

## REVIEW

# IDO1 in cancer: a Gemini of immune checkpoints

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Indoleamine 2, 3-dioxygenase 1 (IDO1) is a rate-limiting metabolic enzyme that converts the essential amino acid tryptophan (Trp) into downstream catabolites known as kynurenines. Coincidentally, numerous studies have demonstrated that IDO1 is highly expressed in multiple types of human cancer. Preclinical studies have further introduced an interesting paradox: while single-agent treatment with IDO1 enzyme inhibitor has a negligible effect on decreasing the established cancer burden, approaches combining select therapies with IDO1 blockade tend to yield a synergistic benefit against tumor growth and/or animal subject survival. Given the high expression of IDO1 among multiple cancer types along with the lack of monotherapeutic efficacy, these data suggest that there is a more complex mechanism of action than previously appreciated. Similar to the dual faces of the astrological Gemini, we highlight the multiple roles of IDO1 and review its canonical association with IDO1-dependent tryptophan metabolism, as well as documented evidence confirming the dispensability of enzyme activity for its immunosuppressive effects. The gene transcript levels for *IDO1* highlight its strong association with T-cell infiltration, but the lack of a universal prognostic significance among all cancer subtypes. Finally, ongoing clinical trials are discussed with consideration of IDO1-targeting strategies that enhance the efficacy of immunotherapy for cancer patients.

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## INTRODUCTION

Within the last decade, there has been incredible success regarding application of immune checkpoint inhibitors, with an emphasis on targeting CTLA-4 and/or PD-(L)1, to improve patient survival for otherwise untreatable melanoma,<sup>1</sup> non-small-cell lung<sup>2</sup> and renal<sup>3</sup> cancers. This progress has motivated medical oncologists and/or tumor immunologists to better understand the regulation, role and functions of co-inhibitory pathways that are expressed by immune and cancer cells.<sup>4</sup> Preclinical and clinical studies have demonstrated a trend of immunotherapeutic combination strategies to confer a greater survival benefit over single-agent approaches.<sup>5,6</sup> There are, however, notable exceptions to the general belief that more is better, and several recent studies have highlighted the fact that multi-therapy approaches do not universally provide an advantage to the host and/or immune system.<sup>7,8</sup> These

considerations reflect the complicated regulatory network that governs the human immune system's response to cancer, its failure to have a 'one-size-fits-all' approach, the toxicities induced by certain immunotherapeutic combinations and the critical need to further understand why checkpoint therapies (i) provide benefits to some patients, but not others; (ii) enhance tumoricidal effects against some cancers, but not others; and (iii) beneficially stimulate immune responses with certain combinations, but not others.

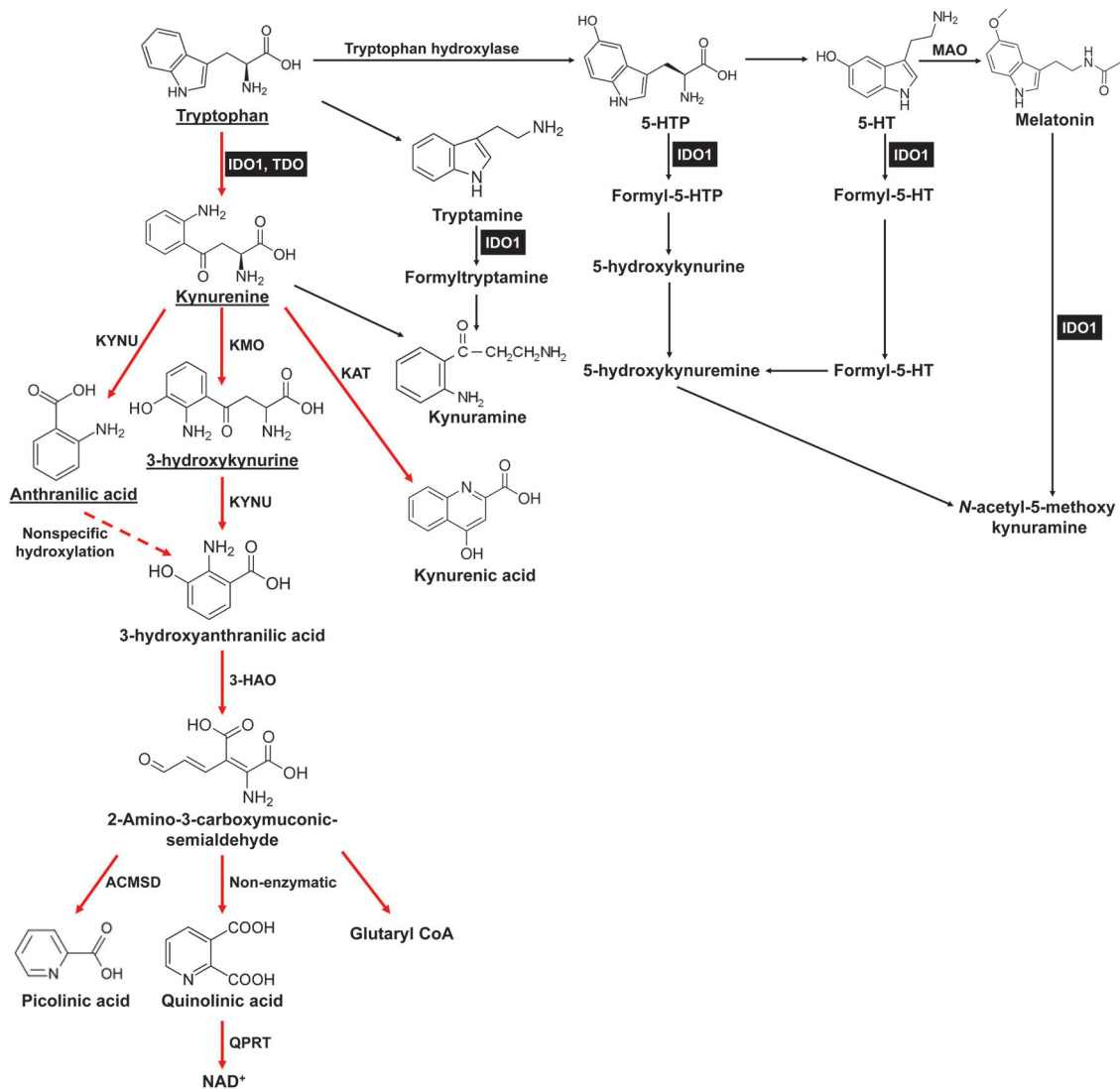
In this review, we focus on the novel immune checkpoint target, indoleamine 2, 3-dioxygenase 1 (IDO1), which is characterized as a rate-limiting metabolic enzyme that converts tryptophan (Trp), into downstream kynurenines (Kyn) (Figure 1). IDO1 is interferon-inducible and has been associated with mediating potentially immunosuppressive effects in cancer.<sup>9,10</sup> While a growing body of data suggest that there is

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**Figure 1** Tryptophan (Trp) catabolic pathways. In addition to being used as a building block for protein synthesis, the majority of dietary Trp (95%) is catabolized via the Trp→Kyn pathway (red arrows). Other minor pathways include conversion to tryptamine or melatonin. Within the Kyn pathway, the underlined metabolites can cross the blood brain barrier (BBB). IDO1 (and TDO) are highlighted in black boxes. ACMSD: 2-amino-3-carboxymuconate semialdehyde carboxylase; 3-HAO, 3-hydroxyanthranilate 3, 4-dioxygenase; IDO1, indoleamine 2, 3-dioxygenase 1; KAT, kynurenine aminotransferase (I, II, III); KMO, kynurenine 3-monooxygenase; KYNU, kynureninase; MAO, monoamine oxidase; QPRT, quinolinic-acid phosphoribosyl transferase; TDO, tryptophan 2,3-dioxygenase.

a lack of therapeutic efficacy when targeting IDO1 alone, there is strong support for combination approaches to provide a synergistic benefit.<sup>11–14</sup> Due to the limited effect(s) of single agents, a growing number of active clinical trials utilize IDO1 as an adjuvant alongside other cancer treatment modalities (Vacchelli et al.<sup>15</sup> and Table 1), which raises the question of whether immunological therapies somehow ‘activate’ IDO1 so that it becomes therapeutically targetable. These developments highlight additional questions that have yet to be answered, including the following: (i) Is enzyme metabolism the sole characteristic that endows IDO1 with immunosuppressive activity? (ii) Why is IDO1 enzyme inhibition not effective as a monotherapy<sup>16</sup> given that IDO1 is highly expressed in a variety of human cancers?<sup>10,17</sup> (iii) Why does coupling radiotherapy and/or chemotherapy tend to synergize with IDO1

inhibition?<sup>18</sup> (iv) How do IDO1 pathway inhibitors that have no effect on converting Trp into Kyn,<sup>19</sup> such as indoximod (dextrorotatory 1-methyl-Trp; D-1-MT), affect IDO1-mediated immune suppression?<sup>20</sup> (v) Do select IDO1 inhibitors confer gain-of-function toxicity under certain therapeutic contexts? (vi) Are there immunosuppressive effects of IDO1 in cancer cells that are independent of enzyme activity and similar to effects that have been reported in innate immune cells?<sup>21</sup>

### TRP DIOXYGENASES

L-Trp, which is the least abundant essential amino acid, can be metabolized via four distinct mechanisms: decarboxylation to tryptamine; protein synthesis; the serotonergic pathway; and the Kyn pathway (Figure 1).<sup>22</sup> Kyn pathway metabolism accounts for ~95% of all mammalian dietary Trp.<sup>23</sup> The first

**Table 1 Ongoing and historical clinical trials that target IDO1 in cancer**

Agent	Indication(s)	Phase	Status	Notes	NCT no.	
Indoximod (b-1-MT)	Metastatic solid tumor	I	Completed	Combined with docetaxel	NCT01191216	
	Solid tumor	I	Completed	Single agent	NCT00567931	
	Metastatic breast cancer	I/II	Active, not recruiting	Combined with vaccine	NCT01042535	
		II	Recruiting	Combined with fulvestrant or tamoxifen	NCT02913430	
	Melanoma	II	Active, not recruiting	Combined with docetaxel	NCT01792050	
		I/II	Recruiting	Combined with ipilimumab (CTLA-4 mAb)	NCT02073123	
		I/II	Recruiting	Combined with gemcitabine and nab-paclitaxel	NCT02077881	
		I/II	Recruiting	Combined with cytarabine, idarubicin	NCT02835729	
	INCB024360	GBM, glioma, gliosarcoma	I/II	Recruiting	Combined with temozolomide, bevacizumab (VEGF mAb) and radiation	NCT02052648
		GBM, glioma, ependynoma, medulloblastoma	I	Recruiting	Combined with temozolomide and radiation	NCT02502708
Prostate carcinoma		II	Active, not recruiting	Combined with sipuleucel-T	NCT01560923	
NSCLC		II	Recruiting	Combined with docetaxel and tergepumatucel-L	NCT02460367	
Advanced neoplasms		I	Completed	As single agent	NCT01195311	
Myelodysplastic Syndromes		II	Completed	As single agent	NCT01822691	
Melanoma		I/II	Recruiting	Combined with ipilimumab	NCT01604889	
		II	Recruiting	Combined with a multipptide-based vaccine	NCT01961115	
Reproductive tract tumors		II	Completed	Compared to tamoxifen	NCT01685255	
		I	Recruiting	Combined with vaccine and cyclophosphamide	NCT02785250	
	I	Active, not recruiting	As single agent	NCT02042430		
	I	Recruiting	Combined with adoptive transfer of NK cells and IL-2	NCT02118285		
	Solid tumors	I/II	Recruiting	Combined with CRS-207 and pembrolizumab (PD-1 mAb)	NCT02575807	
		I/II	Recruiting	Combined with DC-targeted NY-ESO-1 and poly-ICLC	NCT02166905	
	Solid tumors	I/II	Recruiting	Combined with a PDGD1 mAb	NCT02178722	
		I	Recruiting	Alone or combined with pembrolizumab	NCT02862457	
		I	Active, not recruiting	Combined with itacitinib (JAK inhibitor)	NCT02559492	
		I/II	Recruiting	Combined with nivolumab or pembrolizumab and chemotherapy	NCT03085914	
GDC-0919 (formerly NLG-919)	Meta. colorectal cancer	I/II	Recruiting	Combined with MEDI4736 (PD-L1 mAb)	NCT02318277	
	Gastric cancer	I/II	Not yet recruiting	Combined with pembrolizumab and azacitidine	NCT03182894	
	Meta. Pancreatic cancer	II	Not yet recruiting	As single agent	NCT03196232	
	NSCLC, urothelial carcinoma	II	Not yet recruiting	Combined with CRS-207 and pembrolizumab	NCT03006302	
		I	Active, not recruiting	Combined with atezolizumab (PD-L1 mAb)	NCT02298153	
	Solid tumors	I	Completed	Single agent	NCT02048709	
	IDO1 peptide	Locally advanced or metastatic solid tumors	I	Active, not recruiting	Combined with MPDL3280A (PD-L1 mAb)	NCT02471846
		NSCLC	I	Completed	As single agent	NCT01219348
		Melanoma	I	Recruiting	Combined with ipilimumab or vemurafenib (BRAF inhibitor)	NCT02077114
		Melanoma	II	Recruiting	Combined with temozolomide, imiquimod, GM-CSF and survivin peptide	NCT01543464

Table 1 (Continued)

Agent	Indication(s)	Phase	Status	Notes	NCT no.
PF-06840003	GBM or grade III anaplastic glioma	I	Recruiting	As single agent	NCT02764151
BMS986205	Cervical, DLBCL, SCCHN, UC, pancreatic, melanoma, NSCLC Advanced cancer	I	Recruiting	As single agent	NCT02658890
		I	Recruiting	Combined with nivolumab	NCT03192943

Abbreviations: DC, dendritic cell; DLBCL, diffuse large B-cell lymphoma; D-1-MT, dextrorotatory 1-methyl-tryptophan; IDO1, indoleamine 2, 3-dioxygenase 1; GM-CSF, granulocyte-macrophage colony-stimulating factor; NK, natural killer cell; GBM, glioblastoma; IL-2, interleukin-2; mAb, monoclonal antibody; NSCLC, non-small-cell lung cancer; SCCHN, squamous cell carcinoma of head/neck; UC, urothelial carcinoma; VEGF, vascular endothelial growth factor.

rate-limiting step required for conversion of Trp into Kyn is oxidative cleavage of a 2,3-indole ring double bond that forms *N*-formylkynurenine, which is almost immediately converted to *L*-Kyn. Three different Trp dioxygenases have been identified in mammals, including IDO1, tryptophan 2,3-dioxygenase (TDO) and IDO2. Human IDO1 is a monomeric heme-containing protein encoded by chromosome 8p12 and has high enzyme activity for Trp ( $K_m \sim 20 \mu\text{M}$ ).<sup>24</sup> TDO is located on chromosome 4 and forms a tetrameric heme-containing complex with lower enzyme activity for Trp ( $K_m \sim 190 \mu\text{M}$ )<sup>25</sup> compared to IDO1. IDO2 is a genetic paralog of IDO1 and is directly adjacent to IDO1 on the same chromosome.<sup>26</sup> Although it can allegedly convert Trp into Kyn, it has an almost 1000-fold lower enzyme activity ( $K_m \sim 6.8 \text{ mM}$ ).<sup>27</sup>

In addition to the differences in structure and rates of enzyme conversion, the three Trp catabolic enzymes have varying substrate specificities, tissue distribution and regulation of expression. While TDO is only capable of metabolizing the *L*-Trp isomer,<sup>25</sup> IDO1 mediates oxidative cleavage of several indole substrates, including *D*- and *L*-Trp, tryptamine, 5-hydroxy-*L*-tryptophan, serotonin and melatonin (Figure 1).<sup>28</sup> Compared to IDO1 and TDO, the exceptionally low enzyme activity of IDO2 raises the question of whether *L*-Trp is a relevant physiological substrate.<sup>24</sup> Under normal conditions, TDO is primarily expressed in the liver, placenta and brain, which likely reflects its professional role of satisfying the energetic needs required by the body. In contrast, IDO1 is detected throughout mammalian tissues at various levels, including the central nervous system, epididymis, intestine, thymus, respiratory tract, spleen, pancreas, placenta, lens, kidney, myeloid cells and endothelial cells,<sup>17</sup> and has been shown to be increased in select tissues with age.<sup>29</sup> Interestingly, IDO1 is noticeably absent from the liver. Constitutive expression of murine IDO2 mRNA and protein is detected in the liver, epididymis and brain, but little information has been reported for human IDO2 due to the lack of antibody specificity and complexity of human IDO2 transcription.<sup>30</sup> In addition to their expression in normal tissues, IDO1, TDO and IDO2 are selectively expressed in different types of human and mouse cancers.<sup>31</sup> Notably, although IDO1 and TDO are expressed at a high level in many cancer subtypes, a recent gene expression profiling study evaluating two large RNA-sequencing data sets among 31 cancer subtypes revealed negligible expression of IDO2 in the majority of human cancers (>99%), which possibly indicates that IDO2 has a less important role in supporting tumorigenesis due to its reduced expression and/or activity.<sup>30</sup>

Previous mechanistic studies demonstrated that expression of TDO is regulated by glucocorticoid hormones and dietary Trp levels,<sup>32,33</sup> whereas IDO1 is regulated and expressed in response to a variety of inflammatory stimuli, including interferon (IFN)- $\alpha$ ,<sup>34</sup> IFN- $\gamma$ ,<sup>35</sup> lipopolysaccharide (LPS),<sup>36,37</sup> interleukin-1 (IL-1), tumor necrosis factor,<sup>38</sup> CpG oligodeoxynucleotides (CpG-ODN)<sup>39</sup> and prostaglandin-E2 (PGE2).<sup>40</sup> IDO2 is also increased by treating cells with IFN- $\gamma$ , IL-10, LPS and PGE2, although its expression is less robust than that of IDO1.<sup>41</sup> Coincidentally,

activation of the aryl hydrocarbon receptor (AhR), which is a transcription factor that Kyn has been proposed to serve as a physiologically relevant ligand for,<sup>42</sup> has been shown to be upregulated through an IDO2-dependent pathway in dendritic cells (DCs).<sup>43,44</sup>

The involvement of multiple Trp-catabolizing enzymes contributes to Kyn metabolite generation and/or accumulation, which raises a potential challenge for therapeutic strategies that target this metabolic pathway. A clear question for the field is determining whether inhibiting a single player is sufficient for enhancing immune-mediated antitumor effects or whether simultaneous inhibition of all three enzymes is required. A complete answer to this question will likely depend on the following: (i) the type of cancer under investigation; (ii) expression levels and metabolic activity of IDO1, TDO and IDO2; (iii) intratumoral and serological levels of Trp and Kyn; and (iv) cellular origin of the expressed functional gene products. To date, an IDO1–TDO dual inhibitor has been discovered, although the *in vivo* relevance of this agent in antitumor therapy has yet to be revealed.<sup>45</sup> Furthermore, while addressing whether inhibition of all three Trp dioxygenases is interesting, it is important to note that the more limited anatomical expression of TDO as well as the full role of IDO2 may detract from the undeniably immunosuppressive effects of IDO1, which is a clear therapeutic priority for achieving greater immune-mediated antitumor efficacy.

#### KYN PATHWAY AND TUMOR IMMUNE ESCAPE

The relationship between cancer and elevated Trp catabolism was recognized as early as the 1950s.<sup>46</sup> Since Trp is the least abundant amino acid and must be ingested through the diet, IDO1 was originally thought to be part of an ancient, innate mechanism that was designed to slow the growth of neoplastic tissues and/or infectious agents that require Trp stores for continued metabolic activity.<sup>27</sup> In support of this hypothesis, Munn *et al.* demonstrated that female mice pregnant with allogeneic pups and treated with 1-methyl Trp (1-MT) resulted in maternal immune-mediated rejection.<sup>47</sup> Later studies of experimental autoimmune encephalomyelitis suggested that Trp catabolites and their derivatives contribute to a shift in primarily Th1-mediated disease to a Th2-associated non-pathological condition.<sup>48</sup> Collectively, these studies indicate an important role for IDO1 in general mechanisms that support immune tolerance. The role of IDO1 in immune-mediated evasion of cancer was first introduced in 2002 when Friberg *et al.*<sup>49</sup> showed that Lewis lung carcinoma (LLC) cells stimulated a more robust allogeneic T-cell response when cultured in the presence of an IDO1 inhibitor, which commensurately delayed LLC tumor growth after systemic treatment *in vivo*.

Currently, three major hypothetical mechanisms are proposed to explain the role of IDO1 in tumor-associated immunosuppression. First, enzyme activity results in local depletion of Trp, which results in an increase of uncharged transfer RNA in neighboring T cells and activation of the amino-acid-sensitive GCN2 and mTOR stress-kinase pathways.

In turn, GCN2 signaling causes cell cycle arrest and induction of anergy in responding T cells. An additional hypothesis is that downstream Kyns, including L-Kyn, 3-hydroxy-L-Kyn, 3-hydroxyanthranilate (3HAA) and quinolinic acid, have an immune modulatory effect that acts by inducing effector T-cell arrest or apoptosis, both *in vitro* and *in vivo*.<sup>50</sup> Downstream Kyn accumulation may also contribute to the conversion of naive CD4<sup>+</sup> T cells into immunosuppressive FOXP3-expressing regulatory T cells (Treg, CD3<sup>+</sup>CD4<sup>+</sup>CD25<sup>+</sup>FOXP3<sup>+</sup>) by virtue of the interaction between L-Kyn and Ahr.<sup>51</sup> It is important to note, however, that this mechanism is unlikely to represent the majority of Treg in solid tumors, since the infiltrating component is primarily thymus-derived natural Treg (nTreg)<sup>52,53</sup> combined with the absence of Ahr expression in nTreg.<sup>54</sup> Collectively, these mechanisms may play a role in contributing to the suppression of tumor immunity via IDO1 expression in cancer.

The above hypotheses are supported by several lines of experimental evidence, with the majority of observations derived from an *in vitro* cell culture-based investigation. The limitation of cell culture analyses for studying the effects of Trp depletion and/or Kyn accumulation is that there is an obvious lack of physiological relevance unless the observations can be mirrored *in vivo*. This limitation has introduced a potential form of '*in vitro* bias' that leaves researchers with a series of challenges and questions. For example, previous work has demonstrated that to inhibit T-cell proliferation *in vitro*, Trp concentrations are required to be below 0.5–1  $\mu$ M.<sup>55</sup> While this observation is interesting and scientifically well-supported, its physiological significance should be considered. Notably, plasma Trp levels range from 50 to 100  $\mu$ M in humans, and local Trp reservoirs can be rapidly replenished by diffusion and/or active transport across the large amino-acid transporter from surrounding blood vessels. The Trp depletion theory incorporates the premise that non-T cells are more resistant to Trp starvation and is partially explained by the identification of a high-affinity Trp transporter that is selectively expressed on myeloid-derived macrophages, but not in T cells.<sup>56</sup> It is not clear whether other types of IDO1-expressing cells, including tumor cells, use the same mechanism to survive Trp depletion. Further questions arise that need to be answered to determine how downstream Kyns suppresses T cells, with hypotheses suggesting that phosphoinositide-dependent protein kinase 1 (PDK1) acts as a direct target for 3HAA and 3HAA-mediated PDK1 inhibition and is responsible for the induction of type 2 T helper cell (T<sub>H</sub>2 cell) dysfunction and apoptosis.<sup>57</sup>

#### NOVEL ASPECTS OF IDO1 IN CANCER IMMUNITY

The majority of experimental data supporting a non-enzymatic immunosuppressive role for IDO1 is derived from studies in mouse plasmacytoid DCs, which is a type of professional antigen-presenting cell. In these studies, 1-MT was used as an IDO1 enzyme inhibitor to demonstrate that IDO1-mediated immune suppression was independent of Trp catabolism.<sup>58–60</sup> The mechanism of action involved two IDO1-intrinsic immunoreceptor tyrosine-based inhibitory motifs (ITIMs).



Transforming growth factor- $\beta$  (TGF- $\beta$ ) signaling caused phosphorylation of the ITIMs, which triggered noncanonical nuclear factor- $\kappa$ B (NF- $\kappa$ B) activation and phosphorylation of inhibitor for NF- $\kappa$ B subunit alpha and led to further autocrine reinforcement of IDO1 and TGF- $\beta$  expression; ultimately, this signaling led to long-term tolerance of DCs. Although this unique IDO1 signaling pathway was not inhibited by 1-MT, it was abolished in cells lacking IDO1 expression.<sup>61</sup>

The relevance of non-enzyme IDO1 activity has yet to be addressed in a cancer setting. It is therefore unclear whether IDO1 possesses the same signaling circuitry in tumor cells as has been demonstrated in DCs. Coincidentally, our recent work found that the intracranial engraftment of murine glioblastoma cells into syngeneic immunocompetent mice resulted in decreased tumor-infiltrating Treg ( $P < 0.01$ ) and increased animal subject survival ( $P < 0.001$ ) when the brain tumor cells were silenced for IDO1 expression with stably expressing small hairpin RNA.<sup>62</sup> Notably, IDO1-silenced tumor cells kill animal subjects similar to IDO1-wild-type control cells when the cells are engrafted intracranially into mice deficient for either CD4<sup>+</sup> and/or CD8<sup>+</sup> T cells, which suggests that the mechanism regulating survival from tumor cells requires the suppression of IDO1 expression and is immune system-dependent. Interestingly, these outcomes were independent of the IDO1 effects on the Trp and Kyn levels mediated by tumor cells.<sup>63</sup> Instead, the majority of IDO1 metabolism was mediated by non-tumor cells of the engrafted intracranial glioblastoma. Also, IDO1 metabolism did not decrease the endogenous Trp levels within the brain tumor compared to a naive mouse brain without tumor cells. Collectively, these data suggest that IDO1 in tumor cells and non-tumor cells possess different functions that are non-overlapping, which infers a potential difference in targetability with the current generation of IDO1 inhibitors primarily focused on enzyme activity.

One recent study revealed a novel role for IDO1 in which it affects tumor repopulating cell survival via induction of the tumor dormancy program.<sup>64</sup> Unexpectedly, IFN $\gamma$  treatment of differentiated tumor cells led to higher rates of apoptosis via STAT1-dependent signaling. By contrast, when IDO1 and AhR were co-expressed in tumor cells with IFN $\gamma$  treatment, STAT1 signaling was inhibited, which led to suppression of cell death and activation of the tumor cell dormancy program. Mechanistically, IFN $\gamma$  induced high IDO1 and AhR expression as well as increased the Trp transporter levels in tumor-repopulating cells, which did not occur in differentiated cells. Mechanistically, IDO1/AhR pathway activation upregulated the cell cycle inhibitor p27, which diverted tumor-repopulating cells from the pro-apoptotic STAT1-dependent pathway toward the survival dormancy program. Therapeutically, L-1-MT-driven IDO1 enzyme inhibition diminished IFN $\gamma$ -induced dormancy and suppressed tumor growth *in vitro* and *in vivo*. This latest finding further highlights the multi-faceted role of IDO1 in tumorigenesis and its complex mechanism of action with respect to cancer immunotherapy.

## IDO1 AND INNATE IMMUNE MODULATION IN THE TUMOR MICROENVIRONMENT

Over the last decade, interactions between IDO1 and a large range of immune modulators have been discovered. CpG-ODN induces expression of IDO1 through toll-like receptor 9 activation,<sup>39</sup> with similar increases of expression by DNA nanoparticle-mediated activation of the stimulator of IFN genes (STING) adaptor pathway coupled with type I IFN (IFN- $\alpha/\beta$ ) signaling.<sup>65</sup> STING-mediated IDO1 induction in the setting of tumor immune evasion and tumor progression was demonstrated using a STING knockout mouse with engrafted LLC cells.<sup>66</sup> Given the promising results of STING agonists in preclinical tumor immunotherapy<sup>67</sup> and the potential commensurate induction of IDO1 via STING activation, it has become critical to evaluate STING-targeted cancer therapies for their potential synergistic potential with IDO1 inhibitors.

Another mechanism resulting in increased IDO1 expression is mediated through cell apoptosis and/or necrosis, which is a pathological hallmark in many cancers. The balance between immunogenic and tolerogenic cell death determines the outcome of the immune response within the tumor microenvironment.<sup>68</sup> IDO1 appears to play a significant role in maintaining tolerance of apoptotic cells by the following: (i) altering the phenotype of macrophages and neighboring cells through upregulation of IL-10 and TGF- $\beta$  as well as inhibition of IL-1; (ii) inducing phenotypic changes of local cross-presenting DCs; and (iii) recruiting Tregs.<sup>69–71</sup> Furthermore, a subcutaneous injection of apoptotic tumor cells causes activation of IDO1, which induces suppressive phosphatase and tensin homolog (PTEN)-expressing Tregs<sup>72</sup> and indicates that there is a potential role for IDO1 in apoptotic tumor cell-induced immune suppression.

Myeloid-derived suppressor cells (MDSCs) have been recognized as an important group of heterogeneous mediators in cancer that convey potent immunosuppressive effects on T cells.<sup>73</sup> Recent studies have demonstrated that IDO1 is highly induced in tumor-infiltrating MDSCs and is responsible for MDSC-associated activation and/or recruitment of Tregs in human breast cancer, sarcoma and chronic lymphocytic leukemia.<sup>74–76</sup> In addition to its role in immunotolerance, studies utilizing a mouse melanoma model also revealed that tumor-expressed IDO1 recruits and activates MDSCs through a Treg-dependent mechanism,<sup>77</sup> which demonstrates the functional versatility of IDO1 in MDSC-associated immunoevasion.

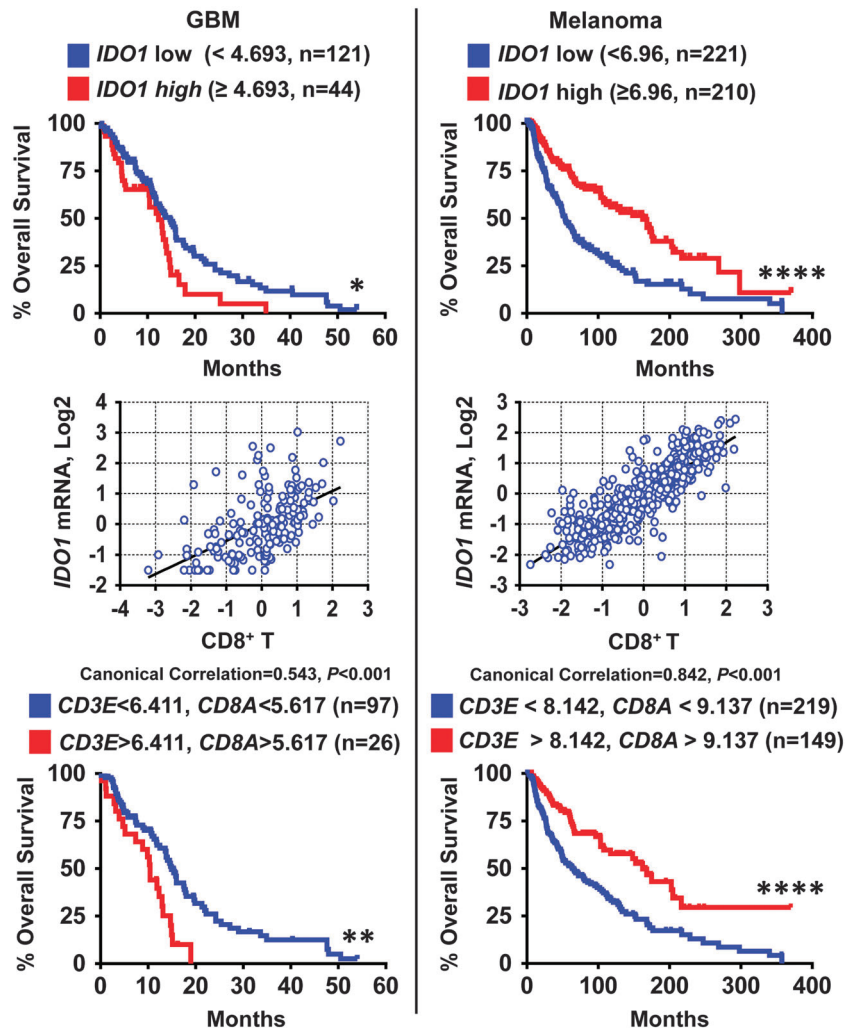
IDO1 is also implicated in the inhibition of T-cell-dependent complement system activation, which was initially reported in an early study of allogeneic mouse fetal rejection.<sup>78</sup> However, the linkage between IDO1 and the complement system in cancer was not discovered until recently. In a study of intracranial mouse glioblastoma, combination radiation and chemotherapy mediated extensive complement deposition when non-brain tumor cell IDO1 was targeted and/or inhibited through pharmacological and/or genetic methods, respectively. Importantly, complement deposition was mechanistically required for the pro-survival effect of an IDO1 pathway inhibitor.<sup>18</sup> Given that we previously demonstrated that non-glioblastoma cells are the predominant mediators of

IDO1-dependent Trp catabolism,<sup>8</sup> the data collectively suggest that metabolically active IDO1 becomes targetable when other forms of cytotoxic therapy are used synergistically and highlights the IDO1-dependent non-tumor cell mechanisms that contribute to immunosuppression in solid tumors.

### IDO1 AND OTHER KEY IMMUNE CHECKPOINTS

An increasing number of recognized immune checkpoints act to coordinately influence the local tumor-immune environment. To obtain the maximal therapeutic benefit with combination approaches that incorporate multiple forms of immunotherapy, a critical question is how IDO1 inhibition will interact with other key modulators of tumor-induced immune

suppression (that is, CTLA-4 and/or PD-1 blockade)? Despite some preclinical cancer studies showing synergy when combining pharmacological IDO1 and CTLA-4/PD-1 blockade,<sup>13,14,79</sup> the molecular mechanism of this relationship remains largely unknown. It has been reported that Treg cell-expressed CTLA-4 upregulates IDO1 expression by DCs,<sup>80</sup> and there is reciprocal activation of Treg cells. In addition, IDO1 upregulates PD-1 expression on Tregs, which contributes to the maintenance of PTEN activity.<sup>72</sup> One of our recent studies also demonstrated that treatment with a dual blockade of CTLA-4 and PD-L1 in glioblastoma-bearing mice resulted in increased tumor *IDO1* mRNA expression commensurate with elevated transcripts of *CD3e*, *CD8α* and *IFNγ*,<sup>63</sup> possibly suggesting that



**Figure 2** The Cancer Genome Atlas analysis reveals distinct correlations between patient survival, *IDO1* transcript levels and markers for tumor-infiltrating lymphocytes between glioblastoma (GBM) and melanoma. Top panel: Kaplan–Meier analysis is based on the mRNA expression level of *IDO1* in GBM (left column) and melanoma (right column). Expression of *IDO1* is divided into low (blue) and high (red) groups as determined by the indicated cutoff value (calculated by Cutoff Finder, Supplementary Material and Methods). The sample size of each group is listed in the parenthesis. Middle panel: canonical correlation analysis (Supplementary Material and Methods) between *IDO1* and tumor-infiltrating CD8<sup>+</sup> T lymphocytes within GBM (left column) and melanoma (right column). Bottom panel: Kaplan–Meier analysis based on the mRNA expression level for the CD8<sup>+</sup> T cell marker genes *CD3E* and *CD8A* in GBM (left column) and melanoma (right column). Expression of *CD3E* and *CD8A* are divided into low (blue) and high (red) groups, which were determined by the indicated cutoff value (calculated by Cutoff Finder, Supplementary Material and Methods). The patient sample size for each group is listed in parentheses. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\*\* $P < 0.0001$ .

tumor-infiltrating effector T cells (CD3<sup>+</sup>CD8<sup>+</sup>) activated by immune checkpoint inhibition increase *IDO1* expression via IFN $\gamma$  stimulation. Our recent analysis of the cancer genome atlas supports this hypothesis and found a correlation between increasing *IDO1* levels with other immune checkpoints, including *PD-1*, *PD-L1*, *PD-L2*, *CTLA-4*, signal transducer and activator of transcription 3, CD39, B- and T-lymphocyte attenuator, lymphocyte-activation gene 3 and FoxP3 in surgically resected human glioblastoma.<sup>81</sup> Taken together, the data suggest that as *IDO1* expression increases in tumors, so do other immune checkpoints. It is therefore possible that combination strategies targeting multiple immune checkpoints may lead to greater synergistic effects in cancer immunotherapy, although the enhancement of host toxicity may also increase as well.

### IDO1 IN DIFFERENT CANCER TYPES: FUNCTIONAL DIVERSITY?

Since the discovery of increased *IDO1* levels in human cancer, there have been several reports correlating *IDO1* expression with poor patient prognosis, including those diagnosed with acute myeloid leukemia,<sup>82</sup> colorectal cancer,<sup>83</sup> non-small-cell lung cancer,<sup>84,85</sup> prostate cancer,<sup>86</sup> ovarian carcinoma,<sup>87,88</sup> endometrial cancer<sup>89</sup> and esophageal cancer.<sup>90</sup> Unexpectedly, high *IDO* expression levels in renal cell carcinoma and hepatocellular carcinoma patients are correlated with better survival outcomes.<sup>91–93</sup> The complexity of outcomes associated with utilizing *IDO1* expression as a stratifying factor for cancer patient prognosis likely reflects the complexity of *IDO1* expression, regulation and functional effects within different types of human cancer. In support of this concept, our analysis of The Cancer Genome Atlas reveals that distinct *IDO1* gene expression correlates with overall survival when comparing patients diagnosed with glioblastoma and melanoma.<sup>81</sup> As shown in Figure 2, higher *IDO1* transcript levels correlate with decreased glioblastoma patient survival, which is diametrically opposed to the correlation between increased *IDO1* mRNA levels and its association with increased survival in melanoma patients. These results are somewhat surprising given the preclinical work suggesting that *IDO1* inhibition synergizes with *CTLA-4* blockade to mediate rejection of mouse melanoma.<sup>14</sup> Further analysis demonstrates that *IDO1* expression positively correlates with gene expression markers for CD8<sup>+</sup> cytolytic T and Tregs, which are both found in glioblastoma and melanoma. Although there is a difference between these cancers regarding their correlation with *IDO1* expression and survival, both diagnoses appear to have a strong correlation between the presence of intratumoral T cells and increased *IDO1* expression. This led us to ask whether there is also a difference between T cell infiltration and survival outcomes. Whereas higher gene expression for cytolytic T cell markers was associated with decreased glioblastoma patient survival (Figure 2), the opposite trend was true for melanoma patients, which reflects the results of published studies.<sup>94,95</sup>

While these data are straightforward for patients diagnosed with glioblastoma, in regard to providing a rationale for

including *IDO1* adjuvant therapy in treatments that enhance T cell-mediated *IDO1* expression increases, there are many questions about melanoma, including the following: (i) Does *IDO1* play a negative role in the tumors of human patients with malignant skin cancer? (ii) Why does increased *IDO1* expression correlate with increased patient survival? (iii) Will the threshold for immunotherapeutic intervention be different between patients diagnosed with glioblastoma and melanoma? (iv) What is the composition of *IDO1* expression by tumor and stromal cells among different malignant subtypes? (v) If *IDO1* possesses different functions among distinct cell types, do these differences contribute to the differences in outcomes between *IDO1* expression and patient survival when glioblastoma and melanoma are compared?

### IDO1 INHIBITORS IN CANCER IMMUNOTHERAPY

To date, *IDO1* inhibitors have been designed, screened, and tested in preclinical models of disease (reviewed in Vacchelli et al.<sup>15</sup>; Röhrig et al.<sup>96</sup>). Currently, no *IDO1*-targeting agent is approved by the Food and Drug Administration as a standalone cancer therapeutic. However, the results of recent phase I–II studies suggest that the *IDO1* pathway modulator indoximod (D-1-MT), the best-in-class *IDO1* enzyme inhibitor INCB024360 (Epacadostat), and the *IDO1* vaccine are well tolerated by cancer patients.<sup>97–101</sup> With confirmation that targeting *IDO1* is safe and well tolerated, the number of trials evaluating *IDO1* inhibition in cancer therapy continue to grow (Table 1). Consistent with preclinical evaluation, the objective response rates for the non-enzyme-targeting *IDO1* pathway inhibitor indoximod has yielded objective response rates of 10–18%.<sup>101,102</sup> Combined with other immunotherapeutics, such as *PD-1*/*PD-L1* or *CTLA-4* inhibitors, this value ranges from 10 to 57% among different cancer types.<sup>103,104</sup> One potential explanation for this wide range of variable response rates is based on the complexity of *IDO1* functions among different cancer types, which suggests that the elucidation of *IDO1* in different cancer subtypes is imperative for its efficacy as a therapeutic target.

Precision medicine initiatives that tailor targeted therapy against *IDO1* may enhance the effectiveness of treatment, but this ideological concept still requires verification in clinical trials and across cancer diagnoses. This effect may also be a moving target since immunotherapies that enhance T-cell infiltration may also increase immunosuppressive molecule expression and activity. Furthermore, to effectively evaluate *IDO1* inhibitors, target validation, pharmacodynamic properties on Trp and Kyn levels, as well as their impact on conformational activity and protein stability, are critical future requirements. While it is easy to measure the Trp and Kyn levels *in vitro*, quantification of *IDO1* metabolism is more challenging *in vivo*. Recent developments for noninvasive *in vivo* metabolite imaging may provide a solution for this technical hurdle.<sup>105,106</sup> It should be noted, however, that systemic Trp levels can be affected by *TDO*, which is expressed in the liver constitutively and induced in some types of cancer,<sup>42</sup> suggesting that evaluation of this amino acid is not necessarily a sole reflection of *IDO1* enzyme activity. Finally,



understanding how IDO1 works in cancer cells, versus non-cancer cells, in terms of the enzyme and signal transduction properties, is essential for targeting the full effects of this pleiotropic mediator of immune suppression.

### CONCLUDING REMARKS

Substantial knowledge of IDO1 and its role in cancer has been generated over the past two decades. However, new questions continue to be raised regarding its full spectrum of function(s). Similar to the astrological description of Gemini, the Trp catabolic function of IDO1 appears to be one feature of a multifunctional player. Cell lines that express IDO1, but do not catabolize Trp, have become important tools for recognizing this phenomenon. Similarly, the IDO1 pathway inhibitor indoximod (D-1-MT), which does not convert Trp to Kyn,<sup>107,108</sup> but is a potentially important and clinically meaningful treatment for cancer patients, has further highlighted the possibility that the non-enzyme activity of IDO1 is a relevant target in cancer immunotherapy. To address some of the questions raised earlier in this review, our group is currently constructing three novel transgenic mouse models that (i) possess a point mutation that nullifies IDO1 enzyme activity; (ii) have a 2-TA linker connecting the C terminus of endogenous IDO1, with an eGFP reporter; and (iii) contain a floxed STOP codon upstream of FLAG-tagged IDO, for future knock-in experiments under tissue-specific promoters. It is our hope that ongoing work by our team and others will answer some of the unanswered questions surrounding IDO1 in malignant cancers and, perhaps, other diseases as well.

### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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