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Inflammatory reprogramming with IDO1 inhibitors: turning immunologically unresponsive ‘cold’ tumors ‘hot’

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Abstract

We discuss how small molecule inhibitors of the tryptophan catabolic enzyme indoleamine 2,3-dioxygenase (IDOi) represent a vanguard of new immunometabolic adjuvants to safely enhance the efficacy of cancer immunotherapy, radiotherapy or 'immunogenic' chemotherapy by leveraging responses to tumor neoantigens. IDO activation in cancer supports inflammatory processes that IDOi can re-program to help clear tumors by blunting tumor neovascularization and restoring immunosurveillance. Studies of regulatory and effector pathways illuminate IDO as an inflammatory modifier. Recent work suggests that coordinate targeting of the Trp catabolic enzymes TDO and IDO2 may also safely broaden efficacy. Understanding IDOi as adjuvants to turn immunologically 'cold' tumors 'hot' can seed new concepts in how to improve the efficacy of cancer therapy while limiting its collateral damage.

Keywords

immunometabolism; immunotherapy

Leveraging cancer therapy with immunometabolic adjuvants

The elucidation of immune escape mechanisms in cancer has yielded clinical breakthroughs that have remarried tumor immunology to the mainstream of cancer research [1, 2]. As one striking illustration, up to 50% of advanced melanoma patients receiving combined immune checkpoint drug therapy (anti-CTLA-4 plus anti-PD1) will exhibit complete responses even though these drugs act only by relieving immune suppression. Thus, it appears that the host

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Conflict of Interest Statement

G.C. Prendergast and A.J. Muller state a conflict of interest as shareholders and G.C. Prendergast as a former grant recipient and compensated member of the scientific advisory board for New Link Genetics Corporation, a biopharmaceutical company that has licensed IDO intellectual property for clinical development from the Lankenau Institute of Medical Research, as described in U.S. Patents Nos. 7705022, 7714139, 8008281, 8058416, 8383613, 8389568, 8436151, 8476454 and 8586636. A. Mondal recently accepted a position at Incyte Corporation which is also engaged in the clinical development of IDO inhibitors. The other authors state no conflict of interest.

Resources

i <http://www.newlinkgenetics.com>

ii <https://www.incyte.com>

immune system in many melanoma patients is latently active and when de-repressed can durably clear neoplastic cells [3]. Is it possible to extend responses in immunologically non-responding ‘cold’ tumors? If so, how? Correlational studies to date associate immune checkpoint drug efficacy with high neoantigen loads in tumors [4, 5]. Thus, strategies to elevate neoantigen load may help turn ‘cold’ tumors ‘hot’, e.g. by combinations with chemotherapy, radiotherapy or inhibitors of DNA damage repair. But it may also prove fruitful to identify adjuvants that can re-program the host inflammatory milieu to enable responses to the lower neoantigen loads of ‘cold’ tumors. Indeed, in considering the breakthroughs in infectious disease in the 20th century, the discovery of adjuvant substances that suitably programmed inflammation to empower vaccines was a pivotal advance. In like manner, creating systemic immunity that is needed to eradicate cancer [6] may rely on adjuvant substances that can suitably program inflammatory processes to re-engage immunosurveillance, i.e. the clearance of heterogeneous, plastic ‘rogue’ cells which arise during natural lifespan as a result of the inescapable interplay of oncogenesis and immunoediting (**Figure 1**). Hallmark metabolic disruptions in cancer point logically to metabolic inhibitors as a wellspring of adjuvant substances to improve cancer treatment. In this review, we survey understanding of IDO1 as an inflammatory modifier and how this understanding suggests ways to fully exploit IDO inhibitors as immunometabolic adjuvants to improve the efficacy and safety of cancer therapy while limiting collateral side-effects and long-term damage in patients.

IDO1 modifies inflammation and immunity

IDO1 is one of three tryptophan catabolic enzymes in mammals, (IDO1, IDO2 and TDO), that catalyze conversion of the essential amino acid tryptophan (Trp) to kynurenine (Kyn). Historical studies of the kynurenine pathway of tryptophan catabolism have defined roles in neurological and immunological functions [7–9]. IDO1 is extrahepatic and inducible in many cell types by interferons and other diverse pro-inflammatory signals. High basal levels of IDO1 are expressed on mucosal surfaces. In contrast, TDO is expressed mainly in liver where it controls Trp homeostasis. IDO2 is also expressed in liver as well as brain, kidney and antigen-presenting cells, but its catalytic activity is relatively weaker and it does not contribute to systemic Trp catabolism [10–12]. In terms of tumoral expression, there remains significant gaps and some conflict in the literature on precisely where these enzymes are expressed and active. In particular, there is conflicting data over whether IDO1 is expressed in normal or tumor-draining lymph nodes (TDLN), and also whether there is an increase in the number of IDO1-expressing cells in TDLN relative to normal nodes. In TDLN from melanoma or breast cancer patients, no difference in the number of IDO1-expressing cells was observed relative to normal nodes [13], in contrast to previous reports [14–17], although evidence of IDO1 activity required for tolerigenic function has been offered [18, 19]. Recently, a knockout-validated, mouse IDO1-specific antibody was reported [20] for murine studies along with Kyn-specific antibodies that may report on IDO/TDO activity [21]. Two caveats here are that nitric oxide produced at tumor sites can inhibit IDO1 activity [22], and Kyn degradation at tumor sites may confound interpretation of IDO/TDO activity. As immunohistological questions concerning the patterns of expression and function are

resolved in more detail in various tissue settings, there is nevertheless general agreement that IDO1 is commonly expressed and active in the tumor microenvironment itself.

IDO1 was initially recognized as an immunomodulator by the discovery that administering 1-methyl-tryptophan (1MT), a weak bioactive inhibitor [23], could trigger T cell-dependent rejection of an allogeneic fetus in pregnant mice [24]. The concept of IDO1 as an immunosuppressive actor was extended by later discoveries that it was a common pathophysiological mediator of tumoral immune escape [25]. As a classical pharmacological target, IDO was ripe for development of small molecule inhibitors [26] with medicinal chemistry groups at New Link Geneticsⁱ and Incyteⁱⁱ ultimately advancing several mechanistically distinct compounds to clinical trials [27]. Efforts to elucidate IDO1 regulatory and effector pathways discussed below have illuminated its major function as a inflammatory modifier in cancer (**Figure 2**).

Regulatory pathways

In response to diverse pro-inflammatory signals, many cells in tumors upregulate IDO1 including malignant cells, stromal cells, lymphocytic cells and dendritic cells. Many studies have identified IDO1 activation by type I and II interferons (IFNs), pathogen-associated and damage-associated molecular patterns (PAMP, DAMP), phorbol myristate acetate (PMA), prostaglandin E2 (PGE2), tumor necrosis factor (TNF α) and transforming growth factor- β (TGF- β) [28, 29]. In malignant cells, a variety of oncogenic signals drive IDO1 expression levels, including Bin1 suppressor attenuation, Jak/STAT, Ras/PKC and Kit [30–33]. Additional control occurs at the level of protein stability, with the E3 ligase-recruiting factor SOCS3 binding phosphotyrosine residues and ubiquitinating IDO1 to drive proteolytic degradation [18, 34].

T cell co-receptor molecules that negatively regulate immune responsiveness (immune checkpoints) are interconnected with IDO1 regulation. Upon engagement, several negative acting co-regulatory receptors on T cells trigger IDO1 expression in dendritic cells (DC) and other antigen-presenting cells via associated co-regulatory receptor ligands ('reverse signaling'). For example, T cell-associated CD80/CD86 or GITR activate CTLA-4 or CD200 on DC, respectively, to increase IDO1 expression and promote T cell tolerance [35, 36]. In DC, type I IFNs mediate the 'reverse signaling' to IDO1 after ligation of CTLA-4 or Toll-like receptors (TLR). Likewise, type I IFNs are critical mediators in the ability to STING to activate IDO1, an event which impacts the growth of tumors of low antigenicity [37, 38]. Notably, PD-1/PD-L1 reverse signaling also induces IDO1. Given that clinical trials of IDO1 inhibitors with anti-PD1 are showing promising results, these shared regulatory connections provide a rationale to evaluate additional combinations of immune checkpoint inhibitors with IDO1 inhibitors.

High constitutive expression of IDO1 observed in many malignant cells may be facilitated in large part by BIN1 suppressor attenuation and COX2-mediated PGE2 production [30, 39–41]. Indeed, an intimate relationship between COX2 and IDO1 appears to exist in cancer-promoting inflammation and immune escape, which genetic studies of IDO1 in the mouse suggest are genetically overlapping [42]. PGE2, a root driver of cancer-promoting inflammation, acts with additional factors such as TNF- α and TLR ligands (PAMP) to fully

induce IDO1 enzymatic activity in DC and other immune cells [28]. IDO1, in turn, participates in a feed-forward loop to support PGE2 generation and inflammation. Recent work underscores the significance of COX2 in activating IDO1 in tumor cells [41]. Accordingly, IDO1 blockade reduces PGE2 levels, whereas PGE2 blockade reduces IDO1 activity [43–45]. This connection is not only involved in T cell anergy but also in the generation of myeloid-derived suppressor cells (MDSC), as indicated by earlier studies [46, 47]. Further, recent work clearly connects IDO1, arginase and polyamines in MDSC accumulation and function [48, 49]. In summary, IDO1 is the focus of numerous major pro-inflammatory pathways in cancer where it acts as a pivotal modifier of disease progression.

Effector pathways

IDO1 activity stimulates three main effector pathways in T cells and other cells responding to Kyn production and locoregional Trp deprivation in the tumor microenvironment. Activation of the stress kinase GCN2 and inhibition of the metabolic kinase mTORC1 mediate the effects of Trp deprivation. The third pathway involves the Trp catabolite Kyn binding to the aryl hydrocarbon receptor (AHR). There also appears to be a fourth pathway, little understood as yet, in which IDO1 acts in a non-catalytic manner as a signaling protein to mediate effects of TGF- β -mediated immunomodulation [19]. Together, these pathways are responsible for mediating the major effects of IDO1 on immunosuppression and neovascularization in cancer.

The starvation stress kinase GCN2 is activated as a result of binding uncharged Trp-tRNA which accumulates due to local IDO1 activation [50]. When activated, GCN2 phosphorylates the key protein translation regulator eIF2, which coordinately retards translation and stimulates the integrated stress response (ISR) pathway [51]. By promoting preferential transcriptional and translational events, the ISR helps cells cope with stress conditions. In T effector cells experiencing local Trp deprivation, GCN2 activation may trigger cell cycle arrest and/or apoptosis, engendering immunosuppressive conditions in a growing tumor. Notably, T cells from GCN2-deficient mice are not suppressed (anergized) by IDO1-expressing DC [50]. A key ISR mediator in this context is the transcription factor CHOP, which alters the activity of the metabolic transcription factor C/EBP β in directing expression of several inflammatory cytokines including IL-6 [52], which in turn is critical for IDO1-mediated MDSC formation and metastatic tumor outgrowth [33]. Notably, genetic deletion of IDO1 attenuates IL-6 production, leading to a defect in MDSC accumulation and function that is associated with reductions in tumor neovascularization and metastatic progression [33, 50]. The GCN2 pathway downstream of IDO1 may have a contextual weighting in different tumors, with preclinical studies suggesting it has less impact in skin cancers but more impact in gliomas [39, 53].

The master metabolic regulatory kinase mTORC1 is networked to sensors of glucose and amino acid levels needed to coordinate cell growth and proliferation. Nutrient deprivation inhibits mTORC1, thus blocking its phosphorylation of the ribosomal kinase S6K, which controls ribosome biogenesis, as well as 4EBP, which regulates translation [54, 55]. Tryptophan deprivation due to IDO1 activity leads to inhibition of mTORC1, thereby stimulating autophagy in an effort to recover Trp from cellular protein stores [56].

Interestingly, this negative regulatory impact can be reversed not only by restoration of L-Trp but also by exposure to 1MT (indoximod), revealing a key mechanism of action of this IDO1 pathway inhibitor now entering Phase III clinical trials [56]. The precise connections between IDO1 activity and mTORC1 repression are not yet defined. However, mTORC1 is controlled by the amino acid sensing kinase GLK1 which acts upstream of mTORC1 in response to Trp catabolism [57]. Also, the T cell receptor regulatory kinase PKC- θ is downregulated in T cells by local IDO1 activity [29, 56]. Both mTORC1 and PKC- θ are regulated by GLK1, suggesting that GLK1 may be a nodal point for Trp catabolic signaling in T cells. In summary, the mTORC1 effector pathway provides a mechanism through which local IDO1 activity can influence signaling by the T cell receptor in activating or tolerizing T cells to antigen.

Kynurenine as well as the downstream catabolites 3-hydroxy-kynurenine (3-HK) and kynurenic acid (KA) each contribute to IDO1-mediated immunosuppression [29, 58]. Though most research has focused on IDO1, TDO and the more recently identified enzyme IDO2 similarly catalyze Kyn production [10, 59]. In a pivotal advance, Platten and colleagues showed that Kyn is a native ligand for the aryl hydrocarbon receptor (AHR) [60], a transcription factor with important roles in modulating inflammation and tumor immunity [61, 62]. Other Trp metabolites may also be AHR ligands. As with increased IDO1 expression, elevated AHR in tumors corresponds with poor prognoses in cancer patients. AHR is expressed in many immune cells including T cells, B cells, macrophages, DC as well as epithelial cells. Genetic studies in mice along with studies in human tumors have shown that Kyn binding to AHR promotes naïve CD4⁺ T helper cell differentiation events that favor Treg phenotypes, while simultaneously blunting differentiation events that lead to Th17 cells [62, 63]. AHR binds DNA through heterodimers with ARNT/HIF-1 β , which also partners with the hypoxia-induced transcription factor HIF-1 α , a critical regulator of malignancy. Interestingly, both IDO1 and IDO2 are AHR target genes that are transcriptionally upregulated by ligand-stimulated AHR signaling in myeloid cells [64, 65]. Thus, IDO1 contributes to a feed-forward expression loop with both COX2 and IDO2 in inflammatory programming of the tumor microenvironment.

IDO1 activity ablates T effector cells and promotes formation of Treg and MDSC

IDO1 is widely expressed in human cancers [13] at multiple sites where it may act to promote immune tolerance (**Figure 4**). While T cells do not express IDO1, they are a primary responder to its activity in the tumor microenvironment. IDO1 exerts both direct and indirect effects on effector T cells through depletion of Trp and production of Kyn and downstream catabolites, which impair both CD8⁺ and CD4⁺ effector T cell function *in vitro* [50, 66]. Although less studied, IDO1 also restricts natural killer (NK) cells, the cytolytic activity of which was found to be limited by Kyn exposure [67, 68], although a recent study has been contradictory [69]. IDO1 also enhances the suppressive activity of T regulatory cells (Tregs). In vitro studies indicate that Kyn signals through AHR to promote the generation of FoxP3⁺ inducible Tregs [70]. Furthermore, Trp depletion can trigger GCN2 to accentuate resident Tregs, upregulating PD-1 and PTEN signaling that is needed to maintain a Treg suppressive phenotype in vitro [71–73]. Excellent reviews surveying mechanisms of

action of IDO1 in mediating T cell tolerance via Treg induction/activation have appeared recently [8, 74].

As major mediators of tumor immunosuppression in many human cancers, MDSCs are immature bone marrow-derived hematopoietic cells which are functionally defined by their ability to suppress T cell activity [75]. MDSC utilize key metabolic pathways to exert their T cell suppressive effects [76, 77]. Notably, IDO1 is critical for MDSC expansion and function (**Figure 4**). In two models of invasive lung tumors, IDO1-deficient mice were resistant to malignant outgrowth and MDSC obtained from these animals were impaired for suppression of both CD8⁺ and CD4⁺ T cells. IL-6 attenuation was a key feature of IDO1 loss, with defective MDSC function and pulmonary metastasis outgrowth in *Ido1*^{-/-} mice each rescued by ectopic expression of IL-6 [33]. Thus, IDO1 controls MDSC suppressor function by controlling the inflammatory milieu. A similar role was established in melanoma-bearing mice, where IDO1 was required to recruit and sustain MDSCs along with the ability to mediate Treg recruitment [78, 79]. In mouse models, IDO1 does not appear to be directly expressed in MDSCs, however, studies in human breast and hematological cancers have identified populations of IDO1-expressing MDSCs with immunosuppressive function [80, 81]. Thus, IDO1 can promote MDSCs through multiple mechanisms by licensing their expansion, tumor recruitment and immunosuppressive prowess.

Stromal cells in tumors can also express IDO1 but their contributions are generally less clear to date. Tumor-associated macrophages and neutrophils (TAMs and TANs) express IDO1 which may contribute to their immunosuppressive roles [82, 83]. Cancer-associated fibroblasts (CAFs) which contribute to the fibrotic component of many tumors may express IDO1 due to locoregional PGE2 production [84, 85]. Lastly, IDO1 is also expressed in the endothelial cells of the tortuous blood vasculature in certain tumors, such as renal tumors where its expression has been associated paradoxically with good prognosis [86].

IDO2 and TDO in inflammation and immunity

Recent studies of a second IDO isoform, designated INDOL1 or IDO2 [10, 11], add a wrinkle to the complex picture of how IDO functions as an inflammatory modifier. IDO2 is less catalytically active than IDO1 but it also has different biochemical requirements for activity that are still being unraveled [87, 88]. One interesting feature of human IDO2 is common occurrence of two genetic polymorphisms that attenuate or abolish enzymatic function in human populations [10]. As summarized in **Figure 3**, mouse genetic studies define IDO2 as an immunomodulator which appears to genetically interact with IDO1 but also to exert unique independent functions [89]. Mouse genetic studies showed that while IDO2 and IDO1 modulate unique inflammatory programs, IDO2 was also required for IDO1-mediated induction of regulatory T cells in an established model [12]. Notably, studies suggest that IDO2 functions in B cells where it supports autoantibody production in a model of autoimmune arthritis where 1MT has therapeutic activity and IDO2 ablation phenocopies this effect [90, 91]. These genetic results support earlier evidence of a role for IDO2 in mediating 1MT responses in some settings [89, 92], an issue which may cloud interpretations in the IDO literature which unsuitably employs 1MT as an IDO1-specific probe.

Observations connecting IDO2 and B cell responses are intriguing in their contrast with IDO1 and T cell responses. There is certainly a complex relationship between autoimmunity and cancer, as reflected in clinical paraneoplastic syndromes and the need to erect a specialized autoimmune response against the ‘altered-self’ of cancer to achieve durable cures [93]. The likelihood of IDO1-IDO2 genetic interaction is suggested by evidence of functional mosaicism in IDO2 expression in hematopoietic cells from IDO1-deficient mice [12]. While IDO2 investigations are still very much in their infancy, efforts to understand their interplay may illuminate the relationship between autoimmune responses that are beneficial – i.e. as produced by cancer immunotherapy – and those that are pathogenic – i.e. as manifested by side-effects in patients receiving cancer immunotherapy. Given that IDO2 may help sustain certain autoimmune responses, the development of small molecule inhibitors to inhibit both IDO1 and IDO2 might expand therapeutic windows at both ends, through increased efficacy via IDO1 inhibition and decreased autoimmune side-effects via IDO2 inhibition.

Like IDO1, TDO has been implicated in immune escape [59, 62, 94], suggesting its inhibition also offers a cancer immunomodulatory strategy [59, 62]. TDO-deficient mice accumulate L-Trp and show neurologic alterations attributable to serotonin elevation [95]. Treating mice with a selective TDO inhibitor produces similar effects and also increases sensitivity to endotoxin-induced shock, supporting a role in inflammatory processes [96]. However, TDO exhibits differences in its inflammatory roles that are not yet understood [97]. The contribution of TDO to immune escape has been established with bioactive inhibitors [60, 98] but with no genetic validation in mice as yet. However, given initial evidence of TDO expression in human cancer and its overlap with IDO1 in certain tumors, there is a sound pharmacological rationale to explore TDO and IDO1/TDO combination inhibitors. Notably TDO has been found to act beyond immune escape to promote tumor cell survival and metastatic capacity in aggressive triple-negative breast cancers [99]. In this study, TDO-induced kynurenine could activate the AhR signaling pathway, and blocking TDO or AhR was sufficient to reduce cell survival and metastasis. Thus, TDO in cancer can also be interpreted as an inflammatory modifier providing a selective advantage beyond simply enabling immune escape, like IDO1.

Inflammatory neovascularization: a newly recognized function of IDO1

Given the contradictory roles of IDO1 and IFN- γ in tumor immunity, it has been difficult to understand why IFN- γ is an upstream inducer of IDO1, indeed the first one to be discovered [100]. It has long been noted that IDO1 might counterbalance IFN- γ in some manner but what this specifically entailed was poorly understood. Although IFN- γ studies in cancer have overwhelmingly focused on its tumor cell directed and immunomodulatory effects, it has also been reported that IFN- γ is anti-angiogenic in tumors [101, 102]. Blood vessel formation during tumor growth is referred to as neovascularization, which, unlike physiologic angiogenesis, involves excessive and disorganized growth of blood vessels similar to that induced by ischemia in certain normal tissues like the retina or lungs. In a mouse model that involved CD4⁺ and CD8⁺ T cell-mediated tumor rejection, it was found that IFN- γ acted primarily through an indirect anti-angiogenic mechanism to enable tumor cell killing [101, 102]. However, the intermediary of this effect was undefined.

Recent work has established IDO1 as an intermediary in mitigating the anti-angiogenic effects of IFN- γ . Initial studies of *Ido1*^{-/-} mice revealed a reduction in blood vessel density in normal lungs [33]. Subsequent studies corroborated the hypothesis that IDO1 promotes inflammatory neovascularization by counteracting the anti-angiogenic activity of IFN γ in 4T1 breast cancer pulmonary metastases and in oxygen-induced retinopathy (OIR) [103], an established model for studying factors contributing to neovascularization [104]. IDO1 ablation by gene deletion, siRNA or IDOi treatment (epacadostat) were sufficient to reduce levels of pathological neovascularization in the OIR model to a similar extent as targeting the angiogenic factor VEGF-A [103]. Notably, normal retinal vascular development that occurs under normoxic conditions was unaffected in *Ido1*^{-/-} mice. Likewise, physiological re-vascularization that occurs along with neovascularization in the OIR model was not impaired, indeed, it was increased, suggesting that IDOi may offer therapeutic utility to overcome the indiscriminate targeting of normal compensatory re-vascularization which is a detrimental effect of anti-VEGF therapy.

The likelihood that IDO1 acts as a negative feedback on the anti-angiogenic activity of IFN γ was confirmed in mice lacking both IFN γ and IDO1, which exhibited wild type levels of neovascularization. Further, *Ifn γ* ^{-/-}*Ido1*^{-/-} mutant mice developed and died from pulmonary metastases at rates similar to wild type mice and accelerated relative to *Ido1*^{-/-} mice [103]. In contrast to IFN γ , IL-6 is a pro-angiogenic inflammatory cytokine that is important for ischemia-induced neovascularization in lungs [105]. IL-6 deletion was found to phenocopy IDO1 deletion in terms of neovascularization in the OIR and 4T1 metastasis models, delaying 4T1 metastasis development and improving survival similar to *Ido1*^{-/-} mice. Furthermore, *Ifn γ* ^{-/-}*Il6*^{-/-} mutant mice exhibited wild-type levels of neovascularization in both the OIR and 4T1 metastasis models, while developing and dying from pulmonary metastases at a rate comparable to wild-type mice [103]. Overall, these results established that IDO1 is a critical intermediary between IFN γ and IL-6 in promoting a pro-angiogenic inflammatory state. A major implication of this work is that IDOi may have anti-angiogenic activity in cancer.

The new perspective on IDO1 as an modifier of inflammatory neovascularization is consistent with recent work on IL-6. Defective angiogenesis stimulated by IL-6 is independent of VEGF-A [106]. Upregulation of STAT3 signaling downstream of IL-6 is an important resistance mechanism for anti-VEGF therapies [107]. Thus, IDOi may be able to target this alternate pathway to broaden the effect of anti-VEGF therapies in the same way that IDOi can leverage immunogenic responses. Indeed, IDOi may have unrecognized utility for treating a variety of neovascularization-associated diseases beyond cancer, e.g., ‘wet’ macular degeneration and other retinopathies. In such settings, VEGF targeting is approved but is not optimal, due to the indiscriminate suppression of compensatory intraretinal re-vascularization and resulting non-responsiveness in many patients. Thus, a mechanistic rationale exists to evaluate IDOi as therapy for ocular diseases of abnormal neovascularization, possibly as a topical formulation (e.g. eye drops) rather than an anti-VEGF injectable. In summary, these findings add a new dimension to the potential utility and breadth of application of IDOi with a new mechanism-based feature to take into consideration in designing clinical oncology trials.

Therapeutic approaches to block IDO function

While proceeding through clinical development, IDO1 inhibitors have been termed immune checkpoint therapies - cancer immunotherapy in a pill - but these agents would seem to be better understood as immunometabolic adjuvants which modulate inflammatory processes. This conceptualization yields a broader perspective on the significance of this new class of drugs in medicine, which offer logistical advantages for combinations with biological or cell-based alternatives delivered intravenously for immunomodulation [26]. **Figure 5** summarizes the first set of mechanistically distinct compounds to enter clinical trials which block IDO1 function in different ways. **Table I** provides greater detail of all IDOi reported in preclinical or clinical development from pharmaceutical industry. Many clinical trials are ongoing with the ones of chief interest involving drug combinations where preclinical work argues IDOi may be most useful (<http://clinicaltrials.gov/ct2/results?term=IDO&Search=Search>). Careful examination of the phenotype of *Ido1*^{-/-} mice suggests that IDOi may not be free of side-effects but may be much less toxic than immune checkpoint drugs or traditional or targeted chemotherapy [108]. A detailed review on the preclinical discovery and development of the first IDOi to be translated to clinic – indoximod, epacadastat and navoximod – will appear elsewhere [27]. There also have been several excellent comprehensive reviews with detailed surveys of medicinal chemistry, pharmacological and biological considerations [8, 59, 74, 109–112]. Here we provide a brief overview of IDOi discovery with regard to biological milestones in proof of concept for cancer therapy.

Indoximod

The IDO1 inhibitor most often deployed in the literature is the simple agent 1-methyl-D,L-tryptophan (1MT), a low specificity and low potency inhibitor with a K_i of 34 μ M [23, 113]. The L isomer, actually a weak substrate for IDO1, is responsible for the weak inhibitory activity insofar as the D isomer neither binds nor inhibits IDO1 [29]. Early studies showed that 1MT could weakly inhibit the growth of tumor cells engrafted into syngeneic hosts [114, 115], with subsequent work showing that 1MT was far more efficacious if combined with immunogenic chemotherapy [30]. However, it quickly became clear to molecular pharmacologists that 1MT is not a valid inhibitor of IDO1 enzyme activity and therefore not suited to address the candidacy of the IDO1 enzyme as a therapeutic target. Nevertheless, 1MT is commonly used as an IDO1 probe in preclinical studies, and detailed investigations of its antitumor activity led to successful clinical translation of the D racemer (now known as indoximod), as a clinical drug candidate with unique properties [27]. As alluded to above, indoximod has a unique mechanism of action, acting with high potency to restore mTORC1 activity in T cells in an environment of IDO1-mediated Trp depletion [56]. The finding that mTORC1 interprets indoximod – essentially a D-Trp analog – as a mimetic of L-Trp is pivotal. Since IDO1 is not expressed in T cells, but exerts its effects from a neighboring cellular milieu, indoximod acts differently from true IDO1 enzyme inhibitors and may act by directly targeting T cells. It is also important to point out that if indoximod acts downstream of IDO1 in the mTORC effector pathway it may be active against tumors driven by any tryptophan catabolic enzyme(s) and may also be less prone to drug resistance that may be expected for a IDO1-specific enzyme inhibitor. Recent clinical data indicates that indoximod

safely heightens the efficacy of anti-PD1 in melanoma patients [116], consistent with preclinical data [117], suggesting that mTORC1 restoration may be a sufficient cause of the antitumor effects of IDO1 blockade [116]. D-amino acids are used for signaling by gut bacteria and the potential interface of IDO1 with the gut microbiome is intriguing to consider in light of D-Trp and its mimetic indoximod as possible PAMP molecules [118, 119]. Further mechanistic investigations of indoximod might yield important new therapeutic directions.

Enzyme Inhibitors

The discovery of a BIN1-IDO1 linkage in immune escape prompted initiation of an IDO1 inhibitor screening program by our group at Lankenau in 2002. Several structural classes of bioactive inhibitors were identified which all displayed similar biological properties, namely, antitumor effects reliant upon T cell function and IDO1 targeting [30, 120–124]. Among the bioactive inhibitors identified, MTH-Trp was the first to clearly demonstrate that IDO1 enzyme inhibition exerted antitumor properties [30], a finding corroborated and extended later with additional structurally distinct classes of IDO1 inhibitors [120–122, 124]. Overall, preclinical genetic and pharmacological proof of concept was achieved, validating IDO1 blockade as a therapeutic approach in cancer based on modulation of inflammation, adaptive immunity and neovascularization [31, 33, 103].

A phenylimidazole chemotype deemed most promising from this effort was explored by Kumar and colleagues through structure-based drug design to probe the IDO1 active site entry region, the active site interior, and the heme iron-binding group [123]. Subsequently Kumar moved to NewLink Genetics Corporation where the medicinal chemistry effort yielded an imidazoisindole series of Trp non-competitive inhibitors that was developed independently. From this root navoximod was ultimately generated as a clinical lead compound [125, 126]. Structural information demonstrated that the ring planes of the phenylimidazole and imidazoisindole classes were oriented slightly differently in the active site [127], consistent with distinct SAR. Navoximod inhibits human IDO1 with an EC₅₀ of 75 nM and a 10- to 20-fold selectivity against TDO in cells [125], suggesting its possible utility in tumors expressing both IDO1 plus TDO. In mice, its administration reduced plasma Kyn levels by ~50% and enhanced vaccine responses against B16 melanoma or the efficacy of anti-PD-L1 against EMT6 mammary carcinoma in a manner associated with elevated CD8⁺ T/Treg ratios, plasma interferon- γ levels, and activated intratumoral macrophages and DC [128]. Early clinical studies showed it to be well tolerated up to 600 mg twice daily with some evidence of cancer control [126, 129].

A hydroxyamidine chemotype that emerged from an IDO1 inhibitor discovery program started in 2004 at Incyte Corporation led ultimately to the development and translation of the Trp competitive inhibitor epacadostat (INCB024360) as a clinical lead agent. A recent review details the development of this compound [130]. SAR studies defined hydroxyamidine as essential for enzyme inhibitory activity based on its direct binding to the heme iron in the active site. Further chemical refinement yielded the bioactive preclinical proof-of-concept compound INCB14943 [131] with additional work engaged to generate the pharmacologically superior clinical lead compound INCB024360/epacadostat [130].

Epacadstat exhibits an inhibitory potency of $EC_{50}=12$ nM in cells against human IDO1 with >100-fold selectivity against TDO. Its administration to co-cultures of human allogeneic lymphocytes and DC or tumor cells promoted the growth of effector T cells and NK cells, reduced conversion of naïve T cells to Tregs and increased numbers of CD86^{high} DC [132]. In tumor-bearing syngeneic mice, epacadastat inhibited plasma Kyn levels ~90%, reduced tumor growth and leveraged the antitumor effects of anti-CTLA4 or anti-PD-L1 [133]. In clinical studies, epacadostat is generally well tolerated at doses of >100 mg twice daily with some evidence of cancer control [134]. Efficacy is currently being studied in several tumor types in combination with anti-PD-1 or anti-PD-L1 antibodies with early data providing evidence of favorable clinical activity [135].

Other IDO1 enzyme inhibitors have been registered in the patent literature [109] with little information in the biomedical literature as yet. Identification of a benzenesulfonylhydrazide series of inhibitors [136] seeded work yielding a potent IDO1 inhibitor of $EC_{50}=68$ nM in cells and 59% oral bioavailability which produced significant tumor growth delay without body weight loss [137]. Several pharmaceutical companies report compounds in late-stage preclinical development or early clinical testing. BMS-986205 is an irreversible suicide inhibitor of highest potency ($EC_{50} = 2$ nM in cells) that appears to crosslink to IDO1 in the active site. This compound is reported to have superior pharmacokinetics relative to epacadostat and navoximod. In 2015, BMS-986205 entered first-in-man studies in solid tumors as monotherapy or in combination with the PD1 antibody nivolumab (NCT02658890). PF-06840003 is a Trp non-competitive, non-heme binding IDO1 inhibitor (Wythes et al, SITC 2016, poster 253). This compound is predicted to have favorable human PK based on prolonged half-life that may allow single-dose daily administration along with the ability to penetrate the CNS and treat brain metastases. In a preclinical study, PF-06840003 enhanced the antitumor efficacy of anti-PD1/PDL1 axis blockade and a trial was initiated in 2016 in patients with malignant glioma (NCT02764151).

IDO1 vaccines

In a provocative set of studies, Andersen and colleagues have shown how IDO1 is spontaneously recognized by specific CD8⁺ T cells present in humans [138]. Indeed, IDO1-reactive CD8⁺ T cells appear to act as specific CTLs that can recognize and kill IDO1-expressing cells [139]. IDO2 and TDO may also share these features [140, 141]. These observations have prompted efforts to explore IDO1 peptides as anti-cancer vaccines, as examined in a recent clinical trial where early evidence was obtained of long-lasting disease stabilization and a partial response against liver metastasis in metastatic lung cancer patients, in the absence of notable toxicity [142]. Employing such an approach, one potential clinical strategy could be to combine IDOi with IDO1 vaccines, with the IDOi promoting IDO1-specific T cells that are functional at the site of the tumor. This combination may be self-reinforcing, as inflammation induced by IDO1-specific T cells might stimulate IDO1 activity at the tumor which could be blocked by the IDOi treatment, thereby producing a synergistic effect. Here a caveat should be noted of the possibility of elevating the risk of autoimmunity due to chronic ablation of IDO1 via vaccine-based approaches either alone or in combination with IDOi.

Leveraging anticancer responses by inflammatory reprogramming with IDO1 inhibitors

Conceptualizing IDO1 as an immunometabolic adjuvant suggests new perspectives on how to use IDO1 inhibitors to heighten anticancer efficacy in a wide range of treatment contexts, including contexts where increasing immunogenicity does not or can not suffice. **Figure 6** summarizes the different aspects of IDO1 inhibitors which may be beneficial in different treatment contexts.

Combinations with anti-PD1 drugs are the most exciting currently, based on established preclinical and emerging clinical evidence [27]. Here the chief interest is to extend the disease control rate (DCR) in greater numbers of patients, ideally without intensifying their autoimmune side-effects as occurs in combination with anti-CTLA4 [3]. Melanoma, non-small cell lung carcinoma (NSCLC), mismatch repair-deficient colon tumors and PD-L1hi urothelial tumors exhibit the highest rates of complete response (CR) as a gold standard for DCR. Early clinical data from melanoma patients suggests indoximod and epacadostat extend the DCR for anti-PD1 therapy to a similar extent as anti-CTLA4 without increasing side-effects [116, 135]. Tumors that exhibit fewer and less robust responses to anti-PD1 include small cell lung carcinoma (SCLC), head and neck squamous cell carcinoma (HNSCC), hepatocarcinoma, triple-negative breast, ovarian and renal cancers. By altering the inflammatory phenotype, IDO1 inhibitors may extend responses in these settings of favorable or weak immune checkpoint response by relieving locoregional suppression on TILs, CD8+ T cells and lower PD-L1 exposure (**Figure 7**). In checkpoint non-responsive tumors, including non-MMR deficient colorectal, pancreatic, mutant p53/RAS, and prostate tumors, the coordinate properties of IDO1 inhibitors to blunt tumor angiogenesis, block MDSC function and reprogram the inflammatory character of CAF-driven reactive stroma may prove crucial in some patients to broaden immunogenicity. Indeed, the low antiogenicity of tumors which tend to respond poorly to immune checkpoint drugs has been reported to be elevated by the DNA sensor STING, through a mechanism that involves IDO1 upregulation [38]. Accordingly, IDO1 inhibitors might open up the ‘virgin territory’ of low-antigenicity tumors by acting as an adjuvant to ‘put on board’ an inflammatory trigger to heighten neoantigen responses, broadening a niche where IDOi may be quite useful. In prostate cancer, a >2-fold extension in patient survival was reported in a Phase II combination study of indoximod with the dendritic cell vaccine sipuleucel-T (Provenge®) [27], illustrating how IDOi might be able to leverage responses in otherwise immunologically ‘cold’ tumors.

IDO1 blockade can deepen responses to ‘immunogenic’ chemotherapy and radiotherapy [30, 143], although such combinations have yet to be pursued as energetically as immune checkpoint therapy so far. Here benefits of the combination may relate to mutagenesis and cell death produced by chemo/radiotherapy, thereby yielding higher antigenicity to which the adaptive immune system can react. In this scenario, chemo/radiotherapy produces a vaccine effect through the wave of tumor neoantigens released by damaged tumor tissue. IDO1 inhibition would similarly leverage the effect as above to strengthen a weakly inflamed or non-inflamed tumor microenvironment. The anti-angiogenic and inflammatory reprogramming effects of IDOi may be critical in non-inflamed (or

incorrectly inflamed) tumors, not only in speeding immunogenic responses (as observed in chemotherapy-IDOi combinations in preclinical models [30, 39]), but also as an adjuvant that ‘puts on board’ a clearing inflammatory trigger that can heighten tumor immunogenicity.

Further applications of IDOi can be envisioned to leverage anti-angiogenic therapy in immunologically ‘cold’ tumors where immune checkpoint therapy or ‘immunogenic’ chemotherapy is used, for example, in gliomas or MMR competent colorectal tumors. In settings where anti-angiogenic therapy is employed, IDOi adjuvancy may offer leverage to both therapies. Indeed, we envision that combination therapies which capture both the immunomodulatory and anti-angiogenic facets of IDOi adjuvancy may be most effective. Further, in considering of how to heighten responses to tumor neoantigens, we envision that IDOi adjuvancy may safely empower combinations of DNA repair inhibitors with immune checkpoint therapy or STING agonists, the activity of which may be blunted by IDO1 activity in tumors [37, 38]. In summary, IDOi may offer tools to loose all-out attacks while focusing the inflammatory fires which are unleashed on tumors relative to normal tissue.

Concluding Remarks

In the exuberance to develop and leverage immune checkpoint modalities, IDOi have generated the most interest in melanoma trials with plans to expand testing in lung, head and neck, bladder, renal, ovarian and other tumor types. Early clinical data from melanoma trials of anti-PD1 plus IDOi suggest a high level of efficacy as achieved with anti-PD1 + anti-CTLA-4 combinations but without the acute side-effects. These exciting aspects of the clinical data succinctly capture the nature of an immune adjuvant: a substance that influences inflammation with little therapeutic effect on its own but a powerful effect when added to an immunogenic therapy.

Conceptualizing IDOi as immunometabolic adjuvants opens a new perspective on how to use them to widen efficacy in other contexts, including those where increasing immunogenicity either can not or will not suffice for positive outcomes. **Figure 7** and the **Outstanding Questions** box summarize opportunities and issues in using IDOi as immunometabolic adjuvants. These questions build upon the concept that IDO1 metabolically ‘flavors’ an inflammatory state that enables immune escape and neovascularization in a tissue, and the concept that IDOi re-program this state to restore immunosurveillance and blunt neovascularization, creating an inflammatory state that can facilitate rather than antagonize cancer cell clearance by immunogenic modalities, including immunotherapy, radiotherapy, chemotherapy or their combinations.

In future work, it will be important to evaluate to what extent blocking TDO and/or IDO2 may widen efficacy and reduce inherent resistance or risks of acquired resistance to IDO1 blockade. IDO2 poses an intriguing subject in connection to autoimmune side-effects produced by immune checkpoint drugs (including durably in survivors). We envision that combinatorial targeting of IDO1, IDO2 and TDO in a single modality may open therapeutic windows for many regimens, both by increasing efficacy and decreasing autoimmune or other chronic inflammatory side-effects which affect patients and survivors. In their potential

to leverage less expensive generic standards of care using traditional chemotherapy and radiotherapy, IDOi may also support cancer treatment in both developing and developed nations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. IDO1 activation is an late-stage event in oncogenesis and immunoediting

Oncogenesis produces cells normally eradicated by immune surveillance due to neoantigen presentation. This process imposes a selection for cells that can escape control. Genetic instability in neoplastically transformed cells facilitates the evolution of escape mechanisms, including a selection for IDO1 dysregulation due to loss of control by the tumor suppressor BIN1, which is often attenuated in cancer cells [144] due to aberrant RNA splicing patterns abolishing its suppressor function [145–149] or to altered gene methylation patterns extinguishing its expression [150–154]. Resulting skirmishes with tumor cells ‘sculpt’ the immune system to equilibrium, a dynamic yet dormant state of disease, with iteration of escape mechanisms ultimately enabling progressive disease. In the model, genetic alterations initiate tumorigenesis, but the ultimate course to clinically apparent disease is dictated by activation of locoregional modifiers such as IDO1 which influence the host response and act as the etiologic cause of progressive disease with actionable relevance.

Figure 2. Overview of IDO1 regulatory and effector pathways

IDO1 expression is induced at the cellular sites and through the signaling pathways noted, leading to locoregional Trp deprivation plus production of the catabolic product Kyn. IDO1 also has a non-catalytic signaling function that can be stimulated by TGF- β [19]. Responding cells interpret Trp insufficiency through the mTORC1 and GCN2/eIF-2 pathways, whereas Kyn acts as a native ligand for xenobiotic receptor AHR, with the respective downstream effects on critical pathways for different responding cells shown. IFNs, interferons; ONCs, oncogenic ligand-receptor signaling complexes; PAMP, pathogen-associated molecular pattern (e.g. LPS or CpG ligands for Toll-like receptors [TLR]); DAMP, damage-associated molecular pattern (e.g. extracellular HMGB1 or ATP as ligands for TLR4 or A2A receptors, respectively); B7, T cell co-receptor-ligand complexes (e.g. CD80-CTLA4 or PD1-PDL1) which stimulate ‘reverse signaling’ into APC, including IDO1 induction [29].

Figure 3. Phenotypes of IDO1 and IDO2 nullizygous mice

The figure summarizes evidence that IDO1 requires IDO2 function to induce a PD1-dependent population of Treg cells in mice [12]. It also contrasts phenotypes of *Ido1*^{-/-} and *Ido2*^{-/-} mice highlighting differences in pathogenic character relevant to cancer and autoimmunity, the latter of which includes desirable and undesirable features that would be separated in an ideal therapeutic response.

Figure 4. Sites of IDO expression and action in cancer

IDO expression has been documented in a variety of cells in tumors and tumor-draining lymph nodes (and other metastatic sites) including malignant cells as well as other stromal, vascular and immune cells indicated. Both Trp deprivation and Kyn production mediated by IDO has been implicated in inflammatory processes and the generation of antigenic immune tolerance (immune escape). The figure summarizes the general effects that have been described on T cell function at each site. APC, antigen-presenting cell (e.g. dendritic cell); MDSC, myeloid-derived suppressor cell; TAM, tumor-associated macrophage; TAN, tumor-associated neutrophil; Teff, T effector cell; Treg, T regulatory cell.

Figure 5. Distinctive mechanistic properties of IDO inhibitors in clinical trials

Tryptophan catabolism proceeds through pathways leading to serotonin or NAD production, the latter through the Kyn pathway which handles ~95% of Trp catabolism in mammals. Epacadostat is >100-fold selective against IDO and displays Trp competitive inhibitory kinetics. Navoximod is ~20-fold selective against IDO and exhibits Trp non-competitive inhibitory kinetics. BMS-986205 is a high-potency suicide inhibitor that irreversibly binds and inactivates IDO1. None of these compounds inhibits IDO2 appreciably. Indoximod is a Trp mimetic which mTORC1 interprets as L-Trp under conditions of high Trp catabolism and autophagy. It also indirectly disrupts IDO2 activity. This compound reverses the disruptive influence of mTORC1 suppression in T cells that results from Trp deprivation generally (i.e. it is a pan-IDO/IDO pathway inhibitor acting downstream on the mTORC effector pathway).

Figure 6. IDO inhibitors as immunometabolic adjuvants to turn ‘cold’ tumors ‘hot’ to immunotherapy, radiotherapy and ‘immunogenic’ chemotherapy
IDO1 inhibitors promote adaptive immunity by relieving immunosuppression of tumor-infiltrating lymphocytes (TILs) including CD8+ T effector cells and natural killer cells. Restraints on the production and anti-neovascular effects of IFN γ mediated by IDO1 are diminished [33, 103]. Further, IDO1 mediation of PD-L1 reverse signals in APC are diminished (Figure 2). In the inflammatory tumor stroma, IDO1 inhibitors block neovascularization, deprive reactive stromal CAF of IDO1 activity, and blunt the production and suppressive activity of MDSC. Preclinical studies show that IDOi potentially leverage the efficacy of ‘immunogenic’ radiotherapy and chemotherapeutic drugs, including many DNA damaging drugs [30, 143]. Through these multimodal effects, IDO1 inhibitors act as immunometabolic adjuvants to empower ‘immunogenic’ therapy or frank immunotherapy approaches in otherwise weakly or non-responding tumors as noted.

Figure 7. IDO inhibitors reprogram cancer inflammation and improve therapy

Multiple applications are suggested in various combinations with tolerance blockade (immune checkpoint inhibitors), active immunotherapeutic interventions (vaccines or CART [chimeric antigen receptor T cells]), classical or targeted chemotherapy, and anti-angiogenic therapy or radiotherapy.

Table 1

IDO1 inhibitors in pharmaceutical development.

Name	Incyte	NewLink	BMS <i>Flexus</i>	NewLink	Pfizer <i>ITEos</i>	Merck <i>IO-Met</i>	Roche <i>Curadev</i>
Structure						Unknown <i>Patent Published</i>	Unknown <i>Patent Published</i>
Mechanism	Catalytic Inhibitor	Stimulates mTORC1 Downstream of IDO/TDO	Suicide Inhibitor	Catalytic Inhibitor	Catalytic Inhibitor	Unknown	Unknown
Inhibitory Kinetics	Tryptophan Competitive	NA	Irreversible	Tryptophan Non-Competitive	Tryptophan Non-Competitive	Unknown	Unknown
Cell-Based Potency	12 nM	NA	2 nM	75 nM	1100 nM	Unknown	Unknown
TDO Selectivity	> 100-fold	Non-Selective	> 100-fold	10–20 fold	> 100-fold	> 100-fold	~5-fold
Clinical Entry	2012	2008	2015	2015	2016	NA	NA
Program Status	Phase 3 Enrolled	Phase 3 Enrolling	Phase 2 Enrolling	Phase 1B Complete	Phase 1 Enrolling	Preclinical	Preclinical