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Targeting indoleamine-2,3-dioxygenase in cancer: Scientific rationale and clinical evidence

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ABSTRACT

Immunotherapy through immune checkpoint blockers (ICBs) is quickly transforming cancer treatment by improving patients' outcomes. However, innate and acquired resistance to ICBs remain a major challenge in clinical settings. Indoleamine 2,3-dioxygenases (IDOs) are enzymes involved in tryptophan catabolism with a central immunosuppressive function within the tumor microenvironment. IDOs are over-expressed in cancer patients and have increasingly been associated with worse outcomes and a poor prognosis. Preclinical data have shown that combining IDO and checkpoint inhibition might be a valuable strategy to improve the efficacy of immunotherapy. Currently, several IDO inhibitors have been evaluated in clinical trials, showing favorable pharmacokinetic profiles and promising efficacy. This review describes the mechanisms involved in IDO-mediated immune suppression and its role in cancer immune escape, focusing on the potential clinical application of IDO inhibitors as an immunotherapy strategy for cancer treatment.

Contents

Abbreviations: AEs, Adverse events; AhR, Aryl hydrocarbon receptor; APCs, Antigen presenting cells; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; CTLA-4, Cytotoxic T-lymphocyte antigen 4; DC, Dendritic cell; DLTs, Dose-limiting toxicities; D-1-MT, 1-Methyl-D-tryptophan; GCN2, General control nonderepressible 2; IDO, Indoleamine 2,3-dioxygenase; ICBs, Immune checkpoint blockers; IFN, Interferon; IL, Interleukin; KYNase, Kynureninase; NK, Natural killer; NSCLC, Non-small cell lung cancer; MDSCs, Myeloid-derived suppressor cells; mPFS, Median progression free survival; MTD, Maximal tolerated dose; mTOR, Mammalian target of rapamycin; ORR, Overall response rate; PD-1, Programmed cell death protein 1; PD-L1, Programmed death-ligand 1; RCC, Renal cell carcinoma; SCCHN, Squamous cell carcinoma of head and neck; TGF, Transforming growth factor; Teff, T effector; TNBC, Triple negative breast cancer; TMZ, Temozolamide; Treg, T regulatory; tRNA, Transfer ribonucleic acid; UC, Urothelial carcinoma; 1-MT, 1-Methyl-DL-tryptophan.

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1. Introduction

In the last decade of breakthrough discoveries, unraveling the complex crosstalk between cancer cells and immune system have led to the development of novel therapeutic strategies capable of effectively enhancing anti-tumor immune responses. Immune checkpoint blockers (ICBs) are monoclonal antibodies that restore tumor-specific T cell cytotoxic activity through restriction of inhibitory molecules [e.g., programmed cell death protein 1 (PD-1), programmed death-ligand 1 (PD-L1), cytotoxic T-lymphocyte antigen 4 (CTLA-4)] hard-wired in the immune system. ICBs have shown unprecedented clinical efficacy in several types of cancer including non-small cell lung cancer (NSCLC), melanoma, renal cell carcinoma (RCC), urothelial carcinoma (UC) and Hodgkin lymphoma, where they represent the current standard of care (Antonia et al., 2017; Borghaei et al., 2015; Brahmer et al., 2015; Ferris et al., 2016; Garon et al., 2015; Herbst et al., 2016; Larkin et al., 2015; Motzer et al., 2015; Reck et al., 2016; Rittmeyer et al., 2017; Robert et al., 2015). Despite their efficacy and the potential long-term response, innate and acquired resistance to ICBs represent an important hurdle in achieving maximal benefit of these drugs (Gong, Chehrazi-Raffle, Reddi, & Salgia, 2018).

Among the pathways involved in cancer innate and adaptive immune tolerance, the catabolism of tryptophan has increasingly been recognized as playing a fundamental role (Mbongue et al., 2015). Indoleamine-2,3-dioxygenases (IDOs) are monomeric and heme-containing intracellular enzymes that catalyze the first rate-limiting reaction in the oxidative metabolism of indolic compounds, that is the transformation of L-tryptophan to N-formyl-L-kynurenine, which in turn leads to the depletion of local tryptophan and accumulation of kynurenines and their derivatives (Ball et al., 2007; Mbongue et al., 2015). This results in a highly tolerogenic microenvironment characterized by reduced T effector (Teff) lymphocytes and natural killer (NK) cells, and an increased number of functionally active T regulatory (Treg) cells and myeloid-derived suppressor cells (MDSCs) (Lob, Königsrainer, Rammensee, Opelz, & Terness, 2009; Moffett & Namboodiri, 2003). Aberrant IDO expression is involved in a wide spectrum of human diseases including infections, autoimmune diseases, atherosclerosis, obesity and depression (Fatokun, Hunt, & Ball, 2013; Yeung, Terentis, King, & Thomas, 2015). Recently, IDOs have been recognized as an immune evasion mechanism responsible for cancer development and progression, as well as for the promotion of tumor-associated neoangiogenesis (Prendergast et al., 2014). Consistently, preclinical data indicate that the pharmacological inhibition of IDOs can revert tumor-induced immunosuppression and induce anti-cancer responses (Yentz & Smith, 2018). On the heels of these data, a different IDO inhibitors have been designed and are already under clinical evaluation with encouraging results.

The aim of this review is to provide a comprehensive overview of the role of IDOs in cancer biology, focusing on the clinical potential of targeting IDOs to improve the efficacy of currently available immunotherapeutic agents.

2. Indoleamine 2,3-dioxygenases and immune tolerance

Indoleamine 2,3-dioxygenase-1 (IDO1), indoleamine 2,3-dioxygenase-2 (IDO2) and tryptophan 2,3-dioxygenase (TDO) are intracellular heme-dioxygenases that cleave the aromatic indole ring of the essential amino acid tryptophan (Mbongue et al., 2015). This enzymatic reaction is the first and rate-limiting step in the tryptophan catabolism and leads to the production of different degradative products, collectively known as kynurenines (Mbongue et al., 2015). The expression of TDO is highly conserved across different species including both prokaryotes and eukaryotes, whereas IDO1 and IDO2 expression is restricted to eukaryotes (Yuasa et al., 2009).

IDO1 was first isolated from rabbit intestine homogenates (Yamamoto & Hayaishi, 1967). Subsequently, the human *IDO1* gene was identified on chromosome 8 (Burkin et al., 1993), and a second gene located on chromosome 8, downstream of the *IDO1*, encoding IDO2 was identified (Metz et al., 2007). IDO1 and IDO2 share a high amino acid homology (approximately 43% of sequence identity) (Van Baren & Van den Eynde, 2015). Of note, both IDO1 and IDO2 are not constitutively expressed in most cells but can be induced by different inflammatory stimuli (i.e., pathogens, cytokines and lipopolysaccharides) (Mbongue et al., 2015). The inducible expression of IDO1 is ubiquitous, whereas IDO2 expression appears to be restricted to liver, small intestine, spleen, placenta, thymus, lung, brain, kidney and colon (Metz et al., 2007). Nonetheless, only a highly specific subset of professional antigen presenting cells (APCs) is specialized for a rapid high-grade upregulation of both IDO1 and IDO2 in response to exogenous inflammatory stimuli (Munn & Mellor, 2013). Among pro-inflammatory cytokines, interferon (IFN)-γ is one of the primary inducers of IDO1 expression, yet it seems to only marginally contribute to IDO2 inducible expression (Watcharanurak et al., 2014; Prendergast, Metz, Muller, Merlo, & Mandik-Nayak, 2014). Other cytokines including interleukin (IL)-1β, IL-2 and tumor necrosis factor (TNF)-α may potentiate the IFN-γ-mediated induction of IDO1 expression. On the other hand, different anti-inflammatory cytokines such as IL-4, IL-10 and transforming growth factor (TGF)-β have been reported to inhibit IDO1 induction by IFN-γ (Badawy, 2017). Additionally, a wide spectrum of signaling pathways involving Toll-like receptors (TLRs), tumor necrosis factor receptors (TNFRs), interferon beta receptor (IFNBR), interferon gamma receptor (IFNGR), TGF-β receptors (TGFBRs) and the aryl hydrocarbon receptor (AhR) are now being recognized as being capable of either inducing or maintaining IDO1 expression (Mbongue et al., 2015; Opitz et al., 2011; Mimura & Fujii-Kuriyama, 2003). The *IDO1* gene promoter contains different nucleotide sequences [i.e., interferon sequence response-like elements (ISRE), palindromic gamma-activated sequences (GAS) and non-canonical nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB) consensus sequences] that allow the regulation of gene expression (Puccetti & Grohmann, 2007). At the transcriptional level, *IDO1* expression can be promoted by different transcription factors including the forkhead box O3 (FOXO3) and interferon regulatory factor 8 (IRF-8), while it is suppressed by the DNAX activation protein of 12 kDa (DAP12) (Dejean et al., 2009; Orabona et al., 2006). In contrast, *IDO2* expression has been reported to be primarily induced by the interferon regulatory factor 7 (IRF-7) (Prendergast, Metz, et al., 2014). Regarding post-transcriptional regulation, two immunoreceptor tyrosine-based inhibitory motifs (ITIMs) are known to suppress the cytokine signaling 3 (SOCS3)-dependent proteasomal degradation of IDO1 protein in the presence of IL-6 (Orabona et al., 2008). In addition, nitric oxide (NO) has recently been reported to play an important role in the post-translational control of IDO1 levels via a direct interaction with the enzyme and by promoting its proteasome-mediated degradation (Samelson-Jones & Yeh, 2006).

The role of IDO1 in the modulation of immune responses was first postulated in the 1970's, when it was reported that the exposure to bacterial lipopolysaccharides induced IDO1 expression in murine lung (Davar & Bahary, 2018). This finding initially fueled the hypothesis that IDO1 had a crucial function in promoting innate immunity against infections. However, in 1998 Munn et al. reported that placental IDO1 was critical in preventing the maternal immune system from attacking fetal tissues during pregnancy, suggesting a more sophisticated role for this enzyme in modulating immune responses (Munn et al., 1998). Subsequently, several lines of research corroborated the hypothesis that IDO1 exerts a crucial role in the suppression of adaptive immunity.

By contrast, the role of IDO2 in the regulation of the immune response is still poorly understood. Although functional differences between IDO1 and IDO2 have been reported, IDO2 also shows a degree of redundancy relative to IDO1 functioning in controlling immune responses. Consistently, *IDO1* gene deletion has been reported to induce a compensatory upregulation of *IDO2* expression in mice (Prendergast, Metz, et al., 2014). Nonetheless, there is preliminary evidence showing

that IDO2, but not IDO1, may exert some pro-inflammatory effects as well. Accordingly, in a mouse model of autoimmune arthritis, *IDO2* gene deletion has been associated with a delayed onset and decreased severity of joint inflammation (Merlo et al., 2014). IDO2 has also been shown to promote autoantibody production in a mouse model of systemic lupus erythematosus (Bilir & Sarisozen, 2015). However, further studies are necessary to elucidate the physiological role of IDO2 in immunity and any discordance between IDO1 and IDO2 activities.

Increasing evidence shows that IDO1 promotes immune tolerance by regulating the proliferation, differentiation and activity of different immune cells (Bilir & Sarisozen, 2015). Specifically, it has been reported that IDO1 exerts its immune regulatory function through either an enzyme-dependent action (i.e., depletion of tryptophan due to tryptophan conversion into kynurenines) or an enzyme-independent action (i.e., direct intracellular signaling in IDO1-expressing cells) (Yeung et al., 2015). Due to its intracellular location, IDO1-associated metabolic effects may be either autocrine or paracrine in nature (Yeung et al., 2015). Both IDO1-expressing APCs and immune cells may sense either environmental tryptophan depletion through amino acid sensing signal transduction pathways or secreted kynurenines through the AhR pathway (Fig. 1) (Fallarino, Grohmann, & Puccetti, 2012). Importantly, tryptophan depletion in T cells promotes a stress response via activation of the ribosomal kinase general control nonderepressible 2 (GCN2) kinase (Moon, Hajjar, Hwu, & Naing, 2015). Of note, intracellular tryptophan starvation leads to the accumulation of uncharged tryptophan transfer ribonucleic acid (tRNA). GCN2 senses the binding of uncharged tRNA to ribosomes and induces the integrated stress response (ISR) to amino acid withdrawal limiting or altering protein translation. In CD8 + T cells, IDO1-induced activation of GCN2 leads to cell cycle arrest and functional anergy (Moon et al., 2015). On the other hand, the activation of GCN2 in CD4+ T cells inhibits Teff cells activity and promotes *de novo* Treg cell differentiation and activation (Moon et al., 2015). In addition, a decrease in tryptophan levels has also been proven to inhibit Teff cell proliferation and promote the induction of apoptosis via downregulating the mammalian target of rapamycin complex 1 (mTORC1) (Bilir & Sarisozen, 2015). Moreover, upon binding to kynurenines, AhR may also induce immunosuppressive responses by decreasing the immunoreactivity of dendritic cells (DCs) through their conversion into tolerogenic DCs, and by promoting T cell differentiation into forkhead box P3 (FOXP3) positive Treg cells (Harden & Egilmez, 2012). Besides, kynurenines may exert a direct cytotoxic effect on activated T cells (Terness et al., 2002).

Lastly, IDO1 intracellular signaling has recently been shown to promote a sustained immunosuppressive microenvironment via the TGF-β pathway (Fallarino et al., 2012). In fact, IDO1 is phosphorylated through the recruitment of Src homology 2 domain tyrosine phosphatases (SHP)-1/−2 to ITIMs and activates different downstream pathways leading to TGF-β production (Fallarino et al., 2012). High TGF-β levels disrupt the balance between pro-inflammatory and anti-inflammatory immune responses and promote immune cell shift toward a regulatory phenotype (Fallarino et al., 2012). In humans, increased IDO1 expression and activity have been reported in a wide spectrum of pathological conditions including infections, autoimmune and allergic diseases (Fatokun et al., 2013; Yeung et al., 2015).

3. Indoleamine 2,3-dioxygenase pathway in cancer

As previously mentioned, IDO1 and its catabolic pathway promote immunotolerance to "non-self" antigens in tissue microenvironments. This effect is mediated through the depletion of tryptophan, which is essential for the proliferation and clonal expansion of T cells, and through the production of suppressive metabolites that induce T lymphocytes apoptosis (Fallarino et al., 2003; Moffett & Namboodiri, 2003; Munn, Sharma, Ron, & Mellor, 2005). Tumors turn the immunosuppressive function of IDO1 to their own advantage in order to resist restriction by the host immune response. Mounting evidence demonstrates that IDO1 is constitutively over-expressed in many types of cancers where it plays a key role in fostering immune suppression (Fallarino et al., 2003; Theate et al., 2015). High expression levels of IDO1 and kynurenines in cancer inhibit NK cell function, prevent Teff cell activation, stimulate Treg cells, promote activation and differentiation of tolerogenic DCs, and promote the expansion and activation of MDSCs

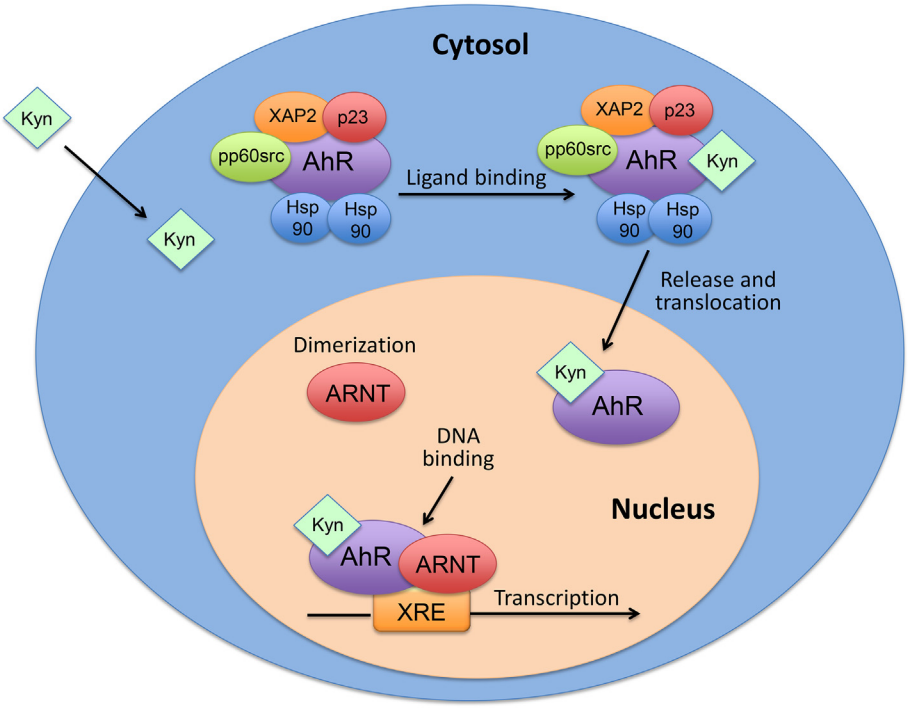

Fig. 1. Representation of kynurenyne-AhR pathway. IDO catabolism of tryptophan generates kynurenines, endogenous ligands of AhR. AhR is a ligand-activated transcription factor that regulates gene expression. AhR is assembled with heat shock protein 90 chaperones in an inactive form. Upon binding with its ligand, the kynurenine-AhR complex undergoes structural modification and translocates to the nucleus, where it forms a heterodimer with the AhR nuclear translocator (ARNT).

(Harden & Egilmez, 2012; Moon et al., 2015). All these properties make IDO1 a potent immunoregulatory enzyme capable of creating a suppressive microenvironment in human tumors which contributes to growth and survival of cancer cells. Elevated levels of IDO1 have been found to correlate with a reduction of tumor-infiltrating T lymphocyte-mediated promotion of tumor escape mechanisms in murine models of cancer (Uyttenhove et al., 2003). Several studies have shown that IDO1 expression in either cancer cells or in tumor-associated cells is associated with a more aggressive cancer phenotype, worse clinical outcomes and poor prognosis in multiple tumor types including ovarian carcinoma, colorectal carcinoma, B-cell lymphoma, breast cancer and NSCLC (Brandacher et al., 2006; Creelan et al., 2013; Ferns et al., 2015; Kozuma et al., 2018; Ninomiya et al., 2011; Okamoto et al., 2005; Pan et al., 2008). In contrast, IDO1 expression levels in patients with RCC and hepatocellular carcinoma positively correlated with better survival outcomes, which suggest that prognostic significance of IDO levels might partly depend on the cancer type (Grohmann, Fallarino, & Puccetti, 2003; Ishio et al., 2004; Riesenberg et al., 2007).

Mechanistically, three main hypotheses have been proposed to explain the association between IDO1 over-expression and cancer immune escape. First, the increased enzymatic activity of IDO1 can lead to a critical depletion of tryptophan which translates into an increased amount of uncharged tRNA in intratumoral T cells and an increased activation of the amino acid sensitive GCN2 and mammalian target of rapamycin (mTOR) stress-kinase pathways, which cause cell cycle arrest and T cell anergy (Grohmann et al., 2003; Wainwright, Dey, Chang, & Lesniak, 2013). A second hypothesis suggests that downstream kynurenines, including L-kynurenine, 3-hydroxy-L-kynurenine, 3-hydroxyanthranilate (3HAA) and quinolinic acid, can induce cell cycle arrest or apoptosis of Teff cells in light of their immunomodulatory properties (Grohmann et al., 2003; Wainwright et al., 2013). Lastly, it has been proposed that kynurenines accumulation can contribute to the switch of naive CD4+ T cells into immunosuppressive FOXP3-positive Treg cells, as a consequence of the interaction between L-kynurenine and AhR (Grohmann et al., 2003; Wainwright et al., 2013). The exact contribution of each mechanism to cancer immune escape remains to be determined; however, it is likely that all of them cooperate simultaneously and synergistically in the development of immune tolerance in the tumor microenvironment (Fig. 2). Finally, although these hypotheses have been corroborated by different experimental studies, it should be noted that most of the evidence produced so far derives from *in vitro* cell culture-based experiments. This limitation certainly brings up some issues about the actual biological significance of these studies.

Several novel aspects of IDO1 role in cancer immunity have recently been unveiled, including its non-enzymatic activity and its involvement in tumor-associated angiogenesis. Although most of the evidence on the non-enzymatic immunosuppressive activity of IDO1 derives from studies in mouse plasmacytoid DCs or other DC populations (Bessede et al., 2015; Munn & Mellor, 2013), it has recently been shown that the intracranial engraftment of murine glioblastoma cells into syngeneic immunocompetent mice resulted in decreased tumor-infiltrating Treg cells ($P < .01$) and increased animal survival ($P < .001$) when the glioblastoma cells were silenced for *IDO1* expression with stably expressing small hairpin ribonucleic acid (RNA) (Wainwright et al., 2012). Noteworthy, these outcomes were independent of the IDO1-mediated effects on the tryptophan and kynurenine levels produced by the tumors, suggesting an IDO1-mediated immunosuppression which does not rely on its enzymatic activity. In contrast, the enzymatic activity of IDO1 was mostly exerted in non-tumor cell types of the engrafted intracranial glioblastoma, suggesting the existence of non-overlapping non-enzymatic immunosuppressive activity of IDO1 in cancer cells (Zhai et al., 2017). Moreover, IDO1 acts as a key player at the regulatory interface between IFN-γ and IL-6 by promoting new blood vessels sprouting during tumor-driven neoangiogenesis (Mondal et al., 2016). Different molecular pathways involved in angiogenesis (Ricciuti, Foglietta, Bianconi, Sahebkar, & Pirro, 2017; Ricciuti et al., 2017) may be modulated by IDO1; specifically, IDO1 may induce the activation of the Janus kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) pathway and the production of matrix metalloproteinase (MMP)-2, MMP-9 and other inflammatory mediators including ciclooxygenase (COX)-2, hypoxia inducible factor (HIF)-1α and IL-6 (Su, Zhang, Liu, & Cao, 2017).

4. Targeting indoleamine 2,3-dioxygenases in cancer

Due to its pivotal role in regulating tumor immune evasion, IDOs have been increasingly advocated as potential anti-cancer therapeutic targets during the last decade. On this basis, numerous preclinical studies have evaluated whether IDO inhibition could enhance anti-tumor immune response, alone or in combination with different drugs (Fig. 3). Also, some IDO inhibitors, either as monotherapy or combination therapy, have entered clinical testing (Table 1). In most cases these drugs have arisen as safe valid options in anti-cancer immunotherapy. However, some clinical studies have failed to show their efficacy. Possible reasons of this failure may be related to the fact that tumor cells or immune cells in the tumor microenvironment may express other Trp-degarding enzymes beyond IDOs (i.e., TDO), thereby potentially escaping from immune surveilance despite IDO inhibition (Pilotte et al., 2012). Thus, regarding the general approach of inhibiting IDOs for cancer therapy, it remains to be determined whether IDO-selective inhibitors will be sufficient, as TDO, which catalyzes the same reaction as IDOs, is expressed in multiple tumors and may promote the inhibition of specific immune responses. To address this issue, dual IDO/TDO inhibitors are currently being developed. Here, we discuss the available data on five IDO inhibitors (i.e., indoximod, navoximod, epacadostat, BMS-986205, PF-06840003) and on a dual IDO1/TDO inhibitor (i.e., SHR9146) which have been investigated in clinical trials.

4.1. 1-MT/indoximod

The racemic compound 1-methyl-DL-tryptophan (1-MT) represents the most extensively investigated non-selective IDO inhibitor (Cady & Sono, 1991). Initial studies on mouse models demonstrated that 1-MT inhibited IDO activity, delayed tumor growth and operated synergistically with chemotherapy in mediating tumor regression (Friberg et al., 2002; Muller, DuHadaway, Donover, Sutanto-Ward, & Prendergast, 2005; Uyttenhove et al., 2003). The effect of 1-MT was lost in immunodeficient recombination activating gene 1 (RAG1) knockout mice, suggesting that its anti-cancer activity was immune-mediated, and primarily based on an enhanced T cell response (Muller et al., 2005).

The occurrence of cell-type specific variations in the activity of the 1-MT isomers has been reported, so that the majority of anti-tumor activity has been ascribed to the D-isomer (D-1-MT) (Hou et al., 2007). Soon after, several preclinical studies showed that D-1-MT was unable to inhibit IDO1 activity but restrained IDO2 activity more potently, and was effective in inhibiting IDO-expressing tolerogenic DCs (Metz et al., 2007; Lob et al., 2008; Lob et al., 2009). It is currently hypothesized that D-1-MT interferes with downstream effectors of the IDO1 pathway along with its inhibitory action on IDO2. The mammalian target of rapamycin (mTOR)-1 is a tryptophan sufficiency signal for T cells and other immune cells that regulate cellular growth and differentiation by "sensing" amino acids and other metabolic components in the microenvironment (Peter, Waldmann, & Cobbold, 2010). Tryptophan depletion mediates mTOR-1 inhibition thus identifying mTOR as an IDO1 effector mechanism (Metz et al., 2012). D-1-MT acts as a potent tryptophan mimetic molecule and is able to restore mTOR-1 signaling in T cells, hence rescuing their function and anti-tumor effect (Metz et al., 2012).

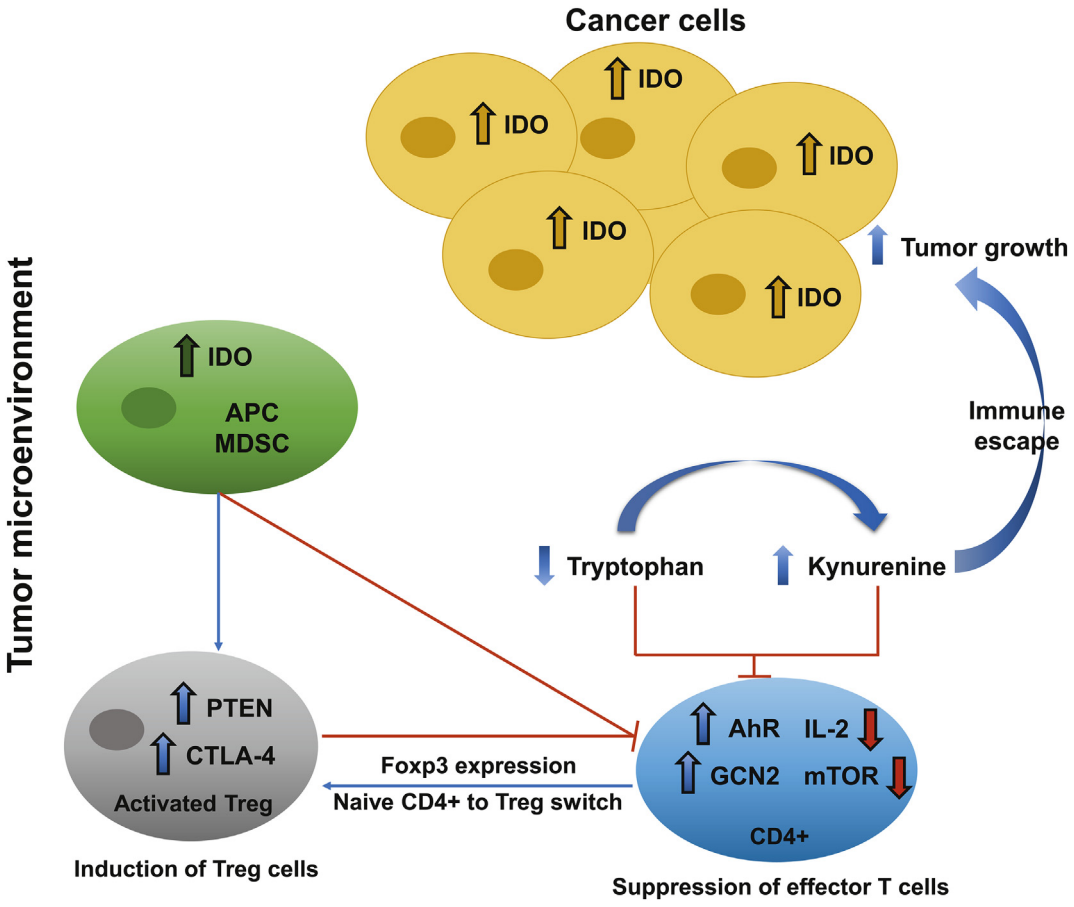

Fig. 2. Immunosuppressive activity of kynurenines in tumor microenvironment. The production of kynurenines by the IDO enzymatic activity of tumor cells, APCs and MDSCs results into the development of an immunosuppressive tumor microenvironment. Kynurenynes play a pivotal role in Treg cell induction through the expression of CTLA-4 and PTEN, the suppression of effector T cells via mTOR and IL-2 downregulation, the AhR upregulation and the induction of CD4+ T cells to switch in Foxp3 positive Treg cells. The resulting microenvironment promotes cancer cells immune escape, ultimately leading to tumor growth and progression.

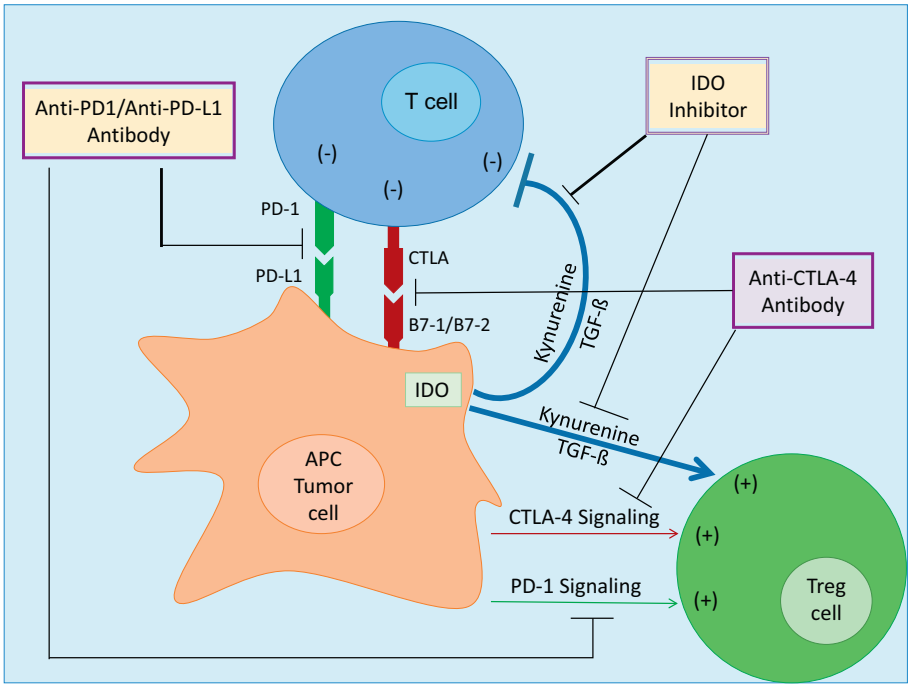

Fig. 3. Rational of combination therapy with different types of immune checkpoint blockers. PD-1 and CTLA-4 activation inhibit Teff cell proliferation and promote Treg cell proliferation. IDO signaling pathway induces tumor immune escape by suppressing Teff cells and activating Treg cells. The combination of IDO inhibitors with anti-PD-1 or anti-CTLA-4 agents might be more effective in reversing immune tolerance and augmenting anti-tumor immune response.

Table 1
Summary of clinical trials of IDO inhibitors in cancer.

Drug name	Clinical trial identifier	Trial status	Study characteristics	Cancer type
NLG-8189/indoximod	NCT01191216	Completed	Phase I (indoximod in combination with docetaxel)	Advanced solid malignancies
	NCT00567931	Completed	Phase I	Advanced solid malignancies
	NCT02835729	Recruiting	Phase I (indoximod in combination with idarubicin and cytarabine)	Newly diagnosed AML
	NCT02502708	Recruiting	Phase I (indoximod in combination with TMZ or conformal radiation therapy)	Pediatric population with WHO grade III and IV gliomas, ependymomas, medulloblastomas, or other primary central nervous system tumors.
	NCT02077881	Active, not recruiting	Phase I/II (indoximod in combination with gemcitabine and nab-paclitaxel)	Metastatic pancreatic adenocarcinoma
	NCT02073123	Active, not recruiting	Phase I/II (indoximod in combination with ipilimumab, nivolumab or pembrolizumab)	PD-1 and CTLA-4 naïve advanced or metastatic melanoma
	NCT02052648	Active, not recruiting	Phase I/II (indoximod in combination with TMZ/TMZ and bevacizumab/TMZ *plus* stereotactic radiosurgery)	Recurrent (TMZ resistant) glioma patients
	NCT02460367	Unknown	Phase I/II (indoximod in combination with tergenpumatucel-L and docetaxel)	Advanced previously treated NSCLC
	NCT01042535	Completed	Phase I/II (indoximod in combination with Ad. p53 DC vaccine)	Metastatic solid tumors and invasive breast cancer
	NCT03301636	Recruiting	Phase II/III (indoximod or placebo in combination with pembrolizumab or nivolumab)	Unresectable stage III or metastaic melanoma
	NCT01560923	Active, not recruiting	Phase II (indoximod in combination with sipuleucel-T)	Asymptomatic or minimally symptomatic patients with metastatic castration-resistant prostate cancer
NLG-919/GDC-0919/RG-6078/navoximod	NCT02048709	Completed	Phase I	Relapsed/refractory solid tumors
	NCT02471846	Active, not recruiting	Phase I (navoximod in combination with atezolizumab)	Locally advanced, recurrent, or metastatic incurable solid tumors
INCB024360/epacadostat	NCT01195311	Completed	Phase I	Advanced tumors
	NCT01685255	Completed	Phase II (epacadostat versus tamoxifen)	Biochemical-recurrent only epithelial ovarian cancer, primary peritoneal carcinoma, or fallopian tube cancer
	NCT02178722	Active, not recruiting	Phase I/II (epacadostat in combination with pembrolizumab)	NSCLC, melanoma, UC, RCC, SCCHN, TNBC, advanced ovarian cancer
	NCT02327078	Active, not recreuiting	Phase I/II (epacadostat in combination with nivolumab)	Select advanced solid tumors and lymphoma
	NCT02752074	Active, not recruiting	Phase III (epacadostat or placebo in combination with pembrolizumab)	PD-1 naïve melanoma
	NCT02785250	Recruiting	Phase I (epacadostat in combination with DPX-Survivac and low-dose cyclophospamide)	Recurrent ovarian cancer
	NCT03006302	Recruiting	Phase II (epacadostat in combination with pembrolizumab, and CRS-207, with or without cyclophosphamide and GVAX pancreas vaccine)	Recurrent metastatic pancreas adenocarcinoma
	NCT02166905	Recruiting	Phase I/II [epacadostat in combination with DEC205 mAb-NY-ESO-1 fusion protein (CDX-1401) given with adjuvant Poly-ICLC]	Patients in remission with epithelial ovarian, fallopian tube, or primary peritoneal carcinoma whose tumors express NY-ESO-1 or LAGE-1
	NCT02318277	Recruiting	Phase I/II (epacadostat in combination with durvalumab)	Selected tumors
	NCT03361865	Active, not recruiting	Phase III (epacadostat or placebo in combination with pembrolizumab)	Cisplatin-ineligible UC
	NCT03260894	Active, not recruiting	Phase III (epacadostat *plus* pembrolizumab versus standard of care)	Locally advanced or metastatic RCC
	NCT03322540	Recruiting	Phase III (epacadostat *plus* pembrolizumab versus pembrolizumab *plus* placebo)	Metastatic NSCLC expressing high levels of PD-L1
	NCT03358472	Active, not recruiting	Phase III (epacadostat *plus* pembrolizumab versus pembrolizumab monotherapy versus EXTREME regimen)	Recurrent or metastatic head and neck squamous cell carcinoma
BMS-986205	NCT02658890	Recruiting	Phase I/II (BMS-986205 in combination with nivolumab or with nivolumab and ipilimumab)	Advanced solid tumors
	NCT03329846	Active, not recruiting	Phase III (BMS-986205 in combination with nivolumab versus nivolumab alone)	Naïve locally advanced and metastatic melanoma
	NCT03386838	Completed	Phase III (BMS-986205 in combination with nivolumab versus EXTREME regimen)	Recurrent or metastatic head and neck squamous cell carcinoma
	NCT03417037	Withdrawn	Phase III (BMS-986205 in combination with nivolumab with/without chemotherapy versus chemoterapy)	Stage IV or recurrent NSCLC
PF-06840003	NCT02764151	Active, not recruiting	Phase I	Malignant gliomas
SHR9146	NCT03208959	Recruiting	Phase I	Advanced solid tumors

AML: acute myeloid leukemia; CTLA—4: cytotoxic T-lymphocyte antigen 4; DC: dendritic cell; IDO: indoleamine-2,3-dioxygenase; LAGE-1: cancer-testis antigen 2; NSCLC: non-small cell lung cancer; NY-ESO-1: New York esophageal squamous cell carcinoma 1; PD-1: programmed cell death protein 1; PD-L1: programmed death-ligand 1; RCC: renal cell carcinoma; SCCHN: squamous cell carcinoma of head and neck; TNBC: triple-negative breast cancer; TMZ: temozolamide; UC: urothelial carcinoma; WHO: Worls Health Organization.

Indoximod is the first IDO inhibitor with an advanced clinical development program. Early phase trials showed that indoximod was safe and well tolerated (Soliman et al., 2014; Soliman et al., 2016). Twenty-two patients with advanced solid malignancies were evaluable in a dose escalation trial of indoximod *plus* docetaxel. Patients received docetaxel 60–75 mg/m^2 every three weeks in association with indoximod 300–2000 mg twice daily continuously. The combination of indoximod *plus* docetaxel did not show increased toxicity over docetaxel alone. Adverse events (AEs) occurring with the highest frequency included grade 1 anemia, fatigue, and hyperglycemia (41%, 45%, and 38%, respectively) while common grade 3/4 toxicities included neutropenia and febrile neutropenia (both 13%). One grade 5 colitis was caused by mesenteric ischemia leading to sepsis and was not related to the study treatment. The recommended dose for the subsequent phase II portion of the trial was 1200 mg twice daily of indoximod *plus* 75 mg/m^2 of docetaxel. Four patients (18%) had a partial response, that in two cases lasted >6 months (Soliman et al., 2014). In a dose escalation trial of indoximod monotherapy in 48 patients with advanced solid cancers, the drug was well-tolerated, no significant dose-limiting toxicities (DLTs) were recorded and the maximal tolerated dose (MTD) was not reached at indoximod 2000 mg twice/day. The majority of AEs were grades 1 and 2, with fatigue, anemia, anorexia, dyspnea, cough, and nausea being the most common. Regardless of attribution to the study drug, serious AE included central nervous system ischemia, hypophysitis, fracture, bowel obstruction, urinary tract infection, encephalomyelitis, ileus, pain, pleural effusion, confusion, and weakness. Two deaths reported after 30 days from the last dose of treatment were attributed to disease progression while no grade 5 toxicities were reported. All treatment discontinuations were due to disease progression and were not attributed to toxicities. Three patients who previously received an anti-CTLA4 treatment (ipilimumab) developed hypophysitis at indoximod 200 mg once daily. No additional cases of hypophysitis were recorded when patients who received a prior ICB therapy were excluded from the trial. No objective responses were observed among evaluable patients (Soliman et al., 2016).

Reports from phase II studies of indoximod have shown encouraging results. An interim analysis of 104 patients with metastatic pancreatic cancer (treatment naïve or after first line therapy) treated with the combination of indoximod *plus* gemcitabine and nab-paclitaxel showed an overall response rate (ORR) of 46% (48/104), including one patient with complete response (CR). Immunological correlation studies were performed demonstrating a significant increase in intratumoral CD8+ cell density among responding patients compared with non-responders. No significant toxicities were reported, with fatigue, nausea, and anemia being the most frequent. Median overall survival (mOS) was 10.9 months (Bahary et al., 2018) [NCT02077881]. Several ongoing trials are evaluating the safety and efficacy of indoximod in combination with different immunotherapy approaches. In metastatic melanoma patients, indoximod in association with the PD-1 pathway inhibitor pembrolizumab showed an ORR of 52% (31/60) with 8% being CRs (5/60). These response rates are similar to those reported with the approved combination of nivolumab *plus* ipilumumab, but with a significantly lower toxicity burden (Zakharia et al., 2017) [NCT03301636]. In the same setting, another phase II trial is currently ongoing with indoximod in association with an anti-PD1 (pembrolizumab or nivolumab) or an anti-CTLA4 (ipilimumab) drug [NCT02073123]. In metastatic breast cancer patients, indoximod was tested in association with an adenoviral p53-transduced DC vaccine. The combination was safely administered. Most AEs, with the exception of anemia, nausea, constipation, and lymphopenia, were not attributed to the study treatment and none of the toxicities required treatment discontinuation. The reported grade 5 dyspnea was a respiratory failure event, which was due to disease progression. From the efficacy standpoint, no response was observed during the vaccination treatment period (Soliman et al., 2018). In metastatic castration-resistant prostate cancer patients, treatment with the DC vaccine sipuleucel-T (i.e., Provenge) followed by indoximod was well tolerated and no increased toxicity of the investigational arm (sipuleucel-T followed by indoximod alone) over the control arm (sipuleucel-T followed by placebo) was reported. Significant improvements were observed in radiographic and clinical progressions, with a > 2-fold increase in median progression free survival (mPFS) in the investigational arm compared to the control arm (sipuleucel-T followed by placebo); mOS was not reached at the time of interim analysis (Jha et al., 2017) [NCT01560923]. IDOs are expressed in 50% to 90% of glioblastomas and an ongoing phase I/II trial is exploring the safety and efficacy of indoximod in this setting. The phase I part found a significant MTD for indoximod in combination with temozolomide (TMZ) at 1200 mg twice daily with a good tolerability [headache, diarrhea, vomiting, nausea, fatigue, and dizziness were reported as the most frequent (>25%) AEs and only one grade 3 AE (fatigue) was recorded]; the phase II part of the study was aimed at evaluating the efficacy of the association of indoximod *plus* TMZ versus the standard front line-therapy with bevacizumab/TMZ *plus* stereotactic radiosurgery (Colman et al., 2015) [NCT02052648]. The combination of indoximod with tergenpumatucel-L and docetaxel is being explored in an ongoing a phase I/II trial of advanced NSCLC patients, but no data are available at this time [NCT02460367]. With respect to hematological malignancies, indoximod is currently being evaluated in combination with standard remission induction and consolidation therapy in patients with acute myeloid leukemia (AML). Preliminary evidence shows no significant increases in toxicity. In particular Emadi et al. reported that the majority of AEs were known toxicities associated with chemotherapy and/or AML disease processes. Of the 15 serious AEs, all were considered unrelated or unlikely to be related to indoximod. The all grade AEs occurring in ≥5% of patients and attributed to indoximod were abdominal pain, hyperhidrosis, diarrhea, fatigue, headache, nausea, vomiting, and asthenia. One patient discontinued the study due to febrile neutropenia, which was attributed to cytarabine and line infection. No grade 5 events were reported (Emadi et al., 2017) [NCT02835729]. Noteworthy, a first in-children phase I trial of indoximod is currently recruiting patients with brain tumors including World Heath Organization (WHO) grade III/IV glioma, relapsed/refractory ependymoma or medulloblastoma. Its results are eagerly awaited [NCT02502708]. A novel prodrug of indoximod with improved pharmacokinetic properties is currently studied in an ongoing phase I trial in patients with solid tumors (Mautino et al., 2017) [NCT03164603].

Overall, a better understanding of indoximod mechanism of action and immunoregulatory function may increase its potential use through new rational combinations.

4.2. NLG-919/GDC-0919/RG-6078/navoximod

NLG-919 is a potent non-competitive IDO1 inhibitor. It is orally bioavailable with a favorable pharmacokinetic and toxicity profile (Mautino et al., 2013). Preclinical studies in syngenic mouse tumor models showed that navoximod enhanced vaccine response in the B12F10 model of human melanoma and improved the entity and duration of response in association with PD-L1 inhibition in solid tumors (Mautino et al., 2013). Immunological analysis showed an improved CD8+ to Treg cell ratio and evidence of an increased maturation and antigen presentation capacity by DCs and APCs when navoximod was combined with PD-1 blockade (Spahn et al., 2015).

The encouraging preclinical data prompted a phase Ia study of navoximod monotherapy (Nayak et al., 2015) [NCT02048709] and a phase Ib, open-label, multicenter and global study of the combination of GDC-0919 and the anti-PD-L1 atezolizumab in patients with locally advanced or metastatic solid tumors [NCT02471846]. The phase Ia portion of the study showed a good tolerability of the study treatment. Regardless of causality, the most common AEs were fatigue, cough, decreased appetite, nausea, pruritus, vomiting, increased aspartate aminotransferase (AST)/alanine aminotransferase (ALT) and dyspnea. One case of grade 2 AST/ALT elevation was attributed to the study drug

along with a grade 4 lower gastrointestinal hemorrhage. No AEs led to treatment discontinuation. Also the combination with atezolizumab was generally well-tolerated with no grade 4 and 5 AEs attributed to the study drugs. Treatment-related grade 3 toxicities were reported in 13% of patients and included nausea, rash, sepsis, pneumonitis and fatigue. In one case a grade 3 pneumonitis, deemed to be related to the study drugs, led to treatment discontinuation. Preliminary efficacy data on 45 patients showed 4 (9%) patients with partial response (PR) and 11 (24%) patients with stable disease (SD) (Burris et al., 2017). Pharmacodynamic studies demonstrated a dose-dependent reduction in plasma kynurenines that was consistent with systemic modulation of IDO1 (Burris et al., 2017).

4.3. INCB024360/epacadostat

Epacadostat is a potent and selective tryptophan-competitive inhibitor of IDO1 enzymatic activity (Koblish et al., 2010). In co-culture systems, epacadostat was able to stimulate T CD8+ and NK cell growth, to reduce Treg cell and to increase DC activation, all necessary features for T cell engagement and activation during an anti-tumor response (Liu et al., 2010). These results were confirmed in syngenic tumor models where INCB024360 significantly increased IFN-γ production by antigen-specific T cells, decreased Treg population, selectively increased activated DCs, and impeded tumor growth in a dose- and lymphocyte-dependent fashion (Jochem et al., 2016; Koblish et al., 2010; Liu et al., 2010).

The first dose escalation phase I trial of epacadostat enrolled 52 patients with advanced solid malignancies. INCB024360 was well tolerated at doses of up to 700 mg twice daily. The most common AEs, all grades combined, were fatigue (69.2%), nausea (65.4%), decreased appetite (53.8%), and vomiting. Seven patients (13.5%) discontinued the therapy because of AEs, including pain, hepatic infection, pneumonia, radiation recall pneumonitis, dyspnea, hypoxia, fatigue, nausea and vomiting. Among these, only radiation pneumonitis and fatigue were considered DLTs but once dose levels were expanded MTD was not confirmed. No grade 4 elevations of AST or ALT were observed and two cases of grade 3 AST/ALT increase were not attributed to the study drug. Pharmacodynamics analysis showed that INCB024360 effectively normalized kynurenine levels, displaying the maximal inhibition of IDO1 activity at doses ≥100 mg twice/day. Although no ORR was detected, a SD lasting ≥16 weeks was observed in 7 of 52 patients (Beatty et al., 2017). A randomized phase II study assigned patients with biochemically recurrent epithelial ovarian cancer, primary peritoneal carcinoma or fallopian tube cancer to epacadostat or tamoxifen. Treatment with epacadostat did not raise safety concerns, though no significant difference in efficacy was found between the two treatment arms (Kristeleit et al., 2017).

Combination treatments of epacadostat with immunotherapy in different cancer types have led to encouraging results and several clinical trials are ongoing in this setting. An initial phase I/II study of ipilimumab *plus* epacadostat reported cases of clinically significant transaminitis and colitis, blunting the enthusiasm for this combination and favoring the association of epacadostat with PD-1 or PD-L1 inhibitors in a subsequent clinical trial (Gibney et al., 2015). ECHO-202/KEYNOTE-037 was a phase I/II trial that investigated the safety and efficacy of epacadostat *plus* pembrolizumab in patients with advanced solid tumors including melanoma, NSCLC, squamous cell carcinoma of head and neck (SCCHN), RCC, UC, triple negative breast cancer (TNBC) and ovarian carcinoma. The combination was well tolerated. The most common (≥15%) all grade treatment-related AEs were fatigue, rash, arthralgia, pruritus, diarrhea, and nausea; grade 3/4/5 toxicities were observed in 18% of patients, with skin rash (8%) and increased lipase (3%) being the most frequent events. No treatment-related grade 5 toxicities were reported. Epacadostat 100 mg twice/day continuously and pembrolizumab 200 mg every 3 weeks were selected for the phase II part of the study (Gangadhar et al., 2016) [NCT02178722]. The preliminary efficacy results showed a striking response rate across different cancer types. Of 36 efficacy-evaluable patients with SCCHN, over 80% received at least 1–2 prior lines of treatment; the ORR in this group was 34% regardless of human papilloma virus (HPV) status (Hamid et al., 2017; Hamid et al., 2017). Similarly, efficacy data from 19 patients with RCC with 0–1 prior treatment and no prior checkpoint inhibitor showed an ORR of 47% (Lara et al., 2017). The NSCLC cohort enrolled patients with prior platinum-based therapy and no prior checkpoint inhibitor treatment. Among 40 evaluable patients, the ORR was 35% and reached 43% in patients with ≥50% PD-L1 tumor proportion score and ≤ 2 prior lines of therapy (Gangadhar et al., 2017). In the UC setting, the combination of epacadostat and pembrolizumab improved the ORR (37%) compared with previously reported results with a PD-1 pathway inhibitor monotherapy (Smith et al., 2017). With respect to the melanoma cohort, 64 patients were enrolled and 54 were evaluable for response. The ORR was 56% regardless of PD-L1 expression and *BRAF* mutation status, the mPFS was 12.4 months, while at the time of analysis the mPFS for PD-1 treatment-naïve patients was not yet reached (Hamid et al., 2017). Not surprisingly, the response rates with the combination of epacadostat and pembrolizumab in patients with ovarian carcinoma and TNBC did not improve the discouraging results already obtained with a PD-1 pathway inhibitor monotherapy in the same settings (Spira et al., 2017; Kwa & Adams, 2018; Nanda et al., 2016). Importantly, no safety concerns were raised during the phase II part of the trial across all tumor types.

The interim analysis of the first phase III trial of epacadostat was recently presented. ECHO-301/KEYNOTE-252 [NCT02752074] is a phase III, randomized, double-blind study evaluating the efficacy of epacadostat *plus* pembrolizumab versus placebo *plus* pembrolizumab in patients with untreated, unresectable or metastatic melanoma. Enrolled patients were treatment-naïve with the exception of *BRAF* mutated patients who could have received a prior BRAF/MEK inhibitor treatment. Despite the initial enthusiasm, preliminary results showed no greater clinical benefit for the combination therapy over pembrolizumab alone across all subgroups [mPFS 4.7 vs 4.9 months, respectively; hazard ratio (HR) = 1.00]. The safety profile of this combination was consistent with the results from early phase trials. (Long et al., 2018). Several other phase III studies of epacadostat in different cancer subtypes are currently ongoing and results are eagerly awaited [NCT03361865, NCT03374488, NCT03260894, NCT03358472].

The PD-1 inhibitor nivolumab was also evaluated in association with epacadostat in the phase I/II trial ECHO-204. Preliminary results in 241 patients, including those in the phase II part, showed no DLTs and the combination was well tolerated up to a maximum epacadostat dose of 300 mg. The most common treatment-related AEs (≥15%) across dose escalation levels were rash, fatigue, and nausea. Rash was also the most common grade 3/4/5 treatment-related AE. Treatment discontinuation due to toxicity occurred in 713% of patients. The study reported no treatment-related deaths. The ORRs were promising in the subset of patients with SCCHN and melanoma (Perez, Riese, Lewis, Saleh, & Berlin, 2017).

Other immunotherapy strategies using epacadostat are under clinical investigation including vaccines [NCT02785250, NCT03006302, NCT03493945] and other immunostimulatory agents (DEC-205/NYESO-1 fusion protein CDX-1401) [NCT02166905].

4.4. BMS-986205

BMS-986205 is a highly selective, potent and irreversible IDO1 inhibitor with a favorable pharmacokinetic profile (Siu et al., 2017). In a phase I/II trial, BMS-986205 was dose-escalated up to 200 mg once daily in patients with previously treated metastatic cancer. BMS-986205 was administered as monotherapy once daily for 2 weeks followed by the addition of nivolumab at 240 mg intravenously every 2 weeks. The trial is still ongoing but an interim report showed good tolerability profile with high and rapid reduction of plasma levels of

kynurenines (Siu et al., 2017) [NCT02658890]. An update on safety data across all tumor cohorts and efficacy in the advanced bladder cancer cohort showed that BMS-986205 *plus* nivolumab had a safety profile similar to that of nivolumab monotherapy. Treatment-related AEs (all grades) were reported in 51% of patients with 12% being grade 3–4. The most common toxicities of any grade were fatigue (13%) and nausea (10%). Sixteen patients (4%) discontinued treatment due to AEs related to treatment, and one patient died due to grade 5 myocarditis. Promising results in the bladder cancer cohort were reported (Tabernero et al., 2018). The safety profile of the same combination is currently evaluated in another ongoing clinical trial involving patients with different tumor types [NCT03192943]. A phase II study to evaluate nivolumab or nivolumab *plus* BMS-986205 with or without Bacillus Calmette-Guérin (BCG) in BCG-unresponsive non-muscle invasive bladder cancer patients has recently been started [NCT03519256]. Importantly, two phase III trials of BMS-986205 in combination with nivolumab in SCCHN [NCT03386838], and in NSCLC [NCT03417037] have failed. Possible reasons of this failure may be related to the fact that these tumors or immune cells in their tumor microenvironment may express other Trp-degrading enzymes, such as IDO2 and TDO, which cannot be target by BMS-986205. Another, phase III trial of BMS-986205 in combination with nivolumab is expected to be completed in 2020. The aim of this study is to verify if BMS-986205 combined with nivolumab is more effective versus nivolumab alone in unresectable or metastatic treatment-naïve melanoma [NCT03329846]. Other ongoing trials are investigating the safety profile of BMS-986205 in combination with PD-1 pathway inhibitor or CTLA-4 inhibitor and relatlimab [a lymphocyte-activation gene 3 (LAG-3) inhibitor] in different types of solid tumors [NCT03459222; NCT02996110, NCT02750514; NCT02935634].

4.5. PF-06840003

PF-06840003 is a non-competitive IDO1 inhibitor with a good *in vivo* efficacy in combination with immune checkpoint inhibitors, a favorable pharmacokinetic profile and a remarkable blood brain barrier penetration (Tumang et al., 2016). The first-in-human study of this compound is ongoing in patients with central nervous system malignancies [NCT02764151].

4.6. SHR9146

To date, one orally bioavailable, highly potent, novel dual IDO1/TDO inhibitor, namely HTI-1090 (SHR9146), has entered clinical evaluation in solid tumors [NCT03208959]. This study is expected to close in March 2019. Regarding the scientific rational of the dual IDO/TDO inhibition, some crucial issues deserve attention. First, dual IDO/TDO inhibitors are likely to be effective only if their target pathways are expressed and active in the tumor microenvironment. Thus, patient stratification aimed at evaluating tumor IDO/TDO expression would be important in order to predict the clinical usefulness of the dual IDO/TDO inhibition. Second, it is currently unclear if the complete blockade of Trp catabolism may elicit toxicity issues, since TDO is highly expressed in liver. Therefore, targeting IDOs and TDO downstream pathways, such as AhR, may appear another promising approach for blocking immunosuppression mediated by the activation of the kynurenine pathway in tumors.

5. Conclusion

Immunotherapy has had a striking impact on cancer patients treatment, providing in some cases unprecedented survival rates. Unfortunately, despite the activity that different ICBs have shown in the clinical setting, only a minority of patients respond to treatment, and among responders the development of resistance to treatment often occurs. In this scenario, the identification of potential companion targets that might boost the efficacy of existing immunotherapeutic agents is of primary importance to improve clinical outcomes in patients with cancer. IDO1 has increasingly been reported to impair immune effector function by inducing tryptophan starvation and by generating the AhR endogenous agonists kynurenines (Harden & Egilmez, 2012; Moffett & Namboodiri, 2003; Moon et al., 2015). Recent data have also demonstrated that IDO1 is frequently overexpressed in patients with cancer and preclinical evidence has confirmed that IDO1 overexpression in cancer cells leads to escape from immune surveillance, ultimately promoting cancer growth and progression (Bilir & Sarisozen, 2015; Moffett & Namboodiri, 2003). Therefore, the therapeutic inhibition of IDO1 may offer a new treatment modality to improve efficacy of immunotherapy or delay the emergence of resistance. Different IDO inhibitors have been investigated and are under investigation in clinical trials. Indoximod, a non-selective IDO inhibitor has shown significant efficacy results particularly in combination with ICBs. More recently, second-generation selective IDO1 inhibitors have been designed to potently inhibit the activity of IDO1 with minimal off-target interactions. Some of them have already entered clinical trials and results of various ongoing clinical studies are awaited.

Importantly, all IDO inhibitors entered in clinical trials have shown a good tolerability either alone or in combination with standard chemotherapy regimens or different ICBs. Of note, the challenge of fully assessing increased toxicity from an investigational compound added to agents with high toxicity burden (such as docetaxel or gemcitabine/nabpaclitaxel) or with specific toxicity patterns (such as ICBs) within small phase I trials is difficult. However, the ongoing phase II or randomized phase III combination trials in different cancer types will allow a more adequately powered and meaningful assessment of the safety profile of IDO inhibitors.

Regarding the proposed approach of inhibiting IDOs for cancer therapy, it remains to be determined whether IDO inhibition will be sufficient, as TDO, which catalyze the same reaction as IDOs, is over-expressed in several tumors. Results from a recent ongoing clinical study using a dual IDO1/TDO inhibitor are awaited to evaluate this issue.

It is worth mentioning that, along with IDO inhibitors, other therapeutic approaches have been recently developed to target the kynurenines pathway. Engineered kynureninase (KYNase) derived from bacteria converts kynurenines into the immunological inactive anthranilic acid in a more efficient manner compared with endogenous KYNase (Stone et al., 2015; Triplett et al., 2017; Zhang et al., 2017). In the CT26 mouse model of colon carcinoma, KYNase monotherapy resulted in a sustained anti-tumor response (Zhang et al., 2017). From a molecular standpoint, treatment with KYNase led to intra-tumoral CD8+ accumulation and proliferation, and increased production of INF-γ in the tumor microenvironment (Stone et al., 2015). Of note, KYNase has also shown to decrease cell growth and prolong survival when combined with ICBs in B16F10, CT26 and 4 T1 tumor models (Zhang et al., 2017).

An additional strategy that is currently being explored is the development of AhR antagonists. Theoretically, the pharmacological inhibition of AhR might reverse immune tolerance and enhance the anti-tumor efficacy of immune cells. Consistently, preclinical data seems to support this hypothesis (Boitano et al., 2010; Hall et al., 2010; Parks et al., 2014; Prud'homme et al., 2010; Wagner et al., 2016; Wang, Wyrick, Meadows, Wills, & Vorderstrasse, 2011; Zhang et al., 2012). However, the development of AhR antagonists for potential clinical use is still in its infancy and further studies are required to better understand the multiple roles of AhR in the interaction between cancer and immune cells.

With a promising clinical efficacy and predictable safety profile, IDO inhibition of represents an innovative way to boost the efficacy of anti-cancer immunotherapy. Several clinical trials of IDO inhibitors in combination with either chemotherapy or ICBs are currently underway and are expected to further expand the therapeutic armamentarium for patients with cancer.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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