Immune Effects of Chemotherapy, Radiation, and Targeted Therapy and Opportunities for Combination With Immunotherapy

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Abstract

There have been significant advances in cancer treatment over the past several years through the use of chemotherapy, radiation therapy, molecularly targeted therapy, and immunotherapy. Despite these advances, treatments such as monotherapy or monomodality have significant limitations. There is increasing interest in using these strategies in combination; however, it is not completely clear how best to incorporate molecularly targeted and immune-targeted therapies into combination regimens. This is particularly pertinent when considering combinations with immunotherapy, as other types of therapy may have significant impact on host immunity, the tumor microenvironment, or both. Thus, the influence of chemotherapy, radiation therapy, and molecularly targeted therapy on the host anti-tumor immune response and the host anti-host response (ie, autoimmune toxicity) must be taken into consideration when designing immunotherapy-based combination regimens. We present data related to many of these combination approaches in the context of investigations in patients with melanoma and discuss their potential relationship to management of patients with other tumor types. Importantly, we also highlight challenges of these approaches and emphasize the need for continued translational research.

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ANTIGEN RECOGNITION AND T-CELL ACTIVATION

Antigen Presentation

Antigen presentation is a process allowing presentation at the cell surface of peptides reflecting the current state of the cell for recognition by the immune system. These peptides may be presented on major histocompatibility class I (MHC I) molecules by all nucleated cells to CD8^{+} T lymphocytes,[1] or by the MHC II molecules exclusively expressed by antigen-presenting cells (APCs) such as macrophages, B lymphocytes, and dendritic cells to CD4^{+} T lymphocytes.[1] Classically, MHC I molecules present antigens derived from the intra-cellular space, whether it be self-proteins in healthy cells, viral proteins in infected cells, or malignant and mutated proteins in cancer cells. On the other hand, MHC II molecules classically present peptides derived from the digestion of extracellular necrotic cells and cell debris and therefore paint a picture of the state of the immediate microenvironment rather than of the APC itself. Alternately, APCs are capable of cross-presentation, a process through which they may present exogenously derived antigens on MHC I molecules for recognition by CD8^{+} T lymphocytes[2] (Figure 1A).

The Immunological Synapse

The interface between APC and T cells is complex, requiring the proximity of multiple ligands to trigger proper T-cell activation. This interface is termed the immunological synapse,[3] and is comprised of the T-cell receptor (TCR), which binds to the MHC I or II molecule on APCs in unison with the CD8 (MHC I) or CD4 (MHC II) co-receptors, as well as the interaction between the T-cell–expressed CD28 molecule and its CD80/CD86 co-stimulation ligand on APCs.[4] It is crucial that both signal 1 (TCR–MHC interaction) and signal 2 (CD28–CD80/CD86 interaction)[5] be engaged for proper initial T-cell priming by APCs, although subsequent activation of T cells may occur in absence of signal 2.[6,7] Proper activation of T cells results in lysis of infected (or otherwise targeted) cells through production of cytotoxic proteases such as granzyme B and perforin at the immunological synapse,[8] as well as through interaction between Fas and Fas-ligand, which results in apoptosis of targeted cells[9] (Figure 1B).

T-Cell Signaling

T-cell signaling occurs upon formation of the immunological synapse, and binding of the TCR to the peptide-presenting MHC molecule expressed on the APC. Recruitment of the CD8 or CD4 co-receptor associated to the intracellular Lck kinase promotes CD3ζ phosphorylation at the TCR and subsequent ZAP-70 phosphorylation.[10] In turn, this results in recruitment of the linker for activation of T cells (LAT),[11] which promotes downstream signaling through the mitogen activated protein kinase (MAPK) pathway,[12] T-cell activation, and cytokine production. Furthermore, ligation of the CD28 co-stimulation molecule by CD80/ CD86 on APC results in phosphoinositide-3-kinase (PI3K) signaling and in subsequent survival and proliferation of T cells[13] (Figure 1C).

Contraction of T-Cell Responses

A prolonged T-cell response could have devastating consequences, causing damage to healthy cells and organs following pathogen clearance and eventually resulting in persistent auto-immunity. Accordingly, upon T-cell activation, the inflammatory environment generated by the immune response results in induction of expression of immunomodulatory proteins such as programmed cell death protein 1 (PD-1),[14] cytotoxic T-lymphocyte–associated protein-4 (CTLA-4),[15] lymphocyte activation gene-3 (LAG-3),[16] and T-cell immunoglobulin mucin-3 (Tim-3),[17] which cause inhibition of T-cell function and subsequent T-cell anergy and contraction upon ligation. Accordingly, the number of antigen-reactive T cells drastically decreases following pathogen clearance, thereby decreasing chances of damage due to an overdrawn immune response. Importantly, a certain limited number of antigen-specific T cells remain in circulation following contraction in order to ensure T-cell memory and future responses to the same infectious agent (Figure 1D).

RATIONALE FOR COMBINATION STRATEGIES WITH IMMUNOTHERAPY IN CANCER THERAPY

Though monotherapy regimens for cancer have yielded some success, there are significant limitations with regard to response rates and duration of therapy.[18] Based on these limitations and some provocative preclinical evidence for potential synergy of immunotherapy with other treatment modalities,[19] there is now tremendous enthusiasm for combination strategies in cancer therapy. However, rational design of these combination strategies requires a deep understanding of the effects of each therapy alone (and in combination) on host antitumor immunity.

Given the growing success of immunotherapy regimens across cancer types, there is significant interest in combining immunotherapeutic approaches with standard and novel agents to exploit potential synergy. The premise behind this is that several treatments may make a tumor more immunogenic, thus enhancing the effects of immunotherapy when these strategies are given in combination. Immunogenicity of tumors may be enhanced by increased antigen and MHC class I expression on tumors, but may also be enhanced by favorable changes to the tumor microenvironment (such as by increasing vascular permeability and immune infiltrate, or by positively modulating cytotoxic T lymphocytes [CTLs] within the tumor) (Figure 2). A number of therapies are currently being investigated in combination with immunotherapy including radiation therapy, chemotherapy, and a new wave of vaccines; although cytotoxic chemotherapy is the prototype.

CLINICAL EVIDENCE OF IMMUNE EFFECTS OF CHEMOTHERAPY

The concept of combining immunotherapeutic approaches with conventional chemotherapy is highlighted in the treatment of melanoma, where a number of different regimens have been tested. One of these regimens, termed "biochemotherapy", has shown promise in single-center studies. Specifically, the combination of cisplatin, vinblastine, and dacarbazine (CVD) was given with interleukin-2 (IL-2) and interferon (IFN)-α and was associated with response rates approaching 50%.[20–22] However, when randomized, multicenter studies were

performed, response rates were lower and outcomes, namely overall survival, were not superior to either combination or single-agent chemotherapy[23,24] Newer regimens, including nab-paclitaxel, are now being studied[25] (NCT00970996).

COMBINATIONS OF CHEMOTHERAPY WITH IMMUNE CHECKPOINT INHIBITORS

With the discovery of therapeutic immune checkpoint inhibitors, efforts to combine these agents with chemotherapy were pursued very early in their clinical development. At the same time, preclinical work continued to describe the effects of various cytotoxic agents on the immune system generally and on the tumor immune microenvironment. For example, chemotherapy with an agent such as gemcitabine, was associated with apoptosis that increased tumor antigen presentation and "cross-priming" of CTLs.[26] Additionally, gemcitabine was shown to selectively target and suppress humoral immune elements, but have very little to no effect on cytotoxic immunity, while cyclophosphamide efficiently led to the depletion of regulatory T cells.[27,28] These data would predict that chemotherapy may enhance the effects of anti-CTLA-4 antibodies, and this was, in fact, shown to be the case in vivo. Several groups have shown synergy with a variety of combination chemotherapy-immunotherapy regimens in preclinical models with multiple tumor types.[29–31]

There have also been a number of clinical trials combining ipilimumab (monoclonal antibody targeting CTLA-4) with chemotherapy. The largest was one of the two registration trials of ipilimumab comparing the combination of ipilimumab with dacarbazine versus dacarbazine alone in treatment-naïve patients with unresectable or metastatic melanoma.[32–34] While the response rates were similar in the patients receiving combination versus single-agent therapy (15% v 10%), the hazard ratios for overall survival (OS) were 0.72 (P<.001), and 0.76 (P<.001) for progression-free survival (PFS), both favoring ipilimumab plus dacarbazine.[34] Unfortunately, there were no data in this trial comparing the combination versus single-agent ipilimumab; though despite this, the results of this trial helped support the approval of single-agent ipilimumab for the treatment of patients with stage IV melanoma. Another large, randomized phase II study enrolled 204 chemotherapy-naïve patients with non-small cell lung cancer (NSCLC) to receive six cycles of carboplatin and paclitaxel (CT), four doses of ipilimumab plus six cycles of CT (commenced concurrently), or two cycles of CT followed by four cycles of CT plus ipilimumab (phased ipilimumab arm). The phased ipilimumab-containing arm had the highest immune-related PFS (irPFS) and immune-related best overall response rate (irBORR), although this antitumor activity did not appear to be associated with a significant OS benefit.[35] Additionally, a phase II study randomized 130 patients with chemotherapy-naïve extensive-stage disease small cell lung cancer (SCLC) to CT, concurrent ipilimumab plus CT, or phased ipilimumab.[36] As in the NSCLC study, treatment with phased ipilimumab was associated with improvement in irPFS. Several non-randomized studies have been performed with ipilimumab and chemotherapy and are summarized in Table 1.

Combination studies of anti–PD-1 inhibitors and chemotherapy have also recently commenced and early data are beginning to emerge. For example, a phase I dose–de-

escalation trial of nivolumab with multiple regimens (gemcitabine-cisplatin, pemetrexed-cisplatin, and CT) in chemotherapy-naïve patients with NSCLC was recently presented at the 2014 American Society of Clinical Oncology (ASCO) annual meeting and showed that response rates across four cohorts ranged from 33%–47% without unexpected toxicities.[37] A number of other trials are ongoing evaluating the combination of PD-1/PD-L1 inhibitors with chemotherapy.

TOXICITY OF COMBINED CHEMOTHERAPY AND IMMUNOTHERAPY

With the introduction of immune checkpoint inhibitors to the clinic, a new set of toxicities, specifically, immune-related adverse events (irAEs), have emerged. Side effects of these irAEs range from minimal to lethal and require a completely different management approach. Ipilimumab, in particular, is associated with grade 3–5 toxicity in 10%-45% of patients, depending on dose, whether maintenance therapy was allowed, the clinical setting (adjuvant V previously treated metastatic disease), and whether it was given as a single agent or in combination with other immune therapies, chemotherapies, or molecularly targeted therapies.[34,38]

As a single agent, the dominant toxicities are dermatologic (pruritus, rash, or Stevens Johnson Syndrome), gastrointestinal (ranging from mild diarrhea to frank and severe colitis associated with intestinal perforation), hepatic (typically elevated transaminases, very rarely hepatic failure), endocrine (hypophysitis, thyroiditis), neurologic (mononeuritis such as facial nerve palsy, Guillian-Barre syndrome), and renal (nephritis).[33,39,40] Additionally, in most clinical trials, fatal toxicity was seen in approximately 1%-2% and most often from inflammatory colitis with associated intestinal perforation.[38] However, one of the more remarkable findings from studies of CTLA-4 antibodies has been the change in toxicity pattern when combined with other agents compared to single-agent ipilimumab or tremelimumab.

The aforementioned phase III trial of dacarbazine with or without ipilimumab offers an interesting example of how the distribution of toxicities may be shifted when CTLA-4 inhibitors are combined with chemotherapy.[34] As a single agent, the rate of grade 3–5 ipilimumab-related gastrointestinal toxicity (diarrhea or colitis) is approximately 8%-23%,[33] but in this trial it was 4%.[34] In contrast, the rate of hepatic toxicity was much greater in this clinical trial with 20%-30% of patients experiencing a grade 3 or 4 elevation of their alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) (eg, hepatitis) with the combination, compared to the usual rate of grade 3/4 ipilimumab-related hepatitis of 3%-7%. A phase II trial of ipilimumab plus fotemustine corroborated the pattern that chemotherapy augments hepatic toxicity but attenuates colonic toxicity. Specifically, the most common irAE was hepatitis (~38%) with 14% being grade 3 or 4, while the rate of grade 3/4 diarrhea/colitis was only 5%.[41] Interestingly, the phase II trials of ipilimumab plus CT in patients with either NSCLC or SCLC showed different rates of hepatic toxicity in the combination arm when compared across trials, and similarly low rates of colitis in each arm of both trials. In the SCLC study, the rate of grade 3/4 hepatitis was 43% (18/42) in the combination arm, 18% (7/42) in the phased arm, and 0% in the chemotherapy alone arm.[36]

In the NSCLC study, the rate of grade 3/4 hepatitis was low across all arms (approximately 3% in each arm).[35]

CUTTING EDGE: TARGETED THERAPY EFFECTS ON IMMUNE MICROENVIRONMENT

Over the past 15–20 years, numerous oncogenic mutations have been described in cancer that contribute to their malignant potential through increased growth and invasiveness, resistance to apoptosis, and increased angiogenesis.[42] Treatment of cancers with pharmacologic agents targeting these mutations represents one of the most significant advances in cancer therapy in decades, and these forms of therapy may demonstrate high response rates but are often limited by a relatively short duration of response.[43] This is highlighted in melanoma, where pharmacologic inhibition of the BRAF oncogene (which is mutated in tumors from approximately half of patients with advanced melanoma) results in responses in the vast majority of patients.[44,45] However, responses to therapy are of limited duration, with most patients progressing on therapy within a year. Numerous molecular mechanisms of response and resistance to BRAF-targeted therapy have been identified,[39,46–56] and there is now growing evidence that there may be immune mechanisms of response and resistance[57–60] as well, providing a potential avenue to improve responses by combining molecularly targeted therapy with immunotherapeutic approaches.

In Vitro Evidence

In vitro studies have demonstrated that hyper-activation of the MAPK pathway in melanoma is associated with down-regulation of melanoma anti-gens,[61] which is likely due to transcriptional repression of microopthalmia-associated transcription factor (MITF). Additional in vitro studies showed that exposure of melanoma cell lines and fresh tumor digests to a BRAF inhibitor was associated with a significant increase in melanoma antigen expression (up to 100-fold).[62] Critically, this increase in antigen expression was associated with an enhanced recognition by antigen-specific T lymphocytes. Another key feature to these studies is that enhanced antigen expression and reactivity of T cells was also seen when BRAF–wild-type melanoma cells were treated with a MEK inhibitor, although culturing T lymphocytes in the presence of a MEK inhibitor completely abrogated the response and inhibited T-cell proliferation.[62] Conversely, selective BRAF inhibitors have no detrimental effect on T cell function. The findings of the deleterious effects of MEK inhibition on T-cell function have been corroborated in other studies,[63] and have important implications when considering combining MEK inhibitor–containing regimens with immunotherapeutic agents. Interestingly, selective BRAF inhibitors may actually potentiate T-cell function through paradoxical MAPK signaling.[64]

Evidence of an Immune Response in Patients on Targeted Therapy

Based on this early in vitro evidence, there was intense interest in studying the immune effects of BRAF inhibitors in early phase trials in patients with metastatic melanoma. To do this, investigators performed tumor biopsies pretreatment and shortly after initiating treatment with a BRAF inhibitor, and again at time of progression (when feasible). In these studies, treatment with a BRAF inhibitor was associated with a significant increase in

melanoma antigen expression in tumors within 2 weeks of starting therapy,[57] corroborating the in vitro findings. In addition, biopsies demonstrated a dramatic increase in CD8^{+} T-cell infiltrates within 10–14 days of initiating treatment with a BRAF inhibitor.[57,65] This increase in T-cell infiltrate was associated with a more favorable tumor microenvironment, a decrease in immunosuppressive cytokines and vascular endothelial growth factor (VEGF), and an increase in markers of cytotoxicity.[57–59] There was also a concurrent increase in the expression of immunomodulatory molecules (such as PD-1, Tim-3, and PD-L1)[57] in the tumor microenvironment, which was likely related to IFN-γ release from the infiltrating T cells.[66] Alternatively, this increase in immunomodulatory molecule expression may represent an immune-mediated mechanism of resistance to BRAF-targeted therapy.[67,68] Sorting out the nature and kinetics of the T-cell infiltrate that develops following BRAF inhibitor therapy is highly clinically relevant, and may provide the key to development of rational regimens for combining immune checkpoint inhibitors with molecularly targeted agents in the treatment of patients with advanced melanoma.

The mechanism through which BRAF inhibitors elicit an immune response is not completely clear, though this is an area of intense study. A key question is whether this represents an antigen-specific response versus a non-specific immune infiltration into a dying tumor mass. There is evidence suggesting an active, antigen-specific immune response in the setting of BRAF inhibition. Specifically, TCR sequencing studies have demonstrated that the T-cell infiltrate is polyclonal before treatment and more clonal during therapy,[69] suggesting a narrowing of the immune repertoire in response to BRAF-targeted therapy. An intriguing finding in these studies was that patients who had a good response to BRAF inhibitor therapy seemed to have a pre-existing set of dominant immune clones, which were not present in patients who had a poor response to therapy.[69] Although melanoma antigen expression is increased in tumors, the T-cell response is not likely to be solely directed against these shared melanoma antigens. Studies are underway to evaluate the ability of infiltrating T cells induced by molecularly targeted therapy to recognize tumor specific neoantigens.

Preclinical Models Exploring Synergy of Targeted Therapy and Immunotherapy

Initial studies exploring potential synergy of targeted therapy and immunotherapy were performed in murine models. Several different combination strategies were used in these studies, including combining BRAF-targeted therapy with either adoptive cellular therapy[59,70] or immune checkpoint blockade.[19] The majority of studies showed synergy with enhanced responses to therapy,[19,59,70,71] although one study did not support this interpretation.[72]

Through these murine studies, we have gained additional insights into the immune mechanisms of response to BRAF-targeted therapy. The immune infiltrate seems to be related to a decrease in VEGF, which is a direct consequence of inhibiting mutated BRAF and the MAPK pathway.[59] Perhaps the strongest evidence for the role of an immune response to BRAF-targeted therapy is an absolute requirement for CD8^{+} T cells to achieve a response. Two independent studies demonstrated that depletion of CD8^{+} T cells completely

abrogated the response to BRAF-targeted therapy,[19,71] suggesting that an immune infiltrate was not only present, but was required for an adequate response to therapy.

Interestingly, recent studies exploring synergy with targeted therapy and immune checkpoint blockade demonstrated that although there are infiltrating T cells in the setting of treatment with BRAF-targeted therapy, these T cells are not completely functional.[19] However, the addition of immune checkpoint blockade to a backbone of BRAF-targeted therapy is associated with a dramatic increase in infiltrating CD8$^+$ T cells which demonstrate an activated and cytolytic phenotype.[19] Together, these data provide provocative evidence for synergy of targeted therapy and immunotherapy, and these concepts are being investigated in ongoing clinical trials both in patients with melanoma and other tumor types.

Clinical Trials Exploring Synergy of Targeted Therapy and Immunotherapy

Based on the growing evidence for potential synergy of targeted therapy and immunotherapy in preclinical and murine studies, clinical trials are currently underway to evaluate these combination regimens. Early trials focused on combining BRAF-targeted therapy with cytokine-based therapy (NCT01754376, NCT01683188, NCT01603212); however, there are now several trials combining BRAF inhibitor-based targeted therapy with immune checkpoint blockade (NCT01767454, NCT01940 809, NCT01656642, NCT02130466, NCT02027961, NCT02224781) (Table 2). Though response data are not mature on these trials and it is unclear if synergy will be seen, important information has been gained already. Namely, we have learned that complexities exist in combining these strategies, as highlighted by a clinical trial combining vemurafenib (a BRAF inhibitor) with ipilimumab (a monoclonal antibody targeting the CTLA-4 molecule). In this trial, the first cohort of patients received full dose vemurafenib at 960 mg orally twice daily for one month as a single agent prior to administration of ipilimumab at 3 mg/kg intravenously. Dose-limiting toxicity (DLT) of grade 3 transaminase elevations was noted in four of six patients within 5 weeks after the first dose of ipilimumab.[73] A second cohort of patients was then enrolled and treated with a lower dose of vemurafenib (720 mg orally twice daily) with ipilimumab at 3 mg/ kg. Hepatotoxicity was again observed, and the trial was closed to accrual. Of note, all hepatic adverse events were asymptomatic and were reversible either with temporary discontinuation of both study drugs or with administration of corticosteroids.[73]

There is also an ongoing trial combining dabrafenib (a BRAF inhibitor) with or without a trametinib (a MEK inhibitor) in combination with ipilimumab for patients with BRAF-mutant melanoma (NCT01767454). Data regarding this trial were recently reported at the annual ASCO meeting in June 2014, with seven patients enrolled on dabrafenib + trametinib + ipilimumab and 12 patients enrolled on dabrafenib + ipilimumab. Of note, there was no DLT in the patients receiving dabrafenib + ipilimumab, but two cases of colitis with perforation were noted in the cohort treated with dabrafenib + trametinib + ipilimumab (leading to suspension of accrual in this cohort).[74] Trials sequencing these therapies are currently underway, as are additional studies combining different targeted therapy and immunotherapy regimens (Table 2). Taken together, these results highlight the potential for additive to synergistic anti-tumor effects and the complexity in combining these regimens.

Evidence of an Immune Response in Targeted Therapy for Other Cancers

Investigators are now exploring immune effects of targeted therapy for other cancer types. The hypothesis behind this stems from the fact that oncogenic mutations may affect anti-tumor immunity in other cancers as well. An example of this is in gastrointestinal stromal tumors (GIST), where up to 80% of tumors harbor a mutation in c-kit.[75] Oncogenic mutations in c-kit lead to signaling down the MAPK and PI3K pathways, with downstream effects similar to those mediated by BRAF mutations.[75]

The immune effects of targeted therapy for GIST have been studied by a group from Memorial Sloan Kettering Cancer Center, which demonstrated that successful treatment with imatinib (a c-kit inhibitor) in a murine model was dependent on the presence of CD8^{+} T cells.[76] The group also showed that the addition of immune checkpoint blockade to imatinib enhanced immune infiltrates in tumors as well as survival in the same model.[76] This concept is now being studied in clinical trials for patients with c-kit mutant tumors, where imatinib treatment is being combined with ipilimumab immunotherapy (NCT0 1738139). The effects of other agents are being studied in other histologies, and clinical trials are either in development or are underway.

Combinations of immune and angiogenesis targeting agents have also been studied for the better part of a decade. Initial studies were principally in patients with renal cell cancer where agents targeting vascular endothelial growth factor (VEGF), such as bevacizumab, and VEGF receptor tyrosine kinase inhibitors such as sorafenib, sunitinib, pazopanib, and axitinib, have shown sufficient activity to lead to FDA approval. In fact, the phase III trial that led to the approval of bevacizumab in renal cell carcinoma (RCC) randomized patients to interferon plus bevacizumab versus interferon alone as part of the Cancer and Leukemia Group B (CALGB) 90206 study. The trial demonstrated improved PFS and response rate with the combination.[77,78] However, a subsequent phase II trial of the combination of bevacizumab plus high-dose IL-2 in patients with RCC showed no apparent increase in response rate or durable response rate compared to historical data with single-agent IL-2.[79]

More recently, the combination of immune checkpoint inhibitors with VEGF pathway blockers has been evaluated primarily in patients with either melanoma or RCC with more compelling results. In melanoma, the combination of ipilimumab with bevacizumab in a dose-escalation phase I trial was associated with a 17% response rate, a 67% disease control rate, and a 25-month median OS.[80] Based on these results, a randomized phase II intergroup trial is now accruing patients (NCT01950390). In kidney cancer, the results from a phase I trial of nivolumab plus either sunitinib or pazopanib, were presented at the 2014 ASCO annual meeting and showed a response rate of approximately 50% in each arm (17/33 treated with nivolumab plus sunitinib, 9/20 treated with pazopanib plus nivolumab). However, similar to what had been observed with chemotherapy, the addition of VEGF receptor inhibitors to PD-1–based blockade was associated with enhanced hepatotoxicity. Nonetheless, building on this encouraging anti-tumor data, there are now several trials looking at anti–PD-1/PD-L1 treatment plus anti-VEGF therapy, (NCT02210117, NCT02133742, NCT01984242) and an interest in exploring more selective VEGF pathway inhibitors such as bevacizumab and axitinib in the hopes of reducing hepatotoxicity.

RADIATION THERAPY, THE IMMUNE SYSTEM, AND THE ABSCOPAL EFFECT

Combining immunotherapy with radiotherapy is another area of great interest. The foundation of this approach rests on the premise that localized radiotherapy will promote tumor antigen release, enhancing tumor-specific targeting by the adaptive immune system.[81] Although durable responses to radiotherapy are rare, most patients derive some measurable benefit from this treatment.

Immune Effects of Radiation Therapy

Radiotherapy effectiveness is thought to occur through numerous biological mechanisms. These include the creation of significant levels of clustered DNA damage, including complex double-strand breaks (DSB), which helps slow or kill tumor cells by limiting DNA damage repair.[82,83] Radiotherapy has long been viewed as immunosuppressive, in part due to lymphocyte sensitivity to direct radiation.[84–86] However, the radiation therapy-induced pro-inflammatory response, which includes the promotion of immunity through the release of damage associated molecular patterns (DAMPs) and toll-like receptors, more recently has been described and provides evidence to the contrary.[87–92] Additionally, sublethal irradiation of tumors may result in increased expression of MHC class I antigen or tumor-associated antigens in melanoma cells and gastric adenocarcinoma cells, respectively.[93–95]

Preclinical studies have also demonstrated the important role the immune system plays in treatment response to radiotherapy. Lee et al demonstrated that the efficacy of high-dose ablative radiotherapy was mediated by CD8+ T cells.[96] Additionally, Perez and colleagues suggested that ex vivo irradiated melanomas demonstrated an increase in tumoral CD8+ T-cell infiltrate and dendritic cell-mediated phagocytosis that was responsible for the decrease in metastatic disease observed in mice with irradiated tumors.[97]

Clinical Evidence of the Immune Effects of Radiation Therapy

Anecdotal reports of patients treated with radiotherapy support the existence of both local and systemic radiation-induced immune-modulatory effects. The term "abscopal effect" was coined in 1953[98] and refers to the ability of radiation therapy to produce effects at sites distant to the radiation field. In 1975, Kingsley et al published a case report of a 28 year old man with melanoma and diffuse nodal disease who received 14.4 Gy in 12 fractions to right inguinal involvement and subsequently experienced a complete and durable response in all nodal chains.[99] Similar effects have been reported in patients with melanoma,[40,99–102] NSCLC,[103] Merkel cell carcinoma,[104] hepatocellular carcinoma,[105] and RCC.[106,107] The abscopal effect can also be observed in mouse models. However, T-cell–deficient or CD8+ T-cell–depleted mice lack the abscopal effect, supporting the concept that radiation-induced distant effects may be mediated by immune cells and the two treatment approaches might work synergistically.[91,108,109]

In the treatment of melanoma, radiation-induced immune modulation has been described after depigmentation following irradiation within the target area as well as in non-irradiated areas and was associated with durable disease regression.[110,111] Immune analysis of the

peripheral blood, depigmented skin, and metastasis demonstrated the presence of specific CD8^{+} T and B cells that could respond to melanocyte-derived antigens (MDA–MART-1 or gp100).[110] Interestingly, depigmentation (aka, vitiligo) has been suggested to be a sign of effective radiotherapy and/or immunotherapy.[110,112,113]

Clinical Evidence of Synergy With Radiation Therapy and Immunotherapy

Immune checkpoint inhibitors such as anti–PD-1 and anti-CTLA4 have been successful in inducing effective and durable anti-tumor immunity. However, the response has been limited to a subset of patients.[38,114] Early clinical evidence suggests that responses to immune checkpoint blockade may be augmented through combination with radiation therapy. Postow and colleagues reported a case of a patient who was being treated with maintenance ipilimumab and exhibiting slow disease progression. The patient experienced regression of metastatic melanoma following the initiation of concurrent radiotherapy. Following three 9.5-Gy fractions to an area near the spine, an abscopal effect was observed in distant splenic lesions and lymph nodes leading to near complete regression of disease.[100] The authors also demonstrated a temporal association of antibody response to NY-ESO-1 antigen, peripheral blood immune cells and an increase in antibody responses to other antigens after radiation initiation.[101] This anecdotal evidence is corroborated by a case reported by Hiniker et al in which the authors treated a patient with melanoma with ipilimumab and concurrent radiotherapy (54 Gy in three fractions), leading to a complete regression in all metastatic lesions.[115] This enhanced response to radiation therapy may even be seen after patients exhibit disease progression on immune checkpoint blockade. Grimaldi et al demonstrated an abscopal response in 11 of the 21 patients with melanoma; nine had partial responses (43%) and two maintained stable disease (10%).[102]

Clinical Trials Combining Radiation Therapy and Immunotherapy

There is now ongoing interest in combining immune checkpoint blockade with radiotherapy in other cancers. In a phase I/II study in metastatic prostate cancer, 50 men were given 4- to 10-mg/kg doses of ipilimumab plus 8-Gy fractions to each metastatic lesion for 3 weeks.[116] This led to one complete response and six cases with disease stabilization. This approach is being tested in a randomized phase II trial (NCT01689974) in patients with prostate cancer and in other tumor types. Studies combining anti–PD-1 antibodies and radiotherapy are also underway, though it is too early to comment on the efficacy of this approach.

Cytokine therapy has also been tested in combination with radiation with mixed results.[117–119] Intralesional injections of 3–5 million units of IFN-β three times weekly preceding radiotherapy (5 days a week for a total of 40–60 Gy) demonstrated complete (70%) or partial remission in all 21 patients with metastatic melanoma and a median survival of 10 months.[120]

The combination of IL-2 and radiotherapy is also being explored. The earliest results from the National Cancer Institute demonstrated tolerability of 10–20 Gy of radiotherapy before IL-2 but no increase in clinical efficacy.[121] More recently, Seung and colleagues initiated a trial of stereotactic body radiation therapy (SBRT) followed by high-dose IL-2 in patients with metastatic melanoma or RCC.[122] In this phase I trial, patients received one, two, or

three doses of SBRT (20 Gy per fraction), with the last dose administered 3 days before starting the standard high-dose IL-2 regimen. Eight of the 12 enrolled patients had a Response Evaluation Criteria in Solid Tumors (RECIST)-defined anti-tumor response (one complete and seven partial responses) in non-irradiated target lesions. Blood-based immune monitoring showed an increase in CD4^{+} memory effector cells.[122] Further trials are underway in patients with either renal cell carcinoma or melanoma in which three daily doses of 6–12 Gy will be given concurrently with high-dose IL-2 with a primary objective of studying immunological effects.[123]

PUTTING IT TOGETHER: NOVEL COMBINATIONS WITH IMMUNE TARGETED THERAPY

Prior to 2010, there were no randomized, phase III trials in patients with melanoma showing an improvement of OS. Over the past 5 years there have been single-agent studies of immune checkpoint inhibitors (ipilimumab), BRAF inhibitors (vemurafenib, dabrafenib), MEK inhibitors (trametinib), chemotherapy (nab-paclitaxel), and vaccines (TVEC) showing improvements in overall survival versus a control arm. Over this same period, combination strategies have also shown remarkable benefit (OS, PFS) compared to single agents, approved agents, or contemporary controls including ipilimumab plus dacarbazine (DTIC) versus DTIC, high-dose IL-2 plus gp100 vaccine versus high-dose IL-2, ipilimumab plus nivolumab (compared to contemporary rates of 1- and 2-year OS), vemurafenib plus cobimetinib versus vemurafenib, dabrafenib plus trametinib versus dabrafenib (and versus single-agent vemurafenib), and ipilimumab plus TVEC (high response rate, limited toxicity). Needless to say, it has been a half-decade of dramatic advances that have transformed the treatment of melanoma patients and provided hope to a patient demographic that historically was afforded little. The major questions are no longer "How can we trigger immune responses in more patients?", "How do we sensitize patients to chemotherapy?", or "How can we effectively target melanoma?", but rather "How can we safely combine effective therapies?" and "How can we best sequence cytotoxic treatments (either via molecularly targeting or with traditional chemotherapy) with immune therapy?" These questions, while specifically being asked in reference to melanoma, are appropriate for almost every disease where immunotherapy is proving to be effective. With expected approvals of anti-PD-1 inhibitors in NSCLC and with emerging data in treating head and neck cancer, bladder cancer, kidney cancer, triple-negative breast cancer, and Hodgkin disease with PD-1/PD-L1 pathway inhibitors, the approach to sequencing standard cytotoxic or molecularly targeted therapies with immune therapies or the development of treatment regimens that incorporate combinations of immune therapies and standard therapies now being extensively explored in patients with melanoma will be applicable to these diseases as well.

The Case for Sequencing

An alternative to combining other treatment modalities with immunotherapy is the administration of the distinct modalities in sequence. Theoretically, this could be both simpler and less toxic in practice, this may not be the case. When evaluating the merits of sequencing two treatment modalities, it is imperative to take into consideration toxicity of a particular sequence in addition to determining its effectiveness. While predicting toxicity of

combination therapy is fraught with challenges, predicting the toxicity of a sequence of therapies is equally after high-dose IL-2 is associated with similar toxicity of ipilimumab not given after high-dose IL-2 and is as likely to be effective.[38,124] Conversely, high-dose IL-2 given after ipilimumab is associated with a markedly increased risk of intestinal perforation (3/22 patients) than either single-agent high-dose IL-2 or ipilimumab (8/1797 and 4/198, P =0.002 and .024, respectively).[125] This example highlights the concept that a previous treatment may prime an individual to have a more (or less) robust reaction (either efficacy or toxicity) to a subsequent therapy.

With a number of immune and molecular targeted therapies entering the clinic, it is important to determine if there is an ideal sequence for these two modalities. In melanoma, two distinct datasets showed that outcome appeared to be better if ipilimumab was given before single-agent BRAF inhibitor or combined BRAF-MEK inhibitor therapy as opposed to ipilimumab after BRAF-targeted therapy.[126,127] While selection bias may explain this difference in part (patients with rapidly progressing disease are more likely to be offered a BRAF inhibitor than ipilimumab and are more likely to have a worse outcome than patients with slower progressing disease), there are data suggesting that this phenomenon may be related to changes in the tumor-immune microenvironment. Namely, at the time of progression on BRAF inhibitors, the presumably immunologically favorable effects on the tumor microenvironment (increased antigen expression, infiltration of CD8$^+$ T cells, etc) are gone.[67] Another explanation for these data is that patients exhibiting disease progression on molecularly targeted therapy do not have sufficient time to respond to immune therapy.[128] Whether differences in sequencing will be seen with PD-1/PD-L1 inhibitors and BRAF-targeted therapy in BRAF mutant melanoma or with PD-1/PD-L1 inhibitors and chemotherapy or oncogene-targeted (eg, EGFR, ALK) therapy in NSCLC or VEGFR tyrosine kinase inhibitors therapy in patients with RCC requires further exploration.

The Case for Combinations

One of the key principles in oncology and microbiology is that therapeutic resistance more commonly occurs with single-agent therapy than with multi-drug regimens. In fact, the development of chemotherapy regimens with non-overlapping dose limiting toxicities for the treatment of childhood leukemia still stands as the most important development in oncology; serving as the exemplar in the chemotherapy age that led to the development of curative regimens in both adult and childhood acute leukemia, Hodgkin and non-Hodgkin lymphoma, and testicular cancer, as well as leading to adjuvant therapy regimens improving surgical cure rates in colon cancer, breast cancer, and lung cancer. It is conceivable that in the future development of immune-immune targeted, or immune-oncogene targeted, or immune-targeted chemotherapy regimens will become standards of care. The results from the first combined checkpoint inhibitor study, ipilimumab plus nivolumab, highlight both the promise and challenge of these types of studies.[129]

Concluding Thoughts

The approach to incorporating novel immunotherapy regimens into the treatment armamentarium for various cancers will likely be different across malignancies and even across subgroups of patients with a specific malignancy. Needless to say, there is work to be

done, and medical and radiation oncologists treating a wide spectrum of diseases will be carrying out trials to sort this out over the next several years and decades. What is clear is that efforts must be made to collect tissue and blood, both to determine which patients are most likely to benefit from specific treatment paths and to understand the effects of these therapies on tumor-immune microenvironment with the ultimate aim of gaining insight as to why they do or do not work. This is a large job that will require collaboration among investigators, surgeons, pathologists, bench researchers, pharmaceutical companies, and regulatory bodies to successfully carry it out. However, given the transformative potential of combined modality, immunotherapy-based treatment regimens, this is a task worthy or pursuit.

Acknowledgments

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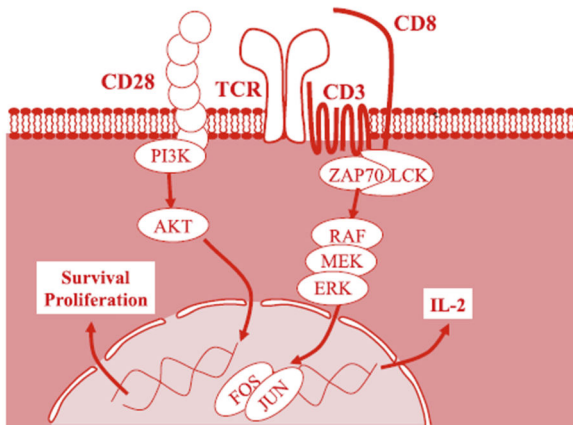
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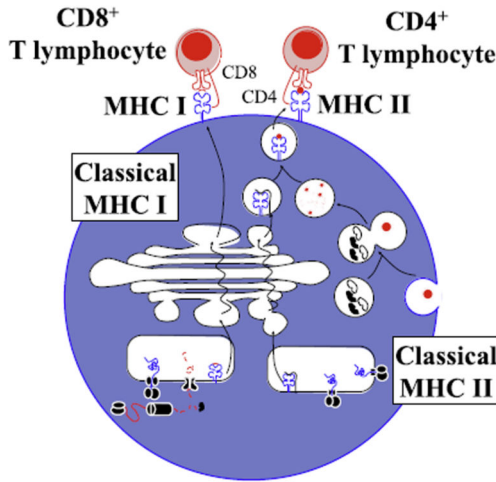
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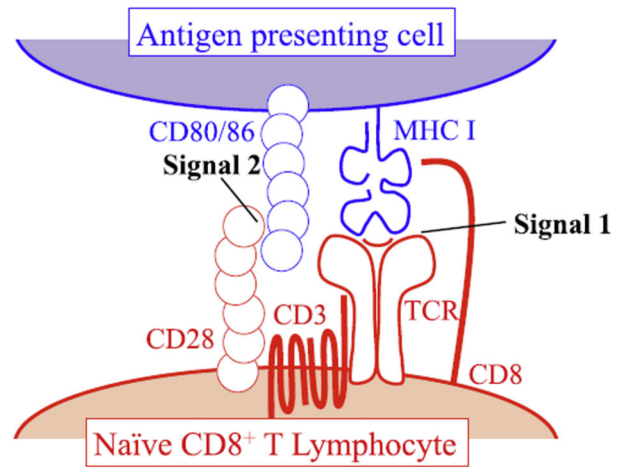
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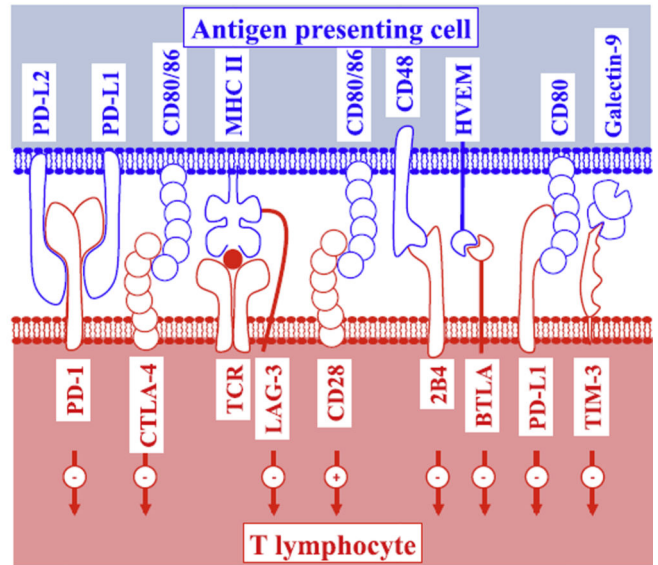
D) Immunomodulatory receptors

Figure 1.

T-cell activation is regulated by antigen presentation, as well as stimulatory and inhibitory co-receptors. (A) Overview of the MHC I and II antigen presentation and processing pathways. Canonical pathways of MHC I antigen presentation allow display of intracellular proteins following processing and transport to circulating CD8^{+} T lymphocytes. Canonical MHC II antigen presentation allows display of antigens derived from the extracellular environment to circulating CD4^{+} T lymphocytes. (B) Signals required for proper T-cell activation. Both CD4^{+} and CD8^{+} T cells are activated following interaction with signal 1 provided through presentation of MHC I or II/peptide complexes at the cell surface with the T-cell receptor (TCR), as well as signal 2 supplied by interaction of CD80/CD86 costimulation molecules expressed on antigen-presenting cells (APCs) with CD28 expressed on T cells. Only in presence of both signals will naive T cells be activated. Following

activation, T cells may recognize and kill cells presenting only signal 1 (MHC/peptide). (C) Signaling pathways engaged following interaction of T cells with signals 1 and 2. T cells stimulated through the TCR engage MAPK pathway signaling, which results in production of cytokines such as IL-2. Signal 2 targets PI3K/AKT signaling, through which T-cell survival and proliferation result. Both signals are crucial for initial T-cell activation. (D) Overview of the receptors and ligands expressed on T cells and APCs, which may influence T-cell activation upon interaction. Several of these receptors may be expressed on T cells following T-cell activation as a mechanism to inhibit T-cell activation and prevent autoimmune disease.
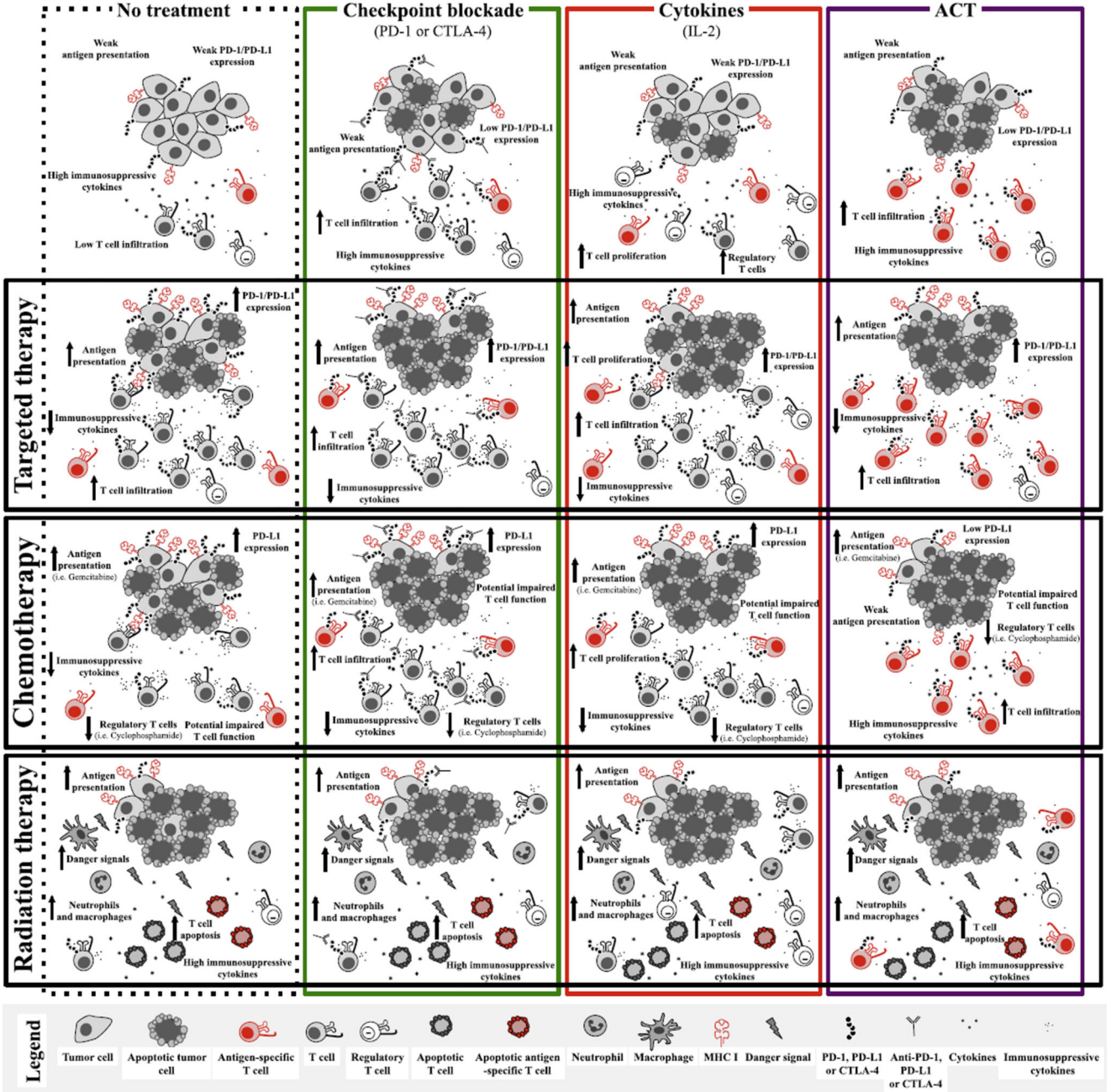
Figure 2.
Impact of immunotherapy, targeted chemotherapy, and radiation therapy on tumors and the immune system as monotherapy and in combination. The top row depicts known impacts of immunotherapies through targeting checkpoint inhibitors, cytokine treatment, or adoptive cell therapy on tumor mass, and immune cell function in comparison to without treatment (top left). The left column depicts the known effects of other forms of therapy, such as targeted therapy, chemotherapy, and radiation therapy, on tumor mass and immune cell function in comparison to without treatment (top left). Intersections between forms of

treatment represent the extrapolated effect on combining immunotherapies with other forms of therapy, based on known effects of regimens individually.

Table 1

Ongoing Clinical Trials of Combined Molecularly Targeted Therapy and Immunotherapy

Target Therapy and Checkpoint Blockade	Targeted Therapy and Cytokine Therapy	Targeted Therapy and Adoptive Cell Therapy
Dabrafenib +/− trametinib + ipilimumab (NCT01767454; NCT01940809)	Vemurafenib + high-dose IL-2 (NCT01754376; NCT 01683188)	Vemurafenib + tumor-infiltrating lymphocytes (NCT00338377; NCT01585415; NCT01659151)
Vemurafenib + anti–PD-L1 (MPDL3280) (NCT01656642)	Vemurafenib + IL-2 (infusional 96 hour) + IFN-α (NCT01603212)	
Dabrafenib + trametinib + anti–PD-1 (MK-3475) (NCT02130466)	Vemurafenib + pegylated IFN (NCT01959633)	
Trametinib +/− dabrafenib + anti–PD-L1 (MEDI4736) (NCT02027961)	Vemurafenib + high-dose IFN-α2b (NCT01943422)	
Dabrafenib + trametinib followed by ipilimumab + nivolumab or vice versa (NCT02224781)		
Anti-PD-L1 (MPDL3280A) +/− bevacizumab versus sunitinib in advanced RCC. (NCT01984242)		
Nivolumab plus sunitinib, pazopanib, or ipilimumab in subjects with metastatic RCC (NCT01472081)		
Pembrolizumab plus axitinib in advanced RCC (NCT02133742)		

Table 2

Results of Clinical Trials Evaluating the Combination of Cytotoxic Chemotherapy and Ipilimumab in Melanoma and Lung Cancer

Regimen	Disease	No. Enrolled	Response Rate (%)	Median PFS (mo)	Median OS (mo)	Reference
DTIC	Melanoma	502	10.3	~2.8	9.1	34
DTIC plus IPI			15.2	~2.8	11.2	
CT	Extensive stage, SCLC	130	53	5.3	9.9	36
CT plus IPI			47	5.7	9.1	
Phased IPI			71	6.4	12.9	
CT	NSCLC	204	14	4.2	8.3	35
CT plus IPI			21	4.1	9.7	
Phased IPI			32	5.1	12.2	
IPI	Melanoma	59	33	n/a	n/a	33
IPI plus DTIC			33			
IPI plus CT			28			
Fotemustine plus IPI	Melanoma	86	29	5.3	13.3	41
IPI	Melanoma	72	5.4		11.4	32
IPI plus DTIC			14.3		14.3	

Abbreviations: PFS, progression-free survival; OS, overall survival; DTIC, dacarbazine; IPI, ipilimumab; CT, carboplatin and paclitaxel; SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer.