Reimagining IDO pathway inhibition in cancer immunotherapy via
downstream focus on the tryptophan-kynurenine-aryl hydrocarbon axis

Running title: Trp-Kyn-AhR immunotherapy

Brian W. Labadie¹, Riyue Bao²,³ and Jason J. Luke¹,⁴*

¹Department of Medicine, ²Department of Pediatrics, ³Center for Research Informatics, ⁴Section of Hematology/Oncology, University of Chicago, Chicago, Illinois

*Corresponding Author:

Jason J. Luke, MD
Assistant Professor of Medicine
University of Chicago
5841 S. Maryland Ave. MC2115
Chicago, IL 60637
Telephone: 7738347961
Fax: 7737020963
jluke@medicine.bsd.uchicago.edu

Keywords: Immunotherapy, Tryptophan, Kynurenine, IDO, Aryl hydrocarbon receptor

Funding: J JL is supported by Department of Defense Career Development Award (W81XWH-17-1-0265), The National Cancer Institute (P30CA014599-43S), the Arthur J Schreiner Family Melanoma Research Fund, J. Edward Mahoney Foundation Research Fund and Brush Family Immunotherapy Fund as well as support from Center for Research Informatics of The University of Chicago Biological Science Division and The Institute for Translational Medicine/CTSA (NIH UL1 RR024999).

Conflicts of Interest: J JL Consultancies to: 7 Hills, Actym, Amgen, Array, AstraZeneca, Bristol-Myers Squibb, Castle, CheckMate, Compugen, EMD Serono, Gilead, Ideaya, Janssen, Merck, NewLink, Novartis, Palleon, RefleXion, Syndax, Tempest, WntRx with research support from AbbVie, Array, Boston Biomedical, Bristol-Myers Squibb, Celldex, CheckMate, Corvus, Delcath, Five Prime, Genentech, Immunocore, Incyte, Leap, MedImmune, Macrogenics, Novartis, Pharmacyclics, Palleon, Merck, Tesaro, Xencor

Word count: 3302

Figure/table count: 4

Supplemental Tables: 2
Abstract

Significant progress has been made in cancer immunotherapy with checkpoint inhibitors targeting programmed cell death protein 1 (PD-1)–programmed death-ligand 1 signaling pathways. Tumors from patients showing sustained treatment response predominately demonstrate a T cell-inflamed tumor microenvironment prior to, or early on, treatment. Not all tumors with this phenotype respond however and one mediator of immunosuppression in T cell-inflamed tumors is the tryptophan-kynurenine-aryl hydrocarbon receptor (Trp-Kyn-AhR) pathway. Multiple mechanisms of immunosuppression may be mediated by this pathway including depletion of tryptophan, direct immunosuppression of Kyn and activity of Kyn-bound AhR. Indoleamine 2,3-dioxygenase 1 (IDO1), a principle enzyme in Trp catabolism, is the target of small molecule inhibitors in clinical development in combination with PD-1 checkpoint inhibitors. Despite promising results in early phase clinical trials in a range of tumor types, a phase III study of the IDO1-selective inhibitor epacadostat in combination with pembrolizumab showed no difference between the epacadostat-treated group versus placebo in patients with metastatic melanoma. This has led to a diminution of interest in IDO1 inhibitors however other approaches to inhibit this pathway continue to be considered. Novel Trp-Kyn-AhR pathway inhibitors such as Kyn degrading enzymes, direct AhR antagonists and tryptophan mimetics are advancing in early stage or pre-clinical development. Despite uncertainty surrounding IDO1 inhibition, ample preclinical evidence supports continued development of Trp-Kyn-AhR pathway inhibitors to augment immune-checkpoint and other cancer therapies.
Introduction

It has long been understood that cancer cells express antigens that are recognized and prompt elimination by the immune system. Despite clinical trial evidence that cancer vaccines led to efficient antigen presentation with subsequent priming and infiltration of cytotoxic T cells into tumors, regression of tumors only occurred in a small subset of patients (1). This finding led to the prediction that important barriers downstream of initial T cell priming must exist that limit meaningful tumor elimination (2). It is now appreciated that evasion of immune mediated elimination occurs through multiple mechanisms, including immuno-editing, decreased antigen presentation, and importantly, local immunosuppression in the tumor microenvironment (3,4).

Tumor analysis from patients with metastatic melanoma receiving vaccine and cytokine therapies suggested a paradigm of two broad phenotypes characterized by the presence or absence a T cell-inflamed tumor. T cell-inflamed tumors are characterized by tumor infiltrating lymphocytes, a type-I/II interferon (IFN) transcriptional profile, and high degree of expression of immunosuppressive mechanisms. In contrast, a non-T cell-inflamed tumor is observed to have a low inflammatory signature and the absence of tumor infiltrating lymphocytes (5-7).

Immunotherapy in the treatment of solid malignancies has evolved significantly over the past decade with the emergence of monoclonal antibodies against programmed cell death protein 1 (PD-1)–PD-L1 and cytotoxic T-lymphocyte associated antigen 4 (CTLA4), delivering meaningful clinical benefit across multiple solid tumors. Patients more likely to benefit from checkpoint immunotherapy include those with tumors demonstrating a high density of somatic mutations, elevated PD-L1 expression and/or are enriched with IFNγ transcriptional profiles (8-13). However, a subset of tumors identified as having a T cell-inflamed tumor phenotype do not respond to checkpoint immunotherapy, suggesting other immunosuppressive mechanisms contribute to limiting immune-mediated tumor regression besides the PD-1/L1 axis. Preclinical studies have identified multiple immunosuppressive mechanisms that are present in the T cell inflamed tumors, including, but not limited to, extrinsic inhibition by regulatory cell populations such as forkhead box P3 (FoxP3)-positive regulatory T cells (Tregs) and metabolic mechanisms of immune suppression such as the tryptophan-kynurenine-aryl hydrocarbon receptor (Trp-Kyn-AhR) pathway (14,15).

Specific clinical focus has increasingly centered on the immunosuppressive actions of tryptophan catabolism as regulated by indoleamine 2,3-dioxygenase 1 and 2 (IDO1/IDO2),
tryptophan 2-3-dioxygenase (TDO) and kynureninase as well as downstream signaling of tryptophan catabolites as agonists of AhR. In preclinical models, heightened activity of the Trp-Kyn-AhR pathway has been linked to impairment of antitumor immunity and tumor growth (16,17). More recent characterization AhR has demonstrated multiple mechanisms by which it facilitates a tolerogenic immune environment (18). Inhibition of the Trp-Kyn-AhR pathway has become an attractive therapeutic target and the focus of substantial biotechnology and pharmaceutical effort. Currently, several IDO1, combination IDO1/TDO inhibitors, AhR inhibitors as well as a recombinant kynureninase are in clinical development or late pre-clinical testing.

**Immunomodulatory Role of Trp-Kyn-AhR Pathway**

The Trp-Kyn-AhR pathway, in which the essential amino acid tryptophan is converted to Kyn and other secondary metabolites, is the primary route of tryptophan catabolism (19). Three enzymes catalyze the rate-limiting step of tryptophan catabolism to kynurenines: IDO1, IDO2, and TDO. The enzyme kynureninase hydrolyzes 3-hydroxykynurenine to 3-hydroxyanthranilic acid in the production of nicotinamide adenine dinucleotide. Regulation of these enzymes is notably divergent, with IDO1 being influenced by the interplay of IFNγ and interleukin (IL)-6 as compared with TDO being regulated by tryptophan, cholesterols, and prostaglandin E2 (20,21). The regulation and role of IDO2 is uncertain. Immunosuppression associated with Kyn was first described in experiments that demonstrated increased tryptophan catabolism limits allogeneic fetal rejection in mice (22).

In tumors, IDO1 is expressed by stromal cells of the TME and is induced by IFNγ as a result of CD8+ T cell infiltration and activation of other immunosuppressive pathways (7,23,24). TDO is ectopically expressed by tumor cells in certain malignancies (25). Kynurenines act as potent agonists of AhR, a ligand-gated transcription factor that is expressed in many immune cells and mediates a wide range of immunomodulatory effects (26,27). Elevated IDO1 and TDO activity and Kyn levels are associated with increased tumor grade and poor prognosis in many cancers (28).

Several mechanisms have been proposed to explain the role of the Trp-Kyn-AhR pathway in tumor-associated immunosuppression. T cells are exquisitely sensitive to local depletion of tryptophan in which low tryptophan levels suppress mTORC pathways and activate
general control nondepressible 2 (GCN2) kinase leading to cell cycle arrest and anergy of infiltrating T cells via eIF-2-dependent pathways (Figure 1) (29-31). However, recent studies have questioned the significance of this mechanism (32,33). Accumulation of kynurenines induce effector T cell arrest and lead to binding of AhR. This results in nuclear translocation and promotion of FoxP3 transcripts and IL-10, eventually producing regulatory T cell populations (18,34-37). In-vitro studies of AhR-deficient lung dendritic cells demonstrate failure to promote Treg development and an increase Th2 cell differentiation and pro-inflammatory responses to allergen exposure (38). AhR suppresses innate immunogenicity of antigen presenting cells and promotes IL-10 production by natural killer cells (Figure 2) (39-41). In addition, the Kyn-AhR interaction has been shown to upregulate PD-1 expression by CD8+ T cells via transcellular signaling mechanism in the tumor microenvironment (42).

Prominent IDO1/TDO Inhibitors and Trp-Kyn Pathway Inhibitors in Clinical Development

Several biochemical strategies exist to inhibit the Trp-Kyn-AhR pathway. IDO1 knockout mice demonstrate no clinical phenotype, in contrast to the inflammatory phenotype observed for knockouts of the immune checkpoints CTLA-4 and PD-1, and thus IDO1 inhibitors have predominantly been used in combination with other treatment modalities (43,44). Selective-IDO1 enzyme inhibitors such as epacadostat, NLG-919, and BMS-986205 either compete with tryptophan for the catalytic site of IDO1 or bind the enzyme with very high affinity (44-47). In contrast, the tryptophan mimetic indoximod appears to have pleiotropic effects on downstream Kyn-AhR pathway signaling and has been shown to relieve immunosuppressive signaling normally induced by tryptophan depletion (48,49). AhR inhibitors and recombinant kynureninase have more recently entered clinical development and will be discussed below.

A primary pharmacodynamic measure reported for selective-IDO1 inhibitors in clinical trials was reduction in peripheral blood Kyn levels. Initial peripheral blood Kyn suppression data demonstrated approximately 50% reduction suggesting other enzymes contribute to the production of systemic kynurenine, such as TDO. To date, assessment of intra-tumoral Kyn has not been consistently collected or reported in clinical trials (50,51). Figure 3 describes the prominent IDO, TDO inhibitors and Trp-Kyn pathway inhibitors currently in clinical development.
IDO1, TDO and Trp-Kyn-AhR Inhibition in Combination Treatment

Association between the Trp-Kyn-AhR pathway and PD-1/L1 was suggested by the observation that both pathways are induced by IFNγ signaling in the tumor microenvironment (7,14). Indeed, across 30 human solid tumors from The Cancer Genome Atlas (TCGA) database, we have observed that the gene expression of IDO1 was strongly correlated with the expression of PD1 across increasing level of IFNγ responsive gene expression from non-T cell-inflamed to highly T cell-inflamed tumors (Figure 4A). In contrast, expression of IDO2, TDO2, KNYU, AHR and GCN2 (alias EIF2AK4) does not appear to correlate with PD1 expression or demonstrate IFNγ responsiveness on a transcriptional level as strongly as IDO1 (Figure 4B).

Despite early observations for lack of monotherapy activity of selective-IDO1 inhibitors (52) combination strategies utilizing IDO1 inhibitors were quickly advanced. Indeed, IDO1 and PD-1/L1 inhibitor combinations appeared to show great promise in early phase clinical trials across multiple tumor types (Suppl Tables 1, 2).

A substantial literature also supports the potential utility of inhibition of the IDO pathway in conjunction with other anti-cancer modalities. Studies of IDO pathway blockade with radiation, chemotherapy and tumor vaccines suggest an improvement relative to those treatments alone (53,54). Several clinical trials evaluating combinations across these modalities are ongoing (Suppl Table 1).

Epacadostat

Epacadostat, a competitive, selective inhibitor of IDO1, reached the most advanced stage of development, with the recent early termination of ECHO-301/Keynote-252; a phase III clinical trial in combination with pembrolizumab in metastatic melanoma. At median follow up of 14 months, patients treated with epacadostat plus pembrolizumab demonstrated a progression free survival of 4.7 months versus 4.9 months in those treated with pembrolizumab plus placebo (HR=1.00; CI, 0.83-1.21; P=0.517.) The overall response rate was 34.2% versus 31.5% in the epacadostat plus pembrolizumab and placebo plus pembrolizumab groups respectively. Treatment related adverse events occurred in 79.3% of patients receiving epacadostat plus pembrolizumab versus 81.0% receiving placebo plus pembrolizumab and Grade >3 treatment-related adverse events occurred in 21.8% versus 17.0%, respectively (55). These negative results
were unexpected as preclinical studies and early phase clinical trials of this combination in as many as 14 different solid tumors showed encouraging results (Suppl Table 1). Multiple hypotheses have been advanced to explain the seeming discrepancy between early phase clinical trial success of epacadostat and the failure of the late phase ECHO-301 trial. A non-exhaustive list of possible explanations to differentiate early vs late phase results could include differences between the treatment populations, inappropriately low dosing of epacadostat and incomplete suppression of intra-tumoral Kyn.

Regarding the patient populations for these studies, the patient characteristics appeared to be relatively similar from the early (ECHO-202) to late phase (ECHO-301) melanoma studies across multiple variables including but not limited to performance status of zero (77 vs 76%), M1c staging (55 vs 61%), elevated lactate dehydrogenase (37 vs 32%) and no prior therapy (71 vs 89%), respectively (55,56). As IDO1 expression is intimately linked to IFNγ gene expression, the baseline quality of the T cell-inflamed tumor microenvironment may be of relevance to the results. To date, this is not well characterized in either study. In ECHO-301 IDO1 expression was not an inclusion criterion (though PD-L1 expression was a randomization stratification factor) with only PD-L1 status as a surrogate from the early phase studies. Given the seeming complete lack of activity between epacadostat and placebo in ECHO-301, some have wondered whether further study should be given to the patients treated in the early phase study. Despite the lack of obvious clinical differentiators, perhaps the tumors from these patients were disproportionately T cell-inflamed and thus much more likely to respond to pembrolizumab and possibly epacadostat.

Other less commonly controlled for differences of potential clinical significance could exist between the early to late phase trial populations also however. One of these would be to note that the early phase trial was conducted at a select number of sites in the United States while the late phase study was predominately international (Australia, Europe, Asia, South America) with a lesser accrual in the United States. It has been observed in previous trials of melanoma that outcomes appeared to be substantially different between these populations in the phase III setting (57). Consideration might be given to whether dietary or environmental exposures could be variable in these different localities especially given the canonical role of AhR as a xenobiotic sensor that is responsive to signals from the gut microbiome (18). With an evolving literature supporting the microbiome as a potential influencer of PD1 antibody response (58), focus on
diet, medication use and microbiome contents may be important variables to track in clinical trials moving forward.

Regarding dosing of epacadostat, 100 mg was taken forward out of the phase I study despite higher dose levels being tolerable and no maximum tolerated dose being established (59). This dose was chosen by Incyte as it was deemed that maximal inhibition of IDO1 activity was observed at doses of $\geq 100$ mg with a relative plateau in decrease of kynurenine level in peripheral blood at higher doses. This point of the most appropriate dose is debated however particularly given that published modeling of IDO inhibition only reaches approximately 50-70% at the 100 mg dose (60). It is somewhat notable that an early phase study of epacadostat with nivolumab was simultaneously pursued using 300 mg of epacadostat (61) and published modeling implies a higher likelihood of IDO1 inhibition at this dose (60). The optimal dose of epacadostat continues to be explored in on-going clinical trials.

Perhaps of most relevance to the failure of ECHO-301 however is the open question of intra-tumoral pharmacodynamics. While peripheral blood monitoring of kynurenene was reported for epacadostat in phase I, to date no intra-tumoral data has been released from any clinical trial. Given the lack of consensus surrounding whether limiting tryptophan depletion versus suppression of Kyn acts as a primary mechanism of immunosuppression, this data is essential to inform the field surrounding next steps. Even very low levels of canonical AhR ligands, such as Kyn and dioxin, can activate AhR associated gene expression and recent preclinical studies have suggested kynureninase and direct AhR inhibitors have higher potency relative to IDO1 selective inhibitors (62-64). If suppression of Kyn is indeed the dominate mechanism it is very possible that IDO1 inhibition alone may be inadequate to drive intra-tumoral levels consistently low enough to alleviate the immunosuppressive effects of Kyn-activated AhR, including production of IL-10 and suppression of type I IFN (41).

**Other Selective-IDO1 Inhibitors**

Pivotal studies of BMS-986205, an irreversible IDO1 inhibitor developed by Flexus Biosciences and Bristol Meyers Squib, have been predominately scaled back in wake of epacadostat’s late-stage trial failure though a randomized study in bladder cancer is still planned. Relative to epacadostat, BMS-986205 demonstrates higher potency based on IC50 in IDO1 expressing cell lines. In contrast to the lack of such data for epacadostat, analysis of 39 paired
pre- vs on-treatment tumor samples across various tumor types from the Phase I trial of BMS-986205 plus nivolumab demonstrated decreased Kyn levels (and mostly near zero levels on-treatment) and increased the percentage of proliferating CD8+ T cells (65).

Genentech has recently terminated rights to NewLink’s NLG-919 (navoximod/GDC-0919), another selective inhibitor of IDO1.

**Tryptophan Mimetics**

Indoximod, the D-enantiomer of 1-methyl-tryptophan, has demonstrated inhibition of the IDO pathway as a tryptophan mimic. Indoximod limits IDO-mediated immunosuppression by at least two mechanisms including 1) serving as an artificial Trp-sufficiency signal that prevents activation of GCN2 and inhibition of mTORC1 and 2) modulation of AhR-dependent transcriptional activity (66). Indoximod increased activity and proliferation of CD8+ T cells by limiting tryptophan-depletion mediated mTORC1 suppression (66). mTORC1 activation has been associated with ICOS expression, a T cell coregulatory receptor seen on tumor-infiltrating T cells that has been associated with clinical response (67). In an AhR-dependent manner, indoximod was shown to stimulate CD4+ T cell differentiation to Th17+ helper T cells, inhibit FoxP3 T regulatory cells and downregulate expression of IDO in dendritic cells (66).

Data from a single-arm phase 2 trial of indoximod plus anti-PD1 in advanced melanoma achieved an ORR of 56% and CR in 19% with low rates of high-grade immune-related adverse events (68). Multiple phase II and III trials combining indoximod with other current modalities of treatment, including chemotherapy, cancer vaccines, and checkpoint immunotherapy are ongoing (Suppl Tables 1, 2).

**Dual IDO1/TDO Inhibitors**

Analysis of tumor and immune cells by immunohistochemistry revealed differences in the expression of IDO1 and TDO among tumor types, suggesting the potential for a possible advantage with dual IDO and TDO inhibitors in certain tumors (69). Dual IDO/TDO inhibitors such as RG70099 decrease serum Kyn levels by approximately 90%. IOM-E and IOM-D are selective-IDO1 and dual IDO1 and TDO inhibitors, respectively. Preclinical studies have revealed a promising pharmacokinetic profile. Significant in vivo efficacy was observed in mouse pancreatic adenocarcinoma cells treated with IOM-E, the selective-IDO1 inhibitor, in
combination with gemcitabine and abraxane. Particular efficacy in preclinical lung cancer models has also been seen (70). Several companies have disclosed pre-clinical programs surrounding the development of dual IDO/TDO inhibitors though the current status of these programs is in flux in wake of ECHO-301. It is worth pointing out however that in murine models, complete IDO/TDO inhibition results in significant alteration of Trp metabolism which has raised concern over potential neurologic toxicity (seizures) from dual IDO1/TDO inhibition (71).

**AhR Inhibitors**

Inhibition of AhR signaling with small molecule antagonists interferes with the downstream immunomodulatory effects irrespective of the source of Kyn production. Early inhibitors in this class have been shown to block nuclear translocation of AhR and enhanced production of IFNγ, TNFα, IL-2 and reduction in tumor associated M2-like macrophages. In mouse models, AhR inhibitors have demonstrated activity as monotherapy, a notable contrast to IDO1 inhibitors. Furthermore, enhanced activity is seen with AhRi combination with anti-PD-1 (72). Several biotechnology companies including Hercules Pharmaceuticals, Ideaya Biosciences and KYN Therapeutics have disclosed the development of AhR inhibitors.

**Kynurenine Degrading Enzymes**

Recombinant Kyn degrading enzymes, kynureninase or KYNase, have been shown to reduce Kyn levels in IDO1, TDO and IDO1/TDO dual positive cancer cells without impact on systemic tryptophan levels. Preclinical studies of a recombinant PEG-KYNase in established tumor models have demonstrated inhibition of tumor growth and increase in tumor infiltrating effector T cells as monotherapy. Synergistic activity with anti-PD1 has also been demonstrated (73,74). An ongoing development program of recombinant kynureninase has been disclosed by KYN Therapeutics. Intra-tumoral injection of engineered *E. coli* strains that metabolize kynurenine have demonstrated reduction in *in vivo* kynurenine levels and generation of antitumor response (75).
Conclusions and Future Directions

The degree of tumor inflammation, as assessed by the presence of type I/II IFN signaling and infiltrating effector T cells, is associated with an improved response to checkpoint immunotherapy (8-11,13). However, despite robust T cell inflammation, a considerable percentage of tumors progress by virtue of multiple immuno-suppressive mechanisms (14,15). Upregulation of the Trp-Kyn-AhR pathway has been identified as one such mechanism. The immunosuppressive effect of this pathway is believed to be mediated by Trp depletion, T cell cycle arrest mediated by Kyn cytotoxicity and activation of immune-tolerogenic AhR.

Recent studies have cast doubt on the Trp-depletion mechanisms (32,33) and highlighted the potent immunosuppressive activity of intra-tumoral Kyn and AhR signaling. To this end, in the evaluation of Trp-Kyn-AhR inhibitors, reduction of extracellular Kyn within the tumor microenvironment or downstream AhR transcriptional programs should be emphasized as major pharmacodynamic endpoints. Indeed, a major concern pertaining to the clinical evaluation of selective-IDO1 inhibitor epacadostat was the absence of intra-tumoral Kyn biomarker analysis. Prior studies have demonstrated serum Kyn:Trp correlate with response to anti-PD-1 (76,77), however it is unclear if serum kynurenine is a surrogate for intra-tumoral Kyn.

Despite the failed experience of epacadostat in unselected melanoma patients, a strong translational rationale still exists for targeting of the Trp-Kyn-AhR pathway in conjunction with immunotherapy. Alternative mechanisms to achieve intra-tumoral Kyn reduction are currently being investigated. Comprehensive inhibition of kynurenine production by dual IDO1/TDO inhibitors and/or degradation of Kyn molecules by recombinant kynurenine degrading enzymes may provide more robust intra-tumoral Kyn reduction. Alternatively, inhibition of AhR may serve to alleviate the immunosuppressive TME regardless of the source of Kyn production. Novel agents in each of these classes are approaching phase I studies and pre-clinical experiments have shown promising results (72-75).

A contrarian view to acknowledge surrounding this pathway would be that in a T cell-inflamed tumor, inhibition of Trp-Kyn-AhR may be insufficient to elicit further antitumor immune response due to the presence of further escape mechanisms (6,7,15). In these settings, this pathway may fail to make an inflamed environment even more inflamed. However, it may be that targeting downstream in the pathway could mediate the induction of the T cell-inflamed tumor microenvironment in previously non-inflamed tumors given studies suggesting regulation.
of type I IFN response by AhR (41). This concept awaits further investigation and biospecimens from phase I studies of AhR antagonists will be especially interesting in this regard.

Cancer immunotherapy has advanced significantly with the development of CTLA-4 and PD-1–PD-L1 inhibitors. Progress in understanding the biology underlying the T cell-inflamed tumor microenvironment suggest that the Trp-Kyn-AhR pathway and PD-1–PD-L1 signaling are both associated with IFNγ response however mediate independent mechanisms of immunosuppression. Combinatorial therapies may thus benefit a subset of patients. Despite uncertainty surrounding selective-IDO1 inhibition, ample preclinical evidence supports continued development of Trp-Kyn-AhR pathway inhibitors to augment immune-checkpoint and other cancer therapies. Novel Trp-Kyn-AhR inhibitors have demonstrated promising pre-clinical activity and as new candidates undergo lead optimization and early evaluation, lessons learned from recent IDO1 inhibitor failure must guide the field moving forward.

Acknowledgments

Prior to the failure of ECHO-301 and near complete re-work of the manuscript, medical writing and editorial support were provided by Jeremy Kennard, PhD, and Shannon Davis of Infusion Communications, Middletown, CT, with funding by Incyte Corporation, Wilmington, DE.
Figures

Figure 1.
Tryptophan depletion-dependent signaling. Depletion of tryptophan suppresses activity in the mTORC1 signaling pathway, leading to autophagy in T cells, and releases GCN2-mediated phosphorylation of eIF-2, inducing cell cycle arrest and death in T cells.

Figure 2. IDO1-Kynurenine-AhR signaling in TME immunosuppression. A, IDO1 in tumor cells, dendritic cells, and fibroblasts. TDO in hepatocytes are the rate-limiting enzymes in the conversion of tryptophan to Kynurenine and Kynurenine derivatives. Kynurenine binds to and activates the AhR, a ligand-activated transcription factor, in regulatory T cells, NK cells and dendritic cells. B, Activation and nuclear translocation of the AhR (1) in dendritic cells induces synthesis and release of IL-10 and inhibits IFNβ signaling, (2) in NK cells induces synthesis and release of IL-10 and IFNγ, and (3) in Tregs promotes Treg development. C, Tregs and IL-10 promote immunosuppression within the TME, whereas inhibition of IFNβ by AhR releases regulation of immunosuppression from inhibitory IFNβ signaling. In addition, both IL-10 and IFNγ promote IDO1 activity, establishing a positive feedback loop for IDO1-Kynurenine-AhR signaling.

Figure 3. Trp-Kyn pathway inhibitors in current or prior clinical development

Figure 4. Expression of PD1 is positively correlated with immunotherapy relevant target genes across solid tumors from TCGA. (A) Heatmap of Pearson’s product-moment correlation
coefficient \( r \) between \( PD1 \) and immune target genes by tumor type. Immune target genes were separated into those strongly or less correlated with \( PD1 \) expression. **Methods**: level 3 RNA-Seq data (release date February 4, 2015) were downloaded for 30 solid tumor types from TCGA and processed as previously described (87). Acute myeloid leukemia, diffuse large B-cell lymphoma, and thymoma were excluded because of high tumor intrinsic immune cell transcripts. Skin cutaneous melanoma had both primary and metastatic samples available, whereas the other 29 cancers had only primary tumors available. A non-comprehensive list of 171 immune molecules representative of the interactions between tumor cells and immune cells in the tumor microenvironment were selected and correlated with \( PD1 \) (alias \( PDCD1 \)) gene expression. For each tumor type, Pearson’s \( r \) was computed between each immune molecule and \( PD1 \) and used for clustering the genes by hierarchical unsupervised clustering with Euclidean distance. Two distinct groups are shown, consisting of (1) strongly correlated genes and (2) less correlated genes. (B and C) Correlation plots of \( PD1 \) vs \( IDO1, IDO2, KYNU, TDO2, AHR \) and \( GCN2 \) (alias \( EIF2AK4 \)) (highlighted in red in A) in (B) metastatic melanoma and (C) NSCLC. Patients were categorized into T cell-inflamed (red), non-T cell-inflamed (blue) and intermediate groups using a defined T cell-inflamed gene signature (87). Each data point represents one patient. NSCLC = non-small cell lung carcinoma.
REFERENCES:


Figure 1:

[Diagram showing the relationship between tryptophan depletion and mTORC1 inhibition leading to autophagy, cell cycle arrest, and T cell death.]

Key:
- IDO1
- Kynurenine
- Tryptophan depletion
- mTORC1
- Autophagy
- GCN2
- Phospho-eIF-2
- Cell cycle arrest
- Cell death
- T cell
Figure 2:
Figure 3:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Company</th>
<th>Target</th>
<th>Structure</th>
<th>Mechanism</th>
<th>Dosing</th>
<th>Human IDO1 enzymatic assay (IC50) (μM)</th>
<th>Human IDO1 cell-based assay (IC50) (μM)</th>
<th>Human TDO enzymatic activity</th>
<th>Phase of Development</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indoximod</td>
<td>Newlink</td>
<td>Tryptophan</td>
<td></td>
<td>Stimulates mTOR kinase to reduce T-cell autophagy</td>
<td>1200 mg BID (79)</td>
<td>&gt;2.5 μM (HeLa cells) (46)</td>
<td>&gt;30 μM (Human DCs)</td>
<td>Nonselective</td>
<td>III</td>
<td></td>
</tr>
<tr>
<td>Epacadostat</td>
<td>Incyte</td>
<td>IDO1</td>
<td></td>
<td>Competitive inhibition of IDO1 (46)</td>
<td>100 mg BID (59)</td>
<td>72 nM (46)</td>
<td>7-23 nM (46)</td>
<td>&gt;100-fold (46)</td>
<td>II</td>
<td></td>
</tr>
<tr>
<td>BMS986205</td>
<td>Bristol Meyers Squib</td>
<td>IDO1</td>
<td></td>
<td>Irreversible inhibition of IDO1 (79)</td>
<td>150 mg QD (80)</td>
<td>1nM (HEK293 cells)</td>
<td>&gt;2 μM (n)</td>
<td>HEK293 cells)</td>
<td>(44)</td>
<td>II</td>
</tr>
<tr>
<td>Navoximod</td>
<td>Newlink</td>
<td>IDO1</td>
<td></td>
<td>Noncompetitive inhibition of IDO1</td>
<td>50–800 mg BID (81)</td>
<td>28 nM (82)</td>
<td>75 nM (82)</td>
<td>10–20-fold (79)</td>
<td>Ib</td>
<td></td>
</tr>
<tr>
<td>PF-06840003</td>
<td>iTeos</td>
<td>IDO1</td>
<td></td>
<td>Noncompetitive inhibition of IDO1 (83)</td>
<td>250–500 mg BID (84)</td>
<td>120 nM (83)</td>
<td>1100 nM (83)</td>
<td>&gt;100-fold (83)</td>
<td>I</td>
<td></td>
</tr>
<tr>
<td>KHK2455</td>
<td>Kyowa Hakko Kirin Co</td>
<td>IDO1</td>
<td></td>
<td>Competitive inhibition, apo-configuration (85)</td>
<td>1 mg QD (86)</td>
<td>14 nM (85)</td>
<td>&gt;100-fold (85)</td>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RG70099</td>
<td>Roche</td>
<td>IDO1/TDO</td>
<td></td>
<td>Competitive inhibition</td>
<td>Unspecified (69)</td>
<td>16 nM (69)</td>
<td>12 nM (69)</td>
<td>6-fold (69)</td>
<td>Preclinical</td>
<td></td>
</tr>
<tr>
<td>IOM-E</td>
<td>Merck</td>
<td>IDO1</td>
<td></td>
<td>Unknown</td>
<td>Unspecified (70)</td>
<td>100 nM (70)</td>
<td>&gt;100-fold (70)</td>
<td>Preclinical</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IOM-D</td>
<td>Merck</td>
<td>IDO1/TDO</td>
<td></td>
<td>Unknown</td>
<td>Unspecified (70)</td>
<td>365 (70)</td>
<td>10 nM (70)</td>
<td>Preclinical</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 4:

A

Strongly correlated
HLA–DPA1, HLA–DPA2, HLA–DPA4, HLA–DPA5, HLA–DQA1, HLA–DQB1, HLA–C, HLA–DRB1, HLA–DQA2, HLA–DQB2, HLA–DMB, ITGB2, CD4, CD86, HAVCR2, CD40LG, CD69, HLA–DOB, CD79A, CXCL10, CD72, KLRK1, LTA, IFNG, BTLA, FOXP3, TNFRSF9, STAT4, CD244, SIRPG, CD3E, CD3D, CCL5, ICOS, CD8A, CD27, TIGIT, CD247, LAG3, ITGAL, TBX21, CD3G, CXCL9, CTLA4, CXCR3, SIGLEC1, CSF1R, HLA–DQA2, CCR2, IFNB1, IFNG, IFNA1, IL17A, IL13, TNFSF4, TYK2, IL23A, CX3CL1, CLEC4C, IL12A, STAT3, TGFB3, CD93, XBP1, ST6GAL1, GATA3, IL1B, IL6, CCL20, A4GALT, LAYN, TNFSF9, ICOSLG, TMEM173, KYNU, IL18, LY75, IFNK, IL5, MME, IFNB1, IFNW1, CEACAM8, IL4, NDUFA2, RORC, KIR3DL3, IL17F, MST1R, MICA, STAT6, BCL6, AHR, IL1A, KRT20, ARG1, VTCN1, NT5E, NCAM1, CD24, EIF2AK4, ARG2, VEGFA, JAK1, STAT5B, RORA, SMAD3, EDNRB, MAGEH1

Less correlated
TLR9, FCER2, IL8RA, CCR6, IDO2, PD1, LIFR, IFNA13, HMGB1, CD24, EIF2AK4, ARG2, VEGFA, JAK1, STAT5B, RORA, SMAD3, EDNRB, MAGEH1

B

Metastatic melanoma

C

NSCLC

© 2018 American Association for Cancer Research
Reimagining IDO pathway inhibition in cancer immunotherapy via downstream focus on the tryptophan-kynurenine-aryl hydrocarbon axis

Brian W. Labadie, Riyue Bao and Jason J. Luke

Clin Cancer Res  Published OnlineFirst October 30, 2018.

Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-18-2882

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2018/10/30/1078-0432.CCR-18-2882.DC1

Author Manuscript
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://clincancerres.aacrjournals.org/content/early/2018/10/30/1078-0432.CCR-18-2882.
Click on "Request Permissions" which will take you to the Copyright Clearance Center’s (CCC) Rightslink site.