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REVIEW

Enhancing antitumor response by combining immune checkpoint inhibitors with chemotherapy in solid tumors

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Background: Cancer immunotherapy has changed the standard of care for a subgroup of patients with advanced disease. Immune checkpoint blockade (ICB) in particular has shown improved survival compared with previous standards of care for several tumor types. Although proven to be successful in more immunogenic tumors, ICB is still largely ineffective in patients with tumors that are not infiltrated by immune cells, the so-called cold tumors.

Patients and methods: This review describes the effects of different chemotherapeutic agents on the immune system and the potential value of these different types of chemotherapy as combination partners with ICB in patients with solid tumors. Both preclinical data and currently ongoing clinical trials were evaluated. In addition, we reviewed findings regarding different dosing schedules, including the effects of an induction phase and applying metronomic doses of chemotherapy.

Results: Combining ICB with other treatment modalities may lead to improved immunological conditions in the tumor microenvironment and could thereby enhance the antitumor immune response, even in tumor types that are so far unresponsive to ICB monotherapy. Chemotherapy, that was originally thought to be solely immunosuppressive, can exert immunomodulatory effects which may be beneficial in combination with immunotherapy. Each chemotherapeutic drug impacts the tumor microenvironment differently, and in order to determine the most suitable combination partners for ICB it is crucial to understand these mechanisms.

Conclusion: Preclinical studies demonstrate that the majority of chemotherapeutic drugs has been shown to exert immunostimulatory effects, either by inhibiting immunosuppressive cells and/or activating effector cells, or by increasing immunogenicity and increasing T-cell infiltration. However, for certain chemotherapeutic agents timing, dose and sequence of administration of chemotherapeutic agents and ICB is important. Further studies should focus on determining the optimal drug combinations, sequence effects and optimal concentration–time profiles in representative preclinical models.

Key words: chemotherapy, checkpoint inhibitors, cancer, neoplasms, chemoimmunotherapy

Introduction

Drug development in oncology is shifting from targeting intrinsic properties of cancer cells to the tumor microenvironmental and the immune system of the host. Boosting T-cell memory may lead to more durable anticancer responses than seen with conventional anticancer therapy [1]. Endogenous anticancer response can be enhanced by blocking inhibitory checkpoint molecules. These checkpoint molecules function by dampening immune cells, a mechanism that prevents auto-immunity. Tumors utilize checkpoint inhibition in order to prevent T-cell-mediated tumor cell killing, by upregulating the ligands of checkpoint inhibitors, such as PD-L1. Activating checkpoint inhibition pathways turns T-cells anergic and leads to T-cell exhaustion.

Approved drugs for immune checkpoint blockade (ICB) include the anti-PD1 antibodies nivolumab and pembrolizumab, the anti-PD-L1 antibodies atezolizumab, avelumab and durvalumab, and the anti-CTLA-4 antibodies ipilimumab and tremelimumab. ICB has been approved for use in a wide range of tumors, including melanoma, non-small cell lung cancer (NSCLC), renal cell cancer, Merkel cell cancer, Hodgkin's lymphoma, urothelial cancer and mismatch repair deficient (dMMR)/microsatellite instability high (MSI-H) tumors [1–5].

Extensive research has been carried out identifying factors contributing to response to ICB (Table 1). Currently approved biomarkers for ICB are PD-L1 expression and dMMR/MSI-H tumor status [42, 43]. In practice, lactate dehydrogenase (LDH) and tumor mutational burden are also commonly used to select patients that are thought to benefit from ICB treatment [6, 8]. Other biomarkers that have been identified include but are not limited to, tumor-infiltrating lymphocytes (TILs), CD8+ T-cells, T-cell receptor clonality and IFN-γ-related gene signatures [6, 9, 10, 21, 24, 33, 37].

It is thought that ICB has the highest likelihood of success in tumors that have an inflamed phenotype [44, 45]. These inflamed phenotypes typically have a tumor microenvironment with functional CD8+ TILs, functional antigen presentation machinery proteins, and T-helper type 1 cytokines and chemokines such as IFN-γ and IL-2 [27, 29, 46]. While there is an active immune response, inhibitory factors may also be present. Potential inhibitory factors are large densities of Tregs, MDSCs, and anti-inflammatory T-helper type 2 cytokines, such as TGF-β and IL-10 [47].

Other immunological phenotypes that can be found in the tumor microenvironment include a phenotype which is completely deprived of immune cells (immune desert), or a phenotype in which the immune cells are unable to infiltrate the tumor properly (immune-excluded tumors) [46, 48, 49]. These tumors lack infiltration of competent T-cells, rarely express checkpoint inhibitor molecules, have a low mutational load, and have low expression of antigen presentation machinery markers [48]. These two phenotypes rarely respond to ICB monotherapy [19]. In order to convert these immune deserts or immune-excluded tumors into inflamed tumors, combination therapy with either other immunotherapies or different treatment modalities [50], including chemotherapy, may be an option [48].

Chemotherapy was previously thought to be solely immunosuppressive, but recent data show that it may also possess immunostimulatory properties [51, 52]. It has the potential to induce favorable immunogenic conditions within the tumor microenvironment, which may be difficult to achieve by just targeting immune cells [51, 52]. In this review, we describe these immunomodulatory effects for different classes of chemotherapy. Each compound exerts unique immunological effects, which may be either beneficial or detrimental to treatment with ICB. Furthermore, this review discusses the compounds and treatment schedules in ongoing combination studies.

Immunomodulatory effects of chemotherapy

Chemotherapy comprises a large group of molecules which target proliferating cells. Although chemotherapy predominantly affects cancer cells, proliferating benign cells such as immune cells may also be affected. For this reason, it was long assumed that chemotherapy is merely immunosuppressive. Indeed, chemotherapy may lead to myelosuppression and leukocytopenia. However, recent findings demonstrate that many forms of chemotherapy also exhibit immunostimulatory effects. Here, we discuss the immunomodulatory effects of the four main groups of chemotherapy: topoisomerase inhibitors, antimicrotubule agents, alkylating agents and antimetabolites (Figure 1 and supplementary Table S1, available at *Annals of Oncology* online). We searched PubMed for preclinical and clinical trials published before 13 December 2018. Interim analysis and early-release publications of American Society of Clinical Oncology (ASCO) and European Society for Medical Oncology (ESMO) were also reviewed. Only articles in English were included. The search terms were 'immune checkpoint inhibitors', 'anti-PD-(L)1', 'anti-CTLA-4', and the names of the ICB available to date, 'immunomodulation' and the specific actors of the immune responses, 'topoisomerase inhibitors', 'antimicrotubule agents', 'alkylating agents and antimetabolites' and the specific agents per group. We only discuss chemotherapeutic compounds which are used for the treatment of solid tumors. Abstracts were reviewed and relevant articles were assessed in full.

Topoisomerase inhibitors

Topoisomerase inhibitors block the action of topoisomerases, enzymes controlling topological changes in DNA structures. Type I topoisomerases cut one strand of a DNA double helix, whereas type II topoisomerase cut both strands. Important topoisomerase inhibitors in the treatment of solid cancers of which immunomodulatory effects are described include topoisomerase I inhibiting camptothecin derivatives and topoisomerase II inhibiting anthracyclines.

Camptothecin derivatives. Irinotecan and topotecan are camptothecin derivatives commonly used in the treatment of a wide variety of solid tumors. Preclinical findings suggest that they may enhance T-cell recognition of tumor cells. In melanoma, they are capable of upregulating tumor-specific antigens. *In vitro* models demonstrated that treatment with topoisomerase I inhibitors led to increased expression of the antigens melan-a/MART-1 and TP53INP1. Overexpression of these antigens led to improved recognition of tumor cells by T-cells, and subsequently increased T-cell-mediated killing of these tumor cells [53, 54]. Another *in vitro* experiment revealed upregulation of the danger-associated molecular patterns (DAMPs) high mobility group box 1 protein (HMGB1) and heat shock protein 70 (HSP70) after irinotecan treatment [55]. DAMPs have the potential to induce dendritic cell maturation leading to an inflammatory antitumor response. Tumor cells surviving topotecan treatment have upregulated major histocompatibility complex I (MHCI) and Fas expression, making them more sensitive to effector T-cell killing [56, 57].

Clinical studies determining the impact of camptothecin derivatives and individual drug doses and schedules on the immune system are limited in number. Small studies have been carried out monitoring changes in immune cell subsets in patients undergoing treatment. Camptothecin derivatives appear to impact the

Table 1. Predictive factors for checkpoint inhibition therapy

Type	Predictive factor	Effect	Cancer type	Checkpoint inhibitor	Reference
Clinical	Clinical condition	High ECOG performance status is predictive for poor OS	Melanoma, NSCLC	Nivolumab, pembrolizumab, ipilimumab	Nakamura et al. [6], Bagley et al. [7]
	Clinical chemistry	High LDH is predictive of poor OS	Melanoma, TNBC	Nivolumab, ipilimumab	Nakamura et al. [6] Nanda et al. [8], Loi et al. [9], Martens et al. [10]
		High C-reactive protein is predictive of poor OS	Melanoma	Nivolumab, ipilimumab	Nakamura et al. [6], Simeone et al. [11]
		High levels of soluble CD73 is associated with poor OS and PFS	Melanoma	Nivolumab	Morello et al. [12]
Tumor	Tumor mutational burden	High mutational load correlates with improved OR, PFS and OS, and durable clinical benefit	Various	Pembrolizumab, nivolumab, ipilimumab, atezolizumab	Hugo et al. [13], Rizvi et al. [14], Snyder et al. [15], Rosenberg et al. [16]
	Mismatch repair status	Mismatch repair deficiency correlates with response	Any solid tumor with mismatch repair deficiency	Pembrolizumab	Le et al. [17]
	Tumor PD-L1 expression	PD-L1 expression correlates with response	Various tumor types	Pembrolizumab, nivolumab, atezolizumab	Gettinger et al. [18]; Herbst et al. [19] Fuchs et al. [20]
	Viral etiology	Human papilloma virus positivity correlates with response	Head and neck cancer	Pembrolizumab	Chow et al. [21]
		Epstein-Bar virus positivity correlates with response	Gastric	Pembrolizumab	Kim et al. [22]
Immunological	Tumor-infiltrating lymphocytes	Baseline peritumoral and intratumoral PD-1 expression on $CD8^+$ T-cells correlates with response and improved survival	Melanoma	Pembrolizumab, nivolumab	Vilain et al. [23]
		High level of stromal TILs correlates with response	Melanoma, TNBC	Pembrolizumab	Loi et al. [9], Tumeh et al. [24]
		PD-L1 expression on TILs correlates with response	Various tumor types	Atezolizumab, pembrolizumab	Herbst et al. [19], Dirix et al. [25], Tumeh et al. [24]
		High baseline FoxP3 and IDO expression correlates with clinical activity	Melanoma	Ipilimumab	Hamid et al. [26]
		More clonal TCR repertoire correlates with response	Melanoma	Pembrolizumab	Tumeh et al. [24]
	Peripheral blood	Low number baseline $Ki67^+EOMES^+CD8^+$ T-cells is associated with relapse	Melanoma	Ipilimumab	Wang et al. [27]
		High percentage of baseline memory $CD45RO^+CD8^+$ T-cells correlates with improved survival	Melanoma	Ipilimumab	Tietze et al. [28]
		Lower baseline level of peripheral NK cells correlates with improved survival	Melanoma	Ipilimumab	Tietze et al. [29]
		High neutrophil-to-lymphocyte ratio is predictive of poor OS	NSCLC, melanoma	Nivolumab, ipilimumab	Bagley et al. [7], Cassidy et al. [30]
		Low absolute and relative lymphocyte count is predictive of poor OS	Melanoma	Nivolumab, ipilimumab	Nakamura et al. [6], Simeone et al. [11], Martens et al. [10]
		Low leukocyte count at baseline correlates with response	Melanoma	Ipilimumab	Gebhardt et al. [31]
		Low neutrophil count is associated with improved OS	Melanoma, NSCLC	Ipilimumab, nivolumab	Bagley et al. [7], Ferrucci et al. [32]
			Melanoma	Ipilimumab	

Continued

Table 1. *Continued*

Type	Predictive factor	Effect	Cancer type	Checkpoint inhibitor	Reference
		Low baseline MDSCs correlates with improved OS			Kitano et al. [33], Sade-Feldman et al. [34]
		High levels of serum IFN-γ, IL-6 and IL-10 are associated with response	Melanoma	Nivolumab	Yamazaki et al. [35]
		High frequencies of circulating Tregs is associated with improved OS	Melanoma	Ipilimumab	Martens et al. [10]
		Low absolute monocyte count correlates with OS	Melanoma	Ipilimumab	Martens et al. [10]
		High frequency of $CD14^{+}CD16^{-}HLA\text{-}DR^{high}$ monocytes correlates with response	Melanoma	Nivolumab, pembrolizumab	Krieg et al. [36]
	Immune gene expression	Upregulated IFN-γ signaling correlates to better response rates and better PFS rates	Head and neck cancer, NSCLC, melanoma, urothelial cell cancer	Pembrolizumab, nivolumab, atezolizumab	Ribas et al. [37], Ayers et al. [38], O'Donnell et al. [39], Prat et al. [40] Fehrenbacher et al. [41]
		Upregulated T-helper type 1 gene expression at baseline correlates to response	Various	Atezolizumab	Herbst et al. [19]
		Upregulated genes regarding antigen presentation machinery correlates to better response rates and better PFS rates	Head and neck cancer, NSCLC, melanoma, urothelial cell cancer	Pembrolizumab, nivolumab	O'Donnell et al. [39], Prat et al. [40]
		Upregulated genes regarding T-cell cytotoxic function correlates to better response rates and better PFS rates	Head and neck cancer, NSCLC, melanoma, urothelial cell cancer	Pembrolizumab, nivolumab	O'Donnell et al. [39], Prat et al. [40]

ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; MDSC, myeloid derived suppressor cell. NK cells, natural killer cells; NSCLC, non-small-cell lung cancer; OS, overall survival; PFS, progression-free survival; TCR, T-cell receptor; TILs, tumor-infiltrating lymphocytes; TNBC, triple negative breast cancer; Tregs, regulatory T-cells.

composition of immune cells in peripheral blood little, compared with other chemotherapeutic drugs. Topotecan treatment did not significantly impact absolute lymphocyte count nor T-cell and B-cell numbers in ovarian cancer patients with advanced disease [58]. However, the naive T-cell subpopulation was decreased upon treatment in chemotherapy naive patients, whereas the proportion of memory T-cells remained the same [58].

Anthracyclines. Anthracyclines are topoisomerase II inhibitors capable of inducing immunogenic cell death (ICD), a form of apoptosis which can induce an effective antitumor immune response through activation of DCs and the subsequent activation of specific T-cell responses. It is characterized by the expression of DAMPs, such as calreticulin, ATP, HMGB1 and HSP70 [59, 60]. *In vitro* studies demonstrated that DAMPs could be detected after 12 h of treatment and remained elevated through 24 h [61]. The dosage needed for induction of ICD, however, was generally higher than the dose needed for cytotoxicity [62]. ICD may also lead to the production of immunostimulatory cytokines, such as IFN-γ [62]. Inhibition of caspase, or depletion of DCs or CD8+ T-cells may abolish anthracycline-mediated antitumor immune response [63]. Doxorubicin, epirubicin and idarubicin are all known to induce ICD [52].

Apart from ICD, other immunomodulatory effects of anthracyclines have been investigated as well. For instance, anthracyclines are able to elicit an immune response in a similar manner as induced by viral pathogens [64]. An *in vivo* experiment using fibrosarcomas in mice demonstrated that intratumoral doxorubicin increased levels of transcripts associated with viral infections, including IFN-stimulated genes, genes involved in the recruitment and activation of leukocytes, and *Cd274* (encoding PD-L1). Anthracyclines have also been shown to selectively deplete immunosuppressive cells. Administration of 5 mg/kg doxorubicin intraperitoneally Q3W may lead to decreased MDSCs numbers *in vivo*, which in turn lead to increased numbers of CD4+ and CD8+ T-cells, as well as increased expression of IFN-γ, granzyme B and perforin [65]. Epirubicin impairs the function of Tregs by blocking the interaction between FoxP3 and the NF-κB subunit p65 *in vitro* [66]. This has resulted in blocking Treg-mediated suppression of CD8+ T-cells.

The potential negative effects of anthracyclines on the immune system have been investigated in small studies. A single dose of epirubicin appeared to not significantly decrease blood

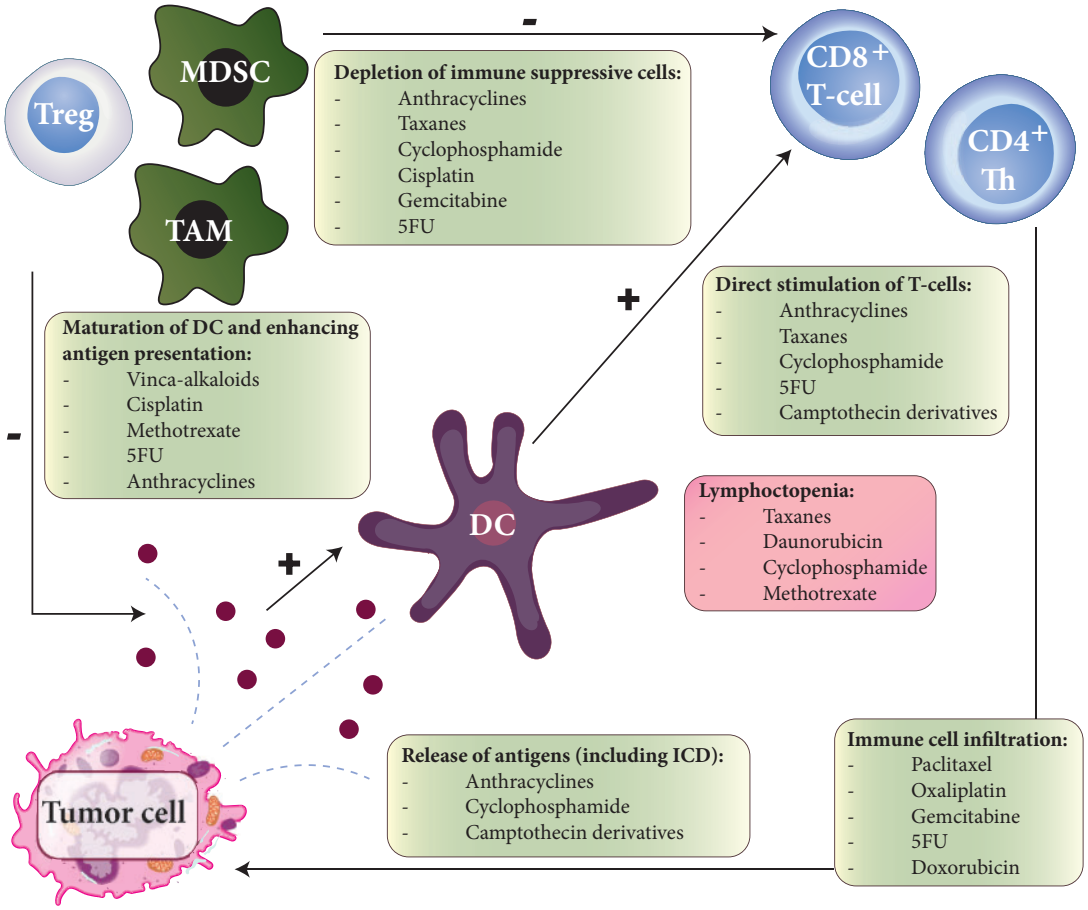

Figure 1. Immunomodulatory effects of chemotherapy. DC, dendritic cells; 5FU, 5-fluorouracil; ICD, immunogenic cell death; MDSC, myeloid derived suppressor cell; TAM, tumor-associated macrophages; Th, T-helper cell; Tregs, regulatory T-cells.

lymphocyte numbers [67]. Daunorubicin has been shown to induce cell death in both resting and active peripheral blood lymphocytes after 20 h of incubation. This may be a potential negative effect for ICB combination [68]. Assessment of dose and schedule dependency of the aforementioned effects in cancer patients is warranted.

Antimicrotubule agents

Antimicrotubule agents exert neoplastic effects by disrupting microtubules. The most widely used antimicrotubule agents are the taxanes and vinca-alkaloids.

Taxanes. Docetaxel and paclitaxel are the most commonly used taxanes in the treatment of cancer. Taxanes are known for inducing leukocytopenia, depleting both lymphocytes and neutrophils, which has been described previously in a model [69]. Given as a 3-weekly standard of care, taxane-induced leukocytopenia typically starts 10 days after infusion and restores to baseline levels ~3 weeks after infusion. Neutrophils are depleted more than lymphocytes [69], thereby improving the neutrophil-to-lymphocyte ratio to a more favorable one for ICB treatment [30]. However, various types of lymphocyte subsets are depleted, including CD3+, CD4+, CD8+, CD56+ and CD45RO+ cells [70]. As some of these cells are positively correlated with ICB response [28], further research is necessary to understand whether and how leukocytopenia affects ICB outcome.

Taxane treatment reduces the number of lymphocytes, but it is debatable whether they impact the functionality of cytotoxic T-cells. One study found that T-cell-mediated cytotoxicity was found to be impaired upon paclitaxel treatment [71]. In contrast, another study found no effect, [72], while others even found that taxane treatment led to increased NK and lymphokine activated killer cell activity [73]. Additionally, pro-inflammatory cytokines, such as IFN-γ, IL-2, IL-6 and GM-CSF were found to be upregulated after six cycles of standard taxane treatment [73].

Taxanes appear to selectively reduce immunosuppressive cells. Both docetaxel and paclitaxel have been shown to selectively decrease Treg and MDSC numbers, while unaffecting CD4+ and CD8+ viability [74–77]. Not only the number, but also the inhibitory function of Tregs is diminished. Expression of FoxP3, one of the key regulators of the immune system, was lowered in PBMCs which were incubated with paclitaxel for 24 h [77]. Another study found the anti-inflammatory cytokine IL-10 to be significantly decreased in patients with advanced disease after 4 weeks of paclitaxel treatment [72].

Taxane treatment may lead to induction of TILs [78]. A small prospective study showed that in breast cancer patients, tumors were non-inflamed before treatment. However, after four

treatment cycles of 200 mg/m^2 Q2W neoadjuvant paclitaxel treatment, surgery was carried out and one-third of the patients demonstrated immune infiltrates in their tumor biopsies. Interestingly, only the patients with a partial or complete response demonstrated TILs after treatment.

Vinca-alkaloids. Few studies have been carried out to assess the impact of vinca-alkoloids on the immune system. Vincristine suppresses the activity of immunosuppressive tumor-associated macrophages, whilst upregulating pro-inflammatory cytokines, and downregulating PD-L1 in PBMCs of healthy donors [79]. Vinorelbine generates reactive oxygen species and nitrogen species *in vivo*, which kills a significant number of immune cells [80], an effect which might negatively impact outcome of ICB. Vinblastine was identified as a compound capable of inducing maturation of DCs in an *in vitro* drug screen. Subsequent *in vivo* experiments revealed that administration of vinblastine enhanced CD8+ clonal expansion and cytotoxic function [81]. Here, vinblastine was administered subcutaneously twice, with 1 week between injections.

Alkylating and platinum-based anticancer agents

Alkylating agents inhibit a.o. the transcription of DNA into RNA, thereby stopping protein synthesis.

We discuss the most widely used compounds of this class of drugs: cyclophosphamide, dacarbazine and platinum-based chemotherapeutic drugs.

Cyclophosphamide. Cyclophosphamide is extensively investigated for its immunomodulating effects. Similar to anthracyclines, cyclophosphamide is capable of inducing ICD [52]. Furthermore, cyclophosphamide may trigger DC homeostasis [82]. Mice which were injected with a single dose of intraperitoneal 100 mg/kg cyclophosphamide exhibited tumor cell death with immunogenic features, tumor infiltration and engulfment of apoptotic tumor cells by DCs, and subsequent cross priming of CD8+ T-cells by DCs.

The dosage may be crucial when combining cyclophosphamide with immunotherapy. While higher dosages of cyclophosphamide induce myelosuppression [83], metronomic low dosing may boost the immune system [84–86]. Patients receiving a daily dose of 100 mg oral cyclophosphamide showed decreased numbers and percentages of Treg cells, whereas there was no significant decrease in other lymphocyte counts [85]. Interestingly, doubling the dose of cyclophosphamide depleted all lymphocyte subpopulations. Next to decrease of Treg cells, effector functions are increased in patients receiving a metronomic dose. Both NK and T-cell activity were increased [85]. Furthermore, *in vivo* experiments show that a single low dose of cyclophosphamide leads to a shift from T-helper type 2 to type 1 cytokines, with enhanced IL-2 and IFN-γ production, and decreased IL-10 and TGF-β production after treatment compared with pretreatment [86]. A clinical trial investigating a modified vaccine Ankara-5T4 (MVA-5T4) further demonstrated the potential abilities of cyclophosphamide to deplete Treg cells. Patients were randomized to receive 50 mg twice daily cyclophosphamide or not, and MVA-5T4 or not [87]. In both cyclophosphamide group and the cyclophosphamide plus MVA-5T4 group, FoxP3 Treg cells were depleted. These depletions were noted at week three of treatment, which was associated with a longer progression-free survival (PFS). In the cyclophosphamide only group, Treg numbers returned to baseline at day 29. Although various studies illustrated positive effects of metronomic low dosing of cyclophosphamide, other studies showed no difference or even increase in Treg levels upon treatment [88, 89].

Next to the dosage and schedule, the tumor type may play a role as well in triggering a drug induced-immune response. Treg depletion was observed in breast cancer and CRC [87, 90], but not in melanoma patients [91]. Cyclophosphamide eliminated MDSCs in CRC [92], but in prostate cancer patients, metronomic cyclophosphamide treatment led to an increase of MDSC [91].

Dacarbazine. Dacarbazine is currently only used in melanoma patients for which the newer therapies are contra-indicated or who progressed on other therapies. Dacarbazine upregulates NKG2D receptors in human melanoma cells, which leads to activation of NK cells and release of IFN-γ. Increased levels of IFN-γ results in upregulation MHCI-expression on tumor cells, which is necessary for the recognition by T-cells [93].

Platinum derivatives. Well-known platinum derivatives are cisplatin, oxaliplatin and carboplatin. Cisplatin is the best studied platinum derivative regarding immunomodulatory effects. Cisplatin was shown to upregulate tumor cell expression of PD-L1, e.g. in head and neck squamous cell carcinoma patients receiving standard cisplatin treatment [94]. This suggests a post-exposure anticancer T-cell response, hampered by coinciding PD-L1 expression. High doses of cisplatin significantly reduced IFN-γ production by T-cells *in vitro* [95], and reduced the cytotoxicity of NK cells in ovarian cancer patients [96]. Lower doses impaired T-cell function less significantly [95]. Conversely, aside from the immunosuppressive effects through PD-L1 upregulation, cisplatin has been shown to have immunostimulatory properties as well, demonstrated by upregulation of MHC class I expression on antigen presenting cells [97, 98], recruitment of effector cells to the tumor site, triggering their proliferation [99], and downregulation of the immunosuppressive microenvironment by depleting MDSCs and Tregs [100].

Less data are available regarding the effect on the immune system of oxaliplatin and carboplatin. Oxaliplatin induces upregulation of PD-L1 on DCs [101], and carboplatin PD-1 mRNA expression [102]. Oxaliplatin may induce novel T-cell infiltration of the tumor. A single dose of oxaliplatin increased immune-cell infiltration in a CRC mouse model [103]. Furthermore, oxaliplatin is a known ICD inducer [104] and upregulates DAMPs [105].

Clearly, also of this class of oncolytics optimal dose and schedule for boosting ICD needs to be further established.

Antimetabolites

Antimetabolites interfere with essential biochemical pathways for DNA synthesis, often acting as a substitute or competitor of the natural substrates in physiological metabolism. We focused on the compounds that are often used in solid tumors: gemcitabine, methotrexate and 5-fluorouracil (5FU).

Table 2. Clinical combination trials

Drugs immunotherapy	Chemotherapy	Regimen	Tumor type	Reference	Major findings/issues
Atezolizumab	Nab-paclitaxel	Atezolizumab 800 mg Q2W (d1, 15) and nab-paclitaxel 125 mg/m^2 Q1W (d1, 8, 15) in cycles of 4 weeks	TNBC	Adams et al. [116]	Median PFS 5.5 months, OS 14.7 months
Atezolizumab	Carboplatin, paclitaxel, nab-paclitaxel	Atezolizumab 15 mg/kg (in a later amendment 1200 mg flat dose) + carboplatin AUC6 + paclitaxel 200 mg/m^2 Q3W(Arm C), atezolizumab + carboplatin + pemetrexed 500 mg/m^2 Q3W(Arm D), or atezolizumab + carboplatin Q3W + nab-paclitaxel 100 mg/m^2 QW (Arm E) for four to six cycles followed with atezolizumab maintenance	NSCLC	Liu et al. [117]	Confirmed ORRs were 36% Arm C, 68% Arm D (one complete response [CR]) and 46% Arm E (four CRs). Median PFS was 7.1, 8.4 and 5.7 months, respectively. Median OS was 12.9, 18.9 and 17.0 months, respectively
Atezolizumab	Carboplatin, etoposide	Carboplatin AUC5 + etoposide 100 mg/m^2 Q3W + atezolizumab 1200 mg or placebo Q3W	NSCLC	Horn et al. [118]	The median OS was 12.3 months in the atezolizumab group and 10.3 months in the placebo group (hazard ratio for death, 0.70; 95% CI 0.54–0.91; *P*=0.007). The median PFS was 5.2 and 4.3 months, respectively (hazard ratio for disease progression or death, 0.77; 95% CI 0.62–0.96; *P*=0.02)
Atezolizumab, durvalumab, nivolumab or pembrolizumab	Carboplatin, pemetrexed, bevacizumab, docetaxel, ramucirumab, vinorelbine, gemcitabine, paclitaxel	Any ICB before any chemotherapy	NSCLC	Grigg et al. [119]	Durable responses after treatment. Lack of control arm
Atezolizumab	Carboplatin, paclitaxel, bevacizumab	Atezolizumab 1200 mg + carboplatin AUC6 + paclitaxel 200 mg/m^2 (Arm A) or atezolizumab + bevacizumab 15 mg/kg + carboplatin + paclitaxel (arm B) versus bevacizumab + carboplatin + paclitaxel (Arm C) i.v. Q3W for four or six cycles per investigator discretion, then maintenance atezolizumab, atezolizumab + bevacizumab or bevacizumab, respectively	NSCLC	Reck et al. [120]	Clinically meaningful PFS benefit with atezolizumab + bevacizumab + chemotherapy versus bevacizumab + chemotherapy. Approved for first-line treatment of metastatic non-squamous NSCLC
Avelumab	Carboplatin, gemcitabine	Two cycles of induction avelumab before combining carboplatin-gemcitabine plus avelumab for six cycles	Urothelial cell cancer	Vida et al. [121]	Induction phase with ICB. 'Priming the immune response before chemotherapy could prevent the detrimental effect of chemotherapy on immune cells; reduction of tumor burden with chemotherapy

Continued

Table 2. *Continued*

Drugs immunotherapy	Chemotherapy	Regimen	Tumor type	Reference	Major findings/issues
					may allow immunotherapy to be more effective'
Avelumab	Cisplatin	Avelumab 10 mg/kg on day 1 of the lead-in phase; days 8, 25 and 39 of the chemoradiation therapy phase; and Q2W for 12 months during the maintenance phase Cisplatin 100 mg/m^2 i.v.: days 1, 22 and 43 of the chemoradiation therapy phase. Radiation therapy 70 Gy/35 fractions/7 weeks	Head and neck cancer	Yu et al. [122]	No results yet
Durvalumab	Nab-paclitaxel, cyclophosphamide, epirubicin	Durvalumab 1.5 g or placebo Q4W. placebo monotherapy (0.75 g) was given for the first 2 weeks (window phase), and durvalumab/placebo + nab-paclitaxel 125 mg/m^2 QW for 12 weeks, followed by durvalumab/placebo + epirubicin/cyclophosphamide Q2W for four cycles	TNBC	Loibl et al. [123]	Combination therapy resulted in a high CR rate in TNBC. Induction therapy with durvalumab seemed beneficial
Durvalumab	Eribulin	A fixed dose of durvalumab (1.12 g) is given on day 1 of each cycle The starting dose is 1.1 mg/m^2 with dose escalation to 1.4 mg/m^2 on days 1 and 8 Q3W	TNBC	Landry et al. [124]	No results yet
ICB	Carboplatin, paclitaxel, temozolomide, nab-paclitaxel	Not specified in abstract/poster	Melanoma	Aguilera et al. [125]	Patients who received chemo-immunotherapy had a median OS of 5 years (95% CI 2–NR) versus 1.8 years (95% CI 0.9–2; *P*=0.002) for those who received either ICB (*n*=9) or chemotherapy alone (*n*=15), with ORR of 61% versus 17% (*P*=0.001), respectively
Ipilimumab	Dacarbazine	Ipilimumab 10 mg/kg + dacarbazine 850 mg/m^2 or dacarbazine + placebo Q3W followed by dacarbazine monotherapy	Melanoma	Robert et al. [126]	Combination therapy resulted into a higher OS (11.2 versus 9.1 months). In another phase II study ipilimumab monotherapy resulted into an OS of 11.1 month, even in pretreated patients [127]
Ipilimumab	Dacarbazine	Ipilimumab 3 mg/kg Q4W alone or with dacarbazine 250 mg/m^2/day up to 6×5 day cycles	Melanoma	Hersh et al. [128]	Objective response rate was 14.3% versus 5.4% for the combination therapy. OS was 20.9 and 16.4, respectively
Ipilimumab	Paclitaxel, carboplatin	Concurrent: 4× ipilimumab 10 mg/kg + paclitaxel 175 mg/m^2 + carboplatin AUC6	NSCLC	Lynch et al. [129]	Only phased regimen leads to improved PFS compared with control

Continued

Table 2. *Continued*

Drugs immunotherapy	Chemotherapy	Regimen	Tumor type	Reference	Major findings/issues
		followed by 2× placebo + paclitaxel + carboplatin Phased: 2× placebo + paclitaxel + carboplatin followed by 4× ipilimumab + paclitaxel + carboplatin. Control: placebo + paclitaxel + carboplatin			
Ipilimumab	Gemcitabine, cisplatin	2× gemcitabine 1000 mg/m^2 + cisplatin 70 mg/m^2 followed by 4× ipilimumab 10 mg/kg + gemcitabine + cisplatin	Urothelial cell carcinoma	Galsky et al. [130]	No changes in composition and frequency of peripheral immune cells after gemcitabine. Expansion of CD4+ cells after combination therapy. No ipilimumab monotherapy cohort
Ipilimumab	Paclitaxel, carboplatin	Phased ipilimumab paclitaxel + carboplatin followed by 4× 10 mg/kg ipilimumab + paclitaxel + carboplatin	NSCLC	Govindan et al. [131]	No prolonged OS compared with chemotherapy alone
Ipilimumab, nivolumab	Trabectidin	Ipilimumab (1 mg/kg Q12W), nivolumab (3 mg/kg Q2W), and escalating doses of trabectedin (1.0, 1.3, 1.5 mg/m^2 Q3W)	STS	Gordon et al. [132]	No results yet
Nivolumab	Gemcitabine, cisplatin, pemetrexed, paclitaxel, carboplatin, docetaxel	Arm A: nivolumab 10 mg/kg + gemcitabine 1250 mg/m^2 + cisplatin 80 mg/m^2 Arm B: nivolumab + pemetrexed 500 mg/m^2 + cisplatin 75 mg/m^2 Arm C: nivolumab + paclitaxel 200 mg/m^2 + carboplatin AUC6 + bevacizumab 15 mg/kg Arm D: nivolumab + docetaxel 75 mg/m^2	NSCLC	Kanda et al. [133]	Full dose was given. Difficult to compare response rates: different lines of treatment, different and low patient numbers
Nivolumab	Gemcitabine-cisplatin, pemetrexed-cisplatin, paclitaxel-carboplatin	10 mg/kg nivolumab gemcitabine-cisplatin, 10 mg/kg nivolumab pemetrexed-cisplatin, 10 mg/kg nivolumab paclitaxel-carboplatin 5 mg/kg nivolumab paclitaxel-carboplatin	NSCLC	Rizvi et al [134]	Chemotherapy doses not defined. Most promising results in nivolumab-paclitaxel-carboplatin group
Nivolumab	Carboplatin	Carboplatin (AUC6) with or without nivolumab (360 mg) Q3W	TNBC	Garrido et al. [135]	No results yet
Nivolumab	Paclitaxel	Nivolumab (Q4W, 3 mg/kg on days 1 and 15 for level 1 and 1 mg/kg for level −1) with fixed doses of paclitaxel and ramucirumab (Q4W, 80 mg/m^2 on days 1, 8 and 15 and 8 mg/kg on days 1 and 15, respectively)	Gastric cancer	Nishina et al. [136]	No results yet

Continued

Table 2. *Continued*

Drugs immunotherapy	Chemotherapy	Regimen	Tumor type	Reference	Major findings/issues
Nivolumab	Paclitaxel	Nivolumab 240 mg/body on day 1, 15, paclitaxel 90 mg/m^2 on day1, 8, 15 and bevacizumab 10 mg/kg on day 1, 15 Q4W	HER2 negative breast cancer	Ozaki et al. [137]	No results yet
Nivolumab	Cisplatin, pemetrexed or cisplatin, gemcitabine	Cisplatin 75 mg/m^2 Q3Wx3 plus either pemetrexed 500 mg/m^2 Q3Wx3 or gemcitabine 1250 mg/m^2 d1, d8 Q3Wx3 plus nivolumab 360 mg Q3Wx3	NSCLC	Evans et al. [138]	No results yet
Nivolumab	Trabectidin	Trabectedin (1.5 mg/m^2) Q3W, and nivolumab (3 mg/kg) Q3W	STS	Chawla et al. [139]	Paired administration of trabectedin and nivolumab is safe, and that this combined chemo-/immuno-therapy approach may have synergistic activity
Nivolumab	Cisplatin, pemetrexed	Cisplatin (75 mg/m^2), pemetrexed (500 mg/m^2) and nivolumab (360 mg) Q3W	Mesothelioma	Fujimoto et al. [140]	No results yet
Pembrolizumab	Paclitaxel, doxorubicin, cyclophosphamide, carboplatin	Cohort A: pembrolizumab 200 mg Q3W + nab-paclitaxel 100–125 mg/m^2 QW followed by Q3W pembrolizumab 200 mg + cyclophosphamide 600 mg/m^2 + doxorubicin 60 mg/m^2. Cohort B: pembrolizumab 200mg Q3W + nab-paclitaxel 100–125 mg/m^2 QW + carboplatin AUC6 Q3W followed by Q3W pembrolizumab 200 mg + doxorubicin 60 mg/m^2 + cyclophosphamide 600 mg/m^2	TNBC	Bhatti et al. [141]	Both regimens showed promising antitumor activity with manageable toxicity. Addition of carboplatin resulted in more grade 3 or 4 toxicities, mainly neutropenia
Pembrolizumab	Gemcitabine, docetaxel, nab-paclitaxel, vinorelbine, irinotecan, liposomal doxorubicin	Arm 1: pembrolizumab 2 mg/kg Q3W + gemcitabine 1000 mg/m^2 D1 and D8 Q3W Arm 2: pembrolizumab + gemcitabine 900 mg/m^2 D1 and D8 + docetaxel 75 mg/m^2 D8 Q3W Arm 3: pembrolizumab + gemcitabine 1000 mg/m^2 + nab-paclitaxel 125 mg/m^2 D1 and D8 Q3W Arm 4: pembrolizumab + gemcitabine 1000 mg/m^2 + vinorelbine 25 mg/m^2 D1 and D8 Q3W Arm 5: pembrolizumab + irinotecan 300 mg/m^2 Q3W Arm 6: pembrolizumab + liposomal doxorubicin 30 mg/m^2 Q3W	All solid tumor types	Weiss et al. [142]	All advanced solid tumor types included. Full doses of chemotherapy are used. Recommended phase II dose determined as maximum tolerated dose. Partial responses observed in arm 3–6
Pembrolizumab	Carboplatin, pemetrexed,	Carboplatin AUC5 mg/ml + pemetrexed 500 mg/m^2 Q3W for four cycles followed by	NSCLC	Papadimitrakopoulou et al. [113];	All drugs administered on same day. Improved efficacy over chemotherapy alone.

Continued

Table 2. *Continued*

Drugs immunotherapy	Chemotherapy	Regimen	Tumor type	Reference	Major findings/issues
		optional pemetrexed 500 mg/m^2 ± pembrolizumab 200 mg Q3W for 2 years		Langer et al. [143]	Approved for first-line treatment of metastatic non-squamous NSCLC
Pembrolizumab	5FU, cisplatin	Pembrolizumab 200 mg Q3W + cisplatin 80 mg/m^2 Q3W + 5FU 800 mg/m^2 Q3W or capecitabine 1000 mg/m^2 b.i.d. Q3W	Gastric cancer	Bang et al. [20]	Full dose given. Promising antitumor activity irrespective of PD-L1 expression
Pembrolizumab	Carboplatin, gemcitabine	Pembrolizumab 200 mg Q3W, and carboplatin (AUC2) + gemcitabine (800 mg/m^2) ondays 1 and 8	TNBC	Obeid et al. [144]	Two out of the three patients showed effective immune stimulation
Pembrolizumab	Eribulin	Eribulin 1.4 mg/m^2 on days 1 and 8, pembrolizumab Q3W	TNBC	Tolaney et al. [145]	Median PFS 4.2 months, OS 17.7 months
Pembrolizumab	Capecitabine or paclitaxel	Pembrolizumab 200 mg Q3W and first- or second-line paclitaxel (80 mg/m^2 QW) or oral capecitabine (2000 mg b.i.d., weekly 1 on/1 off)	TNBC	Page et al. [146]	Three out of the nine patients showed a partial response, of whom two had metastatic disease
Pembrolizumab	Pemetrexed, cisplatin, carboplatin	Pemetrexed and a platinum-based drug + 200 mg pembrolizumab or placebo Q3W for 4 cycles followed by 35 cycles of pembrolizumab or placebo + pemetrexed	NSCLC	Gandhi et al. [147]	OS at 12 months was 69.2% in the pembrolizumab-combination group versus 49.4% in the placebo-combination group, regardless of PD-L1 status. PFS survival was 8.8 versus 4.9 months, respectively. The incidence of grade 3 rAEs was comparable between the two groups
Pembrolizumab	Carboplatin, nab-paclitaxel	Pembrolizumab at 200 mg/week, carboplatin AUC6 Q3W and paclitaxel at 200 mg/m^2 Q3W or nanoparticle albumin-bound (nab)-paclitaxel at 100 mg/m^2 QW for four cycles versus the same chemotherapy plus placebo	NSCLC	Paz-Ares et al. [148, 149]	Improved overall survival (15.9 versus 11.3 months), response rates, and duration of response (PFS if 6.4 versus 4.8 months) in the group with chemo-immunotherapy compared with chemotherapy alone. Approved for first-line treatment of metastatic squamous NSCLC
Pembrolizumab	Docetaxel or gemcitabine	Pembrolizumab 200 mg and either docetaxel 75 mg/m^2 (arm A) or gemcitabine 1000 mg/m^2 on days 1 and 8 (arm B) Q3W	Urothelial cell cancer	Parikh et al. [150]	Arm A had an ORR of 50% and DCR of 67%, whereas arm B had an ORR of 33% and DCR of 50%. Median PFS was 4.8, 5.7 and 3.7 months for the overall cohort, arm A, and arm B, respectively
Tremelimumab	Gemcitabine	Gemcitabine (1000 mg/m^2 on days 1, 8 and 15 of each 28-day cycles) + tremelimumab (6, 10 or 15 mg/kg) on day 1 of each 84-day cycle for a maximum of four cycles	Pancreatic cancer	Aglietta et al. [151]	Full dose gemcitabine, MTD of tremelimumab 15 mg/kg. Two partial responses

AUC, area under the curve; b.i.d., twice daily; CI, confidence interval; CR, complete response; D, day; DCR, disease control rate; 5FU, 5-fluorouracil; ICB, immune checkpoint inhibition; MTD, maximum tolerated dose; NSCLC; non-small cell lung cancer; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; Q×W, every×weeks; rAE, related adverse events; STS, soft tissue sarcoma; TNBC, triple negative breast cancer.

Gemcitabine. The immunomodulating properties of gemcitabine are mainly investigated when applied at the standard dose. Administration of this dose decreases the number of MDSCs, while enhancing cross-presentation of malignant antigens [106]. In pancreatic cancer patients, standard dose gemcitabine led to the depletion of Tregs, which lasted until 2 weeks after the last dose of chemotherapy [107]. Interestingly, no other lymphocyte subtypes significantly decreased after treatment. In ovarian cancer, a single dose of gemcitabine increased the CD8+ T-cell tumor infiltration and PD-L1 expression both *in vitro* and *in vivo* [107, 108]. This effect was observed during the first 5 days after treatment, but not after 2 weeks of treatment [102]. Due to this time-dependent effect, ICB could best be given 1 week after gemcitabine administration. The impact of dose on immunomodulatory effects require further investigation.

Methotrexate. Methotrexate targets rapidly dividing cells by inhibiting the formation of nucleotides, thereby impairing proliferation. Although high-dose methotrexate causes bone marrow suppression [109], low-dose methotrexate has shown immunostimulating properties. In an *in vitro* experiment, low-dose non-cytotoxic concentrations of methotrexate boosted the maturation of DCs by upregulating CD40, CD80 and CD83 [110]. In return, the DCs stimulated proliferation of T-cells [110], which could lead to a greater antitumor response. This suggests that low-dose methotrexate could be used as an immunostimulating agent. However, more research evaluating the impact of methothrexate on the immune system is needed to confirm whether it is indeed a suitable combination partner for ICB, as the currently available data are too limited.

5-Fluorouracil. 5FU functions as antimetabolite of pyrimidine by inhibiting the synthesis of DNA and RNA. 5FU is the most extensively investigated oncolytic compound for its immunomodulating effects.

A standard dose of 5FU may exert immune stimulatory effects, e.g. by facilitating antigen uptake by DCs. In an *in vitro* experiment, DCs were incubated with a gastric cancer cell line which was pretreated with 5FU. The isolated DCs showed higher IL-12 production when incubated with the gastric cancer cell line pretreated with 5FU compared with the control. Subsequently, the cytotoxic T-lymphocytes generated by these DCs showed higher cytotoxicity compared with the control [111]. Furthermore, 5FU also selectively kills MDSCs *in vivo*, while sparing the other lymphocyte subtypes [108]. Effects were seen in the spleen and tumor of mice, 5 days after the intraperitoneal injection. Selective depletion of MDSCs was associated with greater CD8+ T-cell tumor infiltration and T-cell-dependent antitumor responses.

Combination therapy

Various studies investigating combination therapy with chemotherapy and checkpoint inhibitors have been carried out. Both *in vivo* and clinical studies are showing promising results [103, 112, 113].

In vivo experiments allow for swift testing of different regimens by varying both the doses and the order of administration of the drugs. One study tested three different regimens using the combination of gemcitabine and ipilimumab in non-immunogenic mouse models [114]. Gemcitabine was given either 15 days before anti-CTLA-4, concomitantly, or 3 days after anti-CTLA-4. Synergistic effects were only observed in the concomitant regimen, while omitting the first dose of gemcitabine drastically decreased antitumor effects. In another *in vivo* study combining cyclophosphamide and anti-CTLA-4 similar results were obtained [115]. Immunological antitumor responses were seen when cyclophosphamide was given 1 day before anti-CTLA-4 treatment. However, when reversing the order, CD8+ T-cells underwent massive apoptosis and antitumor effects of anti-CTLA-4 were attenuated. These findings suggest that indeed there is a need for a chemotherapy induction phase before administering ICB.

An overview of clinical trials of which data are available is presented in Table 2. Combination therapy in the clinic is mostly well tolerated, and durable responses have been observed in various trials. Currently, three combinations have been approved for first-line treatment, all for advanced NSCLC [120, 147, 148].

In the majority of clinical trials, chemotherapy and ICB are administered concurrently and at full doses. Few trials have explored the optimal dose, or sequence of administration, while preclinical data have shown that these parameters might affect outcome. For example, an induction phase of chemotherapy can modify the tumor microenvironment thereby optimizing it for ICB [152]. A study in metastatic triple negative breast cancer (TNBC) patients investigated induction therapy with various types of chemotherapy [153, 154]. For the induction phase, low doses of chemotherapy were given for 2 weeks: 50 mg daily cyclophosphamide, twice 40 mg/m^2 cisplatin or twice 15 mg doxorubicin. Response rates with chemotherapy appear higher in the cohorts where low-dose chemotherapy was used as induction, compared with nivolumab alone. Thus far, response rates appear most promising in the doxorubicin and cisplatin induction arms. Biomarker analysis carried out in this trial showed that indeed upon treatment with these two compounds, upregulation is found in key immunological pathways associated with response to anti-PD-1, and this effect is further increased after nivolumab administration. Furthermore, the number of intratumoral T-cells as well as the T-cell clonality is found to be higher after treatment with these drugs, compared with no induction phase [155]. Another study that investigates the impact of order of administration is a large phase II study of ipilimumab with paclitaxel and carboplatin in NSCLC patients [129]. Three different regimens were tested: a phased regimen in which chemotherapy is given before ipilimumab, a concurrent regimen, and a control group of placebo and chemotherapy. The primary end point of improved PFS was only met in the phased regimen, suggesting again that there is indeed a need for a chemotherapy induction phase.

Conversely, a potential immunotherapy induction phase may also be useful. This type of induction could prevent the adverse effects of chemotherapy on the immune system and could improve the overall response rate of combination therapy [121]. A study in TNBC showed that an induction phase with durvalumab followed by combination therapy of weekly nab-paclitaxel for 12 weeks followed by four cycles of combination therapy with epirubicin and cyclophosphamide resulted into a higher

pathological CR rate when compared with chemotherapy alone (53.4% versus 44.2%, respectively) [123]. As there was no chemotherapy induction arm in this trial, it remains to be elucidated whether an immunotherapy or chemotherapy induction phase is most effective.

Discussion

Conclusions and future perspectives

Checkpoint blockade therapy is effective in a variety of tumor types. However, to further increase the number of suitable tumor types, ICB may be combined with compounds which are able to convert non-inflamed tumors into inflamed ones. This in return may render these tumors more sensitive to ICB therapy. Preclinical studies demonstrate that the majority of chemotherapeutic drugs has been shown to exert immunostimulatory effects, either by inhibiting immunosuppressive cells and/or activating effector cells, or by increasing immunogenicity and increasing T-cell infiltration. Although preclinical data have proved to be useful for identifying immunomodulating effects, extrapolation to the clinic should be done cautiously. For example, drug concentrations used in these experiments and drug exposure over time often do not correspond to observed exposure in the clinic. Preferably, preclinical experiments should mimic as much as is possible the clinical situation. An additional potential confounder is that the majority of studies investigate the immunomodulating effects of chemotherapy in peripheral blood and not in the tumor microenvironment. Although some peripheral factors contribute to a response to ICB, intratumoral immunological factors such as CD8+ T-cell infiltration, PD-L1 expression and IFN-γ secretion may be even more crucial and representative of observed effects. Therefore, it is warranted to further investigate the impact of chemotherapy in the tumor micro-environment. For this, it will be essential to draw pre- and on-treatment tumor biopsies during clinical trials, as they may reflect changes in the immunological status of the tumor better than peripheral markers.

In addition to choosing the ideal drugs for combination, it is crucial to investigate the optimal regimen for combination treatment. Current practice is that full-dose chemotherapy is administered with ICB on the same day. However, preclinical research suggests that for certain chemotherapeutic agents timing and sequence of administration of both modalities is important. Furthermore, during combination treatment, chemotherapy is now often administered at the maximum tolerated dose. For the majority of chemotherapeutic compounds, treatment at these doses results in bone marrow toxicity and may lead to altered immune cell function, while metronomic doses have been shown to augment immunotherapeutic activity [85, 156, 157]. Early signs of improved outcome of combined modality of chemotherapy and ICB in patients encourage more advanced approaches in identifying representative preclinical models, optimal drug combinations, sequence effects and ideal concentration–time profiles. This outcome should be the template for translation to clinical proof of concept studies, which should incorporate extensive pre- and on-treatment biomarker assessment, which may leverage pivotal studies, ultimately leading to novel standards of care.

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