

# Marrying Immunotherapy with Chemotherapy: Why Say IDO?

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## Abstract

**Activation of the tryptophan catabolizing enzyme indoleamine 2,3-dioxygenase (IDO) in cancer cells facilitates immune escape. A recent study now shows how small-molecule inhibitors of IDO can be used to leverage the efficacy of traditional chemotherapeutic drugs that are used to treat cancer in the clinic. By promoting antitumor immune responses in combination with cytotoxic chemotherapy, IDO inhibitors may offer a drug-based strategy to more effectively attack systemic cancer.** (Cancer Res 2005; 65(18): 8065-8)

## Background

During the breakdown in cellular physiology that accompanies malignant tumor development, cancer cells develop certain emblematic characteristics that include inherent cellular properties (cell intrinsic) as well as properties defined through interaction with the host environment (cell extrinsic). Fundamental cell-intrinsic characteristics of cancer cells include immortalization, growth signal self-sufficiency, insensitivity to growth inhibitory signals, and apoptosis resistance, whereas fundamental cell-extrinsic characteristics include the capacity for angiogenesis, invasion, metastasis, and immune escape. Establishment of the importance of immune escape to malignant progression has been relatively recent (1). Indeed, studies of the cell-extrinsic traits of cancer have, in general, tended to lag behind studies of the cell-intrinsic traits, because the former can not be easily evaluated in simple tissue culture systems. Moreover, these processes are generally associated more with epigenetic changes and modifier effects than with mutation of the classically defined oncogene and tumor suppressor pathways that have, until recently, been the major focus of research in molecular cancer biology.

The interactions between developing tumors and the immune system are complex and dynamic. On the one hand, inflammation provides a host of protumorigenic factors and suppression of immune responses can actually promote tumor regression in some model systems (2). On the other hand, cancer cells are also subject to immune surveillance with pressure on tumors to evade or subvert the immune response that tumor antigens should elicit (3). The development of immunotherapeutic strategies has focused predominantly on stimulating or supplementing immune effector cells. It is becoming increasingly apparent, however, that immune tolerance may be dominant in cancer patients and that it will be essential to breach established immune suppressive mechanisms for immunotherapy to be effective (1).

One strategy of immune escape that is used by cancer cells (Fig. 1) has been adapted from a mechanism that normally exists to prevent maternal immune response to paternal fetal antigens that are

present during gestation (4). An inescapable consequence of sexual reproduction among histoincompatible individuals is that some means to circumvent maternal immunity must be hardwired into the system to protect the allogeneic fetus. The catabolic enzyme indoleamine-2,3 dioxygenase (IDO; EC 1.13.11.42) has been implicated in providing immune protection to the developing conceptus. IDO catalyzes the initial step in the degradation of tryptophan in the pathway leading to biosynthesis of NAD<sup>+</sup>. Activation of IDO in placental trophoblast cells has been proposed to lead to the establishment of immune tolerance through either localized depletion of tryptophan or accumulation of toxic catabolites. This process is immune suppressive because T cells undergoing antigen-dependent activation are exquisitely sensitive to local tryptophan catabolism, which can cause them to arrest in G<sub>1</sub>, become anergic, or die (5–7). In a key experiment, treatment of pregnant female mice with 1-methyl-tryptophan, a small-molecule inhibitor of IDO, has been shown to promote T cell-mediated destruction of allogeneic but not syngeneic concepti (4). IDO has also been more generally implicated in CTL-associated protein-4 (CTLA-4)-induced immune tolerance mediated through reverse B7 signaling *in vivo* (8).

## Immune Escape in Cancer: Modulation of Indoleamine-2,3 Dioxygenase Expression by Bin1

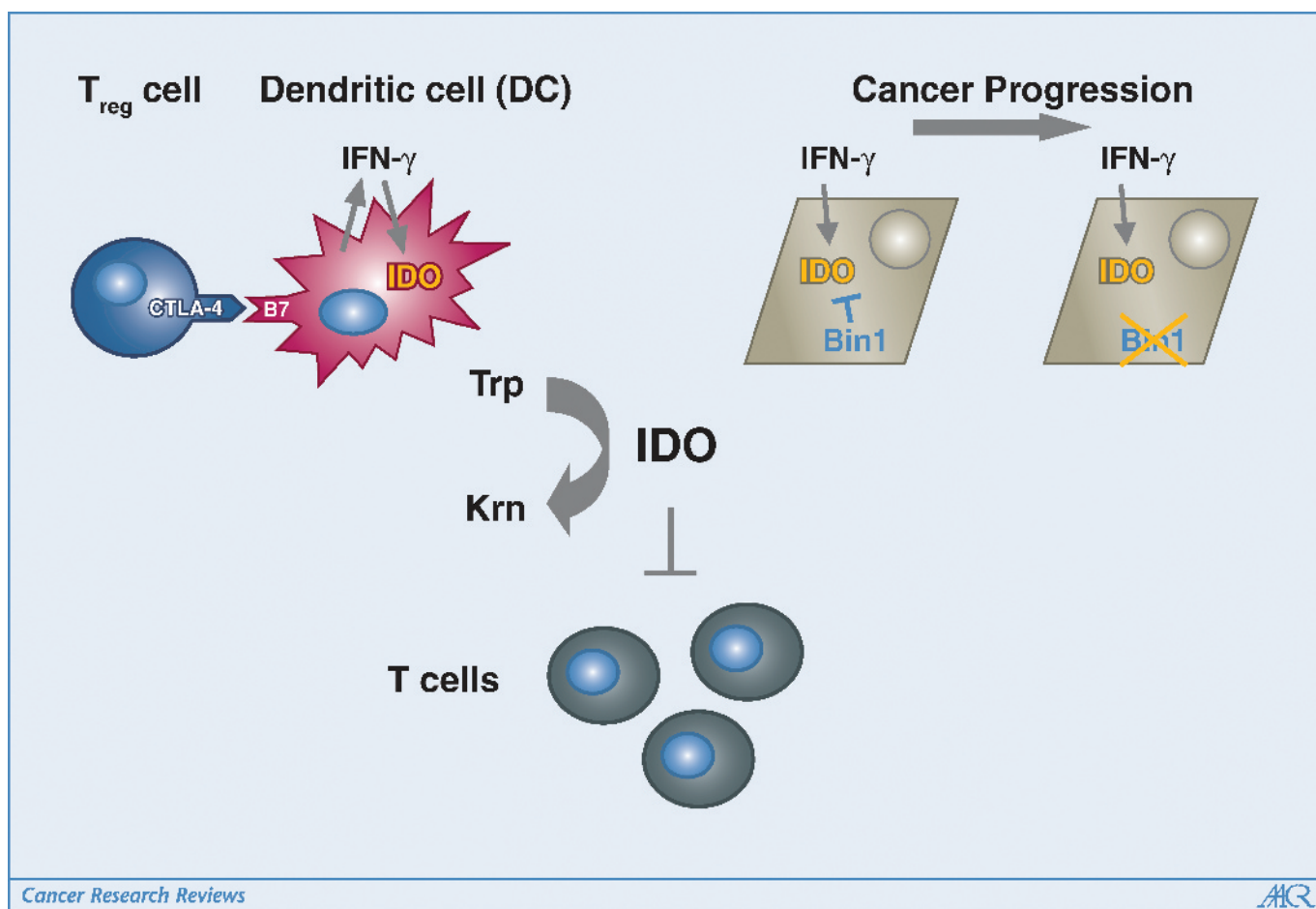
A connection between elevated urinary tryptophan catabolites and bladder cancer was first reported in the 1950s (9). Since then, elevated levels of IDO-generated catabolites have been associated with a number of malignancies. This phenomenon was initially thought to be a tumoricidal consequence of IFN- $\gamma$ , which stimulates expression of IDO in cells (10). However, a radical rethinking of the significance of IDO in cancer has been engendered by its implication in the prevention of allogenic conceptus rejection and by the evidence that IDO is overexpressed in most tumors and/or tumor-draining lymph nodes (11–13). How does IDO become deregulated in cancer cells? One possible answer has emerged from studies of a gene called *Bin1*, a cancer suppressive gene that seems to limit cancer to a large extent by limiting immune escape.

*Bin1* was initially identified in a two-hybrid screen for c-Myc-interacting proteins (14). Along with the *Bin3* gene, *Bin1* is one of two related genes that are conserved through evolution to yeast and that define a family of adapter proteins characterized by a unique fold termed the BAR domain (14, 15). Frequent loss or attenuation of *Bin1* occurs in advanced breast cancer, prostate cancer, melanoma, astrocytoma, neuroblastoma, and colon cancer (16–19).<sup>1</sup> At least 10 different *Bin1* splice isoforms exist in mammalian cells of which two are ubiquitously expressed, whereas the remainder are restricted to specific terminally differentiated tissues including neurons and skeletal muscle cells. The different splice isoforms exhibit different patterns of subcellular localization and cancer suppressive activity, arguing that they have different functions. A precedent for BAR adapter proteins with dual trafficking and transcriptional functions

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<sup>1</sup> K. Xie et al., unpublished observations.



**Figure 1.** Mechanisms of IDO-induced tumoral immune escape. IDO expression in local immune stroma and directly in tumor cells has been implicated in promoting immune tolerance. IDO is upregulated in antigen-presenting dendritic cells (DC) by autocrine IFN- $\gamma$  released as a result of T<sub>reg</sub> cell-induced CTLA-4/B7-dependent cell-cell signaling. Local tryptophan catabolism limits the proliferation and survival of T cells that would otherwise be activated by tumor antigens on the APC. This mechanism may operate in tumor-draining lymph nodes. In tumor cells, attenuation of Bin1 leads to superactivation of IDO expression by IFN- $\gamma$ , directly suppressing activation of T cells in the local tumor environment. Blocking IDO activity systemically with small molecule inhibitors (e.g., 1-methyl-tryptophan) reverses T-cell suppression that occurs as a result of tryptophan catabolism in both settings.

has been established through studies of APPL, a Rab5-binding endosomal protein that translocates to the nucleus upon epidermal growth factor stimulation to associate with the NuRD/MeCP1 nucleosome remodeling and transcriptional repression complex (20). Likewise, the ubiquitously expressed Bin1 splice isoforms, which encode its anticancer properties, have been implicated in both endosomal trafficking and transcriptional repression (21, 22). The possibility that Bin1 adapter proteins may affect pathways leading to the nucleus has garnered additional support based on possible involvement in the trafficking of signal transducer and activator of transcription (STAT) and nuclear factor- $\kappa$ B (NF- $\kappa$ B) transcription factors (23, 24).

Studies aimed at understanding how Bin1 restricts tumor outgrowth identified immune tolerance established through IDO deregulation as a likely mechanistic explanation (25). Deleting the *Bin1* gene from cells resulted in superinduction of IDO gene expression by IFN- $\gamma$ . *In vitro* transformation of *Bin1*-null and *Bin1*-expressing primary mouse embryo keratinocytes with *c-myc* and mutant *Ras* oncogenes produced cell lines with similar *in vitro* growth properties. However, when these cells were grafted s.c. into syngeneic animals, the *Bin1*-null cells formed large tumors, whereas the *Bin1*-expressing cells formed only indolent nodules.

This dichotomy reflected a difference in immune response to the cells, as *Bin1*-expressing cells produced rapidly growing tumors when introduced into either athymic nude mice or syngeneic mice depleted of CD4<sup>+</sup>/CD8<sup>+</sup> T cells. Treatment of mice with the small-molecule IDO inhibitor 1-methyl-tryptophan suppressed the outgrowth of *Bin1*-null MR KEC tumors in syngeneic mice, but had no effect on tumor growth in mice lacking T cells (either nude mice or immunodepleted syngeneic animals). Taken together, these findings indicated that the deregulation of IDO, which accompanies *Bin1* loss in these cells, promotes tumorigenicity by enabling immune escape. The frequent *Bin1* attenuation and IDO overexpression observed in human cancers warrants further evaluation of the relationship between these two events.

### Cooperation of Indoleamine-2,3 Dioxygenase Inhibitors with Chemotherapy

The *Bin1*-IDO studies prompted us to evaluate IDO inhibitors as potential anticancer agents. This effort revealed that immune modulation via IDO inhibition can significantly increase the efficacy of a variety of traditional chemotherapeutic drugs. In several preclinical models of cancer, single-agent therapy with an IDO inhibitor is only marginally efficacious, at best slowing tumor growth

(11, 12, 25). In contrast, regression of established tumors can be achieved by combining an IDO inhibitor with a cytotoxic chemotherapeutic drug (25). In the MMTV-*neu* transgenic mouse model of breast cancer (harboring the *c-neu* proto-oncogene controlled by the mouse mammary tumor virus promoter), which closely resembles human ductal carcinoma *in situ*, established tumors refractory to single-agent therapy underwent regression when enrolled on the combination regimen. This response could not be explained by drug-drug interactions that might raise effective exposure to the cytotoxic agent, and it was dependent on T-cell immunity because depletion of CD4<sup>+</sup> T cells abolished the efficacy of the combination therapy. These results offer an initial step in validating IDO as a drug development target in the context of a cytotoxic combination treatment modality.

As a possible drug development target, IDO has a number of appealing features. First, as a single-chain catalytic enzyme with a well-defined biochemistry, IDO is highly tractable for developing small-molecule inhibitors compared with most other therapeutic targets in cancer. Second, the only other enzyme that catalyzes the same reaction, TDO2, has a more restricted expression and substrate specificity, mitigating “off-target” issues posed by novel agents. Third, bioactive and orally bioavailable “lead” inhibitors exist that serve as useful tools for preclinical validation studies. Fourth, an *Indo* gene “knockout” mouse has been reported to be viable and healthy (26), indicating that IDO inhibitors will be unlikely to produce unmanageable mechanism-based toxicities (although promotion of inflammatory conditions would remain a valid concern). Fifth, pharmacodynamic evaluation of IDO inhibitors can be done easily by examining the blood serum levels of tryptophan and kynurenine, the chief substrate and downstream product of the IDO reaction, respectively. Lastly, small-molecule inhibitors of IDO likely offer substantial logistical and cost advantages relative to biological or cell-based therapies that aim at modulating immunity. IDO inhibitors may be useful not only in cancer but also in other pathologic settings, where it is desirable to relieve immune suppression and/or break immune tolerance (e.g., chronic viral infections).

## Future Perspective

One general question raised by the work on combining IDO inhibitors with cytotoxic agents is how an immunotherapy can effectively enhance the efficacy of chemotherapy. As detailed elsewhere (27), there are at least six critical factors for inducing an antitumor immune response that might be augmented by cytotoxic chemotherapy including antigen threshold, antigen presentation, T-cell response, T-cell traffic, target destruction, and generation of memory. Consensus is lacking as to whether chemotherapy affects immune responsiveness through direct disruption of toleragenic mechanisms or indirectly through tumor cell killing. In some experimental settings, tumor cell killing by cytotoxic agents has been shown to be critical for cooperativity with no evidence of direct effects on cross-presentation by antigen-presenting cells (APC) or on endogenous immune responsiveness (27). The finding that tumor cells killed by alkylating agents such as cyclophosphamide are more effective at activating APCs, when compared with tumor cells killed by antimetabolites or freeze thaw (28), suggests some specificity to this mechanism of immune stimulation. IFN- $\gamma$  can reportedly sensitize resistant tumor cell lines to apoptosis induction by cytotoxic agents independent of their p53 status (29). In this way, immunotherapy might cooperate with chemotherapy to augment

tumor cell killing and indirectly generate additional proinflammatory signals. On the other hand, there is a long history of cyclophosphamide treatment preferentially neutralizing the suppressor arm of the immune system to enhance antitumor responses (30), and such a mechanism of action has been suggested for other cytotoxic agents as well (31). Recently, there has been a growing realization that it is precisely these tolerizing mechanisms that must be overcome for an immunotherapeutic strategy to be successful (1). In this context, both an IDO inhibitor and a cytotoxic agent might be acting as complimentary immunotherapies. Studies have indeed shown that when enhancement of antitumor T-cell responses by immunotherapy with CTLA-4 antibodies (CTLA-4 blockade) was combined with subtherapeutic doses of chemotherapy that shifted the cytokine profile to that of a Th1 response, this potentiated the treatment of established tumors in a mouse model and correlated with enhanced Th1 responsiveness in the treated mice (31). In this context, it is interesting to note that IDO has been proposed to be a downstream effector for the induction of CTLA-4-mediated immune tolerance (8).

IFN- $\gamma$  may provide a key to understanding how the complex interplay between tumor and stroma is affected by IDO activity and inhibition. A number of reports argue that IFN- $\gamma$  suppresses tumor outgrowth. Likewise, IDO activity can have antitumor consequences and its up-regulation by IFN- $\gamma$  may significantly contribute to the negative effect of IFN- $\gamma$  on tumors (10). These observations seem to run counter to the idea that IDO contributes positively to tumorigenesis, but this interpretation ignores the inherently complex and evolving nature of the interaction between developing tumors and the host immune system. IFN- $\gamma$  has been directly implicated in the process called immune editing, whereby the immunogenic environment of the host provides positive selection for reduced tumoral immunogenicity (3). Specifically, IFN- $\gamma$  signaling contributes to an immune-based host environment that suppresses tumor incidence but which can also drive formation of tumors that are more highly aggressive within an immune context (33). At early stages of tumor development, IDO up-regulation by IFN- $\gamma$  may be detrimental. However, if tumor cells can adapt to the tryptophan poor environment, then keeping IDO under IFN- $\gamma$  control could give tumor cells the flexibility of turning IDO off and thereby mitigating its negative consequences in the absence of elevated IFN- $\gamma$  levels that would signal an active Th1 response.

Alternatively, because IDO acts as the rate-limiting enzyme in NAD<sup>+</sup> biosynthesis, one can also envision scenarios in which constitutive expression of IDO in cancer cells is intrinsically beneficial (e.g., under hypoxic conditions that tend to confer drug resistance). Notably, poly(ADP-ribose) polymerase (PARP)-mediated NAD<sup>+</sup> consumption drives “programmed necrosis” independent of the major apoptotic effectors p53, Bax, Bak, and caspases in cancer cells that have become dependent on glycolysis to maintain ATP levels (34). If tumor cells turn on the NAD<sup>+</sup> biosynthesis pathway, they may be able to override sensitization to PARP. In targeting the rate-limiting step for NAD<sup>+</sup> biosynthesis, IDO inhibitors would be expected to cooperate with chemotherapeutic drugs by reestablishing the sensitivity of tumor cells to PARP activation by these drugs. Unlike apoptosis, this necrotic form of cell death is highly proinflammatory potentially incorporating an immune component into the therapeutic response as well. By raising these issues, studies of IDO inhibitor cooperativity with chemotherapy should not only provide insights into the mechanistic basis for this new therapeutic approach but may also afford a deeper understanding of the complex contextual relationship between cancer cells and the multifaceted immune/stromal environment.

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