Immunochemical approaches to monitor and modulate the adaptive immune system

Luimstra, J.J.

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The future of cancer immunotherapy: opportunities for small molecules

Jolien J. Luimstra[1], Jacques Neefjes[1], Jannie Borst[2], and Huib Ovaa[1]

[1]Department of Cell and Chemical Biology, Leiden University Medical Center, Leiden, The Netherlands
[2]Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, The Netherlands

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Abstract

Cancer immunotherapy has demonstrated remarkable successes, by inducing systemic anti-tumor T cell responses. However, treatment is extremely costly and effectivity is limited by several bottlenecks that require strategic interventions. Relatively new to cancer immunotherapy are small-molecule drugs that target defined pathways or cells involved in immune activation and -suppression. Chemical drugs harbor unique properties that allow systemic administration and targeting of extra- and intracellular targets. They may activate complementary pathways and help overcome tolerance and immune suppression to induce effective anti-tumor responses. Synergistic effects may be achieved by combining immunotherapy with conventional therapies and/or new small-molecule chemotherapeutics.

Engaging immune pathways to treat cancer

Immunotherapy has recently become the fourth pillar of cancer treatment, next to surgery, chemotherapy and radiotherapy. Biomedical research has previously delivered personalized cancer treatment with drugs that target critical signaling molecules in cancer cells. However, efficacy of these targeted therapies is severely hampered by acquired resistance of clonally diverse tumor cell populations. Immunotherapy presents a unique approach with the capacity to tackle the problems of genetic **heterogeneity** (see Glossary) in cancer, since the immune system has inherently evolved to deal with genetic **heterogeneity** of microorganisms. The same immunotherapy treatment can act on a great variety of different cancer types, because it relies on tumor-extrinsic mechanisms. The aim of cancer immunotherapy is to activate a systemic, tumor-specific **cytotoxic T lymphocyte (CTL)** response. Ideally, a **CTL** response is raised that can eradicate (occult) metastases, also in cases where only the primary tumor has been diagnosed[1]. Often such a response is suppressed by tumor cells through upregulation of coinhibitory receptors, or checkpoints, that dampen the T cell response (Box 1). Current clinically approved checkpoint inhibitors are antibodies targeting cytotoxic T lymphocyte-associated protein 4 (CTLA-4) or programmed death 1 (PD-1). These powerful antibodies have shown remarkable results by restoring anti-tumor immune responses, but often lead to adverse immune-related events, which are treatment-limiting and may even result in mortality. For further advance, we need new strategies based on insights into the molecular basis of immunity, as well as cancer cell biology. Such therapies should increase tumor killing efficiency without increasing damage to healthy tissue. Here, we provide our perspective on the future of immunotherapies, with an emphasis on the contribution to be expected from the field of (bio)chemistry.

Box 1. How current immunotherapy with checkpoint blockade works

Immunity to cancer may be therapeutically promoted by antibody-based inhibition of membrane receptors that dampen T cell responses ("checkpoints"), a discovery for which James Allison and Tasuku Honjo received the Nobel Prize for Physiology or Medicine in 2018. The leading immunotherapeutic monoclonal antibodies (mAbs) block the interaction between PD-1 (programmed death 1) and its ligands PD-L1/L2, or CTLA-4 (cytotoxic T lymphocyte-associated antigen 4) and its ligands CD80/CD86 (B7-1/B7-2)[89-91], and proved efficacious in the treatment of immunogenic tumors, including melanoma and lung cancer[92-94]. PD-1 is associated with the tyrosine phosphatase SHP-2 that can dephosphorylate CD3 components and CD28 and thereby block TCR-signaling and CD28 costimulation[95]. CTLA-4 binds and downregulates CD80 and CD86 and thereby blocks CD28 costimulation[6,96]. Both checkpoints can act during T cell priming as well as in the tumor microenvironment (TME) and their exact division of labor is not yet clear[97,98]. Combined PD-1 and CTLA-4 blockade proved synergistic in late stage melanoma, suggesting different mechanisms of action[99].

Three bottlenecks in the immune response against cancer

Ideally, a 'cancer immunity cycle' is operational, wherein tumor cells are recognized by T cells and eradicated before they grow out or metastasize[2]. However, whether tumor cells can be recognized as "non-self" presents the first bottleneck (Fig. 1). Clonal deletion of self-reactive T cells during their development in the thymus (**central tolerance**) serves to avoid auto-immunity, but at the same time limits the T cell repertoire able to recognize tumor cells. As a prerequisite for clearance, tumor cells must therefore be different from non-transformed cells. Virus-induced cancers carry foreign proteins and will therefore be antigenic[3]. In addition, cancer such as melanoma, lung cancers and **microsatellite-instable** colon cancer feature tumors harboring a high mutational load and therefore express so-called **neoantigens**: peptides encompassing those mutations towards which no **central tolerance** has developed. These types of tumors are particularly sensitive to checkpoint blockade[4,5]. Other cancer types may carry alternative types of antigens towards which a naïve T cell repertoire is present. Many of such tumor antigens likely remain to be discovered.

Tumor-derived proteins are taken up by professional antigen-presenting cells, in particular dendritic cells (DCs), and processed into peptides subsequently presented to T cells in secondary lymphoid organs by major histocompatibility complex class I and class II (MHCI and MHCII) molecules. In order to become activated, $CD8^+$ or $CD4^+$ T cells need to recognize peptide-MHC complexes by their T cell receptor (TCR). Additional signals required to undergo clonal expansion and effector- and memory differentiation are delivered by specific

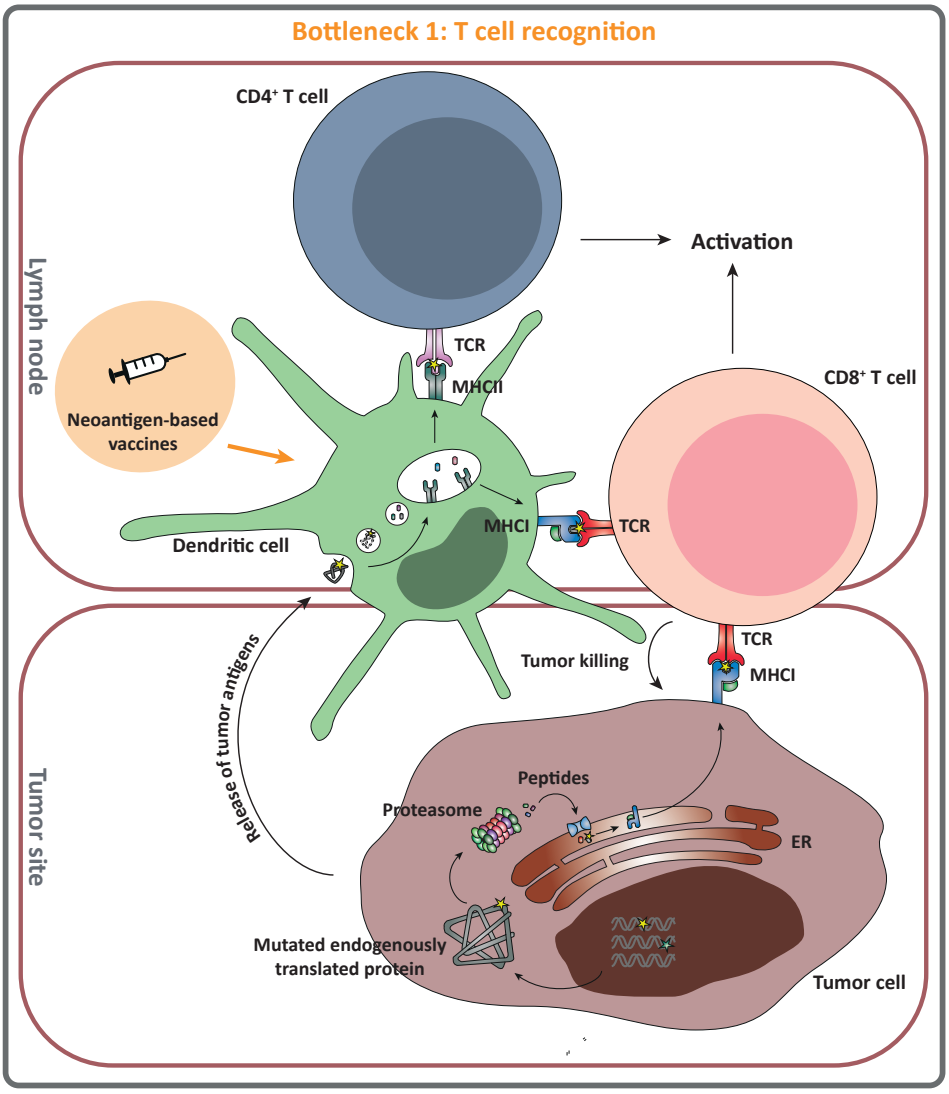

Figure 1. **The first bottleneck in the T cell response against cancer is the presence of tumor-specific T cells in the patient**. The tumor cells must generate (neo)antigens that can be presented by MHCI respectively MHCII and recognized by naïve $CD8^+$ and $CD4^+$ T cells in the patient. Neoantigen recognition leads to tumor cell lysis and release of tumor antigens. When taken up and processed by dendritic cells these contribute to the activation of T cells. Neoantigen-based vaccines boost this step in anti-tumor immunity. MHC, major histocompatibility complex; TCR, T cell receptor

costimulatory molecules and cytokines (Fig. 2). Such molecules are expressed by DCs upon pattern recognition receptor (PRR) activation by **pathogen**- or **danger-associated molecular patterns** (**PAMPs** and **DAMPs**, respectively), in concert with specific cytokines, such as type I interferons (IFNs). Tumors often do not supply these activating signals and therefore fail to activate DCs. Thymic **regulatory T cells** (t**Treg**) furthermore attenuate DC signals, in particular by downregulating costimulatory ligands CD80 and CD86 on DCs[6]. The **peripheral tolerance** that is thus imposed as a safeguard against auto-immunity constitutes the second bottleneck in the T cell response to cancer.

In case tumor-specific $CD4^+$ and/or $CD8^+$ T cells are activated by tumor-derived antigen and other signals, they differentiate into helper (Th) and cytotoxic effector cells, which exit lymphoid organs and travel via the blood to the tumor site. There, they are attracted to extravasate into the tumor tissue by chemokine signals. The tumor micro-environment (TME) and the signals it exudes in concert with the T cell response may lead to state of **immunosuppression**. The TME may present physical barriers that exclude the T cells and/or be immunosuppressive, thus erecting the third bottleneck to anti-cancer immunity (Fig. 3). It may express molecules such as IDO (indoleamine 2,3-dioxygenase) and PD-L1 (programmed death ligand 1) to directly inhibit effector T cell function, but also cytokines such as TGF-β (transforming growth factor β) and IL-10 (interleukin 10) to promote **Treg** cell expansion and function, thereby inhibiting effector T cell responses[7]. Optimal cancer treatment must ensure that all three bottlenecks, i.e. **central tolerance**, **peripheral tolerance** and tumor-associated **immune suppression** are overcome, so that tumor cells are detected and properly attacked by **CTLs**, ideally without invoking auto-immunity.

Therapeutic vaccination with (neo)antigens

The aim in therapeutic vaccination is to prime tumor-specific naïve T cells, thereby developing or augmenting T cell responses against tumors[8]. This approach can work in tumors that either do not raise a tumor-specific T cell response by themselves, or raise an ineffective one, and thus remain devoid of T cell infiltration. The advantage is that such "**cold" tumors** have likely not yet developed an immune-suppressive environment, for this generally occurs through a dialogue with activated T cells[9]. Since the advent of genome-wide DNA sequencing of cancers, the focus in therapeutic vaccination development has been on **neoantigens**: mutated self-antigens that arise from tumor-specific somatic DNA mutations[10]. While other tumor-associated antigens may lead to toxicity in healthy tissues that also express the target, **neoantigens** are exclusive to tumor cells and are hence actively pursued in cancer immunotherapy[11].

In addition to pattern recognition receptor (PRR) signals and/or inflammatory cytokines, $CD4^+$ T cells provide the help that DCs need for the initiation of effective primary and memory $CD8^+$ T cell responses[12-15]. For this reason, the most successful peptide vaccines to date are long peptides (around 40 amino acids in length) or antigen-encoding mRNA or DNA encompassing both MHCI and MHCII epitopes, thus activating both $CD8^+$ and $CD4^+$ T cells[16]. These vaccines have shown therapeutic promise in treatment of early stages of (virus-induced) cancer, but not in later stages. The inferred importance of $CD4^+$ T cells is illustrated by Sahin et al., who showed that 60% of elicited T cell responses were $CD4^+$ upon vaccination with RNAs that each encode five mutated long peptide sequences predicted for MHCI binding[17]. Likewise, Keskin et al. also observed prominent $CD4^+$ T cell responses in a phase Ib glioblastoma trial after administering a personalized **neoantigen** vaccine consisting of 20 long peptides[18]. Although vaccination successfully induced systemic and intratumoral **neoantigen**-specific immune responses, all patients eventually relapsed, indicating other challenges, including **immune suppression**, still pose significant bottlenecks. Enhancing activation of DCs by adjuvants

Therapeutic cancer vaccines as monotherapy will fail to induce potent anti-tumor responses, because they lack costimulatory signals. The use of adjuvants that activate DCs via PRRs, such as toll-like receptors (TLRs) helps overcome **peripheral tolerance**[19]. Biological adjuvants such as CpG, poly IC:LC (polyinosinic and polycytidylic acid) or (incomplete) Freund's adjuvant, are regularly included in both preventive and therapeutic vaccine, and synthetic approaches provide ample opportunities for further improvement[20-23]. An excellent example of a vaccine aimed to overcome both bottleneck 1 and 2 was recently described by Zom et al., who synthesized a dual synthetic long peptide conjugate that triggers two PRRs: NOD2 (nucleotide-binding oligomerization domain-containing protein 2) and TLR2[20,24,25].

Small molecules as DC activating, immunomodulatory drugs

Chemical drugs are potentially superior to biologicals because of their tissue-penetrating capacities and considerably lower production costs compared to mAbs[26]. They can target both intracellular proteins and cell-surface receptors, while therapeutic antibodies are restricted to membrane proteins and secreted proteins. Moreover, their half-lives are shorter, allowing more acute action, potentially reducing the chance of systemic adverse effects. We will highlight some of the most potent examples in the next paragraphs.

The first small-molecule immune-oncology drug approved by the FDA was

imiquimod, an imidazoquinoline derivative commonly used in the treatment of genital warts and approved for treatment of basal cell carcinoma[27]. Its target is TLR7, a PRR that binds conserved **PAMPs**, such as double-stranded RNA, lipopolysaccharide (LPS) or unmethylated CpG DNA[28]. Most TLRs are located on the cell surface, but TLR3, 7, 8 and 9 are located in endosomal compartments[29]. A small-molecule TLR8 agonist, motolimod (VTX-2337), has demonstrated anti-tumor activity in recurrent or metastatic squamous cell carcinomas of the head and neck (SCCHN) by stimulating natural killer (NK) cell activation, enhancing antibody-dependent cell-mediated toxicity and through the induction of Th1-polarizing cytokines[30]. A subset of treated patients demonstrated even higher responses in combination with cetuximab (anti-EGFR (endothelial growth factor)) or chemotherapy[31,32]. Imiquimod, motolimod and resiquimod, a relative of imiquimod that targets TLR7 and TLR8, were tested in a number of clinical trials for treatment of solid tumors, often as adjuvants to vaccination. The search for small molecules targeting other (and preferably multiple) TLRs continues with the help of high-throughput screening of drug libraries in cell-based assays[33].

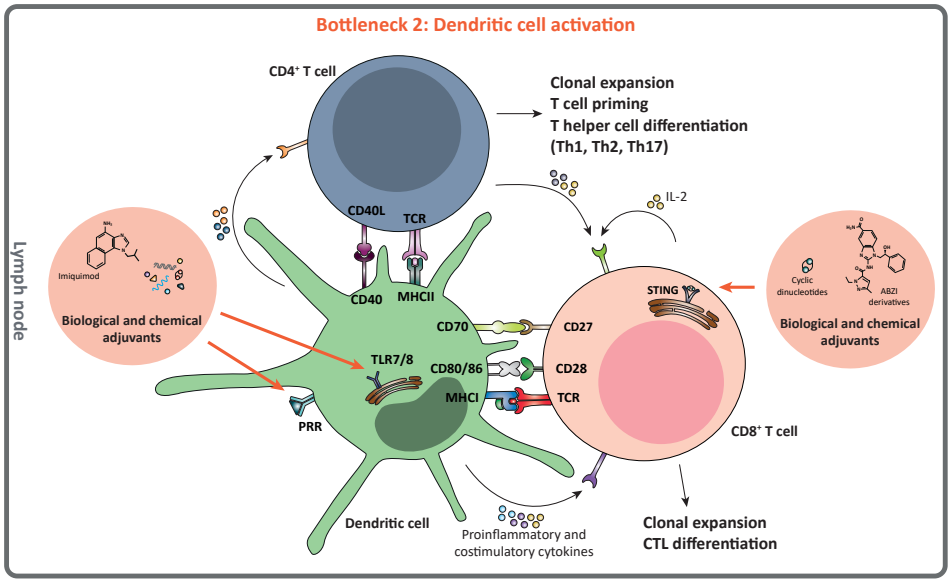

Figure 2. **The second bottleneck in anti-tumor T cell immunity is dendritic cell activation.** Dendritic cells must receive activating signals, such as DAMPs and PAMPs, in order to supply the costimulatory signals needed for priming expansion and differentiation of T cells. Tumors generally lack these activating signals and fail to prompt costimulation, even when their antigens are recognized by T cells. DC activation of can be induced by adjuvants targeted at extracellular or intracellular PRRs. CTL, cytotoxic T lymphocyte; DAMP, danger-associated molecular pattern; MHC, major histocompatibility complex; PAMP; pattern-associated molecular pattern; PRR, pattern recognition receptor; TCR, T cell receptor; Th, T helper

Other PRRs, such as NOD-like receptors (NLRs), C-type lectin receptors (CLRs) or RIG-I-like receptors (RLRs) have been less extensively studied than TLRs, but agonists targeting these families likely also enhance immune responses[34].

Another innate sensor implied in anti-tumor immunity is STING (stimulator of interferon genes), a PRR on the ER membrane that binds cyclic dinucleotides derived from cytosolic DNA converted by cGAS (cyclic-GMP-AMP synthase)[35]. The cGAS/STING pathway leads to type I IFN production, which promotes DC activation and T cell priming, as shown in response to tumors in mice[36]. This finding put STING on the map as a target for cancer immunotherapy, in addition to being a potent adjuvant. Intratumoral injection of small-molecule STING agonist DMXAA (5,6-dimethylxantheone-4-acetic acid, Vadimezan) demonstrated specificity and efficacy in controlling established and distant tumor progression in mice[37]. However, this drug was ineffective in humans because human and mouse STING are structurally different[38-40]. Considerable efforts to create derivatives of DMXAA that are active against human STING are ongoing[41]. In a high-grade serous carcinoma mouse model, a cyclic dinucleotide STING agonist combined with anti-PD-1 mAb increased systemic tumor responses to chemotherapy[42]. In a phase I clinical trial (NCT03010176) intratumoral vaccination with Merck's cyclic dinucleotide STING agonist, MK-1454, did not show remissions in monotherapy and 25% responders in combination with pembrolizumab (anti-PD-1). Aduro Biotech's STING agonist ADU-S100 is also a cyclic dinucleotide, chemically modified to enhance stability and increase efficacy[37]. ADU-S100 monotherapy led to a partial response in two out of the 40 patients enrolled and stable disease in 11 patients. The safety and efficacy as single agent and in combination with ipilimumab (anti-CTLA-4) are under investigation in an ongoing phase I study (NCT02675439) (http://investors.aduro.com/news-releases/news-release-details/aduro-announces-first-patient-dosed-phase-1-study-adu-s100?ID=2386898&c=242043&p=irol-newsArticle). The drug is currently being tested in a phase Ib clinical trial (NCT03172936) in combination with spartalizumab (former PDR001), an anti-PD-1 antibody developed by Novartis. Preliminary results presented at the 2019 American Society of Clinical Oncology (ASCO) meeting were disappointing. Five out of 83 patients achieved confirmed responses - one patient with a complete response (CR) and three with partial response (PR) among PD-1 naïve triple-negative breast cancer (TNBC) patients, and two with PR among previously immunotherapy-treated melanoma patients (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). A phase II trial combining ADU-S100 and anti-PD-1 for first-line treatment of PD-L1-positive recurrent or metastatic HNSCC is now recruiting (NCT03937141).

Recently, three related small-molecule STING agonists based on amidobenzimidazole (ABZI) were reported[43]. In contrast to dinucleotides, which

are rapidly degraded by phosphodiesterases in the body and therefore have to be injected intratumorally, these chemical compounds can be delivered intravenously[44,45]. Systemic administration renders them suitable for treatment of less accessible solid tumors and potentially achieves systemic efficacy. The most potent compound described in this study binds three human and one mouse STING alleles with high affinity and proved efficacious in mouse models after i.v. injection. In contrast to the reported successes, other studies showed that upregulation of cGAS/STING signaling enhanced carcinogenesis and induced immune checkpoint IDO in poorly immunogenic tumors, dampening the immune response and promoting tumor growth[46-48]. These contradicting findings highlight the complexity of the various signaling pathways, but also indicates new avenues for combination treatment.

Targeting the TME to relieve cancer-associated immune suppression

The TME can render T cells dysfunctional and attenuate the efficacy of immunotherapy[49]. Checkpoint inhibition can help to overcome $CD8^+$ T cell dysfunction, in part perhaps because responsiveness depends on specific T cell differentiation states[50,51]. Elucidating and targeting **immune suppression** and -evasion mechanisms can help to improve clinical outcomes. Small-molecule drugs may again be used to specifically target suppressive factors and to induce or restore immune reactivity in the TME. A number of small molecules that block the PD-1/PD-L1 interaction have been described, although none have been approved by the FDA. Curis' CA-170, claimed to inhibit PD-L1, PD-L2 and VISTA, is currently being tested in a phase I clinical trial to treat patients with advanced tumors and lymphomas (NCT02812875)[52].

One of the main advantages of small molecules is the fact that they can enter the cell, while mAbs cannot. A promising target in this context is RORγt (retinoic acid receptor-related orphan receptor gamma), a transcription factor involved in the pro-inflammatory IL-17 pathway. A large number of RORγt antagonists is under investigation for treatment of autoimmune and inflammatory disorders[53,54]. In contrast, RORγt agonists can induce production of cytokines and chemokines, decrease proliferation of **Tregs** and revoke **immunosuppression** by tumor cells[55,56]. Specifically, RORγt agonists have demonstrated enhanced activity, proliferation and survival of Th17 ($CD4^+$) and Tc17 ($CD8^+$) cells in vitro[57-59]. Two phase II trials have been designed to test the effects of these agonists in human. One of these trials aims to investigate the responses to RORγt agonist LYC-55716 (Lycera) in six solid tumor types (NCT02929862) and the other aims to test the safety and tolerability in combination with anti-PD-1 antibody pembrolizumab (NCT03396497). It

is unclear what the effects on tumor control will be, since the presence of Th17 cells has been associated with a poor prognosis in a number of cancer types[60-62]. In these cases, RORγt antagonists may provide therapeutic benefit, but inhibitor design proves complicated because of RORγt's large and lipophilic ligand binding domains[63]. One of the main adverse effects of stimulating this transcription factor is the occurrence of autoimmune disorders, such as inflammatory bowel disease[64]. Taking into account that the 'classical' checkpoint inhibitors anti-CTLA-4 and anti-PD-1 also induce autoimmune disorders, the combination may strongly induce side effects. The ongoing trials will tell.

Another emerging TME target in immunotherapy is IDO1, a tryptophan catabolic enzyme found to induce **immune suppression** and -evasion through

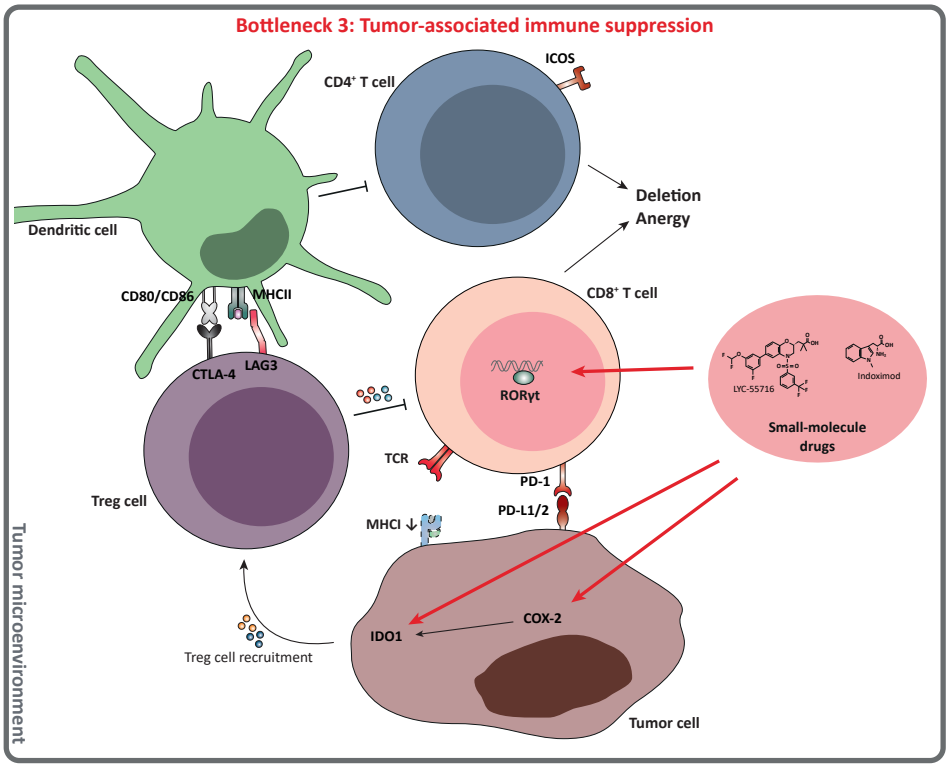

Figure 3. **Tumor-associated immune suppression presents a third bottleneck in anti-tumor T cell activity.** Tumors often establish an immunosuppressive environment through upregulation of inhibitory checkpoint molecules, such as PD-L1 (programmed death ligand 1), downregulation of MHCI or expression of regulatory T cell (Treg)-recruiting cytokines. Suppression can be relieved by small-molecule drugs targeted at relevant mechanisms. CTL, cytotoxic T lymphocyte; CTLA-4, cytotoxic T lymphocyte-associated antigen 4; LAG-3, lymphocyte-activation gene 3; MHC, major histocompatibility complex; PD-1, programmed death 1; PRR, pattern recognition receptor; TCR, T cell receptor.

the expansion of **Treg** cells[65,66]. IDO1 is the most broadly expressed of three enzymes (together with IDO2 and tryptophan 2,3-dioxyenase (TDO)) involved in the first step of the kynurenine pathway. Indoximod was the first IDO1 inhibitor to be tested in humans, but with confusing results[67]. The exact mechanism by which indoximod operates is not fully elucidated, but it has been suggested that the drug inhibits mTORC1, a downstream effector of IDO1, and not IDO1 itself. Despite promising results from multiple phase I/II trials, epacadostat, a direct IDO1 inhibitor, failed to show increased benefit in combination with anti-PD-1 in a stage III clinical trial, but the search for new inhibitors continues[68-71]. Inhibition of IDO1 alone frequently results in resistance by upregulation of IDO2 and TDO, hence broad-spectrum inhibitors targeting all three may provide most benefit[72].

One of the drivers of IDO1 expression is cyclooxygenase (COX)-2, a fairly unexplored target in immunotherapy, but a common target of non-steroidal anti-inflammatory drugs (NSAIDs)[73]. COX-2 catalyzes the synthesis of prostaglandins, lipid compounds involved in many physiological processes in response to injury and inflammation. Expression of this enzyme has been associated with several cancers and consequently celecoxib, an NSAID that inhibits COX-2 as well as IDO1, is being explored as anti-cancer therapeutic[74-76]. Buzharevski et at. developed analogues of celecoxib and showed a potent cytostatic effect on melanoma and colon cancer cell lines[77]. Concurrent inhibition of COX-2 and EGFR was previously found to have synergistic effects and recently Tang et al. reported the dual inhibition of COX-2 and EGFR, by melafolone, a naturally occurring flavonoid[78,79]. They demonstrated improved PD-1 blockade in lung cancer by downregulating PD-L1 and normalizing tumor vasculature by downregulating VEGF or TGF-β. These examples of drugs that harbor dual activity against tumor-associated molecules are very promising.

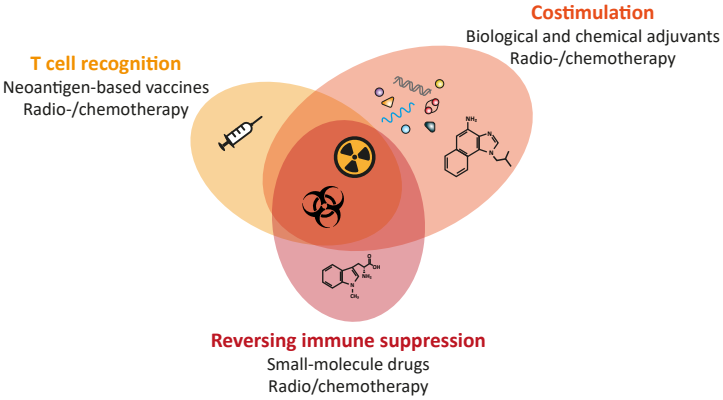

Figure 4. **Neoantigen-based vaccines, adjuvants, small-molecule drugs and conventional chemotherapeutic drugs each work at different stages of immunity.** A combination of strategies can boost T cell immunity and overcome tumor-associated immune suppression.

Concluding remarks

Immunotherapy has demonstrated potent anti-tumor T cell responses in treatment of various cancer types; however, only in a subset of patients and often accompanied by severe adverse events and systemic toxicity. Opportunities for improvement are provided by small-molecule drugs. Their pharmacokinetics allow systemic administration without the rapid degradation often hampering effectivity of biological drugs. Due to their tissue-penetrating capacities small molecules can be directed at both extra- and intracellular targets to inhibit relevant immunosuppressive pathways activated by tumor cells. Studies aimed at elucidating the molecular mechanisms responsible for tumor-associated **immune suppression** will greatly contribute to the advance of this relatively unexplored area of drug development. By combining chemical drugs with conventional strategies, cancer therapies can be improved to overcome the bottlenecks in anti-cancer T cell response, ideally achieving synergistic effects. Strategic combinations in immunotherapy will include (checkpoint) antibodies with diverse forms of therapeutic vaccination[80,81], but also combination therapies with (targeted) drugs, conventional chemotherapy or radiotherapy[82]. Both RT and CT may induce **immunogenic cell death**, resulting in release of tumor antigens and other danger signals that in turn can activate DCs via innate receptors, such as TLRs or the cGAS/STING pathway, and they modulate the TME[83-86]. These therapies operate at the level of all three bottlenecks (Fig. 4), thereby creating an immune-activating environment and as a result enhance the effect of immunotherapies, potentially rendering even **cold tumors** susceptible to immunotherapy[87,88]. In a way, the tumor then functions as its own vaccine, inducing a systemic anti-tumor response.

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Glossary

Central tolerance: the absence of self-reactive T cells to avoid autoimmunity. T cells that recognize self-antigens are deleted during negative selection in the thymus.
Cold tumor: non-immunogenic tumor devoid of tumor-infiltrating lymphocytes and hence less sensitive to immunotherapy.
Cytotoxic T lymphocyte (CTL): $CD8^+$ killer T cell that recognizes intracellular alterations, in the context of major histocompatibility class I (MHCI) complexes that are expressed on all tissues and thus also on a wide variety of tumor types.
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 (PRRs) induces innate immunity and DC maturation.
Heterogeneity: phenotypical variations between tumor cells, often of genetic origin, that affect therapy response and hamper treatment design.
Immunogenic cell death: form of cell death that, in contrast to apoptosis, results in the release of immune-stimulating factors, such as danger-associated molecular patterns (DAMPs) or tumor antigens.
Immunosuppression: inhibition of immunity induced by tumor cells to escape elimination. Often mediated by induction of Treg cells, upregulation of inhibitory checkpoints or downregulation of activating signals.
Microsatellite instability: genetic predisposition to mutation caused by the loss of DNA mismatch repair activity.
Neoantigen: tumor antigen arising from somatic DNA mutations, so that no central tolerance has been raised. T cells can recognize these antigens and attack tumor cells expressing them.
Pathogen-associated molecular patterns (PAMPs): molecules not found on vertebrates that trigger innate immunity by binding pattern recognition receptors. Classic PAMPs are dsRNA, endotoxins or bacterial cell wall constituents.
Peripheral tolerance: suppression of self-reactive immune cells in the periphery that have escaped central tolerance, for example through suppression by Tregs or induction of anergy.
Regulatory T cell (Treg): subset of $CD4^+$ T cells that modulate the immune response by suppressing effector cells.

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