Is there a clinical future for IDO1 inhibitors after the failure of epacadostat in melanoma?

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Running title

Future of IDO1 inhibition in the clinic

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Abstract

Indoleamine-2,3 dioxygenase 1 (IDO1) contributes to tumor immunosuppression by enzymatically degrading tryptophan, 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 will 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-CTLA-4 antibody ipilimumab, have dramatically changed the prognosis of patients with metastatic melanoma (Postow et al 2015, Robert et al 2015, Robert et al 2011, Taube et al 2015). After first-line pembrolizumab, about 47% of the 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 pro-tumoral inflammation (Figure 1) (Munn & Mellor 2016). IDO1 expression is normally restricted to 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 pro-tumoral 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, a number of 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 in advanced melanoma patients (Mitchell et al 2018). The initial results of this trial, which were presented at ASCO2018, did not show any benefit in patients treated with epacadostat and pembrolizumab as compared to pembrolizumab alone (Long et al 2018a). The trial was stopped and the whole clinical development of IDO1 inhibitors experienced a serious setback, with a number of 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 determining the path(s) forward in the clinical development of IDO1 inhibitors for cancer therapy. We will start this review by summarizing the initial phase I/II epacadostat trials as well as the phase III ECHO-301 trial.

We will then discuss a number of reasons that could explain the outcome of ECHO-301, starting from the biology of the target IDO1. This will lead us to a number of 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 value around 10 nM, while it does not inhibit IDO2 or TDO, 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 as 2.4-3.9 hour 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 *in vitro* 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 (NCT01604889), nivolumab (ECHO-204, NCT02327078) or pembrolizumab (ECHO-202/KN-037, NCT02178722).

In NCT01604889, 7 patients with metastatic melanoma received ipilimumab at 3 mg/kg every 3 weeks on 4 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 6 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 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 2 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, epacadostat was combined with pembrolizumab in 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. 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 epacadostat plus pembrolizumab, 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 twice daily 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 co-primary endpoints were progression-free survival (PFS) and overall survival (OS). Seven hundred and six patients were randomized, including 72.5% with PD-L1 positive tumor and 44.5% with a BRAF V600 mutation. The baseline characteristics were in general well balanced between the two groups. However, some non-statistically significant differences disadvantaging the epacadostat + pembrolizumab (E+P) arm versus the pembrolizumab (P) arm were observed. These included increased baseline LDH (123/354, 34.7% (E+P) vs. 113/352, 32.1% patients (P)), M1c disease stage (64.4% vs. 60.8% patients), brain metastases (5.4% vs. 4.0%), prior adjuvant therapy (9.6% vs. 6.5%) and prior lines of therapy (13.6% vs. 11.9%). The PFS at 6 months was remarkably identical for the E+P and P 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 the combo in patients with a low tumor burden (Long, 2018a). The OS at 1 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 E) were lower than in the Keynote-006 (47% for E) 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 enrol, sometimes repeatedly, to 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 non-randomized 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. 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 will be 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 of epacadostat twice daily that was chosen in ECHO-301 seems to rely firstly on the hepatic toxicities observed when combining ipilimumab and 300 mg epacadostat twice daily. Ipilimumab alone is known to cause autoimmune hepatitis, and increased hepatic toxicity was observed when it was combined with several drugs including DTIC, 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 appears to be autoimmune in nature, and 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 twice daily, 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 2/3 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 to explain 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 2019). 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 gliobastomas (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 on the role of IDO1 in cancer reported a higher expression of IDO1 in tumor-draining lymph nodes (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 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 non-inflamed 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 in which *IDO1* expression is correlated –or not– with a signature of exposure to interferon-gamma. We chose *CXCL9* as a major interferon-gamma target gene. We mined the whole transcriptome data from the TCGA, encompassing a large series of tumors (Cancer Genome Atlas Research 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 (blue dots: tumors with no or few T cells), 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 expressing or not PD-L1 in their tumor had no different outcome (Long et al 2018a). Because IDO1 and PD-L1 are co-expressed in melanoma, this suggests that stratification for IDO1 expression would not have changed outcome 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 with regard to 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, IDO2?

Two other enzymes can degrade tryptophan along the kynurenine pathway and potentially contribute to tumor resistance to immune rejection: tryptophan-dioxygenase (TDO, encoded by gene *TDO2*), and indoleamine 2,3-dioxygenase 2 (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-KO 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 a number of 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 has remained unclear until the recent development of highly specific TDO monoclonal antibodies, which allowed a refined characterization of TDO-expressing cells in human tumors (Hoffmann et al 2019). 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. 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 that is sufficient to suppress immune responses. In addition, the activity of TDO is characterized by a higher Km as compared to IDO1 (van Baren & Van den Eynde 2015a), and the stability of TDO is impaired at low tryptophan concentrations (Lewis-Ballester et al 2016) (Klaessens 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. And both tryptophan depletion and kynurenine production seem to contribute to immunosuppression (Fallarino et al 2006, Schramme et al 2019). 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. Answer to this question would require an evaluation of TDO expression in the tumors from patients who did not respond to epacacodostat 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 result from a duplication of the IDO1 gene. Two IDO2 alleles are found in equal proportions of Caucasians, one of which having 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 supra-physiological concentrations of tryptophan, with a kcat 30x lower and a Km about 300x higher than that of IDO1 (Fatokun et al 2013, Pantouris et al 2014). Whether IDO2 plays an important role in physiological conditions remains unclear at the moment (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 from patients whose tumor progressed under this therapy.

Combination with anti-PD1 therapy: is it the best choice?

The immunosuppressive mechanisms triggered by tryptophan catabolism are multiple, and it remains unclear to date 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 tRNA that are not coupled to amino acids, as happens in case of amino acid depletion (Munn et al 2005). GCN2 then phosphorylates 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 PD-1 expression on activated T lymphocytes (Liu et al 2018). PD-1 expression is normally triggered by T-cell activation, but further increased PD-1 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 PD-1 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-PD-1, as both drugs act on the same pathway: blocking IDO1 would reduce PD-1 expression on tumor-infiltrating T cells, but this would not add any benefit if the PD-1/PD-L1 axis is already fully blocked by anti-PD-1. Synergy was, however, 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 PD-1 pathway by anti-PD-1 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-PD-1/PD-L1. Among candidates are cancer vaccines (Blair et al 2019) and other checkpoint inhibitors, except maybe ipilimumab given the toxicities observed with this combination.

Aryl hydrocarbon receptor (AhR) 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 a number of 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 considered as a potential factor to explain 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).

Non-catalytic effects of IDO1?

Besides its enzymatic properties, IDO1 appears to have a 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-beta. The pathway involves the phosphorylation of ITIM motives 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-beta. 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. Would this be 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 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/AhR 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 PEGylated 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. Clinical trials are currently launched 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 a number of 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 translational studies that will help 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 a number of key questions as outlined above. This would have allowed 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 towards understanding what happens in patients, before launching new phase III trials. A number of such proposals are detailed in the text above and summarized in Table 1.

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Disclosure of Potential Conflicts of Interest

Benoit Van den Eynde is co-founder of, has ownership interest in, and is Chairman of the Scientific Committee of iTeos Therapeutics.

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Figure Legends

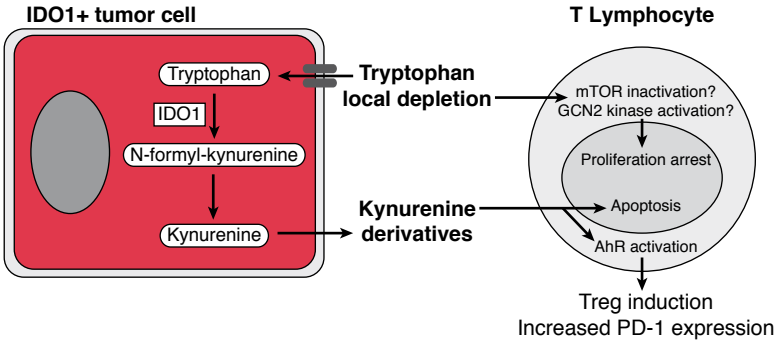
Figure 1. Proposed mechanisms by which IDO1-mediated tryptophan degradation inhibits local T-cell responses in tumors. See text for details.

Figure 2. Two different mechanisms account for IDO1 expression in tumors, allowing them to resist T-lymphocyte attack. Left: IDO1 is constitutively expressed in tumor cells (intrinsic resistance). Right: IDO1 is induced in tumor cells and other cell types by IFN-gamma released by neighboring activated T or NK cells in a negative feedback loop (adaptive resistance). (**A**) Schematic view. (**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.

Figure 3. The proportion of IDO1-positive tumors with weak T-cell infiltration and activity vary greatly from one tumor type to the other. The expression values are taken from the TCGA transcriptome data, and are displayed in Log10 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. The number of tumor samples for each tumor type is shown in the lower right corner of the corresponding panel. X values are levels of expression of the *CXCL9* gene, a marker of IFN-gamma activity. Y values represent *IDO1* gene expression. Each dot is a tumor sample, colored according to its T-cell content (blue: no or few T cells, defined as *CD3E* gene expression level < 25,000, grey: T-cell rich, *CD3E* ≥ 25,000). Some tumor types, such as colorectal adenocarcinomas and skin melanomas have their *IDO1* expression proportional to IFN-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 (+/- one log) the linear regression line (dashed line) from the colorectal carcinoma 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, rather 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*. C. = carcinoma.

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, and 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-PD-1/PD-L1 therapy
Mechanism of IDO1 expression in melanoma is adaptive	Focus on tumor types with constitutive IDO1 expression
Epacadostat and pembrolizumab acting on the same pathway, therefore potentially not synergistic	Combine IDO1 inhibitor with other immunotherapy approaches
Compensatory expression of TDO or IDO2	Use dual inhibitors
Signaling activity of IDO1 potentially not affected by epacadostat	Develop new IDO1 inhibitors that also block its signaling activity
Epacadostat activates AhR, 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)



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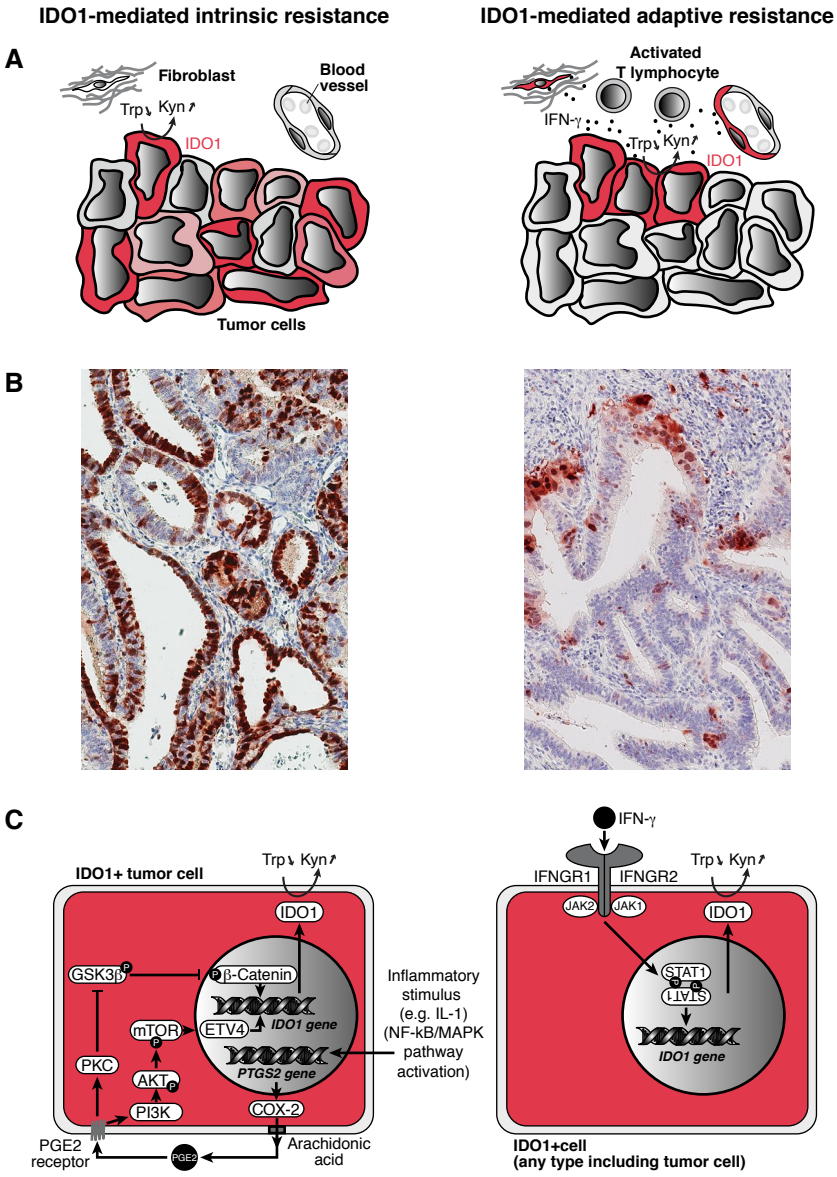
See text for details.



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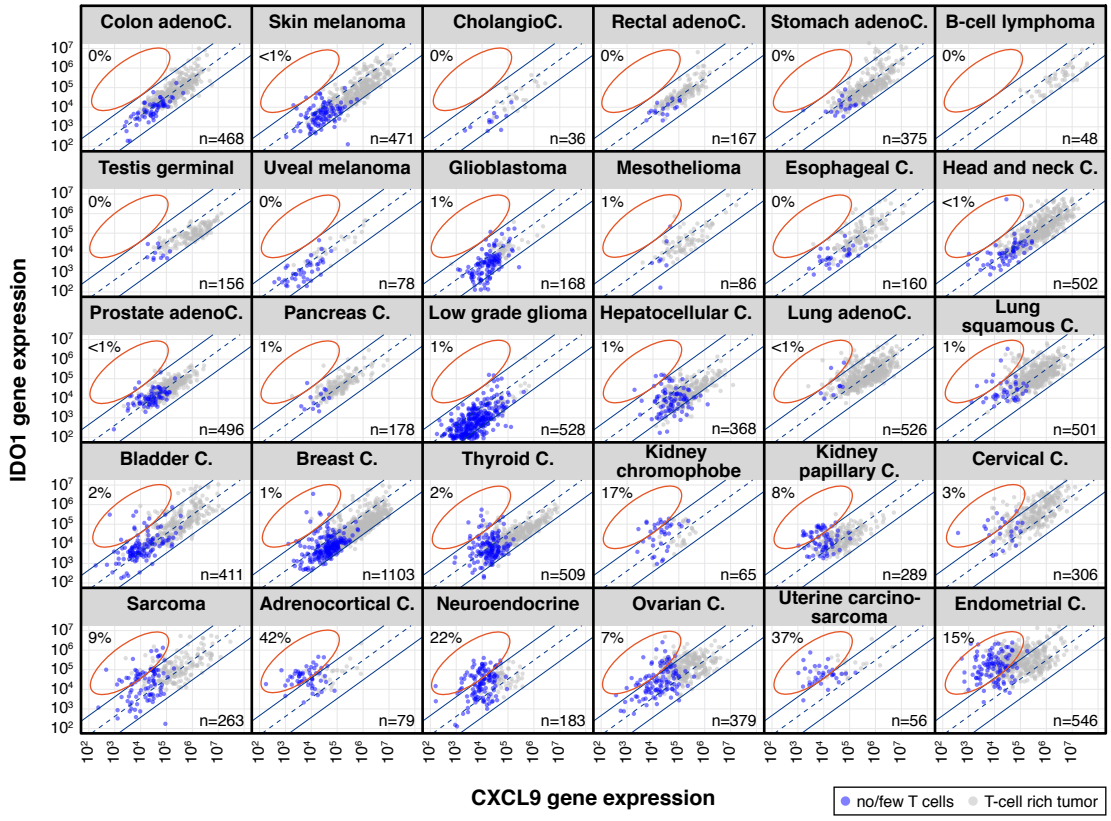

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