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# Indoleamine 2,3-dioxygenase (IDO) inhibitors and cancer immunotherapy

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## Abstract

Strategies for unlocking immunosuppression in the tumor microenvironment have been investigated to overcome resistance to first-generation immune checkpoint blockade with anti-programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) and anti-cytotoxic *T*-lymphocyte associated protein 4 (CTLA-4) agents. Indoleamine 2,3-dioxygenase (IDO) 1, an enzyme catabolizing tryptophan to kynurenine, creates an immunosuppressive environment in preclinical studies. Early phase clinical trials investigating inhibition of IDO1, especially together with checkpoint blockade, provided promising results. Unfortunately, the phase 3 trial of the IDO1

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

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inhibitor epacadostat combined with the PD-1 inhibitor pembrolizumab did not show clinical benefit when compared with pembrolizumab monotherapy in patients with advanced malignant melanoma, which dampened enthusiasm for IDO inhibitors. Even so, several molecules, such as the aryl hydrocarbon receptor and tryptophan 2,3-dioxygenase, were reported as additional potential targets for the modulation of the tryptophan pathway, which might enhance clinical effectiveness. Furthermore, the combination of IDO pathway blockade with agents inhibiting other signals, such as those generated by *PIK3CA* mutations that may accompany IDO1 upregulation, may be a novel way to enhance activity. Importantly, IDO1 expression level varies by tumor type and among patients with the same tumor type, suggesting that patient selection based on expression levels of IDO1 may be warranted in clinical trials.

#### **Keywords**

Indoleamine 2; 3-dioxygenase; Immune checkpoint inhibitor; Aryl hydrocarbon receptor; Immuno-oncology; Precision medicine; Immunotherapy

### Introduction

The development and approval of immune checkpoint (programmed cell death protein 1 [PD-1], programmed death-ligand 1 [PD-L1], cytotoxic *T*-lymphocyte associated protein 4 [CTLA-4], and lymphocyte-activation gene 3 [LAG-3]) inhibitors resulted in dramatic changes in the landscape of cancer therapy. Still, most patients treated with an immune checkpoint inhibitor, especially with a monotherapy approach, will demonstrate either primary or acquired resistance to these treatments, which has led to clinical research focusing on therapeutic options utilizing combination strategies with immune checkpoint inhibitors and other agents [1–4]. To date, multiple mechanisms of resistance have been proposed. In particular, tryptophan catabolism was suggested as having an important role in contributing to resistance to immunotherapy [5].

Tryptophan is essential for protein synthesis and cell survival. It is catabolized to its metabolites including kynurenine and kynurenic acid, which usually serve as neurotransmitters and molecules in cell signaling pathways [6]. Reports have also shown upregulation of tryptophan catabolism in response to the inflammatory status induced by autoimmune diseases [7,8]. These studies suggest that modulating the tryptophan pathway may be important for cancer immunotherapy. Still, to date, clinical studies with indoleamine 2,3-dioxygenase (IDO) inhibitors, have been disappointing, despite the crucial impact of IDO on tryptophan metabolism and on immunosuppression (Fig. 1 and Supplemental Table 1).

Here, we review the important role of tryptophan metabolism in the immune system orchestra, and the biological implications for optimizing the effectiveness of IDO inhibitors.

#### Biological role of tryptophan metabolism and indoleamine 2,3-dioxygenase (IDO)

Three enzymes–indoleamine 2,3-dioxygenase (IDO) 1, IDO2, and tryptophan 2,3dioxygenase (TDO)– degrade tryptophan to its downstream metabolites, resulting in

enhanced levels of immunosuppressive cells [9–22]. As a result, the role of IDO1 in cancer cells has been investigated as an attractive therapeutic target (Fig. 2).

Studies have revealed that IDO1 is an immunosuppressant in the tumor microenvironment and is related to tumor progression [10]. IDO1 regulates tryptophan metabolism by catabolizing tryptophan to kynurenine, the first step of tryptophan degradation. Reduced tryptophan levels are associated with poor clinical outcomes among multiple cancer types, consistent with the premise that regulation of tryptophan metabolism plays an important role in cancer survival or progression [11,12]. Decreased levels of tryptophan and increased expression of IDO1 correlate with an increase in the level of immunosuppressive cells such as regulatory T cells and myeloid-derived suppressor cells (MDSCs), a decreased level of tumor infiltration lymphocytes and NK cells, and upregulation of PD-1 in cytotoxic T cells [10,13,15,23]. Moreover, even with higher levels of tumor-infiltrating CD8 +T cells, the cancer genome atlas (TCGA) data analysis showed higher IDO1 expression was associated with worse clinical outcomes in colorectal cancer [16]. Several potential mechanisms mediating differentiation to regulatory T cells are through activation of the stress response kinase, general control non-derepressible 2 (GCN2), in the setting of tryptophan depletion, and activation of the aryl hydrocarbon receptor (AhR) by increased tryptophan metabolites in the tumor microenvironment [17–19]. Clinically, the increased expression of regulatory T cells was found in the context of increased expression of IDO1 in dendritic cells in patients with cervical cancer, and a high level of IDO1 in peripheral monocytes was associated with poorer outcomes in early-stage malignant melanoma [20,21]. These results suggest that higher levels of IDO1, not only in tumor cells but also inside the tumor microenvironment, contribute to the immune evasion by cancer cells.

Tryptophan catabolites also exert their immunosuppressive effect by activating the AhR in cancer cells and by suppressing the signaling pathway of cytotoxic lymphocytes, leading to decreased function of cytotoxic T cells [10,24]. Tryptophan metabolites, such as kynurenine, kynurenic acid, cinnabarinic acid, indole-3-pyruvic acid (I3P), indole-3-acetic acid, and indole-3-carboxaldehyde, have a role as ligands to the AhR [19,25–29]. The activated AhR induces the accumulation of tumor-associated macrophages and regulatory T cells, and tolerogenicity of MDSCs, making the tumor microenvironment more immunosuppressive and enabling the escape of cancer cells [22,30,31]. Moreover, differentiation to regulatory T cells via activation of AhR occurs in the environment where anti-inflammatory cytokines such as TGF-  $\beta$  and IL-10 are produced by dendritic cells with an immunosuppressive feature [22]. Conversely, knockdown of AhR in the oral cancer cell model leads to a decrease in the expression of PD-L1 positive tumor-infiltrating CD8 + T cells and an increase in the number of cytotoxic CD8 + T cells [32]. However, the AhR is also known to induce interleukin-6 (IL-6) in the tumor microenvironment synergistically [26,33]. IL-6 is one of the main cytokines observed around cancer cells and has a pro-inflammatory role leading to tumorigenesis, cell proliferation, angiogenesis, and invasiveness [34]. Upregulation of AhR increases the production of IL-6, resulting in the activation of STAT-3, which in turn leads to the generation of IDO1, creating an autocrine AhR-IL-6-STAT-3 signaling loop that maintains IDO1 expression in human cancer cells [35].

Taken together, ample evidence of the role of IDO1 as an immunosuppressive molecule in the tumor microenvironment supports therapeutic strategies to target the tryptophan-IDO1-kynurenine pathway, using IDO1 inhibitors, perhaps combined with other systemic therapies, such as cancer vaccines and or established checkpoint blockade agents [36].

#### **Development of IDO inhibitors**

Promising results in preclinical models and early phase clinical trials: IDO

inhibitors, which generally suppress IDO1 or inhibit IDO1 and TDO concurrently, were applied in the clinic after IDO1 was found to exert immunosuppressive roles in the tumor microenvironment and to be associated with tumor progression in preclinical models [10,37].

IDO1 deficiency was correlated with a decrease in the incidence and proliferation of hepatocellular carcinoma in mouse models, with suppressed invasion of regulatory T (Treg) cells in the liver [38]. In a mouse model of lung cancer, ablation of IDO1 resulted in a reduction in tumor burden, improvement in survival of MDSCs, and infiltration of PD-1 + CD8 + T cells in the tumor microenvironment [37]. Knockout of IDO1 in a mouse model of melanoma cells also revealed enhancement of therapeutic efficacy of PD-1/PD-L1 inhibitors and CTLA-4 inhibitors, suggesting synergistic efficacy of IDO1 inhibition with checkpoint inhibitors [5]. Through these preclinical studies, IDO1 inhibition gradually became an established target combined with other immunotherapeutic strategies [39,40].

As studies supported the strategy of targeting tryptophan catabolism mediated by IDO and TDO, various IDO1 and TDO inhibitors have been identified and entered into clinical trials (Supplementary Table 1) [6,41]. The IDO1 inhibitor epacadostat, which competes with tryptophan for IDO1 binding, was initially developed in the clinical setting after it was shown to boost antitumor effects by enhancing the function and proliferation of T- and NK-cells [42,43]. Unfortunately, epacadostat monotherapy was not impressive in regards to antitumor activity, but was well tolerated in a phase 1 study among patients with advanced cancer [44]. However, since synergistic effects with immune checkpoint inhibitors were observed in preclinical models [5,40], the combination of epacadostat with immune checkpoint inhibitors (anti-CTLA-4 inhibitor and anti-PD-L1 inhibitor) was investigated in early-phase clinical trials with promising results [39,45]. Subsequent phase 1/2 (ECHO-202/KEYNOTE-037) trial, an open-label and single-arm study with escalating doses of epacadostat, to evaluate epacadostat plus pembrolizumab (anti-PD1) in patients with advanced solid tumors, showed relatively high objective response rates (ORR = 40.3 %, n = 25/62), and adequate anti-tumor efficacy was seen, especially in patients with malignant melanoma (ORR = 61.9 %, n = 13/21) [46]. Unfortunately, phase 3 trials did not confirm the benefit (see next section) [47,48].

In addition to epacadostat, the IDO pathway modulator indoximod, and its prodrug NLG802, were developed. Although the precise mechanism of action remains controversial, these agents are known to modulate the IDO pathway, in contrast to IDO1 inhibitors, which directly inhibit the activity of IDO1 [49]. The combination of docetaxel and indoximod was well tolerated in patients with advanced solid tumors in a phase 1 trial [50]. Indoximod was subsequently evaluated with taxanes in patients with breast cancer in a randomized

phase 2 trial, but it failed to meet its primary endpoint; the progression-free survival (PFS, 6.8 months in indoximod plus taxane vs 9.5 months in placebo plus taxane) [51]. BMS-986205 (Linrodostat), another IDO1 inhibitor, was the first agent to demonstrate potent reduction of plasma kynurenine level in a clinical trial; a subsequent clinical trial evaluating BMS-986205 with nivolumab (anti-PD1) reported a promising ORR (34 %, n = 10/29 in an advanced bladder cancer cohort) [52,53].

Several IDO pathway inhibitors/modulators have been evaluated in clinical trials (summarized in Supplementary Table 1). Many of these trials showed disappointing results, either with the use of IDO inhibitor monotherapy or when combined with other agents in a randomized setting.

Failure in phase 3 KEYNOTE-252/ECHO-301 trial: Because of the encouraging results in early-phase trials such as the ECHO-202/KEYNOTE-037 phase 1/2 trial (epacadostat plus pembrolizumab in patients with advanced solid tumors) [46], the combination of epacadostat 100 mg twice daily with pembrolizumab 200 mg once every 3 weeks was compared with placebo plus pembrolizumab (anti-PD1) in a large phase 3, ECHO-301/KEYNOTE-252 trial, in advanced melanoma. However, this trial showed that epacadostat plus pembrolizumab did not improve PFS and overall survival when compared to pembrolizumab alone. Subgroup analysis based on the level of PD-L1 by immunohistochemistry (IHC) did not show differences in PFS between treatment groups [47]. Additionally, with a 1 % positivity threshold, ~ 90 % of tumors stained IDO1 positive, and IDO1 positivity did not correlate with the outcome; no other IDO1 positive thresholds were examined. This trial could not identify the right biomarker to predict the efficacy of the investigational treatment. Moreover, the prespecified endpoints such as pharmacokinetics and pharmacodynamics of epacadostat were not analyzed due to a lack of predictive factors or biomarkers, which made it difficult to address the reasons for the failure of epacadostat plus pembrolizumab in patients with advanced malignant melanoma in the ECHO-301/ **KEYNOTE-252** trial.

#### **Current status of IDO inhibitors**

**Multiple negative phase 3 trials:** Along with the ECHO-301/KEYNOTE-252 trial, several phase 3 trials were conducted to evaluate the efficacy of the combination of epacadostat and pembrolizumab for a variety of cancer types. However, these trials were halted or underwent a setback to phase 2 trials after the failure of the ECHO-301/KEYNOTE-252 trial. The partial or full results of these trials were reported and ORR in each trial is summarized in Fig. 1. Although ORR with IDO1 inhibitor use was relatively higher in patients with cisplatin-ineligible urothelial carcinoma (ORR = 31.8 % in epacadostat plus pembrolizumab, 24.5 % in placebo plus pembrolizumab) or recurrent advanced urothelial carcinoma (ORR = 21.4 % in epacadostat plus pembrolizumab, 9.5 % in placebo plus pembrolizumab), no apparent clinical benefit was observed in patients treated with the combination of IDO1 inhibition and an immune checkpoint inhibitor or other agents in similar or other types of cancer (NCT03260894, NCT03322540, NCT03322566, NCT03358472, NCT03361865, NCT03374488) (Fig. 1 and Supplementary Table 1) [51,54]. Phase 3 trials evaluating another IDO1 inhibitor, BMS-986205, with the anti-PD1 nivolumab

in patients with malignant melanoma, head and neck cancer, and non-small cell lung cancer were subsequently halted (NCT03329846) (NCT03386838) (NCT03417037). One phase 3 study, which evaluates the combination of BMS-986205 with or without nivolumab in patients with muscle-invasive bladder cancer is ongoing (NCT03329846).

**Current trials re-evaluating IDO inhibitors:** While larger combination IDO1/immune checkpoint inhibitor trials did not demonstrate efficacy, phase 1 and 2 trials are ongoing to uncover efficacy for patients with advanced malignancies. Epacadostat, for example, is being evaluated in the preoperative setting in combination with chemoradiation for rectal cancer (NCT03516708), PD-1 inhibition (retifanlimab), radiation, and bevacizumab for recurrent glioma (NCT03532295), and with retifanlimab or other therapies in advanced endometrial cancer (NCT04463771). Studies assessing indoximod (IDO pathway modulator) and BMS-986205 (IDO1 inhibitor) are ongoing and further evaluation is pending (Table 1).

# Future perspectives: How can we optimize IDO inhibitors in the cancer immunotherapy era?

The negative result of the ECHO-301/KEYNOTE-252 trial raised questions about the usefulness of targeting IDO metabolism in cancer immunotherapy. Previously, several possible causes of the disappointing observations were proposed: insufficient inhibition of IDO1, no selection of patients based on IDO1 expression, lack of consideration for expression of other molecules including TDO2, and inadequate blockade of the IDO1 downstream pathway [41]. Here, we discuss possible reasons for failure in previous clinical trials, and suggest potential new tactics for targeting tryptophan catabolism in cancer immunotherapy.

#### Ensuring adequate blockade of tryptophan catabolism in the tumor

**microenvironment:** In the ECHO-301/KEYNOTE-252 trial, the dose of epacadostat was set at 100 mg twice daily. This dosing was based on several phase 1 studies evaluating epacadostat as monotherapy or as part of the combination with ipilimumab (anti-CTLA4) or pembrolizumab (anti-PD1). In a phase 1 study assessing epacadostat as monotherapy, sufficient inhibition of IDO1 was achieved when epacadostat was dosed at 100 mg or more, twice daily [45]. The dose of epacadostat combined with ipilimumab was evaluated at 25–300 mg twice daily in a phase 1/2 trial for patients with advanced melanoma [55]. A dose of 100 mg twice daily was chosen when combined with pembrolizumab in a phase 2 trial but dose-dependent efficacy was not evaluated in these early phase trials officially [46]. Although clinical activity was seen in different doses of epacadostat in these trials and the ECHO-301/KEYNOTE-252 trial selected 100 mg twice daily dose based on the result of the phase 2 trial, there is a question if 100 mg twice daily is the best dose or not [46]. Indeed, CTLA-4 inhibitors, PD-1/PD-L1 inhibitors, and cancer vaccine therapy induced an increase in IDO1 expression and metabolic activity, implying that a higher dose of IDO1 inhibitors might be needed when combined with immunotherapy [56–58].

Another question is whether IDO1 inhibitors actually block the activity of IDO1 and change tryptophan and kynurenine levels in cancer cells. Pharmacokinetics and pharmacodynamics analyses in the phase 1/2 ECHO-202/KEYNOTE-037 trial showed 50 % inhibition

of IDO1 when epacadostat was given at 100 mg twice daily [46]. However, this trial and the phase 3 ECHO-301/KEYNOTE-252 trial did not measure intratumoral or serum tryptophan and kynurenine levels before and during the treatment. One study revealed an association between an increased tryptophan level with the activation of CD8 + T cells in a mouse model, suggesting the importance of periodic measurement of tryptophan metabolites before and during treatments [59]. Additionally, IDO1 blockade might be insufficient to suppress the production of tryptophan derivatives that are ligands of AhR. Activation of AhR suppresses anti-tumor immunity and induces tumor progression, and thus, tryptophan metabolites need to be fully reduced to exert the efficacy of IDO pathway inhibitors. However, a recent study revealed interleukin-4-induced-1 (IL4I1) as a stronger activator of AhR than IDO1 and TDO2 [28]. Through the production of metabolites such as I3P, IL411 activates AhR, leads to an increase in Tregs and MDSCs, and suppresses the anti-tumor immunity. It was also shown in this study that immune checkpoint blockade induced both IDO1 and IL4I1, suggesting that the presence of IL4I1 weakens the degradation of tryptophan metabolites through IDO1 blockade and explains the failure of the ECHO-301/ KEYNOTE-252 trial [28].

In the ECHO-301/KEYNOTE-252 trial, patients treated with adjuvant ipilimumab (7–10 %) and previous BRAF inhibitors (12 %) were included. BRAF inhibitors have a role as an agonist binding directly to AhR, resulting in stimulation of its nuclear translocation. The use of BRAF inhibitors is associated with an increase in the AhR-activated and BRAF inhibitor-persister cells in the malignant melanoma xenograft model [60]. Activation of AhR can lead to an increase in PD-L1-expressing CD8 + T cells and an induction of IDO1 [32,35]. The AhR pathway can also potentially activate IDO2 and TDO2, which may decrease the efficacy of IDO1 inhibitors [31,61]. Thus, the ECHO-301/KEYNOTE-252 trial may have included a subpopulation of patients more resistant to IDO1 inhibition. These insights indicate that inadequate suppression of tryptophan catabolism can be one of the reasons for the primary resistance to IDO1 blockade combined with immune checkpoint inhibitors, and the dynamic interrogation of intracellular tryptophan catabolites may be useful in predicting the outcome of IDO1 inhibitors and developing novel therapeutic strategies.

**Selecting patients based on high IDO1 expression in their cancer:** The presence of IDO1 expression in tumor cells or other cells in the tumor microenvironment may be important when using IDO1 inhibitors. However, the expression pattern of IDO1 varies across tumor types and even within the same tumor type. In the ECHO-301/ KEYNOTE-252 trial, IDO1 status was positive in 62 % of patients treated with epacadostat plus pembrolizumab and in 66 % of those treated with pembrolizumab alone, when IDO1 positivity was defined as a tumor or intra-tumoral immune cell expression higher than 1 % of cells [47]. Assessment of IDO1 expression by IHC in common solid tumors revealed various positive rates in cervical cancer (52–100 %), endometrial cancer (18–94 %), urothelial carcinoma (94 %), ovarian cancer (57–66 %), colorectal cancer (13–90 %), renal cell carcinoma (44–81 %), breast cancer (37 %–46 %), pancreatic carcinoma (37 %), and glioblastoma (8 %) [62]. This heterogeneity of IDO1 positivity is likely due to different methods to determine the expression of IDO1, such as IHC and reverse transcription–

polymerase chain reaction, and various definitions of positivity such as positivity only in cancer cells or in tumor-infiltrating lymphocytes.

To illustrate the IDO1 expression across diverse cancer types, we performed a comprehensive analysis of IDO1 RNA expression across diverse solid tumor types in 514 patients diagnosed with advanced cancer at the Moores Cancer Center at the University of California San Diego (Fig. 3). The percentile of the IDO1 expression based on transcript level in each patient was ranked on a scale of 1 to 100 as previously described [63] (normalized to the reference population of 735 tumors spanning 35 histologies), and classified into low (0–24), moderate (25–74), and high (75–100). The percentage of high IDO1 expression was 17.7 % (91/514 patients) in all cancer types, and was highest in patients with uterine cancer (52.2 %, 12/23), followed by ovarian cancer (37.2 %, 16/43), lung cancer (25 %, 5/20), and esophageal cancer (17.6 %, 3/17). There was high variability of IDO1 RNA expression between and within tumor types.

However, the positivity of IDO1 expression was not set as one of the inclusion criteria in most clinical trials using IDO inhibitors. Subgroup analysis based on IDO1 expression in the ECHO-1/KEYNOTE-252 trial did not show a survival difference between each group (HR = 0.99, 95 % CI: 0.69–1.42), but a threshold of the positivity of IDO1 was set as more than 1 %, and most patients were categorized as positive IDO1 expression. Moreover, information on the PD-L1 expression ratio in patients with positive IDO1 expression was lacking. IDO1 expression was retrospectively analyzed and was not set as a stratification factor before enrolling patients in this trial; therefore, it is unknown if the clinical characteristics of patients with IDO1 expression in each group were equally distributed. Although recent development of molecular-targeted therapy led to biomarker-driven cancer treatments with improved outcomes, the majority of immunotherapy trials are still conducted without setting prespecified biomarkers, which potentially miss the identification of good responders [64]. It is plausible that the selection of patients based on their tumor's expression of IDO is needed in order to optimize IDO inhibitor responsiveness.

**Choosing the most suitable IDO1 inhibitor:** Although mechanisms of action are broadly categorized as IDO1 inhibitors/modulators, available agents might have a different impact on IDO1 inhibition, tryptophan catabolism, and other molecular pathways in the tumor microenvironment. For example, indoximod and navoximod are regarded as IDO pathway modulators, since they are not the actual inhibitors of the IDO1 enzyme, but can exert their effect as a substance mimicking tryptophan [49,65]. This results in iDO-mediated tryptophan deprivation, leading to a revitalization of mTOR signals necessary for the antitumor T cell activity [66]. Therefore, indoximod or navoximod might be able to exert better anti-cancer immunity, especially when combined with T cell-targeting immunotherapy theoretically. The combination of indoximod plus an immune checkpoint inhibitor demonstrated an ORR of 55.7 % among patients with advanced melanoma in a phase 2 trial [67]. However, a phase 1 trial evaluating the combination of navoximod and atezolizumab for patients with advanced solid tumors only showed little clinical benefit (ORR = 9 %, n = 6/66 in the dose-escalation population, ORR = 11 %, n = 10/91 in the dose-expansion cohort) [68].

Another factor that needs to be considered is the actual pharmacodynamics of the IDO1 inhibitors/modulators. Reduction in intra-tumoral kynurenine levels or changes in intratumoral tryptophan/kynurenine ratio might differ in each IDO1 inhibitor. Epacadostat monotherapy inhibits more than 90 % of the plasma kynurenine level when dosed with 100 mg or more twice daily, but pharmacodynamics analysis regarding intratumoral levels of tryptophan metabolites remains scarce [45]. BMS-986205, one of the other IDO1 inhibitors, was associated with a reduction of the intratumoral kynurenine level of up to 90 % when administered either as monotherapy or combined with nivolumab for patients with advanced cancer [52]. Another IDO1 inhibitor, PF-0684003 demonstrated an 80% reduction of the intratumoral kynurenine level in a mouse model [57]. Recently, LY338196 also showed a 76 % and 67 % decrease in the kynurenine level in plasma and cancer cells when dosed as monotherapy or in combination with a PD-L1 inhibitor (LY3300054) for advanced cancer [69]. In contrast, indoximod and navoximod were not associated with a significant reduction in the intratumoral kynurenine level in phase 1 trials [50,65]. Further studies are needed to elucidate the association between clinical efficacy and changes in levels of intratumoral tryptophan metabolites.

**Targeting TDO2 or IDO2:** Other enzymes in addition to IDO1, including IDO2 and TDO2, are potentially important regulators of tryptophan catabolism in the tumor microenvironment. Although BMS-986205, an IDO1 inhibitor, demonstrated a sufficient reduction in plasma kynurenine level and T cell proliferation without having activities against IDO2 and TDO2 in preclinical models, several reports revealed the association of TDO2 or IDO2 with cancer immunity and clinical phenomena [70]. TDO2 is seen in multiple cancer types as an immunosuppressive molecule and catabolic enzyme of tryptophan, leading to tumor progression [25,71]. Tryptophan degradation through TDO2 leads to the production of immunosuppressive kynurenine, resulting in the AhR activation and inhibition of T cells [25]. TDO2 is also associated with epithelial-to-mesenchymal transition through activation of the AhR pathway, leading to invasion and metastasis of hepatic cellular carcinoma in a cell model [72]. In IDO/TDO-overexpressing tumors, the active AHR pathway through kynurenine is observed, leading to the promotion of Treg and tumor-associated macrophages, and creating an immunosuppressive environment and resistance to immune checkpoint inhibitors [73]. In addition, TDO2, rather than IDO1, is clinically correlated with a poorer outcome in patients with renal cell carcinoma treated with an immune checkpoint inhibitor [74]. Blockade of TDO2 improved antitumor T cell activity and dendritic cell function, leading to regression of tumor nodules in a mouse model [75]. Therefore, inhibition of TDO2 could be a promising strategy for cancer immunotherapy.

Targeting IDO2 would be another option to augment the efficacy of the IDO1 blockade. IFN $\gamma$  is the major cytokine to induce IDO1 through the JAK-STAT pathway but it also induces IDO2 [76]. If IDO1 was blocked, the signal from IFN $\gamma$  would potentially increase the level of IDO2 in a cancer cell, probably resulting in a diminishment of the effect of IDO1 inhibitors. Therefore, IDO2 inhibition would be a meaningful way to revoke the resistance to IDO1 blockade; however, inhibition of IDO2 has not been established because of the difficulty in purifying molecules to inhibit IDO2 physiologically [77]. In addition, the efficacy of the tryptophan metabolizing process by IDO2 is presumably less than that

by IDO1 and, thus, it is uncertain if inhibition of IDO2 opens avenues to overcome the resistance to IDO1 blockade [78]. Currently, inhibition of both IDO1 and TDO2 is evaluated in phase 1 clinical trials. One study reported the development of pan- and IDO1/TDO2 inhibition in mouse and human cell models [79]. A recent preclinical study evaluating a dual IDO1/TDO inhibitor showed effective blockade of the kynurenine pathway and kynurenine-AhR signaling, resulting in a reduction in migration and invasion of pancreatic carcinoma cells in mice [80]. A phase 1 study assessing M4112, the first dual inhibitor of IDO1 and TDO2 evaluated in the clinical setting, reported safety in patients with advanced cancer but this agent was not associated with a reduction in plasma kynurenine level, resulting in termination of the trial [81]. A recent study explored the compounds which can potentially block both IDO1 and TDO2 and 10 compounds were confirmed to inhibit IDO1 and TDO2 [82]. Only a few agents are undergoing evaluation in phase trials, but these results could expedite the developmental process of dual IDO1 and TDO2 inhibitors in the future.

Blocking the aryl hydrocarbon receptor (AhR) pathway: The AhR is a liganddependent transcription factor that mediates many of the biological and toxicological actions of a variety of hydrophobic natural and synthetic chemicals. Tryptophan catabolites are known ligands of AhR, and the AhR pathway is associated with the accumulation and proliferation of immunosuppressive cells in the tumor microenvironment. Activation of the AhR pathway is related to viability, migration, and proliferation of cancer cells, and antagonists of AhR lead to a reduction in tumor aggressiveness [83]. AhR is also associated with the induction of IDO2 and TDO2, which might result in mitigation of the efficacy of IDO1 inhibition [61,84]. Therefore, although the AhR pathway is downstream of iDO-kynurenine signaling, blockade of the AhR can theoretically augment the efficacy of IDO1 inhibition. Interestingly, a recent study reported that activated AhR through IDO1-kynurenine signaling induced PD-1 expression on CD8 + T cells in the tumor microenvironment of the ovarian cancer model [85]. This suggests an association of inhibitory checkpoint signaling with the activation of the iDO-AhR pathway, and the AhR inhibition with immune checkpoint blockade might be a better synergistic therapeutic option.

Several companies have developed AhR inhibitors and shown their efficacy in the reduction of activity of immunosuppressive cells and induction of pro-inflammatory response toward tumor cells in preclinical models [86,87]. Phase 1 trials to evaluate AhR inhibitors, such as IK-175 as monotherapy or in combination with nivolumab, and BAY2416964 as monotherapy or in combination with pembrolizumab, have started and are recruiting patients with advanced solid tumor or urothelial carcinoma (NCT04069026) (NCT04200963) (NCT04999202).

**Targeting other pathways or molecules to augment the efficacy of IDO1:** IDO1 can be induced by the activation of several cell signaling pathways such as the prostaglandin E2 (PGE2) pathway. The expression of cyclooxygenase-2 drives the expression of IDO1 and TDO2 in tumor cells through the activation of E prostanoid receptor and protein kinase C and phosphoinositide 3-kinase (PI3K) pathways (Fig. 2) [88,89]. Hence, regulation of

the PGE2 pathway and its related molecules can probably improve the efficacy of IDO1 inhibitors.

A recent study investigating the relationship between the expression of immunoregulatory molecules and mutations in "targetable" molecules showed significant upregulation of IDO1 expression in the presence of phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) mutations (E545K and R88Q) [90]. This could be due to the activation of IDO1 transcription through the EP receptor-PI3K pathway, and suggests that targeting PIK3CA with a combined regimen utilizing a PIK3CA inhibitor and an IDO1 inhibitor may be a reasonable strategy for patients with high IDO1 expression accompanied by *PIK3CA* alterations in order to unlock the immunosuppressive microenvironment [88]. An association between downregulation of IDO1 expression and *BRAF V600E* mutations was also observed, suggesting that there might be less efficacy of IDO1 inhibitors in tumors bearing *BRAF V600E* mutations [90]. Upregulation of IDO1 expression in melanomas resistant to BRAF inhibitors has also been reported [91]. Further investigation is necessary to better understand the complexity of the interaction between the MAPK pathway and tryptophan metabolism.

Another potential strategy to enhance the efficacy of IDO1 inhibitors would be the combination with angiogenesis inhibitors. IDO1-expressing cells are related to an increase in neovascularization and genetic loss of IDO1 is associated with reduction of IL-6 and neovascularization [92,93]. Although clinical trials combining IDO1 inhibitors with vascular endothelial growth factor (VEGF) inhibitors have not been conducted, a phase 2 trial evaluating nivolumab plus the IDO1 inhibitor, BMS986205, will assess changes in inflammatory markers including VEGF before and after the treatment, and give an insight into the clinical effect of IDO1 inhibition on angiogenesis (NCT03854032).

Additionally, the non-enzymic function of IDO1 decreased the survival of animal models with glioblastoma through an increase in complement factor H and its isoform, factor H like protein, independent of tryptophan catabolism [94]. This observation suggests an association between the non-enzymic function of IDO1 and the survival of cancer cells.

**Combining drugs to optimize checkpoint blockade:** IDO1 inhibitors have been mainly evaluated as monotherapy or in combination with PD-1 or PD-L1 inhibitors in clinical trials. Combinations of IDO inhibitors with a CTLA-4 checkpoint inhibitor or a cancer vaccine therapy are under evaluation. Two clinical trials assessed the combination of an IDO1 inhibitor with a CTLA-4 inhibitor, but clinical efficacy was limited. A phase 2 trial that assessed epacadostat with ipilimumab (anti-CTLA4) showed limited clinical activity in patients with malignant melanoma (ORR = 25.6 %, n = 10/39 in immunotherapy-naïve patients; ORR = 0 %, n = 0/11, in patients previously treated with immunotherapy) [55]. In contrast, a phase 2 trial evaluating indoximod with either pembrolizumab, nivolumab, or ipilimumab for advanced melanoma showed promising efficacy (ORR = 55.7 %, n = 39/70) [67]. To augment the efficacy of IDO1 inhibition with checkpoint blockade, the identification of checkpoints and other immune molecules associated with higher IDO1 expression by utilizing an immunogram technique may be in future research [95]. Additionally, a phase 1 study that assessed the safety of ipilimumab with a peptide

vaccine derived from IDO demonstrated the safety but the efficacy of this combination did not exceed that of ipilimumab monotherapy [96]. A recent phase 1/2 trial of an immune-modulatory vaccine against IDO and PD-L1 (IO102-IO103) in combination with nivolumab in metastatic malignant melanoma demonstrated ORR of 80 % (n = 24/30) with a median PFS of 26 months [97]. A phase 2 study evaluating pembrolizumab plus IO102-IO103 for patients with metastatic NSCLC, head and neck cancer, and urothelial bladder cancer has just begun (NCT05077709). Larger studies are warranted to confirm the efficacy of vaccine therapy against IDO combined with a systemic immune checkpoint inhibitor (NCT05155254).

### Conclusions: Potential strategies for future clinical trials

Immuno-oncology is a rapidly expanding field with multiple successes in the treatment of advanced malignancies. Immune checkpoint blockade re-activates the immune system suppressed by the tumor and allows immune cells to perform their function of eradicating cancer cells. Checkpoint blockade with anti-PD-1/PD-L1/CTLA-4 agents produces remarkable responses in a variety of neoplasms. However, many patients do not respond, possibly because of the activation of alternate immunosuppressive pathways. In this regard, it has been recognized that IDO1 and the tryptophan-kynurenine pathway are crucial to immune evasion. As a result, a multitude of IDO1 inhibiting tryptophan analogs, including small-molecule inhibitors and peptide vaccines, are currently being assessed in clinical trials. However, some of these trials have shown disappointing efficacy results. Future optimization of this important area requires ensuring sufficient pharmacologic inhibition of IDO1 by agents used in the clinic, stratifying patients based on IDO1 expression, co-targeting important molecular pathways (such as the PI3K/mTOR signals) that may play a co-dependent role, and suppression of compensatory mechanisms mediated through molecules such as IDO2 or TDO.

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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#### Fig. 1.

Objective response rate (ORR) in selected randomized controlled trials evaluating epacadostat and indoximod. ORR was available from nine randomized controlled trials evaluating either epacadostat or indoximod. No study revealed significant differences in ORR. The vertical axis of the graph shows the National Clinical Trial number and cancer types. The horizontal axis of the graph shows the response rate (0–1). The blue bar illustrates the response rate of IDO inhibitors. The orange bar shows the response rate of the control treatment. The gray bar indicates the response rate of the additional control treatment if the study contains more than two treatment arms. **Abbreviations:** HNSCC, head and neck squamous cell carcinoma; IDO, indoleamine 2,3-dioxygenase: NSCLC, non-small cell lung carcinoma; OFP, ovarian, fallopian tube, and peritoneal; ORR, objective response rate; RCC, renal cell carcinoma; UC, urothelial carcinoma.



#### Fig. 2.

Role of IDO in the tumor microenvironment. The expression of IDO is regulated by signaling pathways such as: (1) the PI3K-AKT-mTOR pathway; (2) the JAK-STAT pathway, which is typically upregulated by inflammatory molecules including PGE2, IFN  $\gamma$ , and IL-6; (3) tryptophan metabolites, which are catabolized from tryptophan by IDO or TDO activate the AhR pathway, leading to accumulation of TAM and Treg, and an increase in tolerogenicity of MDSCs around the tumor cells, making the tumor microenvironment immunosuppressive; (4) tryptophan catabolites, which also increase the expression of PD-1 on the surface of T cells and inhibit the cell signaling inside cytotoxic T cells, resulting in suppression of T cell function towards cancer cells. Abbreviations: AhR, aryl hydrocarbon receptor; AKT, protein kinase B; ATP, adenosine triphosphate; CoA, coenzyme A; COX2, cyclooxygenase 2; ERK, extracellular signal-regulated kinase 1/2; ETV4, ETS variant transcription factor 4; GSK3β, glycogen synthase kinase 3 beta; IDO, indoleamine 2,3-dioxygenase; IFN  $\gamma$ , interferon gamma; IFN  $\gamma$ R, interferon gamma receptor; IL-1, interleukin 1; IL-6, interleukin 6; IL-6R, interleukin 6 receptor; IL-R, interleukin receptor; Kyn, kynurenine; MDSC, myeloid-derived-suppressor cell; MHC1, major histocompatibility complex 1; mTORC1, mammalian target of rapamycin complex 1; NAD+, nicotinamide adenine dinucleotide; NFkB, nuclear factor kappa B; PD-1, programed cell death 1; PD-L1, programmed death-ligand 1; PGE2, prostaglandin E2; PGE2R, prostaglandin E2

receptor; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; PTGS2, prostaglandinendoperoxide synthase 2;STAT1, signal transducer and activator of transcription 1; STAT3, signal transducer and activator of transcription 3; TAM, tumor-associated macrophage; TCR, *T*-cell receptor; TDO, tryptophan-2,3-dioxygenase; TNFa, tumor necrosis factor alpha; Treg, regulatory T cell; Trp, tryptophan.



# High IDO1 expression (%)

#### Fig. 3.

High IDO1 RNA (75 percentile rank) expression rate across cancer types. Different patterns of IDO1 RNA expression based on the primary site of cancer are shown (n = 514). Transcriptomic sequencing was used to evaluate the expression of IDO1 based on RNA transcript abundance normalized to internal housekeeping gene profiles and ranked (0–100 percentile) in a standardized manner to a reference population of 735 tumors spanning 35 histologies. The expression profiles were stratified by rank values into "Low" (0–74), and "High" (75–100) as previously described [63]. The percentage of the population with high expression is shown in this graph. Among diverse types of cancer, RNA expression of IDO1 was highest in patients with uterine cancer (52.2 %, 12/23 patients) followed by ovarian cancer (37.2 %, 16/43), lung cancer (25 %, 5/20), and esophageal cancer (17.6 %, 3/17). Abbreviations: IDO1, Indoleamine 2,3-dioxygenase 1; RNA, Ribonucleic acid.

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Table 1

Current status of development of agents targeting the IDO pathway: Study examples (see also Supplementary Table 1) (data search as of February 27th, 2022 (PubMed and Clinicaltrials.gov)).

Drug	Target	Company	Number of clinical trials registered in NCT (clinicaltrias.gov)	References
Epacadostat (INCB024360)	ШОІ	Incyte	61 7: Active, not recruiting 19: Completed 3: Active, recruiting 13: Terminated 13: Withdrawn 11: Unknown status	[45-47,54,55,98-103]
Indoximod (NLG-8189)	IDO pathway	Lumos Pharma (Previously NewLink)	<b>15</b> 1: Active, not recruiting 10: Completed 2: Active, recruiting 2: Terminated	[49–51,67,104–109]
Navoximod (GDC-0919)	IDOI	Genentech	2 2: Completed	[65,68]
Linrodostat (BMS-986205)	IDOI	Bristol-Myers Squibb	20 5: Active, not recruiting 5: Completed 6: Active, recruiting 1:Terminated 3:Withdrawn	[52,53,110,111]
PF-06840003	IDOI	iTeos Therapeutics (Previously Pfizer)	1 1: Terminated	[112]
NLG802	IDO pathway	Lumos Pharma (Previously NewLink)	1 1: Completed	[49]
KHK2455	IDOI	Kyowa Hakko Kirin	<b>3</b> 1: Completed 1: No longer available 1: Active, not recruiting	[113]
SHR9146 (HTI-1090)	IDO1 and TD0	Jiangsu Hengrui Medicine Co., ltd	2 1: Unknown status 1: Completed	[114]
MK-7162	IDOI	Merck	1 1: Completed	[115]
LY3381916	IDOI	Eli Lilly	1 1: Terminated	[69]
DN1406131	ID01 and TD02	Shanghai De Novo Pharmatech	1 1: Unknown	NCT03641794
IDO vaccine	IDO	Copenhagen University Hospital at Herlev	2 1: Completed 1: Terminated	[116,117]

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) Keterences	[97]	
Number of clinical trials registered in NCT (clinicaltrias.gov	<b>3</b> 1: Completed 1: Not yet recruiting 1: Active, recruiting	
Company	IO Biotech	
larget	IDO and PD-L1	
Drug	IO102-IO103 (vaccine)	

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