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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-1β, 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 IFNγ. 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 IFNα-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	• Elimination of Tregs	Tumor vaccination, ACT	[115]
	• Elimination of MDSCs		[116, 117]
	• Reduction of PD-1 ligand expression		[118–121]
Delivery of tumor antigen	• Upregulation of MHC class I	Tumor vaccination, ACT	[92, 93, 122]
	• Increased cross-presentation		[123]
Activation of antigen presenting cells	• Maturation and activation of DCs	Anti-CTLA-4, tumor vaccination	[12, 124]
Cross-presentation of tumor antigen	• ICD	Tumor vaccination	[15, 80]
T-cell penetration in tumor	• Disruption of tumor stroma	ACT	[125]
	• Upregulation of adhesion molecule on tumor blood vessels		[126]
	• Normalization of tumor vasculature		[127]
T-cell cytotoxicity against tumor cells	• Upregulation of Fas on tumor cell	Anti PD-1/PD-L1, tumor vaccination, ACT	[89]
	• High dose lymphodepleting chemotherapy		[128] [129]
	• Upregulation of MPR by tumor cells		[125]
	• Th1 cytokine production		[130]
	• Increased CTL avidity		[131]
Generation of memory T cells	• 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

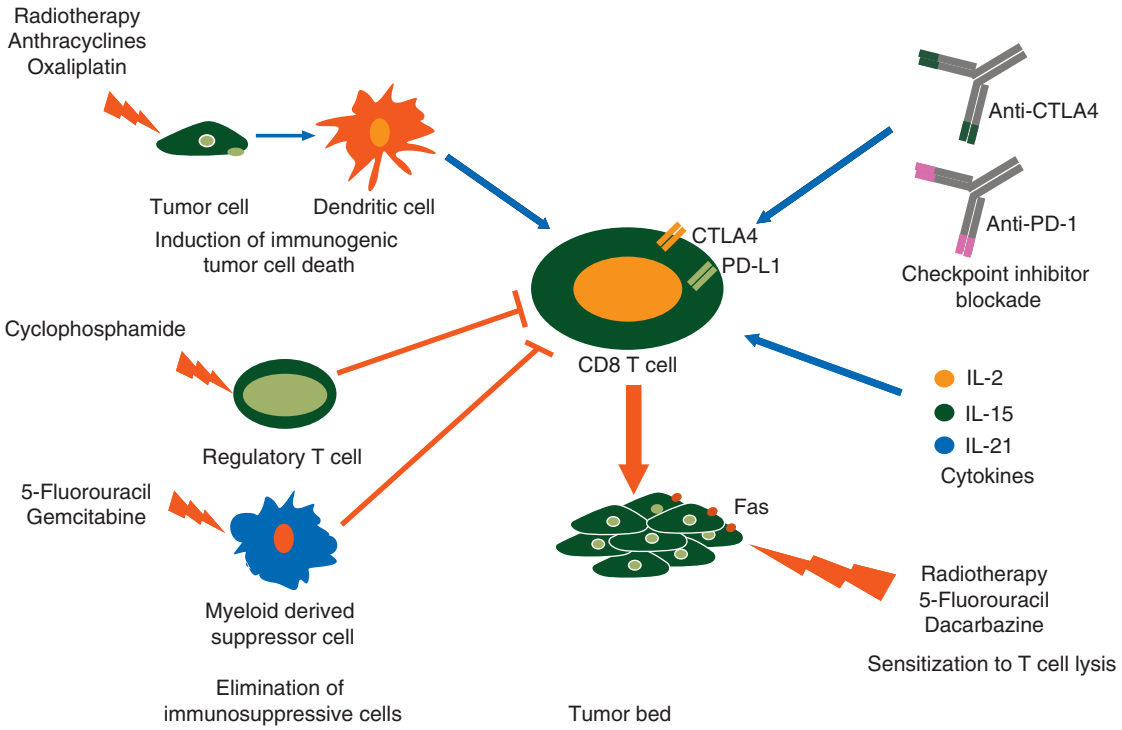

Figure 1. Molecular bases for the rationale to combine immunotherapy with anticancer agents. Conventional chemotherapeutic agents can (1) induce an immunogenic form of tumor cell death, (2) eliminate immunosuppressive cells and (3) sensitize tumor cells to immune effector cells. Immunostimulatory cytokines 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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references

1. Dunn GP, Old LJ, Schreiber RD. The three Es of cancer immunoediting. Annu Rev Immunol 2004; 22: 329–360.
2. Wimmer CD, Angele MK, Schwarz B et al. Impact of cyclosporine versus tacrolimus on the incidence of *de novo* malignancy following liver transplantation: a single center experience with 609 patients. Transpl Int 2013; 26: 999–1006.
3. Robbins HA, Pfeiffer RM, Shiels MS et al. Excess Cancers Among HIV-Infected People in the United States. J Natl Cancer Inst 2015; 107.
4. Shin M, Moon HH, Kim JM et al. Comparison of the incidence of de novo malignancy in liver or kidney transplant recipients: analysis of 2673 consecutive cases in a single center. Transplant Proc 2013; 45: 3019–3023.
5. Salavoura K, Kolialexi A, Tsangaris G, Mavrou A. Development of cancer in patients with primary immunodeficiencies. Anticancer Res 2008; 28: 1263–1269.
6. Fridman WH, Pages F, Sautes-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. Nat Rev Cancer 2012; 12: 298–306.
7. Zhang L, Conejo-Garcia JR, Katsaros D et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med 2003; 348: 203–213.
8. Apetoh L, Smyth MJ, Drake CG et al. Consensus nomenclature for CD8 T cell phenotypes in cancer. Oncoimmunology 2015; 4, doi: 10.1080/2162402X.2014.998538.
9. Andersen MH, Sorensen RB, Schrama D et al. Cancer treatment: the combination of vaccination with other therapies. Cancer Immunol Immunother 2008; 57: 1735–1743.
10. Menard C, Martin F, Apetoh L et al. Cancer chemotherapy: not only a direct cytotoxic effect, but also an adjuvant for antitumor immunity. Cancer Immunol Immunother 2008; 57: 1579–1587.
11. Zitvogel L, Apetoh L, Ghiringhelli F, Kroemer G. Immunological aspects of cancer chemotherapy. Nat Rev Immunol 2008; 8: 59–73.
12. Yu B, Kusmartsev S, Cheng F et al. Effective combination of chemotherapy and dendritic cell administration for the treatment of advanced-stage experimental breast cancer. Clin Cancer Res 2003; 9: 285–294.
13. Weiner HL, Cohen JA. Treatment of multiple sclerosis with cyclophosphamide: critical review of clinical and immunologic effects. Mult Scler 2002; 8: 142–154.
14. Javeed A, Ashraf M, Riaz A et al. Paclitaxel and immune system. Eur J Pharm Sci 2009; 38: 283–290.
15. Apetoh L, Ghiringhelli F, Tesniere A et al. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. Nat Med 2007; 13: 1050–1059.
16. Ghiringhelli F, Apetoh L, Tesniere A et al. Activation of the NLRP3 inflammasome in dendritic cells induces IL-1beta-dependent adaptive immunity against tumors. Nat Med 2009; 15: 1170–1178.
17. Tesniere A, Schlemmer F, Boige V et al. Immunogenic death of colon cancer cells treated with oxaliplatin. Oncogene 2010; 29: 482–491.
18. Lake RA, Robinson BW. Immunotherapy and chemotherapy—a practical partnership. Nat Rev Cancer 2005; 5: 397–405.
19. Melero I, Grimaldi AM, Perez-Gracia JL, Ascierto PA. Clinical development of immunostimulatory monoclonal antibodies and opportunities for combination. Clin Cancer Res 2013; 19: 997–1008.
20. Kalos M, June CH. Adoptive T cell transfer for cancer immunotherapy in the era of synthetic biology. Immunity 2013; 39: 49–60.
21. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature 1998; 392: 245–252.
22. Kirkwood JM, Butterfield LH, Tarhini AA et al. Immunotherapy of cancer in 2012. CA Cancer J Clin 2012; 62: 309–335.
23. Schadendorf D, Ugurel S, Schuler-Thurner B et al. Dacarbazine (DTIC) versus vaccination with autologous peptide-pulsed dendritic cells (DC) in first-line treatment of patients with metastatic melanoma: a randomized phase III trial of the DC study group of the DeCOG. Ann Oncol 2006; 17: 563–570.
24. Wimmers F, Schreibelt G, Skold AE et al. Paradigm shift in dendritic cell-based immunotherapy: from *in vitro* generated monocyte-derived DCs to naturally circulating DC subsets. Front Immunol 2014; 5: 165.
25. Carreno BM, Magrini V, Becker-Hapak M et al. A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells. Science 2015; 348: 803–808.
26. Mitchison NA. Studies on the immunological response to foreign tumor transplants in the mouse. I. The role of lymph node cells in conferring immunity by adoptive transfer. J Exp Med 1955; 102: 157–177.
27. Dudley ME, Wunderlich JR, Robbins PF et al. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. Science 2002; 298: 850–854.
28. Kershaw MH, Westwood JA, Parker LL et al. A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. Clin Cancer Res 2006; 12: 6106–6115.
29. Lamers CH, Sleijfer S, Vulto AG et al. Treatment of metastatic renal cell carcinoma with autologous T-lymphocytes genetically retargeted against carbonic anhydrase IX: first clinical experience. J Clin Oncol 2006; 24: e20–e22.
30. Kalos M, Levine BL, Porter DL et al. T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. Sci Transl Med 2011; 3: 95ra73.

31. Porter DL, Levine BL, Kalos M et al. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. N Engl J Med 2011; 365: 725–733.
32. Maude SL, Frey N, Shaw PA et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med 2014; 371: 1507–1517.
33. Perna SK, De Angelis B, Pagliara D et al. Interleukin 15 provides relief to CTLs from regulatory T cell-mediated inhibition: implications for adoptive T cell-based therapies for lymphoma. Clin Cancer Res 2013; 19: 106–117.
34. Klebanoff CA, Finkelstein SE, Surman DR et al. IL-15 enhances the in vivo antitumor activity of tumor-reactive CD8+ T cells. Proc Natl Acad Sci USA 2004; 101: 1969–1974.
35. Hoyos V, Savoldo B, Quintarelli C et al. Engineering CD19-specific T lymphocytes with interleukin-15 and a suicide gene to enhance their anti-lymphoma/leukemia effects and safety. Leukemia 2010; 24: 1160–1170.
36. Conlon KC, Lugli E, Welles HC et al. Redistribution, hyperproliferation, activation of natural killer cells and CD8 T cells, and cytokine production during first-in-human clinical trial of recombinant human interleukin-15 in patients with cancer. J Clin Oncol 2015; 33: 74–82.
37. Wang G, Tschoi M, Spolski R et al. *In vivo* antitumor activity of interleukin 21 mediated by natural killer cells. Cancer Res 2003; 63: 9016–9022.
38. Zeng R, Spolski R, Finkelstein SE et al. Synergy of IL-21 and IL-15 in regulating CD8+ T cell expansion and function. J Exp Med 2005; 201: 139–148.
39. Hinrichs CS, Spolski R, Paulos CM et al. IL-2 and IL-21 confer opposing differentiation programs to CD8+ T cells for adoptive immunotherapy. Blood 2008; 111: 5326–5333.
40. Singh H, Figliola MJ, Dawson MJ et al. Reprogramming CD19-specific T cells with IL-21 signaling can improve adoptive immunotherapy of B-lineage malignancies. Cancer Res 2011; 71: 3516–3527.
41. Vegran F, Berger H, Boidot R et al. The transcription factor IRF1 dictates the IL-21-dependent anticancer functions of TH9 cells. Nat Immunol 2014; 15: 758–766.
42. Petrella TM, Tozer R, Belanger K et al. Interleukin-21 has activity in patients with metastatic melanoma: a phase II study. J Clin Oncol 2012; 30: 3396–3401.
43. Shankaran V, Ikeda H, Bruce AT et al. IFNgamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity. Nature 2001; 410: 1107–1111.
44. Allison JP, Krummel MF. The Yin and Yang of T cell costimulation. Science 1995; 270: 932–933.
45. Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. Science 1996; 271: 1734–1736.
46. Hodi FS, Butler M, Oble DA et al. Immunologic and clinical effects of antibody blockade of cytotoxic T lymphocyte-associated antigen 4 in previously vaccinated cancer patients. Proc Natl Acad Sci USA 2008; 105: 3005–3010.
47. Hodi FS, O'Day SJ, McDermott DF et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010; 363: 711–723.
48. Zha Y, Blank C, Gajewski TF. Negative regulation of T-cell function by PD-1. Crit Rev Immunol 2004; 24: 229–237.
49. Latchman Y, Wood CR, Chernova T et al. PD-L2 is a second ligand for PD-1 and inhibits T cell activation. Nat Immunol 2001; 2: 261–268.
50. Freeman GJ, Long AJ, Iwai Y et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J Exp Med 2000; 192: 1027–1034.
51. Dong H, Strome SE, Salomao DR et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nat Med 2002; 8: 793–800.
52. Iwai Y, Ishida M, Tanaka Y et al. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. Proc Natl Acad Sci USA 2002; 99: 12293–12297.
53. Tumeh PC, Harview CL, Yearley JH et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature 2014; 515: 568–571.
54. Ribas A, Hodi FS, Callahan M et al. Hepatotoxicity with combination of vemurafenib and ipilimumab. N Engl J Med 2013; 368: 1365–1366.
55. Wolchok JD, Kluger H, Callahan MK et al. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med 2013; 369: 122–133.
56. Robert C, Thomas L, Bondarenko I et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med 2011; 364: 2517–2526.
57. Sherrill B, Wang J, Kotapati S, Chin K. Q-TWiST analysis comparing ipilimumab/dacarbazine vs placebo/dacarbazine for patients with stage III/IV melanoma. Br J Cancer 2013; 109: 8–13.
58. Le DT, Lutz E, Uram JN et al. Evaluation of ipilimumab in combination with allogeneic pancreatic tumor cells transfected with a GM-CSF gene in previously treated pancreatic cancer. J Immunother 2013; 36: 382–389.
59. Santegoets SJ, Stam AG, Lougheed SM et al. T cell profiling reveals high CD4+ CTLA-4 + T cell frequency as dominant predictor for survival after prostate GVAX/ipilimumab treatment. Cancer Immunol Immunother 2013; 62: 245–256.
60. Kwek SS, Dao V, Roy R et al. Diversity of antigen-specific responses induced in vivo with CTLA-4 blockade in prostate cancer patients. J Immunol 2012; 189: 3759–3766.
61. Reck M, Bondarenko I, Luft A et al. Ipilimumab in combination with paclitaxel and carboplatin as first-line therapy in extensive-disease-small-cell lung cancer: results from a randomized, double-blind, multicenter phase 2 trial. Ann Oncol 2013; 24: 75–83.
62. Millward M, Underhill C, Lobb S et al. Phase I study of tremelimumab (CP-675 206) plus PF-3512676 (CPG 7909) in patients with melanoma or advanced solid tumours. Br J Cancer 2013; 108: 1998–2004.
63. Weber JS, Kudchadkar RR, Yu B et al. Safety, efficacy, and biomarkers of nivolumab with vaccine in ipilimumab-refractory or -naive melanoma. J Clin Oncol 2013; 31: 4311–4318.
64. Zhu C, Anderson AC, Schubart A et al. The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity. Nat Immunol 2005; 6: 1245–1252.
65. Sakuishi K, Apetoh L, Sullivan JM et al. Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity. J Exp Med 2010; 207: 2187–2194.
66. Fourcade J, Sun Z, Benallaoua M et al. Upregulation of Tim-3 and PD-1 expression is associated with tumor antigen-specific CD8+ T cell dysfunction in melanoma patients. J Exp Med 2010; 207: 2175–2186.
67. Fourcade J, Sun Z, Pagliano O et al. PD-1 and Tim-3 regulate the expansion of tumor antigen-specific CD8(+) T cells induced by melanoma vaccines. Cancer Res 2014; 74: 1045–1055.
68. Li SY, Liu Y. Immunotherapy of melanoma with the immune costimulatory monoclonal antibodies targeting CD137. Clin Pharmacol 2013; 5: 47–53.
69. Vonderheide RH, Burg JM, Mick R et al. Phase I study of the CD40 agonist antibody CP-870,893 combined with carboplatin and paclitaxel in patients with advanced solid tumors. Oncoimmunology 2013; 2: e23033.
70. Brignone C, Escudier B, Grygar C et al. A phase I pharmacokinetic and biological correlative study of IMP321, a novel MHC class II agonist, in patients with advanced renal cell carcinoma. Clin Cancer Res 2009; 15: 6225–6231.
71. Postow MA, Callahan MK, Wolchok JD. Immune Checkpoint Blockade in Cancer Therapy. J Clin Oncol 2015.
72. Kong YC, Flynn JC. Opportunistic autoimmune disorders potentiated by immune-checkpoint inhibitors anti-CTLA-4 and anti-PD-1. Front Immunol 2014; 5: 206.
73. Downey SG, Klapper JA, Smith FO et al. Prognostic factors related to clinical response in patients with metastatic melanoma treated by CTL-associated antigen-4 blockade. Clin Cancer Res 2007; 13: 6681–6688.
74. Stone HB, Peters LJ, Milas L. Effect of host immune capability on radiocurability and subsequent transplantability of a murine fibrosarcoma. J Natl Cancer Inst 1979; 63: 1229–1235.
75. Lee Y, Auh SL, Wang Y et al. Therapeutic effects of ablative radiation on local tumor require CD8+ T cells: changing strategies for cancer treatment. Blood 2009; 114: 589–595.
76. Demaria S, Formenti SC. Role of T lymphocytes in tumor response to radiotherapy. Front Oncol 2012; 2: 95.
77. Formenti SC, Demaria S. Combining radiotherapy and cancer immunotherapy: a paradigm shift. J Natl Cancer Inst 2013; 105: 256–265.
78. Demaria S, Pilones KA, Vanpouille-Box C et al. The optimal partnership of radiation and immunotherapy: from preclinical studies to clinical translation. Radiat Res 2014; 182: 170–181.
79. Obeid M, Tesniere A, Ghiringhelli F et al. Calreticulin exposure dictates the immunogenicity of cancer cell death. Nat Med 2007; 13: 54–61.

80. Golden EB, Pellicciotta I, Demaria S et al. The convergence of radiation and immunogenic cell death signaling pathways. Front Oncol 2012; 2: 88.
81. Demaria S, Ng B, Devitt ML et al. Ionizing radiation inhibition of distant untreated tumors (abscopal effect) is immune mediated. Int J Radiat Oncol Biol Phys 2004; 58: 862–870.
82. Postow MA, Callahan MK, Barker CA et al. Immunologic correlates of the abscopal effect in a patient with melanoma. N Engl J Med 2012; 366: 925–931.
83. Matsumura S, Wang B, Kawashima N et al. Radiation-induced CXCL16 release by breast cancer cells attracts effector T cells. J Immunol 2008; 181: 3099–3107.
84. Lugade AA, Sorensen EW, Gerber SA et al. Radiation-induced IFN-gamma production within the tumor microenvironment influences antitumor immunity. J Immunol 2008; 180: 3132–3139.
85. Reits EA, Hodge JW, Herberts CA et al. Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy. J Exp Med 2006; 203: 1259–1271.
86. Ruocco MG, Pilones KA, Kawashima N et al. Suppressing T cell motility induced by anti-CTLA-4 monotherapy improves antitumor effects. J Clin Invest 2012; 122: 3718–3730.
87. Chakraborty M, Abrams SI, Coleman CN et al. External beam radiation of tumors alters phenotype of tumor cells to render them susceptible to vaccine-mediated T-cell killing. Cancer Res 2004; 64: 4328–4337.
88. Demaria S, Bhardwaj N, McBride WH, Formenti SC. Combining radiotherapy and immunotherapy: a revived partnership. Int J Radiat Oncol Biol Phys 2005; 63: 655–666.
89. Chakraborty M, Abrams SI, Camphausen K et al. Irradiation of tumor cells up-regulates Fas and enhances CTL lytic activity and CTL adoptive immunotherapy. J Immunol 2003; 170: 6338–6347.
90. Hodge JW, Sharp HJ, Gameiro SR. Abscopal regression of antigen disparate tumors by antigen cascade after systemic tumor vaccination in combination with local tumor radiation. Cancer Biother Radiopharm 2012; 27: 12–22.
91. Newcomb EW, Demaria S, Lukyanov Y et al. The combination of ionizing radiation and peripheral vaccination produces long-term survival of mice bearing established invasive GL261 gliomas. Clin Cancer Res 2006; 12: 4730–4737.
92. Medina-Echeverz J, Berraondo P. Colon cancer eradication after chemoimmunotherapy is associated with intratumoral emergence of proinflammatory myeloid cells. Oncoimmunology 2012; 1: 118–120.
93. Zhang B, Bowerman NA, Salama JK et al. Induced sensitization of tumor stroma leads to eradication of established cancer by T cells. J Exp Med 2007; 204: 49–55.
94. Demaria S, Kawashima N, Yang AM et al. Immune-mediated inhibition of metastases after treatment with local radiation and CTLA-4 blockade in a mouse model of breast cancer. Clin Cancer Res 2005; 11: 728–734.
95. Dewan MZ, Galloway AE, Kawashima N et al. Fractionated but not single-dose radiotherapy induces an immune-mediated abscopal effect when combined with anti-CTLA-4 antibody. Clin Cancer Res 2009; 15: 5379–5388.
96. Verbrugge I, Hagekyriakou J, Sharp LL et al. Radiotherapy increases the permissiveness of established mammary tumors to rejection by immunomodulatory antibodies. Cancer Res 2012; 72: 3163–3174.
97. Chi KH, Liu SJ, Li CP et al. Combination of conformal radiotherapy and intratumoral injection of adoptive dendritic cell immunotherapy in refractory hepatoma. J Immunother 2005; 28: 129–135.
98. Lechleider RJ, Arlen PM, Tsang KY et al. Safety and immunologic response of a viral vaccine to prostate-specific antigen in combination with radiation therapy when metronomic-dose interleukin 2 is used as an adjuvant. Clin Cancer Res 2008; 14: 5284–5291.
99. Slovin SF, Higano CS, Hamid O et al. Ipilimumab alone or in combination with radiotherapy in metastatic castration-resistant prostate cancer: results from an open-label, multicenter phase I/II study. Ann Oncol 2013; 24: 1813–1821.
100. Vacchelli E, Eggermont A, Fridman WH et al. Trial watch: immunostimulatory cytokines. Oncoimmunology 2013; 2: e24850.
101. Vacchelli E, Vitale I, Tartour E et al. Trial watch: anticancer radioimmunotherapy. Oncoimmunology 2013; 2: e25595.
102. Vacchelli E, Vitale I, Eggermont A et al. Trial watch: dendritic cell-based interventions for cancer therapy. Oncoimmunology 2013; 2: e25771.
103. Semeraro M, Vacchelli E, Eggermont A et al. Trial watch: lenalidomide-based immunochemotherapy. Oncoimmunology 2013; 2: e26494.
104. Aranda F, Vacchelli E, Eggermont A et al. Trial watch: peptide vaccines in cancer therapy. Oncoimmunology 2013; 2: e26621.
105. Vacchelli E, Aranda F, Eggermont A et al. Trial watch: tumor-targeting monoclonal antibodies in cancer therapy. Oncoimmunology 2014; 3: e27048.
106. Aranda F, Vacchelli E, Eggermont A et al. Trial watch: immunostimulatory monoclonal antibodies in cancer therapy. Oncoimmunology 2014; 3: e27297.
107. Vacchelli E, Aranda F, Eggermont A et al. Trial watch: chemotherapy with immunogenic cell death inducers. Oncoimmunology 2014; 3: e27878.
108. Aranda F, Vacchelli E, Obrist F et al. Trial watch: toll-like receptor agonists in oncological indications. Oncoimmunology 2014; 3: e29179.
109. Vacchelli E, Aranda F, Obrist F et al. Trial watch: immunostimulatory cytokines in cancer therapy. Oncoimmunology 2014; 3: e29030.
110. Vacchelli E, Eggermont A, Sautes-Fridman C et al. Trial watch: oncolytic viruses for cancer therapy. Oncoimmunology 2013; 2: e24612.
111. Apetoh L, Vegran F, Ladoire S, Ghiringhelli F. Restoration of antitumor immunity through selective inhibition of myeloid derived suppressor cells by anticancer therapies. Curr Mol Med 2011; 11: 365–372.
112. Ghiringhelli F, Apetoh L. Enhancing the anticancer effects of 5-fluorouracil: current challenges and future perspectives. Biomed J 2014.
113. Schlom J, Arlen PM, Gulley JL. Cancer vaccines: moving beyond current paradigms. Clin Cancer Res 2007; 13: 3776–3782.
114. Wheeler CJ, Das A, Liu G et al. Clinical responsiveness of glioblastoma multiforme to chemotherapy after vaccination. Clin Cancer Res 2004; 10: 5316–5326.
115. Lesterhuis WJ, Punt CJ, Hato SV et al. Platinum-based drugs disrupt STAT6-mediated suppression of immune responses against cancer in humans and mice. J Clin Invest 2011; 121: 3100–3108.
116. Ghiringhelli F, Menard C, Puig PE et al. Metronomic cyclophosphamide regimen selectively depletes CD4+CD25+ regulatory T cells and restores T and NK effector functions in end stage cancer patients. Cancer Immunol Immunother 2007; 56: 641–648.
117. Rettig L, Seidenberg S, Parvanova I et al. Gemcitabine depletes regulatory T-cells in human and mice and enhances triggering of vaccine-specific cytotoxic T-cells. Int J Cancer 2011; 129: 832–838.
118. Bolitho P, Street SEA, Westwood JA et al. Perforin-mediated suppression of B-cell lymphoma. Proc Natl Acad Sci USA 2009; 106: 2723–2728.
119. Vincent J, Mignot G, Chalmin F et al. 5-Fluorouracil selectively kills tumor-associated myeloid-derived suppressor cells resulting in enhanced T cell-dependent antitumor immunity. Cancer Res 2010; 70: 3052–3061.
120. Ko HJ, Kim YJ, Kim YS et al. A combination of chemoimmunotherapies can efficiently break self-tolerance and induce antitumor immunity in a tolerogenic murine tumor model. Cancer Res 2007; 67: 7477–7486.
121. Suzuki E, Kapoor V, Jassar AS et al. Gemcitabine selectively eliminates splenic Gr-1+/CD11b+ myeloid suppressor cells in tumor-bearing animals and enhances antitumor immune activity. Clin Cancer Res 2005; 11: 6713–6721.
122. Serrano A, Tanzarella S, Lionello I et al. Rexpression of HLA class I antigens and restoration of antigen-specific CTL response in melanoma cells following 5-aza-2′-deoxycytidine treatment. Int J Cancer 2001; 94: 243–251.
123. Nowak AK, Lake RA, Marzo AL et al. Induction of tumor cell apoptosis in vivo increases tumor antigen cross-presentation, cross-priming rather than cross-tolerizing host tumor-specific CD8 T cells. J Immunol 2003; 170: 4905–4913.
124. Tong Y, Song W, Crystal RG. Combined intratumoral injection of bone marrow-derived dendritic cells and systemic chemotherapy to treat pre-existing murine tumors. Cancer Res 2001; 61: 7530–7535.
125. Ramakrishnan R, Assudani D, Nagaraj S et al. Chemotherapy enhances tumor cell susceptibility to CTL-mediated killing during cancer immunotherapy in mice. J Clin Invest 2010; 120: 1111–1124.
126. Ganss R, Ryschich E, Klar E et al. Combination of T-cell therapy and trigger of inflammation induces remodeling of the vasculature and tumor eradication. Cancer Res 2002; 62: 1462–1470.
127. Shrimali RK, Yu Z, Theoret MR et al. Antiangiogenic agents can increase lymphocyte infiltration into tumor and enhance the effectiveness of adoptive immunotherapy of cancer. Cancer Res 2010; 70: 6171–6180.

128. Ding ZC, Blazar BR, Mellor AL et al. Chemotherapy rescues tumor-driven aberrant CD4+ T-cell differentiation and restores an activated polyfunctional helper phenotype. Blood 2010; 115: 2397–2406.
129. Kaech SM, Tan JT, Wherry EJ et al. Selective expression of the interleukin 7 receptor identifies effector CD8 T cells that give rise to long-lived memory cells. Nat Immunol 2003; 4: 1191–1198.
130. Zhang L, Dermawan K, Jin M et al. Differential impairment of regulatory T cells rather than effector T cells by paclitaxel-based chemotherapy. Clin Immunol 2008; 129: 219–229.
131. Yang S, Haluska FG. Treatment of melanoma with 5-fluorouracil or dacarbazine *in vitro* sensitizes cells to antigen-specific CTL lysis through perforin/granzyme- and Fas-mediated pathways. J Immunol 2004; 172: 4599–4608.
132. Nistico P, Capone I, Palermo B et al. Chemotherapy enhances vaccine-induced antitumor immunity in melanoma patients. Int J Cancer 2009; 124: 130–139.
133. Nencioni A, Beck J, Werth D et al. Histone deacetylase inhibitors affect dendritic cell differentiation and immunogenicity. Clin Cancer Res 2007; 13: 3933–3941.
134. Triozzi PL, Aldrich W, Achberger S et al. Differential effects of low-dose decitabine on immune effector and suppressor responses in melanoma-bearing mice. Cancer Immunol Immunother 2012; 61: 1441–1450.
135. Wolf AM, Wolf D, Rumpold H et al. The kinase inhibitor imatinib mesylate inhibits TNF-{alpha} production *in vitro* and prevents TNF-dependent acute hepatic inflammation. Proc Natl Acad Sci USA 2005; 102: 13622–13627.
136. Wilmott JS, Long GV, Howle JR et al. Selective BRAF inhibitors induce marked T-cell infiltration into human metastatic melanoma. Clin Cancer Res 2012; 18: 1386–1394.
137. Boni A, Cogdill AP, Dang P et al. Selective BRAFV600E inhibition enhances T-cell recognition of melanoma without affecting lymphocyte function. Cancer Res 2010; 70: 5213–5219.
138. Hannesdottir L, Tymoszuk P, Parajuli N et al. Lapatinib and doxorubicin enhance the Stat1-dependent antitumor immune response. Eur J Immunol 2013; 43: 2718–2729.
139. Galluzzi L, Senovilla L, Zitvogel L, Kroemer G. The secret ally: immunostimulation by anticancer drugs. Nat Rev Drug Discov 2012; 11: 215–233.
140. Adotevi O, Pere H, Ravel P et al. A decrease of regulatory T cells correlates with overall survival after sunitinib-based antiangiogenic therapy in metastatic renal cancer patients. J Immunother 2010; 33: 991–998.
141. Terme M, Pernot S, Marcheteau E et al. VEGFA-VEGFR pathway blockade inhibits tumor-induced regulatory T-cell proliferation in colorectal cancer. Cancer Res 2013; 73: 539–549.
142. Ko JS, Zea AH, Rini BI et al. Sunitinib mediates reversal of myeloid-derived suppressor cell accumulation in renal cell carcinoma patients. Clin Cancer Res 2009; 15: 2148–2157.
143. Larmonier N, Janikashvili N, LaCasse CJ et al. Imatinib mesylate inhibits CD4+ CD25+ regulatory T cell activity and enhances active immunotherapy against BCR-ABL- tumors. J Immunol 2008; 181: 6955–6963.
144. Xin H, Zhang C, Herrmann A et al. Sunitinib inhibition of Stat3 induces renal cell carcinoma tumor cell apoptosis and reduces immunosuppressive cells. Cancer Res 2009; 69: 2506–2513.
145. Yu H, Kortylewski M, Pardoll D. Crosstalk between cancer and immune cells: role of STAT3 in the tumour microenvironment. Nat Rev Immunol 2007; 7: 41–51.
146. Amin A, Plimack E, Infante J et al. Nivolumab (N) (anti-PD-1; BMS-936558, ONO-4538) in combination with sunitinib (S) or pazopanib (P) in patients with metastatic renal cell carcinoma (mRCC). ESMO 2014 2014; Abstract 1052PD.
147. Hamzah J, Jugold M, Kiessling F et al. Vascular normalization in Rgs5-deficient tumours promotes immune destruction. Nature 2008; 453: 410–414.
148. Huang Y, Yuan J, Righi E et al. Vascular normalizing doses of antiangiogenic treatment reprogram the immunosuppressive tumor microenvironment and enhance immunotherapy. Proc Natl Acad Sci USA 2012; 109: 17561–17566.
149. Matsumoto S, Batra S, Saito K et al. Antiangiogenic agent sunitinib transiently increases tumor oxygenation and suppresses cycling hypoxia. Cancer Res 2011; 71: 6350–6359.
150. Weng WK, Levy R. Two immunoglobulin G fragment C receptor polymorphisms independently predict response to rituximab in patients with follicular lymphoma. J Clin Oncol 2003; 21: 3940–3947.
151. Bibeau F, Lopez-Crapez E, Di Fiore F et al. Impact of Fc{gamma}RIIa-Fc{gamma} RIIIa polymorphisms and KRAS mutations on the clinical outcome of patients with metastatic colorectal cancer treated with cetuximab plus irinotecan. J Clin Oncol 2009; 27: 1122–1129.
152. Musolino A, Naldi N, Bortesi B et al. Immunoglobulin G fragment C receptor polymorphisms and clinical efficacy of trastuzumab-based therapy in patients with HER-2/neu-positive metastatic breast cancer. J Clin Oncol 2008; 26: 1789–1796.
153. Park S, Jiang Z, Mortenson ED et al. The therapeutic effect of anti-HER2/neu antibody depends on both innate and adaptive immunity. Cancer Cell 2010; 18: 160–170.
154. Stagg J, Loi S, Divisekera U et al. Anti-ErbB-2 mAb therapy requires type I and II interferons and synergizes with anti-PD-1 or anti-CD137 mAb therapy. Proc Natl Acad Sci USA 2011; 108: 7142–7147.
155. Kohrt HE, Houot R, Weiskopf K et al. Stimulation of natural killer cells with a CD137-specific antibody enhances trastuzumab efficacy in xenotransplant models of breast cancer. J Clin Invest 2012; 122: 1066–1075.
156. Disis ML, Wallace DR, Gooley TA et al. Concurrent trastuzumab and HER2/neu-specific vaccination in patients with metastatic breast cancer. J Clin Oncol 2009; 27: 4685–4692.
157. Benavides LC, Gates JD, Carmichael MG et al. The impact of HER2/neu expression level on response to the E75 vaccine: from U.S. Military Cancer Institute Clinical Trials Group Study I-01 and I-02. Clin Cancer Res 2009; 15: 2895–2904.
158. Norell H, Poschke I, Charo J et al. Vaccination with a plasmid DNA encoding HER-2/neu together with low doses of GM-CSF and IL-2 in patients with metastatic breast carcinoma: a pilot clinical trial. J Transl Med 2010; 8: 53.
159. Emens L, Gupta R, Petrik S et al. A feasibility study of combination therapy with trastuzumab (T), cyclophosphamide (CY), and an allogeneic GM-CSF-secreting breast tumor vaccine for the treatment of HER2+ metastatic breast cancer. ASCO Meeting Abstr 2011; 29(15 Suppl): 2535.
160. Disis ML, Dang Y, Bates N. Phase II study of a HER-2/Neu (HER2) intracellular domain (ICD) given concurrently with trastuzumab in patients with newly diagnosed advanced stage breast cancer. Cancer Res 2010; 69(24 Suppl): 5102.
161. Milani A, Sangiolo D, Montemurro F et al. Active immunotherapy in HER2 overexpressing breast cancer: current status and future perspectives. Ann Oncol 2013; 24: 1740–1748.
162. Curran MA, Montalvo W, Yagita H, Allison JP. PD-1 and CTLA-4 combination blockade expands infiltrating T cells and reduces regulatory T and myeloid cells within B16 melanoma tumors. Proc Natl Acad Sci USA 2010; 107: 4275–4280.
163. Woo SR, Turnis ME, Goldberg MV et al. Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape. Cancer Res 2012; 72: 917–927.
164. Lynch TJ, Bondarenko I, Luft A et al. Ipilimumab in combination with paclitaxel and carboplatin as first-line treatment in stage IIIB/IV non-small-cell lung cancer: results from a randomized, double-blind, multicenter phase II study. J Clin Oncol 2012; 30: 2046–2054.
165. Maio M, Di Giacomo AM, Robert C, Eggermont AM. Update on the role of ipilimumab in melanoma and first data on new combination therapies. Curr Opin Oncol 2013; 25: 166–172.
166. Brignone C, Gutierrez M, Mefti F et al. First-line chemoimmunotherapy in metastatic breast carcinoma: combination of paclitaxel and IMP321 (LAG-3Ig) enhances immune responses and antitumor activity. J Transl Med 2010; 8: 71.
167. Demaria S, Pilones KA, Formenti SC, Dustin ML. Exploiting the stress response to radiation to sensitize poorly immunogenic tumors to anti-CTLA-4 treatment. Oncoimmunology 2013; 2: e23127.