Tryptophan metabolism as a common therapeutic target in cancer, neurodegeneration and beyond

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Abstract | L-Tryptophan (Trp) metabolism through the kynurenine pathway (KP) is involved in the regulation of immunity, neuronal function and intestinal homeostasis. Imbalances in Trp metabolism in disorders ranging from cancer to neurodegenerative disease have stimulated interest in therapeutically targeting the KP, particularly the main rate-limiting enzymes indoleamine-2,3-dioxygenase 1 (IDO1), IDO2 and tryptophan-2,3-dioxygenase (TDO) as well as kynurenine monooxygenase (KMO). However, although small-molecule IDO1 inhibitors showed promise in early-stage cancer immunotherapy clinical trials, a phase III trial was negative. This Review summarizes the physiological and pathophysiological roles of Trp metabolism, highlighting the vast opportunities and challenges for drug development in multiple diseases.

L-Tryptophan (Trp) is an essential amino acid that is obtained exclusively from dietary intake in humans. Trp and its metabolites have key roles in diverse physiological processes, ranging from cell growth and maintenance, in which Trp serves as a building block of proteins, to the coordination of organismal responses to environmental and dietary cues, in which Trp metabolites serve as neurotransmitters and signalling molecules[1]. Together, these functions suggest that, during evolution, Trp metabolism has become part of the cellular and organismal communication strategies that align food availability with physiology and behaviour.

The levels of free Trp in the body are determined by food intake and by the activities of several Trp metabolizing pathways. Although a small fraction of free Trp is used for protein synthesis and the production of neurotransmitters such as serotonin and neuromodulators such as tryptamine[2], over 95% of free Trp is a substrate for the kynurenine (Kyn) pathway (KP) of Trp degradation[3–5], which generates several metabolites with distinct biological activities in the immune response and neurotransmission (FIG. 1). The rate-limiting step in the Kyn pathway is the enzymatic conversion of Trp to *N*-formylkynurenine (NFK) by indoleamine-2,3-dioxygenase 1 (IDO1), IDO2 and tryptophan-2,3-dioxygenase (TDO), and depletion of Trp by these enzymes can have fundamental consequences on cellular function and survival[1]. In turn, the activities of these enzymes result in the accumulation of KP metabolites, chiefly Kyn. Kyn may be converted to anthranilic acid (AA) by kynureninase (KYNU) and kynurenic acid (KA) by kynurenine aminotransferases (KATI–KATIII), the latter step being important for controlling the production of neuroprotective KA[2]. Particularly in the brain, Kyn may be transaminated into KA by the mitochondrial aspartate aminotransferase (encoded by *GOT2*)[6]. Independently, kynurenine monooxygenase (KMO) controls the conversion of Kyn to neuroactive and neurotoxic KP metabolites, including quinolinic acid (QA)[2]. QA may be converted to NAD^+ — a key coenzyme in energy metabolism — by certain cell types, but the physiological importance of this de novo production of NAD^+ by the KP is unclear as NAD^+ is produced primarily by salvage (see below). The diverse functions of Trp metabolites in neurophysiology and immunology have been studied extensively and have been well covered in recent reviews[1,2,7].

In humans, KP enzymes and metabolites are localized in different cells and tissues, where their expression is tightly regulated (BOX 1). However, imbalances in the level of Trp and its metabolites have been associated with a wide range of human pathologies, including depression, schizophrenia, autoimmunity, neurodegeneration and cancer[2]. In cancer, aberrant activation of IDO1 and TDO results in suppression of antitumour immunity, whereas in autoimmunity, activation of IDO1 and TDO or the provision of natural or synthetic KP metabolites has therapeutic effects[8]. As many KP metabolites are neuroactive, dysfunction of KP enzymes, often caused by inflammatory insults, can trigger or facilitate diseases of the central nervous system (CNS)[2]. The recent development of antibodies that monitor KP metabolites using immunohistochemistry has enabled the accumulation of KP

Kynurenine (Kyn) pathway (KP). The major pathway in the metabolism of the essential amino acid tryptophan, which contains many immunoactive and neuroactive intermediate metabolites.

Indoleamine-2,3-dioxygenase 1 (IDO1). The first enzyme discovered to initiate immunosuppressive kynurenine pathway metabolism. IDO1 and tryptophan-2,3-dioxygenase (TDO) represent a key intracellular immune checkpoint.

Kynurenine aminotransferases (KATI–KATIII). KATs catalyse the conversion of kynurenine to kynurenic acid (KA) and of 3-hydroxykynurenine to KA and are a drug target for schizophrenia and cognitive impairment disorders.

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Kynurenine monooxygenase
(KMO). A key kynurenine pathway (KP) enzyme and drug target that controls the conversion of kynurenine to neuroactive and immunoregulatory KP metabolites.

Quinolinic acid
(QA). A key kynurenine pathway metabolite accumulating in the brain during inflammation and mediating neuronal death through the activation of *N*-methyl-D-aspartate (NMDA) receptors, a process termed excitotoxicity.

NAD^+
A key coenzyme in metabolic processes and redox reactions regulating cellular fitness, for which the kynurenine pathway is a major source.

Immune checkpoint inhibitors
Antibodies for cancer immunotherapy, which act by neutralizing inhibitory pathways in T cells, thereby enhancing antitumor immunity.

General control non-derepressible 2
(GCN2). A kinase leading to dysfunction of T cells and antigen-presenting cells in response to extreme L-tryptophan shortage (<1 μM).

Aryl hydrocarbon receptor
(AHR). A cytosolic transcription factor originally described as a mediator of xenobiotic detoxification but increasingly shown to mediate important functions of tryptophan metabolism by binding kynurenine and kynurenic acid.

metabolites in tissue to be determined. These tools have demonstrated that Kyn accumulates in IDO1-positive cancers[9] and the excitotoxic metabolite QA accumulates in brain tumours[10,11] and in neurons in neurodegenerative diseases, whereas xanthurenic acid (XA), a modulator of glutamatergic synaptic transmission, localizes to the soma and dendrites of neurons in the healthy brain[12].

The linkage of Trp metabolites to a range of diseases has led to substantial efforts to modulate the KP therapeutically, particularly through inhibition of the key enzymes involved, including IDO1, TDO and KMO (FIG. 1). For CNS disorders, there is growing interest in rectifying the altered rheostat of KP metabolites by targeting specific KP enzymes to achieve a net neuroprotective effect, as well as in the role of Trp and its metabolites in mediating interactions between the gut microbiome and the brain (the 'gut–brain axis')[13]. The influence of the gut microbiome on the absorption and metabolism of dietary Trp is also attracting increasing attention and has potential relevance for CNS disorders as well as irritable bowel syndrome, pancreatitis and diabetes[1]. Finally, in the oncology field, IDO1 inhibitors have been intensively investigated for cancer immunotherapy in recent years, with multiple compounds in clinical trials, typically in combination with other drugs such as immune checkpoint inhibitors[14]. On the basis of promising initial studies, it had been widely anticipated that the leading IDO1 inhibitors would now be approaching regulatory approval, but recent phase III trial terminations have raised questions over the viability of this approach and highlighted the need for greater understanding of the KP.

This Review summarizes current knowledge on the role of the KP in physiology and ageing, including orchestrating the crosstalk between different organs (FIG. 2). The roles of Trp metabolism in a wide range of diseases, including CNS disorders, infectious diseases, autoimmune diseases and cancer, are discussed, along with the associated progress in therapeutically targeting Trp metabolism in each area. Key issues in the clinical translation of drugs that target Trp metabolism, including the use of appropriate diagnostic tools, are highlighted.

Physiological roles of Trp metabolites

Trp metabolites in neuronal function

Trp metabolites of the KP exhibit distinct neuroactive properties (FIG. 3). Whereas QA stimulates excitatory *N*-methyl-D-aspartate (NMDA) receptors, KA acts as an antagonist of all three ionotropic glutamate receptors and is suggested to be an endogenous negative allosteric modulator of α7-nicotinic receptors (α7nAChRs)[15]. Although regional and even cell-type-specific differences in receptor expression and distribution of KP metabolites may explain specific vulnerabilities of neurons to the effects of KP metabolites, it appears that the rheostat of QA to KA is an important determinant of neuronal activity in general and excitotoxicity in particular and that these functions are mediated through competing functions at the NMDA and ionotropic glutamate receptors[2]. Furthermore, cinnabarinic acid (CA) and XA have recently been demonstrated to interact with metabotropic glutamate (mGlu) receptors. Through orthosteric agonism of mGlu4 receptors, CA has been shown to protect against excitotoxic neuronal cell death, whereas XA exerts antipsychotic-like effects in experimental mouse models by activating mGlu2 and mGlu3 receptors[16]. Several lines of evidence suggest that alterations in the balance between these neuroactive KP metabolites may play a role in neurodegenerative and neuropsychiatric diseases on the basis of their neuroactive properties. For instance, QA accumulates in Alzheimer disease (AD) plaques and induces neurodegeneration via NMDA-mediated excitotoxicity, whereas the neuroinhibitory KP metabolite KA is implicated in cognitive dysfunction in schizophrenia[17].

Immunological effects of Trp metabolism

Since the discovery of its immunosuppressive effects[18], a growing body of evidence supports a key role of IDO1 in immune regulation[19]. Activation of TDO, which catalyses the same reaction as IDO1, similarly affects the immune response by inhibiting T cell proliferation, restricting tumour immune infiltration and restraining antitumour immune responses[10,20]. Although the IDO1-related enzyme IDO2 may support IDO1-mediated immune tolerance, the physiological functions of the IDO2 enzyme and its roles in disease conditions involving KP activity are still unclear[14]. Trp degradation is thought to suppress immune cells through the formation of immunosuppressive Trp catabolites[21–24] and by Trp depletion[25,26]. Extreme Trp shortage (<1 μM) leads to accumulation of uncharged tRNAs, which activate the general control non-derepressible 2 (GCN2) kinase pathway, leading to dysfunction of T cells and antigen-presenting cells (APCs)[27]. However, even in situations of forced dioxygenase expression, Trp levels in the local microenvironment do not fall to a level sufficient to activate GCN2[28]. Hence, the immunoregulatory properties of Trp metabolism are mainly a consequence of KP metabolites rather than depletion of Trp[29].

Multiple studies have shown that IDO1 suppresses T cell responses by promoting the activation or differentiation of regulatory T (T_{reg}) cells[30–36]. Kyn was shown to induce T_{reg} cells through activation of the aryl hydrocarbon receptor (AHR)[37], a ligand-activated transcription factor that exerts potent effects on immune cells[38] and is involved in the differentiation of T_{reg} cells[39,40]. Several Trp metabolites produced downstream of IDO1 or TDO, such as Kyn[10,37], KA[41], XA[42] and CA[43], activate the AHR and may contribute to the immune modulation

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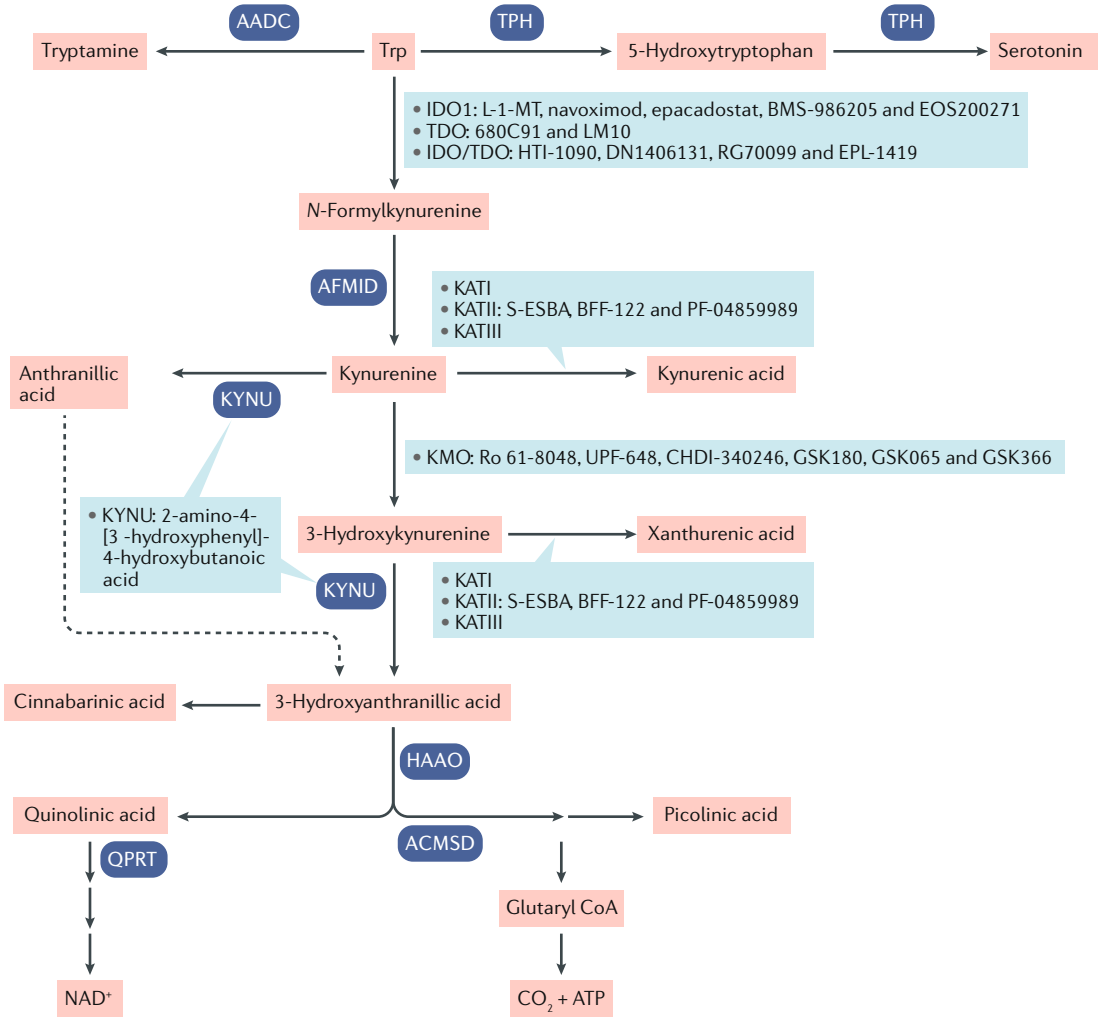


Fig. 1 | **Tryptophan catabolism, key therapeutic targets and drugs in development.** A small fraction of free L-tryptophan (Trp) is used for protein synthesis and the production of neurotransmitters such as serotonin and neuromodulators such as tryptamine. However, over 95% of free Trp is a substrate for the kynurenine (Kyn) pathway (KP) of Trp degradation, which generates several metabolites. Drugs in development and their therapeutic targets within the KP are shown in blue boxes. L-1-MT, 1-methyl-L-Trp; AADC, aromatic-L-amino acid decarboxylase; ACMSD, α-amino-β-carboxymuconate-ε-semialdehyde decarboxylase; AFMID, kynurenine formamidase; HAAO, 3-hydroxyanthranilate 3,4-dioxygenase; IDO, indoleamine-2,3-dioxygenase; KATs, kynurenine amino transferases I–III; KMO, kynurenine 3-monooxygenase; KYNU, kynureninase; QPRT, quinolinic acid phosphoribosyl transferase; TDO, tryptophan-2,3-dioxygenase; TPH, tryptophan hydroxylase.

observed through IDO1 or TDO. Interestingly, whereas Kyn directly binds and activates the AHR, Kyn condensation products do so with high affinity at low picomolar levels[44]. It remains to be investigated if similar mechanisms also apply to other polar metabolites that activate the AHR such as KA. The AHR is also a key factor inducing a regulatory phenotype in DCs[45]. Here, Kyn serves both as an efferent and afferent mediator in the interplay of T cell tolerization[46,47].

Trp metabolites at environmental interfaces

In addition to serving as a nutrient enhancer, Trp plays crucial roles in the balance between intestinal immune tolerance and gut microbiota maintenance. Trp is taken up in the small intestine, but the fraction that reaches the colon can be catabolized by the gut bacteria, resulting in a variety of indole derivatives, which play important roles in key aspects of bacterial physiology such as ecological balance[48] (FIG. 2).

From a 'metabolism-centric' point of view, Trp metabolites may serve as functional complementation to the metabolic capacities of the host and as signalling molecules for fine-tuning host immune responses[34,49]. Other metabolites may serve as signalling molecules for interbacterial communication and quorum sensing, which are particularly important in fighting infections[50]. At the same time, host Trp metabolites may function to shape microbial communities and condition persistence of specific pathogens[51]. Because a large number of metabolites, including those originating from Trp, are shared by distinct taxa, immune sensing of Trp metabolites, or seemly modest alterations of this interconnected metabolic system, can have a substantial effect on the ultimate outcome of an infection[52]. Moreover, recent reports have

shown that in addition to endogenous host-derived Trp metabolites, AHR can bind metabolites derived from bacterial catabolism of Trp, including indole, indole propionic acid, indole acetic acid and tryptamine, that are able to regulate inflammation and disease development both locally and distally in the CNS[53,54].

Trp metabolism as a source of NAD$^+$

As mentioned above, Trp metabolism along the KP is a source for the de novo synthesis of NAD (FIG. 1). As most NAD^+ is produced by salvage, the relevance of NAD^+ generated from the KP in health and disease is not well established[55]. NAD^+ was originally discovered as a coenzyme in metabolic processes and redox reactions, but in recent years a growing number of NAD^+-dependent signalling pathways that involve the consumption of NAD^+ have been identified. Poly-ADP-ribosyltransferases (PARPs) use NAD^+ as the only endogenous substrate for poly-ADP-ribosylation to facilitate the removal of oxidative DNA damage, whereas the NAD^+-dependent deacetylase activity of sirtuins represents a major mechanism of transcriptional regulation[56].

NAD^+ production by the KP is important for embryonic development as loss-of-function variants in 3-hydroxyanthranilic acid (3-HAA) 3,4-dioxygenase (*HAAO*) and *KYNU* in mice and humans results in congenital organ malformations, which were prevented in mice by treatment with nicotinic acid[57]. Although the role of Trp metabolism for NAD^+ synthesis in the liver is well established[58], the contribution of Trp metabolism to NAD^+ formation in other tissues is less clear, but several studies have identified involvement of the KP in NAD^+ formation in brain-resident cells[59–61].

Trp metabolism in ageing

Several food-sensing signalling pathways, which include the insulin/insulin-like growth factor (IIS) pathway and the mammalian target of rapamycin (mTOR) pathway, have been shown to regulate the lifespan of model organisms, and a similar association has been proposed for the KP[62,63]. In humans, the Kyn:Trp ratio, indicative of activity of the pathway, increases with age[64,65]. This increase has been associated with frailty in people over 65 years of age and predicts mortality in people in their nineties[64–66]. Furthermore, a meta-analysis of age-related gene expression changes in the peripheral blood of adult individuals identified the enzyme KYNU as one of the most differentially expressed genes[67]. In follow-up studies in *Caenorhabditis elegans*, knockdown of KYNU by RNA interference (RNAi) prolonged lifespan to a greater extent than that achieved with knockdown of any of the other differentially expressed genes, suggesting an important contribution of KYNU to ageing[67]. Together with independent findings that genetic reduction in the activity of TDO in *C. elegans* and *Drosophila melanogaster*, resulting in a strong increase in the Trp:Kyn ratio, extends lifespan, these studies suggest a causal relationship between the activity of the KP and ageing[63,68,69].

The mechanism by which the KP regulates ageing is not yet known. A role for amino acids, including Trp, in regulating lifespan has been described for different invertebrate and vertebrate animal models, including rats[70–73]. In most of these cases, it is a reduction in Trp availability or a blockade of cellular uptake (for example, as caused by the drug ibuprofen) that prolongs lifespan[70–73]. However, such a mechanism would be counterintuitive to the finding that TDO inhibition, which increases Trp, extends lifespan[69], unless this is associated with a reduced cellular uptake. Moreover, feeding flies with Kyn shortens lifespan, suggesting that metabolite levels downstream in the pathway may also be involved in the regulation of lifespan. The effect of TDO depletion in *C. elegans* to extend lifespan depends on the FOXO transcription factor DAF-16, a mediator

Box 1 | Tissue-specific expression and regulation of kynurenine pathway enzymes

The tissue-specific expression of enzymes in the kynurenine (Kyn) pathway (KP) is best studied for tryptophan-2,3-dioxygenase (TDO) and indoleamine-2,3-dioxygenase 1 (IDO1). In humans, TDO and IDO1 are localized in different cells and tissues and are used in different physiological processes.

TDO. The TDO enzyme is expressed in liver, bone marrow, the immune system, muscle, gastrointestinal tract, kidney and urinary bladder and brain[258], and expression levels are regulated by systemic levels of L-tryptophan (Trp) and corticosteroids[259], which change in response to stress or food; for example, in mice, starvation results in a dramatic decrease in TDO expression[260]. In addition, TDO is induced in glioma cells and in neurons by prostaglandins[103,261]. TDO activity balances the total amount of Trp in the body and is thought to regulate a behavioural response to food availability by initiating the production of bioactive KP metabolites. Such behavioural regulation by the KP has been demonstrated in *Caenorhabditis elegans*, in which the availability of food, as measured by Trp, increases the levels of kynurenic acid (KA)[262]. KA then increases the foraging behaviour via evolutionary conserved neuronal *N*-methyl-D-aspartate (NMDA) receptors until the Trp balance is restored[262]. Fluctuations in levels of KP metabolites in the human brain may similarly regulate behavioural responses, which may be impaired under pathological conditions and lead to behavioural alterations.

IDO1. The IDO1 enzyme is expressed in most tissues at low levels, including cells of the central nervous system (CNS) and macrophages, but not in the liver[3]. The expression and activity of IDO1 in the immune compartment is tightly regulated. It is highly expressed in inflamed tissues, with its expression induced (mainly in myeloid cells) by the pro-inflammatory cytokine IFNγ, interleukin-6 (IL-6) and Toll-like receptor (TLR) ligands[263,264]. IDO1 expression can also be induced through interaction with T cells. The immune checkpoint cytotoxic T lymphocyte-associated protein 4 (CTLA4), which is expressed on regulatory T (T_{reg}) cells, stimulates IDO1 expression in dendritic cells (DCs) through outside-in signalling of the co-stimulatory molecules CD80 and CD86 (REF.[265]). This activation is tightly controlled by suppressor of cytokine signalling 3 (SOCS3), which targets IDO1 for proteasomal degradation. Thus, the degradation of IDO1 appears to represent a default programme to ensure full stimulation of an antigen-specific T cell response[197]. IDO1 is thus an integral part of an immunoregulatory network controlling T cell activation. Here, T_{reg} cells play an important role as both inducers and targets of IDO1-mediated Trp metabolism[134].

IDO1 is also constitutively expressed in the placenta and epididymis, where it has been shown to maintain immune privilege by suppressing T cell responses[18]. Particularly in the lung, elevated levels of IDO1 are thought to prevent the growth of Trp-dependent intracellular pathogens by depleting local Trp. Endothelial IDO1 expression in response to pro-inflammatory stimuli is thought to be involved in the regulation of vascular tone[266].

IDO2. The *IDO2* gene is located immediately downstream of the *IDO1* gene and is believed to possess a more ancestral function. IDO2 mRNA expression is more restricted than IDO1; it is constitutively expressed in human liver, brain, thyroid, placenta, endometrium and testis but can be induced in antigen-presenting cells (APCs) and B cells[14]. IDO2 expression is regulated in APCs and B cells by Kyn binding to the aryl hydrocarbon receptor (AHR). This observation suggests that IDO2 may be part of a cellular restricted feedforward loop of Trp metabolism induced by IDO1 (REF.[14]).

Downstream enzymes. The other enzymes in the KP have been mostly studied in the CNS. Kynurenine monooxygenase (KMO) and kynurenine aminotransferases (KATI–KATIII) are expressed in the brain and induced in several pathological conditions, including traumatic injury[267], viral infection[268] and neurodegeneration[269].

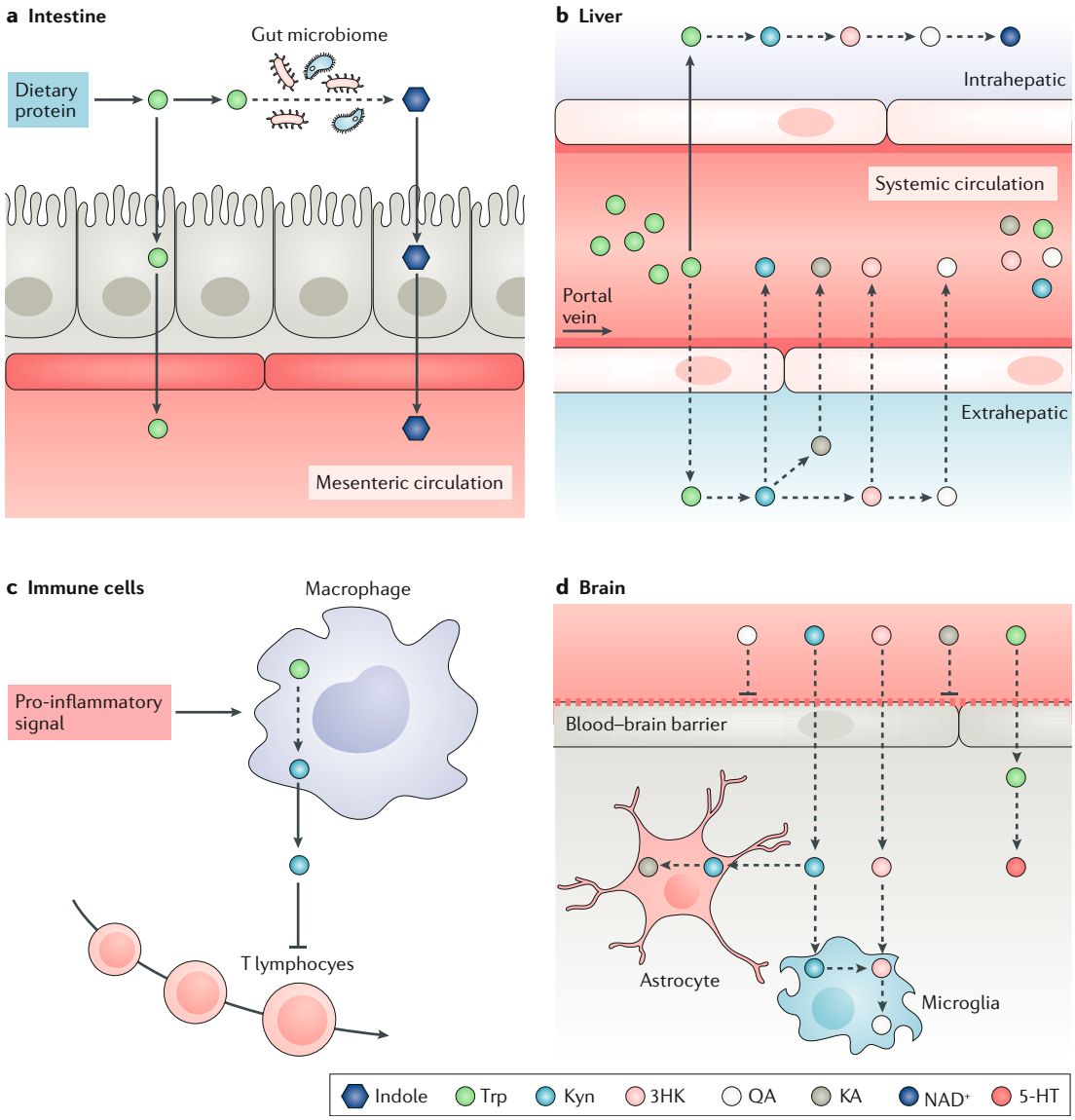


Fig. 2 | **Tryptophan catabolism — key organs involved. a** | Following dietary protein intake, intestinal epithelium cells transport L-tryptophan (Trp) across the apical membrane into the interstitium and mesenteric circulation. Alternatively, intestinal microbiota synthesize and metabolize Trp to indoles and release them into the systemic circulation. **b** | Trp then enters the liver, where most is oxidized to acetoacetyl-CoA and used for the synthesis of NAD^+. Extrahepatic organs that metabolize Trp along the kynurenine (Kyn) pathway (KP), including the kidney, spleen and immune cells, contribute most to circulating levels of Kyn and KP metabolites. **c** | KP metabolites, released by myeloid cells after pro-inflammatory stimulation, suppress T cell responses. **d** | Trp, Kyn and 3-hydroxykynurenine (3HK) are transported across the blood–brain barrier and taken up by astrocytes, microglia and neurons. Astrocytes mainly produce the neuroprotective kynurenic acid (KA) whereas microglia produce neurotoxic KP metabolites such as quinolinic acid (QA). 5-HT, 5-hydroxytryptamine.

of lifespan-regulating pathways such as the IIS pathway that drives the expression of cellular defence pathways, suggesting a role for protection against cellular damage[63]. Interestingly, protection against age-related protein toxicity, which is also induced by depletion of TDO in *C. elegans*, does not depend on DAF-16 and is independent of downstream enzymes in the KP[63]. This observation suggests that the lifespan-extending effect is either a consequence of this protection or is caused by an independent mechanism.

As NAD^+ is emerging as a potential lifespan-extending molecule, alterations in the KP possibly have a lifespan-extending effect via NAD^+ (REFS[74,75]). The longer lifespan in invertebrates, however, is a consequence of reduced KP activity, whereas the prolonged lifespan by external supply of other NAD^+ precursors would argue that an increased KP activity would also be beneficial. More research will be required to understand these seemingly contradictory findings. As knockout mice for IDO1 or TDO are viable[76,77], these models could be valuable in further investigating the lifespan-regulating mechanisms and potential therapeutic targets in the KP. The lifespan-extending effect of KP modulation might arise from a general health benefit rather than a disease-specific effect, similar to that which has been suggested for pathways such as the IIS pathway[62,63].

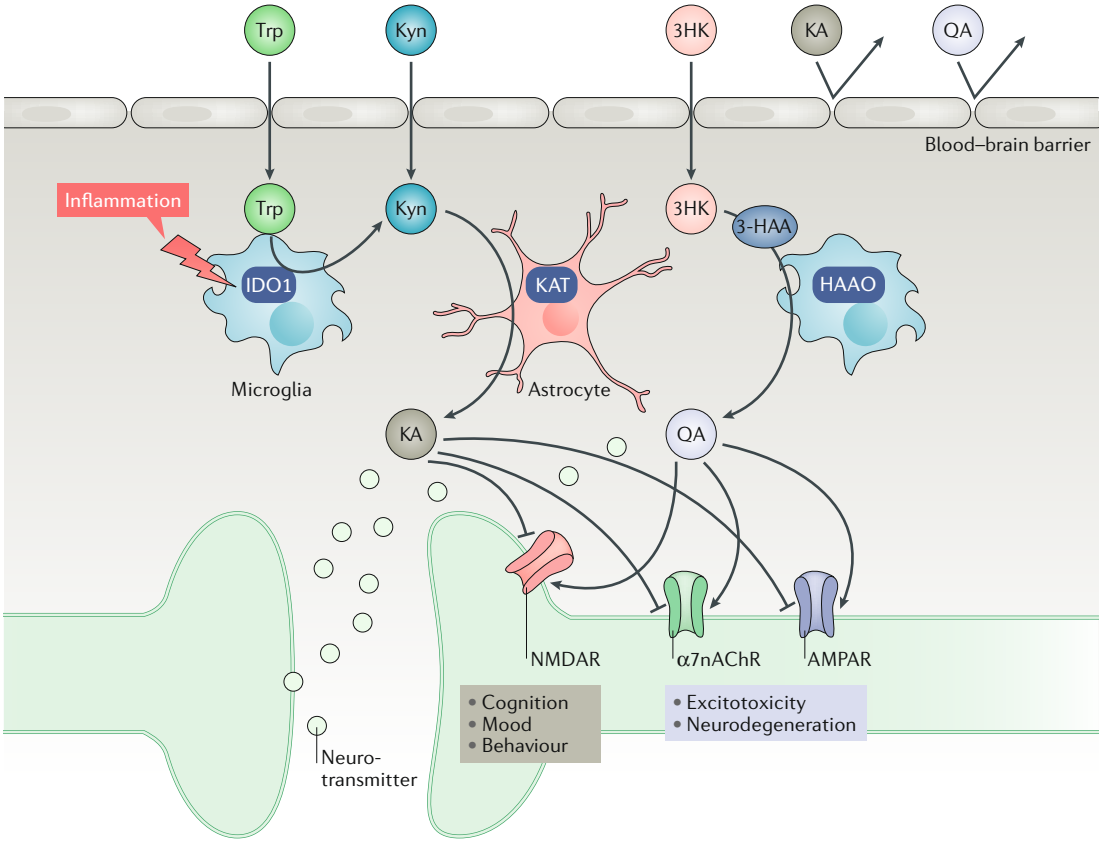


Fig. 3 | **Neuroactivity of tryptophan metabolites.** L-Tryptophan (Trp) is converted to kynurenine (Kyn) by microglial indoleamine-2,3-dioxygenase 1 (IDO1) induced by inflammatory insults. Kyn may be converted to kynurenic acid (KA) by astrocytic kynurenine amino transferase (KAT). KA modulates cognition, mood and behaviour by antagonizing the α7-nicotinic receptor (α7nAChR). In addition, KA is neuroprotective by blocking *N*-methyl-D-aspartate receptors (NMDARs) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs). Kyn is also converted to 3-hydroxykynurenine (3HK), 3-hydroxyanthranilic acid (3-HAA) and quinolinic acid (QA) by microglial 3-HAA 3,4-dioxygenase (HAAO). QA promotes neurodegeneration by inducing excitotoxicity in neurons as an NMDAR and AMPAR agonist.

CNS diseases

Trp metabolism in neurodegenerative diseases

Trp metabolism has been implicated in a variety of neurodegenerative diseases including Huntington disease (HD), AD, amyotrophic lateral sclerosis (ALS) and Parkinson disease (PD). Although the pathophysiological trigger varies, the common denominator of all these diseases is the degeneration of neurons caused by aggregation-prone proteins, resulting in cellular stress and detrimental innate immune reactions. Population-based studies have indicated that with respect to these pathological hallmarks, there is considerable overlap between ageing and neurodegenerative diseases with high intra-individual variability[78]. Although genetic and environmental influences on Trp metabolism are incompletely understood, it is believed that Trp metabolism contributes to both ageing and neurodegeneration and that the mechanisms involved are similar, if not identical. This observation is supported by mouse studies, in which deletion of TDO has been shown to result in enhanced neurogenesis in the hippocampus and subventricular zone[76], possibly counteracting neurodegeneration. Although biomarker studies have shown that Trp metabolism is differentially active in patients with neurodegenerative diseases[79], it is unclear whether this is the result of a primary predisposition or a consequence of neurodegeneration or the collateral innate immune activation. Epidemiological studies suggest that activation of the KP is associated with an increased risk of dementia[80]; however, a clear distinction from physiological ageing is difficult[81]. The sensitivity of the KP to infectious and inflammatory insults clearly compromises its robustness as a marker of neurodegeneration. On the other hand, activation of the KP by inflammation may provide a link between neuroinflammation and neurodegeneration in diseases such as multiple sclerosis[8].

Potential mechanisms of neurodegeneration mediated by Trp metabolism include proteotoxicity through a Trp-dependent mechanism, excitotoxicity through accumulation of neurotoxic Trp metabolites and energy imbalance through depletion of NAD^+ (REFS[1,2,7]). In *D. melanogaster* models of PD and HD, deletion of TDO or KMO results in neuroprotection[82,83].

Trp metabolism in neuropsychiatric diseases

Imbalances in the KP, resulting in an excess of metabolites with specific neuroactive properties, are thought to contribute to diverse neuropsychiatric diseases[15].

Major depressive disorder, for instance, has been causally associated with increased metabolism down the 3-hydroxykynurenine (3HK) branch of the KP, leading to increased brain levels of neurotoxic QA over neuroprotective KA[84]. Similarly, elevated levels of QA in comparison to KA and picolinic acid have also been associated with suicidality[85,86]. Immune activation by psychosocial stress, infections or treatment with cytokines leads to depressive symptoms[87–89]. Depressive-like behaviours are mitigated by IDO1 inhibition or knockout in mice[88], and vulnerability to cytokine-induced depression has been linked to a polymorphism in the IDO1 gene[90]. Systemic IDO1 activation is therefore thought to be involved in the activation of the 3HK branch in depression, but it is currently unclear why KA and QA are not both equally upregulated in response to IDO1 induction.

By contrast, schizophrenia and psychosis appear to arise from increased formation of the NMDA receptor antagonist, KA[15,91]. Elevated levels of KA have been measured in post-mortem brains[92] and the cerebrospinal fluid[93] of patients with schizophrenia. Increased KA levels are associated with cognitive deficits observed in schizophrenia[94,95], whereas reduction in KA formation was related to improved cognitive function[96]. Again, neuroinflammation specifically in the developing brain has been implicated in the cognitive deficits characteristic of schizophrenia[97]. Single-nucleotide polymorphisms in the *KMO* gene are associated with schizophrenia[98,99] and bipolar disorder[100,101], suggesting that the reduced flux down the 3HK branch of the KP may shift Kyn towards KA formation, the accumulation of which has been implicated in these disorders.

However, the rheostat of Trp, Kyn and KP metabolites in the peripheral circulation is highly dynamic and subject to multiple exogenous factors such as infection, diet and drugs, which greatly hampers its reliability as a biomarker, particularly for neuropsychiatric diseases, but also limits the interpretation of epidemiological association studies.

Targeting KP enzymes in CNS disorders

Although clinical trials have focused (and in part still focus) on supplementing or depriving Trp or its metabolites for the treatment of neuropsychiatric disorders, current preclinical efforts in drug development for neurodegenerative and neuropsychiatric diseases have mainly focused on altering the rheostat of neuroactive KP metabolites through inhibition of enzymes involved in the formation of either QA or KA.

Conceptually, all KP enzymes represent potential therapeutic targets, and several studies have investigated the effects of pharmacological inhibition. For instance, the IDO1 inhibitor coptisine has been shown to slow cognitive impairment in a mouse model of AD, although its specificity for IDO1 is unclear[102]. Interestingly, cyclooxygenase inhibition prevents behavioural decline in a similar model of AD by suppressing hippocampal TDO expression[103]. A similar neuroprotective effect was observed when a pharmacological inhibitor of TDO was used[103]. These studies, together with evidence of KP activation in AD and HD patients[79,104], indicate that inhibition of the rate-limiting first enzymatic step in Trp degradation is a potentially viable therapeutic approach to counteract neurotoxicity caused by accumulation of amyloid-forming proteins. Although inhibitors of IDO1 and TDO prevent the production of KP metabolites, this will not directly affect the KA/QA rheostat but block the production of both. Nevertheless, this therapeutic approach is viable as it may prevent the depletion of Trp, which may reduce the proteotoxicity observed in preclinical models[63].

KATs catalyse the conversion of Kyn to KA with the aid of the cofactor pyridoxal-5-phosphate (PLP). KATII is the most prevalent KAT in the mammalian brain and is being pursued as a drug target for schizophrenia and cognitive impairment disorders. As KATII was recently shown to also catalyse the formation of XA from 3HK[105], effects previously attributed to KA on the basis of the inhibition of KATII may also involve XA.

Reversible inhibitors of KATII have been developed (TABLE 1) and include the Kyn analogue (*S*)-4-(ethylsulfonyl)benzoylalanine (*S*-ESBA)[106], which was shown to reduce KA levels in the rat brain but displayed very low activity against human KATII. The fluoroquinolone BFF-122 (REF.[107]), a close analogue of the antibiotic levofloxacin, features a primary amino group that forms a covalent bond to the PLP cofactor as shown by X-ray crystallography. The same mode of inhibition was reported for the highly efficient and selective brain-penetrable irreversible inhibitor PF-04859989 (REF.[108]). However, none of these compounds proceeded to clinical studies, probably owing to the toxicity caused by their irreversible interaction with the PLP cofactor required by the KAT isozymes and all other PLP-dependent enzymes[109]. Major challenges in advancing KATII inhibitors into clinical trials include potential toxicity caused by reductions of brain KA levels, achieving sufficient potency and selectivity and the occurrence of interspecies differences in the potency of KATII inhibitors[110]. For a recent detailed review of KAT inhibitors from a medicinal chemistry perspective, the reader is referred to REF.[109].

With the aim of inhibiting the QA branch of the KP and increasing antagonizing KA levels, KMO inhibitors are under active development[111] (TABLE 1). Information on the crystal structure of KMO has helped generate KMO inhibitors with increased specificity[112,113]. The well-known KMO inhibitor Ro 61-8048 (REF.[114]) has been used in a plethora of preclinical studies demonstrating effects ranging from amelioration of neurodegeneration[115] to reduction in cannabinoid abuse[116]. Another widely used tool compound, UPF-648 (REF.[117]), is a Kyn analogue without an amino group, which is conformationally restricted by a cyclopropyl ring. This compound, as well as the highly efficient oxazolidinone GSK180 (studied in the context of pancreatitis[118]), are so-called type I KMO inhibitors, which mimic Kyn and stimulate the detrimental production of hydrogen peroxide. In a collaborative structure-based medicinal chemistry effort, a new aryl pyrimidine lead compound, CHDI-340246 (REF.[119]), has been developed and evaluated for the treatment of HD. However, chronic treatment with this selective KMO inhibitor did not significantly modify behavioural phenotypes or natural progression in mouse models of HD, although it restored electrophysiological alterations[120].

Table 1 | **Selected small-molecule inhibitors of tryptophan metabolism and modulators of the AHR**

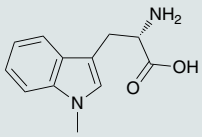
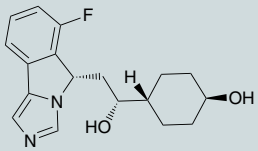
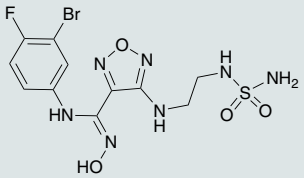
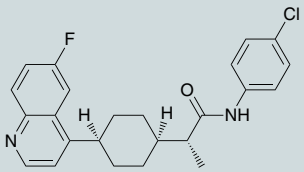
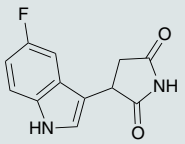
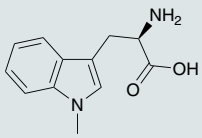
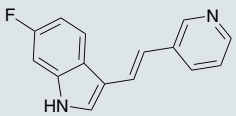
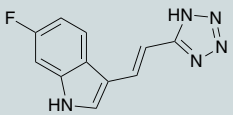
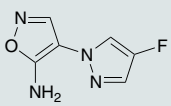
Compound; company	Structure	Comments	Indication/most advanced clinical phase
IDO1 inhibitors			
1-Methyl-L-tryptophan (L-1-MT)		• Trp-competitive inhibitor • Moderate IDO1 inhibition, low specificity • Substrate analogue	Experimental, diverse fields/preclinical
Navoximod/NLG-919; NewLink Genetics		• Based on 4-phenylimidazole scaffold • Forms direct bond to ferric haem iron	Cancer/phase I
Epacadostat/INCB024360; Incyte		• Trp-competitive inhibitor • Forms direct bond to ferrous haem iron	Cancer/phase III
BMS-986205/F001287; Bristol-Myers Squibb (originator: Flexus)		• Irreversible inhibitor • Binds to haem-free apo IDO1	Cancer/phase III
EOS200271/PF-06840003; iTeos Therapeutics		• Noncompetitive kinetics with respect to Trp • Does not form a bond with haem iron • Central nervous system penetration	Cancer/phase I
KHK2455; Kyowa Kirin	No information available	Binds to haem-free apo IDO1	Cancer/phase I
LY3381916; Eli Lilly	No information available	Binds to newly synthesized apo-IDO1 but does not inhibit mature haem-bound IDO1	Cancer/phase I
MK-7162; Merck	No information available	No information available	Cancer/phase I
IDO pathway inhibitors			
Indoximod/NLG8189/1-methyl-D-tryptophan (D-1-MT); NewLink Genetics		Does not inhibit IDO1 in vitro	Cancer/phase II/III
NLG802; NewLink Genetics	No information available	Prodrug of indoximod	Cancer/phase I
TDO inhibitors			
680C91; Glaxo Wellcome		• Nanomolar activity in vitro • Low aqueous solubility • Poor oral bioavailability	Experimental, depression, cancer/preclinical
LM10		• Less potent but better solubility and bioavailability than 680C91 • Investigated in mouse cancer model	Experimental, cancer/preclinical
4-(4-fluoropyrazol-1-yl)-1,2-oxazol-5-amine; Genentech		• Nanomolar cellular activity • Sixfold selectivity over IDO1 • Whole-blood stability • Not proceeding to clinical trials	Experimental, cancer/preclinical
Fused imidazo-indoles; Redx Pharma	No information available	Potent and TDO selective	Experimental/preclinical

Table 1 (cont.) | **Selected small-molecule inhibitors of tryptophan metabolism and modulators of the AHR**

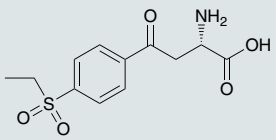
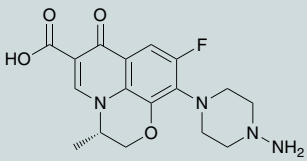
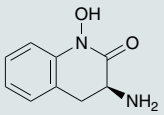
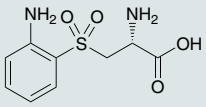
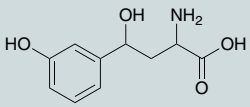
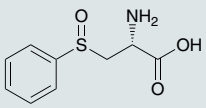
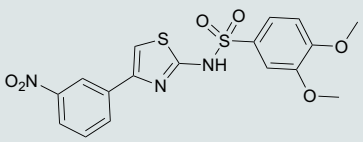
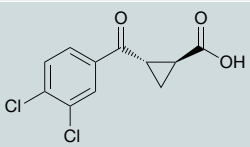
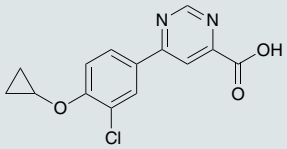
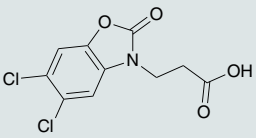
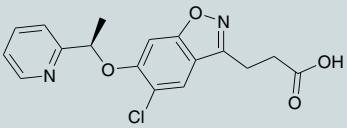
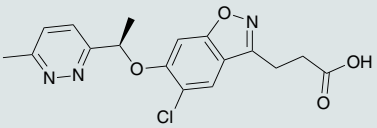
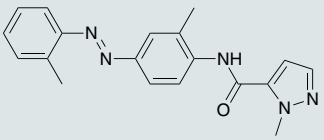
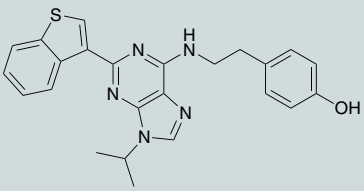
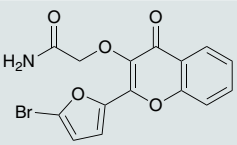
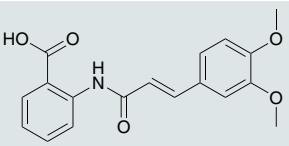
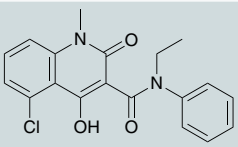
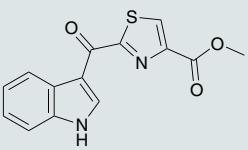
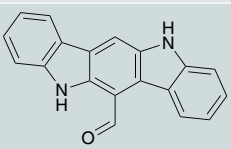
Compound; company	Structure	Comments	Indication/most advanced clinical phase
TDO inhibitors (cont.)			
Indazoles; IOmet Pharma	No information available	Potent and TDO selective	Experimental/preclinical
Dual IDO1–TDO inhibitors			
HTI-1090/SHR9146; Atridia, Hengrui Therapeutics	No information available	Potent, orally bioavailable dual IDO1/TDO inhibitor	Cancer/phase I
DN1406131; Jiangxi Qingfeng Pharmaceutical	No information available	No information available	Cancer/phase I
RG70099; Roche (originator: Curadev)	No information available	Significantly reduces Kyn levels in preclinical tumour models	Cancer/preclinical
EPL-1410; Emcure Pharmaceuticals	No information available	• Good oral bioavailability in rodents • Reduces tumour volume and Kyn:Trp ratio in cancer models	Cancer/preclinical
KATII inhibitors			
(S)-4-(Ethylsulfonyl)benzoylalanine (S-ESBA)		• Reversible inhibitor • Reduces KA levels in rat brain • Low activity on human KATII	Schizophrenia, cognitive impairment disorders/ preclinical
BFF-122		• Analogue of levofloxacin • Forms covalent bond to PLP cofactor of KATII	Schizophrenia, cognitive impairment disorders/ preclinical
PF-04859989; Pfizer		• Highly efficient and selective • Brain penetrable • Forms covalent bond to PLP cofactor of KATII	Schizophrenia, cognitive impairment disorders/ preclinical
KYNU inhibitors			
S-(2-Aminophenyl)-L-cysteine S,S-dioxide		Competitive, covalent inhibitor	Bacterial infection/ preclinical
2-Amino-4-[3′-hydroxyphenyl]-4-hydroxybutanoic acid		• Reversible inhibitor • Selective for mammalian KYNU	Preclinical
S-Phenyl-L-cysteine sulfoxide		• Competitive inhibitor of *Pseudomonas aeruginosa* KYNU • Virulence inhibition in *P. aeruginosa*	Bacterial infection/ preclinical
KMO inhibitors			
Ro 61-8048; Roche		Widely used tool compound	Neurodegeneration, prevention of cannabinoid abuse/ preclinical
UPF-648		Type I inhibitor (non-substrate effector, leads to detrimental H_2O_2 production)	Neurodegeneration/ preclinical
CHDI-340246; CHDI Foundation		Does not significantly modify phenotypes or progression in mouse models of Huntington disease	Huntington disease/ preclinical

Table 1 (cont.) | **Selected small-molecule inhibitors of tryptophan metabolism and modulators of the AHR**

Compound; company	Structure	Comments	Indication/most advanced clinical phase
KMO inhibitors (cont.)			
GSK180; GlaxoSmithKline		Type I inhibitor	Neurodegeneration, pancreatitis/preclinical
GSK065; GlaxoSmithKline		Type II inhibitor (competitive, no H_2O_2 production)	Pancreatitis/phase I
GSK366; GlaxoSmithKline		Type II inhibitor	Pancreatitis/preclinical
AHR antagonists			
CH-223191		• Potent, pure antagonist, does not exhibit agonistic activity • Competitive	TCDD-associated pathology/tool compound
StemRegenin 1 (SR1); Novartis		Expands $CD34^+$ cells from bone marrow of humans, monkeys and dogs but not mice	Haematopoietic stem cell therapy/phase II
CB7993113		Identified by ligand-shape-based virtual screening	Cancer/preclinical
AHR agonists			
Tranilast; Nuon Therapeutics		• Antiallergic • Inducing immune tolerance	Rheumatoid arthritis/ phase II completed 2011
Laquinimod; Teva Pharmaceutical Industries		(Concerto) failed to meet primary end point in relapsing-remitting MS but may be beneficial against neurodegeneration	Relapsing-remitting MS, primary progressive MS, Huntington disease/ phase III
2-(1′H-indole-3,-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE)		• Nontoxic • Immunomodulatory — anticancer functions	Autoimmune neuroinflammation/ preclinical
6-Formylindolo[3,2-b]carbazole (FICZ)		Photoproduct of Trp, natural agonist	Tool compound

AHR, aryl hydrocarbon receptor; IDO, indoleamine 2,3 dioxygenase; KA, kynurenic acid; KATII, kynurenine aminotransferase II; KMO, kynurenine monooxygenase; Kyn, kynurenine; KYNU, kynureninase; MS, multiple sclerosis; PLP, pyridoxal-5-phosphate; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TDO, tryptophan-2,3-dioxygenase; Trp, L-tryptophan.

Structural studies recently deciphered the difference between type I and type II KMO inhibitors[112,121]. The type II KMO inhibitors, GSK065 and GSK366, showed a better drug-like profile than the type I KMO inhibitors owing to their picomolar affinities, increased residence time and absence of peroxide generation[112]. GSK065 has entered a phase I clinical trial for the treatment of pancreatitis under the name GSK3335065 (NCT03245619). Interestingly, peripheral administration of KMO inhibitors is sufficient to affect the CNS KP[120]. Whether penetration of the blood–brain barrier is necessary for a KMO inhibitor to be effective, however, remains a matter of debate[115,122].

Finally, inhibition of the initial rate-limiting KP enzymes IDO1 and TDO, which are induced under inflammatory conditions[123] or chronic psychosocial stress[88], respectively, may also be worth exploring in neurodegenerative and psychiatric diseases. As inhibitors of these enzymes are currently in development for cancer therapy, diverse compounds are available to test these approaches in clinical settings.

Infectious diseases

Trp metabolism in infection

Several lines of evidence have recently revealed a crucial role of Trp metabolism as an important regulator of immune responses in host–pathogen interactions and in shaping host microbiota[51,124–127]. Trp metabolism, by specific Trp metabolic enzymes, is increased at sites of bacterial, viral, fungal and parasitic infections[128]. Normally expressed at low basal levels, an increase in IDO1 is observed in APCs, such as dendritic cells (DCs) and macrophages, in response to several microbial stimuli, including Toll-like receptor (TLR) ligands (for example, lipopolysaccharide (LPS), CpG oligonucleotides and polyinosinic-polycytidylic acid (poly(I:C)))[129–131]. In addition, inflammatory stimuli, such as type I and II interferons[132], tumour necrosis factor (TNF), prostaglandins[133] and membrane-bound molecules[134,135], have been reported to induce IDO1 in specific APC types.

In infectious diseases, IDO1 activity exerts pleiotropic effects, acting as a double-edged sword. Indeed, IDO1 acts to deplete Trp to starve and reprogramme auxotroph invaders[136–139], while at the same time contributing to a Kyn-dependent state of immunosuppression to microorganisms that have not been cleared during acute infection[34,49,140,141] or to those that have been able to reactivate Trp biosynthesis[141]. Accordingly, it has been shown that Trp auxotroph pathogens are hypersusceptible to macrophages activated by $CD4^+$ T cells[141]. Microbial auxotrophy for Trp can be lost in specific environmental conditions. Certain microorganisms can re-acquire the capacity to synthesize this essential amino acid in specific stress conditions[141]. Moreover, microbiota strains that are naturally capable of synthesizing Trp can be expanded during specific infections, providing an additional supply of this essential amino acid in Trp-starved conditions. Recent findings have documented that specific pathogens, such as *Mycobacterium tuberculosis*, can re-acquire the ability to synthesize Trp under stress conditions and thus counteract the antibacterial action driven by IDO1 starvation[141]. In addition, *Chlamydia* has been reported to enter a non-replicative, persistent state in stress conditions caused by local Trp deprivation[142]. Similarly, IDO1-dependent persistence has been documented for other bacterial species, including *Cumuliphoma pneumoniae*[143].

In addition to regulating pathogen load, Trp metabolism through IDO1 activity can also be crucial for restraining immune pathology that would ultimately prevent pathogen eradication[125]. In this regard, recent studies on gut microbiota have found important links between Trp metabolism and the activation of the AHR expressed at mucosal barriers via microbial or bacterial virulence factors acting as specific AHR ligands[51,144,145]. Notably, AHR^+ interleukin-22 (IL-22)-producing group 3 innate lymphoid cells (ILC3s) were induced even in conditions of IDO1 deficiency, owing to the selective expansion of lactobacilli, which produce a Trp metabolite (that is, indole-3-aldehyde) capable of activating AHR and thus induce a state of protective tolerance in models of fungal infection[51].

In contrast to IDO1, less is known about the expression of the IDO1 homologue IDO2 and its functional importance in similar settings. Moreover, the potential role of TDO during infection has also received little attention. TDO expression is increased in the liver of mice challenged with LPS, and TDO-deficient mice are more susceptible to endotoxin challenge[146]. Accordingly, TDO-dependent antimicrobial and immunoregulatory effects have been reported in in vitro studies with *Toxoplasma gondii* and *Staphylococcus aureus* infections[147]. In addition, metabolome analysis revealed changes in TDO activation in patients with primary dengue infection[148].

Accordingly, out of the three different Trp-catabolizing enzymes in host cells, the impact of IDO1 has been addressed in several preclinical models of infection, as reviewed previously[128,149,150]. Specifically, it has been reported that IDO1 suppresses the replication of certain intracellular parasites and bacteria such as *T. gondii*, *Chlamydia* and *Leishmania donovani* in vivo[136–139]. On the other hand, the weak IDO1 inhibitor 1-methyl-L-Trp (L-1-MT) enhanced efficacy of *Chlamydia trachomatis* antibiotic clearance[151], although additional IDO1-independent mechanisms may be involved[129].

IDO1 activity has also been reported to restrain replication of specific viruses, such as human cytomegalovirus (CMV), herpes simplex virus type 2 and vaccinia virus, in vitro[152–155]. However, the situation in vivo may differ in that viral infection may induce IDO1 and the KP to evade host immune responses. Because of their capacity to induce T_{reg} cells[34], the depletion of Trp combined with production of Kyn by IDO1 is an important means to restrain antimicrobial T helper 17 (T_H17) and T_H1-driven inflammation[49,140] (FIG. 4). Therefore, pathogens may hijack the immunosuppressive effects of IDO1 and use them to facilitate their own life cycle. In this regard, uropathogenic *Escherichia coli* (UPEC) induces IDO1 in epithelial cells of the urinary tract[156], and the dampened immune response upon Trp catabolism enables successful colonization by UPEC. Additionally, viruses such as HIV-1 use the immunosuppressive activity of IDO1 to establish HIV chronic infection[157]. Increased activity of the KP has also been associated with progressive liver cirrhosis in patients

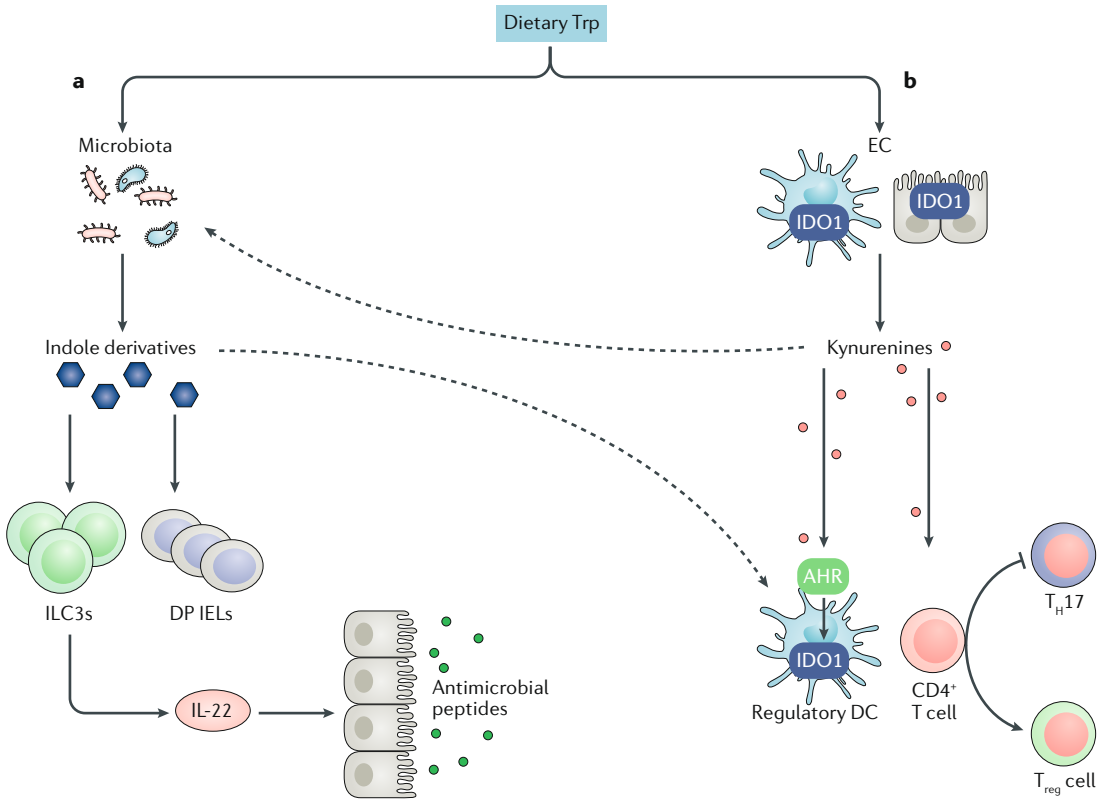


Fig. 4 | **Immunological effects of tryptophan metabolism. a** | The metabolism of L-tryptophan (Trp) is exploited by mammalian host cells and commensals as a source of molecules activating the aryl hydrocarbon receptor (AHR) in different immune cells. Bacterial tryptophanase (ThPA) converts dietary Trp to indoles that are essential for the subsequent production of AHR ligands, which control the generation of $CD4^+CD8\alpha\alpha^+$ double-positive intraepithelial lymphocytes (DP IELs) and group 3 innate lymphoid cells (ILC3s). AHR activation in ILC3s leads to interleukin-22 (IL-22) production, increasing host fitness in response to pathogen infections and immune pathology. **b** | Cytokine release or Toll-like receptor (TLR) activation results in indoleamine-2,3-dioxygenase 1 (IDO1) induction and production of kynurenines in dendritic cells (DCs) and epithelial cells (ECs). AHR engagement by kynurenine leads to the generation of regulatory $IDO1^+$ DCs that promote regulatory T (T_{reg}) cell expansion and suppress T helper 17 (T_H17) responses. Several Trp metabolites are shared by distinct prokaryotic and eukaryotic taxa, and they may serve to accommodate host–microbiota relationships (dotted lines). Graphics were produced with UCSF Chimera[270].

with hepatitis C virus infection[158]. Similarly, influenza A/PR/8/34 (PR8) infection in mice stimulated rapid elevation of IDO1 activity in lungs and lung-draining mediastinal lymph nodes, resulting in increased morbidity, slowed recovery and decreased effector T cell responses in the lungs, although IDO1 induction did not impair virus clearance during primary influenza A infection[159]. In other settings, such as in infection with fungi, IDO1 may be used as an evasion mechanism that establishes commensalism or chronic infection[160].

In this context, the Trp metabolic pathway plays a key role in fostering protective tolerance and is strictly required for the generation of homeostasis with fungi such as *Candida albicans* and *Aspergillus fumigatus*[161]. AHR-activating indole compounds produced from dietary Trp by gut microbiota can regulate the virulence of pathogenic bacteria and thus protect the host by limiting colitis caused by pathogens or chemical stressors[144].

Targeting KP enzymes in infectious diseases

Modulating specific Trp biosynthetic pathways in selected microbial species and targeting the IDO1–AHR–microbiota axis in host cells may represent novel attractive strategies for antibiotic development or for complementing antiviral therapies. However, a more complete understanding of the role of Trp catabolic enzymes or downstream enzymes during specific infections is necessary to inform the utility of therapies aimed at modulating Trp catabolism to eradicate pathogens while maintaining balance with microbiota.

On the basis of the evidence summarized above, it is possible to hypothesize that specific IDO1 blockers may find potential application as adjuvant therapy to improve the efficacy of antiviral drugs but may prove detrimental in fungal infections, where Trp catabolism, largely via IDO1, acts to maintain immune homeostasis and protective tolerance[161]. However, this effect may constitute a potential drawback of using IDO1 inhibitors as antitumour drugs (discussed below). Indeed, in a phase I trial in patients with metastatic solid tumours with the IDO1 pathway modulator 1-methyl-D-Trp (D-1-MT), infections were the most frequent adverse events[162].

Interestingly, a recent study demonstrated that targeted inhibition of KYNU affects *Pseudomonas aeruginosa* gene expression and quorum sensing, suggesting a novel potential anti-virulence strategy[83,163]. Specifically,

S-phenyl-L-cysteine sulfoxide (TABLE 1), having structural similarity to Kyn, inhibited the production of anthranilate, which was critical for *P. aeruginosa* virulence[163].

Autoimmune diseases

Trp metabolism in autoimmunity

Autoimmunity is a consequence of failure to develop central (thymic) tolerance to self and of insufficient maintenance of peripheral tolerance. Trp metabolism in the immune compartment is primarily initiated by IDO1, it representing a target gene of mainly pro-inflammatory stimuli. In this respect, IDO1-mediated degradation of Trp can be viewed as a key feedback mechanism regulating overactive immune responses, a hallmark of autoimmune diseases. The effects of transcriptional activation of IDO1 in inflamed tissues to suppress adaptive immune responses has been expanded from initial observations in the placenta in the maintenance of fetal tolerance[18] to multiple autoimmune diseases[8]. Although IDO1 deficiency does not result in a global autoimmune phenotype associated with deficiency of important checkpoints of T_{reg} cells, it is associated with subtler inflammatory phenotypes[7]. This association may, in part, be due to redundancy in the enzymatic function shared with other dioxygenases[164]. There is increasing evidence that human autoimmune disease is driven by a failure of immune and/or stromal cells to upregulate IDO1 in response to inflammatory stimuli[165–169]. However, potential causes of a constitutive defect in upregulating IDO related to autoimmunity have not been elucidated. Linkage analyses have associated polymorphisms in *IDO1* and *IDO2* genes with severity and risk of Crohn's disease, respectively[170]. By contrast, *IDO2* polymorphisms are not associated with multiple sclerosis[171]. Further studies are required to determine whether constitutive or induced defects in upregulating Trp metabolism in tissues result in tissue-specific autoimmunity.

Many studies in autoimmune disease mouse models of multiple sclerosis, rheumatoid arthritis, lupus and autoimmune diabetes have demonstrated the relevance of Trp metabolism in regulating disease activity. Taken together, these studies indicate that IDO1 is expressed in tissue-resident myeloid cells and limits innate and adaptive immunity to self-antigens and inflammatory pathology[172–178]. Paradoxically, however, in an animal model of spontaneous rheumatoid arthritis, pharmacological inhibition of IDO1 using D/L-1-MT attenuates disease severity, possibly as a result of reduced activation of autoreactive B cells[179]. This finding illustrates the complex immunoregulatory function of IDO1 in autoimmunity, which depends on the cellular compartment (FIG. 4). For instance, the expression of the immunosuppressive cytokine IL-10 in B cells is dependent on IDO1[180], indicating that IDO1 does not simply trigger immunosuppressive mechanisms but orchestrates a complex immunomodulatory response to inflammation. It is important to keep in mind that transcriptional activation and protein expression do not necessarily translate into enzymatic activity as described in human B cells[181]. In this respect, more studies are needed to elucidate the non-enzymatic function of IDO1. In addition, studies drawing key conclusions on IDO1 function using D-1-MT need to be viewed with caution as D-1-MT does not inhibit IDO1 and displays considerable off-target effects resulting in activation of the p38 MAPK pathway[182]. Studies in autoimmune disease models suggest that IDO2, in contrast to IDO1, acts as a promoter of autoimmunity, particularly owing to regulation of humoral immune responses. IDO2-deficient mice display decreased joint inflammation owing to a reduction in pathogenic autoantibodies and antibody-secreting cells[183–185]. Although the relevance for human disease remains unclear[186], these studies highlight the complex and highly compartmentalized regulation of Trp metabolism by dioxygenases.

Although the main effects of IDO1-mediated immune regulation are believed to be driven by activity in the local microenvironment of tissue inflammation[8], systemic activation of Trp metabolism is observed in patients with autoimmune diseases. In patients with Sjogren syndrome, Trp degradation in the serum is augmented and associated with an increased frequency of circulating T_{reg} cells[187]. By contrast, in multiple sclerosis, IDO1 activity in the serum was not significantly different compared with healthy controls, but anti-inflammatory treatment reduced IDO1 activity[188]. As systemic IDO1 activity can be affected by a plethora of unspecific and difficult-to-control stimuli, including infection[29], stress[2] and nutrition[13], attempts to monitor tissue-specific autoimmunity by means of circulating Trp metabolites will be challenging. More detailed analyses of the KP metabolome in serum, however, not only revealed an activation of the KP in patients with multiple sclerosis but also associated the extent of KP activity with disease severity. KP activity may hence serve as a predictive biomarker capable of guiding multiple sclerosis treatment[189].

Targeting Trp metabolism in autoimmune diseases

Efforts to target Trp metabolism therapeutically have mainly focused on developing drugs with Kyn-like properties. Tranilast, for instance, is an AA derivative with AHR agonistic properties capable of inducing immune tolerance and ameliorating disease activity in preclinical models of multiple sclerosis and rheumatoid arthritis[190,191]. However, a phase II clinical trial in patients with rheumatoid arthritis (NCT00882024; TABLE 1) was terminated owing to liver toxicity. Interestingly, laquinimod, a quinoline carboxamide showing structural similarities with KA in development for the treatment of multiple sclerosis, suppresses autoreactive T cell immunity and disease activity in preclinical models of multiple sclerosis in an AHR-dependent fashion[192]. In a series of phase II/III clinical trials in patients with relapsing and progressive multiple sclerosis, laquinimod did not meet the prespecified primary end points, including reduction in relapse rate and disability progression, and was thus discontinued (NCT01707992). Particular endogenous ligands of the AHR are stable enough to be given parenterally in preclinical disease models. 2-(1′H-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE) induces T_{reg} cells and ameliorates autoimmune neuroinflammation in the experimental autoimmune encephalomyelitis (EAE) model by inducing tolerogenic DCs in an AHR-dependent fashion[193].

AHR-activating ligands may also be coupled to autoantigens, thus resulting in the specific targeting of APCs, which are then tolerized to inhibit autoreactive T cell responses suppressing systemic autoimmunity as demonstrated in the EAE model[194].

In addition to the AHR, the relevance of alternative immunoregulatory pathways triggered by KP metabolites is increasingly being recognized. For instance, CA is an endogenous agonist of mGlu4 (and the AHR), which induces T_{reg} cells and ameliorates EAE[195].

Conceptually, Trp metabolism may also be enhanced by systemic administration of Trp, which is quickly metabolized into Kyn after oral gavage. Although this approach results in a differential suppression of T_H17 immunity, this does not translate into amelioration of experimental autoimmune neuroinflammation[196].

Another therapeutic avenue following the recognition of proteasomal degradation as an important mechanism to regulate the immunosuppressive activity of Trp metabolism in autoimmunity[197] is to block IDO1 degradation and thus maintain peripheral tolerance. Indeed, bortezomib, a proteasome inhibitor approved for the treatment of multiple myeloma, prevents IDO1 degradation and ameliorates autoimmune diabetes in preclinical animal models in an IDO1-dependent fashion[198].

IDO2 has only recently emerged as a potential therapeutic target. To date, there are no small molecules with sufficient specificity for IDO2. In preclinical models of autoimmune arthritis, an antibody targeting IDO2 through internalization alleviated disease by suppressing autoreactive T cells and B cells[199]. Newly developed IDO2-specific assay systems and computational structure-based studies may help develop IDO2 inhibitors without cross reactivity to IDO1 (REFS[200,201]).

Finally, IDO1-competent cell-based therapies have been investigated in autoimmune disease models. Although adoptive transfer of mesenchymal stem cells has been shown to suppress clinical disease activity in autoimmune neuroinflammation independent of IDO[202], IDO1 has been shown to be crucially involved in the immunosuppressive properties of mesenchymal stem cells in other autoimmune in vivo disease models[203,204]. IDO1-competent fibroblasts are capable of inducing self-tolerance and mediate remission of autoimmune diabetes when adoptively transferred into non-obese diabetic mice[205]. Similarly, adoptive transfer of IDO1-transduced DCs is therapeutic in autoimmune diabetes in mice[206]. An alternative way to enhance or induce host IDO expression is by local gene therapy. For instance, adenoviral delivery of IDO1 to transplanted organs induces immune tolerance and prevents transplant rejection in rats[207].

Cancer

Trp metabolism in cancer

Several lines of evidence indicate that Trp metabolism can have an important role in cancer, promoting tumour progression by suppressing antitumour immune responses and increasing the malignant properties of cancer cells[10,20,208,209].

First, Trp-degrading enzymes are expressed in multiple cancers. IDO1 is expressed in about 58% of human tumours[210], and its expression is associated with poor clinical outcome in diverse types of cancers including melanoma, gynaecological cancers, colon cancer and haematological malignancies[211]. IDO1 expression is either induced as a counterregulatory mechanism in response to cytokines released from tumour-infiltrating immune cells or its expression is sustained through tumour-intrinsic oncogenic signalling[19]. TDO, which catalyses the same reaction as IDO1, is expressed in glioma, melanoma, ovarian carcinoma, hepatic carcinoma, breast cancer, non-small-cell lung cancer, renal cell carcinoma and bladder cancer and has also been shown to promote tumour progression[10,20,42,212].

Second, reduced systemic Trp levels have been measured in patients with adult T cell leukemia[213], colorectal cancer[214] gynaecological cancers[215], malignant melanoma[216], lung cancer[217] and malignant glioma[10,218]. Elevated concentrations of KP metabolites have less frequently been observed in the blood of patients with these cancers, possibly pointing to more locally restricted changes of Kyn and downstream metabolites in the tumour microenvironment.

Third, there is evidence for a role of Trp degradation in regulating T_{reg} cells and immune cell infiltration in cancer. $FOXP3^+$ T_{reg} cells are found in direct contact with IDO1-expressing DCs in the draining lymph nodes of cervical cancer[219], and IDO1 expression is associated with increased $CD4^+CD25^+FOXP3^+$ T_{reg} cells in patients with metastatic pancreatic ductal adenocarcinoma[220], acute myeloid leukaemia (AML)[33] and non-Hodgkin lymphoma[221]. Furthermore, IDO1 expression correlates with low tumour infiltration of $CD3^+$ T cells, $CD8^+$ T cells and $CD3^+$ and $CD8^+$ T cells as well as $CD57^+$ natural killer cells in patients with colorectal cancer[222], ovarian cancer[223] and endometrial cancer[224], respectively. A recent study has shown that tumour-repopulating cells transfer Kyn to $CD8^+$ T cells, which in turn upregulates programmed cell death protein 1 (PD-1) in an AHR-dependent fashion[225]. Altogether, these observations provide a mechanistic explanation for the role of Trp metabolism in the immune evasion of tumour cells.

Fourth, studies indicate that Trp metabolites can potently promote cancer cell motility and metastasis. For instance, in vitro studies have shown that TDO expression in glioblastoma or breast cancer cells promotes tumour cell migration and invasion[10,42,212]. Similarly, overexpression of IDO1 augmented the motility of lung cancer cells, whereas knockdown reduced motility[165]. This pro-migratory phenotype is also reflected by the promotion of metastasis formation caused by Trp degradation in preclinical models[165–168]. Pharmacological TDO inhibition decreased the number of tumour nodules in the lungs of a mouse model of lung cancer[166]. In addition, IDO1 overexpression in human lung cancer cells implanted into mice increased metastasis formation in the brain, liver and bone[165], whereas IDO1 deficiency reduced metastasis burden and improved survival in mouse models of breast-carcinoma-derived pulmonary metastasis[167,168]. Furthermore, the TDO–AHR signalling axis facilitates resistance to programmed cell death that occurs when anchorage-dependent cells detach from the surrounding extracellular matrix, which constitutes

a critical step for metastasis[212]. Finally, intratumoural IDO1 expression has been shown to correlate with the frequency of liver metastases in colorectal cancer[222], distant metastases in hepatocellular cancer[169] and nodal metastases in endometrial carcinoma[224].

Fifth, there is evidence for a role of NAD^+ generated via the Trp de novo pathway in cancer biology. In mice, impaired Trp metabolism resulting in inhibition of de novo NAD^+ synthesis in the liver promoted hepatic tumorigenesis through DNA damage[226]. In human gliomas, NAD^+ produced de novo from Trp confers resistance to oxidative stress induced by radiochemotherapy[227]. Interestingly, glioma cells and microglia cooperate to produce NAD^+ (REF.[227]). Furthermore, in human cancer cells, IDO1 has been implicated in improving DNA repair and mediating resistance to treatments, such as the PARP inhibitor olaparib, γ-radiation and the chemotherapeutic agent cisplatin, by production of NAD^+ (REF.[228]). Inhibition of Trp metabolism may therefore also prevent treatment resistance via de novo NAD^+ formation; however, this effect may be tissue-specific or cell-specific on the basis of the expression of the KP enzymes necessary for NAD^+ synthesis and thus warrants further investigation.

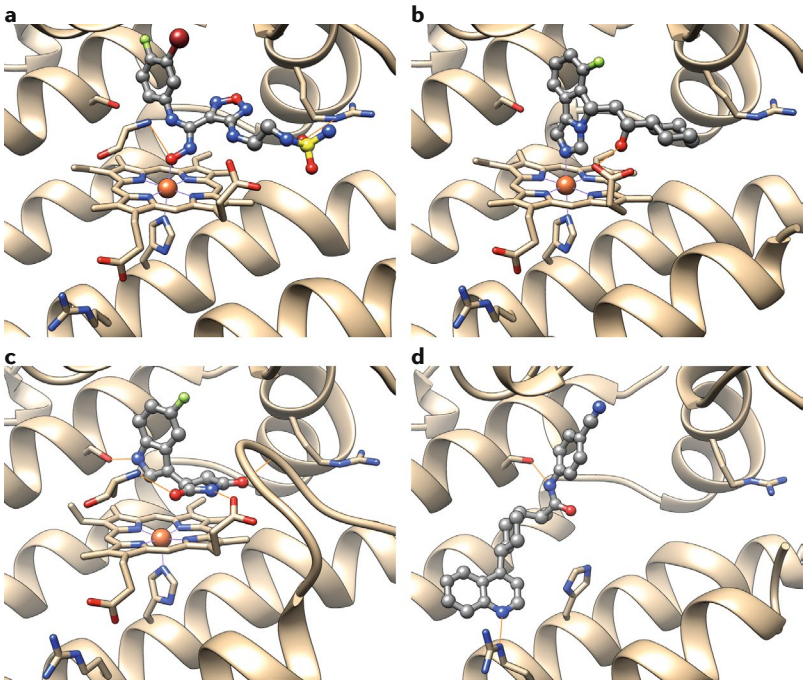


Fig. 5 | **Different binding mechanisms of IDO1 inhibitors. a** | Epacadostat is a reversible L-tryptophan (Trp) competitive inhibitor that preferentially binds to the active ferrous form of indoleamine 2,3 dioxygenase 1 (IDO1) by forming a coordinate bond with the haem iron (PDB ID 5wn8)[271]. **b** | A close analogue of the imidazole navoximod was shown to preferentially bind to the inactive ferric form of IDO1 through a direct iron bond (PDB ID 5ek4)[272]. Navoximod displays reversible noncompetitive inhibitory kinetics with respect to Trp[230] and a moderate 10–20-fold selectivity for IDO1 over tryptophan 2,3 dioxygenase (TDO)[273]. **c** | The Trp analogue EOS200271 does not directly interact with the haem iron and displays noncompetitive kinetics with respect to Trp. EOS200271 forms four hydrogen bonds with the protein and the haem cofactor and induces ordering of a flexible loop, which restricts access to the active site (PDB ID 5whr)[274]. **d** | Co-crystallization of an analogue of the irreversible inhibitor BMS-986205 demonstrates ligand binding to the haem-free apo form of IDO1 (PDB ID 6azw)[275].

Targeting IDO1 and TDO in cancer

On the basis of the tumour-promoting functions of IDO1 and TDO, small-molecule inhibitors of these enzymes have been investigated for cancer therapy (TABLE 1). Various chemical scaffolds for IDO1 and TDO inhibitors have been identified (reviewed in[229–231]). The chemical structures of the clinical-stage IDO1 inhibitors epacadostat (INCB024360), navoximod (NLG-919/GDC919), BMS-986205 (F001287) and EOS200271 (formerly PF-06840003) have been disclosed, and their different modes of IDO1 inhibition have been demonstrated by X-ray crystallography (FIG. 5; TABLES 1,2). Compounds KHK2455, LY3381916 and MK-7162 of undisclosed structures also entered clinical evaluation as IDO1 inhibitors (TABLE 2).

Dual IDO1 and TDO inhibitors are also in development (TABLE 1). HTI-1090 (SHR9146) has entered clinical evaluation as a monotherapy in solid tumours (NCT03208959), whereas DN1406131 is being tested in healthy subjects (NCT03641794) and RG70099 (REF.[232]) from Curadev/Roche and EPL-1410 (REF.[233]) from Emcure are still in preclinical development. TDO inhibitors (which were initially developed as antidepressants to increase systemic Trp levels and thus boost brain serotonin concentrations[234,235]) are also being explored for cancer therapy (TABLE 1) but have not yet reached clinical trials.

In addition, indoximod (NLG8189, D-1-MT) and its prodrug NLG802 (REF.[236]) (TABLE 1) are being investigated in clinical trials (TABLE 2), but unlike L-1-MT[237], they are not IDO1 inhibitors and their mechanism of action, although it appears to be associated with IDO1 expression[238], remains controversial[182,238–241].

The safety, pharmacokinetics and pharmacodynamics of the IDO1 inhibitors epacadostat, navoximod, EOS200271 and BMS-986205 have been studied in patients with advanced solid malignancies and the compounds were well tolerated[242]. As stand-alone therapies, the best overall response was stable disease for both epacadostat[243] and indoximod[244]. However, the greatest therapeutic potential of IDO1 inhibition is expected to be its use in combination with other therapies, and this has been the focus of most phase II and III studies (TABLE 2). Below, we summarize the rationale for various combination strategies, including combination with immune checkpoint inhibitors, other immunomodulators, chemotherapy or radiotherapy, and discuss the progress with each thus far.

Combination with immune checkpoint inhibitors. Clinical evaluation of IDO1 inhibitors is furthest advanced for their combination with monoclonal antibodies targeting immune system checkpoints such as cytotoxic T lymphocyte-associated protein 4 (CTLA4), PD-1 or its ligand (PD-L1), several of which have been approved for the treatment of multiple cancers in recent years on the basis of unprecedented responses in some patients[245]. However, as a considerable proportion of patients do not benefit from checkpoint inhibitors, there is great interest in identifying the molecular basis for the lack of treatment response and treatment resistance, as this knowledge could indicate

Table 2 | **Selected clinical trials with IDO1 inhibitors and pathway modulators**

Indication	Combination partner	Trial ID	Development phase	Status
IDO1 inhibitor: epacadostat (INCB024360)				
Rectal cancer	Pembrolizumab plus chemoradiation	NCT03516708	Phase I/II	Not yet recruiting
Recurrent ovarian cancer	DPX-Survivac vaccine	NCT02785250	Phase I/II	Recruiting
Metastatic pancreatic cancer	Pembrolizumab and CRS207 with or without CY/GVAX pancreas	NCT03006302	Phase II	Recruiting
GIST	Pembrolizumab	NCT03291054	Phase II	Recruiting
Metastatic NSCLC	Pembrolizumab	NCT03322540	Phase II	Active, not recruiting
Metastatic NSCLC	Pembrolizumab and platinum-based chemotherapy	NCT03322566	Phase II	Active, not recruiting
Stage III–IV melanoma	MELITAC 12.1 peptide vaccine	NCT01961115	Phase II	Completed
Unresectable or metastatic melanoma	Pembrolizumab	NCT02752074	Phase III	Active, not recruiting
Cisplatin-ineligible urothelial carcinoma	Pembrolizumab	NCT03361865	Phase III	Active, not recruiting
Recurrent or metastatic SCCHN	Pembrolizumab	NCT03358472	Phase III	Active, not recruiting
Recurrent or metastatic SCCHN	Nivolumab	NCT03342352	Phase III	Withdrawn
IDO1 inhibitor: BMS-986205				
Locally advanced or metastatic solid tumours	Atezolizumab	NCT02471846	Phase I	Active, not recruiting
Pharmacokinetics and metabolism of BMS-986205 in healthy males	N/A	NCT03247283	Phase I	Completed
Advanced and/or metastatic cancers	Nivolumab and ipilimumab	NCT02658890	Phase I/II	Recruiting
MIBC	Nivolumab and chemotherapy	NCT03661320	Phase III	Recruiting
Advanced melanoma	Nivolumab	NCT03329846	Phase III	Active, not recruiting
Recurrent and/or metastatic SCCHN	Nivolumab	NCT03386838	Phase III	Terminated
Advanced and/or recurrent NSCLC	Nivolumab and chemotherapy	NCT03417037	Phase III	Withdrawn
IDO1 inhibitor: navoximod (NLG919/GDC-0919)				
Advanced solid tumours		NCT02048709	Phase I	Completed
IDO1 inhibitor: PF-06840003				
First-in-patient study for malignant gliomas	N/A	NCT02764151	Phase I	Active, not recruiting
IDO1 inhibitor: MK-7162				
Advanced solid tumours	Pembrolizumab	NCT03364049	Phase I	Recruiting
IDO1 inhibitor: LY3381916				
LY3381916 alone or in combination with LY3300054 in solid tumours	LY3300054	NCT03343613	Phase I	Recruiting
IDO1 inhibitor: DN1406131				
Healthy volunteers	N/A	NCT03641794	Phase I	Not yet recruiting
IDO1 inhibitor: KHK2455				
Locally advanced or metastatic solid tumours	Mogamulizumab	NCT02867007	Phase I	Recruiting
Dual IDO–TDO inhibitor: HTI-1090/SHR9146				
Advanced solid tumours	SHR-1210 and apatinib	NCT03491631	Phase I	Not yet recruiting
Advanced solid tumours	N/A	NCT03208959	Phase I	Recruiting
IDO pathway modulator: NLG802				
Advanced solid tumours	N/A	NCT03164603	Phase I	Recruiting
IDO pathway modulator: indoximod (1-methyl-D-tryptophan)				
Acute myeloid leukaemia	Chemotherapy (cytarabine and idarubicin)	NCT02835729	Phase I	Recruiting
Metastatic solid tumours	Docetaxel	NCT01191216	Phase I	Completed
Metastatic or refractory solid tumours	N/A	NCT00567931	Phase I	Completed
Metastatic melanoma	• Checkpoint inhibitors (ipilimumab, nivolumab or pembrolizumab) • Drug: nivolumab • Drug: pembrolizumab	NCT02073123	Phase I/II	Active, not recruiting

Table 2 (cont.) | **Selected clinical trials with IDO1 inhibitors and pathway modulators**

Indication	Combination partner	Trial ID	Development phase	Status
IDO pathway modulator: indoximod (1-methyl-D-tryptophan) (cont.)				
Indoximod with metastatic pancreatic cancer	Gemcitabine and Nab-Paclitaxel	NCT02077881	Phase I/II	Active, not recruiting
Metastatic breast cancer	Adenovirus-p53-transduced dendritic cell vaccine	NCT01042535	Phase I/II	Completed
Unresectable or metastatic melanoma	Pembrolizumab or nivolumab	NCT03301636	Phase II/III	Recruiting

Data accessed from ClinicalTrials.gov database on 23 October 2018. GIST, gastrointestinal stromal tumour; IDO, indoleamine 2,3 dioxygenase; MIBC, muscle-invasive bladder cancer; N/A, not applicable; NSCLC, non-small-cell lung cancer; SCCHN, squamous cell carcinoma of the head and neck.

potential combination therapies to improve responses. Intriguingly, the Kyn:Trp plasma ratio increased in sarcoma patients during treatment with the PD-1 receptor blocking agent pembrolizumab[246], suggesting that IDO1 may be induced by immune checkpoint blockade. Most likely, this induction of IDO1, which is expected to counteract the immunostimulatory effects of immune checkpoint inhibition, is mediated through IFNγ produced by the activated T cells. A preclinical study demonstrated that inhibition of IDO1 slightly enhanced the efficacy of anti-CTLA4, anti-PD-1–PD-L1 and anti-GITR (glucocorticoid-induced TNFR-related protein) therapies[247]. Furthermore, as oncogenic KIT signalling drives IDO1 expression in gastrointestinal stromal tumours (GISTs)[248], IDO1 inhibition by imatinib may partially account for the efficacy of concomitant PD-1–PD-L1 blockade[249]. Furthermore, correlative analyses in a phase Ib study of dasatinib plus ipilimumab suggested that IDO1 suppression may potentially correlate with antitumour efficacy in patients with GISTs, but this finding has to be validated in further studies (for example, NCT03291054)[250].

These findings, albeit slim, sparked extensive clinical investigation of combination therapies of IDO1 inhibitors with immune checkpoint inhibitors (TABLE 2). Following encouraging data from a phase I/II single-arm trial of the combination of epacadostat with pembrolizumab, a phase III trial was performed in patients with unresectable or metastatic melanoma (ECHO 301/KEYNOTE 252; NCT02752074) (TABLE 2). However, the combination of epacadostat with pembrolizumab failed to meet its primary end point in the phase III trial, raising the issue of the validity of single-arm combination trials for clinical decision-making. On the basis of the results of the ECHO-301 trial, the ECHO-305 and ECHO-306 trials of epacadostat in combination with pembrolizumab in lung cancer were converted into randomized phase II trials. However, enrolment in four additional trials of epacadostat in combination with pembrolizumab, and in two trials of epacadostat in combination with nivolumab, was discontinued.

The failure of the ECHO-301 trial resulted in the termination of phase III trials of BMS-986205 in combination with nivolumab in malignant melanoma (NCT03329846), head and neck cancer (NCT03386838) and non-small-cell lung cancer (NCT03417037). However, patients are still being enrolled in phase I and II clinical trials of BMS-986205 in combination with nivolumab or ipilimumab. In addition, the randomization portion of Indigo301 (NCT02073123), a study of indoximod in combination with pembrolizumab or nivolumab for patients with advanced melanoma, has not been initiated.

Although the negative ECHO-301 trial clearly represents a setback in the development of IDO1 inhibitors in cancer immunotherapy[251], it also serves as motivation to utilize clinical trials to learn more about the mechanism of action of IDO1 inhibition in cancer, to develop more sophisticated biomarkers for patient selection and treatment monitoring and to exploit novel targets within this pathway, such as the AHR (TABLE 1).

Combination with other immunomodulators. Investigation of the potential of IDO1 inhibitors in combination immunotherapy is planned to be continued in proof-of-concept trials, including strategies distinct from combinations with PD-1 and PD-L1 antagonists.

Several clinical trials testing the IDO1 inhibitor epacadostat in combination with antitumour vaccines are being conducted (NCT01961115, NCT02785250 and NCT03006302) and may show whether IDO1 blockade boosts the efficacy of antitumour vaccination. The rationale behind this is that upregulation of IDO1 by interferon signalling is involved in multiple immune-related pathways. For instance, activation of TLRs induces IDO1 expression through interferons[7,252,253]. Cytosolic DNA sensing that activates the stimulator of interferon genes (STING) adaptor also upregulates IDO1 via interferons and thus promotes the growth of tumours characterized by low antigenicity[254]. As TLR ligands are employed and STING activators are explored as immune adjuvants for anticancer vaccines, IDO1 induction may constitute an undesired effect by antagonizing the intended immune activation. In addition, immune activation by the vaccination itself may upregulate IDO1.

Several other pathways that suppress antitumour immune responses are also implicated in driving the tumoural expression of Trp-degrading enzymes, including AHR signalling, TGFβ signalling and signal transducer and activator of transcription 3 (STAT3)[211]. Two scenarios can hence be envisioned. If the inhibitors of these pathways are highly efficient and concomitantly fully abrogate the expression of the Trp-degrading enzymes, they may render IDO1 or TDO inhibitors dispensable in this setting. On the contrary, if these drugs do not entirely mitigate the expression of IDO1 and/or TDO, they may synergize with inhibitors of Trp metabolism. By contrast, other therapeutic approaches may induce IDO1 as an undesired effect, suggesting that

a combination of these therapies with IDO1 inhibitors may be beneficial.

Combination with chemotherapy and radiotherapy. Multiple studies have implicated IDO1 in resistance to chemotherapy and/or radiotherapy[208,228,255]. IDO1 is induced as an undesired effect of chemotherapy[256] and radiotherapy[257] in non-small-cell lung cancer. Combination of an IDO1 inhibitor with chemotherapy led to regression of established tumours in the MMTV-*neu* transgenic mouse model of breast cancer — an effect that was dependent on T cell immunity as the efficacy of the combination therapy was abolished in athymic nude mice[208]. Independently of the immune system, downregulation of IDO1 in IFNγ-stimulated tumour cells decreased intracellular NAD^+ levels and increased sensitivity to PARP inhibition, chemotherapeutic agents and irradiation[228]. Inhibition of IDO1 in combination with radiochemotherapy prolonged survival of mice bearing intracranial GL261 gliomas[255]. The combination of chemotherapy, irradiation and IDO1 blockade led to widespread intratumoural complement deposition and C3-dependent tumour destruction[255]. IDO1 inhibition may therefore synergize with radiotherapy and chemotherapy through diverse mechanisms. Combining IDO1 inhibitors with radiotherapy and/or chemotherapy is therefore being tested in clinical trials (NCT03516708, NCT03661320, NCT02077881 and NCT02835729).

Challenges in targeting Trp metabolism in cancer. Although IDO1 inhibitors recently appeared to be on the verge of entering clinical routine, the recent failures of clinical trials with IDO1 inhibitors raise questions regarding the future of this approach for cancer therapy. Currently, it is unclear whether specifics of the clinical trials such as selection of the patient population, dosing, therapy combinations or the target itself led to the clinical failures. Patient stratification certainly is important, as IDO1 inhibitors will work only if IDO1 is present and active. Here, the lack of appropriate clinical tools to monitor IDO1 regulation and activity before and during treatment becomes evident. Clearly, the simple measurements of Trp:Kyn ratios in serum are not sufficient as they are confounded by environmental influences such as infections. In this respect, monitoring tissue Trp metabolism (as outlined in TABLE 3) remains a key challenge for patient stratification, biomarker development and surrogate parameter development for determining biological activity. Here, ratios of KP metabolites, such as Trp:Kyn ratios (for instance, assessed by matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging), may represent a more faithful measure of KP activation. Relating these biomarkers to tissue immune infiltration will provide key insight into tissue-specific mechanisms of KP-mediated immune modulation. This insight may also guide important considerations on dosing of KP inhibitors or other immunomodulatory drugs interfering with KP enzymatic activity and on optimizing combination treatments. Measurement of intratumoural drug and metabolite levels can determine whether sufficient inhibitor concentrations are reached at the target site. Moreover, a better understanding of the crosstalk between IDO1 and potential combination treatments is required.

Regarding the general approach of inhibiting IDO1 for cancer therapy, it remains to be determined whether IDO1-selective inhibitors will be sufficient, as TDO, which catalyses the same reaction as IDO1, is expressed in multiple cancers and may circumvent inhibition of the pathway. Dual IDO1–TDO inhibitors are being explored (TABLE 1) and may solve this issue. However,

Table 3 | **Selected analytical tools for studying tryptophan metabolism**

Analytical tool	Advantages	Limitations	Applications and details
Chromatographic methods	High sensitivity and specificity	• Expensive instrumentation required • Bioanalytical expertise necessary • Medium throughput	• HPLC • GC-MS or LC-MS[276]
Metabolite assays	Amenable to high-throughput assays	Limited specificity and possibility for interference[277]	• Detection of Kyn via reaction with Ehrlich reagent[278,279] • Detection of *N*-formylkynurenine (via reaction with a chemical probe)[280,281] • Screens for drug discovery
Genetically encoded FRET Trp nanosensor	Measurement of Trp levels in living cells	• Demanding experimental setup • Advanced microscopy required	Enables intracellular measurement of Trp levels[282]
ELISA using metabolite-specific antibodies	High throughput, required instrumentation available in standard laboratories	Limited sensitivity	Enables measurement of metabolites in cell culture supernatants[283], plasma[284,285] and serum[286]
MALDI mass spectrometry imaging of Trp metabolites	Enables analysis of the spatial distribution of endogenous metabolite profiles	• Dedicated high-end instrumentation required • Bioanalytical expertise necessary • Medium throughput	Visualization of Trp and Kyn in tissue slides[28,287]
PET	Allows visualization of Trp uptake in vivo	• Patients are exposed to radioactivity • Limited availability • Uncertainty as to how well uptake reflects metabolism	Visualization of Trp uptake in patients[288] and xenograft models[289]

ELISA, enzyme-linked immunosorbent assay; FRET, fluorescence resonance energy transfer; GC-MS, gas chromatography–mass spectrometry; HPLC, high-performance liquid chromatography; Kyn, kynurenine; LC-MS, liquid chromatography–mass spectrometry; MALDI, matrix-assisted laser desorption/ionization; PET, positron emission tomography; Trp, L-tryptophan.

it is currently unclear whether complete blockade of Trp catabolism may elicit tolerability issues. Therefore, targeting pathways downstream of IDO1 and TDO (namely, AHR activation) appears promising. Indeed, diverse pharmaceutical companies are pursuing this approach and AHR inhibitors are currently in preclinical development (TABLE 1). Future studies are required to determine if AHR inhibitors are tolerated and whether they can block immunosuppression mediated by KP activation.

Outlook

The lack of a clear understanding of the exact downstream effector mechanism of immunosuppressive Trp metabolism is a major obstacle in drug development. Although the relevance of Trp depletion as an effector mechanism is clear, and active immunomodulation through the AHR is supported by increasing evidence, further studies are required to uncover potential additional effector mechanisms, determine the relevance of individual KP metabolites and assess the role and therapeutic relevance of additional enzymes in the KP. The delicate regulation of Trp metabolism in the CNS is a key challenge for therapeutic targeting beyond delivering drugs to the CNS. Given the profound compartmentalization of the KP and its sensitivity to unspecific environmental factors, further studies are required to address the important question of whether systemic targeting of KP enzymes is reasonable and sufficient to treat neuropsychiatric and neurodegenerative diseases with sufficient efficacy and without overt toxicity. Finally, therapeutic targeting of Trp metabolism requires concepts beyond the development of enzymatic inhibitors. Here, viral delivery of key enzymes or the development of synthetic KP metabolites may offer additional therapeutic options with improved specificity and efficacy.

The rapidly expanding knowledge on key functions of Trp metabolism in diverse diseases such as neurodegeneration, autoimmunity and cancer has revealed promising therapeutic targets. Although drug development programmes focus mainly on the development of IDO1 and TDO inhibitors in immuno-oncology, there are clearly novel targets and indications rapidly evolving, such as the development of KMO inhibitors in acute pancreatitis. Future efforts must be supported by the implementation of state-of-the-art analytical tools to assess Trp metabolism in a tissue-specific manner. There is a clear compartmentalization of Trp metabolism, which must be considered when developing therapeutic strategies and identifying biomarkers. In addition, with the identification of the relevance of intermediate metabolites and the tight enzymatic regulation of many steps in the KP, the complexity of drug targeting and monitoring increases. Setting aside these challenges, future research and clinical trials will shed further light on the druggability of the KP.

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M.P. has received royalties for patents on aryl hydrocarbon receptor inhibitors and tryptophan metabolites and has received honoraria for advisory board services and research support from Bayer. E.A.A.N., F.F., U.F.R. and C.A.O. declare no competing interests.

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