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The kynurenine pathway presents multi-faceted metabolic vulnerabilities in cancer

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The kynurenine pathway (KP) and associated catabolites play key roles in promoting tumor progression and modulating the host anti-tumor immune response. To date, considerable focus has been on the role of indoleamine 2,3-dioxygenase 1 (IDO1) and its catabolite, kynurenine (Kyn). However, increasing evidence has demonstrated that downstream KP enzymes and their associated metabolite products can also elicit tumor-microenvironment immune suppression. These advancements in our understanding of the tumor promotive role of the KP have led to the conception of novel therapeutic strategies to target the KP pathway for anti-cancer effects and reversal of immune escape. This review aims to 1) highlight the known biological functions of key enzymes in the KP, and 2) provide a comprehensive overview of existing and emerging therapies aimed at targeting discrete enzymes in the KP for anti-cancer treatment.

KEYWORDS

kynurenine pathway (KP), cancer, metabolism, therapeutics, immune evasion

1 Introduction

The kynurenine pathway (KP) has been recognized as a key mediator of tumor immune escape (1–3). Three enzymes, indolamine 2,3-dioxygenase 1 and 2 (IDO1, IDO2) and tryptophan 2,3-dioxygenase (TDO) initiate the first steps in the KP, converting tryptophan (Trp) to N-formyl-kynurenine, which can be further metabolized by arylformamidase (AFMID) yielding the immunosuppressive catabolite kynurenine (Kyn). Whereas TDO is predominately expressed in the liver and accounts for the majority of Trp metabolism, IDO-mediated metabolism predominately occurs secondary to inflammation (4, 5). Upregulation of IDO in tumor cells or antigen-presenting cells leads to Trp depletion and accumulation of its downstream catabolite Kyn in the local tumor

microenvironment (TME), resulting in immunosuppression by inducing T cell anergy and apoptosis and suppressing T cell differentiation (1–3, 6–9). Building upon this underlying biological mechanism, substantial efforts have been dedicated to the development of small molecule inhibitors and agonists targeting the KP as anti-cancer agents to circumvent tumor immune suppression. Among the most investigated IDO inhibitors are epacadostat, navoximod, and indoximod, each of which have been explored as a monotherapy and in combination with immune check-point inhibitors (ICI) and/or chemotherapy (10–21). Despite early success in Phase I/II clinical trials, ECHO-202/KEYNOTE-037 failed to demonstrate additional benefit of epacadostat in combination with pembrolizumab (10) and similar findings have since been reported for navoximod with atezolizumab (19), raising uncertainty about the benefits of targeting IDO1 for anti-cancer treatment.

Yet, it is increasingly recognized that, beyond the well covered Trp-IDO-Kyn axis, other enzymes in the KP along with their associated catabolites can modulate the TME to evade immune surveillance and enable tumor progression (22–26). Moreover, emerging data suggests that KP-related enzymes, such as IDO1, can promote tumor progression through functions that are independent of enzymatic activity (27). The purpose of this review is to 1) highlight the known tumor promotive functions of key enzymes in the KP and 2) provide a comprehensive overview of

existing and emerging therapies aimed at targeting discrete enzymes in the KP for anti-cancer treatment.

2 Role of the kynurenine pathway in cancer development and immune suppression

2.1 Indoleamine 2,3-dioxygenase 1

Indoleamine 2,3-dioxygenase 1 (IDO1) is a rate-limiting enzyme in the metabolism of the essential amino acid Trp into downstream kynurenines (Figure 1) (7). Under physiological conditions, the IDO1 functions to generate cellular nicotinamide adenine dinucleotide (NAD+). However, IDO1 is overexpressed in several cancer types (28, 29) and is prognostic for poor disease-free survival and overall survival (30, 31).

Seminal studies by Munn and colleagues demonstrated that reduced bioavailability of Trp due to IDO overactivation in dendritic cells (DCs) leads to accumulation of uncharged Trp-tRNAs in T cells and that the accumulation of Trp-tRNAs induces integrated stress response kinase (GCN2)-mediated suppression of the translation initiation factor 2a (eIF2a) resulting in subsequent cell growth arrest (6). Tryptophan starvation-mediated activation of GCN2 has also been shown to down-regulate the T cell receptor

FIGURE 1
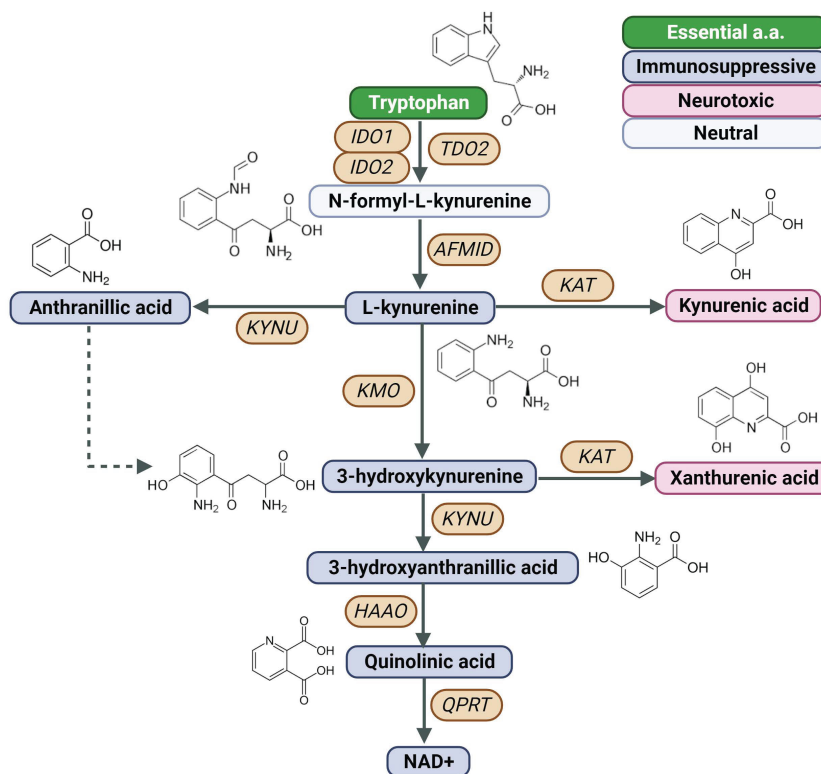Created with BioRender.com. Schematic of the KP, Kynurenine Pathway; AFMID, arylformamidase; HAAO, 3-hydroxyanthranilate 3,4-dioxygenase; IDO1, indoleamine 2,3-dioxygenase 1; IDO2, indoleamine 2,3-dioxygenase 2; KAT, kynurenine aminotransferase; KMO, kynurenine 3-monooxygenase; KYNU, kynureninase; NAD+, nicotinamide adenine dinucleotide; TDO2, tryptophan 2,3-dioxygenase; QPTR, quinolinic acid phosphoribosyl transferase.

(TCR) zeta-chain in naïve T cells, resulting in diminished T cell function (32). Another study demonstrated a GCN2-independent mechanism whereby IDO-mediated catabolism of Trp inhibits immunoregulatory kinases mTOR and PKC resulting in induction of autophagy and immunosuppression (18). Subsequent studies demonstrated that IDO-mediated catabolism of Trp results in the accumulation of Kyn (7) that stimulates ligand-activated transcription factor aryl hydrocarbon receptor (AhR) signaling and promotes the generation of immune-tolerant DCs and regulator T cells (Tregs) (1, 7, 8). To this end, Liu and colleagues demonstrated that interferon-gamma (IFNγ) produced by cytotoxic CD8+ T cells stimulates Kyn release by tumor-repopulating cells (TRCs) and that TRC-derived Kyn is subsequently taken up by CD8+ T cells through TCR-mediated upregulation of solute carrier family 7 member 8 (SLC7A8) and Solute Carrier Family 36 Member 4 (SLC36A4, also known as PAT4), resulting in Kyn-AhR-mediated upregulation of programmed cell death protein 1 (PD-1) (33). Inhibition of IDO1 in CD8+ T cells promoted Trp accumulation and downregulation of PD-1 (34). Another study found that IFNγ secreted by acute myeloid leukemia (AML) cells upregulated IFNγ-dependent genes related to Treg induction, including IDO1, in mesenchymal stromal cells (MSCs). Genetic ablation of IFNγ production by AML cells reduced MSC IDO1 expression and Treg infiltration, thereby attenuating AML engraftment (35).

The IDO1-Kyn-AhR axis has also been shown to impair natural killer (NK) cell and macrophage function. Specifically, Kyn inhibits expression of the natural cytotoxicity triggering receptor 1 (NCR1, also known as NKp46) and killer cell lectin like receptor K1 (KLRK1, also referred to as NKG2D) on NK cells resulting in impaired tumor-killing functions (36, 37). Recently, Fang and colleagues demonstrated that IDO1 regulates expression of ADAM metallopeptidase domain 10 (ADAM10) via IDO1-Kyn-AhR pathway in non-small cell lung cancer (NSCLC) cells and that the induction of ADAM10 downregulates the NKG2D Ligand (NKG2DL). Targeting of IDO1 via RY103, a novel IDO1 inhibitor, improved NK cell-mediate anti-tumor activity in xenograft mouse models of lung cancer (38). Takenaka and colleagues reported that increased IDO-mediated release of Kyn by glioblastoma cells promotes AhR activation in tumor-associated macrophages (TAMs); activation of AhR signaling in TAMs increased expression of the ectonucleotidase CD39 and accumulation of adenosine in the tumor microenvironment (TME) leading to suppression of CD8+ T cell function (39). Interestingly, studies by Campesato and colleagues reported that IDO-Kyn-AhR-mediated immunosuppression depends on the interplay between Tregs and TAMs (40). Using a preclinical mouse model of B16-F10 melanoma overexpressing IDO1 (B16^{IDO}), authors demonstrated that IDO-expressing tumors have marked increases in Treg frequency and upregulated expression of FoxP3 in tumor infiltrating CD4+ T cells compared to B16^{WT}-tumor bearing mice. Concomitant to Tregs was an increased abundance of TAMs (CD11b+F4/80highLy6G−) that accumulated during tumor progression. Gene expression analyses of B16^{IDO}-derived Tregs and TAMs revealed prominent upregulation of AhR and AhR-responsive genes. Depletion of macrophages with αCSF1-R or clodronate liposomes delayed the progression of IDO-

expressing tumors but not wild-type tumors, an effect that could be reversed upon CD8+ T cell depletion. Similarly, specific depletion of Tregs using Foxp3DTR mice significantly abrogated the myeloid-enriched phenotype found in B16^{IDO} tumors, with reduced infiltration of CD11b+ cells and M2-like TAMs after treatment with DT. Co-culture experiments further demonstrated that Treg inhibitor function and expansion of M2-like macrophages was dependent on Kyn-AhR signaling (40).

Whereas IDO1 has largely been studied in the context of Trp-IDO1-Kyn-AhR signaling, IDO1 may also promote tumor progression through non-enzymatic functions (27). To this end, IDO1 can act as a signal-transducing molecule via two immunoreceptor Tyrosine-based inhibitory motifs (ITIMs) in the non-enzymatic domain of the enzyme. Phosphorylation of the ITIMs promotes interaction with SHP family tyrosine phosphatases (41, 42). Moreover, IDO1 can directly bind class IA PI3K regulatory subunits, resulting in IDO1 anchoring to early endosomes and activation of ITIM-mediated signaling (43).

In the context of cancer, using B16-F10 mouse melanoma cells transfected with either the wild-type $Ido1$ gene ($Ido1^{WT}$) or a mutated variant lacking the catalytic, but not signaling activity ($Ido1^{H350A}$), Orecchini and colleagues demonstrated that IDO1 promote tumor growth by increasing SHP-2-mediated activation of Ras and Erk signaling $in vitro$ and $in vivo$. Furthermore, B16^{H350A} tumor bearing mice exhibited markedly enhanced tumor growth, and worse overall survival compared to B16^{wt} or B16^{mock} control mice. Immunophenotyping of tumors indicated that B16^{H350A} tumors had lower IFNγ-producing CD4+ and CD8+ T lymphocytes and a significantly higher percentage of CD4$^+$Fox3p$^+$ Tregs compared to respective controls (27). Zhai and colleagues established IDO-deficient glioblastoma (GBM) cell lines reconstituted with IDO wild-type or IDO enzyme-null cDNA to assess tumor promotive roles of IDO1 independent of its catalytic activity. They found that nonenzymatic tumor cell IDO1 activity increased expression of complement factor H (CFH) and its isoform, factor H like protein (FHL-1) in human GBM, resulting in increased intratumoral Tregs and myeloid-derived suppressor cells, accelerated tumor growth and poor survival (44). These findings highlight an increasingly appreciated role of IDO1 in signal transduction, and, importantly, underscore an alternative mechanism of IDO1-mediated immune suppression that may not be impeded by small molecule IDO inhibitors.

2.2 Indoleamine 2,3-dioxygenase 2

The IDO1 paralogue IDO2 is another enzyme involved in the catabolism of tryptophan to downstream kynurenines. $INDOL1$, the encoding gene for IDO2, is located on chromosome 8p12 near $INDO$ (located on chromosome 8p11), the encoding gene for IDO1 (45). In The Cancer Genome Atlas (TCGA)-PanCancer Atlas transcriptomic datasets, increased tumoral IDO2 is highly correlated with IDO1 expression (Spearman ρ coefficient: 0.59 (95% CI: 0.58-0.61)) (Figure 2), suggesting potential co-regulation (46). In this regard, studies by Kado and colleagues demonstrated that 2,3,7,8-tetrachlorodibenzio-p-dioxin (TCDD)-mediated
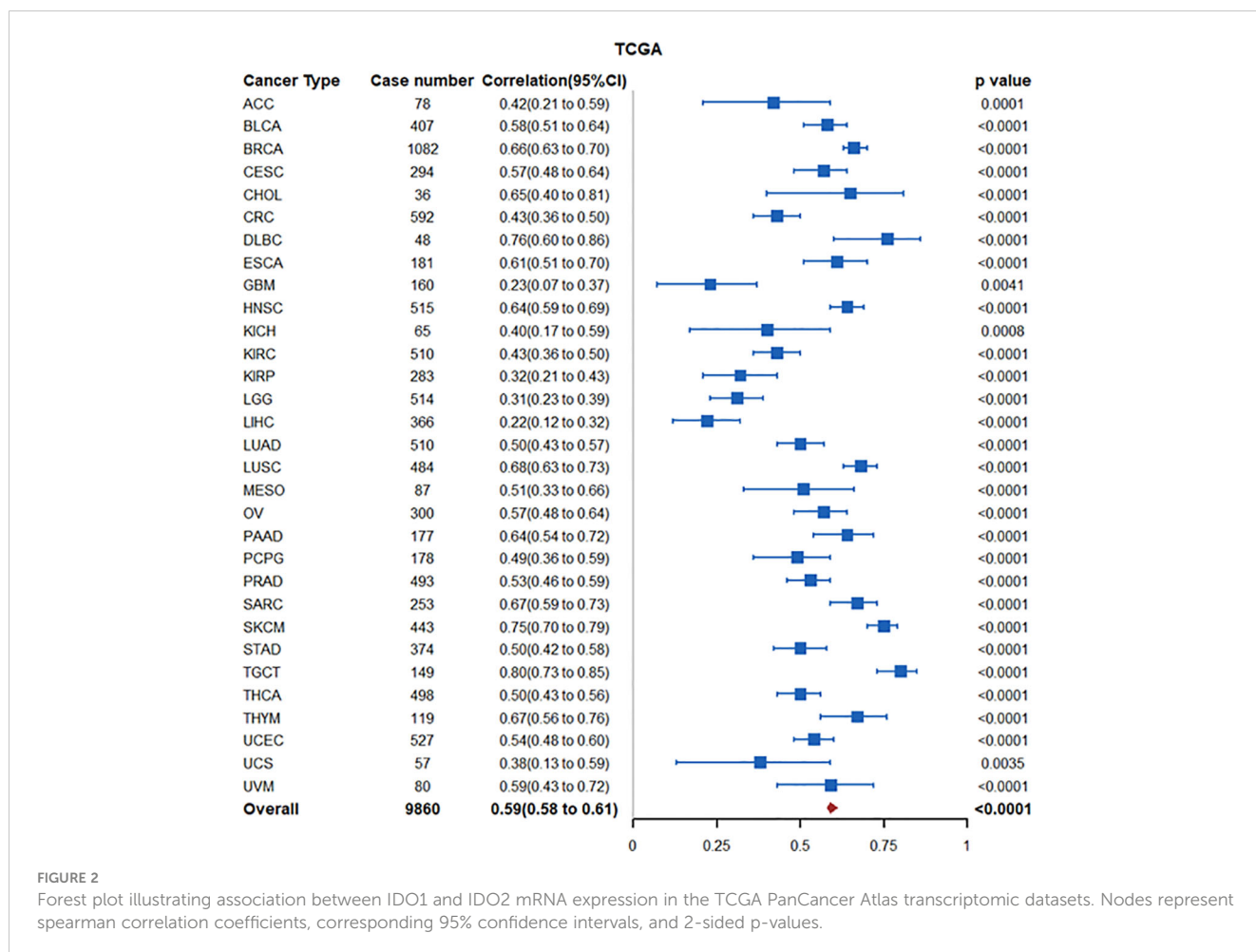
TCGA

Cancer Type	Case number	Correlation(95%CI)		p value
ACC	78	0.42(0.21 to 0.59)		0.0001
BLCA	407	0.58(0.51 to 0.64)		<0.0001
BRCA	1082	0.66(0.63 to 0.70)		<0.0001
CESC	294	0.57(0.48 to 0.64)		<0.0001
CHOL	36	0.65(0.40 to 0.81)		<0.0001
CRC	592	0.43(0.36 to 0.50)		<0.0001
DLBC	48	0.76(0.60 to 0.86)		<0.0001
ESCA	181	0.61(0.51 to 0.70)		<0.0001
GBM	160	0.23(0.07 to 0.37)		0.0041
HNSC	515	0.64(0.59 to 0.69)		<0.0001
KICH	65	0.40(0.17 to 0.59)		0.0008
KIRC	510	0.43(0.36 to 0.50)		<0.0001
KIRP	283	0.32(0.21 to 0.43)		<0.0001
LGG	514	0.31(0.23 to 0.39)		<0.0001
LIHC	366	0.22(0.12 to 0.32)		<0.0001
LUAD	510	0.50(0.43 to 0.57)		<0.0001
LUSC	484	0.68(0.63 to 0.73)		<0.0001
MESO	87	0.51(0.33 to 0.66)		<0.0001
OV	300	0.57(0.48 to 0.64)		<0.0001
PAAD	177	0.64(0.54 to 0.72)		<0.0001
PCPG	178	0.49(0.36 to 0.59)		<0.0001
PRAD	493	0.53(0.46 to 0.59)		<0.0001
SARC	253	0.67(0.59 to 0.73)		<0.0001
SKCM	443	0.75(0.70 to 0.79)		<0.0001
STAD	374	0.50(0.42 to 0.58)		<0.0001
TGCT	149	0.80(0.73 to 0.85)		<0.0001
THCA	498	0.50(0.43 to 0.56)		<0.0001
THYM	119	0.67(0.56 to 0.76)		<0.0001
UCEC	527	0.54(0.48 to 0.60)		<0.0001
UCS	57	0.38(0.13 to 0.59)		0.0035
UVM	80	0.59(0.43 to 0.72)		<0.0001
Overall	**9860**	**0.59(0.58 to 0.61)**		**<0.0001**

0   0.25   0.5   0.75   1

FIGURE 2

Forest plot illustrating association between IDO1 and IDO2 mRNA expression in the TCGA PanCancer Atlas transcriptomic datasets. Nodes represent spearman correlation coefficients, corresponding 95% confidence intervals, and 2-sided p-values.

activation of AhR signaling increased IDO2 expression in wildtype MCF7 breast cancer cells but not in CRISPR-cas9 AhR-knockout MCF-7 cells. Promoter analyses identified short-tandem repeat containing four core sequences of a xenobiotic response element (XRE) upstream of the start site of the human *ido2* gene, reinforcing AhR as an upstream regulator (47). TCDD-mediated AhR activation in DC cells also induced expression of IDO1 and IDO2 (48). Interestingly, Li and colleagues demonstrated that tolerogenic phenotype of IFN-γ-induced IDO2 expression in DCs is maintained via autocrine IDO-Kyn/AhR-IDO loop (49). Thus, in tumors with high IDO1/IDO2 expression, increased IDO2 expression may be driven through the IDO1-Kyn/AhR signaling axis.

With respect to cancer cell functions, small interfering RNA (siRNA)-mediated knockdown of IDO2 inhibited cancer cell proliferation, induced cell cycle arrest in G1 and apoptosis, and reduced cell migration of B16-BL6 melanoma cells *in vitro*. Mechanistically, IDO2 knockdown increased generation of reactive oxygen species (ROS), and decreased generation of NAD + (50).

In the context of the tumor immunophenotype, a pan-cancer analysis of IDO2 in the TCGA transcriptomic datasets revealed IDO2 mRNA expression to be strongly associated with high infiltration of immune cells in the tumor microenvironment, as well as tumor mutational load (TMB), microsatellite instability

(MSI), mismatch repair (MMR), and immune checkpoint related genes (51).

IDO2 may promote tumor immune suppression through the Trp-Kyn-AhR axis. Studies by Yamasuge and colleagues demonstrated that Ido2 knockout (KO) tumors had higher intratumor Trp and reduced Kyn levels compared to wild-type Ido2 tumors in a Lewis lung carcinoma mouse model, indicating Ido2 Trp catalytic activity (52). Moreover, Ido2 KO tumors had significantly higher CD3+ and CD8+ TILs compared to wild-type Ido2 tumors (52).

Yet, it is unlikely that the biological function(s) of IDO2 are completely redundant with that of IDO1. This is reinforced by the fact that the K_m of IDO2 for Trp is markedly lower than IDO1 (K_m 500- to 1000-fold lower), despite both enzymes containing residues necessary for Trp catalytic activity (53, 54). Indeed, studies in autoimmune disease have identified several functions of IDO2 that are independent of IDO1 and Trp catalytic activity. For instance, Metz and colleagues found that IDO1-dependent T regulatory cell generation is defective in $Ido2^{-/-}$ mice. Mechanistically, *Ido2* deficient mice, but not wild-type *Ido2* mice, exhibited lower expression of immune regulatory cytokines, including IFN-α, TNF-α, IL-6, and MCP-1/CCL2 (55). Merlo and colleagues reported that IDO1 mediates T cell suppressive effects whereas IDO2 mediates autoreactive B cell responses to promote

inflammation (56). Using a catalytically inactive IDO2 knock-in mouse model, authors demonstrated that IDO2, but not IDO1, can directly interact with GAPDH, Runx1, RANbp10, and Mgea5 to potentiate an inflammatory response in autoimmune arthritis (57).

The above studies lend to an intriguing question as to whether a concomitant upregulation of tumoral IDO1 and IDO2 may synergize to suppress an anti-cancer immune response, which may lend to additional opportunities for cancer interception (*see Section 2 below*).

2.3 Tryptophan 2,3-dioxygenase

Liver-associated tryptophan 2,3-dioxygenase (TDO2) is another heme-containing enzyme that is involved in the catabolism of Trp to downstream kynurenines (Figure 1) and that has been reported to be frequently overexpressed in various malignancies (58). Elevated tumoral TDO2 expression is associated with poor prognosis in several cancer types including kidney renal papillary cell carcinoma, glioma, testicular germ cell tumors, and uveal melanoma (58) as well as liver (59), colorectal (CRC) (60), and breast cancer (61). Moreover, high TDO2 expression level positively correlated with higher immune infiltration, especially DCs, as well as immune checkpoint-related gene markers, such as LAIR1, CD276, NRP1, CD80, and CD86 (58). Regulation of TDO2 expression has been linked to IL-1β- C/EBPβ-MAPK signaling activities (62).

Like IDO1, TDO2-mediated Kyn promotes tumor immune suppression through AhR-signaling in various cancer types (63, 64). To this end, single-cell RNA sequencing (scRNA-seq) of 13 cancerous tissues identified a subset of myofibroblasts that exclusively expressed TDO2 and that clustered with CD4+ and CD8+ T cells distal to tumor nests. Functional experiments demonstrated that TDO2+ myofibroblasts induce transformation of CD4+ T cells into Tregs and caused CD8+ T cell dysfunction. In a murine model of oral squamous cell carcinoma (OSCC), small molecule inhibition of TDO2 via LM10 attenuated the inhibitory states of T cells, restored the T cell antitumor response, and prevented malignant transformation (65). Interestingly, Schramme and colleagues demonstrated that C57BL/6 TDO-KO mice (66) engrafted with MC38 CRC cells were more sensitive to anti-CTLA4 or anti-PD1 treatment compared to MC38-tumor bearing wild-type C57BL/6 mice. Enhanced efficacy of ICI in C57BL/6 TDO-KO tumor bearing mice was attributed to higher systemic Trp levels, which could be reversed through a Trp-low diet (67). These studies implicate host metabolism Trp independent of cancer TDO2 status as being a determinant of response to ICI.

In addition to its role in immune suppression, TDO2 has also been demonstrated to promote migration and invasion of liver cancer cells via Wnt5a signaling activities (59). Another study demonstrated that TDO2-Kyn-AhR activation in liver cancer cells induces autocrine interleukin 6 (IL-6)-mediated Signal transducer and activator of transcription 3 (STAT3) and Nuclear factor kappa beta (NFκB)/T Cell Immunoglobulin And Mucin Domain Containing 4 (TIM4) signaling to promote tumor progression (68). Inhibition of AhR signaling by PDM2 attenuated tumor

growth in a xenograft model of liver cancer (68). In the context of CRC, deficiency in adenomatous polyposis coli (APC) results in TCF4/β-catenin-mediated upregulation of the TDO2-Kyn-AhR axis to increase glycolysis and drive anabolic cancer cell growth (69). Knockdown of TDO2 in LoVo and HCT116 CRC cells attenuated cell growth, and reduced migration, invasion and colony formation potential through inactivation of TDO2-kynureninase (KYNU)-AhR signaling (60).

2.4 Arylformamidase

Arylformamidase (AFMID) is another downstream KP enzyme that is frequently overexpressed in various malignancies (70), and that catalyzes the hydrolysis of N-formyl-Kynurenine into Kyn and formate (Figure 1) (71). Although the functional role of AFMID upregulation in cancer remains poorly understood, Venkateswaran and colleagues demonstrated that oncogenic MYC increases expression of the Trp importers SLC1A5 and SLC7A5 as well as AFMID in colon cancer. Increased uptake and catabolism of Trp results in the accumulation of Kyn that enables cancer cell proliferation in part through AhR-signaling (72).

Splicing changes in *AFMID* have also been associated with survival and relapse in patients with hepatocellular carcinoma (HCC) (73). Specifically, the switch of AFMID isoforms was found to be an early event in HCC development and was associated with driver mutations in *TP53* and AT-rich interactive domain-containing protein 1A (*ARIDIA*). Authors further found that overexpression of the full-length AFMID isoform leads to higher NAD+ levels, lower DNA-damage response, and slower cell growth in HepG2 cells (73). To this end, Tummala and colleagues found that loss of AFMID expression in SNU-449 HCC cells resulted depletion of NAD+, indicating that Trp catabolism in HCC cells supports *de novo* NAD+ synthesis (74). Additional studies are warranted to better elucidate the functional relevance of AFMID in tumorigenesis and progression; however, one may surmise that the elevation of AFMID underlies increased accumulation and secretion of Kyn.

2.5 Kynureninase

Kynureninase (KYNU) is a pyridoxal phosphate (PLP)-dependent hydrolase that catalyzes the cleavage of Kyn as well as 3-hydroxykynurenine (3HK) into anthranilic acid (AA) and 3-hydroxyanthranilic acid (3HA), respectively (Figure 1) (75). KYNU has been strongly implicated in the inflammation and immune modulation; however, the exact underlying mechanism (s) remain incomplete (76–81).

Recently, our group demonstrated selective enrichment of KYNU, but not other downstream KP enzymes, in lung adenocarcinomas (LUAD) harboring a loss-of-function *KEAP1* mutation (22). Metabolomic analyses confirmed that KYNU was enzymatically functional, as evidenced by the accumulation of AA in conditioned medium of LUAD cell lines. Activation of the Nuclear factor erythroid 2-related factor 2 (NRF2) pathway

through siRNA-mediated knockdown of *KEAP1* or chemical induction with the NRF2-activator CDDO-Me upregulated KYNU at both the mRNA and protein levels with concomitant increases in AA production. Moreover, elevated tumoral KYNU expression was found to be associated with a tumor suppressive immunophenotype and was prognostic for poor overall survival in a tissue microarray (TMA) of LUAD as well as multiple independent LUAD transcriptomic datasets (22). A pan-cancer analyses further revealed upregulation of KYNU to be a prominent feature of NRF2-activated cancers and is associated with tumor immunosuppression and poor prognosis (23). Interestingly, a recent study by Liu and colleagues demonstrated that KYNY-derived 3HA has anti-oxidant properties and that elevations in cancer cell intracellular 3HA pools infer resistance to ferroptosis (82).

Studies by Heng and colleagues similarly found that KYNU was highly upregulated in HER2-enriched and triple-negative (TN) breast cancers (BrCa), leading to increased production of AA and 3HA (81). Interestingly, stimulation of TNBC cell lines with IFNγ resulted in a pronounced 286-fold increase in 3HA and higher serum levels of 3HA was able to distinguish TNBC from the other BrCa molecular subtypes (81). These findings are notable given that 3HA has been shown to enhance the percentage of Tregs, inhibit T-helper (Th)-1 and Th17 cells (24) and suppress antigen-independent proliferation of CD8+ T cells (83). To this end, a large-scale transcriptomic analysis of 2,994 BrCa tumors found a strong correlation between tumoral KYNU with inflammatory and immune responses; elevated KYNU was also linked to BrCa grade and poorer clinical outcomes (78).

In addition to cancer cell-associated upregulation of KYNU, a recent study by Itoh and colleagues found that KYNU is also elevated in cancer-associated fibroblast (CAF)-educated fibroblasts (CEFs). Specifically, authors demonstrated that CAFs induce CEF generation from normal fibroblasts (NFs) via reactive oxygen species (ROS)-induced activation of NFκB signaling, resulting in induction of a pro-inflammatory loop and secretion of asporin (ASPN), a small leucine-rich proteoglycan. ASPN in turn promoted upregulation of IDO1 and KYNU in CEFs, which induced cytocidal effects against CD8+ T-cells and promoted tumor spreading (79).

2.6 Kynurenine 3-monooxygenase

Kynurenine 3-monooxygenase (KMO) is a flavin adenine dinucleotide (FAD)-dependent monooxygenase and is located in the outer mitochondrial membrane that catalyzes the hydroxylation of Kyn into 3-hydroxykynurenine (3HK) (Figure 1) (84). Upregulation of KMO has been reported in several cancer types, including TNBC (85), CRC (86), HCC (87), and astrocytoma (88).

In the context of TNBC, elevated tumoral KMO is associated with worse overall survival and invasive breast cancers have among the highest rates of KMO copy number amplification (81, 89). *In vitro* ectopic overexpression of KMO in TNBC cell lines promoted increased cell growth, colony and mammosphere formation, migration and invasion, as well as increased expression of mesenchymal markers (85). In xenograft models, mice harboring

CRISPR KMO-KD MDA-MB-231 TNBC cells had reduced tumor growth, attenuated capacity for lung metastases, and prolonged overall survival compared to respective control mice. Mechanistically, KMO prevented degradation of β-catenin, thereby enhancing the transcription of pluripotent genes, including CD44, Nanog, Oct4, and SOX2 (85). In support of the aforementioned finding, knockdown of KMO in CRC cells was also reported to decrease expression of cancer stem cell markers CD44 and Nanog, and reduce sphere formation as well as migration and invasion (86). Inhibition of KMO enzymatic activity, using the small molecule inhibitor UPF648, similarly attenuated sphere formation and cell motility of CRC cell lines (86). Interestingly, using immunohistochemistry, flow cytometry, immunofluorescence assay, and transmission electron microscopy, Lai and colleagues demonstrated that KMO is highly expressed on the cell membranes of breast cancer tissues and the cancer cell surfaceome of cell lines (90). Treatment of MDA-MB-231 TNBC cell lines with anti-KMO antibodies reduced the cell viability and inhibited the migration and invasion of the triple-negative (90). Collectively, these findings demonstrate a cancer cell intrinsic function of KMO that promotes tumor aggressiveness and pluripotency.

Notably, induction of KMO in immune cell subtypes has also been linked to dysfunctional anti-tumor activity. Using melanoma-derived cell lines and primary CD4+ CD25- T cell co-cultures, Rad Pour and colleagues demonstrated that activation of CD4+ T cells results in increased production of IFNγ with concomitant increases in Kyn and kynurenic acid that is attributed to reduced KMO expression in CD4+ T cells (25). Accumulation of Kyn and kynurenic acid suppresses CD4+ T cell expansion and viability (25). In the context of multiple myeloma (MM), Ray and colleagues demonstrated that KMO is upregulated in plasmacytoid dendritic cells (pDCs), resulting in immune suppression (26). Specifically, using co-culture models of patient autologous pDC–T–NK–MM cells, authors showed that pharmacological blockade of KMO activates pDCs and triggers both MM-specific cytotoxic T-cell lymphocytes (CTL) and NK cells` cytolytic activity against tumor cells. Combination treatment with R0-61-8048, a potent competitive inhibitor of KMO, and anti-PD-L1 antibody yielded superior anti-cancer efficacy compared to either treatment alone (26).

2.7 Kynurenine aminotransferase

Kynurenine aminotransferase family members (KATI-KATIV) catalyze the transamination of Kyn and 3HK into kynurenic acid (KA) (91) and xanthurenic acid (XANA), respectively (Figure 1) (4). Although limited information exists regarding the functional role of KAT in tumorigenesis, KAT-derived KA is readily detectable in tumor tissues, as well as in blood and urine of cancer patients (92).

Early studies demonstrated that KAT-derived KA exerts anti-proliferative effects by modulating key pathways associated with proliferation, survival, apoptosis, and migration (93–96). Yet, KA is documented to exert immunosuppressive effects via the G-protein-

coupled receptor 35 (GPR35) (97), which may potentiate tumor progression (98). Moreover, KA has also been reported to be a potent agonist of the AhR that synergistically induces IL-6 (99) with potential pro-tumoral effects (100). To this end, a recent pan-tissue study revealed that interleukin-4-induced-1 (IL4I1) more frequently associates with AhR activity than does IDO1 or TDO2. Mechanistically IL4I1-mediated generation of indole metabolites and KAT-derived KA that activated AhR signaling to promote cancer cell motility and suppression of adoptive immunity (101). The above findings underscore the enigmatic role of KAT and KA in tumor progression. Further investigations are needed.

2.8 3-hydroxyanthranilate 3,4-dioxygenase

3-hydroxyanthranilate 3,4-dioxygenase (HAAO) is involved in the synthesis of quinolinic acid (QA) (Figure 1). Expression patterns of HAAO are variable among different cancer types (5, 22, 23); however, studies of endometrial carcinomas revealed promotor hypermethylation of HAAO is prominent and to be associated with microsatellite instability and poor clinical outcomes (102). Hypermethylation of HAAO has also been reported to be higher in prostate cancer tissues compared to adjacent control tissue, and it is associated with a more aggressive phenotype (103).

2.9 Quinolinic acid phosphoribosyl transferase

Quinolinic acid phosphoribosyl transferase (QPRT) is a rate-limiting enzyme in *de novo* NAD+ biosynthesis and catalyzes the conversion of QA to nicotinate ribonucleotide (Figure 1). The cancer cell intrinsic effects of QPRT upregulation have been studied in a variety of cancer types (104–109). For instance, neoplastic transformation in astrocytes is associated with a QPRT-mediated switch in NAD+ metabolism by exploiting microglia-derived quinolinic acid as an alternative source of replenishing intracellular NAD+ pools. Mechanistically, the
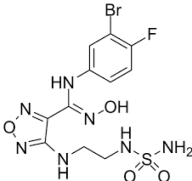
increase in QPRT expression increases resistance to oxidative stress, enabling disease progression (104). Increased expression of QPRT in invasive breast cancer promotes cell migration and invasion through purinergic signaling (105). Another study found down syndrome cell adhesion molecule antisense RNA 1 (DSCAM-AS1) increases QPRT expression in breast cancer cells via competitively binding miRNA-150-5p and miRNA-2467-3p, resulting in increased cell growth, migration, and invasion of estrogen-receptor breast cancer cells (106). Upregulation of QPRT has also been shown to confer resistance to chemotherapy in leukemic cells and ovarian cancer (107, 108). In this regard, Thongon and colleagues evaluated the mechanisms of cancer cell resistance to nicotinamide phosphoribosyltransferase (NAMPT), the rate-limiting enzyme in NAD+ biosynthesis from nicotinamide. In their study, FK866-resistant CCRF-CEM (T cell acute lymphoblastic leukemia) cells were found to have exceptionally high QPRT activity and exhibited an addiction to exogenous Trp to maintain NAD+ pools under stress conditions (109).

Despite the above studies, QPRT has also been shown to be inversely associated with other KP pathway enzymes, suggesting reduced expression in some cancer types (5, 22, 23). For example, studies in renal cell carcinoma revealed QPRT to be downregulated in tumors and loss of QPRT expression led to anchorage-independent growth of RCC cells (110). Thus, the relevance of QPRT in promoting tumor progression is likely to be context dependent.

3 Targeting the kynurenine pathway for anti-cancer treatment

Based on intrinsic malignant properties as well as tumor immune suppression, targeting of the kynurenine pathway has garnered considerable attention, with several small molecule inhibitors being explored for the treatment of solid malignancies as monotherapies or in combination with immunotherapy (Table 1). The below sections highlight existing small molecule inhibitors as well as emergent therapies that target the KP pathway for anti-cancer treatment.

TABLE 1 Existing and emerging therapies that target the Kynurenine Pathway.

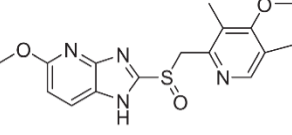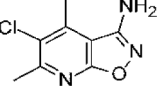| Compound	Structure	Cancer types	Phase
IDO1, IDO2 and TDO2 inhibitors			
Epacadostat		SKCM, RCC, NSCLC, BLCA, SARC, CRC, OVC	Clinical/Phase III
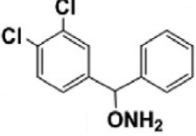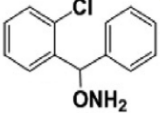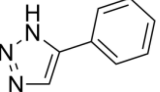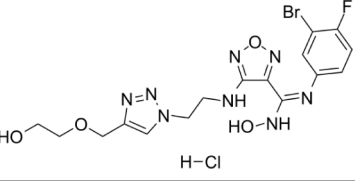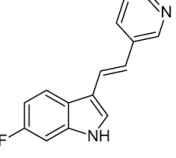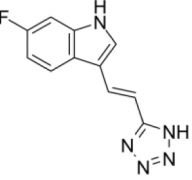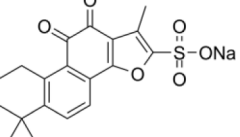
(Continued)

TABLE 1 Continued

Compound	Structure	Cancer types	Phase
Navoximod		Solid tumors	Clinical/Phase I
Indoximod		SKCM	Clinical/Phase II/III
BMS-9862424		OVC	PD
Linrodostat		BLCA,	Clinical/Phase III
YH29407	No information available	CRC	Preclinical
PF-06840003		MG	Clinical/Phase I
KHK2455	No information available	Solid tumors	Clinical/Phase I
LY3381916	No information available	Solid tumors, NSCLC, RCC, TNBC	Clinical/Phase I
MK-7162	No information available	Solid tumors	Clinical/Phase I
IACS-9779		None	*in vitro* PD
IACS-70465		None	*in vitro* PD
IDO1-PROTAC		GBM	Preclinical PD
IU1		CRC	Preclinical
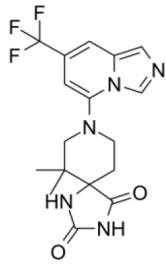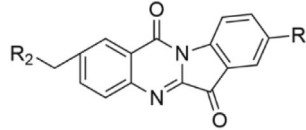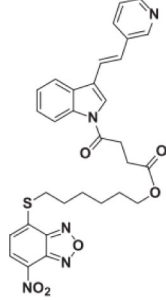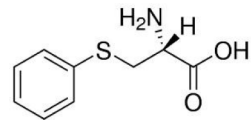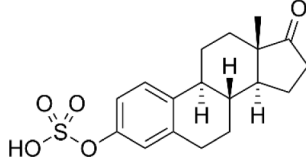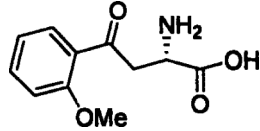
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TABLE 1 Continued

Compound	Structure	Cancer types	Phase
3,4-dichloro aryl ring diaryl hydroxylamine		None	Preclinical PD
2-chloro aryl ring diaryl hydroxylamine		None	Preclinical PD
4-aryl-1,2,3-triazole		None	in vitro PD
Tenatoprazole		None	in vitro
Compound 4t (He X.)		CRC	in vivo
W-0019482		GBM, LLC	Preclinical
680C91		SKCM, CRC, LM, HCC	in vitro
LM10		HCC	in vitro
Sodium Tanshinone IIA Sulfonate		CRC	Preclinical
RG70099	No information available	GBM	Preclinical
M4112	No information available	Healthy subjects	Clinical/Phase I
HTI-1090	No information available	Solid tumors	Clinical/Phase I
DN1406131	No information available	Healthy subjects	Clinical/Phase I

(Continued)

TABLE 1 Continued

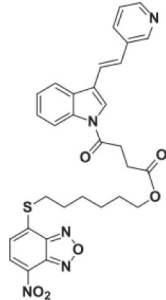| Compound	Structure	Cancer types	Phase
IACS-8968		GBM	Preclinical
EPL-1410	No information available	SKCM, CRC	Preclinical
RY103		GBM, PC	Preclinical
Compound 4 (Hua S.)		HCC	*in vitro*
Compound 5 (Hua S.)		HCC	*in vitro*
KYNU inducers			
S-phenyl-l-cysteine sulfoxide		NA	*in vitro*
Oestrone sulphate		NA	*in vitro*
O-methoxybenzoylalanine		NA	*in vitro*
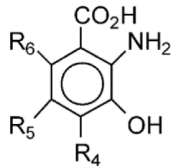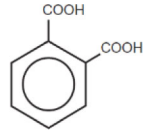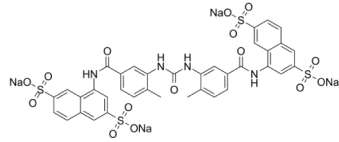
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TABLE 1 Continued

Compound	Structure	Cancer types	Phase
Bensarazide hydrochloride		NA	*in vitro*
PEGylated-KYNU	NA	SKCM, TNBC, CRC	Preclinical
KMO inhibitors			
GSK065		NA	*in vitro*
GSK366		NA	*in vitro*
UPF-648		CRC	*in vitro*
Ro 61-8048		CRC	*in vitro*
GSK3335065		NA	Clinical/Phase I
HAAO inhibitors			
NCR-631		NA	Preclinical

(Continued)

TABLE 1   Continued

Compound	Structure	Cancer types	Phase
3-hydroxyanthranilic acid derivatives	[chemical structure: benzene ring with CO_2H, NH_2, OH, and R_4, R_5, R_6 substituents]	NA	Preclinical
QPRT inhibitors			
Phthalic acid	[chemical structure: benzene ring with two COOH groups]	BRCA	*in vitro*
NF340	[chemical structure]	BRCA	*in vitro*

BLCA, bladder urothelial carcinoma; BRCA, breast cancer; CRC, colorectal cancer; GBM, glioblastoma; HCC, hepatocellular carcinoma; LALM, acute myeloid leukemia; LLC, Lewis lung carcinoma; LM, leiomyoma; MG, malignant glioma; NSCLC, non-small cell lung cancer; OVC, ovarian cancer; PD, pharmacodynamic; RCC, renal cell carcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; TNBC, triple negative breast cancer.

3.1 Indoleamine 2,3-dioxygenase 1

3.1.1 Epacadostat

Epacadostat (INCB024360) is a selective competitive inhibitor of IDO1 that has been explored for the treatment of solid malignancies as both a standalone agent and in combination with immune check-point inhibitor (ICI) therapies (11–16).

Pharmacokinetic and pharmacodynamic studies have demonstrated that, as a monotherapy, epacadostat is generally well tolerated, with a maximal inhibition of IDO1 activity (based on reductions in circulating kynurenine levels) achieved at doses ≥ 100mg BID. Adverse events (AEs) associated with such treatment included fatigue, nausea, decreased appetite, vomiting, constipation, abdominal pain, diarrhea, dyspnea, back pain, and cough (11). In a Phase I clinical study evaluating efficacy of epacadostat in patients with advanced solid malignancies, 18 out of 52 patients achieved stable disease, with 7 patients (13.5%) having stable disease lasting ≥16 weeks (11).

A seminal study, ECHO-202/KEYNOTE-037, evaluated the efficacy of epacadostat in combination with the PD-1 inhibitor pembrolizumab in advanced melanoma, with reported objective response rates (ORR) of 56% and a disease control rates (DCR; complete response (CR) + partial response (PR) + stable disease (SD)) of 78% (111). Despite early success in Phase I and II clinical trials, a Phase III clinical trial (ECHO-301/KEYNOTE-252) did not demonstrate additional benefit of epacadostat + pembrolizumab compared to placebo + pembrolizumab alone in patients with unresectable or metastatic melanoma (10).

ECHO-202/KEYNOTE-037 also reported results from Phase I/II studies evaluating combination treatment of epacadostat + pembrolizumab in sixty-two patients with various advanced solid tumors (13). Antitumor activity was observed in several cancer types, with ORR being observed in 25 (40.3%) of 62 patients.

Epacadostat 100 mg twice a day plus pembrolizumab 200 mg every 3 weeks was recommended for Phase II evaluation. In the Phase II study, an objective response (OR) occurred in 55% (12 of 22) patients with melanoma and in those with non–small-cell lung cancer, renal cell carcinoma, endometrial adenocarcinoma, urothelial carcinoma, and head and neck squamous cell carcinoma (13). For NSCLC, combination treatment of epacadostat + pembrolizumab resulted in a ORR (CR + PR) of 35% (14 out of 40 patients) and a DCR (CR+PR+SD) of 60% (24 out of 40 patients) (112). Similar results were reported for renal cell carcinoma (15) and urothelial carcinomas (16). ECHO-207/KEYNOTE-723, a Phase I/II study of epacadostat plus pembrolizumab and chemotherapy for advanced solid tumors, also demonstrated ORRs of 31.4% across all treatment groups (113). However, a Phase II study evaluating epacadostat + pembrolizumab in 30 patients with selective sarcoma subtypes failed to achieve meaningful antitumor activity, with ORRs of <5% (114).

Epacadostat has also been explored in combination with other ICIs, including ipilimuab, an anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA4) antibody, and nivolumab, an anti-PD-1 antibody (14, 115). To this end, Gibney and colleagues reported the results of a Phase I/II study of epacadostat in combination with ipilimuab in patients with unresectable or metastatic melanoma (14). In this study, immunotherapy-naïve patients (n= 39) achieved an ORR of 26% according to the immune-related response criteria and 23% by the Response Evaluation Criteria in Solid Tumors (RECIST 1.1); no ORR was observed in patients who received prior immunotherapy (14). Combination treatment of epacadostat plus nivolumab in advanced solid tumors achieved respective DCR (CR+PR+SD) of 24%, 28%, and 100% for colorectal cancer, ovarian cancer, and melanoma (115).

3.1.2 Navoximod

Navoximod is an orally available non-competitive inhibitor of IDO1 (17). An open-label Phase Ia study by Nayak-Kapoor and colleagues assessed the safety, pharmacokinetics, pharmacodynamics, and preliminary anti-tumor activity of navoximod as a monotherapy in 22 patients with recurrent/advanced solid tumors (116). Trial-associated adverse events, regardless of causality, included fatigue (59%), cough, decreased appetite, and pruritus (41% each), nausea (36%), and vomiting (27%). Grade ≥3 AEs occurred in 14 of the 22 patients; however, only 2 were related to navoximod. Navoximod was found to be rapidly absorbed (Tmax ~ 1 h) with a half-life of approximately 11 hours, and transiently decrease plasma kynurenine levels. No objective responses were met based on RECIST v1.1 criteria; however, stable disease was observed in 36% of efficacy-evaluable patients (116).

Combination of navoximod plus the anti-PD-L1 inhibitor atezolizumab has also been explored for anti-cancer treatment of solid malignancies (19–21). Results from an open-label Phase Ib trial of 52 patients treated with navoximod plus atezolizumab demonstrated acceptable toxicity profiles; efficacy data available for 45 patients included 4 (9%) patients with a partial response and 11 (24%) patients with stable disease (21). Another Phase I study of navoximod plus atezolizumab in 20 Japanese patients with advanced solid tumors achieved stable disease in 65% (13 out of 20) patients. No dose-limiting toxicities were observed. The recommended dose of navoximod monotherapy was determined as 1000 mg orally BID, and 1000 mg orally BID could be considered in combination with atezolizumab (20). Despite these findings, a larger Phase I study of navoximod plus atezolizumab in 157 patients with advanced solid tumors failed to show benefit of adding navoximod to atezolizumab (19).

3.1.3 Indoximod

Indoximod, also known as 1-methyl tryptophan (1-MT), is an indirect inhibitor of IDO1/IDO2 and is reported to serve as a high-potency Trp mimetic that reverses mTORC1 inhibition and the accompanying autophagy that is induced by Trp depletion in cells (17, 18). Indoximod has been explored for anti-cancer treatment in several Phase I/II clinical trials, both as a monotherapy as well as in combination with chemotherapy (117–120).

Recently, Zakharia and colleagues reported results from a single-arm Phase II clinical trial evaluating the addition of indoximod to standard of care ICI (pembrolizumab, nivolumab, or ipilimumab) approved for melanoma (121). Indoximod was administered continuously (1200 mg orally two times per day), with concurrent ICI dosed per US Food and Drug Administration (FDA)-approved label. A total of 131 patients were enrolled; pembrolizumab was the most frequently used ICI (114 out of 131; 87%). Efficacy was evaluable in 89 patients from the Phase II cohort with non-ocular melanoma who received indoximod combined with pembrolizumab. The ORR for the evaluable population was 51%, with confirm CR of 20% and DCR of 70%. ORR were highest among PD-L1 positive patients (70% compared to 46% for PD-L1 negative patients). Median progression-free survival was 12.4 months (95% CI 6.4 to 24.9) (121). Another recently completed Phase I/II trial (NCT01042535) evaluated an adenovirus-p53 transduced DC vaccine together with indoximod for treatment of metastatic breast cancer. A total of 44 patients were recruited, of which 36 completed the study. The results of this trial are pending.

3.1.4 Others IDO inhibitors

To date, several additional IDO inhibitors have been developed including BMS-986242 (122), linrodostat (BMS-986205) (123, 124), YH29407 (125), PF-06840003 (126–128), KHK2455 (129), LY3381916 (130), and MK-7162. To this end, a Phase I study of PF-06840003 in 17 patients with recurrent malignant glioma demonstrated acceptable toxicity profiles, and with a reported dose-limiting toxicity (DLT) rate of 500 mg BID (131). Disease control occurred in eight patients (47%). Mean duration of stable disease was 32.1 (12.1-72.3) weeks (131). ENERGIZE, a Phase III study of neoadjuvant chemotherapy alone or with nivolumab with or without linrodostat mesylate for muscle-invasive bladder cancer is currently recruiting (128). Phase I clinical trials for KHK2455, (NCT02867007) LY3381916,(NCT03343613- recently terminated) and MK-7162 (NCT03364049) are on-going.

In addition to the above inhibitors, structural elucidation studies have also led to the discovery of imidazopyridines as potent IDO1 inhibitors (132) and substituted oxalamides as novel heme-displacing IDO1 inhibitors (133), as well as the development of first in class IACS-9779 and IACS-70465 inhibitors that bind the IDO1 apoenzyme (134). Anti-cancer efficacy of the mentioned above agents has been demonstrated in preclinical models; however, they have not been explored yet in clinical trials.

Another alternative approach has been to degrade intratumor IDO1. Bollu and colleagues reported the results of a novel IDO1 degrader using proteolysis targeting chimera (PROTAC) technology, which utilizes an E3 ligase complex to ubiquitinate targets for proteasome-mediated degradation. The IDO1-PROTAC resulted in potent cereblon-mediated proteasomal degradation of IDO1, and prolonged survival in mice with established brain tumors (135). Interestingly, studies by Shi and colleagues suggest that IDO1 expression may be regulated post-transcriptionally. Specifically, authors found that overexpression of USP14, a proteosome-associated deubiquitinating enzyme, in CRC cells deubiquitinates the IDO1 at the K48 residue, thus preventing proteasomal degradation (136). Knockdown or pharmacological targeting of USP14 decreased IDO1 expression, independent of AhR signaling, and improved anti-tumor immunity in a MC38 orthotopic syngeneic mouse model of CRC. Combination treatment of IU1, a selective small molecule inhibitor of USP14, and anti-PD-1 resulted in improved anti-killing effects compared to either treatment alone (136). Targeting IDO1 protein stability, rather than enzymatic activity, may thus provide an alternative approach to circumvent IDO1-mediated anti-cancer immunity.

3.2 Indoleamine 2,3-dioxygenase 2 inhibitors

Although the exact crystal structure of IDO2 remains incomplete, its high homology with IDO1 has enabled development of IDO2 inhibitors, including several diaryl

hydroxylamines and 1,2,3-triazoles. For example, diaryl hydroxylamine compounds with a 3,4-dichloro aryl ring substitution exerted potent pan (IDO1/IDO2/TDO) inhibitory function whereas a 2-chloro aryl ring substitution inhibited IDO1 and IDO2 activity with respective IC_{50} values of 18 and 25µM (137). In cellular assays, 4-Aryl-1,2,3-triazole derivatives were also found to inhibit IDO2 function, with the best compound having an IC_{50} value of 50mM for mouse IDO2, and a twofold higher selectivity over human IDO1. Functionally, the 4-Aryl-1,2,3-triazole compounds were found to occupy both the A and B pockets of the IDO active site, which inhibited IDO2 more strongly than IDO1. Notably, the µM activity of the 4-Aryl-1,2,3-triazole compounds were similar to L-1MT (138). Another screening study identified the proton pump inhibitor, tenatoprazole, to exhibit an IC_{50} value of 1.8µM for IDO2 with no observed inhibition of IDO1 or TDO. Similar findings were found with other proton pump inhibitors. Proton pump inhibitors, such as tenatoprazole, were predicted to have one heteroatom coordinating to the heme iron in the active site of IDO2 (139).

A more recent study by He and colleagues reported *in vitro* and *in vivo* findings of their novel dual IDO1/IDO2 inhibitor, 4t. Structurally, 4t is an 1,2,3-triazole with a hydroxyethyl ether substitution with excellent inhibitory activity (IC_{50} values of 28 nM and 144nM for IDO1 and IDO2, respectively). Functionally, the hydroxyethyl ether chain of 4t formed two hydrogen bonding interactions with Tyr244 and Lys366 of IDO2, contributing to improved activity against IDO2. Pharmacokinetic experiments demonstrated favorable profiles, with adequate membrane permeability, high plasma protein binding, and safety. Moreover, 4t exhibited potent anti-tumor activity in a CT26 colorectal xenograft mouse model (140). Although promising, it remains to be determined whether the above mentioned IDO2 inhibitors will yield satisfactory anti-tumor activity in the clinical setting.

3.3 Tryptophan 2,3-dioxygenase inhibitors

There has been a considerable interest in the development of small molecule inhibitors of TDO2 given recent evidence that TDO2 is upregulated in several cancer types and, like IDO, mediates the catabolism of Trp and promote tumor immune suppression (7). To-date, small molecule inhibitors with high affinity to TDO2 are limited (141, 142).

Recently, W-0019482 was identified to be a potent inhibitor of IDO1, resulting in pronounced reductions in plasma and intratumoral ratios of Kyn-to-Trp and delayed growth of subcutaneous GL261-hIDO1 tumors in mice. Synthetic modification of W-0019482 yielded several analogues with either dual or TDO2-selective profiles. Four analogues, SN36458, SN36896, SN36499, and SN36704, were found to be TDO2 selective, exhibiting IC50 values 5.8 to 8.1-fold lower than that measured against IDO1 (143). The utility of these newer generation TDO2 inhibitors for anti-cancer treatment remains to be determined. Studies using HepG2 liver cancer cells demonstrated that TDO2-inhibitors 680C91 or LM10 significantly reduced Trp degradation and that TDO2, but not IDO1, was the primary source

of Kyn production in these cells (144). 680C91 has also been explored in other cancer types including melanoma (145–147), colon (147), leiomyomas (148), and gliomas (149).

Interestingly, Zhang and colleagues reported that sodium tanshinone IIA sulfonate (STS), a sulfonate derived from tanshinone IIA (TSN), reduced the enzymatic activities of IDO1 and TDO2 with a half inhibitory concentration (IC50) of less than 10 µM using enzymatic assays (150). STS markedly decreased Kyn synthesis in IDO1- or TDO2-overexpressing cell lines and reduced the percentage of Forkhead Box P3 (FOXP3) T cells. *In vivo*, STS delayed tumor growth and combination treatment of STS with anti-PD-1 yielded superior anti-cancer efficacy compared to either treatment alone (150).

With regards to dual IDO/TDO2 targeting therapies, preclinical studies demonstrated that RG70099, potent dual small molecule inhibitor (IC_{50} <100nM) (151), efficiently reduced Kyn levels in plasma by ~90%, and >95% in tumors and draining lymph nodes. Notably, compared to conventional IDO inhibitors, RG70099 was able to reduce tumor Kyn levels by >90% in a TDO+ tumor xenograft, reinforcing that RG70099 exerting TDO2 inhibitory activity (151). EPL-1410, a fused heterocycle-based analogue, showed IDO1/TDO2 inhibitor activity in biochemical assays and demonstrated a significant dose dependent pharmacological efficacy in reducing the tumor volume in the syngeneic cancer models of CT-26 colon carcinoma and B16F10 melanoma (152). Recently, a Phase I clinical trial of M4112, an oral small molecule dual inhibitor of IDO/TDO2, in 15 patients with advanced solid tumors reported tolerable toxicity profiles (153). Treatment-emergent adverse events included fatigue, nausea, and vomiting. Dose-limiting toxicities (DLTs) were observed in one patient (grade 3 allergic dermatitis), and one grade 2 QT prolongation that resulted in a dose reduction. Neither the maximum tolerated dose (MTD) nor recommended Phase II dose was achieved (153).

3.4 Kynurenine aminotransferase inhibitors

To date, there exist several selective inhibitors of KAT, however, their applications in the context of anti-cancer treatment has not been readily explored (154–156). Despite this, several cancer types overexpress KAT (5), and, as mentioned in the above sections, KAT-derived KYNA may serve as a ligand for AhR signaling, resulting in tumor immune suppression (101), Evaluation of KAT inhibitors for anti-cancer treatment and potential reversal of immune suppression warrants further exploration.

3.5 Kynureninase inhibitors and pegylated-Kynureninase

Considering recent findings by our group as well as others (22, 23, 78, 79, 81, 83, 157), targeting of KYNU for anti-cancer treatment and reversal of immune suppression warrants investigation. To date, a few kynurenine analogues have been developed that selectively inhibit KYNU enzymatic activity (158). Additional molecules reported to inhibit KYNU include S-phenyl-l-cysteine sulfoxide

(159, 160), oestrone sulphate (161), O-methoxybenzoylalanine (162), and benserazide hydrochloride (163).

However, targeting of KYNU should be met with caution. KYNU mediates the catabolism of Kyn to AA, which is thought to be immunologically inert. Administration of PEGylated-KYNU in immune-competent preclinical models of melanoma, breast, and colon cancer resulted in drastic reductions in tumor KYN levels with concomitant elevations in KYNU-derived AA, resulting in increases in tumor-infiltrating CD8+ T cells and subsequent tumor reduction (8). Moreover, anti-cancer efficacy was further enhanced when combining PEG-KYNase with ICIs (8). Overexpression (OE) of KYNU in modified Chimeric Antigen Receptor (CAR)-T cells yields superior anti-cancer efficacy in a NALM6 acute lymphoblastic leukemia mouse model (164). Compared to KYNU-knockout (KO) CAR T cells, KYNU-OE CAR T cells had higher glucose uptake, increased proliferation in carboxifluorescein diacetate succinimidyl ester (CSFE) assays, and exhibited a higher percentage of effector memory cell and effector CAR T-cells. In cell killing assays, KYNU-OE CAR T cells had higher lytic granules of granzyme B and enhanced cytokine production of IFNγ, compared to KYNU-KO CAR T cells. Importantly, KYNU-OE CAR T cells retained anti-cancer efficacy in the presence of high KYN levels (164). In another study, an organic polymer nanoenzyme (SPNK) conjugated with kynureninase (KYNase) via PEGylated singlet oxygen (1O2) cleavable linker with near-infrared (NIR) photoactivatable immunotherapeutic effects was developed for photodynamic immunometabolic therapy (165). Upon NIR photoirradiation, SPNK generates 1O2 to induce the immunogenic cell death of cancer and released KYNase to degrade KYN in the TME. *In vivo*, SPNK treatment elicited effector T cell tumor infiltration and expansion, enhanced systemic antitumor T cell activity, and prolonged survival of tumor-bearing mice (165).

The potential of KYNU as a direct target of therapy or as the therapy itself (i.e. pegylated-KYNU) remains an active area of exploration.

3.6 Kynurenine 3-Monooxygenase inhibitors

To date, several inhibitors of KMO have been developed, the majority of which share a common pharmacophore containing both an acidic moiety and a mono or 1,2-dichloro substitution of the core phenyl ring (84, 166–169). More recent inhibitors, GSK065 and GSK366, trap the catalytic flavin in a previously unobserved tilting conformation, resulting in picomolar affinities, increased residence times, and an absence of the peroxide production observed with prior inhibitors (166).

Evaluation of KMO inhibitors for anti-cancer treatment remains limited; however, studies by Ray and colleagues demonstrated that Ro 61-8048, a potent, selective inhibitor of KMO (170), activates pDCs and enhances pDC-triggered T cell proliferation and enhanced anti-cancer activity (26). Specifically, in co-culture models of patient pDCs, T cells, or NK cells with autologous multiple myeloma (MM) cells, blockade of KMO via

Ro 61-8048 resulted in a robust MM-specific CD8+ CTL activity as well as significantly increased NK cell cytolytic activity (26). Treatment of CRC cell lines with Ro 61-8048 or UPF 648, another KMO inhibitor, resulted in reduced viability, decreased sphere formation and attenuated cell migration and invasion (86).

The therapeutic potential of KMO inhibitors remains to be determined; however, a prior clinical trial of the KMO inhibitor GSK3335065 was conducted among healthy volunteers. While GSK3335065 rapidly increases kynurenine levels suggesting partial inhibition of KMO activity, the trial was terminated after a serious adverse event (SAE) occurred on the study and a relationship with the study drug could not be excluded (171).

3.7 3-hydroxyanthranilic acid 3,4-dioxygenase and Quinolinic acid phosphoribosyl transferase inhibitors

Presently, there are very few inhibitors available that selectively target HAAO. Existing HAAO inhibitors include NCR-631 (AstraZeneca, Sweden) (172) as well as 4,5-, 4,6-disubstituted and 4,5,6-trisubstituted 3-hydroxyanthranilic acid derivatives, which have been shown to reduce the production of the excitotoxin QA (173, 174). To the best of our knowledge, none of these inhibitors have been evaluated to anti-cancer treatment.

Regarding QPRT, existing small molecule inhibitors include phthalic acid (PA) as well as the P2Y11 antagonist (NF340) (105, 175, 176). To this end, phthalic acid and NF340 were able to attenuate QPRT-enhanced invasiveness pCMV6-QPRT-transfected MDA-MB-231 TNBC cell lines (105). Given that NAD is essential for metabolic and immune homeostasis, QPRT inhibitors for cancer therapy warrants investigation, although should be met with some caution due to potential off target toxicities (177–179).

4 Perspectives

Over the past few decades, there has been considerable advancement in our understanding of the cancer cell intrinsic and complex extrinsic functions of the KP in tumor development and progression (Figure 3). This progress has led to the development of several small molecule inhibitors that exploit multiple facets of the KP to achieve anti-tumor effects and the reversal of tumor-derived immune suppression (Figure 3; Table 1).

Despite these advances, clinical benefit resulting from targeting the KP pathway has been less than anticipated. Limited clinical success is likely to be multifactorial. For instance, advanced stage tumors have already undergone immunoediting to enable immune escape. Benefit of IDO inhibitors, or other KP inhibitors, may be most efficacious during early stages of disease when the immune system iteratively selects and/or promotes the generation of tumor cell variants with increasing capacities to survive immune attack, also referred to as the equilibrium phase (180).

Lack of clinical benefit may also be attributed to patient selection. Tumors that co-express IDO2 and TDO2 may be less responsive to selective IDO1 inhibitors. Consequently, molecular
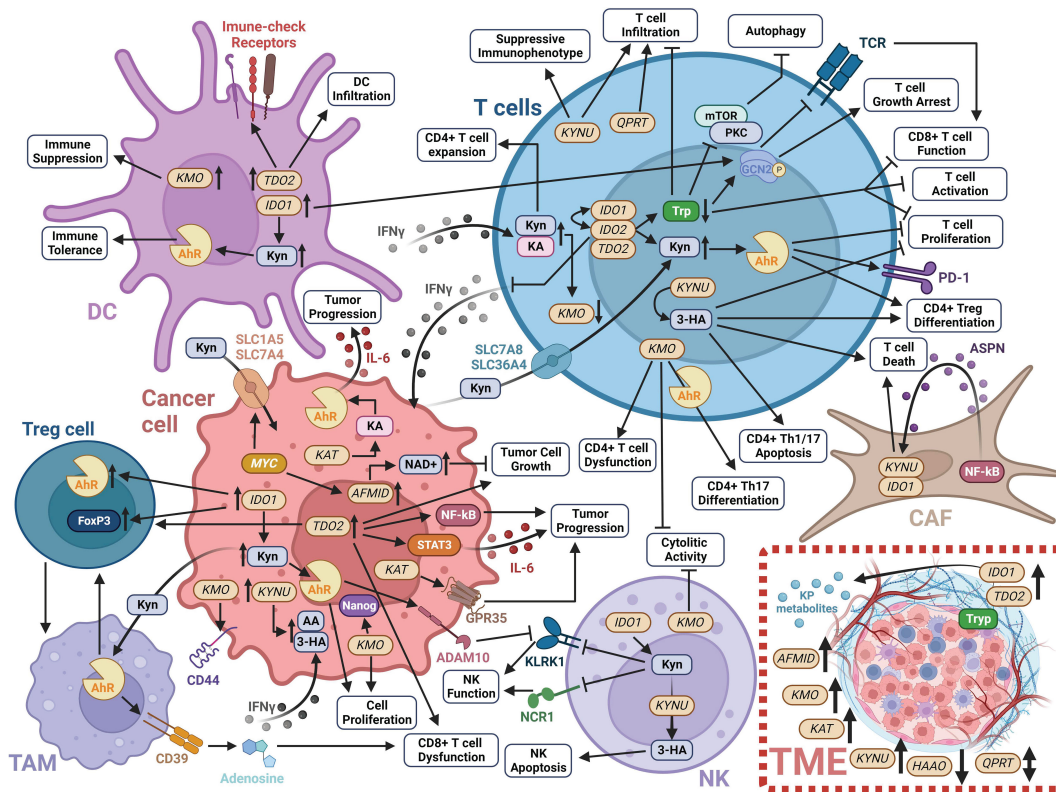
FIGURE 3
Schematic of the impact of KP enzymes and derived metabolites on modulating the tumor immune microenvironment. Detailed information is provided in Section 1 of this review. Created with BioRender.com. 3-HA, 3-hydroxyanthranilic acid; AA, anthranilic acid; ADAM10, ADAM metallopeptidase domain 10; Ahr, Aryl hydrocarbon receptor; AFMID, arylformamidase; ASPN, asporin; CAF, cancer-associated fibroblast; DC, dendritic cell; FoxP3, forkhead box P3; GCN2, general control non-derepressible 2; GPR35, G-protein-coupled receptor 35; HAAO, 3-hydroxyanthranilate 3;4-dioxygenase; IDO1, indoleamine 2;3-dioxygenase 1; IDO2, indoleamine 2;3-dioxygenase 2; IFNγ, interferon-gamma; IL-6, interleukin 6; KA, kynurenic acid; KAT, kynurenine aminotransferase; KLRK1, killer cell lectin like receptor K1; KMO, kynurenine 3-monooxygenase; KP, kynurenine pathway; KYNU, kynureninase; mTOR, mammalian target of rapamycin; NAD+, nicotinamide adenine dinucleotide; NCR1, natural cytotoxicity triggering receptor 1; NF-κB, nuclear factor kappa beta; NK, natural killer cell; PD-1, programmed cell death protein 1; PKC, protein kinase C; SLC1A5, solute carrier family 1 member 5; SLC36A4, solute carrier family 36 member 4; SLC7A5, solute carrier family 7 member 5; SLC7A8, solute carrier family 7 member 8; STAT3, signal transducer and activator of transcription 3; TAM, tumor-associated macrophages; TDO2, tryptophan 2;3-dioxygenase; TME, tumor microenvironment; Tryp, tryptophan; QPTR, quinolinic acid phosphoribosyl transferase.

profiling of tumors with specific evaluation of IDO1, IDO2, and TDO2 expression may better select for patients that would benefit from selective IDO1 inhibitors or from broader pan-IDO/TDO2 inhibitors (Table 1). Recent findings implicating a nonenzymatic role of IDO1 in signaling and immune suppression (44) necessitates that existing or emerging inhibitors should also be evaluated for their effects on catalytic activity in addition to signaling functions to maximize potential for clinical efficacy.

It is also important to consider additional factors that may influence KP activity such as diet (67) and environmental contributors (181, 182). Liang and colleagues demonstrated that nicotine-derived nitrosaminoketone (NNK) upregulates IDO1 via α7 nicotinic acetylcholine receptor (α7nAChR)-mediated activation of the factor c-Jun in lung tumors. In A/J mice, NNK reduced CD8+ T cells and increased Tregs. Moreover, individuals with non-small cell lung cancer who smoked had higher tumoral IDO1 levels and lower Trp/Kyn ratios compared patients who never smoked, further suggesting that smoking impacts KP activity (181).

Exposure to other environmental toxicants can also activate AhR signaling (182), which may upregulate IDO expression resulting in immune suppression (48).

The relevance of other downstream KP enzymes also warrants considerations. This is exemplified by our own findings that tumoral KYNU, but not IDO1, is selectively and frequently upregulated in NRF2 activated tumors and that elevated tumoral KYNU is associated with high immune cell infiltration across several cancer types (22, 23). One may infer that elevated tumoral KYNU promotes tumor immune cell infiltration by reducing levels of Kyn and increasing levels of immune inert anthranilic acid in the TME, as evidenced by studies using PEGylated-KYNU (8). Yet, elevated tumoral KYNU coincides with NRF2 activation, which can promote immune suppression by upregulating PD-L1 (183, 184). Crosstalk between cancer cells and TAMs in the TME can also activate NRF2 in TAMs to reshape the tumor immune microenvironment via multiple mechanisms including suppression of pro-inflammatory cytokines, increasing expression

of PD-L1, macrophage colony-stimulating factor (M-CSF) and KYNU, and accelerating catabolism of cytotoxic-labile heme (185). Upregulation of tumoral KYNU, coupled with NRF2 activation may thus synergize to yield a TME that is highly immune suppressed. Whether therapeutic targeting of KYNU alone or in combination with ICI in NRF2-activated KYNU-high tumors reverses tumor immune suppression remains an area of ongoing inquiry.

In summary, the KP remains an active area of investigation, and insights gained from this study are expected reveal additional promising points actionable metabolic vulnerability within the pathway that can be targeted for anti-cancer treatment.

Author contributions

RL-L: Visualization, Writing – original draft, Conceptualization. RD: Conceptualization, Writing – original draft. JV: Writing – review & editing. AS: Writing – review & editing. EO: Writing – review & editing. SH: Writing – review & editing. JF: Conceptualization, Investigation, Resources, Supervision, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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