Clinical Cancer Research

Molecular Pathways: Targeting IDO1 and Other Tryptophan Dioxygenases for Cancer Immunotherapy

Lijie Zhai[1], Stefani Spranger[2], David C. Binder[2,3], Galina Gritsina[1], Kristen L. Lauing[1], Francis J. Giles[4,5,6], and Derek A. Wainwright[1,7]

Abstract

Indoleamine 2, 3-dioxygenase 1 (IDO1), IDO2, and tryptophan 2, 3-dioxygenase (TDO) comprise a family of enzymes that catalyze the first- and rate-limiting step associated with the catabolic conversion of tryptophan (Trp) into kynurenine (Kyn). Through subsequent enzymatic and spontaneous reactions, Kyn is further converted into the energetic substrates, NAD^+ and ATP, to fuel cellular metabolic functions. Coincidently, the depletion of Trp and accumulation of Kyn has been demonstrated to induce effector T-cell apoptosis/dysfunction and immunosuppressive regulatory T-cell induction, respectively. Similar to other immune checkpoints, IDO1 and TDO are suggested to be important targets for immunotherapeutic intervention. This is represented by the recent growth of efforts to inhibit the Trp-to-Kyn pathway as a means to control immunosuppression. Inhibitors currently in clinical trials, INCB024360, GDC-0919, indoximod, and an IDO1 peptide-based vaccine, are being evaluated for their efficacy against a wide range of cancers including melanoma, glioblastoma, non–small cell lung, pancreatic, and/or breast cancer, as well as metastatic disease. Despite the rapid development of potent clinical grade inhibitors, strategic questions remain. Here, we review the state of the literature with respect to current therapeutic inhibitors of tryptophan catabolism, evaluation of those efforts preclinically and clinically, compensatory changes that occur with therapeutic targeting, as well as newly recognized signaling features that raise critical questions to the field. Given the rapidly evolving interest in determining how IDO1/TDO, and to an unknown extent, IDO2, can be targeted for increasing cancer immunotherapeutic efficacy, we present a brief but comprehensive analysis that addresses critical questions, while highlighting the mechanics that remain to be explored. *Clin Cancer Res; 21(24); 5427–33. ©2015 AACR.*

Background

Cancer immunology and immunotherapy

The immune system is composed of an immediate-acting innate arm comprised principally of granulocyte- and myeloid-lineage cells that quickly respond to cues of inflammation and/or injury, in addition to an adaptive arm, principally comprised of B and T cells that provide specificity and memory. Under normal circumstances, these immunologic arms are mutually dependent on one another for providing defense against infection, injury, and/or malignancy. T cells, which primarily mature following immunologic challenge(s), include $CD4^+$ and $CD8^+$ T lymphocytes that express a wide variety of cytokines based on the context of priming stimuli. Included in the $CD4^+$ T-cell compartment are highly immunosuppressive regulatory T cells (Treg; $CD4^+CD25^+FoxP3^+CTLA\text{-}4^+$) that mature naturally in the thymus (nTreg) or are post-thymically induced from naïve $CD4^+Foxp3^-$ cells into Foxp3-expressing cells (iTreg; refs. 1–3). With respect to solid cancer(s), immunosuppressive mechanisms utilized to evade antitumor immunity include Treg accumulation (4, 5) effector T-cell expression of the PD-1 receptor (6), as well as high PD-L1 levels that localize to multiple types of cells in the tumor microenvironment (7, 8). Therefore, an active effort both clinically and preclinically is needed to develop strategies that reactivate a productive antitumor effector T-cell response, while simultaneously inhibiting immunosuppressive mechanisms.

Recent studies have demonstrated great promise at targeting immunosuppression in cancer, including clinical trials aimed at inhibiting PD-1, PD-L1, and/or CTLA-4 in patients diagnosed with late-stage melanoma, non–small cell lung cancer, and/or renal cell cancer (9–12). Follow-up studies have also shown that the benefit of combined PD-1/CTLA-4 inhibition is not restricted to those patients previously treated with systemic therapy (13). Preclinical work using multiple tumor models in immunocompetent mice further confirms that these immune checkpoint–targeted therapies require effector T cells for antitumor activity, with several studies reporting a coincident neutralization of tumor-infiltrating Treg (14–16). These clinical studies, combined with extensive preclinical validation of combinatorial approaches

[1]Department of Neurological Surgery, Northwestern University Feinberg School of Medicine, Chicago, Illinois. [2]Department of Pathology, The University of Chicago, Chicago, Illinois. [3]Committee on Cancer Biology, The University of Chicago, Chicago, Illinois. [4]Northwestern Medicine Developmental Therapeutics Institute, Northwestern University, Chicago, Illinois. [5]Division of Hematology and Oncology, Northwestern University, Chicago, Illinois. [6]Robert H. Lurie Comprehensive Cancer Center of Northwestern University, Chicago, Illinois. [7]Northwestern Brain Tumor Institute, Northwestern University Feinberg School of Medicine, Chicago, Illinois.

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Corresponding Author: Derek A. Wainwright, 300 E Superior Street-Tarry Bldg 2-703, Chicago, IL 60611. Phone: 312-503-3161; Fax: 312-503-3552; E-mail: derekwainwright@northwestern.edu

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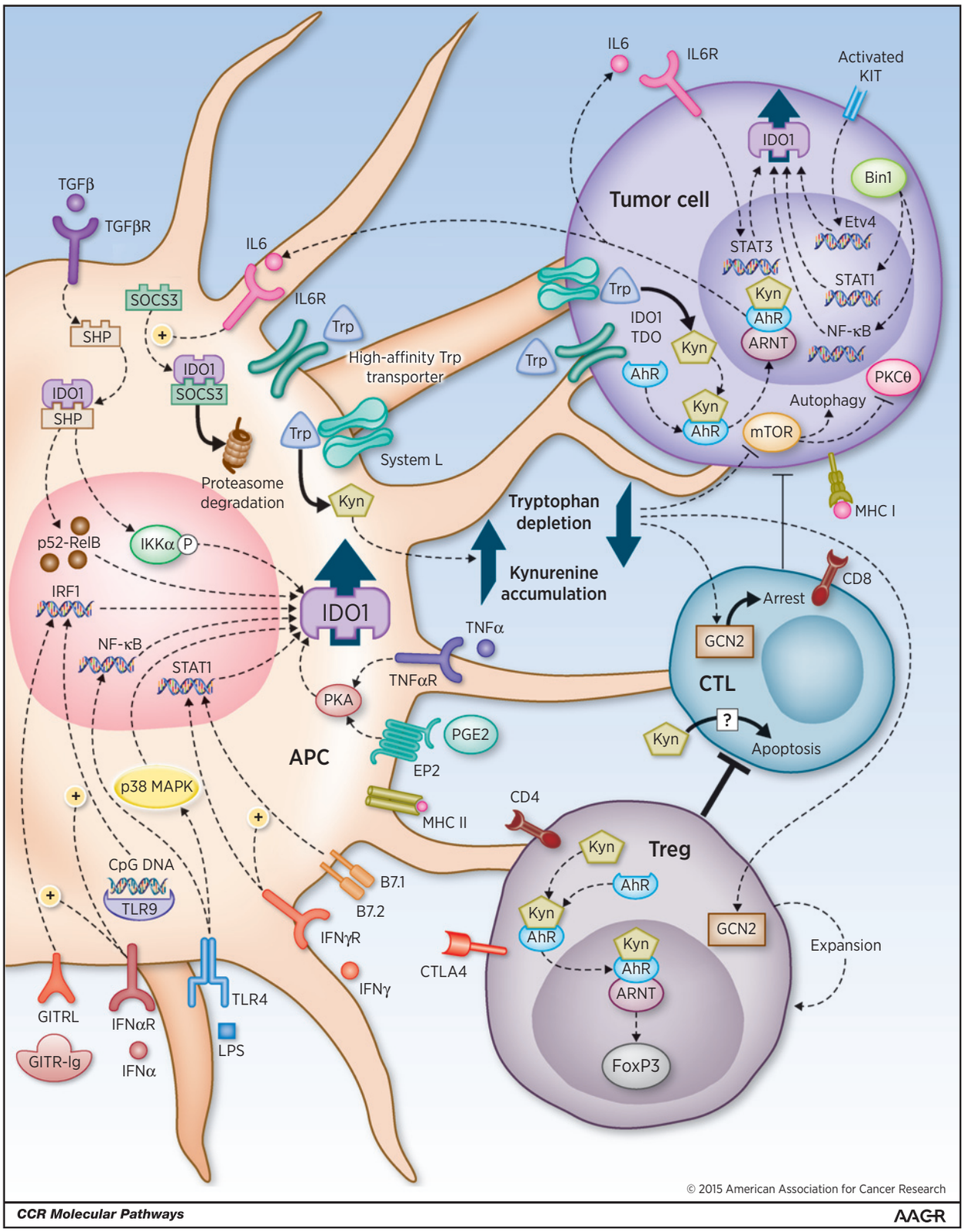

Figure 1.
Signaling pathways associated with tryptophan (Trp) dioxygenases and cancer. The high expression of active IDO1 leads to a commensurately high rate of tryptophan conversion and depletion. (*Continued on the following page.*)

confirm that immunotherapy is a high-value strategy for treating patients with aggressive and immunosuppressive malignancies.

IDO1, TDO, and the Trp→Kyn catabolic pathway

$_{L}$-Tryptophan ($_{L}$-Trp) is used in a variety of anabolic/catabolic processes and metabolized into serotonin, melatonin, protein, and Kyn. IDO1 and TDO are the primary enzymes that catalyze the rate-limiting cleavage of the Trp indole ring 2,3-double bond and incorporation of molecular oxygen. The product of this reaction is *N*-formylkynurenine, which is rapidly and spontaneously converted into $_{L}$-Kyn. The latter catabolite is further converted into downstream intermediates, including 3-hydroxy-$_{L}$-kynurenine (3-HK), 3-hydroxyanthranilate (3-HAA) and quinolinic acid (Quin), which also impact immune responses (17).

Although IDO1 and TDO both catabolize Trp, their quaternary structures (18, 19), expression in normal versus transformed tissue (20, 21) and regulation (22, 23) are quite distinct. While monomeric IDO1 acts on a broad range of substrates and is capable of cleaving both $_{D}$- and $_{L}$-Trp, homotetrameric TDO is enantiomer-specific and only catabolizes $_{L}$-Trp (24). IDO1 expression in adults is relatively limited to lymphoid tissues and placenta (20), whereas TDO is constitutively expressed in liver and brain (25, 26), likely reflecting their primarily immunomodulatory or energy regulating roles, respectively. Until 2007, IDO1 was the only known indoleamine dioxygenase acting at the 2, 3 double bond. Three independent groups then identified the novel paralog, IDO2 (27–29). While the *IDO1* and *IDO2* genes are 43% homologous and found directly adjacent to one another on chromosome 8, the K_m of human IDO1 and IDO2 for $_{L}$-Trp is 20.90 ± 3.95 μmol/L and 6,809 ± 917 μmol/L, respectively, indicating a substantial decrease in activity for the latter enzyme (30). This is particularly interesting given that the residues required for tryptophan catalytic activity are present in both gene products (27). Also notable is that mouse IDO2 has been shown to possess higher enzymatic activity than the human homolog, although the genetic depletion of mouse IDO2 has no impact on systemic Kyn levels (31), a dramatic contrast to the impact of IDO1 deficiency (32).

IDO1 and the stress response

Because of IDO1 expression induced in response to infection, it was originally thought that it serves as an innate immune effector to restrict the amount of Trp required for microbial growth (33). This initial hypothesis was revised by Munn and colleagues, who demonstrated that the *in vivo* administration of an IDO1 inhibitor, 1-methyl tryptophan (1-MT), led to T-cell–dependent fetal allograft rejection (34). Subsequent work demonstrated that IDO1-expressing macrophages, dendritic cells (DC), and tumor cells mediate the inhibition of T-cell proliferation (35–38). IDO1 responses were found to be mediated by downstream stress–response pathways including general control non-depressible 2 (GCN2) and mTOR; both important regulators that sense amino acid sufficiency (Fig. 1). The GCN2 pathway is activated when amino acid deficiency increases overall uncharged tRNA levels, resulting in GCN2 kinase phosphorylation of the alpha subunit of translation initiation factor 2 α (eIF2α) and subsequent inhibition of translation. It was first discovered that GCN2-activated plasmacytoid DC could suppress T-cell proliferation *in vivo* by an IDO1-dependent mechanism (39). It was later discovered that the genetic deletion of IDO1, but not GCN2, prevented skin carcinogenesis in a mouse papilloma model, suggesting that additional critical pathways were downstream of IDO1 activity (40). In support of these findings, Metz and colleagues identified that IDO1-mediated Trp depletion suppressed mTOR, a critically important immunoregulatory kinase (40) that could be reactivated by treatment with $_{D}$-1-MT, a Trp mimetic, *in vitro*.

IDO1-mediated suppression of T-cell activity is hypothesized to rely on the depletion of free Trp. This premise requires cell-specific transport mechanisms that include both the transporter System L, which shuttles Trp and other large hydrophobic amino acids through a low-affinity (K_m = 20–30 μmol/L; ref. 41) interaction, as well as through an independent high-affinity (K_m = 200–300 nmol/L) interaction. Interestingly, the high-affinity transporter is upregulated in differentiated myeloid-derived macrophages (MDM) but not in T cells. In support of the requirement for transport, both Trp and the competitive inhibitor, $_{L}$-1-MT, inhibit Trp uptake into cells, collectively suggesting that competitive IDO1 inhibitors target the transporter and enzyme, simultaneously.

Regulation of IDO1/IDO2/TDO

The literature is replete with redundant pathways that lead to IDO1 expression and activity. Proinflammatory signals including IFNγ, CpG DNA, and LPS are potent inducers of IDO1 expression (33, 42–44). Cytokines, including TNFα, IL6, and IL1β, synergize with each other to dramatically increase IDO1 expression. Other IDO1 modulators include soluble GITR, prostaglandin E2, the oncogene, c-Kit, as well as the tumor suppressor, Bin1 (45). Interesting new data suggests that Wnt5α also mediates IDO1 activity through β-catenin signaling in DC (46), while maintaining continuous expression through an AhR–IL6–STAT3 signaling loop in some cancer cell lines (47). Thus, based on the large number of pathways that modulate and/or sustain IDO1

(*Continued.*) This induces cell-cycle arrest and/or anergy in the effector cytotoxic lymphocyte (CTL) compartment via the eIF2a kinase-dependent GCN2 pathway. Simultaneously, this mechanism also contributes to the activation/maturation of Treg in association with CTLA4-mediated CD80/CD86 coinhibition. Kynurenine (Kyn) directly induces the apoptosis of CTL by an uncharacterized mechanism, while interacting with the aryl hydrocarbon receptor (AhR) in naïve $CD4^+$ T cells, resulting in the induction of $FoxP3^+$ iTreg. AhR interacts with the aryl hydrocarbon receptor nuclear translocator (ARNT) to mediate the specific transcriptional programming. Coincidently, IDO1 nonenzymatically enforces immunosuppression through two intrinsic immunoreceptor tyrosine-based inhibitory motifs (ITIM) in antigen-presenting cells (APC). TGFβ signaling results in the phosphorylation of the IDO1 ITIM, triggering noncanonical NF-κB activation and phosphorylation of IKKα, followed by nuclear translocation of the NF-κB subunits, p52 and RelB and autocrine reinforcement of IDO1 and TGFβ expression. The high-affinity Trp transporter is expressed by APC and tumor cells, with the majority of agonists leading to IDO1 activity demonstrated in APC. Similarly, the oncogene, c-KIT, and tumor-suppressor gene, Bin1, as well as the IL6/AhR/STAT3 signaling loop, have also been shown to impact the regulation of IDO1 in tumor cells. (39, 69–76). Notably, in the presence of IDO1/TDO, Kyn accumulation simultaneously contributes to Treg activation, promoting the disabling and/or apoptosis of CTL, thereby supporting tumor outgrowth by virtue of an unproductive antitumor response. Bin1, Myc box–dependent-interacting protein 1; EP2, prostaglandin receptor E2; Etv4, ETS translocation variant 4; GCN2, general control non-derepressible 2; GITRL, glucocorticoid-induced TNFR-related protein ligand; IRF1, IFN regulatory factor 1; IFNαR, IFNα receptor; IL6R, IL6 receptor; LPS, lipopolysaccharide; PGE2, prostaglandin E2; PKA, protein kinase A; PKCq, protein kinase C theta; SHP, SH2 domain containing protein tyrosine phosphatase; SOCS3, suppressor of cytokine signaling 3; TLR4, toll-like receptor 4; TNFαR, TNFα receptor.

expression/activity, the direct targeting of IDO1, rather than pathways that are up- or downstream, will likely be the most effective modality for controlling the overall impact mediated by this Trp dioxygenase.

Similar to IDO1, TDO mRNA expression has also been found in human tumors (21). Dominant factors that affect TDO expression and/or activity include sex steroid hormones (48) and glucocorticoids (22). New preclinical data also suggest that tumor-infiltrating T cells may regulate TDO expression based on findings from intracranially injected syngeneic murine brain tumors grown in $Rag1^{-/-}$ mice (15). Notably, intraperitoneal injection of mastocytoma cells overexpressing TDO induces potent immunosuppression that can be reversed with a pharmacologic inhibitor of enzymatic activity, leading to immune-mediated tumor rejection ($P < 0.001$; ref. 21).

In contrast, the newest member of the tryptophan catabolic family, IDO2, has yet to be confirmed as a critical contributor to Kyn accumulation and tumor immunity. Notably, while mouse IDO2 possesses some capacity for Trp→Kyn conversion, the human ortholog is devoid of the same enzymatic capacity at physiologic Trp levels (30). Furthermore, transcriptome analysis of 129 human tumor samples and 25 human tumor cell lines has demonstrated limited IDO2 expression (49). As IDO2 was originally cloned from the liver (27), it is still unknown whether there are IDO2 splice variants specific to subtypes of differentiated and/or transformed tissues.

IDO1 and inflammation in tumors

The interactions among inflammation, IDO1, and cancer (50, 51) are noteworthy and raise critical questions regarding how and when to optimally target tryptophan catabolism for therapeutic purposes. Furthermore, despite the presence of antigen-specific T cells within the microenvironment, tumors often escape, immunologically, without loss of antigen expression or presentation (MHC molecule) capacity. This effect is mediated, in-part, through the induction, upregulation, and/or enhanced participation of immunosuppressive T-cell–impairing ligands, CTLA-4 and PD-L1 (52). Similar to PD-L1, IDO1 expression also increases through a response to IFNγ released in the tumor microenvironment (53) as a potent compensatory mechanism contributing to the resistance of productive antitumor immunity (54). Interestingly, only a subset of patients have a T-cell–infiltrating presence within the tumor microenvironment, an observation reported for head and neck and bladder cancer, as well as melanoma, lung adenoma, and glioblastoma (55). A notable observation from those patients treated with the immune checkpoint inhibitor PD-1, correlates a high degree of clinical response to the pre-existence of tumor-infiltrating T cells (56). This observation, paired with the association of IDO1 induction by T-cell–derived IFNγ, leads to the hypothesis that IDO1 inhibitors will be most effective against T-cell–inflamed tumors, either *de novo* or caused by immunotherapeutic intervention. Preclinical studies support this hypothesis, establishing evidence that combinatorial immune checkpoint blockade and IDO1 pathway inhibition provide potent reactivation of tumor-infiltrating T cells and/or decreased tumor-resident immunosuppressive regulatory T cells ($P < 0.01$; refs. 15, 57).

Clinical–Translational Advances

No IDO1 inhibitor is currently approved by the FDA. However, results of recent phase I–II studies suggest that indoximod ($_D$-1-MT), INCB024360, and/or IDO1-targeting vaccines are well tolerated by cancer patients, with clinical anticancer effects in a subset of patients (58, 59). Notably, the number of clinical trials focused on IDO1 has recently grown in size, with many coupling multiple modalities to test the combinatorial benefit (Table 1). These recent reports, in addition to preclinical data suggest that combining tryptophan enzyme targeting with chemotherapy, radiotherapy, and/or immunotherapy may be an effective tool against a wide range of malignancies.

The seminal observation associating IDO1, immunosuppression, and cancer utilized a polyclonal antibody to identify the immunohistochemical frequency of expression among different human malignancies (60). Unexpectedly, recent analyses utilizing a novel monoclonal anti-human IDO1 antibody have demonstrated distinct differences compared with those original observations (20). While it was initially reported that 90% to 100% of human prostate and pancreatic tumors, as well as glioblastoma, were IDO1 positive, the latter study found only 42%, 38%, and 8% of those malignancies positive, respectively. As the antibodies were well vetted in both investigations, these conclusions present a cautionary tale that likely reflects more than simple differences in antibody specificity, but more broadly, the potential for alternative splice variants and/or posttranslational modifications resulting in antigenic variation. Thus, immunohistochemical studies associating IDO1 expression and survival should be interpreted carefully (61). Furthermore, these conflicting findings complicate strategies that would ideally use IDO1 IHC as a prognostic tool for selecting patients who would benefit most from IDO1 inhibition.

Recent work studying the Kyn/Trp ratio in patients with glioblastoma has suggested that analyzing a time point well after surgical tumor resection of 10+ weeks following the procedure, may be prognostically valuable to clinicians planning to enroll patients in immunotherapy trials (62). While this finding requires further validation in a larger patient cohort, it suggests the possibility that IDO1 activity increases well after glioblastoma patients are operated on, as well as highlighting the potential relevance of using a clinical inhibitor against IDO1 systemically. Similarly, the Kyn/Trp ratio was recently validated as a prognostic tool in cervical cancer patients whereby low Trp levels indicated a tumor size greater than 4 cm and metastatic spread to the lymph node (63). Accordingly, high Kyn/Trp ratios in patient sera were associated with lymph node metastasis, FIGO stage, tumor size, parametrial invasion, and poor disease-specific survival, further suggesting the relevance of IDO1 targeting based on a tryptophan catabolic signature. Similar work was recently shown in a clinical study that identified higher Kyn/Trp ratios in T-cell lymphotropic virus type-1 asymptomatic carriers when compared with healthy controls (64). Importantly, the serum Kyn/Trp ratio was a significantly independent detrimental prognostic factor in patients with adult T-cell leukemia/lymphoma. These collective analyses have begun to elucidate the relevance of determining an IDO1 enzymatic "signature" in patient sera, which preliminarily appears to be both prognostically valuable and clinically informative.

Given that the majority of clinical studies aimed at IDO1 inhibition that are currently ongoing have yet to report results, we can gain insight into preclinical analyses that have shown great potential in targeting this immunosuppressive mediator. However, these models possess limited usefulness when considering the potential effects that standard-of-care treatment have on IDO1 activity and/or expression, as well as the potential change of

Table 1. Ongoing and historical clinical trials that target tryptophan catabolism in cancer

Agent	Indication(s)	Phase	Status	Notes	Identifier
Indoximod ($_D$-1-MT)	Metastatic solid tumor	I	Completed	Combined with docetaxel	NCT01191216
	Solid tumor	I	Completed	As single agent	NCT00567931
		I	Terminated	As single agent	NCT00739609
	Malignant glioma	I/II	Recruiting	For recurrent glioma patients	NCT02052648
	Metastatic breast cancer	I/II	Active, not recruiting	Combined with vaccine	NCT01042535
		II	Recruiting	Combined with docetaxel	NCT01792050
	Melanoma	I/II	Recruiting	Combined with ipilimumab	NCT02073123
	Metastatic adenoma of pancreas	I/II	Recruiting	Combined with gemcitabine and nab-paclitaxel	NCT02077881
	Prostate carcinoma	II	Recruiting	Combined with sipuleucel-T	NCT01560923
	NSCLC	II	Not yet recruiting	Combined with docetaxel and tergenpumatucel-L	NCT02460367
INCB024360	Advanced neoplasms	I	Completed	As single agent	NCT01195311
	Myelodysplastic syndromes	II	Active, not recruiting	As single agent	NCT01822691
	Melanoma	I/II	Recruiting	Combined with ipilimumab	NCT01604889
		II	Recruiting	Combined with a multipeptide-based vaccine	NCT01961115
	Reproductive tract tumors	II	Completed	Compared to tamoxifen	NCT01685255
		I	Recruiting	As single agent	NCT02042430
		I/II	Withdrawn	Combined with vaccine therapy	NCT01982487
		I	Recruiting	Combined with adoptive transfer of NK cells and IL2	NCT02118285
		I/II	Recruiting	Combined with DC-targeted NY-ESO-1 and poly-ICLC	NCT02166905
	Solid tumors	I/II	Recruiting	Combined with a PDCD1 mAb	NCT02178722
		I/II	Recruiting	Combined with MEDI4736 (PD-L1 mAb)	NCT02318277
	Previously treated NSCLC	I	Recruiting	Combined with MPDL3280A (PD-L1 mAb)	NCT02298153
GDC-0919 (formerly NLG-919)	Solid tumors	I	Recruiting	As single agent	NCT02048709
	Locally advanced or metastatic solid tumors	I	Not yet open	Combined with MPDL3280A (PD-L1 mAb)	NCT02471846
IDO1 peptide	NSCLC	I	Completed	As single agent	NCT01219348
	Melanoma	I	Recruiting	Combined with ipilimumab or vemurafenib	NCT02077114
	Melanoma	II	Recruiting	Combined with temozolomide, imiquimod, GM-CSF and survivin peptide	NCT01543464

NOTE: Clinical trials were identified on the website clinicaltrials.gov as of 15 July, 2015.

expression between primary and recurrent tumors. Given that inflammation is a primary driver of IDO1 expression, it may be relevant to prognostically stratify tumors that possess a wide range of T-cell–infiltrating heterogeneity, when compared with the primary, versus relapsed malignancy (65, 66).

Concluding Remarks

Our substantial knowledge of the role and expression of IDO1 in cancer has continued to expand over the past two decades, yet critical questions regarding alternative functions regulated by posttranslational modifications, the role that IDO2/TDO plays in the absence or inhibition of IDO1, as well as the impact of tissue-specific alternative splicing, still remain. Most inhibitory strategies against IDO1 focus on disabling enzymatic activity. However, preclinical mouse tumor models suggest that this tactic alone will not lead to effective antitumor immunity, further suggesting that IDO1 inhibition is best suited for combinatorial therapeutic strategies. However, these findings also raise the intriguing, yet unproven possibility that IDO1 subsumes a new/alternative immunosuppressive role when Trp catabolism is abrogated *in vivo*. In support of this hypothesis, it is notable that indoximod ($_D$-1-MT), currently cast as an IDO1 pathway inhibitor, does not inhibit Trp to Kyn catabolism (refs. 67, 68; Supplementary Table S1). This combination of reported observations and untested hypotheses paints a blurry picture of a highly immunosuppressive player in tumor immunity. Unmistakably, IDO1 is a critical mediator that, given the normal limited expression throughout the body, makes it an ideal target for cancer immunotherapy. The central question going forward, thus becomes, how can we best inhibit the activity of this pleiotropic target?

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: L. Zhai, G. Gritsina, F.J. Giles, D.A. Wainwright
Development of methodology: L. Zhai, D.A. Wainwright
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): L. Zhai, D.A. Wainwright
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): L. Zhai, F.J. Giles, D.A. Wainwright
Writing, review, and/or revision of the manuscript: L. Zhai, S. Spranger, D.C. Binder, G. Gritsina, K.L. Lauing, F.J. Giles, D.A. Wainwright
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): K.L. Lauing
Study supervision: D.A. Wainwright

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