

Chapter 2

Overcoming Immune Suppression in the Tumor Microenvironment: Implications for Multi-modal Therapy

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Abstract Effective immunotherapy, whether by checkpoint blockade, vaccines or adoptive cell therapy, is limited in most patients by a fundamental barrier: the immunosuppressive tumor microenvironment. This problem is more than just the suppression of effector T cells, but also includes profound defects in the inflammatory milieu and immunogenic antigen-presenting cells that are required to drive T cell activation. To date, much of the field of immunotherapy has focused on downstream checkpoints that regulate activated T cells, or on vaccination and T cell adoptive transfer to expand the T cell pool. Relatively less attention has been given to regulatory pathways that govern cross-presentation and response to endogenous tumor antigens. But these “upstream” pathways become particularly important in settings where immunotherapy is combined with standard-of-care chemotherapy or radiation therapy, both of which release a wave of tumor antigens. The choice of whether to treat these antigens as tolerizing or immunizing is fundamental to generating an effective immune response against the tumor. In this chapter we consider immunosuppressive mechanisms in the tumor microenvironment from the perspective of factors that may impact the response to antigens from dying tumor cells.

Keywords Indoleamine 2,3-dioxygenase • IDO • Tolerance • Tumor microenvironment • Tumor • Immunotherapy • Checkpoint • Chemotherapy • Radiation

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2.1 Introduction

In this chapter, we will focus on two aspects of tumor immunotherapy that are particularly relevant to pediatrics, but which often receive somewhat less attention in the field. First, our emphasis will be less on the downstream checkpoints that affect activated T cells, and more on the fundamental upstream factors that control the cross-presentation of tumor antigens to T cells in the first place. It is increasingly being realized that this key endogenous antigen-presentation step needs to elicit robust T cell immunity in order for immunotherapy to be successful [1]. Unfortunately, however, the default response in the tumor is frequently T cell suppression and tolerance, rather than an aggressive response to tumor antigens. Thus, one of the important goals of tumor immunotherapy must be to re-configure the suppressive and tolerogenic tumor microenvironment so that it becomes robustly immunogenic for tumor antigen [2, 3].

The second, and conceptually related, focus of this chapter will be on ways in which immunotherapy can be integrated with standard-of-care chemotherapy and radiation-therapy treatment. In adult oncology, the combination of immunotherapy with chemo/radiation therapy is increasingly recognized as a potential opportunity (although currently under-utilized) for achieving valuable synergy [2, 4–6]. In pediatrics, however, it is virtually a requirement that immunotherapy be integrated in combination with the existing standard-of-care treatments. This is because in pediatrics the standard-of-care therapies are often highly effective, and even in relapsed or high-risk disease can still offer significant (albeit reduced) benefit. Thus, if immunotherapy is going to have a major near-term impact on the treatment of children, it will need to extend and enhance the efficacy of our existing treatments, not attempt to replace them. Fortunately, emerging preclinical evidence suggests that both chemotherapy and radiation are not only feasible for combination with immunotherapy, but can be highly synergistic.

2.2 Exploiting Immunotherapy to Create Synergy with Cytotoxic Therapy

2.2.1 *Chemo-Immunotherapy: Beyond Synergy to True Synthetic Lethality*

For a number of years it has been recognized that chemotherapy creates effects that can be exploited to enhance the immune response to tumors [7]. One obvious effect is the release of tumor antigens from dying cells; but, in addition, certain chemotherapy drugs may deplete regulatory T cells [8], or create lymphopenic conditions that favor T cell proliferation and expansion [9]. However, these effects are essentially passive: creating a general milieu in which vaccines or other immunotherapy may work better. A more active role for chemotherapy was revealed with the discovery of so-called “immunogenic cell death” (ICD) [10–12]. When certain preclinical

mouse tumor models are treated with particular chemotherapy drugs, the tumor cells die in a fashion that triggers a spontaneous immune response. Not only does this help prime the immune system against the tumor, but (at least in these particular model/drug combinations) a substantial component of the efficacy of the chemotherapy itself is actually contributed by the immune system [12]. While this was a ground-breaking discovery, in practical terms there are relatively few drugs that elicit ICD, and the effect is highly model-dependent [13]. Thus, while the underlying concept is important, the high-impact clinical role for immunogenic cell death is likely to be in combination with immunomodulatory agents that can enhance and exploit the effect [14]. As we will discuss, when the underlying inhibitory pathways are removed by active immunotherapy, then many chemotherapy drugs may prove to be immunogenic [4].

Ultimately, the goal in combining immunotherapy with chemotherapy is not merely “synergy” in the pharmacologic sense, but rather to generate authentic *synthetic lethality* by the combination. Synthetic lethality describes a combination in which the two agents together recruit an entirely new set of molecular mechanisms, which would not come into play with either agent alone [15, 16]. Thus, for example, in pre-clinical models, our own group has shown that combining a normally ineffective dose of chemotherapy with a specific immune-activating agent (i.e., an agent that blocks a tolerogenic checkpoint to dying tumor cells), allows the ineffective chemotherapy to now cause potent and rapid tumor regression [17]. The mechanism of anti-tumor effect was almost entirely immunologic (T cell dependent), but these immune mechanisms were only triggered if the tumor was also treated with chemotherapy.

2.2.2 The Importance of Endogenous Tumor Antigens

One of the surprising findings of the past several years has been the importance of endogenous tumor antigens in cancer immunotherapy [18]. Prior to the advent of checkpoint-blockade agents, the focus of immunotherapy was often on supplying antigens and T cells exogenously—e.g., via defined vaccines, TIL infusions, TCR-transgenic T cells, or CAR T cells. However, as increasing numbers of patients have been treated with blockade of the CTLA-4 and PD-1/PD-L1 pathways, it has become evident that the best responses are seen in those patients who have many mutational neoantigens in the tumor, and who already have a robust spontaneous immune response prior to treatment [19, 20].

In part this may simply be an artifact of early trials, which use only single-agent checkpoint blockade. In this setting, it is perhaps logical that only those patients who were already spontaneously pre-activated could respond to removing a single checkpoint. This effect may disappear as more powerful combination regimens are employed [21]. But the key take-home point is that the tumor’s own endogenous antigens, cross-presented by the patient’s own APCs to the endogenous T cell repertoire, were the critical factor that drove the anti-tumor response. This emphasizes the importance of endogenous tumor antigens, and the ability to cross-present them in an immunogenic fashion.

This has obvious importance for the immune response to chemotherapy or radiation, which release a wave of endogenous tumor antigens. But even in the case of an exogenous immune intervention, such as an antigen-specific vaccine or T cell adoptive transfer (CAR T cells, etc.), a successful long-term outcome may still depend on generating a response to endogenous tumor antigens [1]. Transferred T cells or defined vaccines are directed against just one or a few antigens. The initial response may be dramatic, but eventual emergence of escape variants is almost inevitable. If, however, during the initial period of robust inflammation and tumor killing, the endogenous host immune system becomes primed to endogenous tumor antigens, then the danger of escape variants is minimized, and long-term tumor control becomes a possibility.

2.2.3 Immunogenic Cell Death Versus Tolerogenic Cell Death: Overcoming Natural Pathways of Tolerance

The preceding general discussion does not tell us how—specifically—to render chemotherapy immunogenic in the clinic. In part this reflects the fact that much still needs to be discovered about the molecular mechanisms of combination chemo-immunotherapy. Also, our current options for immune intervention in the clinic are still somewhat limited, comprising primarily blocking agents against CTLA-4 or the PD-1/PD-L1 pathways, and blockade of the indoleamine 2,3-dioxygenase (IDO) pathway. However, the field is expanding rapidly and the armamentarium is quickly increasing. Thus, a better understanding of the molecular events that regulate the immune response following chemotherapy, in order to exploit this for therapy, has become a subject of some urgency.

In this regard, one fundamental insight emerging recently is the fact that the immune response to dying cells—even normal, non-malignant self cells—is not fixed and inherent. Rather, it reflects a combination of signals generated by the manner in which the cells die (ICD, apoptosis, necrosis etc.), combined with signals from the milieu in which the dying cells are cross-presented by the immune system. These local environmental signals are a very active—and changeable—process. Blocking even one of the tolerogenic signals elicited by apoptotic cells may render dying cells suddenly immunogenic instead. Thus, for example, the tolerogenic IDO pathway is strongly up-regulated by exposure to apoptotic cells [22]. When challenged with apoptotic self cells, normal IDO-sufficient mice remained tolerant, but mice lacking the IDO1 gene rapidly developed lethal lupus-like autoimmunity against self antigens [22–24]. Thus, it was not the nature of the antigens themselves that determined immunity versus tolerance, nor the type of cell death; but rather the ability of the apoptotic cells to elicit the immunosuppressive IDO signal. If this IDO pathway was blocked, then the same cells, and the same self antigens, now became immunogenic.

The relevance of this concept for cancer treatment is that chemotherapy and radiation release a wave of tumor antigens, many of which are potentially immunogenic

[18, 25]. The problem is that these antigens are released into a tumor milieu that is overwhelmingly dominated by immunosuppressive mechanisms. Thus, even though dying tumor cells are potentially immunogenic [6] the actual outcome is usually tolerance and anergy, due to these dominant suppressive mechanisms. If, however, the tolerogenic pathways used by the tumor (such as IDO, Tregs or others) can be identified and blocked at the time of chemotherapy, then the antigens thus released may be treated as immunizing instead of tolerizing. This concept is now well accepted in principle [1, 2], and relevant preclinical studies are beginning to emerge [17], but much of the underlying molecular machinery still remains to be discovered. However, even with our current limited state of knowledge, it is possible to begin to design clinical trials aimed at exploiting the immunogenicity of chemotherapy.

2.3 Negative Regulation in the Tumor Microenvironment

In this section we will briefly discuss several of the key suppressive pathways operating in the tumor. Many of these are discussed in detail elsewhere in this volume, so our focus here is specifically how these inhibitory pathways may affect the cross-presentation and immune response to tumor antigens.

2.3.1 *Regulatory T Cells: Recruitment and Activation*

Regulatory T cells (Tregs) in tumors are an important suppressive population [26]. Physically depleting Tregs [27] or inhibiting the signals that they require [28] rescues anti-tumor immune surveillance. However, it is still unclear how Tregs exert their suppressive function. One important mechanism may be their ability to inhibit tumor-associated antigen-presenting cells [29, 30]. This would be a key leverage point for control of antigen cross-presentation to T cells.

One important unanswered question in the field is why Treg activity is so excessive in the tumor. Many of the Tregs in tumors appear to recognize the same self antigens as in normal tissues [31], but there is a greater degree of constitutive functional activation of Tregs in tumors [32]. Several upstream pathways are known to activate tumor-associated Tregs, includes IDO [32] and neuropilin-1 [33]. Recently, it was shown that when Tregs are activated by IDO they up regulate the PD-1 receptor; PD-1 signaling then maintains the suppressive Treg phenotype long-term, via activation of the downstream PTEN phosphatase [17]. Neuropilin-1 also activates PTEN in Tregs [33], and PTEN has been recently implicated in maintaining normal function and stability of Tregs in the normal immune system [34, 35]. Thus, PTEN may be a centrally-positioned pathway in tumor-induced activation of Tregs. In tumor-bearing mice, ablation or inhibition of the PTEN pathway in Tregs prevented tumors from creating their usual immunosuppressive microenvironment, and this markedly enhanced the immune response to dying tumor cells following chemotherapy [17].

2.3.2 *CTLA-4 and PD-1 Pathways: The Classic T Cell Checkpoints*

CTLA-4 and PD-1 are inhibitory molecules expressed by activated T cells. Clinically, antibodies against these molecules may dramatically enhance the ability of patient's T cells to attack their own tumors, especially in patients who already have a pre-existing anti-tumor T cell response at diagnosis. Anti-CTLA-4 was the first checkpoint-inhibitor to be approved [36]. While responses to single-agent therapy have been limited, combination with PD-1 blockade is encouraging [21]. Clinical benefit was greatest in patients with a high mutational burden in their tumor genome, and a large number of predicted mutational neoantigens [37, 38]. But the effect of CTLA-4 is not limited to tumor-specific neoantigens; patients also showed a significant increase in the frequency of T cells against self antigens associated with melanoma [39]. Thus, blocking CTLA-4 may enhance the ability of the host immune system to respond to endogenous tumor antigens, including even breaking tolerance to shared self/tumor antigens. Therefore, a key take-home message from these studies is that the T cell response to endogenous tumor antigens is not fixed, and can be increased if the relevant suppressive mechanisms can be blocked.

The mechanism of action of CTLA-4 blockade is still unclear [40]. It may lower the activation threshold for effector T cells, and/or it may inhibit or deplete regulatory T cells (Tregs) [40, 41]. Since Tregs can suppress the function of antigen-presenting cells in the tumor [29, 30], blocking CTLA-4 may indirectly enhance cross-presentation of tumor antigens.

PD-1 is a second inhibitory molecule expressed on activated T cells. Some tumor cells constitutively express PD-L1, the counter-ligand for PD-1, and other tumor cells may up-regulate PD-L1 in response to inflammatory signals. In addition, host APCs (dendritic cells (DCs)), Myeloid Derived Suppressor Cells (MDSCs) and macrophages) can express constitutive or inducible PD-L1 or PD-L2, and this may be a biologically important source of inhibition [42]. In certain tumors, blocking PD-1 or PD-L1 can trigger striking clinical responses in a subset of patients. Like CTLA-4 blockade, response to single-agent therapy is more likely in patients who have many neoantigens and a strong spontaneous anti-tumor immune response at baseline [20, 42, 43].

Although there is little published literature on attempts to combine CTLA-4 or PD-1/PD-L1 blockade with chemotherapy, this strategy to date has not met with much obvious success [44]. In this regard it may be important to note that both CTLA-4 and PD-1 are expressed by T cells, and become relevant when the T cells are activated. In the setting of chemotherapy, however, the fundamental immunologic defect may occur much earlier than this, at the level of the tolerogenic milieu and the initial antigen-presentation step. If antigen-presentation is defective or tolerogenic, then the CTLA-4 and PD-1 checkpoints may not even come into play. Thus, CTLA-4 and PD-1 may not be the optimal targets for combination with chemotherapy, because they are too far downstream. That said, if the antigen-presenting milieu after chemotherapy can be improved by blocking upstream checkpoints such as IDO, then the CTLA-4 and PD-1 checkpoints on T cells may now become relevant and useful for combinatorial therapy.

2.3.3 Targeting the IDO Pathway

Indoleamine 2,3-dioxygenase (IDO) is a tryptophan-degrading enzyme expressed by cells in the immune system; it can also be expressed by inflamed tissues (epithelial cells and fibroblasts) and by some tumor cells (reviewed in ref. [45]). The immunologic function of the IDO pathway is both counter-regulatory (suppressing excessive inflammation) and tolerogenic (creating de novo antigen-specific tolerance in T cells). In preclinical models, when the IDO pathway is interrupted mice experience multiple defects in tolerance, including maternal tolerance to the fetus during pregnancy [46–48]; mucosal and other forms of acquired peripheral tolerance [49–56]; and tolerance to apoptotic cells [22, 24]. Conversely, artificial over-expression of IDO can create systemic tolerance de novo [24, 56–58]. IDO also controls inflammation, acting to enhance the tissue-reparative effects of inflammation while limiting the tissue-destructive effects [59–66]. In all of these models, the role of IDO is focused and selective; and IDO-deficient mice do not have the broad, spontaneous defects in self-tolerance that are seen with mice lacking CTLA-4 or Treg cells. (This may be an important consideration in understanding the mild toxicity profile of IDO-inhibitor drugs in the clinic.) However, in settings where IDO is important, it can be a crucial and non-redundant mechanism of immune regulation.

IDO degrades the essential amino acid tryptophan to kynurenine. The IDO gene family includes IDO1 (the main subject of this section) and a related gene, IDO2 [67–69] which is much less well studied. In this chapter, we will use the term “IDO” to refer to IDO1, or to the collective functional IDO enzyme activity. Depletion of local tryptophan by IDO can activate the amino-acid sensitive stress-kinase GCN2. Activation of GCN2 in T cells can inhibit effector T cell proliferation and function, and increase the suppressor function of Tregs [70–72]. In antigen-presenting cells, GCN2 inhibits inflammatory signals [73], and it can profoundly bias antigen-presenting cells toward tolerance induction [24]. In addition to activating GCN2, IDO produces diffusible soluble factors (kynurenine and downstream metabolites) that bind to the aryl hydrocarbon receptor (AhR) [74]. The AhR can enhance Treg function [72, 74], suppress acute inflammatory cytokines such as IL-12 and IL-6 [22, 24, 75] and bias dendritic cells and macrophages toward an immunosuppressive phenotype [24, 75–78]. Taken together, this means that IDO expression can have a profound effect on the nature of the antigen-presentation process in a local tissue site: rendering it tolerogenic when IDO is active, or immunogenic when IDO is blocked [22, 24, 70, 79–81].

2.3.3.1 Activation of Treg Cells by IDO

IDO controls an important activation pathway in Tregs. Resting Tregs are not suppressive, and they must undergo activation in order to become functionally active [82]. This activation step requires both TCR engagement [83] and modulating signals from the local microenvironment [84]. When Tregs are activated by an

IDO-expressing APC, they become highly suppressive [32]. IDO affects Treg activation by inhibiting mTORC2 and Akt signaling in the Treg [17]. This results in up-regulation of PD-1 expression by the Tregs, which then activates the PTEN lipid phosphatase. The PD-1 → PTEN pathway then continues the inhibition of Akt, thus forming a positive feedback loop that maintains on-going Treg suppressor activity as long as the milieu contains PD-ligands. IDO is not the only pathway that can activate PTEN in Tregs: neuropilin-1 (Nrp1) can have a similar activating effect [33]. But IDO may be particularly important in the tumor following chemotherapy or other insult, because it appears that IDO may directly link exposure to apoptotic cells with activation of the potent PTEN-expressing Tregs [17], as discussed in the next section.

2.3.3.2 IDO and Tolerance to Apoptotic Cells

In the normal immune system, IDO is strongly induced by apoptotic cells [22] and appears to play an important role in maintaining tolerance to apoptotic cells. When normal mice are challenged with large numbers of apoptotic thymocytes they remain tolerant, and will even create *de novo* tolerance to foreign antigens introduced on the apoptotic cells [22, 24]. However, if IDO1 is blocked then mice cannot create tolerance to apoptotic cells, and rapidly develop lupus-like autoimmunity against self antigens [22, 24].

In the case of tumors, challenge with apoptotic tumor cells was found to up-regulate IDO, which in turn elicited PTEN-expressing Tregs. These then inhibited immune response to antigens from the apoptotic cells [17]. Even in mice without tumors, targeted deletion of the PTEN gene in Tregs caused loss of self-tolerance when challenged with apoptotic cells (a phenotype essentially identical to mice lacking IDO) [17]. It is still speculative whether these same molecular pathways are operative in human tumors following chemotherapy; but it is clear that tumors are constantly faced with the need to suppress immune recognition of the highly abnormal dying tumor cells. Thus, tumors may “hijack” the normal IDO and PTEN-Treg pathways that inhibit responses to dying cells, and exaggerate them to render dying tumor cells tolerogenic.

2.3.3.3 IDO, PTEN-Tregs and Immunogenic Cell Death

The concept that specific regulatory mechanisms such as IDO and PTEN-Tregs are actively up-regulated by dying cells, and thus prevents the immune system from responding, has significant implications for tumor immunotherapy. In principle, tumor cells should be much more immunogenic than normal cells, since they possess mutational neoantigens, and can also aberrantly over-express self antigens in an immunogenic fashion [18, 25]. It is well known that certain forms of chemotherapy,

in the right tumor models, can create sufficient inflammation to elicit T cell immune responses (immunogenic cell death, as reviewed in ref. [10]). Unfortunately, in most tumors, most chemotherapy drugs do not appear to elicit a robust immune response—at least by themselves. However, this may be an artifact of the exaggerated levels of counter-regulatory suppression, such as IDO and activated Tregs, which are present in the tumor. If these suppressive pathways can be blocked, then immunogenic cell death may actually be much more widespread than currently recognized. Figure 2.1 summarizes the possibility that, during tumor cell death, two sets of signals are created simultaneously: some death occurs by apoptosis, and thus elicits mechanisms of tolerance; while some death is disordered and immunogenic. In the suppressive tumor milieu, however, the inhibitory pathways are exaggerated, and the immunogenic component is therefore dominantly suppressed. If these suppressive signals are blocked, however, then the immune system becomes able to respond to the underlying immunogenic cell death.

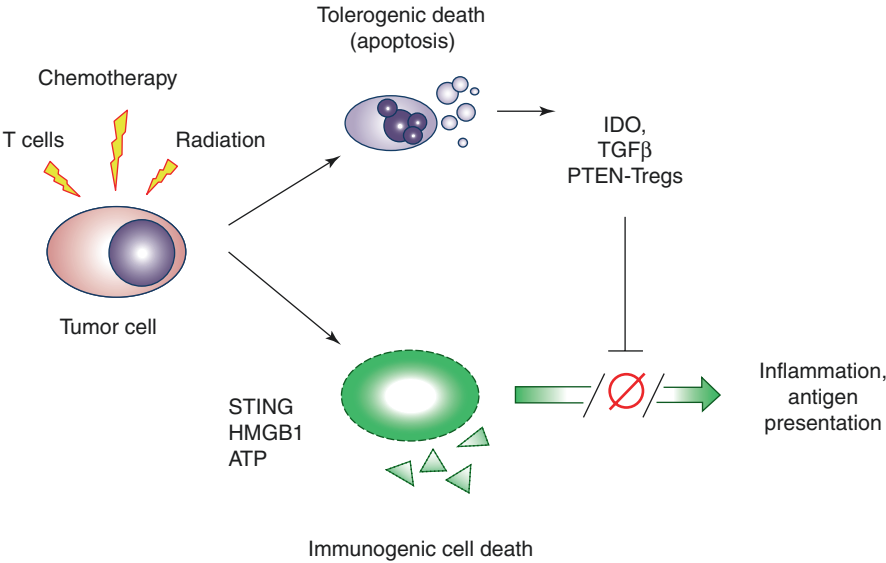


Fig. 2.1 Two possible responses to dying tumor cells. When tumor cells die following exposure to chemotherapy, radiation or killing by cytotoxic T cells, they may be treated by the immune system in one of two ways. Either they die in an immunogenic fashion, releasing inflammatory mediators and triggering cross-presentation of tumor antigens to T cells; or, alternatively, they may die in a tolerogenic fashion, eliciting suppressive signals such as IDO, TGFβ and activated Tregs. In the model shown, we propose that both forms of cell death usually co-exist in the tumor following therapy, but that the tolerogenic pathways are dominant unless they can be blocked or circumvented by immunotherapy

2.3.3.4 Clinical Trials of IDO-Inhibitor Drugs

Inhibitors of the IDO pathway do not kill tumor cells directly. Their role is to permit and enhance immune responses following chemotherapy, radiation or other immunotherapy. Table 2.1 gives a partial listing of current clinical trials of inhibitors of the IDO pathway. Most of the trials are based on either indoximod (1-methyl-D-tryptophan, NewLink Genetics, Inc.) [85–88] or epacadostat (INCB024360, Incyte Pharmaceuticals, Inc.) [89–91]. One of these trials (NCT02502708) is in pediatric

Table 2.1 Clinical trials of IDO-inhibitor drugs

Strategy	Drug	Patients	Trial design	Trial number
Combination with chemotherapy	Indoximod (NewLink Genetics)	Adult	With taxanes in breast cancer	NCT01792050
	Indoximod	Adult	With temozolomide ± radiation in refractory glioblastoma brain tumors	NCT02052648
	Indoximod	Pediatric	With temozolomide ± radiation in pediatric brain tumors	NCT02502708
	Indoximod	Adult	With gemcitabine/abraxane in pancreatic cancer	NCT02077881
	Epacadostat (Incyte)	Adult	With Jak1 inhibitor or PI3Kδ inhibitor	NCT02559492
Combination with checkpoint inhibitors	Epacadostat	Adult	With anti-CTLA-4 (ipilimumab)	NCT01604889
	Indoximod	Adult	With anti-CTLA-4 (ipilimumab)	NCT02073123
	Epacadostat	Adult	With anti-PD-1 (pembrolizumab)	NCT02178722
	Epacadostat	Adult	With anti-PD-L1 (atezolizumab)	NCT02298153
	Epacadostat	Adult	With anti-PD-L1 (MEDI4736)	NCT02318277
	GDC-0919 (Genentech Roche)	Adult	With anti-PD-L1 (atezolizumab)	NCT02471846
Combination with vaccines	Epacadostat	Adult	With NY-ESO-1 vaccine in ovarian cancer	NCT02166905
	Indoximod	Adult	With Hyperacute vaccine (NewLink Genetics) + docetaxel in lung cancer	NCT02460367
	Epacadostat	Adult	With peptide vaccine in melanoma	NCT01961115
	Epacadostat	Adult	With Listeria mesothelin vaccine in ovarian cancer	NCT02575807

patients. No final results have yet been published, so it is too early to assess the benefits of these strategies, but anecdotal results and meeting reports have been encouraging. Additional IDO inhibitors are in the development pipeline, as well as agents that may target IDO2 [92] or TDO [93, 94], two other tryptophan-catabolizing enzymes that may affect tumor immunity [95].

2.3.4 Tumor-Associated Macrophages and Myeloid-Derived Suppressor Cells (MDSCs)

Tumor-associated macrophages (TAMs) are key coordinators of tumor-promoting angiogenesis, fibrous stroma deposition and metastasis formation [96]. In addition, TAMs are also actively suppressive for T cell responses [97]. In part, this immunosuppressive phenotype may simply recapitulate the “reparative” phenotype of macrophages during tissue remodeling [98]. While it is usual to think of wounds as inflammatory, a sterile healing wound (i.e., without a superimposed infection) is actually not a receptive milieu for antigen-specific T cell activation. Sterile wounds are indeed inflammatory, but this is all chronic innate immunity, and any antigens present are purely self antigens. Thus, T cell responses are undesirable, and they are actively suppressed by signals such as TGF β and VEGF, which are high during tissue repair. Tumors resemble “wounds that do not heal” [99], and share many of these features of chronic inflammation with T cell immunosuppression. In addition, tumors contain abnormal signals such as acidosis and hypoxia which may further drive the immunosuppressive phenotype of the associated macrophages [100]. The molecular mechanisms by which TAMs suppress T cell activation in tumors are not yet well defined, but likely include production of VEGF and TGF β , as mentioned above. Whatever the mechanism, destabilization of the intra-tumoral macrophage pool, e.g., by blocking the CSF-1-receptor, significantly impairs tumor growth and enhances anti-tumor immune responses [101, 102].

While TAMs may superficially resemble normal macrophages, in reality the myeloid cells in the tumor are all profoundly disordered [96, 103]. In tumor-bearing hosts, bone-marrow myelopoiesis is altered by factors secreted from the tumor, such as GM-CSF and IL-6, which affects both the monocytic and granulocytic lineages [104, 105]. These abnormal circulating cells become further altered when they are recruited into the tumor microenvironment, with its chronic low-grade inflammation, free-radical flux and constant metabolic stress [106]. The presence of IDO and activated Tregs in the tumor may further enhance the recruitment of these cells, and render them even more suppressive [86]. Once in place, this heterogeneous population of myeloid-derived suppressor cells (MDSCs) creates an immunosuppressive milieu via elaboration of nitric oxide, arginase and reactive oxygen species [96, 107]. Much of the preclinical work on MDSCs has been performed in mouse models, but analogous cells exist in human tumors [108]. The suppressive nature of MDSCs in tumors is completely different from their inflammatory, highly immunogenic counterparts that would normally be created at the site of an infection

(inflammatory monocytes, macrophages and myeloid DCs) [104, 105]. Currently, it is unclear exactly how this abnormal, suppressive state of the myeloid cells in tumors can be reversed. Certain forms of chemotherapy may reduce the number of MDSCs [109]; but, paradoxically, chemotherapy may also elicit a rebound increase in MDSCs [110]. Reducing suppression by MDSCs is a subject of ongoing investigation. Ultimately, however, the most effective approach may be to create a robust pro-inflammatory microenvironment within the tumor by means of immunotherapy: under these conditions the myeloid-lineage cells may now be able to follow their normal, beneficial pathway of differentiation into immunogenic APCs [104].

2.3.5 Overcoming Therapy-Induced Counter-Regulation

The immune system provides potent protection against infection, but it can also be lethal if its activation is not controlled. Hence, the normal immune system contains multiple built-in regulatory feedback loops that control inflammation and limit excessive activation. Thus, for example, pro-inflammatory IFN γ simultaneously up-regulates anti-inflammatory PD-L1 and IDO [111]; while T cell activation spontaneously recruits corresponding suppressive Tregs [84, 112]. In the tumor milieu, these same counter-regulatory mechanisms are operative, and they are often pathologically exaggerated and dominant. As discussed above, this becomes relevant when the immune system must make the decision between immunity and tolerance to apoptotic tumor cells. In addition, pathways of inducible counter-regulation may become important when successful immunotherapy (e.g., a vaccination or CAR T cells) begins to create beneficial inflammation in the tumor. The presence of this desirable inflammation may elicit exaggerated and undesirable counter-regulation, e.g., by up-regulating PD-ligands or IDO [43, 111]. Thus, the efficacy of the initiating therapy is unwittingly compromised by its own success, unless agents are added to block this inducible counter-regulation.

2.4 Implications for the Clinic: Using Chemotherapy as an Immune Adjuvant

2.4.1 Bi-directional Synergy: Chemotherapy as a Vaccine, and the Immune System as a Cytolytic Agent

Based on the forgoing discussion of mechanisms, the field of immunotherapy has a growing opportunity to exploit the synergy (synthetic lethality) between chemotherapy and immunotherapy. As mentioned above, it is now well accepted that chemotherapy has the potential to function as an endogenous “vaccine” when it releases antigens from dying tumor cells [2, 4, 6]. By itself, conventional chemotherapy is typically not highly immunogenic [13]; but as we begin to better understand—and thus become able to block—the inhibitory mechanisms that suppress immune

responses after chemotherapy, our standard chemotherapy agents may become much more immunogenic as part of a combined regimen.

A second, less appreciated aspect of the synergy between chemotherapy and immunotherapy is that the immune system can become a potent downstream effector mechanism for conventional chemotherapy. Historically, when immunogenic cell death was first discovered, there was some speculation that all chemotherapy—even without immunotherapy—might rely on the immune system to cause tumor regression [113]. This turned out to be over-optimistic, and probably applies more to mouse models of transplantable tumor cell lines [114]. However, the situation becomes much different when chemotherapy is combined with active immunotherapy into a dual regimen. Now the immune system is being actively recruited and empowered to provide additional anti-tumor effector mechanisms, which would not normally come into play with standard chemotherapy alone. Thus, the immune system becomes, in effect, a downstream effector arm for the chemotherapy. This effect has been modeled in preclinical mouse systems [17, 115], and there are some encouraging suggestions that it may be translatable into the clinic as well [116, 117].

2.4.2 Targeted Therapies Can Also Release Antigen

The armamentarium of the oncologist is increasing beyond standard chemotherapy, and now includes antibodies, antibody-drug conjugates (ADCs) and selective kinase inhibitors. ADCs target chemotherapy drugs preferentially to tumor cells; they may be very cytolytic and immune-activating, and prime the tumor milieu for response to immunotherapy [118]. The immunologic response to targeted kinase inhibitors is not yet well understood, but when used against a tumor that is heavily dependent on the specific pathway being targeted, then these drugs can cause dramatic tumor regression (although subsequent resistance is a problem). It is not yet known if these targeted agents are as stimulatory for immune responses as conventional chemotherapy, but there is mounting evidence that many (perhaps all) of these agents can be immunogenic [6]. However, because these targeted agents at present play less of a role in pediatrics, our focus will be primarily on conventional cytotoxic chemotherapy.

2.4.3 How Immunosuppressive Is Chemotherapy ... Really?

Every oncologist has a healthy respect for the myelosuppressive potential of chemotherapy; thus, it seems somewhat counter-intuitive to combine chemotherapy with immunotherapy. However, myelosuppression does not necessarily imply a corresponding degree of T cell suppression (which is the relevant attribute for response to immunotherapy). Thus, for example, most chemotherapy regimens for solid tumors—even dose-intensive pediatric regimens—do not require prophylaxis against *Pneumocystis* or invasive fungal infections, in the way that a T cell-immunocompromised

patient would require. While patients may transiently have a low circulating T cell after chemotherapy, their lymph nodes and spleen do not go away, nor do the T cells in the tumor. Indeed, given the chance, the antigen-specific T cells associated with the tumor may be the first to rebound after chemotherapy, because they are responding to the wave of tumor antigens that have been released [119].

The timing and dosage of chemotherapy relative to the timing of immunotherapy must be optimized so as not to suppress the T cell response produced, but appropriate regimens can be designed to accomplish this [120]. Such regimens may be helped by the fact that antigen-specific T cells in the tumor may be non-proliferating at the time of chemotherapy (because they are anergic, or suppressed by the tumor microenvironment), and are thus protected from the effects of chemotherapy. Tumor-associated T cells may only become activated and proliferating later, after the chemotherapy is eliminated and gone. Thus, far from being inimical to immunotherapy, chemotherapy is in fact a ready-made opportunity to be exploited [2, 4].

2.4.4 The Late-Responder Paradox: Immune-Related Response Criteria

When patients are treated with conventional chemotherapy, they typically show one of three patterns: either they respond and are cured; or they fail to respond at all; or they respond for a period of time and then progress despite therapy (i.e., they become resistant). In those patients who do respond, the benefit of conventional chemotherapy is invariably front-loaded: the best response occurs in the initial cycles, and once the tumor begins to progress on therapy (becomes resistant) there is no further benefit from that particular agent or regimen.

In contrast, when tumors are treated with immunotherapy, there can be additional, less familiar patterns of response (Fig. 2.2). The immune system inherently displays memory and recall. Thus, as we know from childhood immunizations, an immune response can become progressively stronger the more times it is exposed to antigen (prime and boost). Therefore, some patients treated with anti-tumor immunotherapy may experience paradoxical late responses, occurring only after many months of treatment. Indeed, there can even be a period of early progression, sometimes lasting several cycles of therapy, before the immune system becomes sufficiently activated to cause tumor regression. This pattern of apparent resistance followed by late regression is essentially never seen with chemotherapy alone. (The specialized case of transient pseudoprogression in brain tumors is actually an effect of successful treatment, not progression [121]). The exact mechanism of these late immunologic responses is still a subject of investigation. It may simply represent progressive “boosting” with repeated treatment; but it also seems likely that late responses reflect a complex shift in the equilibrium between the suppression and immunity in the tumor milieu [1, 122]. Whatever the mechanism, it is important to bear in mind the possibility of late responses when designing clinical trials and stopping rules. This means, for example, the use of appropriate immune-related response criteria [123], which allow for a limited period of initial progression before removing patients from the study.

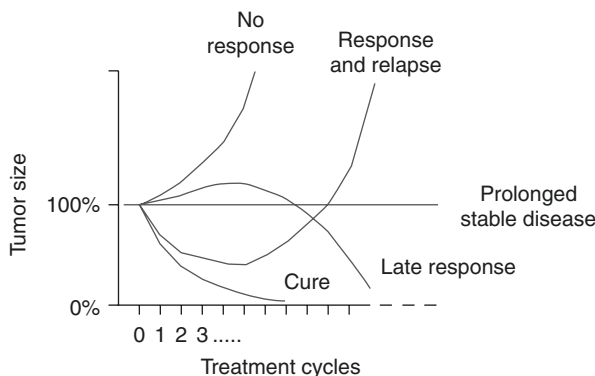


Fig. 2.2 Late responses and stable disease as potential outcomes with immunotherapy. Traditionally, responses to conventional chemotherapy or radiation tend to occur early, if they occur at all. With chemotherapy, for example, the traditional response patterns are either progressive remission induction leading to cure, or an early response followed by subsequent relapse (i.e., the tumor becomes resistant). However, when the immune system participates in the anti-tumor response, due to the addition of immunotherapy to the treatment regimen, then additional patterns of response become possible. Because the immune system can show increasing activation with repeated antigen exposure (immunologic memory), patients receiving immunotherapy may show late responses—sometimes even after multiple cycles of apparent non-response, or even transient progression. Alternatively, the immune system may establish an apparent “equilibrium” state with the tumor, during which residual disease remains, but is prevented from growing by the on-going immune attack. This results in a pattern of long-term stable disease, even though residual tumor is still present. Neither of these latter two patterns would be expected with conventional chemotherapy or radiation, but they are recognized patterns with immunotherapy

As a variant of the late-response pattern, immunotherapy may sometimes simply produce a prolonged period of stable disease, without complete regression (or a pattern of mixed regression of only some lesions). On the occasions when these stable lesions have been biopsied, they often contain an active, on-going immune response. Obviously, in pediatric patients the ultimate goal is cure. But it is important to bear in mind that stable disease in the setting of immunotherapy may actually be a signal of robust immune activation, and an on-going, almost-successful effort at tumor control.

2.4.5 Using Immunotherapy to Reduce the Dose of Chemotherapy

Pediatric malignancies tend to be chemotherapy-responsive, and the cure rate in pediatrics is much higher than in adults. However, this success is often purchased at the price of higher dose-intensity, especially in patients with high-risk or relapsed disease. Indeed, much of the acute and long-term morbidity and impact on quality of life relates to the side effects of chemotherapy—and, specifically, to the need for higher dose-intensity in many settings. But the use of more dose-intense and toxic regimens has been largely driven by the need to extract more efficacies from chemotherapy

regimens used by themselves. Now that immunotherapy agents are becoming available for combination regimens, it may be possible to leverage the immunotherapy to reduce the dose of the conventional chemotherapy. Whether this approach will be successful in the clinic remains to be seen, but preclinical models would suggest that it does not take ultra-high doses of chemotherapy to release immunologically useful amounts of antigen; and, indeed, higher doses of chemotherapy may actually suppress the immune response of interest. Clinically, the dose-reduction needed to reduce side-effects and increase quality of life may not be huge: even a 25–33% dose-reduction from the MTD may be significantly less toxic. One would not intentionally trade loss of efficacy for fewer side-effects, but if adding immunotherapy allows greater efficacy at lower doses, then this would be an important advance for pediatric patients.

2.4.6 Combination Chemo-Immunotherapy Is Particularly Well-Suited to the Pediatric Population

Because childhood malignancies are typically more responsive to chemotherapy, there is more opportunity to treat even relapsed or metastatic disease with curative intent, rather than just for palliation (as often occurs in adults). Thus, second-line or even third-line regimens in children may have a percentage of patients (albeit perhaps small) who can legitimately hope for cure. This is almost never the case in adults. But the consequence of this superior responsiveness to chemotherapy is that it would be inappropriate in children to attempt to forego standard-of-care chemotherapy, and replace it with immunotherapy alone. This may well be appropriate in adults, where immunotherapy often is used in place of chemotherapy [36], because of the dismal prognosis with chemotherapy. However, in children chemotherapy often still holds prospect of benefit. Thus, in order to offer optimal benefit of immunotherapy to the pediatric population, it is important to seamlessly integrate immunotherapy with standard-of-care chemotherapy. This not only allows immunotherapy to be used earlier in the course of the disease, when the patient's immune system is less compromised by extensive pre-treatment and widespread burden of disease; but it also holds the prospect of enhancing the efficacy of the standard chemotherapy itself, and perhaps reducing the dose-intensity and toxicity.

2.5 Rethinking the Possibilities of Radiation: Using Local Radiation to Create an Endogenous Vaccine

2.5.1 Local Radiation and Systemic Immune Responses: The Abscopal Effect

Radiation kills tumor cells by damaging DNA, via a process that depends heavily on generating oxygen free radicals. The effects of radiation are highly inflammatory, and the dying tumor cells are potentially immunogenic [124]. Because radiation is

so pro-inflammatory (whereas most systemic chemotherapy is not); local irradiation may serve as a valuable adjunct to chemo-immunotherapy. By releasing a wave of tumor antigens and creating local inflammation, ablative radiation may serve as an effective “vaccine” against endogenous tumor antigens [125]. However, just as with cell death after chemotherapy, radiation by itself is rarely able to break tolerance to tumor antigens. However, the tolerogenic and suppressive checkpoints after radiation appear very similar to the checkpoints that restrict response to chemotherapy [126]. Thus, when combined with active immunotherapy, the immunologic effects of radiation may become much more pronounced and exploitable [2].

As an example, in 2012, Wolchok and colleagues reported a serendipitous observation in a patient with metastatic melanoma being treated with CTLA-4 blockade, who also happened to receive concurrent palliative local radiation to a single lesion for relief of spinal-cord compression [127]. Unexpectedly, this patient underwent widespread involution of metastatic lesions outside the field of radiation (the so-called “abscopal” effect), with systemic tumor-specific immune activation. Similar anecdotal abscopal effects have been reported with other immunotherapy, and follow-on clinical trials have been initiated in a variety of indications [128]. In another approach, local irradiation of isolated tumor lesions has been combined with intra-lesional injection of an immune adjuvant (TLR-ligand), and this can actively elicit abscopal responses at distant sites [129].

Thus—at least in principal—local irradiation, with its intense inflammation and endogenous “vaccine” effect, has the potential to make an important and non-redundant contribution to multi-modal immunotherapy regimens.

2.5.2 *Designing Radiation Strategies for Maximal Immune Effect*

If radiation is intended as an immune-activating component of a combination immunotherapy regimen (rather than as a single-agent treatment), then the design considerations become somewhat different. Radiation is highly toxic to normal tissues, yet radiation alone can only be curative if every tumor cell is included in the field. Thus, under conventional strategies, radiation fields must deliberately incorporate a margin of normal tissue so as to include microscopic infiltrating tumor cells. It is this need to irradiate margins of normal tissue that accounts for almost all of the acute and long-term toxicities of radiation. Radiating only the middle of the tumor would have little toxicity, but it would also never be curative, since the residual tumor would rapidly regrow. However, as a component of immunotherapy, irradiating only the lesion itself (without margins of normal tissue) is a perfectly usable source of tumor antigens and the “endogenous vaccine” effect. And, if the immunotherapy works at all, it will work on microscopic residual disease, so there is no need to increase the toxicity of radiation in an attempt to secure extensive margins. As a source of antigens and inflammation, hypofractionated radiation (stereotactic radiosurgery, or stereotactic body radiotherapy) may be more inflammatory and immunogenic than a less toxic hyper-fractionated schedule [125].

When radiation is used as a single agent, the decision whether to use it at all is restricted by considerations of toxicity and futility. Thus, re-irradiation must be undertaken with caution (and often may not be possible); and stereotactic radiation of a lesion is futile (other than for palliation) if other metastatic lesions are present. However, when radiation is used as an integral component of active immunotherapy, then the calculus is changed. Toxicity may be much reduced because margins are not necessary; and it is no longer necessary to include every lesion in the radiation field (since the whole goal is the abscopal effect) [127]. Finally, irradiating a key, life-threatening lesion may de-bulk the tumor and buy valuable time to allow the immune system to generate a subsequent response.

Obviously, all of this new hypothetical calculus depends entirely on the ability of the immunotherapy or chemo-immunotherapy to deliver the necessary efficacy. (If not, then the radiation is indeed futile.) However, the experience in multiple adult immunotherapy trials has been that the immune system is indeed capable of delivering very potent anti-tumor effects—including regression of widespread metastatic disease; and it can work in very aggressive and refractory tumors. Thus, the problem is not the inherent potency of the immune system, but rather inducing that efficacy become a reality in all patients. This is the rationale for adding immunogenic radiation to multi-modal immunotherapy.

2.6 Possibilities of Surgery: Can Immunotherapy Help Downstage Tumors for Definitive Resection?

Finally, we consider surgery. Pediatric surgery has reached a superb level of technical competence, but the role for additional surgical intervention in the face of recurrent or metastatic disease remains limited by the problem of futility. However, if immunotherapy is added to the traditional modalities of chemotherapy and radiation, this may also change the calculus for surgery. First, since the immune system is better at dealing with microscopic disease than with bulky disease, up-front de-bulking surgery (even in relapsed disease) may improve the chances of an immune response. And second, regimens that incorporate immunotherapy may create a situation of prolonged stable disease—e.g., a lesion that does not shrink, but does not spontaneously re-grow either (see Fig. 2.2, above). At what point does it become in the patient's interest to resect this remaining stable lesion(s)? In our own immunotherapy trials, we do not yet have an unambiguous answer to this question. The best approach will have to be empirically determined through clinical trials, and may be a judgment call for each patient. But the addition of an immune effector arm, with its potential for late responses and prolonged stable equilibrium, has raised new potential roles for surgery in the management of patients who show evidence of response.

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