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REVIEW

Inhibiting IDO pathways to treat cancer: lessons from the ECHO-301 trial and beyond

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Abstract

With immunotherapy enjoying a rapid resurgence based on the achievement of durable remissions in some patients with agents that derepress immune function, commonly referred to as "checkpoint inhibitors," enormous attention developed around the IDO1 enzyme as a metabolic mediator of immune escape in cancer. In particular, outcomes of multiple phase 1/2 trials encouraged the idea that small molecule inhibitors of IDO1 may improve patient responses to anti-PD1 immune checkpoint therapy. However, recent results from ECHO-301, the first large phase 3 trial to evaluate an IDO1-selective enzyme inhibitor (epacadostat) in combination with an anti-PD1 antibody (pembrolizumab) in advanced melanoma, showed no indication that epacadostat provided an increased benefit. Here we discuss several caveats associated with this failed trial. First is the uncertainty as to whether the target was adequately inhibited. In particular, there remains a lack of direct evidence regarding the degree of IDO1 inhibition within the tumor, and previous trial data suggest that sufficient drug exposure may not have been achieved at the dose tested in ECHO-301. Second, while there is a mechanistic rationale for the combination tested, the preclinical data were not particularly compelling. More efficacious combinations have been demonstrated with DNA damaging modalities which may therefore be a more attractive alternative. Third, as a highly selective IDO1 inhibitor, epacadostat was advanced aggressively despite preclinical genetic evidence of tumors bypassing IDO1 blockade. Indeed, a well-grounded literature starting in 2011 points to targeting strategies that account for both IDO and tryptophan 2,3-dioxygenase as more appealing directions to pursue, including dual inhibitors and inhibitors of nodal downstream effector pathways such as aryl hydrocarbon receptor blockade. Overall, the clinical readout from a single trial with significant limitations is by no means a definitive test for the field. While biomarker information yet to be gleaned from ECHO-301 may yet reveal useful information regarding IDO1 pathway drugs, better rationalized compounds and better rationalized trial designs will be important in the future to accurately gauge medical impact.

Keywords Indoleamine 2,3-dioxygenase · Tryptophan 2,3-dioxygenase · IDO1 · IDO2 · TDO2 · TDO · Immunotherapy · Immunometabolism · Immune checkpoints · Immune adjuvants

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Tryptophan catabolism and tumoral immune escape

The tryptophan catabolic enzymes indoleamine 2,3-dioxygenase-1 (IDO1) and, more recently, tryptophan 2,3-dioxygenase (TDO), have attracted enormous attention and investment as targets for cancer therapy based on preclinical evidence, initially in pregnancy followed by tumor models, of an immunometabolic role in peripheral tolerance as detailed in recent reviews [1–3]. Biochemically, IDO1 and TDO initiate the rate-limiting first step in tryptophan catabolism leading to the generation of kynurenine and its downstream catabolites, referred to in aggregate as the kynurenine pathway. One

outcome of the kynurenine pathway is the de novo synthesis of the central metabolic co-factor nicotinamide adenine dinucleotide (NAD). *IDO1* is a widely expressed, interferon-inducible gene [4]. In contrast, TDO, encoded by the *TDO2* gene, is constitutively expressed in the liver and responsible for metabolizing dietary tryptophan. IDO1 and/or TDO are broadly activated in human cancers [5] where they are implicated in the suppression of local $CD8^+$ T effector (Teff) cells and natural killer (NK) cells, the induction and activation of $CD4^+$ T regulatory (Treg) cells, and the activation, recruitment, and expansion of myeloid-derived suppressor cells (MDSCs) [2]. A possible third player is the more recently identified IDO1-related enzyme IDO2 [6, 7]. Although less well studied, IDO2 has been found to be overexpressed in some human tumors, to functionally enable IDO1-dependent Treg suppressive activity, and to underlie B cell-mediated autoantibody production [8] which is important in the development of certain cancers such as pancreatic cancer [9, 10]. Together, the *IDO1*, *IDO2*, and *TDO2* genes are variably upregulated in neoplastic cells as well as in stromal, endothelial, and innate immune cells of the tumor microenviroment and in tumor-draining lymph nodes where the consequences of their actions are only just beginning to be sorted out.

In cancers, the activation of tryptophan catabolism by any of these three enzymes leads to the locoregional generation of kynurenine and depletion of tryptophan. Three downstream effector pathways have been implicated in the biological responses to these immunometabolic signals which include suppression of cellular and humoral immune responses and promotion of carcinogenesis and tumor cell growth [1, 11, 12]. Kynurenine binds to and activates the aryl hydrocarbon receptor (AHR), a xenobiotic-responsive transcription factor which, upon binding ligand, translocates to the nucleus where it dimerizes with aryl hydrocarbon nuclear translocator (ARNT) and regulates a wide variety of genes that contain xenobiotic response elements (XREs). Meanwhile, tryptophan depletion activates general control non-derepressible 2 (GCN2) [officially designated eukaryotic translation initiation factor 2 alpha kinase 4 (EIF2AK4)] and represses mechanistic target of rapamycin kinase (MTOR). GCN2 is a stress response kinase that responds to uncharged tRNA and phosphorylates eukaryotic translation initiation factor α(EIF2α) to reduce protein synthesis globally but increase the synthesis of specific mRNAs, while MTOR functions within two complexes, TORC1 and TORC2, to integrate multiple threads of information and control cellular metabolism and growth. By catabolizing tryptophan, each of the IDO/TDO enzymes can signal through these various pathways, with the consequent benefit to tumors [13] putting these enzymes under selective pressure to be activated during cancer progression. This challenge of redundancy—a core weakness of any targeted drug development project—forms the rationale for pursuing pharmacologic strategies that minimize bypass opportunities, such as targeting multiple enzymes or nodal effector signals [1, 12]. Such approaches, which we address in more detail below, may, by limiting drug resistance, thus prove superior to specific IDO1 or TDO inhibitors.

Lessons from the anti-PD-1 antibody combination trial ECHO-301

In essence, the ECHO-301 trial was designed as a follow-on confirmatory phase 3 study of a phase 1/2 single-arm combination trial in advanced melanoma, which had provided evidence of encouraging overall response rates and progression-free survival (PFS) that were superior to historical data for pembrolizumab alone in a similar patient population. Despite the failure of epacadostat to improve clinical responses in the subsequent phase 3 trial, there is much to be gleaned from biomarker data yet to be analyzed which is likely to ultimately benefit the IDO/TDO field and the broader field of cancer immunometabolism. Meanwhile, several cautionary notes should be kept in mind when weighing the implications of this one trial result against the overall potential of IDO/TDO inhibitors.

1. The risky decision to initiate a large phase 3 trial based on a small amount of data from an uncontrolled phase 1/2 trial in a patient population where pembrolizumab alone has activity.
2. Uncertainty regarding the sufficiency of the dose of epacadostat employed in ECHO-301 based on the lack of direct pharmacodynamic data on IDO1 inhibition within tumors and doubts raised by the serum analysis as to whether consistent maximal inhibition was being achieved.
3. A paucity of understanding about the best patient population or drug combinations to test.
4. Preclinical evidence that selective IDO1 blockade is insufficient to durably relieve tumor immunosuppression mediated by kynurinine elevation and tryptophan depletion, highlighting the need to take into account the other tryptophan catabolic enzymes TDO and perhaps IDO2 as potential compensatory mechanisms.

As the initial subject of interest in this novel therapeutic space, the IDO1 enzyme has been pursued aggressively, but the determination to rapidly advance clinical trials outpaced an appreciation of accumulating preclinical evidence regarding potential weaknesses of IDO1-selective therapeutic strategies [2]. For example, while IDO1 knockout mice exhibited resistance to the outgrowth of 4T1 breast cancer pulmonary metastases, the delay was transient and tumor growth was accompanied by elevated kynurenine levels likely mediated

by TDO or IDO2 as alternate pathways [14]. Moreover, it is well documented that a variety of engrafted tumor types, including B16 melanoma, that respond to the acute inhibition of IDO1 achieved with small molecule inhibitors will grow unimpeded in IDO1 knockout mice while losing their responsiveness to IDO1 inhibition, suggesting a preferential but not necessarily exclusive reliance on IDO1 that, in its absence, can be bypassed by accessing alternative compensatory immune escape mechanisms. In line with the established idea of "oncogene addiction," this acquired dependence upon IDO1 for immune escape has been conceptualized as "tolerance-gene addiction" [15–17]. Furthermore, genetically knocking out IDO1 provided only a modest increase in the survival benefit for melanoma tumor-engrafted mice treated with immune checkpoint antibodies [18]. Thus, in retrospect, while particular aspects of the ECHO-301 trial design might account for the negative outcome of the study, this outcome also punctuates the need, encouraged by accumulating preclinical evidence, to fundamentally reconsider the utility of IDO1-selective agents for maximizing the effectiveness of this therapeutic approach.

Was the right dose of epacadostat tested in ECHO-301?

From the available data, there is uncertainty as to whether a sufficient dose of epacadostat was tested in ECHO-301. While preclinical evidence supports the functional relevance of IDO1 expressed within tumor cells [19–21], the pharmacodynamic data reported for epacadostat are derived from serum, not tumor. Thus, the phase 3 melanoma trial proceeded based on an assumption that serum measurements were reflective of the steady-state kynurenine levels in tumors. For epacadostat, this was a risky assumption since the compound is an efflux substrate [22], and melanomas (as well as other tumor types) are known to express these drug pumps. Thus, it is conceivable that IDO1 activity in tumor cells was not effectively inhibited in ECHO-301 at the dose tested.

With regard to the epacadostat data from serum, phase 1 studies showed a maximum reduction in kynurenine levels of 50% [23]. Kynurenine is also synthesized by TDO, so a 50% reduction is what one might expect with near-complete IDO1 inhibition as substantiated in IDO1 knockout mice [24]. However, it is concerning that the dose chosen to test in ECHO-301 was 100 mg b.i.d. even though a consistent 50% threshold reduction in kynurenine levels, indicative of maximal inhibition, is not apparent in the reported day 15 pharmacodynamic data until 400 mg b.i.d. [23]. At a minimum, it would be valuable to see a serum time course from ECHO-301 patients in order to evaluate if the 100 mg b.i.d. dose is capable of durable kynurenine lowering so as to gauge the sufficiency of this dose for inhibiting IDO1.

A more potent IDO1 inhibitor, BMS-986205, has also been reported to achieve a 50–60% reduction in serum kynurenine levels (at trough on day 14) at the dose tested with the anti-PD1 antibody nivolumab. Clinical pharmacodynamic data presented at the 2017 ASCO and SITC conferences also included two key pieces of information. First, in melanoma patients who showed poor therapeutic responses in response to nivolumab, a correlation was noted with increased levels of serum kynurenine [25]. Second, in patients co-treated with BMS-986205, an intratumoral pharmacodynamic analysis in selected patients showed a reduced level of locoregional kynurenine in some but not all tumors (possibly due to TDO expression) [26]. Further insights of this nature will help close the current gap in knowledge concerning under what circumstances, if any, a 50% reduction in serum kynurenine is sufficient to achieve positive clinical responses.

Are there better agents to combine with IDO1 inhibitors than immune checkpoint drugs?

Phase 2 clinical data continue to encourage further testing of IDO1 inhibitors in combination with anti-PD1 antibodies in other settings, albeit tempered by the ECHO-301 results [27–30]. Revisiting IDO1 inhibition in combination with anti-CTLA should also be considered given initial evidence of a liver toxicity signal at the 300 mg dose of epacadostat, manifested as significant ALT elevations in five of seven patients [31], which may be indicative of enhanced biological activity that might translate to increased therapeutic efficacy. Alternatively, evidence of tumor regressions in preclinical studies combining IDO1 inhibition with DNA-damaging chemotherapy agents argues for further consideration of this approach [19]. Likewise, radiotherapy and chemoradiotherapy responses were improved by interfering with IDO/TDO pathways [32–34]. Radiotherapy also licenses the ability of IDO1 inhibitors to empower anti-PD1 activity in glioblastoma [35]. In further support of a connection between tryptophan catabolism and DNA damage signaling, IDO1 is regulated by the nucleic acid sensor STING, empowering its ability to drive tumor growth [36]. Recent clinical evidence supports a role for IDO1 status in determining efficacious responses to DNA damaging modalities [37]. In a similar way, common coding sequence variations in the human IDO2 gene that attenuate its enzymatic activity predict improved radiotherapeutic outcomes in pancreatic cancer [38]. Overall, support continues to grow for exploring IDO/TDO pathway inhibitors as immunometabolic tactics to leverage the efficacy of DNA damaging modalities, which are used widely in the oncology clinic and at a more favorable comparative cost relative to immunotherapeutic modalities.

Are IDO1-selective blocking strategies prone to resistance in advanced cancer?

In considering all the preclinical literature, IDO1 can be viewed more precisely not as an immune checkpoint but as an inflammatory modifier that empowers tumor growth via proximal effects on innate inflammatory cells that drive more distal effects on adaptive immune tolerance and neovascularization [2, 3]. Genetic studies demonstrate that IDO1 loss blocks engagement of a pathogenic program(s) of inflammation that helps promote and sustain many cancers, for example, mutant RAS-induced skin or lung cancers that rely on inflammatory stimuli [39, 40]. In inflammation-driven cancers whose growth is inhibited by selective IDO1 blockade, an initial delay in tumor growth is subsequently overcome in conjunction with increased locoregional levels of kynurenine [14]. In these studies where IDO1 is genetically absent, the elevation in kynurenine accompanying tumor growth infers alternate mechanisms involving TDO and/or IDO2. This acquired resistance to IDO1 blockade in mice that initially benefited from IDO1 loss occurred relatively rapidly on a scale of weeks. The ready capability for acquired resistance observed in these studies poses a major concern for the use of IDO1 selective inhibitors to treat advanced cancer patients, especially when they are recruited without any histopathologic or mechanistic basis. In summary, there are preclinical data that justify broader-spectrum approaches to inhibiting both IDO and TDO either directly or through targeting of convergent effector mechanism downstream of these enzymes.

Next-gen approaches: IDO/TDO combi-inhibitors and Kyn-AHR signaling blockade

As an immunometabolic inflammatory modifier, kynurenine pathway-directed tryptophan catabolism has appeal as a therapeutic target [41]. This appeal is tempered, however, by the complicating factor of functional redundancy between IDO1, TDO, and IDO2, which elevates the risk of mechanistic bypass. In one study, for instance, co-expression of IDO1 and TDO has been observed in one sixth of tumors [5]. Due to caveats about the quality of the anti-TDO antibodies currently available, the extent and overlap of TDO expression in tumors is not entirely clear at present. However, in silico RNA-Seq analyses of melanoma and head and neck carcinoma in the TCGA cancer database confirm widespread TDO overexpression in these tumors and frequent co-expression with IDO1 (Fig. 1).

Beyond its direct roles in cancer, TDO is also a logical candidate to mediate resistance to IDO1-selective blockade. TDO has been implicated in multiple tumor types, including melanomas and brain and breast tumors [42, 43]. Studies in breast cancer cells also reveal distinct benefits of TDO overexpression for tumor progression independent of immunosuppression [43], analogous to roles distinct from immunosuppression for IDO1 in tumor neovascularization [44]. With regard to IDO2, although not yet extensively studied in cancer, there is reported evidence of its overexpression in gastrointestinal cancers [45], where IDO1 and TDO are also often overexpressed [5, 21, 46]. In particular, IDO2 is over expressed in pancreatic adenocarcinomas [47] where genetic evidence indicates differing consequences for familial and

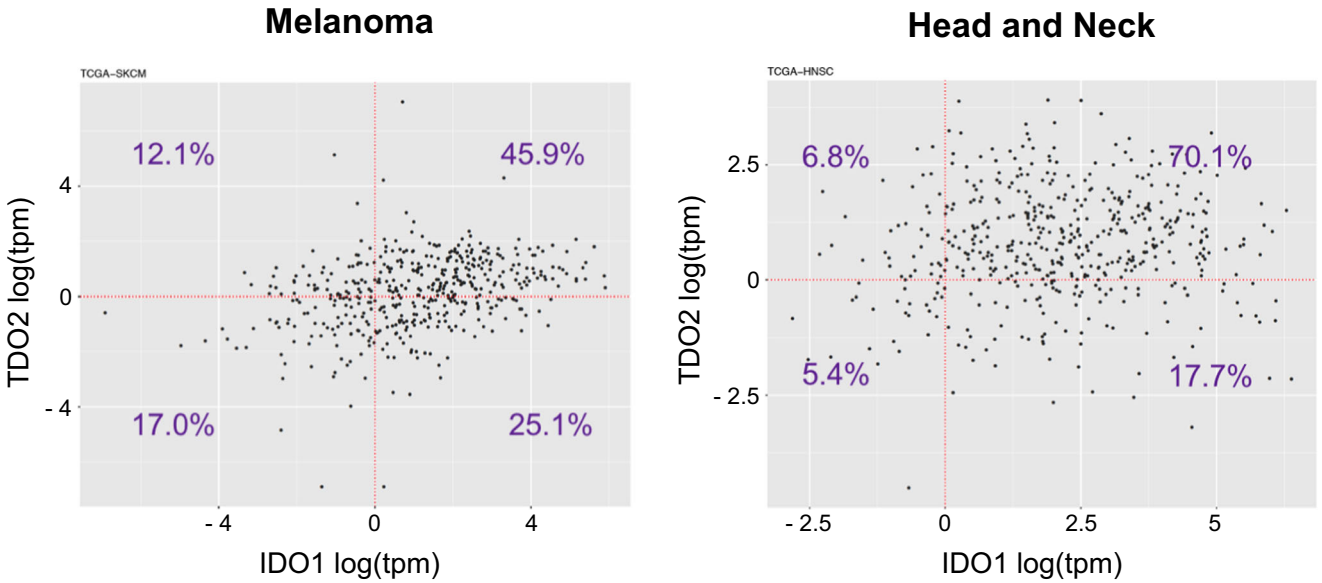

Fig. 1 Frequent overexpression of both IDO1 and TDO in melanoma and head and neck cancers. IDO1 and TDO2 RNA-Seq expression data were analyzed using TCGA database. Expression shown for melanoma and head and neck cancer

sporadic cancer pathogenesis [38, 48]. Taken together, a sound rationale exists to explore IDO1/TDO and possibly IDO1/IDO2/TDO pan-inhibitors, as tactics to more effectively attenuate tryptophan catabolism in human cancers [1].

IDO/TDO enzymes exert their physiological effects via three downstream effector pathways, one mediated by AHR which responds to the tryptophan catabolite kynurenine and the other two mediated by the GCN2 and MTOR kinases which respond to tryptophan deprivation [1]. Preclinical evidence of IDO1-associated immunoregulatory effects mediated through GCN2 [49] and MTOR [50] have been reported. Interestingly, despite having clear IDO1-dependent biological effects, the first and most widely studied "IDO1 inhibitor", 1MT (1-methyl-tryptophan), is actually far too weak to exert any direct effect on the enzyme at concentrations that can be achieved in an animal. Instead, it appears that 1MT acts by interfering with the downstream signaling of IDO1. In this regard, both GCN2 and MTOR have been reported to be inhibited by 1MT [51, 52] although only the study of MTOR inhibition [52] looked specifically at the D stereoisomer which represents the chemical composition of the clinical agent indoximod. A comprehensive review of the ongoing clinical development of indoximod will appear elsewhere [53]. Small molecule inhibitors directly targeting either GCN2 [54] or MTOR [55], which are currently in development, may likewise turn out to be of interest in this regard.

Kynurenine, the most stable tryptophan metabolite which can reach concentrations of > 100 μM in tumors, acts as an endogenous ligand for AHR, a xenobiotic-responsive transcription factor that modulates immune activity [56]. AHR has rapidly emerged as a point of focus in immuno-oncology research as elaborated in a recent review on therapeutic targeting of the IDO/TDO-AHR pathway in cancer [12]. By transducing the common immunometabolic signal resulting from IDO/TDO activation, the kynurenine/AHR interaction provides a downstream effector node that circumvents their enzymatic redundancy. Biologically, the link between kynurenine accumulation and tumor growth in IDO1 knockout mice offers a genetic rationale to target kynurenine levels directly, while further support for this assertion is provided by evidence that kynurenine monooxygenase (KMO)-knockout mice exhibit elevated levels of systemic kynurenine accompanied by increased susceptibility to inflammatory skin carcinogenesis (A.M. and G.P., unpublished data). Conventional small molecule inhibitors that antagonize AHR binding to kynurenine (and other agonist ligands) [57] are currently under development by several companies. Alternatively, an innovative strategy to enzymatically deplete kynurenine levels is also being pursued. The latter strategy employs an injectable biologic based on kynureninase ("kynase") to degrade systemic levels of kynurenine regardless of which catabolic enzyme(s) generated it (Fig. 2) [58]. Specific disruption of the tryptophan catabolism/AHR axis in this manner may avoid

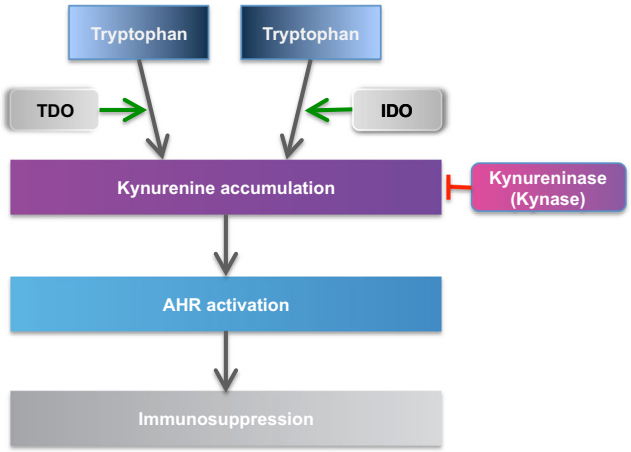

Fig. 2 Kynurenine and AHR as therapeutic targets downstream of IDO/TDO enzymes

potential adverse consequences associated with general AHR inhibition, and recently published preclinical responses observed in different tumor models are encouraging [58]. Investigations in these exciting new areas are expected to proceed rapidly given the ready tractability of AHR for drug discovery and development.

Closing comments

Conclusions regarding the negative outcome from ECHO-301 must be tempered by caveats that reflect uncertainties about particular aspects of the trial design as well as movement in the IDO field since its inception. Biomarker information from the trial is awaited that may shed light on primary concerns about epacadostat dosing. However, fundamental issues raised by preclinical research bear on other questions about the best agents, therapeutic combinations, and clinical designs to test the hypothesis that inhibiting the immunometabolic signals associated with tryptophan catabolism can effectively leverage cancer therapy outcomes. The underlying biology is solidly grounded, and biomarker-driven clinical trials that address the above issues raised by the latest preclinical research are likely to continue to advance the field. More generally, immunometabolic adjuvants will continue to represent one of the most exciting areas for development in coming years.

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Compliance with ethical standards

Conflict of interest G.C.P. and A.J.M. are shareholders and G.C.P. is a former compensated scientific advisor and grant recipient 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. G.C.P. is a compensated scientific advisor for Kyn Therapeutics Inc. which is developing IDO/TDO/AHR pathway antagonists for cancer treatment. A.J.M. is a grant recipient and compensated scientific advisor for I-O Biotech AG which is developing IDO vaccines for cancer treatment. MM is a shareholder in Kyn Therapeutics. Y.Z. is a recipient of research and conference travel support from NewLink Genetics and an advisory board member for Amgen, Roche Diagnostics, Novartis, Eisai, Castle Bioscience, and Exelixis.

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