



# Indoleamine 2,3-dioxygenase (IDO): Only an enzyme or a checkpoint controller?

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## ABSTRACT

Tryptophan (Trp) is one of the important essential amino acids and Kynurenine pathway (KP) is the first and rate limiting step of degrading Trp to active metabolites. Recent studies showed that KP metabolites have antimicrobial and immunosuppressive roles. The main controller enzyme of this pathway is indoleamine-2,3-dioxygenase (IDO). IDO is an intracellular monomeric, heme-containing enzyme that controls the Trp breakdown in the KP. In this review, we discuss IDO functions on cancer progression and prognosis, and possible therapeutic aspects of inhibition of IDO on cancer immunotherapy.

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## 1. Introduction

Tryptophan (Trp) is one of the important essential amino acids and is essential for cell survival, protein synthesis, being a precursor for serotonin and other functional molecules in the brain tissue as niacin and melatonin. Finally, it is a valuable reserve for the kynurenine pathway and works as a coenzyme of NAD and NAD(P).<sup>1–3</sup> Kynurenine pathway (KP) is the first and rate limiting step of degrading Trp to active metabolites such as Kynurenine (Kyn), kynurenic acid, 3-hydroxykynurenine, 3-hydroxyanthranilic acid, picolinic acid and quinolinic acid (Fig. 1).<sup>4</sup> Trp consumption via KP can affect the ongoing reactions for cell growth and other important functions. Recent studies showed that KP metabolites have antimicrobial and immunosuppressive roles.<sup>4,5</sup> The main controller enzyme of this pathway is indoleamine-2,3-dioxygenase (IDO). IDO is an intracellular monomeric, heme-containing enzyme that controls the Trp breakdown in the KP.<sup>6</sup> In this review, we discuss IDO functions on cancer progression and prognosis, and possible therapeutic aspects of inhibition of IDO on cancer immunotherapy.

## 2. Indoleamine-2,3-dioxygenase

IDO is an intracellular cytosolic enzyme that regulates the degradation of Trp to *N*-formylkynurenine as a first step rate

controller.<sup>8</sup> This heme-containing enzyme is encoded by IDO1 gene, and located on chromosome 8.<sup>9</sup> There are two kinds of IDO enzyme, IDO1 and IDO2; both convert Trp to Kyn with different activity rates.<sup>10</sup> IDO2 is more narrowly expressed than IDO1 and has only 3–5% enzymatic activity of IDO1.<sup>10–12</sup> Another type of Trp catabolic enzyme is tryptophan-2,3-dioxygenase (TDO), which has a different distribution than IDO and additionally present in bacteria.<sup>13</sup> In the human body, the IDO enzyme has been expressed from many types of cell such as endothelial cells, antigen presenting cells (APC), fibroblasts, macrophages and DCs.<sup>14,15</sup> While the previous studies investigated IDO enzymes as unique components with similar functions, recent studies showed that there had been several differences between the IDO1 and IDO2, with the latter has been much less studied in the literature.

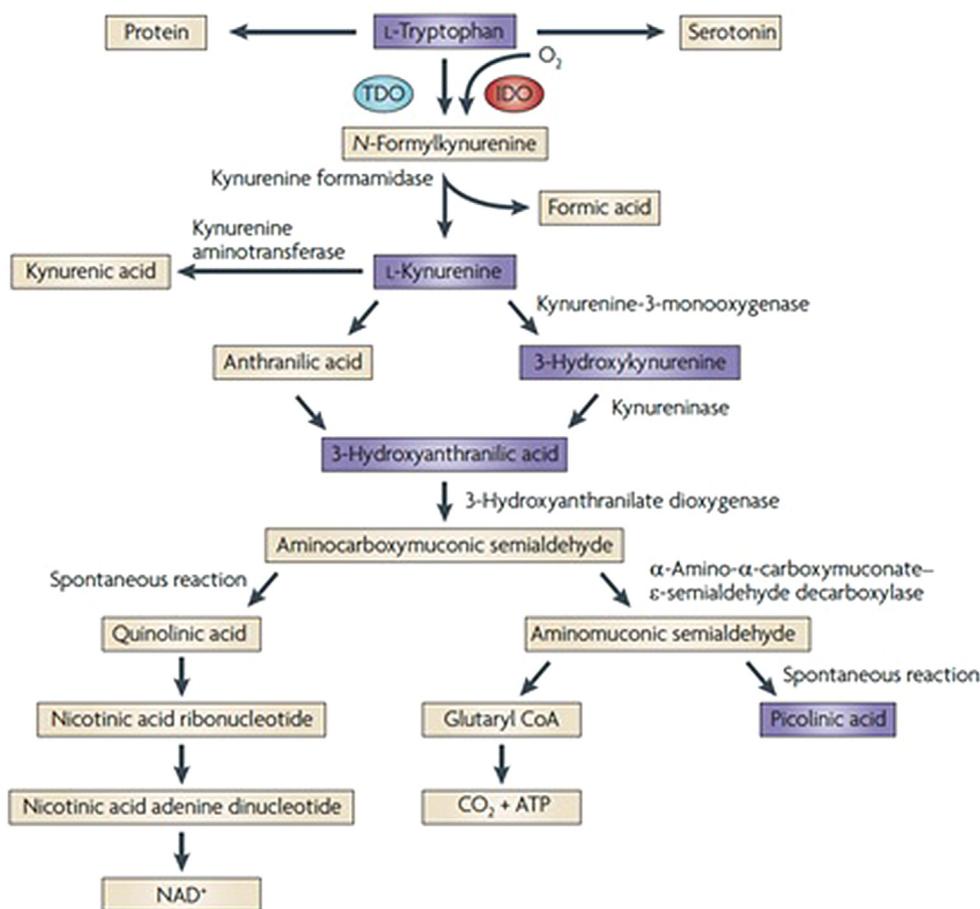
### 2.1. IDO and immune tolerance

IDO plays important roles in carcinogenesis and its progression. It promotes inflammation around tumor tissues (microenvironment), cause immune tolerance by the regulation of natural killers (NK), T cells and T regulatory cells (Tregs) as well as myeloid-derived suppressor cells (MDSC).<sup>16</sup> Complete immunomodulatory effects of IDO have not yet been characterized fully. Immune modulatory actions of IDO mainly result from starving the tumor microenvironment of Trp and increasing the Trp-derived metabolite Kyn. Activated IDO enzyme decreases Trp levels in the tumor microenvironment, and this reduction causes immunosuppressive effects (Fig. 2). More specifically, tryptophan depletion induces cell cycle arrest of T cells and increase their apoptosis by inhibiting the mechanistic target of rapamycin complex 1 (mTORC1), and

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**Fig. 1. IDO mediated tryptophan catabolism in the kynurenine pathway.** Immunologically important metabolites are highlighted in purple. IDO, Indolamine-2,3-dioxygenase; TDO, tryptophan-2,3-dioxygenase. Reprinted with permission from.<sup>7</sup>

inducing a stress response that activates the general control nondepressible-2 (GCN2). It has been shown that below 0.5  $\mu$ M concentrations of tryptophan, T cell proliferation is inhibited significantly.<sup>17</sup> Moreover, increasing Trp metabolites, especially Kyn, is reported to be toxic to lymphocytes and it activates the transcription of aryl hydrocarbon receptor (AHR). This in return induce the CD4<sup>+</sup> T cells differentiation to immunosuppressive Treg cells.<sup>18</sup>

## 2.2. IDO pathway and cancer

The activated IDO pathway has been shown in many pathologic situations including infections, obesity, rejection of transplantations, autoimmunity and atherosclerosis.<sup>15,20,21</sup> Complementing these reports, both *in vitro* or *in vivo* studies showed that IDO pathway has a key regulatory function in immune escape.<sup>22,23</sup> Many cancers including gastrointestinal system, lung, gynecological, and breast cancers, have IDO protein expression while as the healthy tissues don't.<sup>24</sup> The main mechanism of IDO pathway mediated immune escape is the decreasing T cell infiltration into tumor microenvironment; hence, this status concluded with poorer oncologic outcomes.<sup>15</sup> Some studies showed that higher IDO expression is associated with shorter survival in patients with colorectal cancer, small-cell lung cancer, and melanoma.<sup>25–27</sup> Also, increased IDO expression is suggested as one of the drug resistance mechanism against chemotherapeutics after revealing in a study that the ovarian cancer patients with paclitaxel resistance showed higher IDO gene profiles.<sup>28</sup>

## 3. Differences between the roles of IDO1 and IDO2 enzymes

### 3.1. IDO1

Mesenchymal stem cells (MSCs) shows their immune suppressor effects with IDO1 enzyme. In normal situations they can't express IDO protein, however, following inflammatory stimulation by interferon- $\gamma$  (INF- $\gamma$ ) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) they start IDO1 expression that activates their immunosuppression mechanisms.<sup>29</sup> Endothelial cells in blood brain barrier use IDO1 to degrade Trp to Kyn for maintaining their functions.<sup>30</sup> In dendritic cells, INF- $\beta$  and  $\gamma$ , and TNF- $\alpha$  activate the JAK/STAT pathway as well as interferon regulatory factor-3 (IRF3), and all these stimuli cause activation of IDO1.<sup>31</sup> It has been showed in *in vitro* experiments that many human cancer cell lines had IDO1 expression with active enzyme form, but IDO2 was forming in its inactive form.<sup>32</sup> INF- $\gamma$  is one of the strongest activators of IDO1 whereas, it is a weak inducer of IDO2.<sup>33</sup> Activated IDO1 allows Treg cells to activate FoxO3a, and this process upregulates programmed cell death protein-1 (PD-1) expression. Subsequently, PD-1—PTEN pathway inhibits Akt pathway. Furthermore, IDO1 activated Treg cells are potent inducer of PD-L1 and -L2 on DCs.<sup>34</sup>

### 3.2. IDO2

IDO2 is expressed only in a few subsets of tissues including liver and kidney, and some immune cells such as APC and DCs.<sup>35,36</sup> Contribution of IDO2 to immune escape via DCs and Tregs is

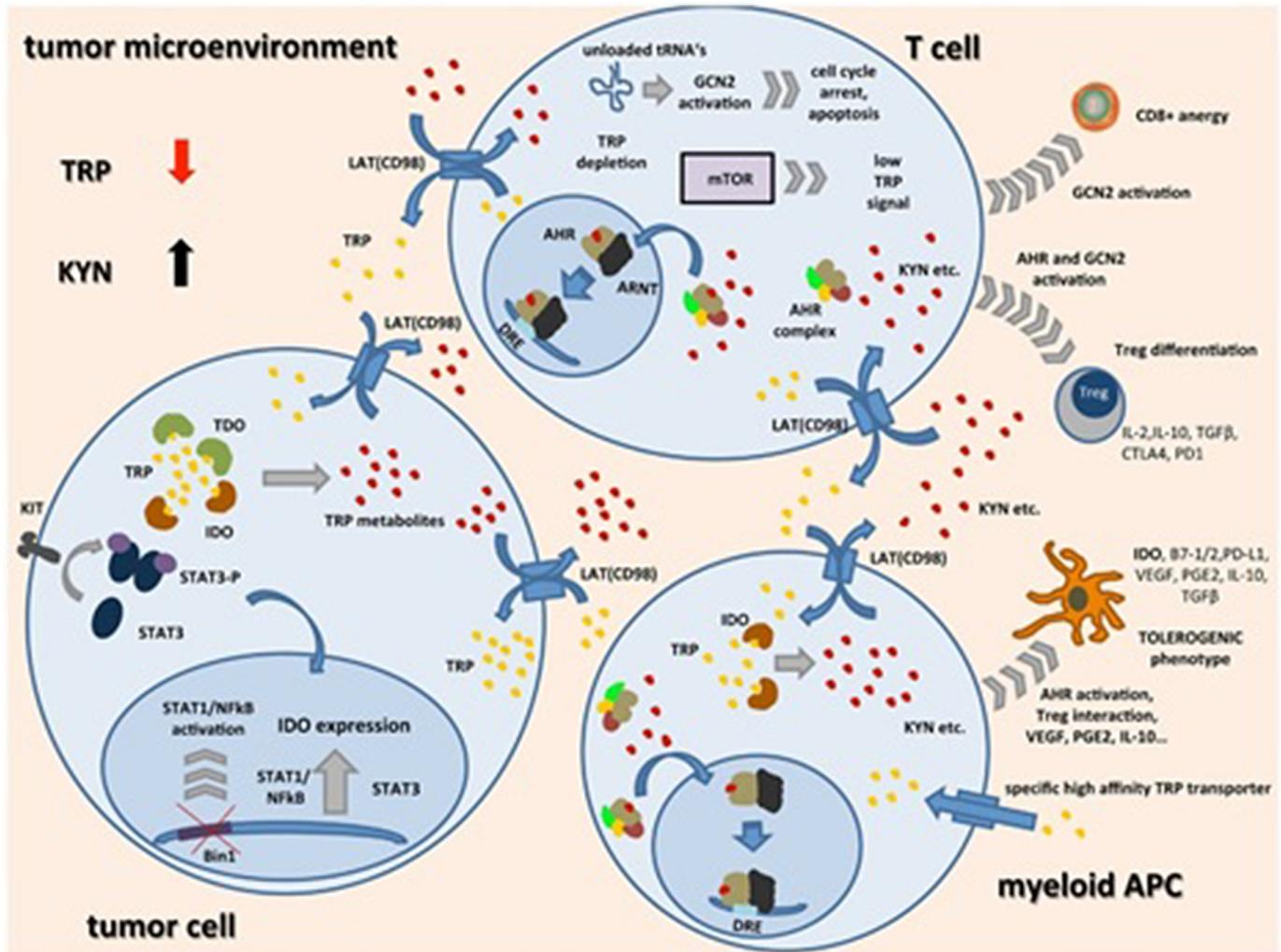


Fig. 2. Schematic representation of KP mediated Trp catabolism and its effects on tumor microenvironment related events. Reprinted from<sup>19</sup> under Creative Commons Attribution Licence (© 2015 Platten, von Knebel Doeberitz, Oezen, Wick and Ochs).

similar to IDO1 but, unlikely to IDO1, it can upregulate peripheral blood cells in patients with inflammatory disease, suggesting that IDO2 has also a role in the inflammatory process.<sup>37</sup> Function of IDO2 in cancer is less clear than IDO1; several cancer types express IDO2 namely pancreatic, gastric and colon cancers, but some cancers do not such as cervical cancer.<sup>32,38</sup> IDO2 has a pro-inflammatory role opposite to IDO1 as supported by a study which showed that IDO2 knock-out (ko) mice had delayed onset or reduced severity of arthritis where as IDO1 ko mice were not.<sup>35</sup> Moreover, another study reported that IDO2 contributed to an autoantibody production in systemic lupus erythematosus model in mice.<sup>39</sup> IDO2 also influences different cytokines compared to IDO1; IDO2 ko and wild type mice showed different levels of interleukin-4, IL-6 and IL-21 but similar levels of INF- $\gamma$  and TNF- $\alpha$ .<sup>35</sup>

#### 4. IDO inhibitors and clinical data

IDO inhibition has uncovered a new treatment option in cancer therapy. Numerous pre-clinical and clinical studies are focusing on several new combinations with chemotherapeutics and IDO1, IDO2 or both enzyme inhibitors. Among the IDO inhibitors, 1-methyl-DL-tryptophan (1-MT) is the most studied inhibitor. It has two isomers,

D- and L-, which of first is a potent inhibitor of IDO2, and the second one is modestly inhibits IDO1.<sup>12,40</sup> IDO inhibitors, which are being investigated on clinical trials, can be summarized as: specific IDO1 inhibitors Epacadostat (INCB024360), NLG-919, PF-06840003 and INCB024360 analogues, specific IDO2 inhibitor indoximod, and IDO1 and IDO2 inhibitor Norharman and 1-MT.<sup>41</sup>

Best to our knowledge, the first phase 1 study of an IDO inhibitor was presented at the ASCO 2014 meeting which involves orally administered NLG-919 (NCT02048709). Following this trial, many studies had been planned and are currently ongoing. In a phase II trial, oral 1200 mg BID indoximod combined with checkpoint inhibitors in stage IV melanoma was studied. In the study 1 patient showed complete response and 6 out of 9 patients were still alive (9–14 month of enrollment) without any severe adverse events (NCT02073123).<sup>42</sup> In another study, indoximod was combined with gemcitabine/nab-paclitaxel in 30 patients diagnosed with stage IV pancreatic cancer. In this study, investigators found a 37% of objective response rate with 1 complete response (NCT02077881).<sup>43</sup>

#### 5. Discussion

Current immunotherapy options have been achieving promising

results. Many patients with cancer types such as melanoma, lung cancer, head and neck cancer and others, can have 30% rate for 5 year survival even for stage IV disease.<sup>44–46</sup> However, more than 50% of these patients under the treatment of immune check point inhibitors will be progressed with a resistance to therapy, so most of the stage IV patients still need new therapeutic approaches. IDO and KP have gained emerging attention in the last couple years after its activity is associated with T-cell apoptosis and decreased migration to the tumor microenvironment. Thus, IDO inhibitors have arisen as promising options and several clinical trials are ongoing however with some concerns. First, the IDO enzyme has two isoform and activity of these isoforms strongly depend to tissue, cells or pathologic sites. Secondly, IDO can be activated with many stimuli. INF- $\gamma$  and TNF- $\alpha$  are the strongest inducers of IDO, especially IDO1, so we can speculate that, if a patient does not have inflamed tumor microenvironment, IDO inhibitors may not work. Third, non-inflamed tumors that are treated with INF- $\gamma$  may have activated immune cells around the tumor microenvironment however this treatment could activate IDO and in this case combining IDO inhibitors could be an effective option to counter this unwanted effects.

Lastly, tumor cells build a cover as a shell around the tumor microenvironment and the current passive drug administration may not work as effectively for penetrating into the solid tumor mass. To bypass this and also several additional challenges such as cancer cell specific efficacy and low healthy tissue distribution, use of nanomedicine based drug delivery systems are gaining significant attention. It should be also noted that IDO is an intracellular enzyme and for any modulator to inhibit its action, effective cytoplasmic delivery is a prerequisite. Moreover, another enzyme with identical bioactivity to IDO is tryptophan-2,3-dioxygenase (TDO). TDO is mainly expressed in liver and many other organs, thus inhibiting non-tumor associated IDO or TDO can also cause significant side effects. Perhaps, cancer cell targeted nanomedicine-based drug therapies that combine small molecule therapeutics or bioactive molecules such as INF- $\gamma$  with IDO inhibitors inside the same nanocarrier could be effective options in this hot-topic area of immunotherapy.

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