Lee, K. H., Cheng, Y., Novello, S., Halmos, B., Li, X., Lubiniecki, G. M., Piperdi, B., Kowalski, D. M. & Investigators, K.-. (2018) Pembrolizumab plus Chemotherapy for Squamous Non-Small-Cell Lung Cancer, *N Engl J Med.* 379, 2040-2051.
50. Karzai, F., VanderWeele, D., Madan, R. A., Owens, H., Cordes, L. M., Hankin, A., Couvillon, A., Nichols, E., Bilusic, M., Beshiri, M. L., Kelly, K., Krishnasamy, V., Lee, S., Lee, M. J., Yuno, A., Trepel, J. B., Merino, M. J., Dittamore, R., Marte, J., Donahue, R. N., Schlom, J., Killian, K. J., Meltzer, P. S., Steinberg, S. M., Gulley, J. L., Lee, J. M. & Dahut, W. L. (2018) Activity of durvalumab plus olaparib in metastatic castration-resistant prostate cancer in men with and without DNA damage repair mutations, *J Immunother Cancer.* 6, 141.
51. Jiao, S., Xia, W., Yamaguchi, H., Wei, Y., Chen, M. K., Hsu, J. M., Hsu, J. L., Yu, W. H., Du, Y., Lee, H. H., Li, C. W., Chou, C. K., Lim, S. O., Chang, S. S., Litton, J., Arun, B., Hortobagyi, G. N. & Hung, M. C. (2017) PARP Inhibitor Upregulates PD-L1 Expression and Enhances Cancer-Associated Immunosuppression, *Clin Cancer Res.* 23, 3711-3720.
52. Deng, J., Wang, E. S., Jenkins, R. W., Li, S., Dries, R., Yates, K., Chhabra, S., Huang, W., Liu, H., Aref, A. R., Ivanova, E., Paweletz, C. P., Bowden, M., Zhou, C. W., Herter-Sprie, G. S., Sorrentino, J. A., Bisi, J. E., Lizotte, P. H., Merlino, A. A., Quinn, M. M., Bufo, L. E., Yang, A., Zhang, Y., Zhang, H., Gao, P., Chen, T., Cavanaugh, M. E., Rode, A. J., Haines, E., Roberts, P. J., Strum, J. C., Richards, W. G., Lorch, J. H., Parangi, S., Gunda, V., Boland, G. M., Bueno, R., Palakurthi, S., Freeman, G. J., Ritz, J., Haining, W. N., Sharpless, N. E., Arthanari, H., Shapiro, G. I., Barbie, D. A., Gray, N. S. & Wong, K. K. (2018) CDK4/6 Inhibition Augments Antitumor Immunity by Enhancing T-cell Activation, *Cancer Discov.* 8, 216-233.
53. Uyttenhove, C., Pilotte, L., Theate, I., Stroobant, V., Colau, D., Parmentier, N., Boon, T. & Van den Eynde, B. J. (2003) Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase, *Nat Med.* 9, 1269-74.
54. Lippitz, B. E. (2013) Cytokine patterns in patients with cancer: a systematic review, *Lancet Oncol.* 14, e218-28.
55. Giraldo, N. A., Sanchez-Salas, R., Peske, J. D., Vano, Y., Becht, E., Petitprez, F., Validire, P., Ingels, A., Cathelineau, X., Fridman, W. H. & Sautès-Fridman, C. (2019) The clinical role of the TME in solid cancer, *Br J Cancer.* 120, 45-53.

56. Prendergast, G. C., Malachowski, W. J., Mondal, A., Scherle, P. & Muller, A. J. (2018) Indoleamine 2,3-Dioxygenase and Its Therapeutic Inhibition in Cancer, *Int Rev Cell Mol Biol.* 336, 175-203.
57. Liu, Y., Liang, X., Dong, W., Fang, Y., Lv, J., Zhang, T., Fiskesund, R., Xie, J., Liu, J., Yin, X., Jin, X., Chen, D., Tang, K., Ma, J., Zhang, H., Yu, J., Yan, J., Liang, H., Mo, S., Cheng, F., Zhou, Y., Zhang, H., Wang, J., Li, J., Chen, Y., Cui, B., Hu, Z. W., Cao, X., Xiao-Feng Qin, F. & Huang, B. (2018) Tumor-Repopulating Cells Induce PD-1 Expression in CD8(+) T Cells by Transferring Kynurenine and AhR Activation, *Cancer Cell.* 33, 480-494 e7.
58. Soliman, H. H., Jackson, E., Neuger, T., Dees, E. C., Harvey, R. D., Han, H., Ismail-Khan, R., Minton, S., Vahanian, N. N., Link, C., Sullivan, D. M. & Antonia, S. (2014) A first in man phase I trial of the oral immunomodulator, indoximod, combined with docetaxel in patients with metastatic solid tumors, *Oncotarget.* 5, 8136-46.
59. 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., Demidov, L., Robert, C., Larkin, J., Anderson, J. R., Maleski, J., Jones, M., Diede, S. J. & Mitchell, T. C. (2019) Epacadostat plus pembrolizumab versus placebo plus pembrolizumab in patients with unresectable or metastatic melanoma (ECHO-301/KEYNOTE-252): a phase 3, randomised, double-blind study, *Lancet Oncol.* 20, 1083-1097.
60. Aung Naing, J. D. P., Gerald Falchook, Benjamin Creelan, John Nemunaitis, Jose Lutzky, Adi Diab, Judy S. Wang, Naomi Laing, Michelle Niewood, Xiaohua Gong, Gongfu Zhou and Manish Patel (2018) Abstract CT177: Epacadostat plus durvalumab in patients with advanced solid tumors: preliminary results of the ongoing, open-label, phase I/II ECHO-203 study.
61. Depth, N. i. (2018) Companies Scaling Back IDO1 Inhibitor Trials, *CANCER DISCOVERY.* 8, OF5.
62. Opitz, C. A., Litzenburger, U. M., Sahm, F., Ott, M., Tritschler, I., Trump, S., Schumacher, T., Jestaedt, L., Schrenk, D., Weller, M., Jugold, M., Guillemin, G. J., Miller, C. L., Lutz, C., Radlwimmer, B., Lehmann, I., von Deimling, A., Wick, W. & Platten, M. (2011) An endogenous tumour-promoting ligand of the human aryl hydrocarbon receptor, *Nature.* 478, 197-203.
63. McGaha, T. L. (2015) IDO-GCN2 and autophagy in inflammation, *Oncotarget.* 6, 21771-2.
64. Fujimoto, J., Kurasawa, O., Takagi, T., Liu, X., Banno, H., Kojima, T., Asano, Y., Nakamura, A., Nambu, T., Hata, A., Ishii, T., Sameshima, T., Debori, Y., Miyamoto, M., Klein, M. G., Tjhen, R., Sang, B. C., Levin, I., Lane, S. W., Snell, G. P., Li, K., Kefala, G., Hoffman, I. D., Ding, S. C., Cary, D. R. & Mizojiri, R. (2019) Identification of Novel, Potent, and Orally Available GCN2 Inhibitors with Type I Half Binding Mode, *ACS Med Chem Lett.* 10, 1498-1503.
65. Hennequart, M., Pilotte, L., Cane, S., Hoffmann, D., Stroobant, V., Plaen, E. & Van den Eynde, B. J. (2017) Constitutive IDO1 Expression in Human Tumors Is Driven by Cyclooxygenase-2 and Mediates Intrinsic Immune Resistance, *Cancer Immunol Res.* 5, 695-709.
66. Pang, L. Y., Hurst, E. A. & Argyle, D. J. (2016) Cyclooxygenase-2: A Role in Cancer Stem Cell Survival and Repopulation of Cancer Cells during Therapy, *Stem Cells Int.* 2016, 2048731.
67. Buzharevski, A., Paskas, S., Sarosi, M. B., Laube, M., Lonneck, P., Neumann, W., Mijatovic, S., Maksimovic-Ivanic, D., Pietzsch, J. & Hey-Hawkins, E. (2019) Carboranyl Analogues of Celecoxib with Potent Cytostatic Activity against Human Melanoma and Colon Cancer Cell Lines, *ChemMedChem.* 14, 315-321.

68. Li, N., Li, H., Su, F., Li, J., Ma, X. & Gong, P. (2015) Relationship between epidermal growth factor receptor (EGFR) mutation and serum cyclooxygenase-2 Level, and the synergistic effect of celecoxib and gefitinib on EGFR expression in non-small cell lung cancer cells, *Int J Clin Exp Pathol.* 8, 9010-20.
69. Tang, H., Liu, Y., Wang, C., Zheng, H., Chen, Y., Liu, W., Chen, X., Zhang, J., Chen, H., Yang, Y. & Yang, J. (2019) Inhibition of COX-2 and EGFR by Melafolone Improves Anti-PD-1 Therapy through Vascular Normalization and PD-L1 Downregulation in Lung Cancer, *J Pharmacol Exp Ther.* 368, 401-413.
70. Sun, X. & Li, Q. (2018) Prostaglandin EP2 receptor: Novel therapeutic target for human cancers (Review), *Int J Mol Med.* 42, 1203-1214.
71. af Forselles, K. J., Root, J., Clarke, T., Davey, D., Aughton, K., Dack, K. & Pullen, N. (2011) In vitro and in vivo characterization of PF-04418948, a novel, potent and selective prostaglandin EP(2) receptor antagonist, *Br J Pharmacol.* 164, 1847-56.
72. Ma, X., Holt, D., Kundu, N., Reader, J., Goloubeva, O., Take, Y. & Fulton, A. M. (2013) A prostaglandin E (PGE) receptor EP4 antagonist protects natural killer cells from PGE2-mediated immunosuppression and inhibits breast cancer metastasis, *Oncoimmunology.* 2, e22647.
73. Miao, J., Lu, X., Hu, Y., Piao, C., Wu, X., Liu, X., Huang, C., Wang, Y., Li, D. & Liu, J. (2017) Prostaglandin E2 and PD-1 mediated inhibition of antitumor CTL responses in the human tumor microenvironment, *Oncotarget.* 8, 89802-89810.
74. Morris, S. M., Jr. (2009) Recent advances in arginine metabolism: roles and regulation of the arginases, *Br J Pharmacol.* 157, 922-30.
75. Rodriguez, P. C., Zea, A. H., Culotta, K. S., Zabaleta, J., Ochoa, J. B. & Ochoa, A. C. (2002) Regulation of T cell receptor CD3zeta chain expression by L-arginine, *J Biol Chem.* 277, 21123-9.
76. Steggerda, S. M., Bennett, M. K., Chen, J., Emberley, E., Huang, T., Janes, J. R., Li, W., MacKinnon, A. L., Makkouk, A., Marguier, G., Murray, P. J., Neou, S., Pan, A., Parlati, F., Rodriguez, M. L. M., Van de Velde, L. A., Wang, T., Works, M., Zhang, J., Zhang, W. & Gross, M. I. (2017) Inhibition of arginase by CB-1158 blocks myeloid cell-mediated immune suppression in the tumor microenvironment, *J Immunother Cancer.* 5, 101.
77. Adams, J. L., Smothers, J., Srinivasan, R. & Hoos, A. (2015) Big opportunities for small molecules in immuno-oncology, *Nat Rev Drug Discov.* 14, 603-22.
78. De Santo, C., Serafini, P., Marigo, I., Dolcetti, L., Bolla, M., Del Soldato, P., Melani, C., Guiducci, C., Colombo, M. P., Iezzi, M., Musiani, P., Zanovello, P. & Bronte, V. (2005) Nitroaspirin corrects immune dysfunction in tumor-bearing hosts and promotes tumor eradication by cancer vaccination, *Proc Natl Acad Sci U S A.* 102, 4185-90.
79. Molon, B., Ugel, S., Del Pozzo, F., Soldani, C., Zilio, S., Avella, D., De Palma, A., Mauri, P., Monegal, A., Rescigno, M., Savino, B., Colombo, P., Jonjic, N., Pecanic, S., Lazzarato, L., Fruttero, R., Gasco, A., Bronte, V. & Viola, A. (2011) Chemokine nitration prevents intratumoral infiltration of antigen-specific T cells, *J Exp Med.* 208, 1949-62.
80. Ohta, A., Gorelik, E., Prasad, S. J., Ronchese, F., Lukashev, D., Wong, M. K., Huang, X., Caldwell, S., Liu, K., Smith, P., Chen, J. F., Jackson, E. K., Apasov, S., Abrams, S. & Sitkovsky, M. (2006) A2A adenosine receptor protects tumors from antitumor T cells, *Proc Natl Acad Sci U S A.* 103, 13132-7.
81. Ohta, A., Kini, R., Ohta, A., Subramanian, M., Madasu, M. & Sitkovsky, M. (2012) The development and immunosuppressive functions of CD4(+) CD25(+) FoxP3(+) regulatory T cells are under influence of the adenosine-A2A adenosine receptor

pathway, *Front Immunol.* 3, 190.

82. Gillespie, R. J., Bamford, S. J., Botting, R., Comer, M., Denny, S., Gaur, S., Griffin, M., Jordan, A. M., Knight, A. R., Lerpiniere, J., Leonardi, S., Lightowler, S., McAteer, S., Merrett, A., Misra, A., Padfield, A., Reece, M., Saadi, M., Selwood, D. L., Stratton, G. C., Surry, D., Todd, R., Tong, X., Ruston, V., Upton, R. & Weiss, S. M. (2009) Antagonists of the human A(2A) adenosine receptor. 4. Design, synthesis, and preclinical evaluation of 7-aryltriazolo[4,5-d]pyrimidines, *J Med Chem.* 52, 33-47.

83. Kerr, W. G. & Chisholm, J. D. (2019) The Next Generation of Immunotherapy for Cancer: Small Molecules Could Make Big Waves, *J Immunol.* 202, 11-19.

84. Huck, B. R., Kotzner, L. & Urbahns, K. (2018) Small Molecules Drive Big Improvements in Immuno-Oncology Therapies, *Angew Chem Int Ed Engl.* 57, 4412-4428.

85. Chang, M. R., Dharmarajan, V., Doebelin, C., Garcia-Ordonez, R. D., Novick, S. J., Kuruvilla, D. S., Kamenecka, T. M. & Griffin, P. R. (2016) Synthetic ROR γ Agonists Enhance Protective Immunity, *ACS Chem Biol.* 11, 1012-8.

86. Hu, X., Liu, X., Moisan, J., Wang, Y., Lesch, C. A., Spooner, C., Morgan, R. W., Zawadzka, E. M., Mertz, D., Bousley, D., Majchrzak, K., Kryczek, I., Taylor, C., Van Huis, C., Skalitzy, D., Hurd, A., Aicher, T. D., Toogood, P. L., Glick, G. D., Paulos, C. M., Zou, W. & Carter, L. L. (2016) Synthetic ROR γ agonists regulate multiple pathways to enhance antitumor immunity, *Oncoimmunology.* 5, e1254854.

87. Ivanov, II, McKenzie, B. S., Zhou, L., Tadakoro, C. E., Lepelley, A., Lafaille, J. J., Cua, D. J. & Littman, D. R. (2006) The orphan nuclear receptor ROR γ directs the differentiation program of proinflammatory IL-17+ T helper cells, *Cell.* 126, 1121-33.

88. Asadzadeh, Z., Mohammadi, H., Safarzadeh, E., Hemmatzadeh, M., Mahdian-Shakib, A., Jadidi-Niaragh, F., Azizi, G. & Baradaran, B. (2017) The paradox of Th17 cell functions in tumor immunity, *Cell Immunol.* 322, 15-25.

89. Wang, R., Yang, L., Zhang, C., Wang, R., Zhang, Z., He, Q., Chen, X., Zhang, B., Qin, Z., Wang, L. & Zhang, Y. (2018) Th17 cell-derived IL-17A promoted tumor progression via STAT3/NF- κ B/Notch1 signaling in non-small cell lung cancer, *Oncoimmunology.* 7, e1461303.

90. Lee, M. H., Tung-Chieh Chang, J., Liao, C. T., Chen, Y. S., Kuo, M. L. & Shen, C. R. (2018) Interleukin 17 and peripheral IL-17-expressing T cells are negatively correlated with the overall survival of head and neck cancer patients, *Oncotarget.* 9, 9825-9837.

91. Kono, M., Ochida, A., Oda, T., Imada, T., Banno, Y., Taya, N., Masada, S., Kawamoto, T., Yonemori, K., Nara, Y., Fukase, Y., Yukawa, T., Tokuhara, H., Skene, R., Sang, B. C., Hoffman, I. D., Snell, G. P., Uga, K., Shibata, A., Igaki, K., Nakamura, Y., Nakagawa, H., Tsuchimori, N., Yamasaki, M., Shirai, J. & Yamamoto, S. (2018) Discovery of [cis-3-((5 R)-5-((7-Fluoro-1,1-dimethyl-2,3-dihydro-1 H-inden-5-yl) carbamoyl)-2-methoxy-7,8-dihydro-1,6-naphthyridin-6(5 H)-yl)carbonyl]cyclobutyl] acetic Acid (TAK-828F) as a Potent, Selective, and Orally Available Novel Retinoic Acid Receptor-Related Orphan Receptor γ Inverse Agonist, *J Med Chem.* 61, 2973-2988.

92. Wang, J., Bhatia, A., Krugliak Cleveland, N., Gupta, N., Dalal, S., Rubin, D. T. & Sakuraba, A. (2018) Rapid Onset of Inflammatory Bowel Disease after Receiving Secukinumab Infusion, *ACG Case Rep J.* 5, e56.

93. Ghosh, M. C., Makena, P. S., Gorantla, V., Sinclair, S. E. & Waters, C. M. (2012) CXCR4 regulates migration of lung alveolar epithelial cells through activation of Rac1

- and matrix metalloproteinase-2, *Am J Physiol Lung Cell Mol Physiol.* 302, L846-56.
94. Onoue, T., Uchida, D., Begum, N. M., Tomizuka, Y., Yoshida, H. & Sato, M. (2006) Epithelial-mesenchymal transition induced by the stromal cell-derived factor-1/CXCR4 system in oral squamous cell carcinoma cells, *Int J Oncol.* 29, 1133-8.
 95. Zhou, W., Guo, S., Liu, M., Burow, M. E. & Wang, G. (2019) Targeting CXCL12/CXCR4 Axis in Tumor Immunotherapy, *Curr Med Chem.* 26, 3026-3041.
 96. Kato, I., Niwa, A., Heike, T., Fujino, H., Saito, M. K., Umeda, K., Hiramatsu, H., Ito, M., Morita, M., Nishinaka, Y., Adachi, S., Ishikawa, F. & Nakahata, T. (2011) Identification of hepatic niche harboring human acute lymphoblastic leukemic cells via the SDF-1/CXCR4 axis, *PLoS One.* 6, e27042.
 97. Uy, G. L., Rettig, M. P., Motabi, I. H., McFarland, K., Trinkaus, K. M., Hladnik, L. M., Kulkarni, S., Abboud, C. N., Cashen, A. F., Stockerl-Goldstein, K. E., Vij, R., Westervelt, P. & DiPersio, J. F. (2012) A phase 1/2 study of chemosensitization with the CXCR4 antagonist plerixafor in relapsed or refractory acute myeloid leukemia, *Blood.* 119, 3917-24.
 98. Taromi, S., Kayser, G., Catusse, J., von Elverfeldt, D., Reichardt, W., Braun, F., Weber, W. A., Zeiser, R. & Burger, M. (2016) CXCR4 antagonists suppress small cell lung cancer progression, *Oncotarget.* 7, 85185-85195.
 99. Chen, Y., Ramjiawan, R. R., Reiberger, T., Ng, M. R., Hato, T., Huang, Y., Ochiai, H., Kitahara, S., Unan, E. C., Reddy, T. P., Fan, C., Huang, P., Bardeesy, N., Zhu, A. X., Jain, R. K. & Duda, D. G. (2015) CXCR4 inhibition in tumor microenvironment facilitates anti-programmed death receptor-1 immunotherapy in sorafenib-treated hepatocellular carcinoma in mice, *Hepatology.* 61, 1591-602.
 100. Steele, C. W., Karim, S. A., Leach, J. D. G., Bailey, P., Upstill-Goddard, R., Rishi, L., Foth, M., Bryson, S., McDaid, K., Wilson, Z., Eberlein, C., Candido, J. B., Clarke, M., Nixon, C., Connolly, J., Jamieson, N., Carter, C. R., Balkwill, F., Chang, D. K., Evans, T. R. J., Strathdee, D., Biankin, A. V., Nibbs, R. J. B., Barry, S. T., Sansom, O. J. & Morton, J. P. (2016) CXCR2 Inhibition Profoundly Suppresses Metastases and Augments Immunotherapy in Pancreatic Ductal Adenocarcinoma, *Cancer Cell.* 29, 832-845.
 101. Katoh, H., Wang, D., Daikoku, T., Sun, H., Dey, S. K. & Dubois, R. N. (2013) CXCR2-expressing myeloid-derived suppressor cells are essential to promote colitis-associated tumorigenesis, *Cancer Cell.* 24, 631-44.
 102. Zhu, H., Gu, Y., Xue, Y., Yuan, M., Cao, X. & Liu, Q. (2017) CXCR2(+) MDSCs promote breast cancer progression by inducing EMT and activated T cell exhaustion, *Oncotarget.* 8, 114554-114567.
 103. Zom, G. G., Khan, S., Britten, C. M., Sommandas, V., Camps, M. G., Loof, N. M., Budden, C. F., Meeuwenoord, N. J., Filippov, D. V., van der Marel, G. A., Overkleeft, H. S., Melief, C. J. & Ossendorp, F. (2014) Efficient induction of antitumor immunity by synthetic toll-like receptor ligand-peptide conjugates, *Cancer Immunol Res.* 2, 756-64.
 104. Zom, G. G., Willems, M., Meeuwenoord, N. J., Reintjens, N. R. M., Tondini, E., Khan, S., Overkleeft, H. S., van der Marel, G. A., Codee, J. D. C., Ossendorp, F. & Filippov, D. V. (2019) Dual Synthetic Peptide Conjugate Vaccine Simultaneously Triggers TLR2 and NOD2 and Activates Human Dendritic Cells, *Bioconjug Chem.* 30, 1150-1161.
 105. Corrales, L., Glickman, L. H., McWhirter, S. M., Kanne, D. B., Sivick, K. E., Katibah, G. E., Woo, S. R., Lemmens, E., Banda, T., Leong, J. J., Metchette, K., Dubensky, T. W., Jr. & Gajewski, T. F. (2015) Direct Activation of STING in the Tumor Microenvironment Leads to Potent and Systemic Tumor Regression and Immunity,

Cell Rep. 11, 1018-30.

106. Shih, A. Y., Damm-Ganamet, K. L. & Mirzadegan, T. (2018) Dynamic Structural Differences between Human and Mouse STING Lead to Differing Sensitivity to DMXAA, *Biophys J.* 114, 32-39.

107. Hwang, J., Kang, T., Lee, J., Choi, B. S. & Han, S. (2019) Design, synthesis, and biological evaluation of C7-functionalized DMXAA derivatives as potential human-STING agonists, *Org Biomol Chem.* 17, 1869-1874.

108. Ramanjulu, J. M., Pesiridis, G. S., Yang, J., Concha, N., Singhaus, R., Zhang, S. Y., Tran, J. L., Moore, P., Lehmann, S., Eberl, H. C., Muelbaier, M., Schneck, J. L., Clemens, J., Adam, M., Mehlmann, J., Romano, J., Morales, A., Kang, J., Leister, L., Graybill, T. L., Charnley, A. K., Ye, G., Nevins, N., Behnia, K., Wolf, A. I., Kasparcova, V., Nurse, K., Wang, L., Puhl, A. C., Li, Y., Klein, M., Hopson, C. B., Guss, J., Bantscheff, M., Bergamini, G., Reilly, M. A., Lian, Y., Duffy, K. J., Adams, J., Foley, K. P., Gough, P. J., Marquis, R. W., Smothers, J., Hoos, A. & Bertin, J. (2018) Design of amidobenzimidazole STING receptor agonists with systemic activity, *Nature.* 564, 439-443.

109. Ghaffari, A., Peterson, N., Khalaj, K., Vitkin, N., Robinson, A., Francis, J. A. & Koti, M. (2018) STING agonist therapy in combination with PD-1 immune checkpoint blockade enhances response to carboplatin chemotherapy in high-grade serous ovarian cancer, *Br J Cancer.* 119, 440-449.

110. Aduro (2019) ([http://investors.aduro.com/news-releases/news-release-details/aduro-biotech-and-novartis-present-results-ongoing-phase-1b?field_nir_news_date_value\[min\]=2019](http://investors.aduro.com/news-releases/news-release-details/aduro-biotech-and-novartis-present-results-ongoing-phase-1b?field_nir_news_date_value[min]=2019)) in

111. Wang, G. L., Jiang, B. H., Rue, E. A. & Semenza, G. L. (1995) Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O₂ tension, *Proc Natl Acad Sci U S A.* 92, 5510-4.

112. Hogenesch, J. B., Chan, W. K., Jackiw, V. H., Brown, R. C., Gu, Y. Z., Pray-Grant, M., Perdew, G. H. & Bradfield, C. A. (1997) Characterization of a subset of the basic-helix-loop-helix-PAS superfamily that interacts with components of the dioxin signaling pathway, *J Biol Chem.* 272, 8581-93.

113. Chiu, D. K., Tse, A. P., Xu, I. M., Di Cui, J., Lai, R. K., Li, L. L., Koh, H. Y., Tsang, F. H., Wei, L. L., Wong, C. M., Ng, I. O. & Wong, C. C. (2017) Hypoxia inducible factor HIF-1 promotes myeloid-derived suppressor cells accumulation through EN-TPD2/CD39L1 in hepatocellular carcinoma, *Nat Commun.* 8, 517.

114. Ruf, M., Moch, H. & Schraml, P. (2016) PD-L1 expression is regulated by hypoxia inducible factor in clear cell renal cell carcinoma, *Int J Cancer.* 139, 396-403.

115. DiNatale, B. C., Smith, K., John, K., Krishnegowda, G., Amin, S. G. & Perdew, G. H. (2012) Ah receptor antagonism represses head and neck tumor cell aggressive phenotype, *Mol Cancer Res.* 10, 1369-79.

116. Courtney, K. D., Infante, J. R., Lam, E. T., Figlin, R. A., Rini, B. I., Brugarolas, J., Zojwalla, N. J., Lowe, A. M., Wang, K., Wallace, E. M., Josey, J. A. & Choueiri, T. K. (2018) Phase I Dose-Escalation Trial of PT2385, a First-in-Class Hypoxia-Inducible Factor-2alpha Antagonist in Patients With Previously Treated Advanced Clear Cell Renal Cell Carcinoma, *J Clin Oncol.* 36, 867-874.

117. Chiche, J., Ilc, K., Laferriere, J., Trottier, E., Dayan, F., Mazure, N. M., Brahimi-Horn, M. C. & Pouyssegur, J. (2009) Hypoxia-inducible carbonic anhydrase IX and XII promote tumor cell growth by counteracting acidosis through the regulation of the intracellular pH, *Cancer Res.* 69, 358-68.

118. Radvak, P., Repic, M., Svastova, E., Takacova, M., Csaderova, L., Strnad, H., Pastorek, J., Pastorekova, S. & Kopacek, J. (2013) Suppression of carbonic anhy-

drase IX leads to aberrant focal adhesion and decreased invasion of tumor cells, *Oncol Rep.* 29, 1147-53.

119. Lou, Y., McDonald, P. C., Oloumi, A., Chia, S., Ostlund, C., Ahmadi, A., Kyle, A., Auf dem Keller, U., Leung, S., Huntsman, D., Clarke, B., Sutherland, B. W., Waterhouse, D., Bally, M., Roskelley, C., Overall, C. M., Minchinton, A., Pacchiano, F., Carta, F., Scozzafava, A., Touisni, N., Winum, J. Y., Supuran, C. T. & Dedhar, S. (2011) Targeting tumor hypoxia: suppression of breast tumor growth and metastasis by novel carbonic anhydrase IX inhibitors, *Cancer Res.* 71, 3364-76.

120. Dubois, L., Lieuwes, N. G., Maresca, A., Thiry, A., Supuran, C. T., Scozzafava, A., Wouters, B. G. & Lambin, P. (2009) Imaging of CA IX with fluorescent labelled sulfonamides distinguishes hypoxic and (re)-oxygenated cells in a xenograft tumour model, *Radiother Oncol.* 92, 423-8.

121. Toure, M. & Crews, C. M. (2016) Small-Molecule PROTACS: New Approaches to Protein Degradation, *Angew Chem Int Ed Engl.* 55, 1966-73.

122. Winter, G. E., Buckley, D. L., Paulk, J., Roberts, J. M., Souza, A., Dhe-Paganon, S. & Bradner, J. E. (2015) DRUG DEVELOPMENT. Phthalimide conjugation as a strategy for in vivo target protein degradation, *Science.* 348, 1376-81.

123. Watt, G. F., Scott-Stevens, P. & Gaohua, L. (2019) Targeted protein degradation in vivo with Proteolysis Targeting Chimeras: Current status and future considerations, *Drug Discov Today Technol.* 31, 69-80.

124. Sun, X., Wang, J., Yao, X., Zheng, W., Mao, Y., Lan, T., Wang, L., Sun, Y., Zhang, X., Zhao, Q., Zhao, J., Xiao, R. P., Zhang, X., Ji, G. & Rao, Y. (2019) A chemical approach for global protein knockdown from mice to non-human primates, *Cell Discov.* 5, 10.

125. Jensen, S. M., Potts, G. K., Ready, D. B. & Patterson, M. J. (2018) Specific MHC-I Peptides Are Induced Using PROTACs, *Front Immunol.* 9, 2697.