

Discovery of IDO1 Inhibitors: From Bench to Bedside

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Abstract

Small-molecule inhibitors of indoleamine 2,3-dioxygenase-1 (IDO1) are emerging at the vanguard of experimental agents in oncology. Here, pioneers of this new drug class provide a bench-to-bedside review on preclinical validation of IDO1 as a cancer therapeutic target and on the discovery and development of a set of mechanistically distinct compounds, indoximod, epacado-

stat, and navoximod, that were first to be evaluated as IDO inhibitors in clinical trials. As immunometabolic adjuvants to widen therapeutic windows, IDO inhibitors may leverage not only immuno-oncology modalities but also chemotherapy and radiotherapy as standards of care in the oncology clinic. *Cancer Res*; 77(24); 6795–811. ©2017 AACR.

Introduction

Present therapies fail many patients with metastatic cancer, generally a terminal stage after the relapse of drug-resistant disease. Tumors display abundant immunogenic antigens yet ultimately escape immune rejection through the evolution of various tactics to evade, subvert, and reprogram innate and adaptive immunity. Over the past decade, the molecular mechanisms enabling immune escape have been unraveled to a sufficient extent that mainstream acceptance of immunotherapy in the field of cancer research has been restored after a historical divorce occurring nearly half a century ago (1, 2). Perhaps the most impactful achievement of work in this area has been to resolve the historical failure of active immunotherapy to treat solid tumors, by revealing that such approaches are generally ineffective due to the dominance in most patients of tumor-mediated immunosuppression (1, 3). One such immune "braking" mechanism that has emerged with broadly useful appeal is mediated by the cytosolic enzyme indoleamine 2,3-dioxygenase-1 (IDO1), which acts in immunometabolism and inflammatory programming through its biochemical function in tryptophan catabolism (4, 5). Indeed, as demonstrated in preclinical models starting over a decade ago, inhibiting the IDO1 enzyme can empower the efficacy of cytotoxic chemotherapy, radiotherapy, and immune checkpoint therapy without increasing their side effects (6–9). In this review of the foundations of IDO1 inhibitor validation and development, we survey key observations that have catapulted the relatively obscure area of tryptophan catabolism to the forefront of experimental cancer therapy.

The IDO1 enzyme is activated in many human cancers in tumor, stromal, and innate immune cells where its expression tends to be associated with poor prognosis (10). Its role in immunosuppression is multifaceted, involving the suppression of CD8⁺ T effector cells and natural killer (NK) cells as well as increased activity of CD4⁺ T regulatory cells (Treg) and myeloid-derived suppressor cells (MDSC; ref. 4). In tumor neovascularization, IDO1 acts as a key node at the regulatory interface between IFN γ and IL6 that shifts the inflammatory milieu toward promoting new blood vessel development (11, 12). As considered below, pharmacologic and genetic studies in autochthonous transgenic and graft mouse models point to a root function for IDO1 in programming an inflammatory tumor microenvironment characterized by a tolerizing immune attitude and a permissive angiogenic context. Accordingly, there are preclinical proofs that IDO1 inhibitors can safely empower the efficacy of cytotoxic or targeted chemotherapy, radiotherapy, immune checkpoint therapy, and cancer vaccines. Furthermore, there is emerging interest in two other tryptophan catabolic enzymes, TDO and IDO2, which also appear to influence inflammatory programs, with ongoing exploration of IDO1 inhibitors that also block TDO and IDO2 to deepen efficacy, limit inherent or acquired resistance to IDO1 blockade, and reduce autoimmune side effects of immune therapy.

IDO1 and Immunosuppression

The discovery of the IDO1 enzyme was rooted in initial observations made in the 1950s in cancer patients where tryptophan catabolism was found to be elevated (13). In 1981, IDO1 was identified as the first IFN-activated enzyme to be described (14), but the precise meaning of this connection was obscure. In 1998, a pivotal conceptual breakthrough emerged from the work of Munn, Mellor, and their colleagues suggesting that IDO1 could mediate immunosuppression based on the preferential sensitivity of T cells to tryptophan deprivation (15, 16). Briefly, they proposed that tryptophan deprivation would impair antigen-dependent T-cell activation in microenvironments where IDO1

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doi: 10.1158/0008-5472.CAN-17-2285

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was active. Initial evidence supporting this concept was offered by studies of how immune tolerance to foreign paternal antigens in pregnant mice could be reversed by a simple tryptophan mimetic that disrupts IDO signaling, 1-methyl-tryptophan (1MT), the administration of which was sufficient to elicit MHC-restricted T cell-mediated rejection of hemiallogeneic concepti presenting foreign paternal antigens (15, 17). Subsequent work developed this concept as a mechanism to defeat immunosurveillance in cancer (as reviewed initially in refs. 18–21). Two pivotal connections were the following. First, IDO1 was found to be broadly expressed in human tumors (22, 23). Second, IDO1 expression in tumor cells was discovered to be linked to the status of *Bin1* (6), a tumor suppressor gene among the most frequently attenuated genes in human cancer (24), due either to aberrant RNA splicing patterns that abolish its tumor suppressor function (25–29), or to altered gene methylation patterns that extinguish its expression (30–34). *Bin1* deficiency in an oncogenically transformed cell was sufficient to facilitate IDO1-mediated immune escape by a cell-intrinsic mechanism (6). Notably, subsequent bone marrow transplant experiments argued that IDO1 acts in nonhematopoietic cells to support inflammatory skin carcinogenesis (35). Together, these observations support the concept that IDO1 can act solely in tumor cells and that its overexpression is sufficient to drive immune escape. The discovery of a link between IDO1 expression and *Bin1* status provided the first sound genetic connection of IDO1 to cancer pathophysiology. As surveyed below, Fig. 1 provides a cartoon summary of the biological impact of IDO1 expression in cancer, whereas Fig. 2 provides an overview of its regulation in expressing cells and the effector signals it generates in downstream responding cells.

IDO1 is an inducible enzyme that is produced widely in response to the pivotal immune regulatory Th1 cytokine IFN γ in various myeloid lineage-derived cells including dendritic cells (DC) and macrophages as well as endothelial cells, mesenchymal stromal cells and fibroblasts (36). Early studies accumulated evidence that IDO1 activity in cellular or tissue environments could suppress the function of T cells (37–41) and NK cells (42), and also that IDO1 was critical for the production and function of Tregs (43) and MDSCs (11). Effector functions for both tryptophan depletion and catabolite production by IDO1 have been described, many focusing on antigen-presenting DCs where IDO1 is upregulated by IFNs, TLR ligands, and other important immune signals (44). Notably, IDO1 expression in a small minority population of DCs enables them to dominantly suppress effector T-cell responses (45, 46). Thenergizing effect of IDO1-mediated tryptophan depletion on T cells requires the stress response kinase GCN2, which is also required for IDO1-induced activation of Tregs (20, 41). Likewise, tryptophan catabolites block T-cell activation and trigger T-cell apoptosis while also promoting IDO1-induced differentiation of CD4⁺ cells into Tregs (through a TGF β -dependent mechanism), with apparent synergistic effects of this effector arm with tryptophan deprivation (47). Although the role of kynurenine and other tryptophan catabolites has been less studied than the effects of tryptophan deprivation, a key advance reported by Platten and colleagues was the discovery that kynurenine acts as a native ligand for the aryl hydrocarbon receptor (AHR), a transcription factor that contributes to proinflammatory programs and xenobiotic responses of great relevance to cancer and inflammatory carcinogenesis (48).

Inflammatory Programming by IDO1 in Cancer

Mouse genetic studies indicate that IDO1 exerts a proximal influence on inflammation that cannot be understood as simply immunosuppressive. It is notable that IDO1 deficiency in mice does not produce rampant inflammation in contrast to deletion of true immunosuppressive functions like CTLA-4 or PD-1. Even when mice were exposed to topical applications of the proinflammatory agent TPA (12-O-tetradecanoylphorbol-13-acetate), no discernable exacerbation in the severity of resulting contact dermatitis occurred (35). In contrast, genetic ablation of IDO1 markedly attenuated the ability of this inflammatory regimen to promote the development of premalignant papillomas following mutagenic initiation with the chemical carcinogen DMBA (7,12-dimethylbenz[*a*]anthracene; ref. 49), while in the absence of inflammatory tumor promotion, the loss of IDO1 had no effect on papilloma development (35). These results suggest that IDO1 functions as a pivotal driver of "tumor-promoting" inflammation, raising the possibility of using IDO1 inhibitors as chemopreventive agents to broaden their utility.

A key aspect of IDO1's ability to establish a tumor-promoting inflammatory milieu is its status as a nodal interface between the inflammatory cytokines IFN γ and IL6. The importance of IFN γ in antitumor immune responses is well established (50) while IL6 is recognized as an important protumorigenic cytokine (51, 52). IFN γ has also been long known to be a primary driver of IDO1 induction (14), while more recent data have shown IDO1 to be involved in the induction of IL6 (11, 12). These findings have suggested a broad conceptual framework in which IDO1 acts as a negative feedback check on IFN γ , mediated at least in part through upregulation of IL6.

Further study indicates that this cytokine connection is particularly relevant to the recently recognized involvement of IDO1 in supporting neovascularization, the abnormal development of new blood vessels associated with tumors as well as ischemia in general. Indeed, IDO1 was shown to be required for neovascularization not only in a mouse model of pulmonary breast cancer metastasis but also oxygen-induced retinopathy, and this was found to be the case for IL6 as well (11, 12). IFN γ can act to eliminate neovascularization as an important aspect of its antitumor activity (53, 54). As predicted by the negative feedback model, the reductions in neovascularization associated with losses of either IDO1 or IL6 were completely reversed by the concomitant loss of IFN γ , while pathophysiologically, the loss of IFN γ correspondingly negated the increased resistance to pulmonary metastasis development associated with losses of either IDO1 or IL6 (11, 12). Such are the hallmarks of a disease modifier, the function of which is contingent on influencing disease context. This distinction has important ramifications for IDO1 as a therapeutic target in contrast to general regulators of immunity (e.g., CTLA-4 or PD-1) or cell growth (e.g., EGFR, BRAF, or TGF β), which are not disease context limited and therefore fraught with therapeutic risk including major side effects. Here, it is notable that IDO1 expression is associated with other immune checkpoints, most notably PD-L1 and CTLA-4, supporting the concept of their joint targeting (55). Although preclinical studies have suggested IDO1 blockade may not

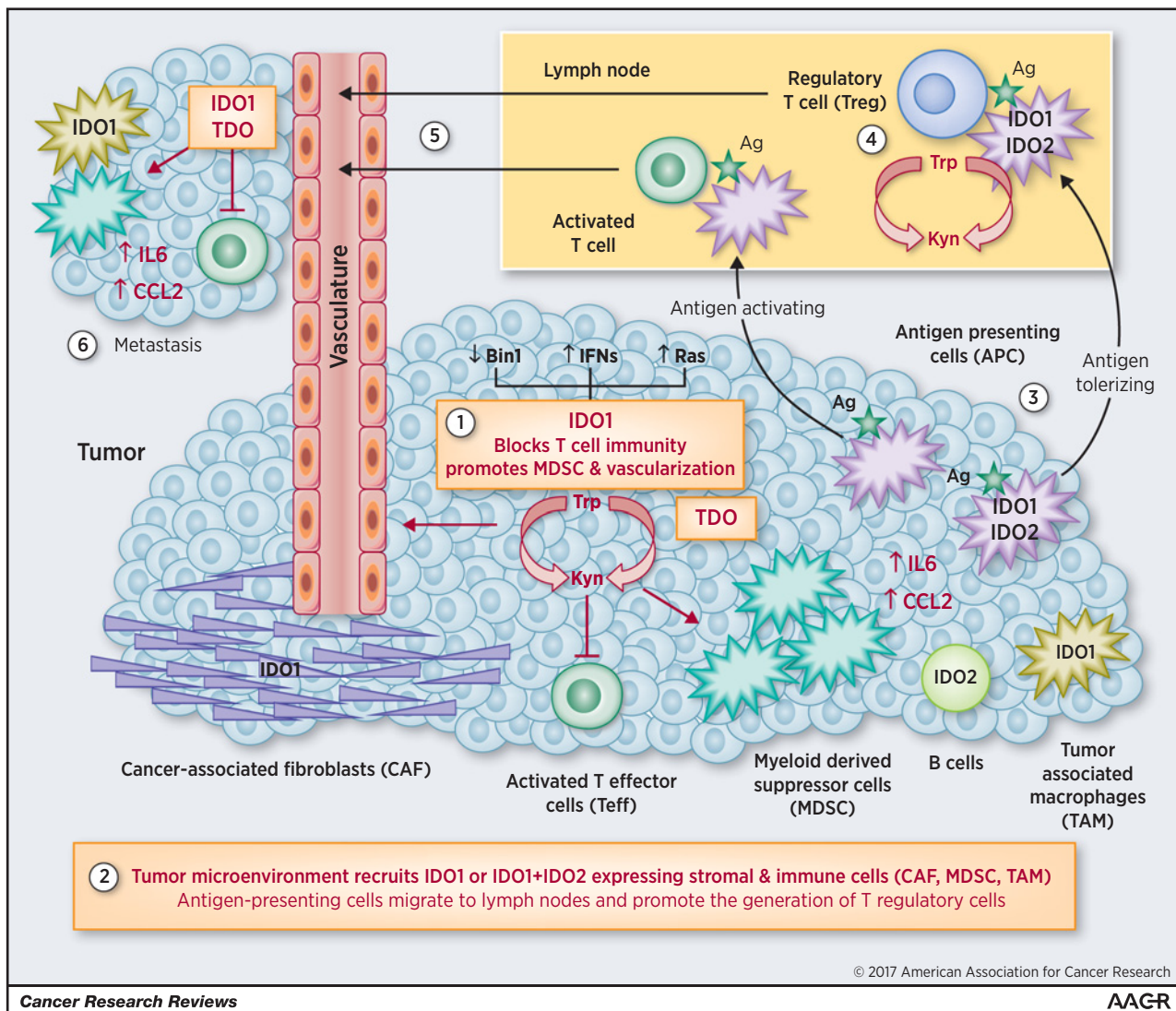


Figure 1.

Impact of IDO1 immunometabolism in cancer. **1**, IDO1 expression patterns in human cancer are complex, occurring heterogeneously in malignant, immune, stromal, and vascular cells within the tumor microenvironment and possibly in antigen-presenting cells (APC) within tumor-draining lymph nodes. TDO and IDO2 are more narrowly expressed than IDO1 in human cancers, with TDO mainly in malignant cells and IDO2 mainly in immune cells. TDO is highly expressed in tumors independently or in parallel with IDO1; it has been ascribed both similar and distinct functions contributing to metastatic progression. IDO2 is expressed in antigen-presenting cells including B cells, where it may influence IDO1 function (86); IDO2 is infrequently overexpressed in tumor cells. Tryptophan catabolism in tumor cells leads to local kynurenine generation and tryptophan depletion in the tumor microenvironment, enabling local suppression of T effector cells (Teff), functional licensing of myeloid-derived suppressor cells and recruitment of the tumor vasculature. **2**, As conditioned by tumor cells, the tumor microenvironment recruits stromal cells expressing IDO1, including cancer-associated fibroblasts, and innate immune cells expressing IDO1 and IDO2, including cancer-associated fibroblasts, myeloid-derived suppressor cells, and tumor-associated macrophages, the latter of which generate IL6 and CCL2 in a manner dependent on local IDO1 activity, positively reinforcing the function of these cells and regulatory T cells that arrive. Tumor antigens absorbed and presented to T cells by antigen-presenting cells that have roved away to a local draining lymph node (**3**) promote the formation of activated T cells or tolerizing T cells (i.e., regulatory T cells), depending on whether the APC expresses IDO1 and perhaps IDO2 (**4**). Antigen-specific T cells leave the lymph node and enter the vasculature (**5**), where they can engage the primary tumor and contribute to the immune attitude of a latent metastatic niche (**6**). CCL2, a potent myeloid cell attractant and prodifferentiation agent, including for MDSCs and TAMs; IL6, the master proinflammatory cytokine IL6, which in tumors helps sustain myeloid-based and lymphoid-based immunosuppression and promotes neovascularization.

be entirely without risk (56), they have been pivotal in establishing that IDO1 provides integral support for tumor-promoting inflammation, thereby helping validate its status as a disease modifier and consequently the rationale for its development as a therapeutic target.

Discovery and Preclinical Development of IDO1 Inhibitors

From a pharmacologic standpoint, IDO1 is very appealing for small-molecule drug development. It is a single-chain

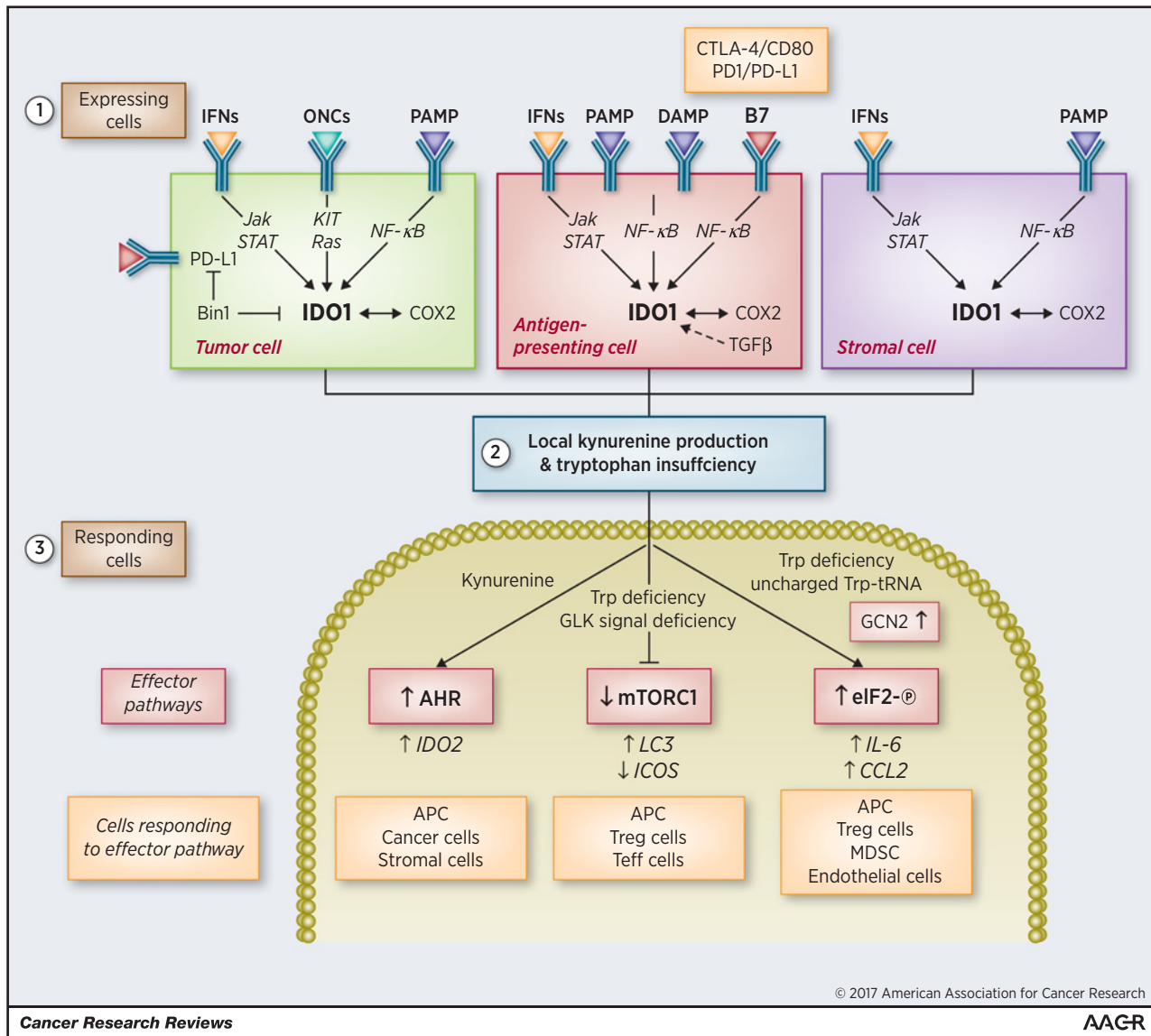


Figure 2.

Sites of IDO1 expression and effector function in tumors. **1**, IDO1 is expressed in tumor cells, inflammatory/antigen-presenting cells, and stromal cells under the diverse controls indicated in different tumor types. In tumor cells, Bin1 attenuation and PGE2 production are key modifiers of IDO1 expression, which is transcriptionally controlled in different tumor settings by the IFN/Jak/STAT, ONC, and PAMP signaling pathways. In inflammatory/antigen-presenting cells, B7, T-cell coreceptor–ligand complexes (e.g., CTLA4-CD80/86 or PD-1-PD-L1), which stimulate signals into T cells and “reverse signals” into antigen-presenting cells. Thus, tolerance mediated by PD-1 and CTLA-4 from regulatory T cells is intertwined with IDO1 upregulation, engendering a feed-forward loop to suppress adaptive immunity. In stromal cells, IDO1 can also be upregulated variably by IFN and PAMP signaling and PGE2 production. **2**, Altogether, IDO1 upregulation in tumor cells and the tumor microenvironment leads to locoregional deprivation of tryptophan and production of its catabolite kynurenine. **3**, Responding cells interpret Trp insufficiency through the mTORC1 and GCN2/eIF-2 pathways, whereas kynurenine acts as a native ligand for the xenobiotic receptor AHR. Downstream effector pathways with responsive target regulators are shown along with the different types of responding cells. APC, antigen-presenting cell; B7, T-cell coreceptor–ligand complexes (e.g., CTLA4-CD80/86 or PD-1-PD-L1), which stimulate signals into T cells and “reverse signals” into antigen-presenting cells; DAMP, damage-associated molecular pattern (e.g., extracellular HMGB1 or ATP ligands for TLR4 or A2A receptors, respectively); eIF-2, a key regulatory factor for mRNA translation initiation; GCN2, a stress response kinase that is activated by binding uncharged tRNA, indicative of amino acid starvation; ONC, oncogenic ligand–receptor signaling complex (e.g., EGFR); mTORC1, a master metabolic regulatory complex that monitors amino acid pools for cell growth or autophagic decisions; PAMP, pathogen-associated molecular pattern (e.g., LPS or CpG ligands of Toll-like receptors [TLR]); Teff, activated effector T cells; Treg, regulatory T cells.

catalytic enzyme with a well-defined biochemistry, one of only a small number of structurally distinct tryptophan catabolizing enzymes (TDO2, IDO2, TPH) with more restricted patterns of expression and substrate specificity compared with IDO1.

Pharmacodynamic measurements of tryptophan and kynurenine, the chief substrate and downstream product of the IDO/TDO reaction, are readily obtained from blood specimens. Bolstering the rationale to develop IDO1 inhibitors, it was

discovered that the anticancer effects of certain targeted drugs can be traced to IDO1 blockade [e.g., imatinib (Gleevec) in GIST treatment; ref. 57]. Several excellent comprehensive reviews have surveyed these medicinal chemistry, pharmacologic, and biological considerations in a detailed manner (5, 58–64).

Here, we survey the preclinical work leading to clinical translation of the IDO pathway inhibitor indoximod and the mechanistically distinct IDO1 enzyme inhibitors epaca-

dostat, navoximod, and BMS-986205. Figure 3 presents a summary of the mechanisms through which these different agents affect tryptophan catabolism by IDO1 and other tryptophan catabolic enzymes. Figure 4A–C introduces key preclinical discovery investigators responsible for early work and reflects a historiography of common roots among founders of the IDO programs at NewLink Genetics and Incyte Inc. Figure 5 presents a timeline of IDO1 preclinical proof of concept and bench-to-bedside milestones from these

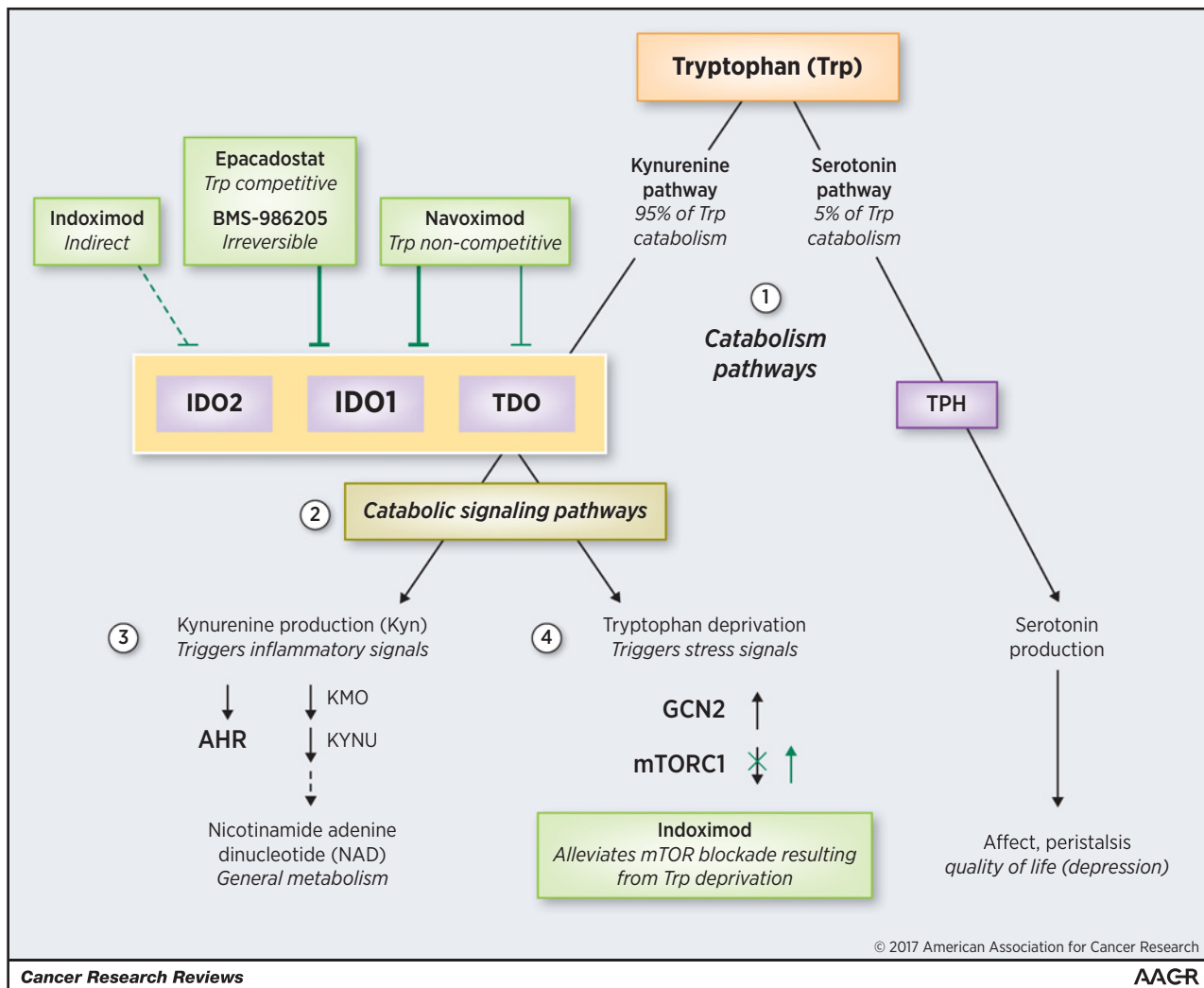


Figure 3.

IDO1 inhibitors and their targets. **1**, Tryptophan (Trp) catabolism proceeds through one of two pathways in mammals, leading to production of nicotinamide adenine dinucleotide or serotonin. The kynurenine pathway accounts for approximately 95% and the serotonin pathway for 5% of tryptophan catabolism, with possible implications on affect and quality of life in cancer patients where the kynurenine pathway is driven by IDO/TDO dysregulation. Epacadostat is >100-fold selective for IDO1 against TDO and represents a highly specific agent with competitive inhibitory kinetics for tryptophan binding. Navoximod is approximately 20-fold selective for IDO1 against TDO and exhibits noncompetitive inhibitory kinetics for tryptophan binding. BMS-986205 is an irreversible inhibitor of IDO1 that is highly specific for that enzyme. None of these agents inhibit IDO2 appreciably. Indoximod is not a direct enzyme inhibitor, and its action is complex; it has been reported to indirectly inhibit IDO2 and/or IDO1 in some settings. Its primary mechanism of action appears to be downstream, in its high potency as a tryptophan mimetic interpreted by mTORC1 as L-tryptophan under conditions of high tryptophan catabolism and autophagy due to tryptophan deprivation by any catabolic enzyme. **2**, The targeted enzyme inhibitors affect both catabolic effector signaling pathways. **3**, Kynurenine functions as a native ligand for the proinflammatory receptor AHR, which activates downstream gene expression. **4**, Tryptophan deprivation triggers starvation-induced signals mediated by upregulation of the general stress kinase GCN2 and downregulation of the mTORC1 complex (which monitors amino acid pools for growth versus autophagy decisions, which are critical for T-cell function).



Figure 4.

Early discovery investigators. **A**, NewLink Genetics team with academic collaborators. Left to right, Alexander Muller (Lankenau), Nicholas Vahanian (New Link), Andrew Mellor (Georgia), David Munn (Georgia), Charles Link (New Link), Mario Mautino (New Link), George Prendergast (Lankenau). The New Link IDO1 program was initiated by Mario Mautino after the company in-licensed founding intellectual property from the Munn/Mellor team at the Medical College of Georgia (now Augusta University) and the Prendergast/Muller team at the Lankenau Institute for Medical Research (LIMR). **B**, Lankenau team. Left to right, George Prendergast, Alexander Muller, James DuHadaway, William Malachowski (Bryn Mawr College). **C**, Incyte and Lankenau. Left to right, Peggy Scherle, a key proponent of the IDO1 program at Incyte, with spouse Alexander Muller, who initiated the IDO1 program at Lankenau with George Prendergast.

teams. Figure 6A–F presents key chemical structures as discussed in the text.

Indoximod (D-1MT; NLG-8189)

By far, the IDO probe most employed in the preclinical literature is the simple racemic compound 1-methyl-D,L-tryptophan (1MT) with a reported K_i for IDO1 of 34 $\mu\text{mol/L}$ (65, 66). The L isomer acts as a weak substrate for IDO1 and is ascribed the weak inhibitory activity observed with the racemate, as the D isomer neither binds nor inhibits the purified IDO1 enzyme (4). Evidence offered in the early 2000s showed that 1MT could weakly retard the growth of cancer cells engrafted into syngeneic hosts (22, 67). However, careful biochemical studies soon made it clear that 1MT is not a valid inhibitor of IDO1 enzyme activity, and that the experiments with 1MT could not address the pivotal question of whether IDO1 enzyme blockade could exert a significant therapeutic effect. Nevertheless, racemic 1MT has continued to serve as a common probe of the IDO pathway in preclinical studies, and further investigations of its antitumor activity led to the eventual clinical translation of the D racemer, now known as indoximod, as a drug candidate with potentially unique effects (Fig. 6A).

In detailed studies of antitumor efficacy in a transgenic model of HER2-driven breast cancer, 1MT exerted little single-agent effect on tumor outgrowth but was found to dramatically empower the efficacy of a variety of chemotherapeutic agents, especially DNA-damaging drugs or taxol, triggering regressions of otherwise recalcitrant tumors (6). This was a pivotal observation in establishing how 1MT may offer a useful tool in cancer therapy. Regressions seen did not reflect drug–drug interaction, that is, by acting to raise the effective dose of the cytotoxic agent, because efficacy was increased without increased side effects. Significantly,

depleting CD4^+ or CD8^+ T cells abolished the effects of not only 1MT but also of another true enzymatic inhibitor of IDO1, consistent with the observed efficacy being dependent on T cell–mediated immunity (6). A later study attributed the majority of the antitumor activity of 1MT to the D racemer, which has no inhibitory effect on IDO1 enzyme activity (7). However, factors in choosing to clinically translate it ultimately included (i) its potency in relieving T-cell suppression by mouse and human IDO1^+ plasmacytoid DCs; (ii) favorable pharmacology and toxicology; (iii) superior preclinical antitumor activity relative to L-1MT in side-by-side comparisons, singly or in combination with chemotherapy; and (iv) its genetic validation based on loss of activity in IDO1-deficient mice (7). The preclinical efficacy of D-1MT/indoximod in combination with chemotherapy led to its inclusion on a selective list of key agents for clinical assessment in 2008 by an NCI immunotherapy workshop (68).

A number of studies have addressed the mechanism of action of indoximod. However, in considering an evaluation of human pharmacokinetics where clinical responses have been noted (69), only one study has provided an explanative mechanism consistent with blood serum levels achieved in clinical trials (70). Specifically, this study revealed that indoximod can resuscitate cellular mTORC1 activity inhibited by tryptophan depletion with an IC_{50} of approximately 70 nmol/L (70). Thus, indoximod acts as a high-potency tryptophan mimetic in reversing mTORC1 inhibition and the accompanying autophagy that is induced by tryptophan depletion in cells. As a central integrator of cell growth signals, mTORC1 receives signals that monitor levels of essential amino acids needed to activate cell growth, including in T cells. Interestingly, indoximod was able to relieve mTORC1 suppression created by tryptophan deprivation with a higher potency than L-tryptophan itself (i.e., at lower concentrations; ref. 70).

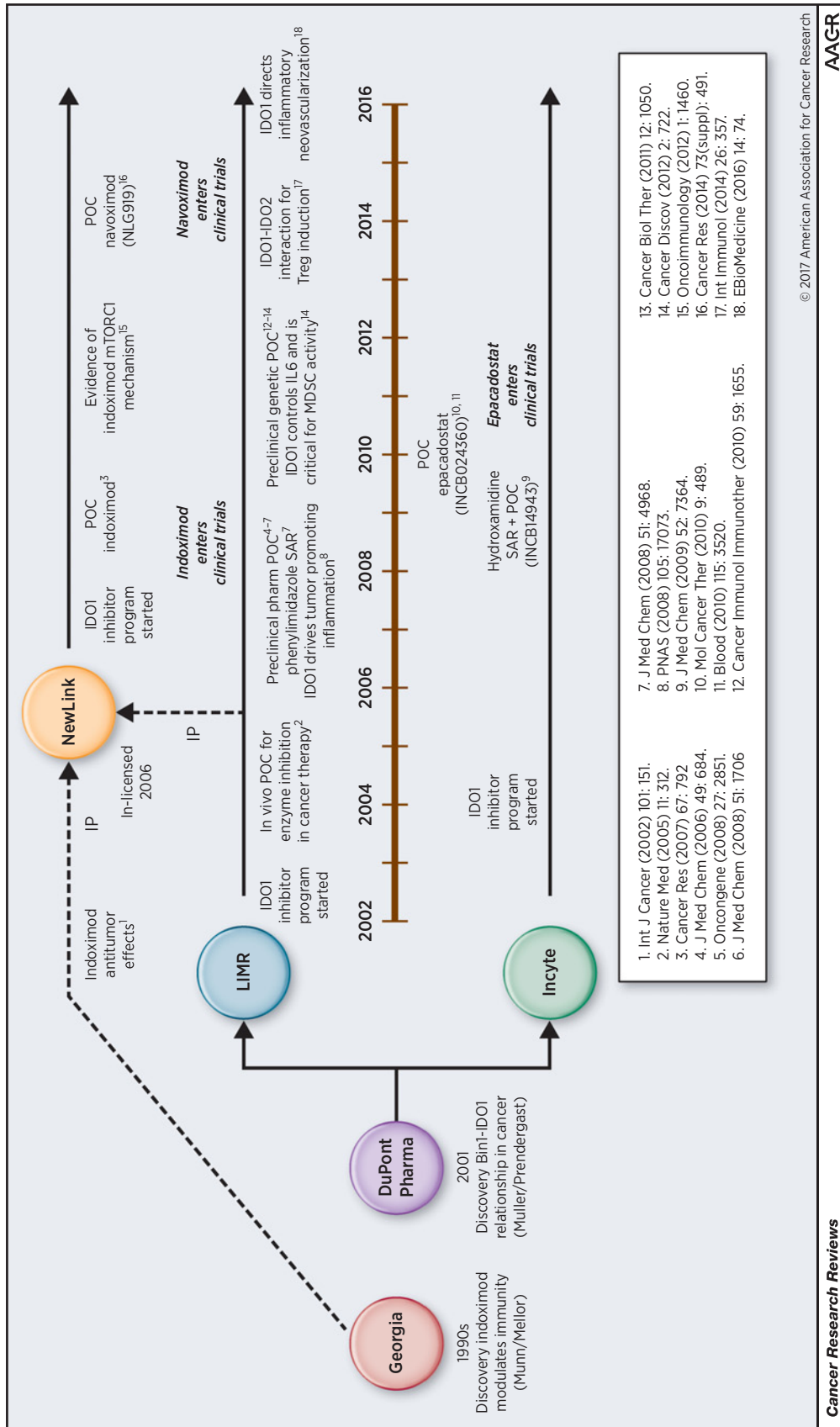
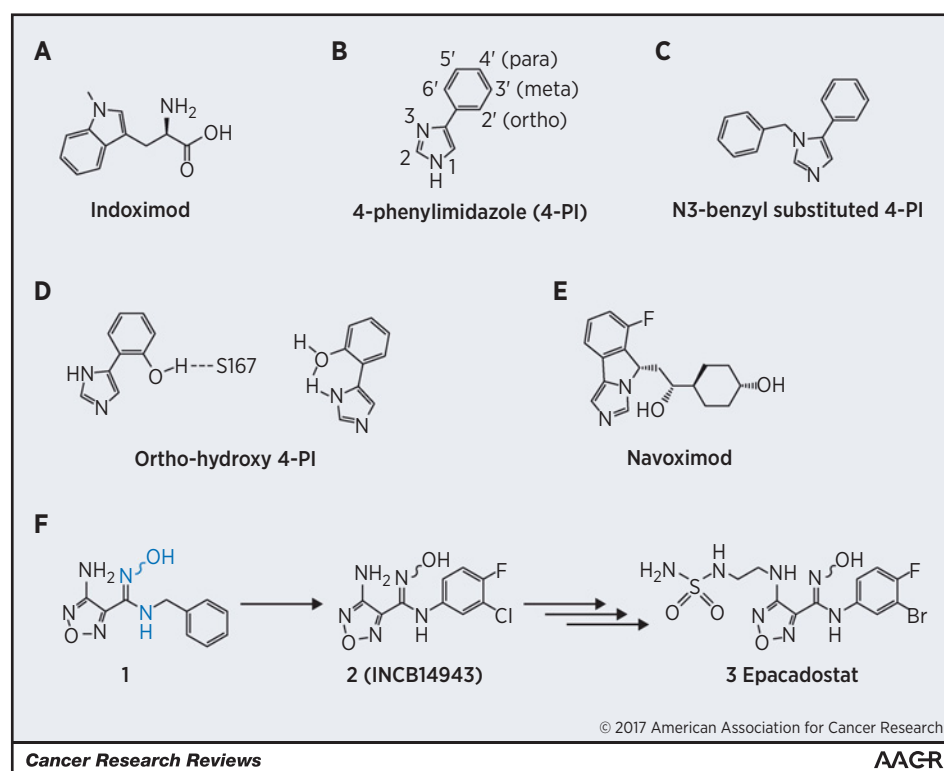


Figure 5. Discovery timelines in preclinical proof of concept. Key milestones are cited in the discovery and preclinical validation of IDO1 as a cancer therapeutic target as referenced in the figure. IP, intellectual property; POC, proof of concept; SAR, structure-activity relationship analysis (for inhibitor class noted).

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**Figure 6.**

IDO1 inhibitor structures and intermediates. **A**, Indoximod. A 1-methyl derivative of D-tryptophan interpreted by mTORC1 in cells as L-tryptophan. **B**, 4-PI. Founding compound of the phenylimidazole chemotype series. **C**, N3-benzyl derivative elaborating this site. **D**, Ortho-hydroxyl modifications, which elaborate potency. **E**, Navoximod (NLG-919), clinical lead from the imidazoisoindole series. **F**, Epacadostat discovery milestones. **1**, Original hit including critical hydroxyamidine (blue). **2**, Preclinical proof of concept lead compound (INCB14943). **3**, Epacadostat (INCB024360) as final clinical lead.

More recent findings confirm this activity in human T-cell cultures, including for indoximod derivatives with superior pharmacokinetic qualities, establishing a unique mechanism of action for this compound acting directly on T cells (71).

The surprising finding that mTORC1 interprets indoximod, essentially a D-tryptophan analogue, as a mimetic of L-tryptophan is a pivotal observation. IDO1 is not expressed in T cells but exerts its effects on them indirectly from a neighboring cellular milieu (Figs. 1 and 2). In acting directly upon T cells, indoximod acts differently from other IDO1 enzyme inhibitors that act outside T cells. Therefore, indoximod use is rationalized in tumors driven by any tryptophan-catabolizing enzyme, and it might be combined productively with frank IDO1 enzyme inhibitors to enhance activity. Recent clinical evidence of the ability of indoximod to safely heighten the efficacy of anti-PD-1 in melanoma patients (72), consistent with preclinical results (8), suggests that mTORC1 restoration may be a sufficient cause of the antitumor effects of IDO1 enzyme inhibitors (72). These findings are interpretable in light of evidence that the T-cell coregulatory receptor ICOS is elevated in T effector cells, which infiltrate melanomas with favorable survival outcomes (73), insofar as ICOS levels are elevated in such cells by mTORC1 activation (74). On the basis of this information, one would predict that therapeutic responses to indoximod occur through mTORC1-mediated ICOS upregulation in tumor-infiltrating T effector cells. In summary, indoximod targets IDO1 signaling indirectly as an IDO pathway inhibitor that appears to act by derepressing mTORC1 in T cells, and perhaps other cells, to produce its antitumor effects.

Phase I studies of indoximod suggest it is well tolerated (69, 75). In a dose escalation study of 22 evaluable advanced

cancer patients receiving taxotere, the coadministration of indoximod was found to be well tolerated to the maximum delivered dose of 1,200 mg twice daily. In this set of patients, 4 partial responses were observed (2 breast cancer, 1 lung adenocarcinoma, 1 thymic tumor), and no drug–drug interactions were noted (69). In a second dose escalation study in 48 advanced cancer patients, an MTD was not reached at the top dose of 2,000 mg twice daily (75). Notably, three patients previously treated with anti-CTLA-4 (ipilimumab) who received 200 mg once daily developed hypophysitis, an autoimmune signature of these inhibitors. In this set of patients, 5 cases of stable disease >6 months were observed. Pharmacokinetic analysis showed a plateau in plasma AUC and C_{max} for indoximod at doses beyond 1,200 mg twice daily. C_{max} occurred at 2.9 hours and the half-life was 10.5 hours. An increase in levels of C reactive protein was noted across multiple dose levels with additional evidence of an increase in tumor antigen autoantibodies (75). Overall, these studies supported the initiation of multiple phase II studies at the dose of 1,200 mg twice daily.

Early reports of phase II trials with indoximod combinations have been provocative. Perhaps most strikingly, administering indoximod with the PD-1 antibody pembrolizumab has been reported to achieve disease control rates in melanoma patients that parallel those produced by the approved combination of nivolumab with the CTLA-4 antibody ipilimumab, but without the high-grade autoimmune side effects seen with this therapy (76, 77). In another phase II trial, in this case in metastatic prostate cancer patients treated with the DC vaccine sipuleucel-T (Provenge), coadministration of indoximod postvaccination led to a >2-fold extension in patient survival (78). In a phase II study of 169 first-line metastatic breast cancer patients treated

with taxotere, coadministration of indoximod did not produce a statistically significant difference in progression-free survival, overall survival, or objective response rate. However, early promising results have been reported from phase IB combination trials where indoximod was combined with standard-of-care chemotherapy in patients with acute myeloid leukemia or pancreatic adenocarcinoma (79, 80), with ongoing phase II trials now proceeding in these disease settings.

Learning more about the distinct mechanism of action of indoximod may yield significant insights. As it relieves a common downstream IDO/TDO effector signal in mTORC1, indoximod is rationalized to attack tumors that overexpress any tryptophan-catabolizing enzyme. Furthermore, its more narrow mechanism of action opens the possibility that relieving mTORC1 blockade in T cells is sufficient for anticancer efficacy by an IDO/TDO enzyme inhibitor, meaning that inhibiting all IDO/TDO function is unnecessary and may even heighten side effects. Further studies of the connections between tryptophan catabolism and mTORC1 control may yield an important new crop of useful immunoregulatory principles. In developing the intriguing research directions opened by indoximod, a prodrug form termed NLG-802 with superior pharmacokinetics has been reported recently that has entered phase I testing (71).

Navoximod (NLG-919)

Building on the discovery of the *Bin1*-IDO1 connection in tumoral immune escape (6), the Prendergast/Muller group embarked in 2002 at the Lankenau Institute for Medical Research (Wynnewood, PA) on a project to genetically and pharmacologically validate IDO1 as a therapeutic target in preclinical models of cancer. This group initiated an IDO1 inhibitor discovery program by screening publicly available indoleamines and indoleamine mimetics and then, in collaboration with Malachowski and colleagues at Bryn Mawr College (Bryn Mawr, PA), synthesizing sets of chemical derivatives for hits of interest. Bioactive inhibitors identified in several structural classes in this manner displayed similar biological properties, namely, antitumor effects that relied upon T-cell function and IDO1 targeting (6, 7, 81–84). Among these bioactive compounds, MTH-*trp* was the first to clearly demonstrate that impeding IDO1 enzyme activity could elicit antitumor effects (6). This pivotal observation was extended by studies of additional structural classes of bioactive inhibitors in establishing a pharmacologic proof of concept for IDO1 as a cancer drug target (7, 81, 82, 84). In one important direction influencing subsequent drug discovery, their investigation of structure–activity relationships for a phenylimidazole chemotype founded in an early crystallographic study provided initial clues to key features of a bioactive IDO1 inhibitor (83, 85). Genetic proof of concept was subsequently achieved in mice lacking IDO1, corroborating evidence of a therapeutic effect for IDO1 blockade in cancer as mediated by effects on inflammation, adaptive immunity, and neovascularization (11, 12, 49). Interestingly, later work also implicated IDO2 in Treg control by IDO1 (86). Together, these pharmacologic and genetic projects were pivotal in preclinical validation of IDO1 as a therapeutic target in cancer.

Emerging from this proof-of-concept project, the phenylimidazole chemotype appeared the most promising with a drug-like profile (83). This series had its roots in 4-phenylimidazole (4-PI) identified originally in 1989 as a weak noncompetitive inhibitor of IDO1 enzyme activity (87). Despite showing noncompetitive

kinetics, early spectroscopic studies confirmed by later crystallographic analyses showed that 4-PI bound the heme iron at the IDO1-active site (87). This work seeded initial structure-based drug design from Malachowski and colleagues (83), as an informative step in later discovery of the imidazoisoindole chemotype and the clinical lead navoximod, as developed independently by the medicinal chemistry team at NewLink Genetics Corporation.

In exploring chemical derivatives of 4-PI, Malachowski and colleagues probed the active site of IDO1 with structural modifications to explore binding interactions within it, namely, (i) the active site entrance region decorated with heme 7-propionic acid; (ii) the active site interior, especially interactions with C129 and S167; and (iii) the heme iron-binding group. Substitutions at the active site entrance region focused on the N-1, C-2, and N-3 positions of the imidazole ring (Fig. 6B). In the 4-PI-bound crystal structure, the entrance region is occupied by an N-cyclohexyl-2-aminoethanesulfonic acid (CHES) buffer molecule, with its alkyl portion making hydrophobic interactions with F163 and F226, and its amino group forming an ion pair with the heme 7-propionic acid. N-1 derivatives lost inhibitory activity, confirming binding of the N-1 nitrogen to the heme iron, and, more importantly, demonstrating that the N-3 nitrogen of the imidazole cannot substitute to bind at the heme iron. However, the N-3 benzyl-substituted derivatives generated were observed to be equipotent to 4-PI, showing that imidazole ring substitutions might be tolerable (Fig. 6C) to situate a hydrocarbon moiety at the active site entrance. The N-3 benzyl-substituted compound suggested the correct imidazole ring location and spatial tolerance, likely occupying the active site entrance where the CHES buffer molecule sits in the IDO1-4-PI crystal structure (87). This insight was supported by studies of brassinin derivatives as IDO1 inhibitors, that is, a heme iron-binding group flanked by two large aromatic or hydrocarbon structures (81).

In probing the active site interior, S167 and C129 were in close proximity to the phenyl ring of 4-PI bound to IDO1 (87). Ortho, meta, and para substitutions of the phenyl ring revealed that ortho-hydroxy (2'-OH) modifications had the greatest impact on potency (Fig. 6D). In exploring this effect, it was found that a 2',6'-dihydroxy-phenyl derivative presenting a hydroxy group to S167 or to N-3 imidazole was roughly equipotent to the 2'-hydroxy derivative, indicating no additional benefit from both events. Retrospectively, this observation may explain the benefits of planarization via a hydrocarbon bridge to replicate the H-bond to the N-3 imidazole. In evaluating the heme iron-binding group, substituting alternative aromatic rings for the imidazole in 4-PI always yielded less potent compounds. Later work illustrated that triazole can substitute for imidazole for presumptive histidine binding (88). In summary, these early studies of the phenylimidazole series of IDO1 inhibitors yielded three insights, namely, that (i) N-3 substitutions suggested the active site could accommodate large hydrocarbon moieties; (ii) an ortho-hydroxyl group was beneficial; and (iii) the imidazole was optimal for binding to the heme iron.

Later development of an imidazoisoindole series incorporated some of these insights but also yielded novel information incorporated into the clinical lead navoximod (Fig. 6E; refs. 89, 90). X-ray structural information shows that the ring planes of the PI and imidazoisoindole series assume similar but not identical positions in the active site (91).

Navoximod has several features of interest defined in preclinical studies. Its potency as an IDO1 inhibitor is $EC_{50} = 75$ nmol/L

in cell-based assays, with only 10- to 20-fold selectivity against TDO (89). Thus, navoximod may exhibit unique activity in tumors expressing both IDO1 and TDO, the latter of potential relevance to intrinsic or acquired resistance to IDO1 blockade (see below). Like earlier compounds in the phenylimidazole series, navoximod exhibits noncompetitive inhibition kinetics. These features contrast with the greater IDO1 specificity and competitive inhibition kinetics displayed by epacadostat, with which a clinical comparison may be informative. Navoximod is orally bioavailable with a superior pharmacokinetic and toxicity profile in the imidazoisoindole series (89). Its oral administration reduced plasma kynurenine levels by approximately 50% in mice and it relieved IDO1-induced T-cell suppression in human cell cultures. In preclinical models, navoximod greatly enhanced vaccine responses against B16 melanoma, reducing tumor size approximately 95% within 4 days of vaccination, and improved the efficacy of anti-PD-1 against EMT6 mammary carcinoma (92), where increased CD8⁺ T/Treg ratios, plasma IFN γ levels, and activated intratumoral macrophages and DCs were noted.

There has been limited clinical study of navoximod as yet. In patients with recurrent/advanced solid tumors and the safety, pharmacokinetic and pharmacodynamic results from a phase IA study have been reported (90). As monotherapy, it was well tolerated at up to 600 mg twice daily on a 21/28-day cycle, with stable disease observed in 7 of 17 patients. Safety, pharmacokinetics, and pharmacodynamics have been evaluated on a continuous dosing schedule (twice daily, 28/28 days), also in combination with the anti-PD-L1 antibody atezolizumab (93).

Epacadostat (INCB024360)

Building on evidence of immunomodulatory effects of 1MT in preclinical graft models (23, 69), Incyte Corporation initiated a project in 2004 to discover small-molecule inhibitors of the IDO1 enzyme, focusing ultimately on a hydroxylamine chemotype series from which the tryptophan-competitive inhibitor epacadostat (INCB024360) was developed as a lead clinical agent. Details regarding the chemistry program that developed epacadostat from earlier compounds in this series have been reported elsewhere (94, 95).

This project was initiated by a high-throughput screen of a proprietary collection of approximately 300,000 compounds, which identified a single viable hit exhibiting selective micromolar-level activity as a tryptophan-competitive inhibitor against the purified IDO1 enzyme and in cell culture (Fig. 6F, 1; ref. 96). This novel chemotype contained hydroxyamine as a unique functional group that structure-activity relationship studies revealed was essential for IDO1 enzyme inhibitory activity. Adsorption spectroscopy supported the hypothesis that the hydroxyamine moiety bound directly to the iron of the heme in the IDO1-active site. Parallel synthesis and a proprietary preparative LC/MS purification technology provided rapid access to hundreds of analogues and afforded a proof-of-concept compound INCB14943 (Fig. 6F). Preclinical efficacy was demonstrated by dosing INCB14943 subcutaneously in a B16 melanoma graft model. Importantly, no tumor growth inhibition was achieved by dosing in immunodeficient *nu/nu* B16 mice, consistent with on-target activity. A full program of medicinal chemistry was engaged to overcome the low oral bioavailability of the lead (95). ADME investigations identified glucuronidation of the hydroxyamine pharmacophore as the main metabolic liability and established *in*

vitro phase II assays to allow medicinal chemistry to test the propensity of subsequent compounds to undergo phase II metabolism.

In analyzing thousands of synthetic derivatives, the most potent compounds with the best oral bioavailabilities unexpectedly required a larger number of hydrogen bond donors and hydrogen bond acceptors and higher polar surface area (PSA) than one would predict to afford high Caco2 permeability and oral bioavailability. The unique chemotype was shown to form several internal hydrogen bonds, thus shielding the polar functionality to allow good membrane permeability. Through a data-centric medicinal chemistry approach focused on improving potency and pharmacokinetics rather than "drug-likeness," INCB24360/epacadostat was ultimately developed as a clinical lead agent (Fig. 6F, 3). As a result of this approach, the calculated properties of epacadostat actually fall outside of the classic rules of drug discovery (Lipinski's Rule of 5 and Veber's PSA Rule; refs. 97, 98), but nevertheless good oral bioavailabilities were observed across all species tested (rodent, canine, primate). In profiling the compound against a panel of enzymes, receptors, ion channels, along with related or unrelated iron-containing heme enzymes (IDO2, TDO2, Cyp P₄₅₀ enzymes), epacadostat was shown to be a highly selective inhibitor for the IDO1 enzyme.

Detailed preclinical studies showed that epacadostat acts as a tryptophan-competitive inhibitor of the catabolic activity of human IDO1 in cell-based assays (IC₅₀ = 12 nmol/L) with >100-fold selectivity exhibited against IDO2 and TDO2. In cocultures of human allogeneic lymphocytes with DCs or tumor cells, epacadostat promoted the growth of effector T cells and NK cells, reduced conversion of naïve T cells to Tregs, and increased the number of CD86^{high} DC (99). Consistent with these effects, administration of epacadostat to tumor-bearing syngeneic mice inhibited kynurenine levels approximately 90% in both plasma and tumor and reduced tumor growth in immunocompetent but not immunocompromised mice, confirming that drug efficacy relies upon functional immunity. Furthermore, in the B16 melanoma model, epacadostat was found to enhance the antitumor effects of anti-CTLA4 or anti-PD-L1 antibodies, where increased IL2 production and CD8⁺ T-cell proliferation were suggestive of more pronounced T-cell activity (100).

In a phase I human study to investigate safety and MTD, pharmacokinetics, pharmacodynamics, and antitumor activity (101), epacadostat was generally well tolerated, effectively normalizing plasma kynurenine levels with maximal inhibition of IDO1 activity at doses of ≥ 100 mg twice daily. No objective responses were detected, although stable disease for ≥ 16 weeks was observed in 7 of 52 patients (101). A randomized phase II study compared epacadostat versus tamoxifen treatment in 42 patients with biochemically recurrent-only epithelial ovarian cancer, primary peritoneal carcinoma, or fallopian tube cancer, where epacadostat was found to be well tolerated but showed no difference in efficacy (102).

Far more exciting are early reports of the results of combining epacadostat with PD1 antibodies in melanoma, head and neck cancer and lung, renal, and urothelial cancers (103). In melanoma, anti-PD-1 combinations (either pembrolizumab or nivolumab) show rates of overall response and disease control similar to those produced by the approved combination of PD-1 and CTLA-4 antibodies (ipilimumab), but without the high-grade autoimmune side effects of combining those antibodies (104, 105). In head and neck cancer, an interim report of 38 heavily pretreated

Table 1. IDO1 inhibitors reported in development from pharmaceutical industry

	NewLink	NewLink	Incyte	BMS Flexus	Pfizer iTeos	Merck IO-Met	Roche CuraDev
Name	Indoximod <i>NLG-8186</i>	Navoximod <i>NLG-919</i>	Epacadostat <i>INCB024360</i>	BMS-986205 <i>F001287</i>	PF-06840003	IOM2983	RG-70099
Structure						Unknown patent published	Unknown patent published
Mechanism	Stimulates mTORC1 downstream of IDO1/TDO	Catalytic inhibitor	Catalytic inhibitor	Suicide inhibitor	Catalytic inhibitor	Unknown	Unknown
Inhibitory kinetics	NA	Tryptophan noncompetitive	Tryptophan competitive	Irreversible	Tryptophan noncompetitive	Unknown	Unknown
Cell-based potency	NA	75 nmol/L	12 nmol/L	2 nmol/L	1100 nmol/L	Unknown	Unknown
TDO selectivity	Nonselective	10–20 fold	>100-fold	>100-fold	>100-fold	>100-fold	~5-fold
Clinical entry	2008	2015	2012	2015	2016	NA	NA
Program status	Phase III	Phase IB	Phase III	Phase II	Phase I	Preclinical	Preclinical

patients suggested that epacadostat could increase rates of overall response and disease control when administered with anti-PD-1, in a manner independent of PD-L1 levels or HPV status, also without any notable increase in side effects. On the basis of such results, the clinical development of epacadostat is currently being advanced aggressively in a total of 14 tumor types (including the above cancers) where it is being coadministered with anti-PD-1 antibodies (nivolumab or pembrolizumab) or anti-PD-L1 antibodies (atezolizumab and durvalumab). A definitive phase III trial in melanoma for the combination with pembrolizumab is reported to be completely enrolled. The similar observations produced in melanoma patients so far by epacadostat or indoximod in combination with anti-PD-1 is extremely encouraging given the different mechanisms of action of these two agents. In summary, clinical validation is emerging for IDO1 and tryptophan catabolic pathways as therapeutic targets to improve cancer management.

BMS-986205 and other IDO1 inhibitors

An increasing number of other IDO1 enzyme inhibitors are being registered in the patent literature, as listed elsewhere (59); however, information on these compounds in the peer-reviewed biomedical literature remains limited. Several IDO1-inhibitory compounds developed in the pharmaceutical industry have been reported to be in later stage preclinical development or early clinical testing (see Table 1 for summary). BMS-986205 is an IDO1 inhibitor licensed by Flexus Inc. to Bristol-Myers Squibb for clinical development. This compound appears to act as an irreversible inhibitor of high potency (~2 nmol/L) with superior pharmacokinetics relative to epacadostat and navoximod. In 2015, BMS-986205 entered a phase I/II study in melanoma patients as monotherapy and in combination with nivolumab (NCT02658890). An interim report noted the compound was well tolerated in patients (106). A second phase I study of the same combination is being conducted in various other types of advanced cancer patients (NCT03192943).

PF-06840003 is a tryptophan noncompetitive, non-heme-binding IDO1 inhibitor licensed by iTeos SA to Pfizer for clinical development (Wythes and colleagues, SITC 2016, poster 253). This compound is predicted to have favorable human pharmacokinetic characteristics, a prolonged human half-life that may allow single-dose daily administration, and CNS penetration properties that may enable efficient access to brain metastases.

In a preclinical study, PF-06840003 enhanced the antitumor efficacy of anti-PD-1/PD-L1 axis blockade. A first-in-patient study was initiated in 2016 in malignant gliomas (NCT02764151).

Other IDO1 inhibitors developed by pharmaceutical groups in late preclinical development, including IOM2983 (Merck/IO-Met) and RG-70099 (Roche/CuraDev), have little information disclosed in the biomedical literature as yet (Table 1). Preclinically, a number of new IDO1 inhibitor scaffolds have been reported, many of which are covered in a recent review (59). Of particular recent note, identification of a benzenesulfonyl hydrazide series of inhibitors (107) yielded compound 40 as a potent IDO1 inhibitor in cells with $EC_{50} = 68$ nmol/L, 59% oral bioavailability, the administration of which produced significant tumor growth delay without body weight loss in mouse tumor models (108).

On the Horizon: TDO and IDO2 in Inflammatory Programming and Therapeutic Utility

Accumulating evidence points to the distinct tryptophan catabolic enzyme TDO as another means of immune escape (62, 109, 110). Thus, TDO inhibition has emerged as a parallel immunomodulatory strategy to attack tumors (111–116), the rationale for which has been reviewed recently by pioneers in this area (62, 110). An early bioactive inhibitor termed 680C91 (111) has been used for mouse studies, but more potent and pharmacologically favorable compounds have been reported (112–115). Deleting the TDO gene in mice (*Tdo2*) causes L-tryptophan to accumulate and these mice show neurologic alterations, possibly due to serotonin elevation (117). Treating mice with 680C91 phenocopies *Tdo2* deletion and increases sensitivity to endotoxin-induced shock, implicating TDO, like IDO1, in inflammatory programming (118). However, there are differences in the inflammatory characteristics conferred by TDO despite a common role of these enzymes in tryptophan catabolism (119). Although enzymological differences may help explain these different roles, they may also reflect differences in locoregional control of kynurenine production or in the availability or efficiency of kynurenine effector mechanisms (e.g., AhR binding, kynurenine pathway catabolic enzymes, etc.). Although there is evidence of a contribution to tumoral immune escape using bioactive inhibitors (48, 113), no genetic proof in mice exists as yet, nor an

understanding of the nature or extent of TDO expression in tumor cells or the tumor microenvironment. In preclinical studies, TDO inhibitors have not exhibited major safety concerns, although the neurologic effects produced by genetic deletion of TDO in the mouse suggest an area of concern in their clinical evaluation (110, 117). With the generation of TDO-deficient mice (117), these animals should be available to investigators for in-depth analysis of this issue of critical importance to consideration of TDO inhibitor development. Nevertheless, despite the current lack of a genetic preclinical proof of concept, there exists a pharmacologic rationale to explore TDO inhibitors along with IDO1/TDO combined inhibitors as next-generation modalities in the field, for which navoximod and RG-70099 represent initial steps from pharmaceutical industry (Table 1).

Beyond its role in immune escape, upregulation of TDO in cancer cells has been found to contribute to tumor cell survival and metastatic prowess as well (120). Anoikis resistance is a key step in metastatic progression (121). In a seminal study of aggressive "triple-negative" breast cancer (TNBC), Richer and colleagues showed how TDO upregulation in cancer cell suspension culture was essential for resistance to anoikis and for metastatic capacity (120). Furthermore, TDO-induced kynurenine production was sufficient to activate the AhR signaling pathway, as is also the case with IDO1, and pharmacologic inhibition or genetic attenuation of either TDO or AhR was sufficient to restore anoikis sensitivity and reduce invasive character. Finally, 680C91 treatment was sufficient to reduce pulmonary metastasis in tumor-bearing mice, and TDO status in clinical TNBC specimens associated with increased grade, ER-negative status, and shorter overall survival (120). These findings extend the concept that TDO can act like an oncogene in cancer cells to directly promote malignant outgrowth, suggesting that upregulation of TDO may provide a selective advantage beyond simply enabling peripheral immune tolerance.

Although less studied, IDO2 is a structural relative of IDO1 also implicated in immunomodulation. The evidence for this function is mainly through studies of autoimmunity to date (122), but IDO2 is anticipated to make contributions to cancer given its overexpression in some solid tumors (123, 124). In mice, *Ido2* gene deletion does not affect embryonic development, hematopoiesis, or immune character, nor does it affect blood levels of tryptophan or kynurenine (86). The tryptophan catalytic activity of IDO2 is weaker than that of IDO1 or TDO, especially for the human enzyme, but it is clear that IDO2 biochemistry also relies upon a different coreductant system in cells (125). Indeed, earlier characterizations of human IDO2 as "inactive" are incorrect, reflecting only nonoptimal biochemical conditions that have been recently improved (S.-R. Yeh, personal communication; ref. 126). The fact that IDO2 deletion does not affect systemic levels of tryptophan likely reflects its far narrower range of expression, relative to IDO1 and TDO, which are expressed both more broadly and more strongly.

Mouse genetic studies offer early evidence of a role for IDO2 in immune tolerance. *Ido2*^{-/-} mice exhibit deficiency in their ability to support IDO1-activated Tregs (86). A role in tolerance has also been suggested by work in human DCs (127). Reciprocally, hematopoietic cells from *Ido1*^{-/-} mice are mosaically deficient for IDO2 function, reinforcing the idea of IDO1-IDO2 interaction in immune control (86). Interestingly, in a model of autoimmune arthritis, the administration of indoximod is therapeutic, phenocopying the amelioration of this chronic inflammatory

disease achieved by *Ido2* deletion (128). Combining indoximod treatment with *Ido2* deletion provided no added benefit, consistent with IDO2 inhibition being the mechanism of action as suggested by earlier evidence that indoximod blunts IDO2 catalytic activity (129). Even more strikingly, the therapeutic effect of indoximod is abolished by *Ido1* deletion consistent with genetic evidence of IDO1-IDO2 interaction (128). Accordingly, common genetic variations in human IDO2, which reduce its catalytic activity, may be relevant to clinical responses to indoximod (129).

Reduced inflammatory disease in *Ido2*^{-/-} mice is associated with diminished levels of autoantibody and has been traced specifically to the lack of IDO2 in B cells, suggesting that IDO2 acts to support B-cell involvement in eliciting inflammation (128, 130, 131). These findings are interesting in light of evidence that certain cancers rely upon B-cell-dependent inflamed states for their development (132, 133). Although IDO2-deficient mice are unchanged with regard to their susceptibility to inflammatory skin carcinogenesis (86), they resist the development of K-Ras-induced pancreatic cancers (G.C. Prendergast and A.J. Muller, unpublished observations) where B cells have been implicated (134, 135). Cancers do not tend to overexpress IDO2, although this has been observed in melanoma and gastric, brain, and pancreatic cancers, in the latter case quite widely (124). The *IDO2* gene is regulated by the AHR (136, 137), which binds Kyn as an endogenous ligand produced by the more active IDO1 enzyme (48). Thus, given genetic evidence of IDO1-IDO2 interactions (86), it is conceivable that locoregional IDO1 activity may, by producing Kyn, increase levels of IDO2 in roving antigen-presenting cells in the tumor microenvironment, perhaps contributing to a tolerized state that engenders IDO1-mediated Treg formation in tumor-draining lymph nodes (4).

Small-molecule inhibitors of IDO2 have been reported (126, 138-141), but they are not bioactive and have yet to be studied *in vivo*. Interestingly, a B cell-penetrating bioactive antibody against IDO2 has been reported that can phenocopy the antiarthritic effects of *Ido2* genetic deficiency in the mouse (131), but this antibody has yet to be tested in cancer models.

Summary and Future Perspectives

As with microbial and viral infections, it is clear that systemic immunity is required for effective immunotherapy in cancer (142). Vaccines against infectious pathogens include the use of adjuvants to program locoregional inflammatory signals needed for effective priming of systemic immunity. These vaccines have the advantage of being administered prophylactically so that systemic immunity can be primed and ready to respond to an infection, whereas cancer immunotherapy involves the treatment of established disease in which dominant tolerogenic mechanisms have already been engaged. Thus, the reprogramming of the tumor-promoting inflammatory state to one that supports the creation of systemic immunity is important to developing any effective immunotherapy regimen. In reprogramming inflammation, IDO1 inhibitors may offer broadly useful "immunometabolic adjuvants" to empower systemic immune responses with immunotherapy, as recent clinical studies focused on immune checkpoint inhibitors suggest, as well as with chemotherapy or radiotherapy. From this perspective, they may be effective in helping convert immunologically "cold" tumors where an inflammatory stimulus is desired, for example, prostate cancers.

Immune combinations of IDO1 inhibitors with pembrolizumab or nivolumab are currently most intriguing. In human melanoma, there is strong evidence of an intertwined relationship between CTLA-4, PD-L1, and IDO1, suggestive of an interrelated signaling network (55). Notably, circulating levels of these markers on plasmacytoid DCs are altered with prior invasion of a sentinel lymph node, where IDO1 expression in the sentinel node correlated to increased numbers of IDO1⁺ peripheral blood mononuclear cells. In identifying the patients most likely to benefit from IDO1 inhibitors, it is tempting to speculate that these markers in the peripheral blood, associated with advanced disease and negative outcome in melanoma, create a rationale for combination treatments directed against these markers to create a desired synergistic response. Vaccine combinations are also appealing to consider (e.g., sipuleucel-T), especially in the context of a coordinate proinflammatory stimulus of the sort created by oncolytic viruses or PAMP/DAMP admixture. Antineovascular effects have yet to be explored in combination with agents that target angiogenesis (e.g., bevacizumab, bortezomib) or the hypoxic environment (e.g., hypoxia-activated prodrugs, HIF-targeting drugs, antimetabolites). In this light, IDO inhibitors may offer a unique tool in certain settings such as brain tumors or leukemia, where antiangiogenic approaches in the cranium or bone marrow clearly pose interest. Finally, combinations with other "immune adjuvant" approaches, radiotherapy, STING agonists, glutamine/glucose starvation signaling, and adenosine signaling, can be envisioned on the basis of emerging mechanistic interfaces yet to be therapeutically explored.

In considering TDO and IDO2, which are currently little understood in cancer, addressing to what extent blocking TDO and/or IDO2 may widen efficacy and reduce inherent resistance or the risk of acquired resistance to IDO1 blockade are additional key questions for the future. In its connections to cancer and autoimmune disease, IDO2 poses an especially intriguing subject to explore, particularly with regard to the use of IDO2 inhibitors to limit autoimmune-related adverse events that immune checkpoint therapies and other cancer therapies can produce, which are often severe and durable in survivors (e.g., arthritis-like joint inflammation, which IDO2 blockade might relieve; refs. 128, 130, 131). As IDO1 inhibitors continue to be evaluated in the clinic, combinatorial targeting of IDO1, IDO2, and TDO to

various degrees in a single modality may afford a special opportunity to safely widen the therapeutic window of many regimens, not only for the new expensive immuno-oncology agents of great current interest, but also the less expensive generic standard-of-care modalities involving chemotherapy and radiotherapy, which are likely to remain workhorses of the cancer clinic for years to come.

Disclosure of Potential Conflicts of Interest

G.C. Prendergast has ownership interest in Incyte Corporation and NewLink Genetics Corporation (including patents) and is a consultant/advisory board member for NewLink Genetics Corporation. W.P. Malachowski has provided expert testimony for NewLink Genetics shares. J.B. DuHadaway has ownership interest (including patents) in U.S. Patents (7705022, 7714139, 8008281, 8383613, and 8476454) and as a stockholder in NewLink Genetics. A.J. Muller reports receiving a commercial research grant from IO Biotech ApS and has ownership interest (including patents) in NewLink Genetics Corp. equities, Incyte Corp. equities, and U.S. Patents 7705022, 7714139, 8008281, 8383613, 8389568, and 8476454.

Acknowledgments

We thank our many colleagues and collaborators and apologize for omitting citations to many primary research reports cited only through reviews due to space limitations. We are grateful to Peggy Scherle and Andrew Combs at Incyte Corporation and Mario Mautino at NewLink Genetics Corporation for their critical comments and contributions to the text. G.C. Prendergast holds The Havens Chair for Biomedical Research at the Lankenau Institute for Medical Research.

Grant Support

G.C. Prendergast recognizes support from the NCI (R01 CA109542, R01 CA191191), the Lankenau Medical Center Foundation and Main Line Health, along with previous support for IDO-related research from the Department of Defense Breast and Lung Cancer Research Programs, Dan Green Foundation, Sharpe-Strumia Research Foundation, and New Link Genetics Corporation. A.J. Muller recognizes support from I-O Biotech, Inc., the Lankenau Medical Center Foundation and Main Line Health, as well as previous support for IDO-related research from the Department of Defense Breast Cancer Research Program, Susan G. Komen for the Cure, and the Pennsylvania Department of Health. W.P. Malachowski recognizes the support of NSF grant CHE-0958996 922 and Bryn Mawr College.

Received August 18, 2017; revised September 23, 2017; accepted November 1, 2017; published online December 15, 2017.

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