Review

Cancer Research

Roles for Innate Immunity in Combination Immunotherapies

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Abstract

Immunity to infectious agents involves a coordinated response of innate and adaptive immune cells working in concert, with many feed-forward and regulatory interactions between both arms of the immune system. In contrast, many therapeutic strategies to augment immunity against tumors have focused predominantly on stimulation of adaptive immunity. However, a growing appreciation of the potential contributions of innate immune effectors to antitumor immunity, especially in the context of combination immunotherapy, is leading to novel strategies to elicit a more integrated immune response against cancer. Here we review antitumor activities of innate immune cells, mechanisms of their synergy with adaptive immune responses against tumors, and discuss recent studies highlighting the potential of combination therapies recruiting both innate and adaptive immune effectors to eradicate established tumors. *Cancer Res; 77(19); 5215–21. ©2017 AACR.*

Introduction

The heterogeneity of human cancers combined with the diversity of mechanisms acting in concert within established tumors to suppress the immune response make it unlikely that any single-agent immunotherapy will elicit meaningful tumor regression in a majority of patients—an expectation that has prompted the search for safe and efficacious combination therapies. The countless number of potential combination therapy permutations possible even within the existing pool of immunotherapy drugs in early clinical testing motivates the need for rational approaches to identify promising combination therapies. Progress in understanding the tumor microenvironment and antitumor immunity has led to the proposal that several functional steps are required for the immune response to eliminate established tumors, including blockade of immunosuppression, promotion of immune infiltration, induction of immunogenic tumor cell death, activation of antigen-presenting cells, and enhancement of effector cell activity (1–3). Identification of these target functional requirements sets the stage for designing combination treatments that address distinct barriers to tumor rejection.

Many studies have focused on combination therapies that promote complementary features of T-cell activity (e.g., treatment with vaccines, antibodies blocking inhibitory receptors, and antibodies agonizing costimulatory receptors) or that synthetically substitute for B cells (antitumor mAbs). However, natural immune responses are never based solely in adaptive immunity; innate immune cells play an important role in complementing the effector activities of $CD4^+$ and $CD8^+$ T cells, and provide unique pathways to bolster an ongoing adaptive response. In this brief review, we discuss pertinent features of innate responses to tumors, and examine findings from recent combination immunotherapy studies that have revealed unexpected important roles for innate immunity in successful antitumor therapies.

Roles of Innate Immune Effectors in Immunotherapy

Cells of the innate immune system are often described as having a dichotomous role in cancer, capable of both promoting and inhibiting tumor growth, depending on the context. There is mounting evidence that innate immune effectors can be driven to impart antitumor immunity both directly and indirectly, given the proper cues (Fig. 1). Dendritic cells (DC) play a critical role in immunotherapy by processing and presenting tumor antigens to T cells, and their role in antitumor immune responses has been discussed in other recent reviews (4–7). Here we focus our discussion here on four key functions of other innate immune cells that can have their own direct antitumor activity.

Direct tumoricidal activities of innate cells

Several innate immune cell populations, appropriately activated, can directly kill tumor cells. Natural killer (NK) cells and NKT cells can recognize cell surface stress ligands and tumor-derived glycolipids expressed by tumor cells, respectively, leading to innate cell activation and tumor cell lysis (4). Macrophages can kill tumor cells through secretion of nitric oxide species (8). Activated eosinophils also exhibit tumoricidal activity through the release of secretory granules containing multiple cytotoxic molecules including membrane-disrupting major basic protein and granzyme A, although mechanisms of tumor cell recognition by eosinophils remain undefined (9). Thus, immunotherapies stimulating these innate immune cell populations have the potential to augment the cytotoxic activities of T cells.

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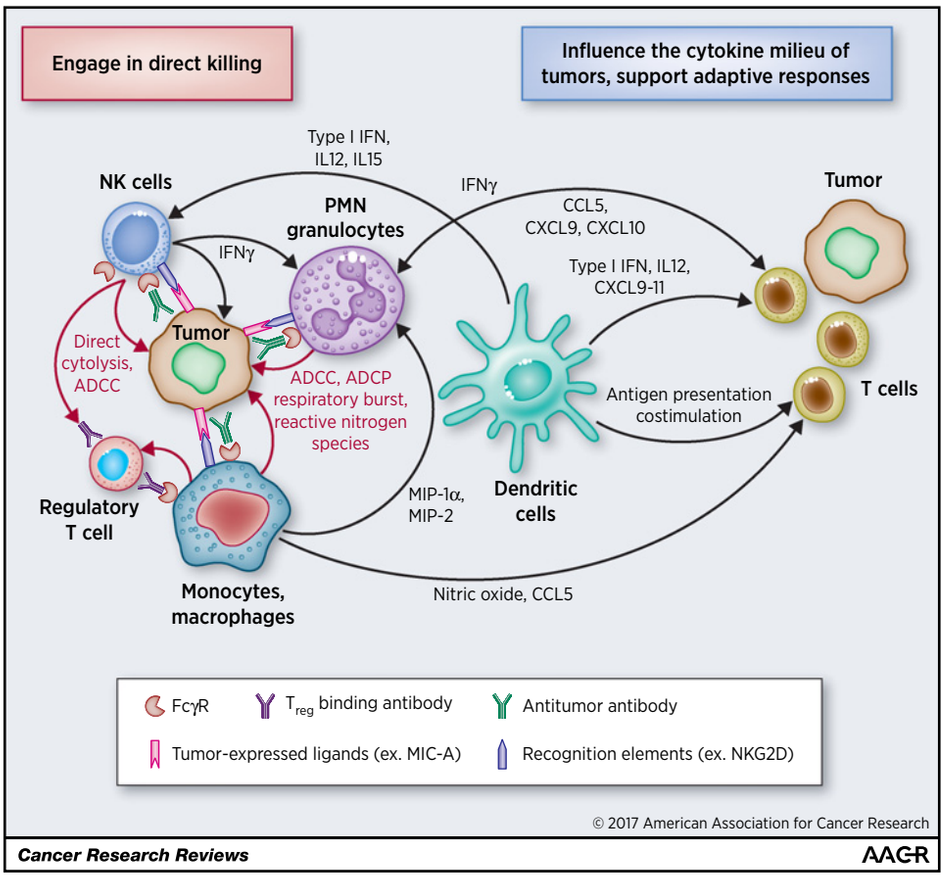

Figure 1.
Roles of innate immune effectors in antitumor immunity. Innate immune effectors including NK cells, polymorphonuclear granulocyte-like neutrophils and eosinophils, macrophages, and monocytes can engage in direct tumoricidal activity or exert Fc-mediated effector functions against antibody-opsonized tumor cells utilizing multiple mechanisms (red). In therapies with antibodies against targets overexpressed by Tregs, like anti-CTLA-4, these FcγR-expressing effectors may also deplete intratumoral Tregs. Tumor-infiltrating innate immune cells may also secrete factors that modulate the cytokine and chemokine milieu of the tumor (blue).

Antibody-mediated killing of tumor cells

Therapeutic mAbs have been approved for use in humans against cancer for nearly two decades (10). Although many of these were originally designed to antagonize oncogenic signaling pathways, it has subsequently been shown that antibodies directed against tumor surface antigens can recruit effector functions of innate immune cells via interaction of the Fc region with FcγR-expressing cells, which can result in direct killing by multiple mechanisms. NK cells, neutrophils, and other myeloid cells can kill through antibody-dependent cellular cytotoxicity (ADCC), a process by which crosslinking of FcγRs by antibody-opsonized tumor cells results in the release of cytotoxic granules that contain perforin and granzyme (10). Both neutrophils and NK cells were shown to be critical for ADCC-mediated elimination of CD52-expressing tumor cells treated with the anti-CD52 antibody alemtuzumab (11), and in separate studies, FcγR-competent neutrophils were shown to be crucial effectors for antibody therapy against syngeneic melanomas in mice (12). In addition to mediating ADCC, macrophages can engulf and destroy tumor cells through antibody-dependent cellular phagocytosis (ADCP; ref. 13). Furthermore, opsonization of tumor cells with antitumor antibodies can also lead to complement-mediated lysis, which has been shown to be important for the mechanism of B-cell depletion with the anti-CD20 mAb rituximab (14). In addition to promoting direct killing, antibodies can form a crucial bridge between these innate responses and adaptive immunity through DCs, which efficiently cross-present antibody-opsonized tumor antigens (5, 6).

Shaping the microenvironment of tumors

Innate immune cells can also play an important role in shaping the cytokine and chemokine milieu of the tumor microenvironment, thus influencing DC activation and differentiation of effector T cells (4). Tumor-infiltrating neutrophils have been shown to engage in proimmunity cross-talk with T cells, secreting inflammatory factors and expressing costimulatory molecules that bolster T-cell function and proliferation in a feed-forward loop (15). Intratumoral innate leukocytes can also undergo productive cross-talk with other innate cells. For example, activated eosinophils promote polarization of intratumoral macrophages toward an M1 phenotype (16). In cetuximab-treated head and neck cancer patients, NK cells were shown to enhance therapy through interactions with DCs, resulting in maturation and priming of antitumor $CD8^+$ T cells (17). This NK-DC cross-talk relied on the interaction of FcγRIIIa on NK cells with antibody-opsonized $EGFR^+$ tumor cells, cytokines released by the cetuximab-activated NK cells, and NKG2D-MICA engagement, which in concert resulted in enhanced DC maturation and cross-presentation to prime EGFR-specific T cells.

In addition, there is mounting preclinical evidence that checkpoint inhibitors targeting molecules expressed at high levels by regulatory T cells (Treg), such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), glucocorticoid-induced TNFR-related protein (GITR), and OX40, may exert their antitumor activity in part by depleting intratumoral Tregs, relieving immunosuppression within the tumor and allowing for immune-mediated tumor destruction. This selective Treg depletion is enacted via ADCC

and/or ADCP by innate immune effectors, and requires an activating antibody isotype. This was first demonstrated for anti-CTLA-4: effective treatment markedly reduced intratumoral Tregs, and depletion was mediated by FcγR-expressing macrophages (18–20). This phenomenon was also expanded to include anti-GITR and anti-OX40 antibodies as well (20, 21).

Recruitment of immune cells to tumors

A final important role of innate cells in the immune response to tumors lies in their capacity to promote additional leukocyte migration to tumors, mediated by several complementary activities. Macrophages polarized toward an M1 phenotype secrete nitric oxide species that activate endothelial cells and chemokines that together promote the recruitment of T cells to tumors (22). Activated eosinophils secrete inflammatory chemokines and also promote normalization of tumor vasculature, which further augments T-cell recruitment to tumors (16).

Combination Treatments Recruiting Innate and Adaptive Immunity

The multiple functions of innate immune effectors in the setting of cancer described above motivate the design of combination immunotherapies that leverage their activities to enhance tumor immunity. A number of combination treatments have now been found to have important innate immune stimulatory features that contribute in important ways alongside adaptive immune effectors to control tumor progression.

Combinations employing antitumor antibodies

Because of their ability to recruit multiple innate immune cell types and enhance cross presentation, antitumor mAbs have been incorporated into a number of combination immunotherapies. Phagocytosis of antibody-opsonized cells is restrained by tumor cell expression of the self-recognition signal CD47, but immunotherapies combining antitumor mAbs with an engineered high affinity form of the natural ligand for CD47 (SIRP-α) were shown to elicit synergistic increases in tumor cell phagocytosis *in vitro* and *in vivo* (23). Carmi and colleagues demonstrated that passively transferred allogeneic Ig antibodies could opsonize tumors and lead to their eradication, if tumors were also treated by intratumoral administration of a TLR3 agonist or CD40L combined with TNFα to activate tumor-associated DCs (24). Although this study focused on the role of antibodies in promoting cross-presentation of antigen by DCs, the initial step of tumor killing/antigen release following opsonization was likely dependent on innate immune-mediated tumor killing. Antibody–drug conjugates (ADC) introduce additional pathways for synergy with immunotherapy agents. Treatment with trastuzumab-DM1, an anti-Her2/maytansine derivative ADC, elicited T-cell infiltration in orthotopic murine Her2-expressing breast tumors as well as patient breast tumors. Motivated by these observations and the DC-activating properties of a drug related to DM1, this ADC was combined with checkpoint blockade (anti-CTLA-4 + anti-PD-1), leading to a dramatic reshaping of the composition and phenotypic status of TILs in the murine model (25): conventional T cells increased in frequency in treated tumors, but also γ/δ T cells, NK cells, and NKT cells; tumor-associated macrophages increased PD-L1 expression but substantially decreased arginase expression. Despite these significant alterations in the innate immune composition of treated tumors, the mechanistic role of innate cells in this therapy were not explored.

Combination therapies reprogramming the tumor microenvironment via innate immune cells

Immunotherapy regimens aiming to drive T-cell responses against tumors are often limited by the lack of activated antigen-presenting cells in tumors and tumor-draining lymph nodes (24). To this end, intratumoral treatment with agonists of pattern recognition receptors and other innate danger sensors expressed by DCs and macrophages has been successfully used to substantially reshape the number and phenotype of tumor-infiltrating leukocytes. For example, intratumoral injection of a lipid-conjugated TLR7 agonist designed for retention at a local injection site led to antitumor efficacy in several tumor models, dependent in part on IFNγ, CD8 T cells, B cells, and tumor-associated macrophages (8). TLR7 agonist therapy activated DCs in tumor-draining lymph nodes and enhanced the recruitment of macrophages with an M1 phenotype to tumors, which were capable of direct nitric oxide–mediated tumor cell killing. Combined treatment with checkpoint blockade (anti-CTLA-4) was highly synergistic, increasing antitumor efficacy and eliciting systemic immunity. In a murine head and neck cancer model, treatment with anti-PD-L1 alone was ineffective, but when combined with intratumoral administration of the STING agonist R_P, R_P dithio-c-di-GMP, the combination elicited significantly more complete tumor rejections than either therapy individually (26). Administration of the STING agonist increased the type I IFN signature of the tumor and draining lymph node, which was concurrent with an increase in dendritic cell transcripts for antigen-processing machinery and surface expression of costimulatory molecules for superior T-cell priming and tumor control. In a murine model of $HER2^+$ breast tumors, when the STING agonist ADU-S100 was given as an intratumoral monotherapy in a HER2-tolerant setting with transgenic neu/N mice, little efficacy was observed (27). Analysis of the antitumor $CD8^+$ T-cell responses showed defective expansion and high expression of exhaustion markers. Combination of intratumoral STING agonism with anti-PD-1 and OX-40 stimulation, however, resulted in enhanced T-cell expansion and activity, resulting in complete clearance in 40% of the treated mice, demonstrating that the combination of STING agonism, checkpoint blockade, and ligation of costimulatory receptors can overcome tumors even in an antigen-tolerant setting.

Radiotherapy also acts to promote a proimmune inflammatory microenvironment in tumors. Local radiotherapy activates DCs and myeloid cells via complement (28), and triggers tumor-associated macrophages to secrete factors normalizing the tumor vasculature and promoting T-cell recruitment to tumors (22). Thus, radiotherapy combined with adoptive cell therapy showed strong synergy and enhanced survival in mouse models of pancreatic cancer (22). Tumor-associated macrophages have also been targeted by immunotherapy: antibody-mediated blockade of CSF1/CSF1R was shown to repolarize tumor-associated macrophages toward a productive antitumor phenotype in pancreatic tumors, and this treatment was highly synergistic with anti-CTLA-4, anti-PD-1, or the combination of these two checkpoint blockade agents (29).

CD39, CD73, and adenosine receptors form an interacting triangle in tumors, with the CD39 and CD73 ecto-enzymes decomposing extracellular ATP to adenosine, which acts to suppress immune reactions on binding to adenosine receptors on T cells. Despite this interrelationship, coinhibition of CD73 via a

mAb and adenosine receptor through a small-molecule adenosine inhibitor was recently shown to exhibit significant synergy in blocking lung metastasis in several tumor models (30). Intriguingly, this response involved expansion of neutrophils and was strongly dependent on NK cells, neutrophils, and activating FcRs in the host.

Immunotherapy agents acting on innate and adaptive immune cells in tandem

A number of immunotherapy agents, particularly immunoregulatory cytokines, act on both adaptive and innate immune cells with antitumor effects. For example, agonistic antibodies against the costimulatory receptor CD137 stimulate both T cells and NK cells. Leveraging the observation that activated NK cells upregulate CD137 expression, Kohrt and colleagues demonstrated in mouse models potent synergy of anti-CD137 and the anti-HER2/neu mAb trastuzumab (31), and later studies showed this observation translated to the EGFR-targeting mAb cetuximab as well (32). A second prominent immunotherapy agent acting on both innate and adaptive immune cells is IL2: IL2 expands T cells and promotes their effector functions but also enhances NK-cell sensitivity to targets, expands NK cells and indirectly expands eosinophils (33). Recently, Zhu and colleagues demonstrated a remarkable synergy of cotherapy employing an antitumor antibody and a long half-life IL2 molecule (34). A coordinated network of antitumor immunity resulting from this therapy was established: macrophages were induced to release the chemoattractant MIP-2, which recruited FcγR-expressing neutrophils, which [along with other polymorphonuclear (PMN) cells] destroyed tumor cells via ADCC. In addition to mediating direct killing themselves, the IL2-activated T cells and NK cells released IFNγ, which bolstered PMN effector function. Interrupting this coordinated network of innate-adaptive interactions, for example with cellular depletions or blocking antibodies, significantly hindered therapy. Expanding on these findings, antibody/IL2 therapy with a potent lymph node targeting vaccine and checkpoint blockade was shown to enable complete elimination of large established tumors in several transplanted tumor models as well as regressions in a genetically engineered melanoma model (35). This four-component immunotherapy relied not only on $CD8^+$ T cells, but also on FcγR-expressing innate effectors, including neutrophils, macrophages, and NK cells. In addition to mediating destruction through ADCC and/or ADCP, an antibody-dependent vaccinal effect was observed in this combination therapy, whereby antigen spreading to T-cell epitopes not included in the vaccine was detected. Thus, therapies effectively recruiting both innate and adaptive immune effectors against tumors show substantial curative potential even against established tumors.

Adoptive cell transfer of innate effectors

Adoptive transfer of T cells is showing remarkable efficacy for the treatment of some hematologic cancers, and there are many efforts underway to expand their activity for other tumor types (36). The refinement of clinical pipelines for implementation of these T-cell–based therapies at scale may result in lower barriers to entry for cell-based therapies utilizing other immune cell types. In particular, NK cells are attractive to consider for combination immunotherapy because they can kill in a non-MHC restricted manner and their cytotoxicity can be directed using antitumor antibodies (10). NK cells integrate inhibitory and activating signals when deciding whether or not to kill a target, and MHC class I is inhibitory to NK cells (37). Because of this feature, NK cells may be particularly well suited to tackle tumors that have downregulated or lost MHC class I expression due to T cell pressure, a known mechanism of resistance to checkpoint blockade (38), and should thus be considered in combination for augmenting immunotherapies that drive T-cell activity.

Adoptive transfer of autologous or allogeneic activated NK cells thus far has shown somewhat disappointing outcomes clinically. In one study, melanoma and renal cell patients received autologous activated NK cells were transferred and no detectable therapeutic benefit was seen (39). Although the NK cells could be detected in circulation for at least 1 week posttransfer, analysis of the transferred NK cells showed defective *ex vivo* killing and loss of expression of the key activating receptor NKG2D. In another study, non-Hodgkin's lymphoma patients received adoptive transfer of allogeneic NK cells in combination with IL2 and rituximab (40). Successful engraftment of NK cells was only detected in one-third of patients treated 1 week following transfer, and all patients showed substantial increases in Treg numbers in response to the IL2 treatment (40). Taken together, these studies suggest that further optimization of the expansion and activation protocol, genetic manipulation, and/or the delivery of improved immunomodulators [e.g., engineered IL2 to decrease Treg expansion (41)] may be necessary to achieve therapeutic benefit with NK-cell-based adoptive transfer studies. To this end, blocking NK-cell checkpoints by targeting inhibitory receptors in the KIR- or LIR/ILT-family members with blocking antibodies may prove fruitful (42). Genetic manipulation may also be beneficial; NK cells have been engineered to express chimeric antigen receptors (43), or separately, higher levels of CD16 and the chemokine receptor CCR7 to improve migration towards CCL19 and ADCC in combination with rituximab, demonstrating NK cells can be genetically engineered to augment trafficking and functional capacity *in vitro* (44).

Another cell type to consider using in combination immunotherapies utilizing adoptive transfer of effectors is γδ T cells. This population is often considered to fall somewhere between innate and adaptive immunity, and they share important features with innate effectors including germline-encoded recognition elements and the ability to exert cytotoxic function in an MHC-independent manner, making them attractive for immunotherapy applications. We refer the reader to a recent review (45) for an overview of the use of γδ T cells in adoptive transfer strategies for the treatment of cancer.

Temporal aspects of combination immunotherapy regimens

Timing of the relative administration for each agent in combination immunotherapies can be crucial, and treatment regimens must be designed with a clear picture of the sequence of events expected in response to the combination. For example, in their studies of anti-CD137 cotherapy with the anti-HER2/neu antibody trastuzumab, Kohrt and colleagues demonstrated that optimal therapeutic responses occurred when the CD137 agonist was administered 1 day after the antitumor antibody (and not before or coincident with the HER2/neu mAb), due to the sequential steps of trastuzumab first binding to Fc receptors on NK cells followed by upregulation of CD137 on the responding cells (31). In a three-component immunotherapy using long half-life IL2, an

antitumor mAb, and IFNα, Tzeng and colleagues demonstrated that the timing of administration of IFNα, acting as a DC maturation stimulus, was critically important for treatment efficacy (46). Optimal responses were only seen when IFNα was delayed following the antitumor mAb/IL2 treatment; this sequential treatment allowed for a bolus of immunogenic tumor debris to be taken up by DCs, which were then matured with IFNα for optimal cross-priming of tumor-reactive T cells. If DCs were matured prematurely by administering IFNα prior to this tumor antigen uptake, therapy failed.

Combination therapies recruiting innate and adaptive immunity in the clinic

Importantly, a number of combination immunotherapies that stimulate both innate and adaptive immunity are currently in clinical trials or have an open path to clinical testing. Innate immune-stimulatory local radiotherapy has been combined with GM-CSF, local intratumoral CpG, or anti-CTLA-4 administration in small clinical studies to increase abscopal responses in patients (47). Rituximab has been combined with PD-1 blockade in a phase II trial in relapsed follicular lymphoma patients; although it was a single-arm trial, the combination was well tolerated and response rates compared favorably to previous trials with rituximab alone in similar patient cohorts (48). Trastuzumab has been combined with an allogeneic cell-based vaccine and cyclophosphamide in $Her2^+$ breast cancer patients (49), and the anti-CTLA-4 antibody tremilumumab has been combined with IFN-α2b in stage IV melanoma patients (50). Trastuzumab-DM1 discussed above has successfully completed clinical trials in breast cancer, as have combinations of anti-CTLA-4 and anti-PD-1 in several cancers, paving the way for the promising preclinical data generated with these three drugs in preclinical models to be tested in patients (25). Similarly, studies of combination therapy with long half-life IL2, antitumor antibodies, and checkpoint blockade, which elicited striking responses in melanoma models in mice, could potentially be translated immediately, using low-dose continuous infusion of IL2, approved anti-PD-1 antibodies, and a melanoma-recognizing anti-TYRP1 mAb that recently completed phase I clinical evaluation (51). Thus, a number of combination therapies with likely synergistic innate and adaptive immune stimulatory activity are either actively being investigated in the clinic or positioned for testing in the near future.

Conclusions

Many preclinical studies and some early clinical trial data support the notion that combination immunotherapies eliciting convergent innate and adaptive immune responses may be capable of enhanced antitumor activity relative to therapeutic strategies focused on single effector cells or arms of immunity. Although the T-cell (and possibly B cell) response to tumors provides an amplifying immune response capable of responding to changes in antigen profiles and the establishment of protective memory, in patients with existing tumors adaptive immunity will often start from a state of poorly-expanded tumor-specific populations that are actively immunosuppressed. Therapies recruiting innate immunity in concert provide an important means to rapidly alter the tumor microenvironment via immediately-responsive innate leukocytes that can be recruited in large numbers from the circulation, or through repolarization of innate cells already present in tumors. Innate immune stimulation can thus provide a supportive window for an adaptive response to be successfully initiated and subsequently supported, just as innate immunity holds infectious pathogens in check and regulates the microenvironment at sites of infection to promote successful adaptive immune-mediated clearance.

An ongoing challenge with moving these concepts into clinical testing is the potential for synergistic increases in toxicity in parallel to increases in antitumor efficacy, and toxicities elicited from combination immunotherapies remain difficult to predict based on single-agent trials or preclinical models. However, progress in managing patients and designing trials to safely identify functional and nontoxic doses of immunotherapy agents are rapidly improving. Compelling data on synergistic innate/adaptive-stimulatory therapies from preclinical models motivates a continued exploration of these approaches and translation to determine which of these strategies are capable of translating to enhanced survival in patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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