

Emerging Opportunities and Challenges in Cancer Immunotherapy

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Abstract

Immunotherapy strategies against cancer are emerging as powerful weapons for treatment of this disease. The success of checkpoint inhibitors against metastatic melanoma and adoptive T-cell therapy with chimeric antigen receptor T cells against B-cell-derived leukemias and lymphomas are only two examples of developments that are changing the paradigms of clinical cancer management. These changes are a result of many years of intense research into complex and interrelated cellular and molecular mechanisms controlling immune responses. Promising advances come from the discovery of cancer mutation-encoded neoantigens, improvements in vaccine development, progress in delivery of cellular therapies, and impressive achievements in biotechnology. As a result, radical transformation of cancer treatment is taking place in which conventional cancer treatments are being integrated with immunotherapeutic agents. Many clinical trials are in progress testing potential synergistic effects of treatments combining immunotherapy with other therapies. Much remains to be learned about the selection, deliv-

ery, and off-target effects of immunotherapy used alone or in combination. The existence of numerous escape mechanisms from the host immune system that human tumors have evolved still is a barrier to success. Efforts to understand the rules of immune cell dysfunction and of cancer-associated local and systemic immune suppression are providing new insights and fuel the enthusiasm for new therapeutic strategies. In the future, it might be possible to tailor immune therapy for each cancer patient. The use of new immune biomarkers and the ability to assess responses to therapy by noninvasive monitoring promise to improve early cancer diagnosis and prognosis. Personalized immunotherapy based on individual genetic, molecular, and immune profiling is a potentially achievable future goal. The current excitement for immunotherapy is justified in view of many existing opportunities for harnessing the immune system to treat cancer. *Clin Cancer Res*; 22(8); 1845–55. ©2016 AACR.

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Introduction

CD8 T lymphocytes, natural killer cells, and certain CD4 T-helper lymphocytes are the only cell types in the organism that acquire the ability to kill sister cells as a mechanism of defense for eradicating or controlling intracellular pathogens. As immunotherapists, our efforts are focused on harnessing and redirecting these cell-killing mechanisms to destroy malignant tissues and thus improve therapeutic efficacy against cancer. In modern oncology, attempts to harness and direct the power of the immune system against cancer are best exemplified by therapeutic vaccines. These are formulations of tumor antigens that are expected to elicit immune responses able to arrest

cancer progression and prevent it from recurring. Vaccine development has required extensive preclinical and clinical research and has unraveled pro- and anticancer immune mechanisms, but has delivered very little to clinical practice (1). This has created skepticism toward cancer immunotherapy among clinical oncologists. In the past 20 years, two lines of research have dramatically changed this unfavorable view of immune therapies: (i) modulation of immune cells with immunostimulatory mAbs (2); and (ii) adoptive T-cell therapy (3).

The development of immunostimulatory mAbs (4) owes much to the pioneering work of James Allison (5), Lieping Chen (6), Tasuko Honjo (7), and Gordon Freeman (8), who discovered the critical role of surface receptor–ligand pairs, now known as checkpoint inhibitors, in downregulating T-cell immunity. Checkpoint inhibition could be interfered with by mAbs able to restore T-cell activation and enable T cells to control cancer progression. This line of research has resulted in unprecedented objective clinical efficacy against cancer starting with CTLA-4 blockade in metastatic melanoma (9, 10) and with PD-1/PD-L1 blockade in non-small cell lung cancer (NSCLC), extending to a growing list of other malignancies, including renal cell carcinoma (11), bladder cancer (12), refractory Hodgkin lymphoma (13), head and neck cancer (14), ovarian cancer (15), MSI colon cancer (16), etc. Table 1 lists recent FDA approvals for clinical use of agents blocking immune checkpoints.

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The other strategy that has improved efficacy of cancer immunotherapy is adoptive transfer of T cells. This field was pioneered by Steven Rosenberg, whose team developed methods for isolation and culture of tumor-infiltrating lymphocytes (TIL) which can be reinfused together with exogenous IL2 to patients rendered lymphopenic by preconditioning regimens (17). Durable response rates of TIL-based adoptive therapies are remarkable and are being replicated in cancer centers worldwide (18). Adoptive T-cell therapy has benefited from Zelig Eshhar's seminal work (19). By engineering T cells with transmembrane receptors encompassing extracellular single-chain Abs and intracellular signaling domains, impressive efficacy has been attained in clinical trials against B-cell-derived malignancies (20). The most successful chimeric receptors pioneered by Carl June and Michel Sadelain include anti-CD19 mAb and the intracellular signaling domains of CD3 ζ plus either CD137 or CD28. Results in pediatric acute lymphocytic leukemia, chronic lymphocytic leukemia, non-Hodgkin lymphoma, and myeloma (21–24) have introduced well-justified optimism for broader applicability of this therapy to hematologic and solid malignancies (20).

Many other recent developments in immunotherapy have contributed to making it "popular" among oncologists and patients. The most promising developments are discussed in this *CCR Focus* and include the following: (i) characterization of nonsynonymous mutations in cancer giving rise to neoantigens (25); (ii) discovery of new checkpoints and other targetable immunosuppressive mechanisms (26); (iii) progress in the field of T-cell trafficking to tumors (27); (iv) an enlarged repertoire of immunologic biomarkers for monitoring responses to therapy and understanding the underlying biology (28); (v) potentiation by immunotherapy of abscopal effects of radiotherapy (see below); and (vi) reinvigoration of therapeutic cancer vaccines by improving tumor antigen presentation and cross-priming (29).

A potential barrier to wide application of immunotherapy has been a concern about toxicities. The concern is legitimate, as most immunotherapies, whether with cells, antibodies, or cytokines, are associated with adverse events. These can be readily managed. However, in cancer, one additional concern is critical, and this is a possibility of accelerated tumor growth as a result of immune therapy. Therapeutic disturbance of the relationship between the tumor and immune system could result in tumor growth, e.g., if reactivated immune cells produce an excess of factors that will favor proliferation of residual tumor cells or cancer stem cells. For this reason, combinatorial therapies designed to first eliminate these cells and then rejuvenate antitumor immunity are under development. More important, the immune system is calibrated to prevent excessive activation that could damage tissues. Hence, regulatory T cells (Treg) and myeloid-derived suppressor cells (MDSC) and other regulatory cells play a key role in maintaining the balance. Its disturbance by reactivating T cells with, e.g., checkpoint inhibitors, is likely to call on regulatory cells to dampen this activation. This is a "rebound effect" that naturally occurs after T-cell activation and leads to expansion of regulatory elements in the immune system. When initiated and/or maintained by therapeutic T-cell activation, this effect could result in temporary or permanent suppression of antitumor activity by endogenous immune regulation. Thus, dis-

turbing the immune balance with the intention of restoring potent antitumor responses might induce resistance to further activation. This and other aspects of interference with the physiology of the immune system by immunotherapies may be one of the major challenges that the field will have to overcome.

Although the use of antibodies in cancer has a relatively long history, and clinicians have learned how to deal with related toxicities, therapies with immune cells are much less familiar to oncologists. The widely prevalent perception that cellular therapies for cancer, e.g., with TILs or chimeric antigen receptor T cells (CART), are difficult to manage and costly has limited the production of cells for therapy and their use to few specialized centers. This perception is persisting despite the fact that technological advances in the production, transport, and delivery to patients of therapeutic cells have made this therapy more affordable, safe, and more widely available. Expectations are that this barrier will disappear, as oncologists become more familiar with cellular therapies and their use.

Current enthusiasm for immunotherapy is justified because overwhelming evidence indicates that it is effective, albeit not in all cases, where conventional therapies were not. Nevertheless, many challenges still exist and will have to be overcome to make it universally available to those patients with cancer who need immune intervention in addition to other therapies.

Immunotherapy Combinations: The Land of Opportunity

Immunotherapeutic synergy defined as a therapeutic effect superior to the additive effect of each of the components in a combination is generally perceived as the most potent engine for progress (30, 31). The first immunotherapy combination that has received FDA approval for metastatic melanoma has been the double CTLA-4 and PD-1 blockade (refs. 32–34; Table 1).

Building on successes of the PD-1/PD-L1 blockade, numerous clinical trials of immunotherapy combinations are in progress (162 entries in ClinicalTrials.gov and more under preparation). Combinations include various immunotherapy agents as well as combinations of immunotherapy agents with standard-of-care treatments (30, 31). It would be very surprising if these combinations do not deliver success. However, in some instances, combinations might give positive results at the expense of safety concerns (32–34) and thus become nontolerable. One promising approach undergoing clinical trials is the combination of costimulatory agents and checkpoint inhibitors. As indicated in Fig. 1, immunomodulation relies on the presence of an ongoing baseline immune response to cancer neoantigens (25) and our abilities to remove the brakes as well as press gas pedals driving this response (35).

Concomitant and sequential use of the palettes of new treatments in various combinations is likely to lead to much needed synergistic efficacy. For instance, recently disclosed results from the combination of an idoleamine-2,3 dioxygenase (IDO) inhibitor and PD-1 blockade with excellent safety and efficacy profiles in a phase I/II trial further justify optimism for this and similar therapeutic strategies (36).

Table 1. Chronologic FDA approvals of novel immunotherapies

| Agent | Target/class | Indication | Evidence | FDA approval date | Pivotal trial references |
|------------------------------------|---|--|----------|--------------------|--------------------------|
| Ipilimumab | CTLA-4 fully human mAb | Metastatic melanoma | OS | March 25, 2011 | (9, 10) |
| Pembrolizumab | PD-1 humanized mAb | Metastatic melanoma | OS | September 4, 2014 | (99, 100) |
| Nivolumab | PD-1 fully human mAb | Metastatic melanoma | OS | December 22, 2014 | (101, 102) |
| Nivolumab | PD-1 fully human mAb | Stage IV NSCLC (squamous), second line | OS | March 24, 2015 | (103) |
| Ipilimumab+nivolumab (combination) | CTLA-4 and PD-1 fully human mAbs | Metastatic melanoma | PFS | September 30, 2015 | (32-34) |
| Pembrolizumab | PD-1 humanized mAb | Stage IV NSCLC, PD-L1 ⁺ <50% | OS | October 2, 2015 | (104) |
| Nivolumab | PD-1 fully human mAb | Stage IV NSCLC (adenocarcinoma), second line | OS | October 9, 2015 | (105) |
| Tamoligene laherparepvec (T-vec) | HSV-1 replicative viral vector engineered to express GM-CSF | Locally advanced or metastatic melanoma accessible for intratumoral delivery | OS | October 27, 2015 | (97) |
| Ipilimumab | CTLA-4 fully human mAb | Surgically resectable melanoma | OS | October 28, 2015 | (106) |
| Nivolumab | PD-1 fully human mAb | Renal cell carcinoma, second line | OS | November 23, 2015 | (11) |

Abbreviations: OS, overall survival; PFS, progression-free survival.

Interestingly, the aforementioned brightest stars in immunotherapy (immunomodulatory mAbs and adoptive T-cell therapy) are clearly synergistic in animal models (37).

Understanding Immunosuppression in the Tumor Microenvironment

The tumor microenvironment (TME) consisting of tumor cells, stroma, vascular elements, and tumor-draining lymph nodes is a milieu in which multiple and complex cellular interactions take place that shape antitumor immune responses and determine eventual efficacy of immunotherapy. The immunosuppressive nature of the TME is well known (38, 39), and the realization that each tumor creates its own, unique TME and orchestrates interactions between various cells present in the TME is likely to individualize our strategies for cancer immunotherapy. Immune cells infiltrating the TME are instructed to preferentially adopt the functional phenotypes and activities that support tumor progression. The instructive signals are delivered by the tumor in the form of soluble factors (cytokines, chemokines, inhibitory factors) or exosomes (virus-size vesicles) which alter the behavior of local or distant immune and tissue cells and/or invite the entry of regulatory immune cells into the tumor milieu. As Table 2 summarizes, many immunosuppressive factors and cells lurk in the TME; however, not all are present in all tumors. For example, some human tumors express COX-2 and secrete PGE₂, others produce adenosine or express IDO, and still others are avid TGFβ or IL10 producers (40). The immunosuppressive profile appears to be related to tumor aggressiveness and determines the presence and degree of T-cell activation or exhaustion/dysfunction prevailing in the TME (26, 40).

Much has been learned recently about Treg and MDSC accumulating in the TME (41–43). Emerging data suggest that these regulatory cells are unlike ordinary "garden variety" T cells or monocytes/macrophages that reside in tissues or blood of normal donors. MDSC in the hypoxic TME are programmed to produce an excess of inducible nitric oxide synthase, ROS, arginase-1, TGFβ, and PGE₂, the factors known

to interfere with differentiation of dendritic cells (DC), effector functions of T cells, and to alter the tumor stroma. A high burden of MDSC in the chronically inflamed TME favors tumor progression (43). Therefore, strategies to eliminate MDSC or block their functions are being actively translated into the clinic, including pharmacologic interference with the major suppressive pathways, e.g., by inhibition of the IDO and tryptophan pathway with indoximod or regulation of the myelopoiesis, e.g., by the administration of all-*trans*-retinoic acid (ATRA) alone or together with IL2 to promote differentiation of myeloid cells. Alternatively, prevention of myeloid cells trafficking to tumors by direct targeting chemokines (including CCL2, CCL3, CCL4, and CCL5) or blocking their production by the tumor can be pursued. Other approaches involve reduction in the frequency or blocking functions of MDSC, e.g., by utilizing chemotherapies, which when delivered at lower doses deplete MDSC and induce antitumor immunity. Not surprisingly, MDSC accumulation in tumors appears to interfere with anti-PD-1 immunotherapy, and targeting of CXCR2⁺ MDSC with antibodies was reported to improve the efficiency of the checkpoint blockade (44). Other approaches already in clinical development involve targeting the CSF1-R (45). Neutralization of MDSC as an adjunct strategy to other immunotherapies is a significant component of the novel antitumor therapeutics.

Treg present in the TME are highly suppressive and, in contrast to other TIL are not dysfunctional. Intratumoral CD4⁺CD25^{hi}CD39⁺FOXP3⁺ Treg upregulate immunosuppressive molecules (e.g., CD39 or TGFβ-associated molecules, LAP, and GARP) and inhibitory receptors (46). Treg isolated from patients' peripheral blood or tumor tissues coexpressed several inhibitory receptors, and their suppressive activity within TILs, far exceeded that of Treg in the periphery (47). As these Treg had high expression levels of PD-1, it was expected that strong negative signaling via this receptor would inhibit Treg functions. However, early studies in mice showed that PD-L1 signaling via PD-1 promoted Treg cell development and functions, synergized with TGFβ to enhance conventional T-cell conversion to iTreg, maintained FOXP3

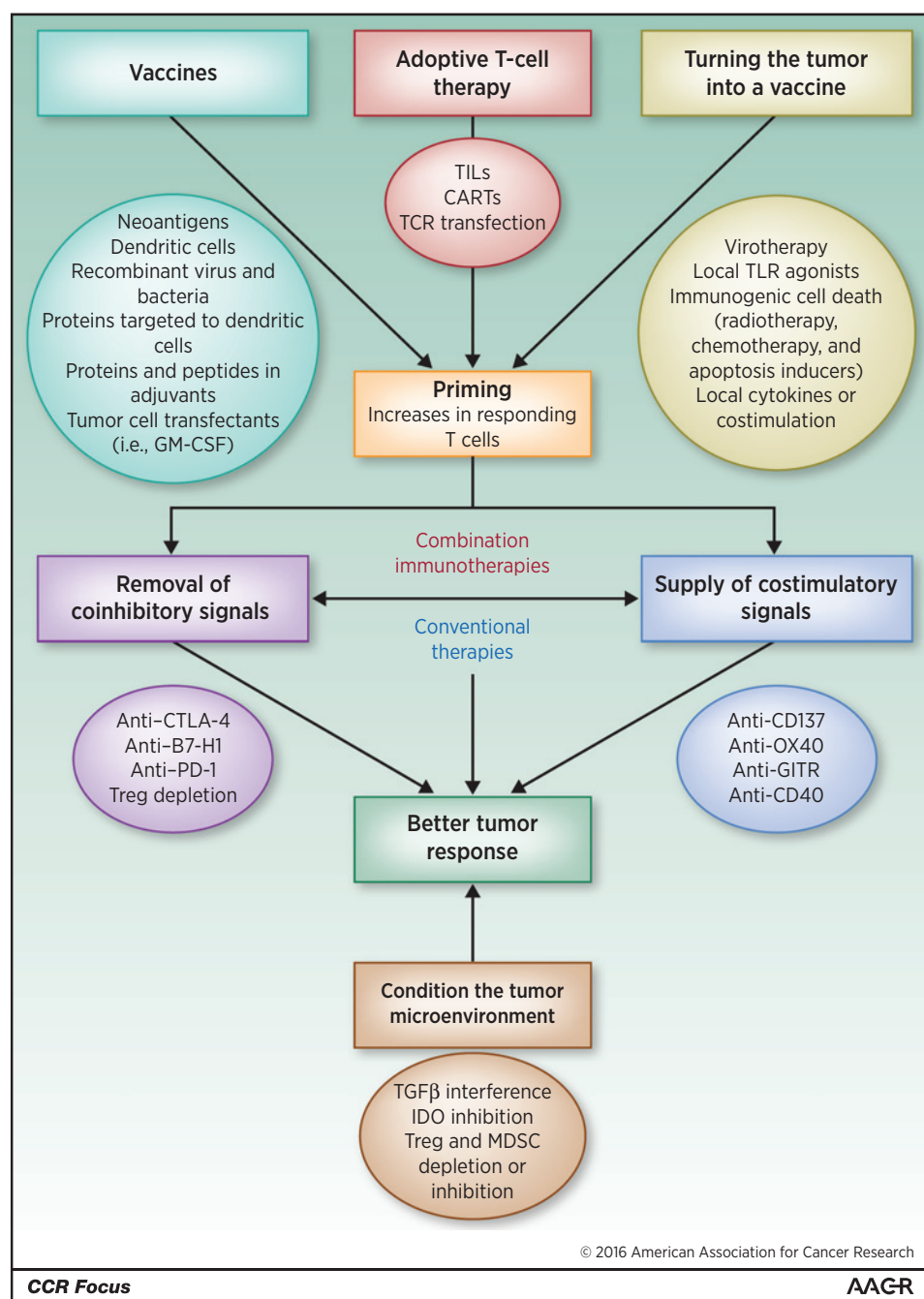


Figure 1. A conceptual palette of immune interventions designed to mix potentially effective combined immunotherapies. For immunomodulatory interventions to be effective, a baseline immune response must be available. Such antitumor responses can be built up by means of vaccines, adoptive cell transfers, or by enhancing tumor tissue immunogenicity using one or more of the listed strategies. Manipulation of the tumor microenvironment appears to be most important to achieve this goal. Adapted from Melero and colleagues (35).

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expression, and increased Treg survival. It appears that PD-1, and perhaps other checkpoint receptors, functions not as inhibitory but as stimulatory receptors in Treg (48). These data suggest that in Treg, PD-1 is programmed to function differently than in conventional T cells. Thus, anti-PD-1 antibodies, which release the break in conventional T cells restoring their functions, would be expected to block Treg-mediated suppression and further enhance antitumor responses benefiting the host. However, there is a concern that in Treg, which overexpress PD-1 in the TME, PDL-1

signaling upregulates PTEN expression, blocks the Akt/mTOR pathway, and activates STAT5/STAT3 signaling (49), leading to expansion of Treg and promoting their suppressive functions. This scenario, based on unique molecular signaling in Treg, implies that anti-PD-1 antibody therapies could have unexpected effects on Treg. Already evidence emerges that ipilimumab targeting CTLA-4 is not completely effective in eliminating Treg by antibody-dependent cellular cytotoxicity (T.L. Whiteside; unpublished data) as suggested in mouse models (50). Depending on conditions prevailing in the TME, the

Table 2. Immunosuppressive factors and cells that contribute to T-cell dysfunction in the TME and immune therapies for restoration of antitumor immune competence

| Factor/cell/cell product | Effects in T cells | Potential immune therapy | References |
|---|--|---|----------------------|
| Inhibitory receptor ligands | T-cell exhaustion via IR signaling plus the TCR-mediated chronic stimulation with TAs | Checkpoint inhibition: partial or complete restoration of T-cell functions translating into clinical responses | (32, 99, 107, 108) |
| Soluble factors: IL10 TGFβ IL35 IDO Galectin-9 Arginase COX-2/PGE ₂ Adenosine Oxygen radicals | Alone or in cooperation with PD-1 inhibits functions of TA-specific CD8 ⁺ T cells by utilizing various relevant molecular pathways (cited in the last column) | Neutralizing Abs; Abs targeting/blocking receptors, pharmacologic inhibitors; selective drug blockade | (109-116) |
| Regulatory cells: iTreg MDSC | Downregulation of effector T-cell functions by contact-dependent or contact-independent delivery of inhibitory proteins, killing-inducing mediators or oxygen radicals | Treg or MDSC depletion or inhibition of their suppressor activities with blocking antibodies, immune checkpoint inhibitors, or pharmacologic agents | (40-42, 44, 115-130) |
| Tumor-derived immunoinhibitory exosomes | Negative signals inhibit T _{eff} functions but promote regulatory cell expansion; inhibitory miRNA transfer | Removal of exosomes (plasmapheresis); blockade of signaling or inhibition of exosome release | (131) |
| MHC class I downregulation/loss; β2-microglobulin inactivation on tumor cells | Interferes with Ag presentation by silencing Ag presenting machinery by tumors and with tumor recognition by T cells | Upregulation of MHC-I expression by IFNs or other immune therapies | (132, 133) |
| Metabolic checkpoints, e.g., glucose deprivation | Limits aerobic glycolysis in TILs; decreases the mTOR pathway activity and the ability to produce IFNγ | Upregulation of metabolites regulating aerobic glycolysis in the TME | (134, 135) |

NOTE: The table lists the best-known inhibitors of T-cell functions in the TME. The list is not comprehensive, as additional blocking factors may be present. Each tumor develops its own unique immunosuppressive signature, and the degree of T-cell dysfunction in the TME varies broadly from one tumor to another depending on the prevailing signature.

Abbreviations: PGE₂, prostaglandin E₂; TA, tumor antigen; TCR, T-cell receptor.

surviving Treg might expand and interfere with benefits of checkpoint inhibitors. Despite many approaches used in the clinic for Treg depletion (reviewed in refs.51, 52), their persistence and resistance to chemotherapies (53) have been a problem. In addition, considerable functional heterogeneity of these cells and their essential role in preventing autoimmunity compel us to think of how to deplete or muzzle "bad" iTreg operating in the TME without sacrificing "good" natural Treg necessary for maintaining homeostasis and keep autoimmunity at bay. Successful management of cancer-associated iTreg remains one of the challenges of cancer immunotherapies today.

Reversal of T-cell Dysfunction at the TME and Checkpoint Inhibitors

There is ample evidence in experimental models and in humans that CD8⁺ T cells become exhausted/dysfunctional upon chronic antigen exposure in the TME. These dysfunctional/exhausted T cells exhibit defective proliferative capacities and cytokine production (54). However, they are not totally inert and appear capable of exerting lytic functions (26). Dysfunctional CD8⁺ T cells upregulate a number of inhibitory receptors (IR)/immune checkpoints (55) that bind to their ligands expressed by tumor cells and antigen-presenting cells (56) in the TME, including PD-1, CTLA-4, Tim-3 (57), LAG-3, BTLA (58), and TIGIT (59). Hence, dual immune checkpoint blockade appears to better enhance T-cell expansion and func-

tions and promotes tumor rejection *in vitro* and *in vivo*. The recent success of dual CTLA-4/PD-1 blockade, which has been approved by the FDA (Table 1), in advanced melanoma underlines the clinical efficacy of such strategy.

Although CD8⁺ TILs in the TME appear to upregulate IRs, they also upregulate a number of activating receptors (AR) like 4-1BB, OX40, and GITR (60). These are members of the TNFR family that can readily costimulate T-cell functions upon ligation. Agonistic mAbs show promising therapeutic effects against cancer mouse models and are under development in clinical trials in mouse models (61-63). At least in preclinical models these agonist agents are strongly synergistic with checkpoint inhibitors (30, 31).

One important question is to determine among cancer patients who is more likely to respond to immunotherapies targeting immunoregulatory pathways and when additional strategies may be needed to induce T-cell responses to tumors. The answer to this question may come from the gene signature studies of metastatic melanoma, which propose to classify tumors into "inflamed" and "noninflamed" phenotypes (28). Although inflamed tumors are spontaneously immunogenic and may be more likely to respond to immune interventions for counteracting the mechanisms of tumor-induced T-cell dysfunction, noninflamed tumors lack tumor-infiltrating T cells and may likely need to be treated with novel targeted therapies (sting agonists, inhibitors of β-catenin pathway) to induce T-cell activation and migration into the tumors (64-66).

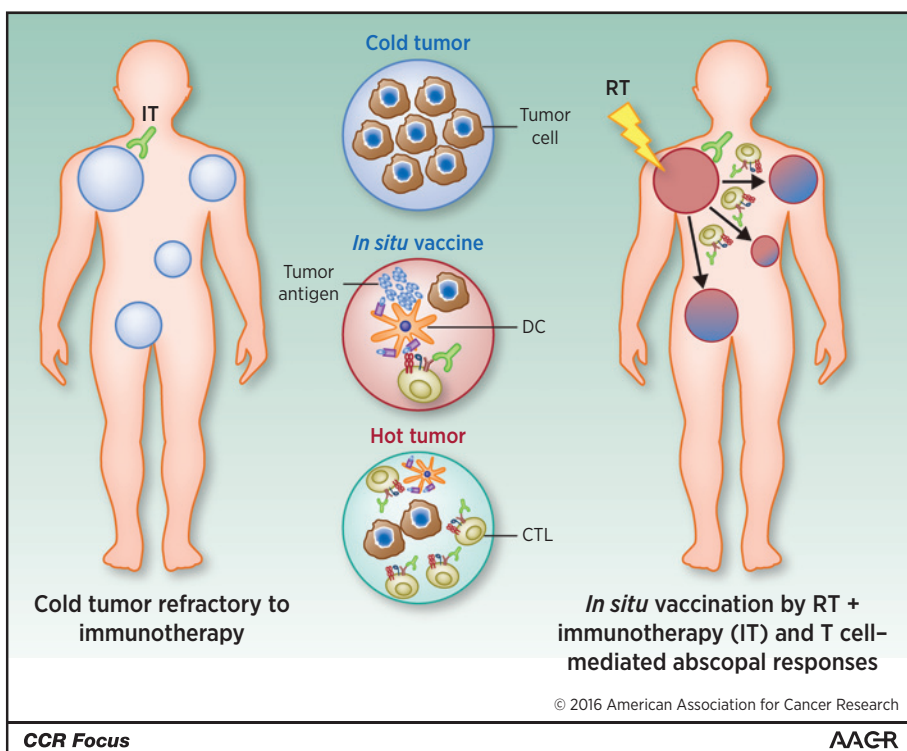


Figure 2. Concept of immune-mediated abscopal effects. Schematic representation of immune-mediated effects. The scheme describes the systemic proinflammatory effects of gamma irradiation of the irradiated tumor lesion well that becomes hot and acts as an *in situ* tumor-attenuated vaccine against distant nonirradiated tumors. Such local response can be enhanced by immunostimulatory mAbs to attain a systemic effect. Exploiting the systemic immune-mediated effects of radiotherapy offers opportunity to maximize the effect of novel immunotherapies. CTL, cytotoxic T cell; RT, radiotherapy.

Radiotherapy and Immune-Mediated Abscopal Effects

The above-mentioned successes of immune checkpoint inhibitors have clearly demonstrated that treating the host immune system in addition to killing the neoplastic cells can be very effective at achieving long-term tumor control. However, responses are limited to patients with some degree of preexisting tumor-reactive T cells infiltrating the tumor. In this context, ionizing radiotherapy, a local cancer treatment used for almost a century to kill cancer cells is finding a new role. The convergence of technological progress in the precise delivery of radiotherapy with improved understanding of the inflammatory signals associated with various cell death pathways triggered by radiation (67, 68) has enabled a conceptual transformation whereby RT is considered a promising partner for immunotherapy due to its ability to induce a cell death that is immunogenic potentially converting the tumor into an *in situ* vaccine (69–71).

The ability of radiotherapy to enlist the help of the immune system against the tumor has important implications not only for improved local control of the irradiated tumor (72, 73), but most importantly for systemic tumor control (ref.74; Fig. 2). The regression of metastases outside the field of radiation after irradiation of one tumor site is known as "abscopal effect." It is a rare but well-documented phenomenon that has been reported more frequently in patients with more immunogenic tumor types (75). Sensing of tumor-derived DNA by tumor-infiltrating DCs activates type I IFN production via the stimulator of IFN genes (STING) pathway, a mechanism critical for generation of spontaneous antitumor T-cell responses to immunogenic tumors (76). Importantly, recent data show that

the same pathway is amplified by radiotherapy (77), providing a possible explanation for the occurrence of abscopal effects. However, the ability of radiotherapy to induce T-cell responses in less immunogenic tumors is limited by immunosuppressive networks operating in the TME. This explains why abscopal effects are very rare. For example, TGFβ is a critical barrier to radiotherapy-induced priming of T-cell responses to multiple endogenous tumor antigens, exacerbated by the conversion of TGFβ from its latent to active form by radiotherapy-generated ROS (78). Other barriers include Treg and MDSC (79, 80). Preclinical studies have demonstrated that multiple immunotherapies that either block immunosuppressive mechanisms or improve immune activation can work in concert with radiotherapy to generate an *in situ* tumor vaccine and induce abscopal effects (81).

Importantly, these preclinical data are beginning to show clinical relevance. Combination of radiotherapy with cytokines that enhance DC numbers and function or TLR agonists that improve immune activation within the irradiated tumor induced abscopal responses in close to 30% of the patients in early clinical trials (82, 83). In another phase I study, a markedly improved response rate to high dose IL2 was seen in melanoma and renal cell carcinoma patients treated with radiotherapy (84). Several trials are ongoing to test radiotherapy in combination with various immunotherapy agents, including OX40 agonist and TGFβ-neutralizing antibodies (85).

Perhaps the most exciting hypothesis being tested in the clinic is that radiotherapy can "raise the roof" of responders to immune checkpoint inhibitors. Extensive preclinical evidence and a growing number of clinical reports in melanoma patients unresponsive to anti-CTLA-4 support this hypothesis

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(86–88). Importantly, a striking synergy of radiotherapy with anti-CTLA-4 has also been seen in a patient with NSCLC, a tumor type in which anti-CTLA-4 alone has no activity (89, 90), raising hope that radiotherapy could be used to extend the benefits of this treatment to multiple tumor types. Recent results of a prospective clinical trial support the synergy of radiotherapy with anti-CTLA-4 in NSCLC (91). However, in another large study in metastatic castrate-resistant prostate cancer, the addition of anti-CTLA-4 to radiotherapy failed to improve responses (92). Although reasons for this difference are unclear, the radiotherapy dose and fractionation used (93), the tumor type or the site chosen for irradiation may all play a role in determining the responses, and need to be further investigated. Several trials testing the synergy of PD-1/PD-L1 targeting agents with radiotherapy are ongoing and will provide important results.

Overall, radiotherapy has a strong appeal as a commonly available, cost-effective treatment to generate T cells specific for neoantigens expressed by each individual patient's tumor (94). Research is ongoing to define the antigenic targets of T-cell responses at the irradiated and abscopal tumor sites, the optimal radiotherapy doses and fractionation, and the optimal partnerships with immunotherapy.

The Road Ahead of Us and Our Patients

In the cancer immunotherapy community, the overall state of mind is optimistic. Much knowledge painstakingly accumulated over the years is driven to clinical translation at an incredibly fast pace. Big pharmaceutical and biotechnology companies are committing their best resources to the field, and we expect good news in the following months and years. In this climate, the following points should be considered:

1. We will be mainly constructing and developing drug combinations based on the success of PD-1 and PD-L1 blockade. And we will especially focus on the nonresponders to PD-1 blockade monotherapy.
2. There are interesting opportunities in targeting engineered biomolecules to the TME (95) and in intratumoral delivery of immunotherapeutic compounds (96).
3. Local and systemic virotherapy (96) will become more widely used as the best way to alert the immune system and render tumors immunogenic hold great promise, especially regarding combinations (30, 31). An agent of this kind based on HSV-1 has recently received FDA approval for melanoma (Table 1) to be used by direct intratumoral injections (97).
4. We will concentrate efforts on strategies to improve therapy of tumors endowed with low antigenicity (25) or those that are refractory to T-cell infiltration (27, 66).
5. We will be developing better, more predictive preclinical models to test immunotherapies including humanized mice implanted with human tumors and human immune systems (98).
6. Access to ever-improving personalized genetic and molecular profiling of tumors together with assessments of the patient's immune status will provide a basis for individualized and potentially more effective selective immunotherapy.
7. Numerous clinical trials will be needed to demonstrate efficacy and learn the biology necessary for building the

most-effective combinations and addressing malignant diseases that are classically considered to be nonamenable to immunotherapy.

8. Acknowledging that our knowledge of the immune system functions in cancer patients is incomplete, we will increase discovery efforts and focus attention on the development of new biomarkers that could improve early diagnosis, serve as surrogates of response to immune therapies, and predict responses.
9. Looking at the impressive Kaplan–Meier survival plots of pivotal immunotherapy clinical trials, we are encouraged to remember that there are many opportunities for making improvements in terms of both patients' survival and the quality of life. Hence, it will be acceptable to take balanced risks in the pursuit of improvements.

Reviews in this *CCR Focus* have been selected to concentrate on the new trends and challenges in cancer immunotherapy. We should "never underestimate the dark side of the force," but if we are doing the right things now, the eyes of our medical students of today will see in their patients things that we would have never dreamt of only 15 years ago.

Disclosure of Potential Conflicts of Interest

S. Demaria is a consultant/advisory board member for Eisai, Lytx Biopharma, and Nanobiotix. H.M. Zarour reports receiving commercial research grants from Bristol-Myers Squibb and Merck. I. Melero reports receiving commercial research grants from Bristol-Myers Squibb and Pfizer and is a consultant/advisory board member for Alligator Bioscience, AstraZeneca, BiOncoTech Therapeutics, Boehringer Ingelheim, Bristol-Myers Squibb, Incyte, and Novartis. No potential conflicts of interest were disclosed by the other authors.

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