

reviews

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Combining immunotherapy and anticancer agents: the right path to achieve cancer cure?

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Recent clinical trials revealed the impressive efficacy of immunological checkpoint blockade in different types of metastatic cancers. Such data underscore that immunotherapy is one of the most promising strategies for cancer treatment. In addition, preclinical studies provide evidence that some cytotoxic drugs have the ability to stimulate the immune system, resulting in anti-tumor immune responses that contribute to clinical efficacy of these agents. These observations raise the hypothesis that the next step for cancer treatment is the combination of cytotoxic agents and immunotherapies. The present review aims to summarize the immune-mediated effects of chemotherapeutic agents and their clinical relevance, the biological and clinical features of immune checkpoint blockers and finally, the preclinical and clinical rationale for novel therapeutic strategies combining anticancer agents and immune checkpoint blockers.

Key words: chemotherapy, radiotherapy, anticancer immunity, immunomodulation, CD4 T cells, CD8 T cells

introduction

The involvement of the immune system in tumor control is now accepted. The ability of innate and adaptive immune cells to detect and eliminate tumor cells was termed ‘cancer immunosurveillance’. However, subsequent studies have shown that immune cells could also facilitate cancer progression by promoting the growth of tumor clones resistant to anticancer immunity. The term ‘cancer immunoediting’ encapsulates the dual activity of the immune system on tumors [1]. The positive effect of the immune system on the control of tumor growth is underlined by the observation that HIV infection or immunosuppressive states induced by genetic deficiency or immunosuppression increase the frequency of solid cancers and hematological malignancies in mice and humans [2–5]. Recent data show that growing tumors are frequently infiltrated by immune cells, notably CD8 T cells and these cells probably contribute to the control of tumor growth in humans because their presence is associated with better outcomes [6–8]. This anti-tumor immune response can be manipulated to enhance tumor immune attack, leading to clinical benefits for cancer patients. Challenging the presiding view that chemotherapeutic agents were immunosuppressive [9–14], we and others have shown that some chemotherapeutic agents could elicit an immunogenic form of tumor cell death that enhances anticancer immune responses

and contributes to the clinical efficacy of these chemotherapies [15–18]. Although the demonstration of immunogenic cell death (ICD) relies on mouse models of intratumor injection of chemotherapy or only one systemic injection of chemotherapy which do not mimic the clinical setting of repetitive systemic injections of high doses of chemotherapy, the findings that patients with genetic deficiencies in molecules involved in the detection of ICD have poorer prognosis under chemotherapeutic treatment underscore the clinical relevance of this concept [15–17]. Recently, the use of monoclonal antibodies (mAbs) blocking key inhibitory receptors of T cells such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death 1 (PD-1) has led to robust anti-tumor immune responses and has yielded clinical benefits across multiple tumor types [19]. In addition, impressive clinical responses were observed upon adoptive transfer of tumor-specific autologous T cells using the generation of T cells that expressed cloned T-cell receptors (TCRs) or chimeric antigen receptors (CAR) [20]. However, despite these recent successes, many patients are not cured. Emerging evidence suggest that combination strategies may be important to achieve deeper tumor responses. Although combination of immune therapy with some conventional cytotoxic chemotherapies can be envisioned, the important question of how to integrate these novel immunotherapy treatments with the current clinical strategy still remains. In this review, we will summarize our knowledge on the immune-mediated effects of chemotherapies and on the mechanisms of action of novel immunotherapies and propose a rationale for the design of synergistic anticancer combinations.

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the emergence of novel immunotherapeutic strategies against cancer

dendritic cells

Owing to their strong ability to initiate and control T-cell responses, dendritic cells (DCs) have long been regarded as attractive candidates for the design of immunotherapy strategies [21]. Initially, DC cancer vaccines consisted of *ex vivo* generated DCs that were loaded with tumor antigens. Although this vaccination strategy has successfully elicited anticancer immune responses in cancer patients, no or very few clinical benefits were noted (reviewed in [22]). This was, for instance, illustrated by the results of a phase III randomized clinical trial comparing the clinical efficacy of DC therapy to dacarbazine in metastatic melanoma patients. The objective response rate for DC therapy was 3.8%, explaining the early discontinuation of the study because of lack of efficacy [23]. Proposed reasons explaining the lack of efficacy of DC vaccines include the use of inappropriate DC maturation cocktail, thus compromising the ability of DC to induce anticancer responses. To circumvent these issues, different strategies are being implemented. Cytokine combinations to favor DC maturation and antigen presentation properties to design more effective DC-based immunization strategies are being tested (reviewed in [22, 24]). A recent study demonstrated that the ability of a DC vaccine to increase, without any associated side effects, the breadth and diversity of melanoma neoantigen-specific T cells further enhances the potential of this approach [25].

adoptive T-cell therapy

Adoptive T-cell therapy for cancer aims to eliminate cancer cells upon administration of T cells into tumor-bearing hosts. In 1955, Mitchison [26] initially demonstrated the feasibility of this approach in mice. In 1966, Southam et al. studied the anticancer activity of leucocytes from tumor-bearing patients against their respective tumor and raised the hypothesis that lymphocytes from cancer patients have a specific inhibitory effect against cancer cells. This provided impetus to exploit T cells from cancer patients to design effective adoptive transfer strategies. Subsequent studies focused on the isolation, expansion and reinfusion of tumor-infiltrating lymphocytes (TILs) into cancer patients. The studies pioneered by Rosenberg and colleagues relied on the culture of tumor-derived lymphocytes in the presence of high doses of IL-2 that were then transferred into tumor-bearing patients. Although encouraging results were noted, side effects impeded the large clinical implementation of this strategy. The safety and efficacy of TIL therapy was improved by implementing preconditioning regimens driving lymphodepletion. The use of a preconditioning regimen relying on cyclophosphamide and fludarabine was shown to eliminate the endogenous lymphocyte repertoire and favor growth and long-lasting persistence of the transferred TILs. This has led to improved responses rate up to 40% [27]. Research in T-cell biology has also improved TIL culture conditions, leading to shorter TIL expansion phase, thereby reducing the time from TIL collection to their reinfusion into cancer patients.

An alternative strategy to target tumor cells using T cells is the engineering of CAR T cells, which are endowed with a specific ability to recognize and kill cancer cells. CARs contain a

fusion protein of light and heavy chains from an antibody, linked to the signaling machinery of the TCR. Such structure enables T-cell activation upon CAR recognition of its target. As CARs are not Major Histocompatibility complex (MHC)-restricted, they are insensitive to tumor-driven immunosuppression mediated through downregulation of MHC molecules. Another advantage of CAR T cells is the possibility of transducing genes that will enhance further T-cell functions upon activation or chemokine receptors that will favor T-cell homing. Although the initial trials in ovarian cancer and renal cell carcinoma using CAR T cells were disappointing because of toxicity and limited T-cell persistence in the tumor microenvironment [28, 29], the use of second-generation CAR T cells in leukemic patients has resulted in remarkable anticancer effects [30, 31]. The success of CAR T-cell therapy in these diseases was associated with a high level of CAR T-cell proliferation following infusion into patients. The feasibility of this approach was further established in 30 patients suffering from relapsed or refractory acute lymphoblastic leukemia. CAR T-cell therapy targeting CD19 led to complete remission in 27 patients and sustained remission was achieved with a 6-month overall survival rate of 78% [32]. This notable efficacy has led to the United States Food and Drug Administration to designate the anti-CD19 CAR T-cell therapy as a 'breakthrough therapy'.

cytokines

The efficacy of IL-2 as an anticancer agent has been investigated in multiple cancer types. It has been shown that high doses of IL-2 could be effective in eliciting anticancer responses in renal cell carcinoma and melanoma. Nevertheless, the overall response rates were low and the associated toxicities were severe (reviewed in [22]). IL-2 was further shown to drive the expansion of regulatory T cells which in turn suppress anticancer immune responses. This has prompted the test of additional cytokines for their anticancer potential upon *in vivo* administration. IL-15 was later identified as an interesting candidate molecule. The anticancer effects of IL-15 have been demonstrated in several preclinical tumor models. The underlying mechanisms have been subsequently identified. IL-15 has been shown to enhance NK cell effector functions. In addition, IL-15 was shown to support the proliferation and effector functions of CD8 T cells in the presence of regulatory T cells, suggesting that IL-15 could preserve the persistence and anticancer functions of T cells in the tumor microenvironment [33]. The ability of IL-15 to activate T-cell functions was further exploited in the context of T-cell therapy. Culture of TILs with IL-15 was indeed shown to improve the quality of CD8 T cells for adoptive therapy [34]. CAR T cells expressing the IL-15 gene featured greater expansion *in vitro* and reduced the cell death rate over control CAR T cells. Upon adoptive transfer in mice, IL-15 expressing CARs showed enhanced anticancer effects *in vivo* [35]. The clinical implementation of IL-15 began in 2009. The safety and efficacy of IL-15 is currently being tested in patients with lymphoma, melanoma, or renal cell carcinoma ([36] and NCT01572493, NCT01385423).

In addition to IL-15, IL-21 is also an immunomodulatory cytokine that is currently being tested for its anticancer activity in humans. Preclinical studies using IL-21-overexpressing

tumors revealed that IL-21 prevented B16 melanoma and MCA205 carcinoma growth and increased mouse survival [37]. In addition, administration of IL-21 was found to control CD8 T-cell expansion and effector functions and to synergize with IL-15, leading to the rejection of large melanoma tumors in mice [38]. The ability of IL-21 to enhance T-cell functionality was shown in the context of adoptive transfer. CD8 T cells cultured with IL-21 enhanced their anticancer activity, leading to rejection of large tumors upon transfer [39]. Similarly, IL-21 enhances CAR T-cell anticancer functions for effective immunotherapy against B-cell malignancies [40]. We have also recently reported that naïve CD4 T cells differentiated into effector Th9 cells in the presence of TGF- β , IL-4 and IL-1 β , secreted high levels of IL-21 and mediated IL-21-dependent anticancer effects against melanoma tumors upon adoptive transfer [41]. IL-21 mediated its anticancer activity through activation of NK and CD8 T cells which in turn controlled tumor progression through IFN γ . The clinical efficacy of IL-21 was investigated in phase I and II trials involving melanoma, renal cell carcinoma and metastatic colorectal cancer patients. In the phase II melanoma trial including 40 patients, the overall response rate to IL-21 was 22.5%, with 9 patients exhibiting partial responses and with 16 who had stable disease [42].

checkpoint inhibitors

A balance between co-stimulatory and inhibitory signals regulates the amplitude and the quality of T-cell responses driven by TCR signaling. T cells require CD28-mediated co-stimulation (also known as signal 2) for the full acquisition of effector functions. However, excessive T-cell activation can result in the loss of self-tolerance, underscoring the importance of immune inhibitory pathways, or immune checkpoints, that regulate T-cell activity. The immunosuppressive tumor microenvironment directly affects the expression of immune checkpoint proteins, thereby favoring resistance to anti-tumor immune response. T cells are essential effectors for cancer immune surveillance, and inhibition of T-cell-dependent anti-tumor response can promote tumor progression [43]. Engagement of the CD28 homologue receptor cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) on T cells by co-stimulatory molecules negatively regulates T-cell activation [44]. Leach et al. [45] have exploited this modulation of T-cell function therapeutically. They showed that administration of neutralizing CTLA-4 antibody into tumor-bearing mice resulted in tumor rejection [45]. In addition, mice that had rejected their tumors following

anti-CTLA-4 treatment were protected against subsequent tumor rechallenge, indicating the establishment of immunological memory [45]. Additional mouse and human studies have validated these results and shown that CTLA-4 blockade triggers anticancer immune responses. Importantly, inhibition of CTLA-4 signaling not only enhances effector T-cell functions, but it also renders effector T cells insensitive to regulatory T-cell-driven suppression. Infusion of anti-CTLA-4 antibodies after vaccination with irradiated, autologous tumor cells secreting GM-CSF (GVAX)-induced anti-tumor immunity but no toxicity in metastatic melanoma patients [46]. The clinical efficacy of anti-CTLA-4 therapy was further confirmed in a phase III clinical trial where ipilimumab, a human mAb against CTLA-4, was shown to enhance the overall survival of metastatic melanoma patients [47]. The demonstrated anticancer activity of ipilimumab (Yervoy) led to its approval by the FDA for the treatment of metastatic melanoma.

Other key inhibitory checkpoints that are relevant in cancer immunotherapy include PD-1 and Tim-3. Expression of the PD-1 receptor is induced in T cells upon activation [48]. Tumor cells can drive T-cell dysfunction because of their expression of PD-1 receptor ligands, PD-L1 and PD-L2 [49–52]. Iwai et al. [52] have shown that transgenic expression of PD-L1 in mastocytoma tumor cells prevented their elimination by CTL and enhanced their invasiveness *in vivo*. Thus, cancer tissues limit the host immune response through PD-1 ligands and their ligation to PD-1 on antigen-specific CD8 T cells, a phenomenon termed adaptive immune resistance. The molecular bases accounting for adaptive immune resistance remain elusive. However, it has been suggested that the therapeutic efficacy of PD-1 blockade is due to the restoration of CD8 T-cell effector function in the tumor microenvironment [53]. Preclinical models have demonstrated that blockade of PD-L1/PD-1 interactions could reinforce anticancer immune responses and promote tumor control [51, 52]. In 2014, pembrolizumab and nivolumab, two anti-PD-1 antibodies, were approved by the FDA for the treatment of advanced melanoma patients (Table 1). Tim-3 is another T-cell inhibitory receptor that was initially identified on fully differentiated Th1 cells. The Tim-3 ligand, galectin-9, induces T-cell death [64]. In the tumor microenvironment, dysfunctional CD8 T cells could be identified by the co-expression of Tim-3 and PD-1. Importantly, the concomitant administration of neutralizing Tim-3 and PD-1 antibodies showed synergistic effects in preventing tumor outgrowth [65]. As Tim-3 and PD-1 expression are associated with tumor antigen-specific CD8+ T-cell dysfunction in melanoma patients and prevent the expansion of tumor antigen-specific

Table 1. Published studies having investigated the effect of combination therapies with checkpoint inhibitors

Therapeutic antibody	Clinical phase	Treatment of	Combinatorial treatment given	References
Ipilimumab (anti-CTLA-4)	I	Melanoma	Vemurafenib	[54]
Ipilimumab (anti-CTLA-4)	I	Melanoma	Nivolumab	[55]
Ipilimumab (anti-CTLA-4)	III	Melanoma	Dacarbazine	[56, 57]
Ipilimumab (anti-CTLA-4)	Ib	Pancreatic cancer	GM-CSF-secreting vaccine	[58]
Ipilimumab (anti-CTLA-4)	I/II	Prostate cancer	GM-CSF-secreting allogeneic vaccine	[59]
Ipilimumab (anti-CTLA-4)	I/II	Prostate cancer	GM-CSF	[60]
Ipilimumab (anti-CTLA-4)	II	Small cell lung cancer	Paclitaxel and carboplatin	[61]
Tremelimumab (anti-CTLA-4)	I	Melanoma	TLR9 agonist PF-3512676	[62]
Nivolumab (anti-PD-1)	I	Melanoma	Peptide vaccine	[63]

CD8 T cells induced by vaccination [66, 67], evaluating the clinical efficacy of anti-Tim-3 antibodies in a clinical setting will be of high interest. Other therapies targeting immune checkpoints are currently in development such as agonist antibodies targeting molecules which activate T cells such as CD137 (BMS-663513) [68], OX40 (MEDI6383) NCT02221960, CD40 (CP870,893) [69] or GITR (TRAX518) NCT01239134 as well as drugs favoring DC activation such as LAG3-Fusion protein (IMP321) [70].

One of the challenging problems with the use of checkpoint inhibitors is the management of autoimmune side effects called immune-related adverse events (irAEs) (for detailed review see [71, 72]). Mild to severe irAEs are observed with ipilimumab in about 60% of patients and in about 15% with anti-PD-1 drugs. irAEs include dermatologic, gastrointestinal, hepatic, endocrine, and other less common inflammatory events. IrAEs are believed to arise from general immunologic enhancement, and temporary immunosuppression with corticosteroids, tumor necrosis factor-alpha antagonists, mycophenolate mofetil or other agents can be an effective treatment in most cases. Interestingly, an association between irAEs and clinical outcome was observed for anti-CTLA-4 therapy [73]. The recent approval of anti-CTLA-4 and anti-PD1 mAbs in the clinic opens a new field in cancer immunotherapy. The discovery that anti-PD-1 mAb treatment could be effective in many types of cancers like melanoma, renal carcinoma, lung cancer, bladder cancer gastric cancer underscores the possible development of immune checkpoint inhibitors in many clinical contexts of solid tumors.

In addition to antibodies targeting checkpoint inhibitors, bispecific antibodies are being developed. These antibodies are artificial proteins that are composed of fragments of two different mAbs and consequently bind to two different types of antigens. This approach is used for cancer immunotherapy, where these proteins simultaneously bind to cytotoxic T cells using CD3 and a tumor cell target. Two drugs are currently available. Catumaxomab consists of one heavy chain and one light chain of an anti-EpCAM antibody and one heavy chain and one light chain of an anti-CD3 antibody as a consequence of which the chimeric protein can bind both EpCAM and CD3. In addition, the Fc-region can bind to an Fc receptor on accessory cells like other antibodies, which has led to calling the drug a trifunctional antibody. This structure allows both T cell and macrophage or DC activation to favor adaptive immune response and tumor cell lysis by immune effectors. This drug could be used to treat patients with ascites with EpCam + tumor cells. Blinatumomab is a bispecific T-cell engager that combines two binding sites: a CD3 site for T cells and a CD19 site to target B cells. The drug works by linking these two cell types and activating the T cell to exert cytotoxic activity on the target cells. Blinatumomab could be used to target malignant B-cell lymphoma/leukemia and make blinatumomab a potential therapeutic option for pediatric and adult B-cell lymphoma or acute B-cell lymphoblastic leukemia.

rationale to combine conventional cancer treatments with immunotherapy

combining radiotherapy with immunotherapy

Accumulating data identifying molecular changes in the tumor microenvironment induced by tumor irradiation have recently

contributed to better understand the contribution of the immune system in the response of the irradiated tumor [74–76] (and reviewed in [77] and [78]). Tumor irradiation can induce the priming of immune response after induction of ICD [15, 79, 80], which could explain the observation of regression of unirradiated distant tumor sites (the so-called abscopal effect) [81, 82]. In addition, irradiation of tumor cells contributes to the effector phase by inducing expression of numerous molecules (MHC I, NKG2D ligands, death receptors, adhesion molecules) able to activate effector immune cells [83–87]. Thus, combining radiation with immunotherapy appears to provide an optimal therapeutic partnership to achieve immune-mediated systemic tumor control [88]. In preclinical models, tumor irradiation induces Fas upregulation by tumor cells, thereby enhancing Fas-dependent CTL killing [89], and the effectiveness of cancer vaccines [87, 90, 91]. This was often accompanied by important tumor influx by CD8+ and/or abscopal effect [90]. Similarly, upregulation of MHC class I molecules by tumor cells following irradiation enhance the anti-tumor effect of adoptive cell therapy (ACT) [92, 93]. Moreover, combining mAbs targeting important immune checkpoints (CD137, CD40, PD-1, CTLA-4) with tumor irradiation has shown promising synergistic activity [94–96]. In humans, localized radiotherapy combined with immunotherapeutic interventions has been shown to increase tumor-specific T-cell number, and encouraging clinical results have been reported in patients with hepatocellular carcinoma or prostate cancer [97–99]. Clinical trials combining radiotherapy with imiquimod (a TLR7 agonist) (NCT01421017), fresolimumab (a mAb that neutralizes TGF- β) (NCT01401062) or ipilimumab (a mAb directed against CTLA-4) (NCT01689974) are actually ongoing, paving the way for the use of radiation as a partner for immunotherapy (Table 2).

combining chemotherapy with immunotherapy

Because most chemotherapeutic agents were regarded as immunosuppressants, combinations between immunotherapy and chemotherapy were long considered as incompatible. However, the emergence of the concept of ICD (discussed above), the observations that some chemotherapies such as cyclophosphamide and 5-fluorouracil can eliminate regulatory immune cell subsets (reviewed in [111, 112]) and some clinical trials results showing that patients treated first with immunotherapy, followed by chemotherapy demonstrated better clinical outcomes than patients that have received chemotherapy alone [113, 114], have prompted scientists and physicians to reassess the potential of combination therapies between chemotherapy and immunotherapy. Subsequent preclinical and clinical investigations have revealed that chemotherapy could enhance the efficacy of immunotherapy through various mechanisms (Table 3 and Figure 1). Chemotherapy can not only improve anti-tumor effects of immunotherapy by overcoming parts of immunosuppression, but also by enhancing cross-presentation of tumor antigens and by supporting better penetration of immune cells in tumor core (Table 3 and Figure 1).

combining targeted therapies with immunotherapy

small molecules. Among targeted therapies, number of tyrosine kinase inhibitors (TKIs), proteasome inhibitors or mTOR

Table 2. Non-exhaustive list of ongoing clinical trials investigating the effect of combination therapies (also reviewed in [100–110])

Therapeutic agent	Cancer	Clinical phase	Combined with	Reference
Imiquimod	Breast cancer with skin metastases	II	Radiotherapy + cyclophosphamide	NCT01421017
Fresolimumab	Metastatic breast cancer	I/II	Radiotherapy	NCT01401062
Ipilimumab	Metastatic melanoma	II	Radiotherapy	NCT01689974
Multipeptide cancer vaccine	Advanced/mRCC	III	Sunitinib	NCT01265901
Peptide-based vaccine	Advanced stage HER2-overexpressing breast cancer	I/II	Adoptive T-cell therapy	NCT00791037
Trastuzumab	High-risk/metastatic HER-2/Neu-overexpressing breast cancer with no evidence of disease	II	Cyclophosphamide, and an allogeneic GM-CSF-secreting breast tumor vaccine	NCT00847171
Trastuzumab	Metastatic breast cancer overexpressing HER-2/NEU	II	DC vaccine given with and vinorelbine	NCT00266110
Trastuzumab	HER2 positive breast cancer	II	Peptide-based vaccine	NCT00343109
Nivolumab or ipilimumab with nivolumab	Resected stages IIIC/ IV melanoma	I	Vaccine combining multiple class I peptides and montanide ISA 51VG	NCT01176474
Anti-PD-1 antibody BMS-936558	Unresectable stages III/IV melanoma	I	Multiple class I peptides and montanide ISA 51 VG	NCT01176461
MPDL3280A	Metastatic melanoma	Ib	Vemurafenib and cobimetinib	NCT01656642
Ipilimumab	Cervical carcinoma	I	Combined with cisplatin and radiation therapy	NCT01711515
Ipilimumab	CrC lymphoma melanoma	I/II	Combined with radiation therapy	NCT01769222
Ipilimumab	Head and neck cancer	I	Combined with cetuximab and radiation therapy	NCT01860430
Ipilimumab	Head and neck cancer	I	Combined with cetuximab and radiation therapy	NCT01935921
Ipilimumab	Hodgkin's lymphoma	I	Combined with brentuximab vedotin	NCT01896999
Ipilimumab	Leukemia lymphoma	n.a.	Combined with lenalidomide	NCT01919619
Ipilimumab	Lymphoma	I	Combined with rituximab	NCT01729806
Ipilimumab	Melanoma	n.a.	Followed by lymphodepletion, TIL infusion and IL-2	NCT01701674
Ipilimumab	Melanoma	0	Combined with radioembolization	NCT01730157
Ipilimumab	Melanoma	I	Combined with SrS or wBrT	NCT01703507
Ipilimumab	Melanoma	I	Combined with dabrafenib ± trametinib	NCT01767454
Ipilimumab	Melanoma	I	Combined with Ny-eSO-1-targeting vaccine ± montanide	NCT01810016
Ipilimumab	Melanoma	I	Combined with BCG	NCT01838200
Ipilimumab	Melanoma	I/II	As single agent or combined with oncolytic virotherapy	NCT01740297
Ipilimumab	Melanoma	II	Combined with cyclophosphamide	NCT01740401
Ipilimumab	Melanoma	II	As single agent or combined with IFN α -2b	NCT01708941
Ipilimumab	Melanoma	II	Combined with nivolumab	NCT01783938
Ipilimumab	Melanoma	II	Combined with paclitaxel	NCT01827111
Ipilimumab	Melanoma	III	Combined with nivolumab	NCT01844505
Ipilimumab	Melanoma	III	Combined with lambrolizumab	NCT01866319
Ipilimumab	Melanoma	Iv	Combined with high-dose IL-2	NCT01856023
Ipilimumab	NSCLC	I	Combined with carboplatin, cisplatin and paclitaxel	NCT01820754
Ipilimumab	Pancreatic cancer	II	As single agent or combined with a GM-CSF-secreting vaccine	NCT01896869
Ipilimumab	Prostate cancer	I	Combined with sipuleucel-T	NCT01832870
Ipilimumab	Prostate cancer	II	After sipuleucel-T treatment	NCT01804465
Ipilimumab	Advanced solid tumors	I	Combined with imatinib	NCT01738139
Ipilimumab	Advanced solid tumors	I	Combined with lirilumab	NCT01750580
Ipilimumab	Advanced solid tumors	I	Combined with lenalidomide	NCT01750983
Ipilimumab	Advanced solid tumors	I/II	Combined with nivolumab	NCT01928394
Lambrolizumab	Melanoma	II	Combined with ipilimumab	NCT01866319
Lambrolizumab	Advanced solid tumors	II/III	As single agent or combined with conventional chemotherapy	NCT01840579
Lirilumab	Advanced solid tumors	I	Combined with nivolumab	NCT01714739

Continued

Table 2. *Continued*

Therapeutic agent	Cancer	Clinical phase	Combined with	Reference
Lirilumab	Advanced solid tumors	I	Combined with ipilimumab	NCT01750580
MeDI4736	Advanced solid tumors	I	Combined with tremelimumab	NCT01975831
Nivolumab	Melanoma	II	Combined with ipilimumab	NCT01783938
Nivolumab	Melanoma	III	Combined with ipilimumab	NCT01927419
Nivolumab	Melanoma	III	Combined with ipilimumab	NCT01844505
Nivolumab	NSCLC	II	Combined with azacitidine ± entinostat	NCT01928576
Nivolumab	Advanced solid tumors	I	Combined with lirilumab	NCT01714739
Nivolumab	Advanced solid tumors	II	As single agent or combined with immunotherapy	NCT01968109
Nivolumab	Advanced solid tumors	I/II	As single agent or combined with ipilimumab	NCT01928394
Tremelimumab	Hepatocellular carcinoma	I	Combined with rFa and TaCe	NCT01853618
Tremelimumab	Advanced solid tumors	I	Combined with MeDI4736	NCT01975831
Urelumab	Non-Hodgkin's lymphoma, chronic lymphocytic leukemia	I	Combined with rituximab	NCT01775631

Table 3. Main mechanisms by which conventional anticancer therapies could synergize with immunotherapy

Step of the anti-tumor immune response	Effects of conventional anti-tumor treatment on immune system	Potential synergistic activity with immunotherapy	References
Elimination of immunosuppressive cells	<ul style="list-style-type: none"> • Elimination of Tregs • Elimination of MDSCs • Reduction of PD-1 ligand expression 	Tumor vaccination, ACT	[115] [116, 117] [118–121]
Delivery of tumor antigen	<ul style="list-style-type: none"> • Upregulation of MHC class I • Increased cross-presentation 	Tumor vaccination, ACT	[92, 93, 122] [123]
Activation of antigen presenting cells	<ul style="list-style-type: none"> • Maturation and activation of DCs 	Anti-CTLA-4, tumor vaccination	[12, 124]
Cross-presentation of tumor antigen	<ul style="list-style-type: none"> • ICD 	Tumor vaccination	[15, 80]
T-cell penetration in tumor	<ul style="list-style-type: none"> • Disruption of tumor stroma • Upregulation of adhesion molecule on tumor blood vessels • Normalization of tumor vasculature 	ACT	[125] [126] [127]
T-cell cytotoxicity against tumor cells	<ul style="list-style-type: none"> • Upregulation of Fas on tumor cell • High dose lymphodepleting chemotherapy • Upregulation of MPR by tumor cells • Th1 cytokine production • Increased CTL avidity 	Anti PD-1/PD-L1, tumor vaccination, ACT	[89] [128] [129] [125] [130] [131]
Generation of memory T cells	<ul style="list-style-type: none"> • Promotion of long-term memory 	Tumor vaccination	[132]

inhibitors have been shown to influence immune response against cancer cells, mostly by affecting T-cell or DC functions [133–139], but also by depleting Tregs or myeloid derived suppressor cells (MDSCs) as discussed above [140–142]. Thus, a randomized phase III clinical trial is presently testing IMA901, a multipeptide cancer vaccine (preceded by a single low dose of cyclophosphamide), in combination with sunitinib in first-line metastatic renal cell carcinoma (mRCC; NCT01265901). This constitutes one of the examples of this new strategy of chemo-immunotherapy combining targeted therapy, immunotherapy and immunogenic chemotherapy schedule. TKIs may disrupt signal transducer and activator of transcription (STAT) signaling pathways, thus potentially decreasing immunosuppression by Tregs, MDSCs or DCs, making combinations with mAbs blocking immune checkpoints also quite attractive [115,143,144]. STAT activation can also control the expression of several immunosuppressive molecules (like PD-L1), providing further

rationale for combinations [145]. Numerous clinical trials are actually testing anti-PD-1/PD-L1 mAbs with TKIs, especially in mRCC patients, with encouraging preliminary results [146]. Of note, emerging data demonstrate that the normalization of tumor neovasculature by anti-angiogenic agents could improve endogenous and vaccination-induced anti-tumor immune responses [147–149].

tumor-targeting mAbs. Contribution of the immune response, especially through antibody-dependent-cellular cytotoxicity, has been demonstrated for the clinical efficacy of therapeutic mAbs, like rituximab [150], cetuximab [151] and trastuzumab [152]. Preclinical studies also have shown that trastuzumab is able to stimulate adaptative anti-tumor immunity [153, 154] and that combination of trastuzumab with anti-PD-1 and anti-CD137 can synergize [154, 155]. Some of these studies also suggest that the synergy between anthracyclines and trastuzumab could be

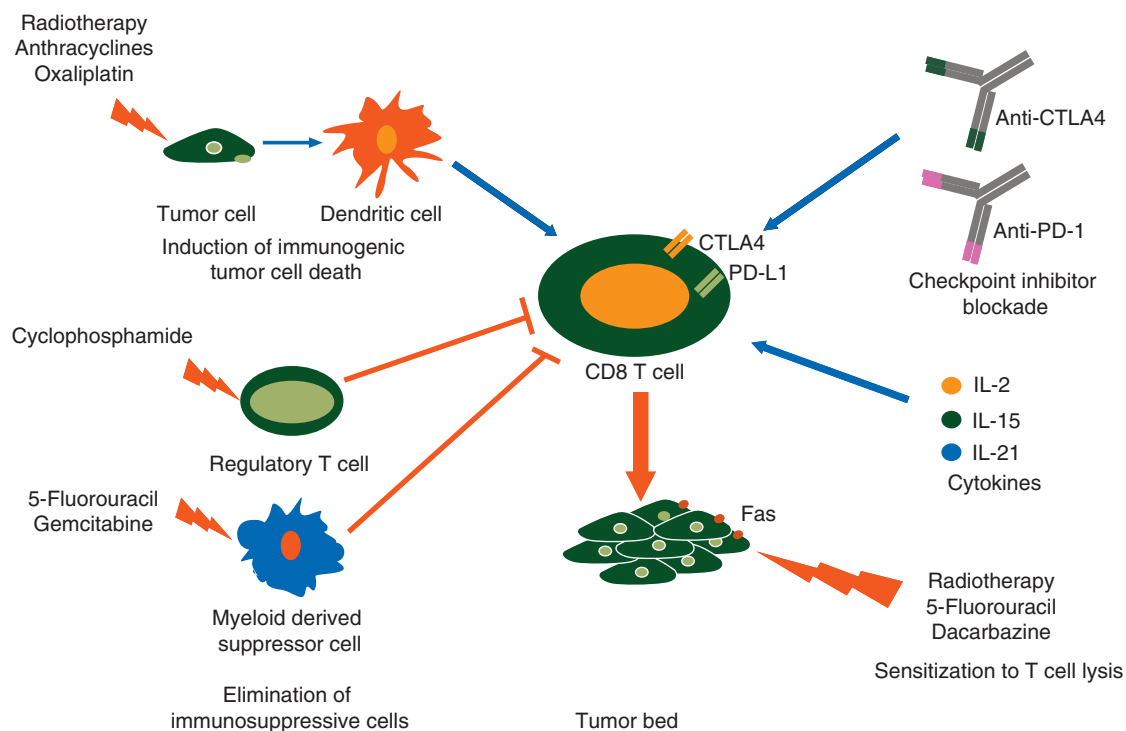


Figure 1. Molecular bases for the rationale to combine immunotherapy with anticancer agents. Conventional chemotherapeutic agents can (1) induce an immunogenic form of tumor cell death, (2) eliminate immunosuppressive cells and (3) sensitize tumor cells to immune effector cells. Immunostimulatory cytokines and checkpoint inhibitor blockers promote CD8 T-cell activation and prevent their subsequent dysfunction in the tumor microenvironment. Combination therapies thus not only target tumor cells, but also enhance CD8 T-cell activation, ultimately resulting in enhanced anticancer effects.

explained in part by increased anti-tumor immune response [154]. Combinatorial approaches of tumor vaccines with passive immunotherapy have been developed in HER2-overexpressing breast cancer. In metastatic breast cancer patients previously treated with trastuzumab, association of HER2-based peptide vaccine and trastuzumab resulted in boosting and prolongation of T-cell response against HER2, with an estimated progression free survival of 33% at 3 years [156]. Preliminary results on the clinical efficacy of this combination [157–160], and its superiority compared with vaccination alone [157], have also been reported by other groups. Ongoing clinical trials incorporating immunization with trastuzumab, with or without chemotherapy, are currently ongoing (NCT00791037, NCT00847171, NCT00266110, NCT00343109) [161].

immune checkpoint inhibitors. Preclinical, but also recent clinical evidences suggest that mAbs targeting inhibitory immune checkpoints can be used in combination. Concurrent PD-1 blockade with mAb blocking CTLA-4, LAG3 or TIM-3 has shown preclinical signs for anti-tumor synergy without significant toxicity [65, 162, 163]. A recent study tested the combination of nivolumab (anti-PD-1 mAb) with ipilimumab (anti-CTLA-4 mAb) in patients with advanced melanoma, at a concomitant or sequential schedule (ipilimumab followed by nivolumab). A total of 53 patients received concurrent treatment. The objective-response rate for all patients in the concurrent group was 40%, and at the maximum doses that were associated with an acceptable level of severe adverse events (nivolumab at 1 mg/kg and ipilimumab at 3 mg/kg) 53% of patients had an objective

response, all with tumor reduction of 80% or more. However, grade 3 or 4 adverse events occurred in over half of the patients but were generally reversible [55]. Checkpoint blockade has also been combined with standard doses and regimens of cytotoxic chemotherapy. Signs of potential efficacy have been reported with a combination of ipilimumab with sequential chemotherapy in non-small cell lung cancer (NSCLC) patients [164], or in metastatic melanoma [165]. A recent clinical study conducted in 30 metastatic breast cancer patients showed that the combination of the immune checkpoint modulator IMP321 (recombinant soluble LAG3/Ig fusion), preceded by standard dose weekly paclitaxel is feasible, and is followed by objective response rates of 50%, and immune activation of NK cells, as well as durable effector memory CD8+ T-cell responses [166]. Moreover, synergy of anti-CTLA-4 associated with chemotherapy and radiotherapy has recently been reported [167], and the combination of mAbs blocking PD-1/PD-L1 with therapeutic vaccines or targeted anticancer agents (BRAF inhibitor vemurafenib) is actually being explored in melanoma (NCT01176474 and NCT01176461) and advanced metastatic cancer patients (NCT01656642), respectively (Table 2).

conclusions

For immunologists, it has become clear that immunotherapeutic strategies should engage multiple effector mechanisms to overcome the immunosuppressive mechanisms of cancer. For patients with metastatic cancer or larger burden of disease, a single therapeutic agent is unlikely to be effective, and immunotherapy should be combined with conventional cancer treatments, with

the aim of not only to reduce tumor load, but also to abrogate immune tolerance and to enhance anticancer immune responses (Table 3 and Figure 1). This opens a field for fundamental and clinical research to better develop the concept of chemoimmunotherapy, especially to design the optimal choice, schedule and dose of therapeutic associations. As the success of these combined approaches may rely on the crosstalk between cancer cells, tumor stroma and the patient's immune system, three major considerations appear crucial for implementation of chemoimmunotherapy efficient combinations. First, cancer-bearing patients who may benefit from such combinations must be properly selected using appropriate biomarkers, which underscores the crucial need for predictive biomarkers that can be introduced in the clinical routine. Second, the optimal choice of combinations along with the schedule and dosage of administration of each component of the chemoimmunotherapy treatment remain to be determined, which highlights the need to develop clear immune biological and clinical parameters that allow for rapid go/no-go decisions. Finally, it is important to keep in mind that cancer patients are often heavily co-medicated with a number of drugs for symptom management as well as over-the-counter products and supplements, which can also affect the immune system. Future research and clinical trials that will rationally sequence immunomodulators, cancer vaccines and conventional treatments of cancer will benefit from taking into account these important aspects.

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disclosure

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