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Review

Rationally combining immunotherapies to improve efficacy of immune checkpoint blockade in solid tumors

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ABSTRACT

With the widespread application of immune checkpoint blocking antibodies (ICBs) for the treatment of advanced cancer, immunotherapy has proven to be capable of yielding unparalleled clinical results. However, despite the initial success of ICB-treatment, still a minority of patients experience durable responses to ICB therapy. A plethora of mechanisms underlie ICB resistance ranging from low immunogenicity, inadequate generation or recruitment of tumor-specific T cells or local suppression by stromal cells to acquired genetic alterations leading to immune escape. Increasing the response rates to ICBs requires insight into the mechanisms underlying resistance and the subsequent design of rational therapeutic combinations on a per patient basis. In this review, we aim to establish order into the mechanisms governing primary and secondary ICB resistance, offer therapeutic options to circumvent different modes of resistance and plea for a personalized medicine approach to maximize immunotherapeutic benefit for all cancer patients.

Contents

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1. Introduction

For many years, directing our immune system to target cancer was minimally effective in generating durable clinical responses. T-cell responses induced by often inferiorly formulated and designed vaccines were not powerful enough to overcome the many barriers posed by advanced solid tumors [1,2]. However, following the unprecedented results of 're-invigorating' T cells in a proportion of metastatic cancer patients by blocking immune inhibitory checkpoints, tumor immunotherapy has regained its position at the forefront of cancer treatment today [3]. To this date, the most studied and manipulated immune checkpoints on T cells are the receptors T lymphocyte associated antigen 4 (CTLA-4) and programmed cell death protein 1 (PD-1). Targeting CTLA-4 and the PD-1-PD-L1-axis with antagonistic antibodies has proven to be highly efficacious in a proportion of cancer patients (Fig. 1). The finding that a subgroup of patients has a pre-existing but dysfunctional anti-tumor immune response that can be therapeutically restored, prompts further investigation into what constitutes tumor immunity and precludes response to immunotherapy.

2. Current state of immune checkpoint blockade (ICB) in advanced cancer

Immune checkpoints are receptors expressed by T cells that upon ligation by their respective ligands regulate immune cell effector functions and proliferation thereby maintaining tolerance to self-antigens and ensure immune homeostasis [4,5]. Blocking inhibitory checkpoints using antagonistic antibodies may 'release the brakes' on T cells, including those cells specific for tumor antigens.

CTLA-4 is upregulated by T cells following recognition of cognate antigen by antigen presenting cells (APCs) in the lymph node [6]. The structure of CTLA-4 is nearly identical to the costimulatory receptor CD28 but interacts with much higher affinity for its ligands CD80/CD86 (B7-1/B7-2) expressed by the APC [7]. In contrast to CD28 stimulation, CTLA-4 has an inhibitory effect on effector T cells by causing cell cycle arrest [6,7]. Additionally, regulatory T cells (Tregs) constitutively express high levels of CTLA-4 on their cell surface, further facilitating their immune suppressive potential [8]. Antibodies directed towards CTLA-4 may therefore also act by decreasing Treg frequencies in blood and tumor via antibody dependent cytotoxicity (ADCC) [9,10].

Besides CTLA4, activated T cells express PD-1, and the coupling of PD-1 to programed cell death ligand 1 (PD-L1, also called B7-H1) or PD-L2 (B7-DC) restrains T-cell effector function and proliferation [11]. PD-L1 is expressed on tumor cells (constitutively due to oncogenic signaling or in response to interferons), myeloid cells including APCs, and PD-L2 is solely expressed by APCs [12]. It has recently been shown that both PD-L1 on host myeloid cells and on tumor cells is a prerequisite for anti-PD-1-therapy efficacy [13]. PD-1 was previously thought to attenuate T-cell receptor (TCR) -signaling but recent insights have firmly established the inhibitory role of PD-1 on downstream CD28-signalling in T-cells, further emphasizing the importance of proper (local) co-stimulation for T-cell function [14,15].

Thus far, four ICBs are FDA approved; anti-CTLA-4 (ipilimumab), anti-PD-1 (pembrolizumab and nivolumab) and anti-PD-L1 monoclonal antibodies (atezolizumab). Response rates vary between 11 and 40% depending on tumor type with PD-1 blockade yielding superior responses at a more favorable toxicity profile compared to CTLA-4 inhibition [16–20]. It has been suggested that the discrepancy in toxicities between ICBs can be explained by the

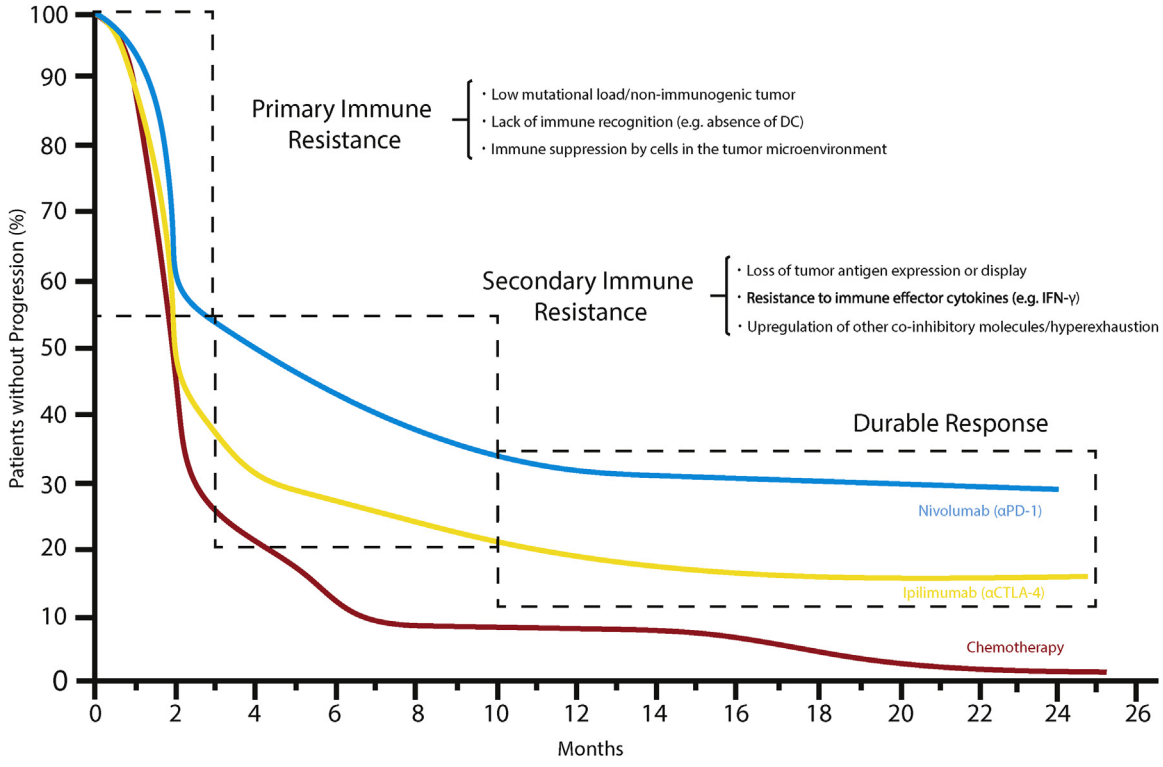

Fig. 1. Progression-free survival curves for chemotherapy, anti-PD-1- and anti-CTLA-4- checkpoint blockers; primary and secondary resistance to immune checkpoint blockade (ICB) therapy precludes patients from achieving durable responses and long-term survival. When patients do not respond to ICBs immediately following start of treatment they experience primary immune resistance. When patients do respond initially but relapse over time, secondary resistance to ICB-treatment has developed. PFS-curves have been derived from the following clinical trials investigating ICB-efficcacy in metastatic melanoma: Robert et al. NEJM 2011, Schachter et al. ASCO #9504 2016.

time of checkpoint engagement in the T-cell response. The PD-1/PD-L1 axis has been proposed to operate later during the effector phase of a T cell, resulting in a more confined response whereas CTLA-4 acts on the lymph node during T-cell priming [21]. These temperospatial differences between ICBs are being exploited by combining anti-CTLA-4 and anti PD-1/PD-L1 in the clinic. Combining ipilimumab (anti-CTLA4) and nivolumab (anti-PD-1) in BRAF wild-type melanoma patients was efficacious in reaching its primary endpoint of progression free survival [22]. Although primary analysis showed a significant advantage of combination therapy over both monotherapies, recent follow-up data report a 2-year survival rate of 64% in the combination treated group compared to 59% survival in αPD-1 monotherapy treated patients. Notably, the difference in serious adverse event rate was considerable (58% vs 21%) suggesting limited clinical value of this immunotherapy combination [23]. In other solid tumors including non-small-cell lung cancer (NSCLC) and renal cell carcinoma, response rates of ICB monotherapy are more modest ranging from 15 to 20% [24–29]. Reasons underlying this heterogeneity in response rates shall be further addressed in the following sections.

Despite the significant progress that has been made with ICB across multiple tumor types, still much remains to be gained. Recent insights into tumors from initial and durable responders and non-responders to ICB have offered novel insights into tumor-immune interactions and the prerequisites for establishing effective and durable anti-tumor immunity. A complete understanding of these processes is still lacking but with knowledge of basic (tumor-)immunological principles and the implementation of innovative diagnostics, rational therapeutic combinations can be designed to improve ICB response rates in advanced cancer patients.

3. Mechanisms underlying primary and secondary resistance to ICB

Despite the success of ICBs, only a minority of patients experience durable responses to ICB therapy. The remainder of patients do not respond at all (primary resistance) or initially respond but relapse over time (secondary resistance) (Fig. 1). A plethora of mechanisms underlie ICB resistance. Primary as well as secondary resistance to ICB results from an intricate interplay between immune cells, other stromal cells (e.g. cancer associated fibroblasts (CAF), endothelial cells) and tumor cells, all together composing the tumor microenvironment (TME). In general, primary resistance occurs when tumors lack an endogenous adaptive and functional immune infiltrate (this includes the pre-existence of an irreversibly 'hyper-exhausted' T-cell response incapable of responding to ICB). Secondary resistance recapitulates all the adaptive mechanisms which takes place subsequently to therapeutic pressure resulting in the failure to *maintain* an effective anti-tumor response. It has to be noted that the proposed distinction between primary and secondary immune resistance is pragmatic and useful in most causes of resistance but in reality, multiple opposing phenomena may be at play and some (such as an immune suppressive TME) may act throughout the course of ICB treatment.

3.1. Primary resistance to ICB

Primary resistance to ICB can result from the absence of a functional immune response to poorly immunogenic tumor (Fig. 2). Tumors with a high non-synonymous mutational load are more likely to display neo-antigens that could be considered foreign to the immune system and thus possibly immunogenic [30,31]. Therefore, it is not surprising that cancer types with the highest mutational loads generally have high response rates to ICB (melanoma, NSCLC) [32]. Also, subtypes of tumors characterized by deficiencies in mismatch repair genes, as is the case for microsatellite instable colon cancers, respond markedly better to ICB compared to their microsatellite stable counterparts [33]. However, even within the same tumor type, high mutational load in tumors was shown to at least partially predict response to both anti-PD1- and CTLA4-inhibition further supporting the importance of tumor mutational landscape and concomitant immunogenicity in determining ICB efficacy [31,34,35]. But does an increased neo-antigen load also necessarily lead to enhanced cytolytic T-cell responses in tumors? A seminal study by Rooney et al. shows that increased neo-antigen load, and in some tumors the presence of viral genes, was indeed associated with enhanced cytotoxic T-cell activity [36]. In line with these findings positive correlations between anti-CTLA-4 therapy efficacy and the presence of a pre-existing immune response together with a high mutational- and neo-antigen load in melanoma have been found [35]. A similar prerequisite for ICB-efficacy was found in melanoma patients where a pre-existing CD8+ PD1+ T-cell infiltrate in the invasive tumor margin and center predicted response to pembrolizumab (anti-PD-1 antibody) treatment [37]. High mutational load and/or expression of neo-antigens alone does not seem to fully predict response to ICB, and others have shown that expression of other antigens such as cancer tests antigens and tumor associated (overexpressed) antigens may also contribute to tumor immunogenicity [38]. These data demonstrate that endogenous immune reactivity characterized by cytolytic T cells in the tumor constitutes a basic requirement for ICB efficacy.

Another major reason for primary ICB resistance is the immune-privileged tumor micro-environment, characterized by the paucity of infiltrating tumor-specific T cells. The existence of this so-called 'non-inflamed' tumor derives from the inadequate generation or recruitment of tumor-specific T cells, or the physical inability of immune cells to reach the tumor. In order to induce a functional immune response, innate immune recognition and subsequent priming of tumor-antigen specific T cells in the lymph node is imperative [39,40]. Interrogation of the the TCGA database by Gajewski and colleagues to identify factors associated with a T-cell inflamed tumor phenotype failed to detect an association between a T-cell inflamed tumor and mutational burden [41]. However, they did find strong positive correlations between T-cell infiltration and presence of DC-related genes emphasizing the importance of DC-mediated anti-tumor immunity over solely tumor antigenicity. In accordance with these data, others have found intratumoral DCs to be critical for establishing tumor immunity, with tumors being capable of actively subverting DC-accumulation or function in vivo [42]. One such cause of immune ignorance that could be at play is a mutated β-catenin/Wnt-signaling pathway in tumor cells, which causes a decrease in chemokines known to be crucial for DC-homing to the tumor [43]. Such mutations could present a significant downside to having a high mutational burden, and could provide an explanation for the heterogeneity observed in ICB efficacy in high mutation tumors [41]. Interestingly, other mutations in key oncogenic pathways are currently being identified that impede immune cell-infiltration and/or function in the tumor (e.g. mutations in PTEN, MYC etc.) [44,45]. Re-establishing immune surveillance by skewing myeloid precursors to the DC-fate, targeting oncogenic pathways or promoting DC-function may be essential in sensitizing patients to ICB.

Moreover, the amount of intratumoral T effector cells could determine the potential of ICB therapy to induce robust anti-tumor response. T effector cells can be mechanically excluded by a psychical barrier consisting of thick extracellular matrix produced by stromal cells (e.g. CAFs) [46]. CAFs can also exclude T cells through coating of cancer cells with CXC chemokine ligand-12

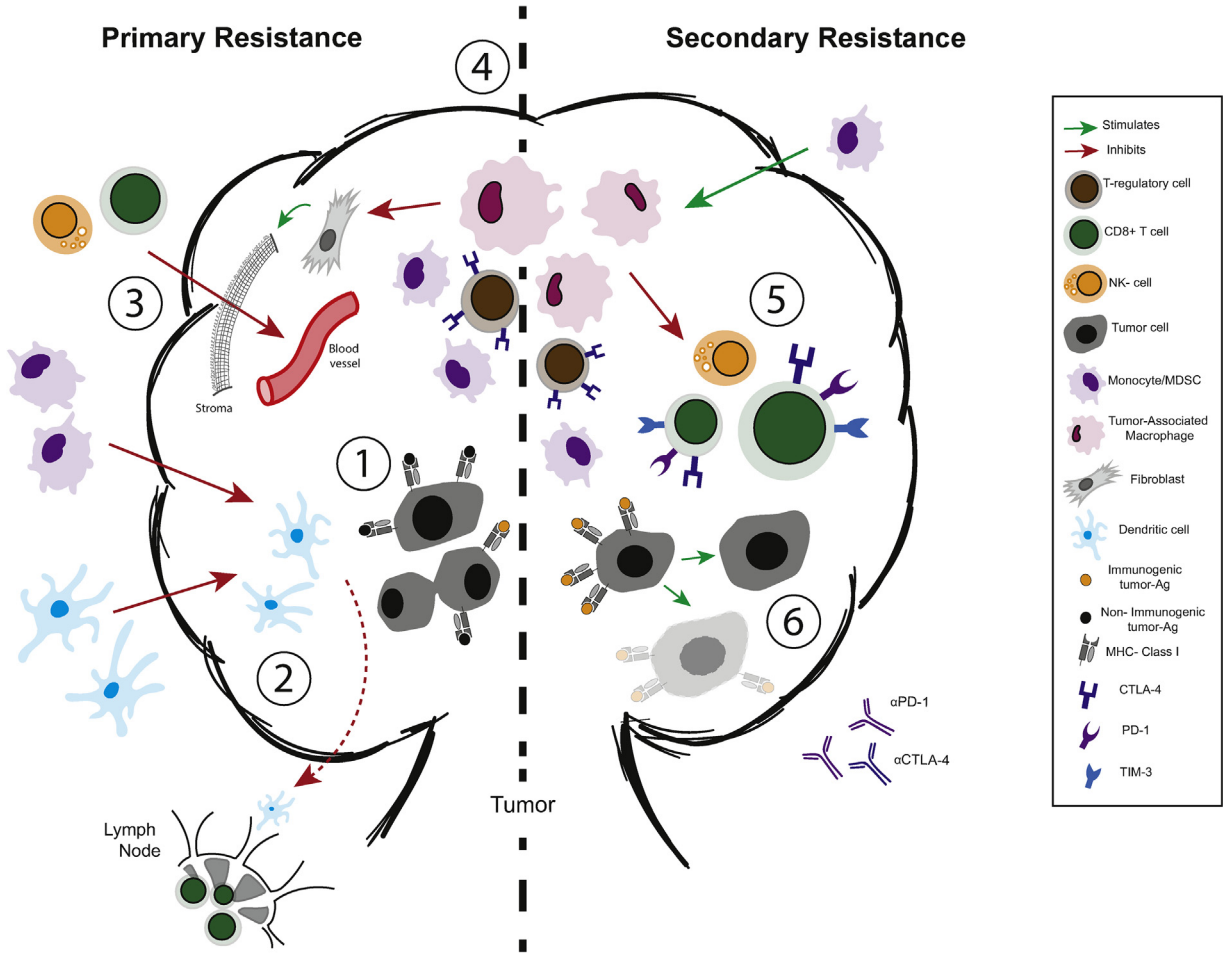

Fig. 2. Different processes underlying primary and/or secondary resistance to checkpoint blocking antibodies in solid tumors; Primary resistance can result from the absence of a functional immune response to a poorly immunogenic tumor. The magnitude of resistance is influenced by differences in: (1) non-synonymous mutational load and neo-antigen expression, (2) the presence of intratumoral dendritic cells capable of antigen trafficking and presentation, (3) the generation or recruitment of tumor-specific T cells and (4) immune inhibition by inhibitory immune cell populations in the TME. Continuous therapeutic pressure may result in the development of secondary (acquired) resistance. Mechanisms include (5) upregulation of other co-inhibitory molecules and (6) loss of tumor (neo)antigen expression.

(CXCL12) [47]. Furthermore, the abnormal vasculature in the TME expressing high endothelial Fas-ligand promotes intravascular T cell apoptosis [48]. In addition, effector T cells will need to express the proper integrins in order to bind to the tumor endothelium, egress and exert their function. Changing the route of vaccination was shown to modulate integrin expression on T-cells and improve homing to the tumor tissue [49].

Finally, immune resistance can also be achieved by the preferred attraction of immune inhibitory cells to the TME. Tregs, tumor associated macrophages (TAMs) and myeloid derived suppressor cells (MDSCs) often populate the TME where they exert several immune inhibitory properties, making it difficult for T-cells to sustain their anti-tumor effector responses, especially in the setting of ICB [50].

Tumors can recruit, induce and expand Tregs capable of suppressing (ICB-induced) anti-tumor T cells via competition for key survival factors (CD80/86 co-stimulatory signals, IL-2) and suppressive cytokines (e.g. IL-10, TGF-β, IL-35). As Tregs are much more potent in binding these survival factors by means of constitutive CTLA-4 and IL-2-receptor (CD25) expression, CD8+ T cells are shortly outcompeted. Tregs were found to be involved in limiting αPD-1-efficacy as depletion of these cells improved responses to therapy in several solid tumor mouse models [51].

TAMs contribute to a majority cancer hallmarks including neo-angiogenesis, metastasis, chronic inflammation and immune suppression [52]. Skewing or depleting TAMs could therefore affect multiple critical steps in oncogenesis and abrogate different modes of immune resistance [53]. TAMs display an alternatively activated 'M2'-phenotype known to be critical in controlling tissue homeostasis and wound healing [52]. In the tumor, however, this phenotype is undesirable as it enables potent T-cell inhibition via cytokines (e.g. IL-10), depletion of key metabolites (expression of arginase, IDO) or by contact inhibition (e.g. via PD-L1) [52]. This TAM-phenotype is also critical in determining ICB efficacy as an innate 'wound healing' and immune suppressive gene signature was found to optimally predict non-responders prior to αPD-1 treatment [54]. Recently, Arlauckas et al. identified another mechanism whereby TAMs can limit αPD-1 therapy efficacy. They found TAMs to capture PD-1 targeting antibodies on the T-cell surface thereby considerably limiting the duration of drug efficacy [55].

Similar to TAMs, MDSCs can potently inhibit T-cell function but they can also indirectly contribute to an immune suppressive TME by differentiating into TAMs or skewing them to an M2-phenotype [56]. MDSCs are the epitome of chronic and systemic immune modulation by a tumor that secretes numerous molecules capable of skewing myelopoiesis (e.g. GM-CSF, IL-6, VEGF etc.) [56].

The presence of these immune inhibitory cells in most patients tumors suggests that a balance exists whereby ICB-responsive anti-tumor T cells are in equilibrium with immune suppressive

cells in the TME [57]. In line with this hypothesis is data from αPD-1- and αCTLA-4-treated patients tumors showing increased presence of memory T cell- and (activated) DC gene signatures in ICB responders, in contrast to MDSC, Treg and monocyte signatures in the non-responding patients [58]. Findings ways to shift this balance preferably from both sides will be key in improving ICB responsiveness.

3.2. Secondary ICB resistance

Over time, relapse will occur in a majority of patients initially responsive to ICB therapy. A possible phenomenon underlying secondary resistance are new mutations acquired by tumor cells that have expanded under continuous therapeutic pressure (immune editing) and have eventually grown out (immune evasion) (Fig. 2).

Tumor intrinsic mutations that have evolved over the course of ICB-treatment can have highly variable consequences to tumor-immune interactions. It has been known for several years that loss of antigen display by tumor cells due to mutations in the antigen-processing machinery (e.g. TAP) or proteins involved in antigen presentation (β2-microglobulin, HLA) can cause lack of recognition by CD8+ T-cells following immunotherapy [59]. Recently, similar mutations were detected in patients who relapsed following αPD-1 ICB [60]. Another pathway that can be silenced by mutations following ICB is the interferon-gamma receptor (IFNGR) pathway, consisting of the IFNGR, JAK1/JAK2 and STAT1 which promotes transcription of interferon-induced genes [61]. The cytokine IFN-γ is known to have dichotomous immunological properties by inducing apoptosis of tumor cells, blood vessel disruption and upregulation of MHC-expression on the one hand, but expression of IDO, PD-L1 and other co-inhibitory markers on the other hand [61–64]. These co-inhibitory molecules including LAG-3 and TIM-3 synergize with CTLA-4 and PD-1 in promoting T-cell exhaustion [65,66] and are known to be upregulated following initiation of ICB therapy [67]. Inactivating mutations in the IFNGR-pathway have been documented in patients and are hypothesized to occur in settings of checkpoint blockade which leaves tumors cells exposed only to the anti-tumor properties of IFN-γ, causing selective pressure [60,63]. Paradoxically, chronic exposure to interferons including IFN-γ was found to also induce immune resistance due to PD-L1-dependent and -independent mechanisms [64]. This may occur in settings of chronic (ICB-induced) inflammation where the pro-tumor functions of IFNs prevail over the anti-tumor ones, leading to immune resistance.

Besides specific mutations in immune-related pathways, tumors may lose neo-antigens and thereby escape immune control. In two melanoma patients, immunogenic neo-antigens were lost during tumor progression indicating immune-editing [68]. Immune editing was also reported in NSCLC patients whose lesion(s) initially responded to PD-1-inhibition but later progressed. The relapsed tumors were devoid of several mutations encoding for neo-antigens that were present prior to treatment [69].

4. Therapeutic interventions aimed at (re-)sensitizing tumors to ICB

Increasing the response rates to ICB will require rational combinations of conventional anti-cancer therapies and other

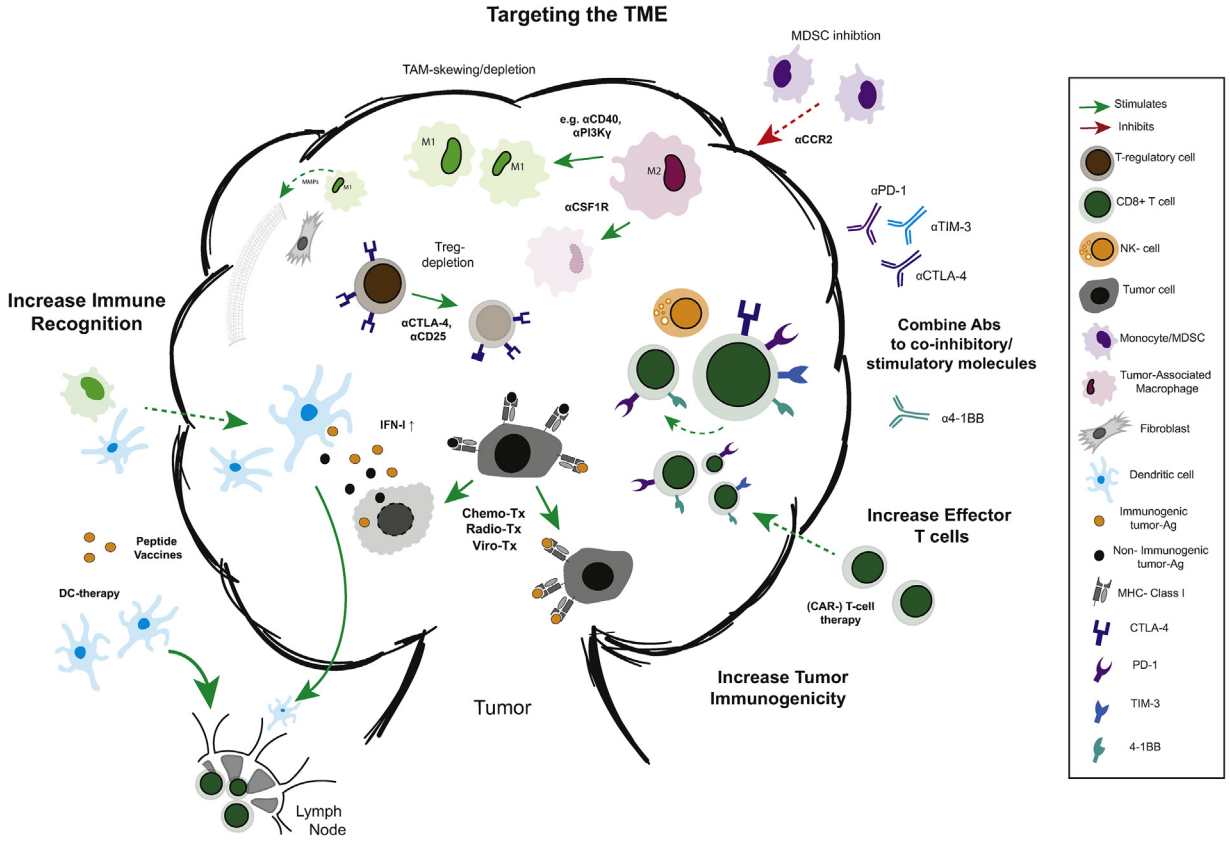

Fig. 3. Therapeutic options to target immune resistance; sensitizing tumors to checkpoint blockade therapy can be achieved by increasing immune recognition, targeting the TME to remove immune suppression, combining antibodies to co-inhibitory/stimulatory molecules on T cells, increasing effector T cells and by increasing tumor immunogenicity.

immunotherapies on a per patient basis to optimally prime the tumor for ICBs to have effect. As many of these therapies act by alleviating both primary and secondary forms of immune resistance they shall be addressed per individual class of therapy (Fig. 3).

4.1. Modulating the T-cell: novel immune checkpoints involving co-inhibition/co-stimulation

Following the discovery of PD-1 and CTLA-4, numerous other co-inhibitory molecules on the T-cell surface have been characterized and shown to contribute to T-cell exhaustion [5]. It could therefore be beneficial or even necessary to target multiple inhibitory molecules at the same time to attempt reversal of exhaustion [70]. It should be noted, however, that T-cell dysfunction in cancer is a multifactorial process depending on many factors besides co-inhibitory receptor signaling [5]. Moreover, co-expression of multiple inhibitory molecules besides PD-1, including LAG-3 and TIM-3 indicates a state of 'hyperexhaustion' that is not recoverable by ICB-treatment [71]. Upregulation of co-inhibitory molecules has been shown to occur in mice and humans following PD-1-inhibition (TIM-3, LAG-3↑) [67] and in case of anti-CTLA-4-treatment (VISTA, PD-L1↑) [72]. These findings provide a clinical incentive to combine different ICB-therapies to potentially sensitize tumors previously thought to be ICB-resistant (e.g. prostate cancer).

Paradoxically, dysfunctional T cells in the TME are known to express co-stimulatory receptors simultaneously with co-inhibitory molecules such as 4-1BB (CD137), ICOS and OX40, suggesting a possible balance that can be therapeutically exploited [72,73]. Preliminary data from pre-clinical mouse models indeed show benefit of combining agonistic antibodies to co-stimulatory molecules with antagonistic antibodies targeting co-inhibitory molecules [73,74]. It may therefore be beneficial to 'push the pedal' by targeting co-stimulatory molecules on the hand, and 'release the brakes' using co-inhibitory checkpoint blocking antibodies on the other hand to fully exploit T-cell effector function.

4.2. Chemotherapy, radiotherapy and oncolytic viruses: aiming to re-establish anti-tumor immunity

Many conventional anti-cancer therapies such as chemo- and radiotherapy, including oncolytic viral therapy was previously thought to principally act by arresting tumor cell proliferation and causing cell death. However, novel insights have led to a change in paradigm where many of these 'traditional' anti-cancer treatment strategies are now appreciated to function at least partially by modulating the immune system [75,76]. As mentioned before, a major contributor to primary ICB-resistance is lack of functional DCs in the tumor capable of priming T cells in lymphoid organs. Both radiotherapy and certain classes of chemotherapy, but also several oncolytic viruses are capable of causing immunogenic cell death (ICD) which increases antigen availability to dendritic cells in the TME [76]. Besides releasing antigens, tumors cells release damage associated molecular patterns (DAMPs) that are capable of attracting and stimulating innate immune cells to subsequently phagocytose cellular debris and present antigen to tumor-specific T cells [75–78]. A thorough appraisal of the various immune modulating functions of the different classes of chemotherapy, and to a lesser extend radiotherapies, is beyond the scope of this review. However, it is important to note that even drugs within the same class of chemotherapies e.g. oxaliplatin and cisplatin, may have different effects on the immune system, be it ICD or enhanced expression of co-stimulatory markers on APCs, respectively [77,79].

Chemo- and radiotherapy have also been shown to upregulate type I interferons in the tumor microenvironment, thereby attracting T cells by increased chemokine production in case of anthracyclines [80], or by activating dendritic cells critical for adaptive immune induction [81]. Therapy elicited type I interferons can also improve responses in the setting of secondary ICB resistance where MHC-molecules on the tumor cell surface are downregulated, but can be potently re-expressed when exposed to type I interferon [82]. Reinstating immunity following primary or secondary immune resistance by conventional therapies has been shown to (re-)sensitize tumors to ICB therapy [83,84]. In a study by Twyman-Saint Victor et al., melanoma patients received radiation on one index lesion followed by systemic CTLA-4-blocking antibodies. Besides a few responses including one patient with abscopal responses (regression of unirradiated distant tumors), the majority of patients progressed [10]. They went on further to show that upregulation of PD-L1 on the tumor following radio-immunotherapy significantly abrogated effective immune responses, which could be reversed by administering PD-1-inhibiting antibodies. Similar phenomena also occur in the setting of oncolytic viral therapy where virus treatment is able toinflame immunologically silent tumors and upregulate immune checkpoints that could be targeted by ICB [85,86]. It has to be noted that several studies have also reported negative effects of radiotherapy on anti-tumor immunity including the increase of immune suppressing cells in the TME (Tregs, MDSCs and TAMs) [75]. Also in patients receiving radiotherapy, immune monitoring of blood showed increased myeloid cell and decreased lymphoid cell counts and immune reactivity following radiotherapy in contrast to standard chemotherapy [87,88]. Some of these discrepancies may be caused by opposing biological pathways underlying different radiation regimens as was recently reported by Demaria et al., showing that multiple low-dose irradiation cycles synergized with αCTLA-4 antibodies in contrast to one single higher dose of radiotherapy in pre-clinical tumor models. Lower doses of radiation induced local type I IFN-production and concomitant recruitment of DCs, whereas high dose irradiation activated a cytosolic DNA-degradation pathway, preventing immune induction [89]. Novel mechanisms underlying these divergent effects of radiotherapy will have to be addressed and may involve modification of thetreatment schedule and dose (fractionated or high dose) and the requirement for future combination strategies (e.g. TME targeted depletion, ICB).

4.3. Cytoreduction by surgery: an (neo-)adjuvant role for ICB in treating locally advanced disease?

The addition of immunotherapy to conventional cytoreductive surgery may improve patient survival by extending recurrence free survival following (incomplete) tumor resection. From an immunological perspective, the major advantage of surgery is the reduction of tumor- and associated antigen load. Chronic antigen exposure is known to be a main contributor to exhaustion of effector T cells and occurs already early in tumorigenesis [90]. The persistence of T cell exhaustion could eventually lead to the irreversibility to reinvigorate T cell function with ICB therapy [71,90]. Moreover, increased tumor size correlates with extended immune suppression [91], suggesting that manually reducing tumor size could alleviate immune inhibition and T-cell exhaustion. Whether ICB should be administered in an adjuvant or neo-adjuvant setting has been recently investigated in murine breast cancer models. In these models Liu et al. showed superiority of neo-adjuvant anti-PD-1 therapy over adjuvant treatment in the context of surgery [92]. Mice treated with neo-adjuvant ICB had significantly longer recurrence free survival due to higher frequencies of circulating tumor specific memory T cells capable

of surveying the body for micro-metastasis [92]. The reported immune response kinetics resemble what is observed in the setting of acute infection, where a decrease in antigen load following clearance of the pathogen supports induction of a proper memory T-cell pool [93]. Furthermore, recent insights into biomarkers associated with response to αPD-1 therapy have implicated elevated CD8+ PD1+ T-cell proliferation in a setting of low tumor load to be predictive of response [94]. It is possible that in the future, surgery may fulfil a pivotal role in establishing such a setting in the case of extensive tumor burden. However, it should be noted that surgery may also induce the influx of immunosuppressive cells abrogating T-cell function as part of a systemic 'wound healing response' [95] (De Goeje, Aerts, unpublished results).

4.4. Immunotherapy: passive and active immunization approaches to induce novel immune responses

Primary immune resistance to ICB can result from the inability or lack of endogenous DCs capable of priming anti-tumor T cells (non-inflamed tumor) or the presence of tumor infiltrating T cells that are either irreversibly exhausted or not specific for tumor-antigens [21,71]. In these cases, novel immune responses need to be induced that in time can be further enhanced by checkpoint blockade.

Tumor vaccines enable induction of novel immune responses or reinstate pre-existing immune responses towards a specific or wide array of tumor antigens formulated in the vaccine [1]. Although cancer vaccines offer significant advantages including high specificity, a favorable safety profile, of-the-shelf applicability and the premise of life-long anti-tumor immunity, clinical efficacy is often limited in overt cancer [2]. Several studies have highlighted the importance and power of neo-antigen specific immune responses in establishing tumor control [30,96]. Exploiting novel tools from the field of cancer immunogenomics enables the characterization of immunogenic neo-antigens that can be subsequently produced and incorporated into personalized vaccines [97,98]. Several trials are underway investigating the safety and clinical efficacy of these personalized vaccines [97].

Besides peptide vaccines, it is possible to circumvent endogenous antigen presentation and expose in vitro cultured autologous dendritic cells to tumor antigens and stimuli [99]. This form of immunotherapy called DC-therapy was found to be safe, capable of inducing anti-tumor immune responses and effective in a subgroup of advanced cancer patients [2,100]. Additionally, DC-immunotherapy was shown to induce epitope spreading, eliciting novel T-cell responses specific to antigens not formulated in the vaccine, and capable of inducing both CD8+ and CD4+ T-cell responses in vivo [101]. Both forms of active immunization were found to synergize with checkpoint blockade therapy in pre-clinical tumor models, possibly by eliciting a new pool of T cells that is susceptible to re-invigoration in a (PD-L1 high) tumor [102,103]. In case of tumors lacking a functional antigen-presentation pathway (mutations in TAP, low MHC-I; secondary immune resistance), it may be possible in the future to vaccinate with TEIPPs (T cell epitopes associated with impaired peptide processing), as these antigens are selectively presented in settings of abnormal antigen processing such as cancer [104].

Instead of actively inducing endogenous anti-tumor T-cell responses using (DC-)vaccines, one can directly infuse large numbers of tumor antigen-specific T-cells derived from resected tumor tissue (TIL-therapy) or from PBMCs following genetic modification TCR-engineered or chimeric antigen receptor (CAR) T-cell therapy [105]. These forms of therapy are currently revolutionizing the field of hemato-oncology with the implementation of CD19-specific T cells, and have yielded anecdotal results in solid tumors [106]. However, as the majority of cancer patients are not eligible for TIL-therapy, and safe and effective targets for engineered T cells are still lacking as well as the challenges in T-cell penetration and persistence for most solid tumors, T-cell therapy still has a long road ahead.

4.5. Targeting key players of the tumor microenvironment – making an example of TAMs

We recently identified TAMs to be critically involved in determining the exhaustion status of vaccine-induced T-cells, as tumor infiltrating T cells expressed lower levels of the co-inhibitory molecules PD-1, LAG-3 and TIM-3 following M-CSFRi-mediated TAM-depletion [127]. As this PD-1 low/intermediate expressing phenotype is particularly sensitive to re-invigoration by PD-1-blocking antibodies [71], M-CSFR-inhibition enhanced the efficacy of ICB in mouse of models of pancreatic cancer [107].

Besides depleting TAMs (e.g. by targeting the M-CSF-receptor or homing receptors such as CCR2), skewing of TAMs to a more pro-inflammatory 'M1' phenotype may be even more efficacious in inducing tumor regression. Skewing of TAMs by CD40-agonistic antibodies was shown to result in loss of desmoplasia and induction of tumor regression in combination with gemcitabine in pancreatic cancer patients and pre-clinical models of PDAC [108,109]. Similar observations were made following pharmacological inhibition of PI3Kγ in multiple tumor models, where PI3Kγ was identified as a key molecular switch governing the M2 macrophage phenotype [110,111]. Skewing of TAMs could therefore ameliorate primary immune resistance caused by mechanical obstruction of T-cell infiltration by the collagen-rich stroma [112]. In support of this are the markedly increased T-cell numbers in tumors treated with PI3Ky-inhibition or CD40-agonistic antibodies [111,113]. Importantly, resistance to ICB in pre-clinical models could be overcome by combination with both TAM-skewing compounds, highlighting the role of myeloid cells in perturbing anti-tumor immunity and ICB-efficacy [114,115]. As PD-1 is thought to act primarily on T-cells at the effector site, it is tempting to speculate whether skewing of TAMs to a M1-phenotype could provide B7-costimulatory molecules capable of binding CD28 on T-cells in the tumor. As PD-1-blockade could enable proper signaling through the CD28-B7-axis, this could provide another explanation for the observed synergy between these different forms of immunotherapy.

The composition of the TME varies extensively between different tumor types, requiring tailored approaches to target specific immune populations [116]. Besides TAMs, other myeloid cells such as neutrophils, MDSCs and tolerogenic DCs but also regulatory T cells can pose significant obstacles to the generation of effective anti-tumor immunity. In line with TAM-targeting therapies, strategies aimed at depleting MDSCs (e.g. anti-CXCR2 or −CCR2 antibodies, multikinase inhibitors e.g. cabozantinib) [117–119] or Tregs (Fc-optimized aCD25-antibodies) [120] all synergize with ICB-therapies.

5. A personalized medicine approach to optimally stratify and treat cancer patients with ICB

At present, the identification of predictive factors determining the response to ICB treatment has remained difficult. Extensively reviewed biomarkers such as PD-L1 on tumor- and myeloid cells have failed to deliver robust results across multiple cancers [121]. Similar to PD-L1, tumor mutational load has been found to contribute to ICB-response but its discriminative value remains insufficient [41]. A more holistic and complete characterization of

the tumor and its TME will likely improve the accuracy of current predictive markers [58]. This may include assessing the presence of a CD8+ T-cell infiltrate in combination with the PD-L1 status of a tumor to further delineate whether a tumor might be sensitive to ICB or that other therapies are required to prime the immune system first [122].

Assessing primary immune resistance can be achieved by employing novel tools in immunogenomics including next-generation sequencing on baseline tumor samples [98]. Using genome-wide approaches or eventually specified sets of genes corresponding to specific resistance modules, it will be possible to determine both the tumor antigen- and immunological landscape of tumors [36]. Recently discovered multiplex immunohistochemistry tools will offer localization of certain cell types on often already available paraffin embedded tissue to further aid patient stratification [123]. Elegantly, optimized pipelines designed to predict neo-epitopes using the aforementioned techniques offer the opportunity for personalized immunotherapy using vaccines and TCR-modified/CAR-T-cell approaches [97].

In contrast to primary tumor tissue which is readily available upon disease diagnosis, samples acquired during and after ICB treatment are often difficult to obtain, thereby limiting monitoring of treatment over time. As several groups have demonstrated the predictive value of tumor tissue early during course of treatment [124,125] it will be challenging to find more non-invasive biomarkers that can guide immunotherapy. Attempts have been made to define such markers in peripheral blood of patients yielding promising results by characterizing proliferating PD-1+ CD8+ T-cells following αPD-1 treatment [94,126]. Extending the scope to other circulating immune cells such as myeloid cells could further improve the sensitivity of these analysis.

6. Conclusion

A recent appreciation of the role our immune system plays in tumors has led to the widespread implementation of immune modulating drugs such as ICBs for the treatment of advanced cancer; with unprecedented clinical success. However, as the majority of patients fails to demonstrate durable responses, rational combinations of conventional- and novel anti-cancer therapies will need to be employed on an individualized basis to ensure the best possible responses.

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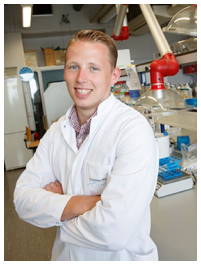

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