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Is There a Clinical Future for IDO1 Inhibitors After the Failure of Epacadostat in Melanoma?

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Keywords

epacadostat, indoleamine-2,3 dioxygenase, IDO1 inhibitor, melanoma, pembrolizumab, immunosuppression

Abstract

Indoleamine-2,3 dioxygenase 1 (IDO1) contributes to tumor immunosuppression by enzymatically degrading tryptophan, which is required for T cell activity, and producing kynurenine. Small-molecule inhibitors, such as epacadostat, have been developed to block IDO1 activity. In preclinical models, they can restore antitumoral T cell immunity and synergize with immune checkpoint inhibitors or cancer vaccines. Based on encouraging clinical results in early phase trials, a randomized phase III study (ECHO-301/KN-252) was launched in metastatic melanoma to test the benefit of adding epacadostat to the reference pembrolizumab therapy. The result was negative. We briefly review the clinical trials that investigated epacadostat in cancer patients and discuss possible explanations for this negative result. We end by suggesting paths to resume clinical development of compounds targeting the IDO1 pathway, which in our view remains an attractive target for cancer immunotherapy.

INTRODUCTION

Immune checkpoint inhibitors such as the anti-PD1 antibodies nivolumab and pembrolizumab, and the anti-CTLA4 antibody ipilimumab, have dramatically changed the prognosis of patients with metastatic melanoma (Postow et al. 2015; Robert et al. 2011, 2015; Taube et al. 2015). After first-line pembrolizumab, about 47% of patients show a clinical response, and their median survival raises to 38.7 months (Long et al. 2018b). The absence of benefit in the remaining patients could be attributed to the paucity of relevant tumor antigens or to local immunosuppressive mechanisms other than PD-L1. Over the last 15 years, tryptophan catabolism has emerged as an important mechanism of tumoral immune resistance (van Baren & Van den Eynde 2015b). Tryptophan is rapidly degraded into kynurenine by indoleamine-2,3 dioxygenase (IDO1), as the first and rate-limiting step of tryptophan catabolism along the kynurenine pathway. The resulting local tryptophan depletion, combined with the effects of kynurenine and its metabolites, impairs the proliferation of effector T lymphocytes and induces regulatory T cells, tolerogenic dendritic cells, and protumoral inflammation (**Figure 1**) (Munn & Mellor 2016). IDO1 expression is normally restricted to the placenta and lung endothelium, mucosal cells in the female genital tract, and myeloid cells in lymphoid tissues (Theate et al. 2015). It is strongly induced in inflammatory tissues by interferon-gamma and thereby contributes to the counter-regulatory effects of this cytokine on the immune response and the shaping of protumoral inflammation (Mellor et al. 2017, Mondal et al. 2016, Smith et al. 2012). IDO1 is also frequently expressed in human tumors, and tumoral IDO1 was shown to protect mouse tumors from immune rejection (Theate et al. 2015, Uyttenhove et al. 2003). In multiple preclinical tumor models, pharmacological inhibitors of IDO1 were shown to increase the therapeutic efficacy of cancer vaccines, immune checkpoint inhibitors, or chemotherapy (Blair et al. 2019, Holmgaard et al. 2013, Muller et al. 2005, Spranger et al. 2014, Uyttenhove et al. 2003). On this basis, several IDO1 inhibitors have been developed and are currently under clinical development (Platten et al. 2019). The most advanced is epacadostat, a highly specific IDO1 inhibitor, which has already been tested in several clinical trials (Beatty et al. 2017). Encouraging results observed in a phase I/II trial in combination with anti-PD1 have prompted the launch of a large phase III trial, named ECHO-301/KN-252, testing epacadostat in combination with pembrolizumab (E+P) in advanced melanoma patients (Mitchell et al. 2018). The initial results of this trial, which were presented at the ASCO (American Society of Clinical Oncology) 2018 Annual Meeting, did not show any benefit in patients treated with E+P as compared to pembrolizumab alone (Long et al. 2018a). The trial was stopped and the whole clinical

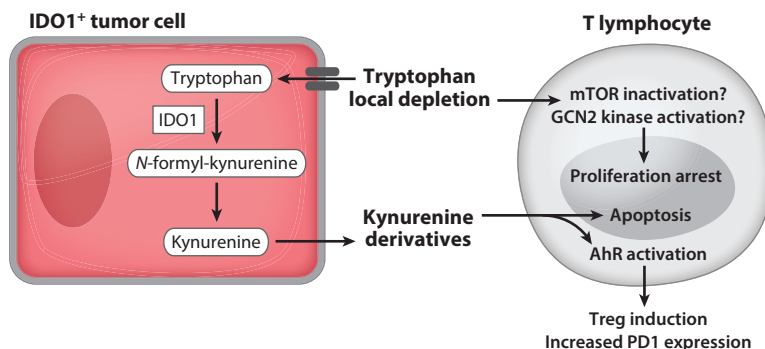


Figure 1

Proposed mechanisms by which IDO1-mediated tryptophan degradation inhibits local T cell responses in tumors. Abbreviations: AhR, aryl hydrocarbon receptor; Treg, regulatory T cell.

development of IDO1 inhibitors experienced a serious setback, with several phase III trials put on hold or converted into phase II (Platten et al. 2019).

The key points we need to address now are the reasons for the clinical failure of epacadostat in melanoma and whether IDO1 remains a relevant immuno-oncology target. This could help us determine the path forward in the clinical development of IDO1 inhibitors for cancer therapy. We begin by summarizing the initial phase I/II epacadostat trials as well as the phase III ECHO-301 trial. We then discuss several reasons that could explain the outcome of ECHO-301, starting from the biology of the target IDO1. This leads us to several considerations indicating that the clinical development of IDO1 inhibitors deserves to be resumed.

EPACADOSTAT: CLINICAL RESULTS

Epacadostat (INCB024360) is an oral drug that competitively blocks the tryptophan-degrading activity of IDO1 with an IC₅₀ (half-maximal inhibitory concentration) value around 10 nM, while it does not inhibit IDO2 (indoleamine 2,3-dioxygenase 2) or TDO (tryptophan 2,3-dioxygenase), two other tryptophan-degrading enzymes (Liu et al. 2010). It has antitumor activity as a monotherapy in a few immunocompetent—but not in immunodeficient—models (Koblish et al. 2010). However, in most models, the antitumor activity of epacadostat is best seen in combination with other immunotherapy agents (Spranger et al. 2014). A first-in-human study was conducted in 52 metastatic patients (Beatty et al. 2017). In this dose-escalation phase I trial, doses ranging from 50 mg once daily to 700 mg twice daily (BID) were tested. Epacadostat was well tolerated and the maximal tolerated dose was not reached. The half-life of the drug was determined to be 2.4–3.9 h by pharmacokinetic analysis. Plasma kynurenine concentrations are often elevated in cancer patients, suggesting tumor-associated IDO1 activity. These levels decreased at all doses of epacadostat, reaching a plateau of 50% decrease from baseline at day 15, starting at the dose of 300 mg BID. None of the 52 patients showed a clinical response. Stable disease lasting more than 16 weeks was seen in 18 patients (32%). Based on these results, and on preclinical studies showing a synergy between IDO1 inhibition and immune checkpoint blockade (Spranger et al. 2014), three phase I/II trials were initiated to investigate the combination of epacadostat with either ipilimumab (<https://www.clinicaltrials.gov/> identifier NCT01604889), nivolumab (ECHO-204, NCT02327078), or pembrolizumab (ECHO-202/KN-037, NCT02178722).

In NCT01604889, seven patients with metastatic melanoma received ipilimumab at 3 mg/kg every three weeks on four occasions and epacadostat at 300 mg BID (Gibney et al. 2019). Five patients developed significant increases in serum alanine amino-transferase (ALT) levels, two of whom required steroid therapy after treatment discontinuation, resulting in ALT normalization. The protocol was put on hold for six months and amended to explore lower doses of epacadostat. Only 2 of the 50 newly included patients received the full 12-week treatment, at the dose of either 25 mg or 50 mg of epacadostat BID. The objective response rate measured by RECIST (Response Evaluation Criteria in Solid Tumors) was 18%, and stable disease was observed in 26% of the patients. Further development was stopped to favor the combination with anti-PD1 therapy.

In the phase II part of the ECHO-204 trial, nivolumab (240 mg every two weeks) was combined with epacadostat at 100 mg BID or 300 mg BID (Perez et al. 2017). The response rate of the 50 patients included was 62%, including 6/8 patients who received 100 mg and 25/42 who received 300 mg (Daud et al. 2018, Perez et al. 2017). Toxicities were more frequent after higher doses of epacadostat (Daud et al. 2018). Grade 3 treatment-related adverse events were observed in 48% of the patients receiving epacadostat at a dose of 300 mg BID and 13% with epacadostat at 100 mg BID. The most common adverse events were rash, pneumonitis, and ALT increase.

In ECHO-202, E+P was given to patients with advanced solid tumors (Mitchell et al. 2018). Sixty-two patients were included and received at least one dose of study treatment. Four dose levels of epacadostat were studied: 25, 50, 100, and 300 mg BID, in 4, 20, 18 and 20 patients, respectively, always in combination with pembrolizumab at 2 mg/kg every three weeks. The treatment was generally well tolerated, with 24% of grade 3/4 toxicities, consistent with an anti-PD1 monotherapy. No grade 3–4 ALT increase was observed even with epacadostat at 300 mg BID. Time-averaged inhibition of IDO1 enzymatic activity predicted by pharmacokinetics showed that all patients at the 100 and 300 mg BID dose levels had at least 50% inhibition. At 100 mg BID, 9 patients out of 15 had 70% but none had 90% inhibition. At 300 mg BID, only 6 out of the 19 patients showed 90% IDO1 inhibition. Twenty-one patients had metastatic melanoma and were evaluable for antitumoral effect. An impressive response rate of 57% (12/21 patients) was observed, which compared favorably to historical cohorts of melanoma patients receiving pembrolizumab only (47%) (Long et al. 2018b). These responses were observed at all dose levels of epacadostat.

Based on the high frequency and severity of adverse events observed after epacadostat combined with ipilimumab or with nivolumab, and the acceptable safety profile and encouraging antitumoral effect of E+P, it was decided to run a randomized phase III trial comparing pembrolizumab at a fixed dose of 200 mg every three weeks with either epacadostat at 100 mg BID or a placebo (ECHO-301/KN-252) (Long et al. 2018a). The main inclusion criteria were cutaneous unresectable stage III or stage IV melanoma, good performance status, and no active brain metastases. Anterior adjuvant treatment with ipilimumab or interferon-alpha was allowed. Two stratification factors were used: tumoral PD-L1 expression and BRAF V600 mutational status. The coprimary endpoints were progression-free survival (PFS) and overall survival (OS). In all, 706 patients were randomized, including 72.5% with PD-L1-positive tumors and 44.5% with a BRAF V600 mutation. The baseline characteristics were in general well balanced between the two groups. However, some not statistically significant differences disadvantaging the E+P arm versus the pembrolizumab arm were observed. These included increased baseline LDH (123/354, 34.7% E+P patients versus 113/352, 32.1% pembrolizumab patients), M1c disease stage (64.4% versus 60.8% patients), brain metastases (5.4% versus 4.0%), prior adjuvant therapy (9.6% versus 6.5%), and prior lines of therapy (13.6% versus 11.9%). The PFS at six months was remarkably identical for the E+P and pembrolizumab arms: 45.8%. No significant difference between the two treatment arms was seen when comparing stratified subgroups. Interestingly, we see a trend for a higher activity of E+P in patients with a low tumor burden (Long et al. 2018a). The OS at one year was identical in both groups: 74%. It will be interesting to analyze follow-up data on OS at later time points, but it is unlikely that the message will change. These PFS and OS rates are in line with the results of the treatment-naïve patients treated with pembrolizumab as monotherapy in the Keynote-006 study (Long et al. 2018b). However, the response rates observed in the ECHO-301 trial (34.2% for E+P, 31.5% for pembrolizumab monotherapy) were lower than in the Keynote-006 (47% for pembrolizumab monotherapy) and ECHO-202 (57% for E+P) trials.

JUMPING FROM UNSELECTED SMALL PHASE I TRIAL TO A LARGE RANDOMIZED PHASE III TRIAL

The outcome of ECHO-301 illustrates the high risk of skipping the usual steps between phase I and phase III. Because they are not randomized, phase I trials are prone to selection biases that may confound the results, particularly in the case of combinations with compounds that are active as single agents, as is the case for pembrolizumab. One such selection bias can result from the fact that patients recruited for phase I trials often have a slowly progressive disease that allows them to enroll, sometimes repeatedly, in phase I trials, which usually impose some recruitment delays

because of the dose escalation. In the case of ECHO-202, 19 of the 22 melanoma patients enrolled were treatment naïve, but they could still have had a more indolent disease. In any case, a nonrandomized study on a small group of patients, which requires comparison with historical response rates of pembrolizumab alone (47% in melanoma), can be misleading and normally calls for a confirmatory study—typically a randomized phase II trial—before embarking in a large phase III trial. Such confirmatory study could also be an opportunity to stratify patients to increase the chance of detecting activity. In this case, for example, one could stratify patients for resistance to anti-PD1 therapy or for expression of IDO1 in the tumor, as discussed below. No such stratification was performed in ECHO-301, nor was any confirmatory phase II performed.

The importance of clinical trial design is illustrated by the development of anti-CTLA4 antibodies. The first phase III trial, involving tremelimumab, proved negative in classical response assessments and was abandoned early, while a second phase III trial, which involved ipilimumab, was adapted to the slow pace of response to this class of drugs and proved positive (Hodi et al. 2010, Hoos 2016). Tremelimumab development was recently resumed and suggests a clinical efficacy similar to that of ipilimumab (Comin-Anduix et al. 2016).

DOSING OF EPACADOSTAT: WAS IT SUFFICIENT TO FULLY BLOCK IDO1 ACTIVITY?

The dose of 100 mg BID of epacadostat that was chosen in ECHO-301 seems to rely firstly on the hepatic toxicities observed when combining ipilimumab and 300 mg BID epacadostat. Ipilimumab alone is known to cause autoimmune hepatitis, and increased hepatic toxicity was observed when it was combined with several drugs including DTIC (dacarbazine), the BRAF inhibitor vemurafenib, and nivolumab (Postow et al. 2015, Ribas et al. 2013, Robert et al. 2011). Of note, the severe hepatotoxicity observed after ipilimumab and epacadostat treatment appears to be autoimmune in nature, and this can be seen as an argument in favor of immune effects of epacadostat, which, in turn, might be predictive of antitumoral effects. The second argument for the choice of the dose is that the response rate in ECHO-202 did not show major differences between the four dose cohorts, including at the low dose levels of 25 and 50 mg BID. However, the small size of these cohorts does not allow to exclude a sampling imbalance in favor of responses obtained by pembrolizumab alone. In addition, we know from phase I data that the enzymatic inhibition of IDO1 by epacadostat is dose dependent (Beatty et al. 2017). Indeed, low doses could induce only a minor reduction in the production of kynurenine in the serum of treated patients. It is only at 100 mg BID that we see the inflection point of the pharmacodynamic curve. A plateau with 50% reduction in the production of kynurenine is only observed at the dose of 300–400 mg BID, and at these dose levels only one third of the patients achieve 90% inhibition of IDO1 (Beatty et al. 2017). It is possible that this rate of inhibition was not sufficient to fully block the immunosuppressive effects of IDO1 in the tumor. This can be estimated by measuring kynurenine modulation in the tumor using paired biopsies. In another phase I study combining nivolumab with BMS986205 (another IDO1 inhibitor), a 50–60% reduction of kynurenine was observed in the serum of all treated patients, but only two thirds of them showed a significant reduction of kynurenine in the tumor (Luke et al. 2017). For epacadostat, there is no data on modulation of kynurenine levels in the tumors of treated patients. Thus, insufficient inhibition of the target is a potential reason for the negative results of ECHO-301. Future trials should consider increasing the dose of epacadostat to achieve full target coverage. New IDO1 inhibitors are in clinical development, and some of them appear more potent than epacadostat (Platten et al. 2019). They should be dosed in such a way that they fully inhibit IDO1 activity. This is usually monitored by measuring systemic kynurenine, whose production is largely dependent on IDO1 activity (Schramme et al. 2020). Serum

kynurenine levels are very low in healthy individuals, but are increased in patients bearing IDO1-expressing tumors. In such patients, serum kynurenine is therefore a sensitive pharmacodynamic marker, but caution should be taken when using this marker in patients bearing IDO1-negative tumors, such as glioblastomas (Reardon et al. 2017, Theate et al. 2015), as kynurenine levels are not elevated in these patients in the first place. Obviously, it would be of great interest to have pharmacodynamic results in the melanoma patients of the ECHO-301 trial. Unfortunately, no pharmacokinetic/pharmacodynamic sampling was performed in the trial.

SELECTION OF PATIENTS FOR TUMORAL IDO1 EXPRESSION?

For a pharmacological inhibitor of IDO1 to be clinically efficient against cancer, its target must be expressed in the tumor of the treated patients. It would therefore make sense to select patients with an IDO1-expressing tumor for testing IDO1 inhibitors. Although initial studies reported a higher expression of IDO1 in tumor-draining lymph nodes as compared to tumors (Munn et al. 2002), this was not confirmed in subsequent studies that demonstrated the prominent expression of IDO1 in the tumors, either in tumor cells themselves or in stromal cells (Theate et al. 2015, Uyttenhove et al. 2003). Moreover, mechanistic studies confirmed the dominant role of tumoral IDO1 in preventing response to immunotherapy (Spranger et al. 2014). It was shown that IdO1 inhibitors, when combined with checkpoint inhibitors, favored tumor rejection in mice even when lymphocytes were prevented from egressing the lymph nodes (Spranger et al. 2014).

A comprehensive analysis showed that IDO1 is expressed in most human tumors, with a few exceptions such as glioblastoma (Theate et al. 2015). This expression, however, can be heterogeneous and can be observed in tumor cells, stromal cells, or endothelial cells, or combinations thereof. Stromal expression of IDO1 is usually observed in tumors that are rich in immune infiltrates. Because *IDO1* transcription is strongly induced by interferon-gamma, IDO1 expression in inflamed tumors likely results from interferon-gamma produced by tumor-infiltrating lymphocytes. Consistently, transcriptomic studies reported a strong correlation between CD8⁺ T cell infiltration and *IDO1* expression in melanoma (Spranger et al. 2013). This is similar to *CD274* (the gene encoding PD-L1), which is also inducible by interferon-gamma and whose expression is also correlated with CD8 in melanomas (Obeid et al. 2016). This represents a mechanism of adaptive resistance, in which the tumor reacts to infiltrating T cells by producing immunosuppressive factors PD-L1 and IDO1 (Taube et al. 2015).

However, in other tumor types, including endometrial and ovarian cancers, IDO1 expression can be observed in noninflamed tumors and is confined to tumor cells themselves (Theate et al. 2015). This constitutive expression represents a mechanism of intrinsic immune resistance, which can prevent the accumulation of tumor-infiltrating lymphocytes and contribute to the fact that these tumors are cold. In a preclinical study, human lymphocytes failed to infiltrate and reject xenografts of the IDO1-expressing human ovarian carcinoma cell line SKOV3 implanted in immunodeficient mice reconstituted with human lymphocytes. Importantly, IDO1 inhibition with epacadostat turned these cold tumors into hot tumors that were then rejected (Hennequart et al. 2017). The signaling mechanism accounting for constitutive expression of IDO1 in human tumors was shown to rely on autocrine production of PGE2 by these tumors, which express high levels of COX2 as a result of overactive MAPK signaling (Hennequart et al. 2017). The concept of IDO1 acting either as an adaptive or intrinsic resistance mechanism in tumors is illustrated in **Figure 2**.

To assess which of these two mechanisms of IDO1 expression prevails in which type of tumor, we considered that we could analyze a large set of transcriptomic tumor data and separate tumors for which *IDO1* expression is correlated with a signature of exposure to interferon-gamma from

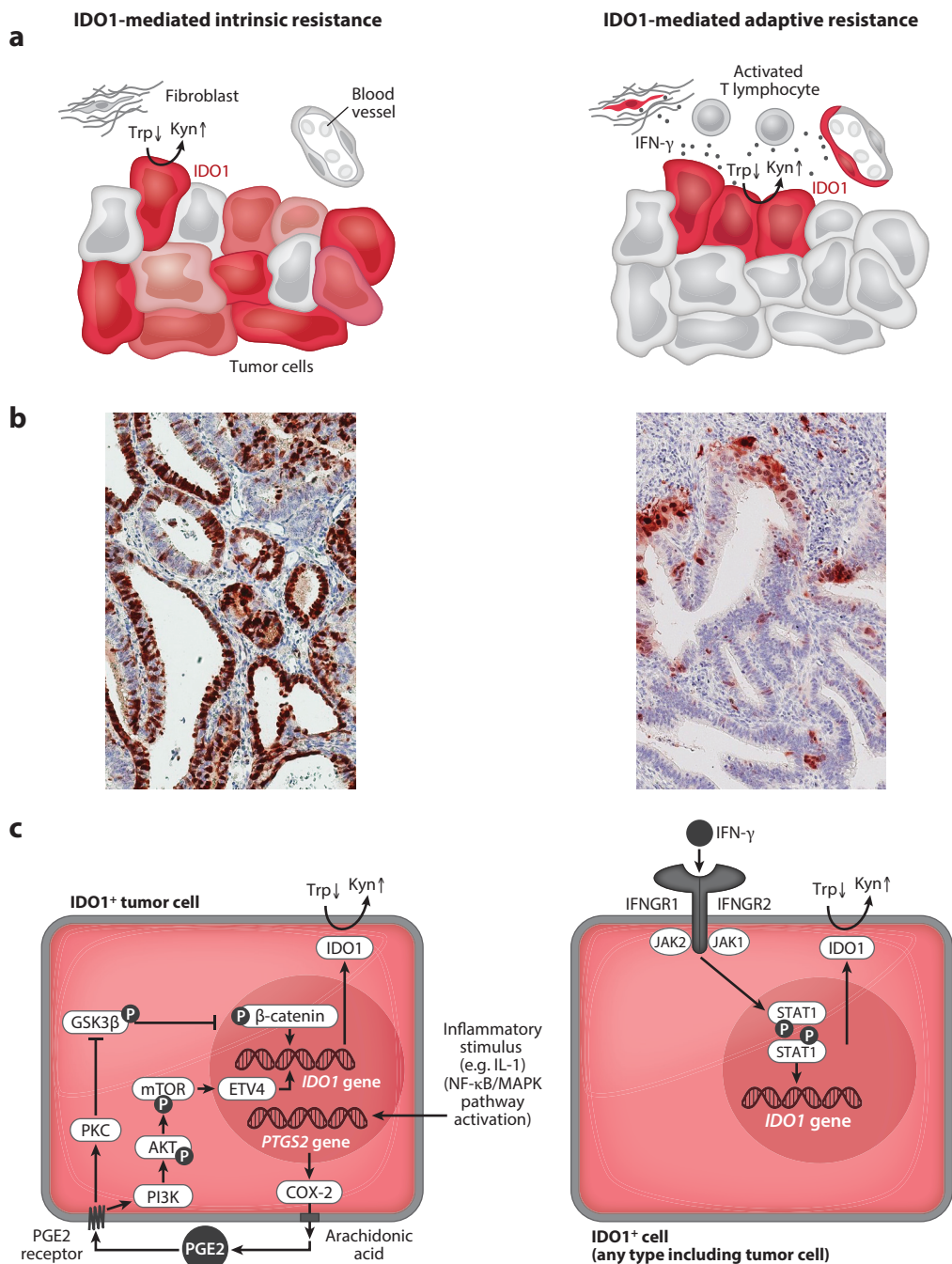


Figure 2

Two different mechanisms account for IDO1 expression in tumors, allowing them to resist T lymphocyte attack. (*Left column*) IDO1 is constitutively expressed in tumor cells (intrinsic resistance). (*Right column*) IDO1 is induced in tumor cells and other cell types by IFN- γ released by neighboring activated T or NK cells in a negative feedback loop (adaptive resistance). (*a*) Schematic view of the two types of IDO1-mediated resistance. (*b*) Illustrative histology view of an endometrial carcinoma (*left*) and cervical carcinoma (*right*) tissue section stained for IDO1 (dark red). (*c*) Molecular mechanisms accounting for IDO1 expression. Abbreviations: IFN- γ , interferon-gamma; Kyn, kynurenine; NK, natural killer; Trp, tryptophan.

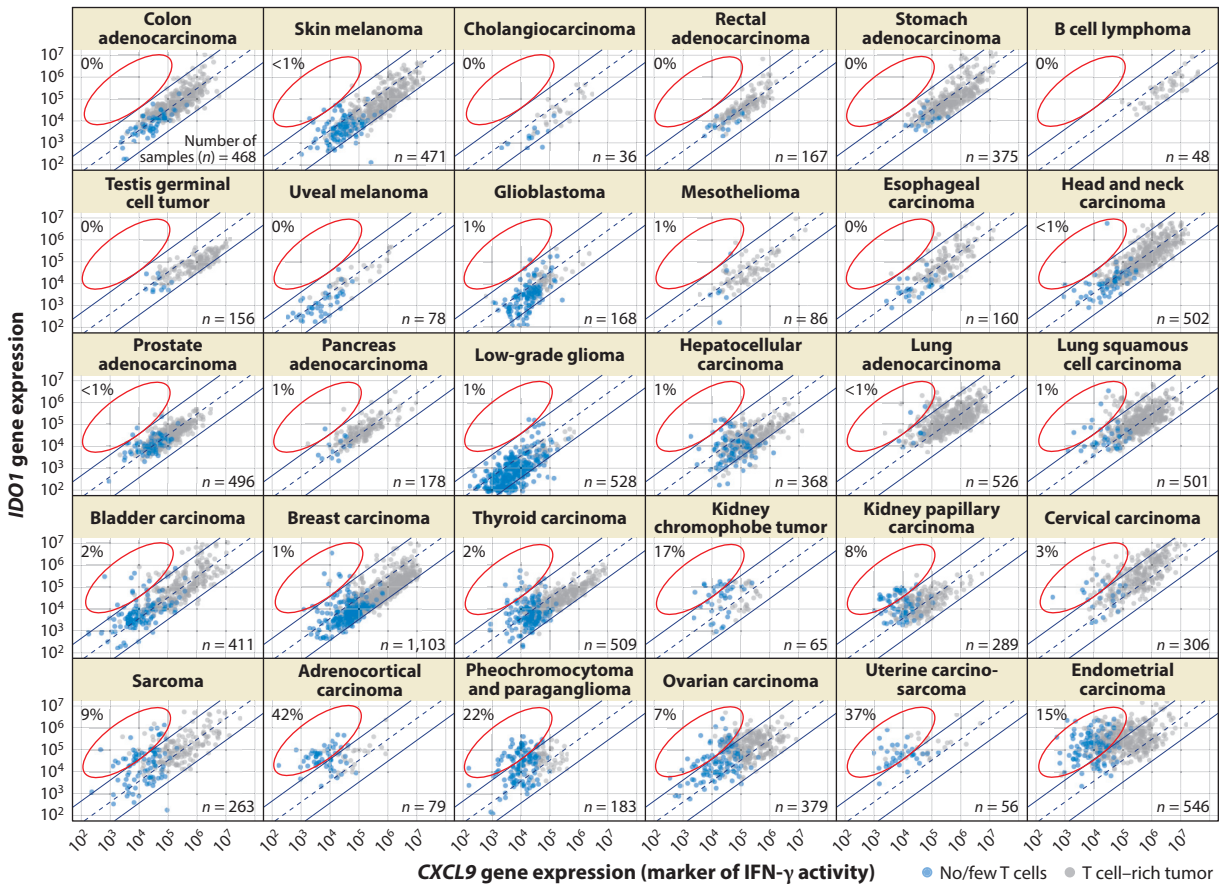


Figure 3

The proportion of *IDO1*-positive tumors with weak T cell infiltration and activity varies greatly from one tumor type to another. The expression values are taken from TCGA (The Cancer Genome Atlas) transcriptome data and are displayed in a log 10 scale of normalized FPKM-UQ values (fragments per kilobase transcript length per million reads, adjusted to upper quartile level for each sample). Each panel is a tumor type. Each dot is a tumor sample, colored according to its T cell content (*blue*, no or few T cells, defined as a *CD3E* gene expression level below 25,000; *gray*, T cell rich, defined as *CD3E* \geq 25,000). Some tumor types, such as colon adenocarcinomas and skin melanomas, have their *IDO1* expression proportional to IFN- γ (interferon-gamma) activity, mainly reflecting adaptive *IDO1* induction in these tumors. Most of these tumors can be found between the two parallel plain lines in each panel, which were obtained by expanding (± 1 log) the linear regression line (*dashed line*) from the colon adenocarcinoma series, which was then taken as reference and applied to all panels. In contrast, other tumors such as endometrial carcinomas display high *IDO1* associated with weak *CXCL9* expression and often poor T cell infiltration, reflecting constitutive tumoral *IDO1* expression in cold tumors. These tumors appear as blue dots above the upper plain line and their proportion is indicated in the upper left corner of each panel. Most of them are included in the red ellipse. Two TCGA tumor types were excluded: renal cell carcinomas because of their frequent vascular expression of *IDO1*, and thymomas because of their tumoral expression of *CD3E*.

those for which *IDO1* expression is not so correlated. We chose *CXCL9* as a major interferon-gamma target gene. We mined the whole transcriptome data from the TCGA (The Cancer Genome Atlas), encompassing a large series of tumors (Cancer Genome Atlas Res. Netw. et al. 2013), and we correlated the expression of *IDO1* and *CXCL9*. To identify cold tumors, we also used the level of *CD3E* transcripts, which are specific to T lymphocytes. The results are shown in **Figure 3**. They confirm a strong correlation between *IDO1* expression and interferon-gamma

activity in tumors such as colorectal carcinomas and melanomas, but also in many other tumor types. However, they also identify some tumor types with a high proportion of samples expressing high *IDO1* but much less *CXCL9*. Moreover, these tumors often display very low T cell infiltrates, in line with the notion that constitutive *IDO1* expression is associated with cold tumors. This is observed mostly in gynecological tumors (mainly endometrial and ovarian carcinomas), and in less frequent tumors such as adrenocortical, neuroendocrine, and kidney chromophobe tumors.

IDO1 expression was not evaluated in the tumors from the melanoma patients enrolled in ECHO-301. One can indeed reason that *IDO1* expression can be induced in all tumors once T cells are boosted by the checkpoint inhibitor and start producing interferon-gamma. As mentioned above, *IDO1* expression in melanoma is mostly adaptive and associates with high PD-L1 expression. In ECHO-301, patients with differing PD-L1 expression in their tumor did not have different outcomes (Long et al. 2018a). Because *IDO1* and PD-L1 are coexpressed in melanoma, this suggests that stratification for *IDO1* expression would not have changed outcomes either.

This notion that *IDO1* expression in melanoma is mostly adaptive and associated with PD-L1 expression and high T-cell infiltration raises interesting considerations regarding the outcome of ECHO-301. It means that *IDO1*-expressing melanomas, which are those in which we expected to see a clinical benefit of adding an *IDO1* inhibitor to the checkpoint inhibitor, are typically tumors with a high T cell infiltrate. Because it is now clear that inflamed tumors respond better to checkpoint inhibitors than cold tumors (Jacquelot et al. 2017, Madonna et al. 2018, Postow et al. 2015, Robert et al. 2015), this factor may have reduced the therapeutic window to see the benefit of the combination. Such benefit might be easier to detect in tumors that express *IDO1* constitutively, which are typically cold tumors. Future clinical trials could therefore focus on tumor types in which *IDO1* expression is constitutive, selecting or stratifying patients for *IDO1* expression by tumor cells.

COMPENSATORY EXPRESSION OF OTHER TRYPTOPHAN-DEGRADING ENZYMES: TDO AND IDO2?

Two other enzymes can degrade tryptophan along the kynurenine pathway and potentially contribute to tumor resistance to immune rejection: TDO, encoded by the gene *TDO2*, and IDO2. It has been proposed that resistance to *IDO1* inhibition in ECHO-301 might result from compensatory overexpression of TDO or IDO2 (Muller et al. 2019). This possibility also emerged from observations of increased kynurenine production in lung metastases developing in *Ido1*-knockout mice (Smith et al. 2012).

TDO is expressed at a high level in normal liver and contributes to maintaining a stable level of tryptophan in the blood by degrading excess dietary tryptophan (Kanai et al. 2009). Transcriptomics studies showed that *TDO2* was also expressed in several human tumors (Opitz et al. 2011, Pilotte et al. 2012). Several human tumor lines also express *TDO2* in a constitutive manner, and *TDO2*-transfected mouse tumor cells resist immune rejection, which can be restored upon treatment with a TDO inhibitor (Pilotte et al. 2012). The level of TDO expression in human tumors and the identity of TDO-expressing cells have remained unclear until the recent development of highly specific TDO monoclonal antibodies, which allowed for a refined characterization of TDO-expressing cells in human tumors (Hoffmann et al. 2020). The results confirmed the expression of TDO in the majority of human tumors. In hepatocarcinoma, TDO expression is mostly observed in tumor cells, in line with the expression in normal hepatocytes. However, in most other tumors, TDO expression is restricted to a subset of cells of the tumor vasculature, which

were identified as pericytes. Besides hepatocarcinoma, the highest proportions of TDO-expressing cells were observed in glioblastoma and melanoma metastases. However, they only represent a few percent of the cellular content of those tumors. It appears quite unlikely that these rare TDO-expressing cells can catabolize tryptophan at a level sufficient to suppress immune responses. In addition, the activity of TDO is characterized by a higher K_M (Michaelis constant) than IDO1 (van Baren & Van den Eynde 2015a), and the stability of TDO is impaired at low tryptophan concentrations (Lewis-Ballester et al. 2016; S. Klaessens, V. Stroobant, D. Hoffmann, L. Pilotte, M. Gyrd-Hansen, et al., manuscript in preparation). Therefore, while tumoral TDO can produce kynurenine when exposed to high tryptophan concentrations, it appears unlikely that it can degrade tryptophan to very low concentrations. Both tryptophan depletion and kynurenine production seem to contribute to immunosuppression (Fallarino et al. 2006, Schramme et al. 2020). Therefore, while a role of TDO in tumoral immune resistance is likely in hepatocarcinoma, such a role is less likely in other tumor types. However, we do not know what triggers TDO expression, and it cannot be excluded that compensatory TDO overexpression is induced upon IDO1 inhibition and contributes to explain the results of ECHO-301. Answering this question would require an evaluation of TDO expression in the tumors from patients who did not respond to epacadostat combined with anti-PD1. Were this to be true, this would be a strong case in favor of developing dual IDO1/TDO inhibitors, some of which have entered clinical testing (Platten et al. 2019).

The other enzyme that could contribute to resistance to IDO1 inhibition is IDO2. Human IDO2 is encoded by a gene that appears to have resulted from a duplication of the IDO1 gene. Two IDO2 alleles are found in equal proportions of Caucasians. One of these alleles has a polymorphism in the predicted catalytic site that prevents enzymatic activity (Metz et al. 2007). The other allele does show some activity in degrading tryptophan; however, this activity is extremely low and can only be detected in vitro in the presence of supraphysiological concentrations of tryptophan, with a k_{cat} (catalytic rate constant) 30 times lower and a K_M about 300 times higher than that of IDO1 (Fatokun et al. 2013, Pantouris et al. 2014). Whether IDO2 plays an important role in physiological conditions is presently unclear (Jusof et al. 2017). In addition, although murine *Ido2* is expressed in some tissues (Jusof et al. 2017), the expression of IDO2 in normal and tumoral human tissues is very low, raising doubts about its relevance (van Baren & Van den Eynde 2015b). Here also, one cannot exclude a compensatory increase of IDO2 expression in tumors that resisted epacadostat/anti-PD1 combination, but this would require the analysis of IDO2 expression in tumors that progressed under this therapy.

COMBINATION WITH ANTI-PD1 THERAPY: IS IT THE BEST CHOICE?

There are multiple immunosuppressive mechanisms triggered by tryptophan catabolism, and it is presently unclear whether tryptophan depletion or kynurenine production is the driver immunosuppressive factor. It appears that a combination of both is required to achieve full immunosuppression, which is exerted through at least three mechanisms (**Figure 1**) (Lemos et al. 2019). One involves the activation of the GCN2 pathway, which is sensitive to an increase in tRNAs (transfer RNAs) that are not coupled to amino acids, as happens in the case of amino acid depletion (Munn et al. 2005). GCN2 then phosphorylates the translation initiation factor eIF2A, resulting in a shutdown of protein translation. However, the role of GCN2 in IDO1-mediated immunosuppression was recently questioned (Sonner et al. 2016). As a second mechanism, tryptophan depletion impairs mTOR activation, which is required for efficient protein translation (Cobbold et al. 2009, Metz et al. 2012). In a third mechanism, kynurenine appears to contribute to immunosuppression by favoring differentiation of regulatory T cells and tolerogenic dendritic cells

through a mechanism that depends on the aryl hydrocarbon receptor (AhR) (Fallarino et al. 2006, Gutierrez-Vazquez & Quintana 2018, Mezrich et al. 2010). Some kynurenine derivatives can also induce apoptosis of effector T lymphocytes (Terness et al. 2002). Recently, kynurenine was also shown to increase PD1 expression on activated T lymphocytes (Liu et al. 2018). PD1 expression is normally triggered by T cell activation, but further increased PD1 levels are found on exhausted T cells. Kynurenine was shown to contribute to these increased levels in an AhR-dependent manner (Liu et al. 2018). This finding might be relevant to the interpretation of the ECHO-301 results. Indeed, if overexpression of PD1 induced by kynurenine is a dominant immunosuppressive effect of tryptophan catabolism, it may not be surprising to see no synergy of IDO1 inhibitors with anti-PD1, as both drugs act on the same pathway: Blocking IDO1 would reduce PD1 expression on tumor-infiltrating T cells, but this would not add any benefit if the PD1/PD-L1 axis is already fully blocked by anti-PD1. However, synergy was observed in mouse tumor models (Spranger et al. 2014), although not in others (Blair et al. 2019). But it is not unlikely that inhibition of the PD1 pathway by anti-PD1 antibodies is more complete in humans than it is in mice, so that mouse models may have a window for improvement that could be absent in humans. If that holds true, then further clinical trials should explore combinations of IDO1 inhibitors with immunotherapy agents other than anti-PD1/PD-L1. Among candidates are cancer vaccines (Blair et al. 2019) and checkpoint inhibitors other than perhaps ipilimumab given the toxicities observed with this combination.

ARYL HYDROCARBON RECEPTOR ACTIVATION BY EPACADOSTAT?

As mentioned above, part of the immunosuppressive effects of tryptophan catabolism are mediated via AhR, which can be activated by kynurenine and its metabolites (Gutierrez-Vazquez & Quintana 2018). A recent study reported that several IDO1 inhibitors, including epacadostat, can also activate AhR (Moyer et al. 2017). Although activation by epacadostat was relatively weak and not observed in all experimental settings, these results raise the possibility that epacadostat might have two opposing activities: inhibiting IDO1 activity to remove immunosuppression and at the same time activating AhR to favor immunosuppression. Although this might be a potential explanatory factor of the outcome of ECHO-301, it remains unclear why such an effect was not observed in preclinical models, even those that used immunodeficient mice reconstituted with human lymphocytes (Hennequart et al. 2017).

NONCATALYTIC EFFECTS OF IDO1?

Besides its enzymatic properties, IDO1 appears to have signaling activity, which was described in mice by the group of Ursula Grohmann (Albini et al. 2017, Pallotta et al. 2011). These long-term effects were observed in dendritic cells and result in a tolerogenic phenotype of these cells when exposed to an environment rich in TGF- β . The pathway involves the phosphorylation of immunoreceptor tyrosine-based inhibition motifs (ITIMs) that are present in IDO1 and then recruit phosphatases such as SHP2, which mediate the signaling activity and induce further expression of IDO1 and TGF- β . It is unclear at this stage whether this signaling activity is also active in human dendritic cells and whether it is relevant for tumoral immunosuppression. If this were the case, it would open the possibility that some IDO1 inhibitors, which were selected for their ability to block the catalytic activity of IDO1, may not in fact block its signaling activity. To shed light on the outcome of ECHO-301, it might be of interest to determine whether epacadostat does block IDO1 signaling activity. If it does not, there may be interest in developing compounds that block both the catalytic and the signaling activity of IDO1.

OTHER MEANS OF BLOCKING THE TRYPTOPHAN/KYNURENINE/ARYL HYDROCARBON RECEPTOR PATHWAY?

As mentioned above, there is a wealth of scientific arguments supporting the relevance of tryptophan catabolism in tumoral immunosuppression and tolerance. Yet there are several players in this pathway, and it could be beneficial to hit additional targets in the pathway, in addition to or in place of IDO1 (Platten et al. 2019). Besides TDO inhibitors, which are mentioned above, AhR inhibitors are being developed (Platten et al. 2019) and may be promising, even though AhR remains a complex pathway whose activation may lead either to immunotolerance (regulatory T cells) or to inflammation (Th17 cells) depending on the ligand (Quintana et al. 2008). Interesting results were also reported recently in mouse tumor models with peritumoral injections of recombinant PEG(polyethylene glycol)-ylated kynureninase, an enzyme that degrades kynurenine. Local kynurenine depletion increased T cell infiltration into tumors and improved the therapeutic efficacy of checkpoint inhibitors or cancer vaccines (Triplett et al. 2018).

Another alternative approach to block the IDO1 pathway is to repress IDO1 expression in tumors. As mentioned above, we have shown that constitutive IDO1 expression in human tumors is driven by an autocrine PGE2 loop triggered by COX2 and can be repressed with the COX2 inhibitor celecoxib (Hennequart et al. 2017). By repressing IDO1 expression, celecoxib can heat up cold human tumor xenografts in humanized mice and induce their rejection (Hennequart et al. 2017). This opens the possibility of using celecoxib in combination with immunotherapy in patients with tumors expressing IDO1 constitutively. A benefit of such an approach is that repressing IDO expression would affect both the catalytic and the signaling activity of IDO1. Clinical trials are currently underway in our institution to test this possibility, starting with a window-of-opportunity design with paired biopsies to confirm IDO1 repression and heating up of cold tumors with celecoxib (NCT03896113, NCT03864575).

CONCLUSION

The IDO1 pathway remains a relevant target to block in order to improve the efficacy of cancer immunotherapy. This relevance is supported by several preclinical mouse tumor models and by evidence in humans demonstrating a worse survival of patients bearing IDO1-expressing tumors (Yu et al. 2018). In preclinical models, IdO1 inhibitors were shown to increase the therapeutic efficacy of checkpoint inhibitors, cancer vaccines, or even chemotherapy (Blair et al. 2019, Holmgaard et al. 2013, Muller et al. 2005, Uyttenhove et al. 2003). This was shown not only with murine tumors, but also with human tumors grafted into immunodeficient mice reconstituted with human lymphocytes (Hennequart et al. 2017). The latter result was important to demonstrate that not only murine but also human lymphocytes were sensitive to IDO1-mediated suppression and could be rescued with epacadostat. However, we need to develop more efficient ways of blocking this pathway and monitoring its inhibition in patients. We also need to understand what is the best clinical setting to see the benefits of IDO1 inhibition. Most importantly, we need to design clinical trials that allow for translational studies to help us understand what happens in patients. Indeed, the most frustrating aspect of ECHO-301, besides the negative outcome, is the lack of sample collection for translational research, which could have provided answers to several key questions, as outlined above. This would have allowed us to learn from failure and design more efficient trials or approaches for the future. In the absence of such data, we have to consider the biology of the IDO1 pathway and make rational proposals to resume smaller-scale trials, oriented toward understanding what happens in patients, before launching new phase III trials. Several such proposals are detailed in the text above and summarized in **Table 1**.

Table 1 Potential reasons for the negative outcome of ECHO-301 and possible solutions

Possible causes	Possible solutions
Insufficient IDO1 inhibition by epacadostat in the tumor	Increase dose of epacadostat or use more potent IDO1 inhibitors; carefully monitor pharmacodynamic markers
No selection of patients for tumoral IDO1 expression	Select or stratify patients according to tumoral IDO1 expression
No selection for patients refractory to immunotherapy	Select patients who failed anti-PD1/PD-L1 therapy
The mechanism of IDO1 expression in melanoma is adaptive	Focus on tumor types with constitutive IDO1 expression
Epacadostat and pembrolizumab act on the same pathway and are therefore potentially not synergistic	Combine IDO1 inhibitor with other immunotherapy approaches
Compensatory expression of TDO or IDO2	Use dual inhibitors
Signaling activity of IDO1 is potentially not affected by epacadostat	Develop new IDO1 inhibitors that also block its signaling activity; repress IDO1 expression
Epacadostat activates AhR (aryl hydrocarbon receptor), which drives immune suppression	Use IDO1 inhibitor without AhR-activating effect or combine with AhR inhibitor
The tryptophan-kynurenine-AhR pathway is insufficiently blocked by IDO1 inhibitors	Combine or replace with drugs that block other steps of the pathway (e.g., AhR inhibitors or recombinant kynureninase)

DISCLOSURE STATEMENT

Benoit Van den Eynde is cofounder of, has ownership interest in, and is a Scientific Advisory Board member of iTeos Therapeutics. The other coauthors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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