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The immune regulation in cancer by the amino acid metabolizing enzymes ARG and IDO

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Some enzymes degrading amino acids have evolved in mammals to dampen immune responses and maintain peripheral tolerance. The enzymes metabolizing L-arginine and L-tryptophan are particularly powerful, contributing to restrain immunity towards fetal tissues and establish neonatal tolerance. Solid tumors can hijack these formidable pathways to construct a microenvironment highly unfavorable to anti-tumor T lymphocytes able to recognize them, one of mechanisms for their immune evasion. In this review, we analyze emerging concepts in the cross-talk between cells expressing these enzymes, their immune regulatory functions and pharmacological approaches that can target them to enhance cancer immunotherapy.

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Introduction

Cancer immunotherapy has profoundly changed the outcome of cancer patients. However, recent evidence indicates that immunotherapy, including novel antibodies blocking T cell checkpoints, is successful in a proportion of patients. For example, the efficacy of Nivolumab, an anti-PD1 monoclonal antibody that has been approved by the Food and Drug Administration (FDA) for the treatment of patients with advanced non-small cell lung cancer, is far from optimal with a response rate of 15–20% [1]. Therefore, there is an urgent need to identify and remove tumor microenvironment cues that limit T cell functionality. One emerging strategy, which is being tested in clinical trials (Tables 1–3), is based on the pharmacological modulation of enzymes that degrade amino acids.

Arginase in the tumor microenvironment

L-arginine is a non-essential amino acid that is involved in the synthesis of many metabolites, such as nitric oxide (NO) and polyamines (putrescine, spermidine, spermine), as well as in protein post-translational modification and immune regulation [2]. Therefore, L-arginine availability can intervene in different aspects of reprogramming energy metabolism, recently identified as a new hallmark of cancer [3]. High levels of intracellular L-arginine not only favor the T cell fitness, activation, differentiation and function by neutralizing the Warburg effect [4] through the shift from glycolysis to oxidative phosphorylation (OXPHOS), but also support the generation of central memory-like T (T_{CM}) cells with superior survival capacity and anti-tumor activity [5••]. On the contrary, L-arginine depletion profoundly suppresses T cell immune responses by decreasing cyclin D mRNA translation and blocking the activity of several cyclin-dependent kinases, which are essential for the cell cycle advancement from G_0/G_1 to S-phase [6] as well as promoting T-cell anergy/paralysis via the down regulation of the T cell receptor (TCR) ζ chain [7]. Arginase (ARG) is the key enzyme that catalyzes L-arginine towards the downstream byproducts L-ornithine and urea. In mammals, two distinct ARG enzymes are present: ARG1 and ARG2. These two enzymes share 58% homology and catalyze the same biochemical reaction but they are distinguished by different cellular expression, molecular regulation and subcellular localization [8]. ARG1 is a constitutive cytosolic enzyme mainly expressed in the liver [9], erythrocytes [10] and human tertiary neutrophil granules where it is inactive but becomes active after its extracellular release [11,12]. In mouse, ARG1 is also present in other immune cells such as mouse lung-resident group 2 innate lymphoid cells [13], macrophages and dendritic cells (DCs) [14]. In association with the gamma amino butyric acid signaling pathway, ARG1 drives both neural maturity and neuroblastoma development [15]. ARG2 is a mitochondrial enzyme preferentially expressed in the lactating mammary glands, kidney and prostate [9]. It is activated also in the CD71-expressing erythroid cell subset in both neonatal mice and human cord blood, conferring them immunosuppressive and tolerogenic function post-birth [16].

Table 1

ARG and IDO1 inhibitors: from bench to beside

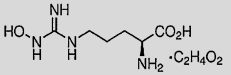
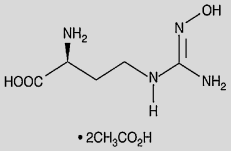
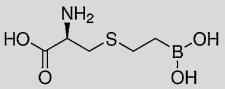
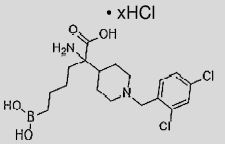
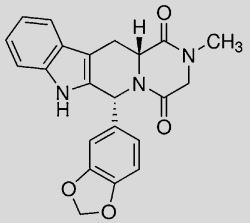
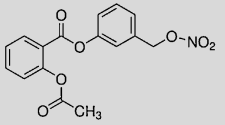
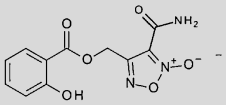
Enzyme inhibitor	Chemical structure	Chemical formula	Cas-number	Validation of immunomodulation effect
N^{G}-hydroxy-L-arginine [NOHA] (ARG Inhibitor)		C6H14N4O3 · C2H4O2	53598-01-9	• In human prostate carcinoma organ cultures, NOHA rescued the T cell proliferation [19]. • No clinical trials on cancer patients.
N^{ω}-hydroxy-nor-Arginine [nor-NOHA] (ARG Inhibitor)		C6H14N4O3 · C2H4O2	1140844-63-8	• *In vitro* nor-NOHA blocked suppressive activity of MDSCs. • *In vivo* nor-NOHA administration in tumor-bearing mice promoted a dose-dependent T cell-mediated anti-tumor response [53]. • Nor-NOHA was tested in a phase I clinical trial (NCT02009527) to evaluate the effect of arginase inhibition in ischemia-reperfusion injury; as well as in phase I and II clinical trial (NCT02687152) to test the therapeutic effect on controlling microvascular endothelial dysfunction in patients affected by type 2 diabetes. • No clinical trials on cancer patients.
S-(2-Boronoethyl)-L-cysteine hydrochloride [BEC hydrochloride] (ARG Inhibitor)		C5H12BNO4S · XHCL	63107-40-4	• *In vitro* BEC hydrochloride decreased arginase activity [54]. • The inhibitor administration in association with immunotherapy (adoptive cell transfer) limited cancer progression in tumor-bearing mice [54]. • No clinical trials on cancer patients.
CB-1158 (ARG Inhibitor)		C18H27BCl2N2O4	1345810-21-0	• CB-1158 in combination with standard treatment promoted a better control of tumor growth in preclinical tumor settings [57]. • CB-1158 is currently in a Phase I clinical trial in solid tumor patients.
Phosphodiesterase 5 inhibitors [Tadalafil, Sildenafil] (ARG transcription Inhibitor)		C22H19N3O4	171596-29-5	• Inhibitor administration in tumor-bearing mice blocked MDSC function favoring a spontaneous anti-tumor response [56]. • Inhibitor administration in tumor-bearing mice increased the therapeutic impact of adoptive T cell therapy [56]. • Inhibitor treatment of peripheral blood mononuclear cells isolated from multiple myeloma patients resulted in increased T cell proliferation *in vitro*. • Tadalafil is currently in Phase II clinical trial in tumor patients.
NCX 4016 [Nitroaspirin] (ARG transcription Inhibitor)		C16H13NO7	175033-36-0	• *In vivo* administration of nitroaspirin in tumor-bearing mice blocked MDSC function restoring immunotherapy efficacy [55]. • NCX4016 was tested in a phase II clinical trial (NCT01256775) to improve the functional capacity of peripheral arterial disease patients affecting atherosclerosis progression; as well as in phase I clinical trial (NCT00331786) to prevent colorectal cancer.
AT38 ([3-(aminocarbonyl) furoxan-4-yl] methyl salicylate) (ARG transcription Inhibitor)		C11H9N3O6	148:553081	• *In vivo* administration of AT38 to tumor-bearing mice controls the generation of reactive nitrogen species in tumor microenvironment favoring a massive T cell infiltration [27]. • No clinical trials on cancer patients.

Table 1 (*Continued*)

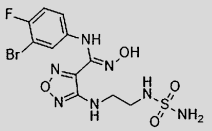
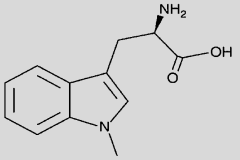
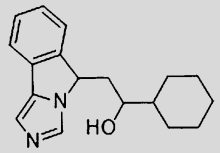
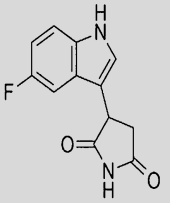
Enzyme inhibitor	Chemical structure	Chemical formula	Cas-number	Validation of immunomodulation effect
INCB024360 [Epacadostat] (IDO inhibitor)		C11H13BrFN7O4S	1204669-58-8	• INCB024360 promoted the proliferation of several immune cells, including NK cells, and T-lymphocytes, as well as the production of IFN-γ in tumor-bearing mice. • NCB024360 administration in tumor-bearing mice induced a contraction of tumor-associated Tregs [61]. • INCB024360 is currently in Phase I, II and III clinical trials in tumor patients.
NLG8189 [Indoximod,1-methyl-D-tryptophan] (IDO inhibitor)		C12H14N2O2	110117-83-4	• NLG8189 administration in tumor-bearing mice limited the expansion of tumor-associated Tregs [59]. • NLG8189 enhanced the adaptive immunologic response induced by DC vaccine in preclinical models [60]. • INCB024360 is currently in Phase I and II clinical trials in tumor patients.
GDC-0919 (IDO inhibitor)		C18H22N2O	1402836-58-1	• GDC-0919 restored the physiological tryptophan levels that favor, NK cell and T lymphocyte proliferation and induces a specific cytotoxic immune response against IDO1-expressing tumor cells. • GDC-0919 is currently in a Phase I clinical trial in solid tumor patients.
PF-06840003 (IDO inhibitor)		C12H9FN2O2	198474-05-4	• PF-06840003 reversed IDO1-associated T-cell anergy *in vitro*. • PF-06840003 inhibited tumor growth in multiple preclinical syngeneic mouse tumor models in combination with immune checkpoint inhibitors [64]. • PF-06840003 is currently in a Phase I clinical trial in solid tumor patients.

Table 2

Clinical trials involving ARG inhibitor (source: https://clinicaltrials.gov/)

Enzyme inhibitor	Trial number	Phase	Cancer	Status	Combined treatment	Primary outcome
CB-1158 (ARG inhibitor)	NCT02903914	I	Advanced and metastatic solid tumors	Recruiting	Nivolumab	• Safety and tolerability
NCX 4016 (ARG transcription inhibitor)	NCT00331786	I	Colorectal Cancer	Completed		• Effects of NCX 4016 on aberrant cryptic foci multiplicity after the second dose at 6 months
Tadalafil (ARG transcription inhibitor)	NCT01858558	I	Multiple Myeloma	Recruiting	Prevnar vaccine, Lenalidomide	• Progression free survival
	NCT01374217	I	Multiple Myeloma	Terminated	Lenalidomide dexamethasone	• Anti-tumor efficacy
	NCT02544880	I, II	Head and Neck Cancer	Recruiting	Anti-Tumor MUC1 Vaccine	• Safety and tolerability

Table 3

Clinical trials involving IDO inhibitors (source: https://clinicaltrials.gov/)

Enzyme inhibitor	Trial number	Phase	Cancer	Status	Combined treatment	Primary outcome
INCB024360 (Epacadostat)	NCT01685255	II	Reproductive tract tumors	Completed	Compared to Tamoxifen	Progression free survival
	NCT02166905	I, II	Reproductive tract tumors	Recruiting	DEC-205/NY-ESO-1 Fusion protein CDX-1401	Maximum tolerated dose (I) and progression free survival (II).
	NCT02118285	I	Reproductive tract tumors	Recruitment suspended, pending amendment	NK cells, IL-2	Maximum tolerated dose
	NCT01822691	II	Myelodysplastic syndromes	Completed		Overall response rate
	NCT02042430	Pilot study	Reproductive tract tumors	Ongoing		Number of participants with an increase in $CD8^+$ T cells and its correlation with IDO expression.
	NCT01961115	II	Melanoma	Recruiting	Multipeptide Melanoma Vaccine (MELITAC 12.1)	Changes in the concentration and number of $CD8^+$ TILs
	NCT02785250	I	Reproductive tract tumors	Recruiting	DPX-Survivac, cyclophosphamide	Safety, cell mediated immunity and evaluation of treatment-induced changes in TILs
	NCT02575807	I, II	Reproductive tract tumors	Recruiting	CRS-207	Number of patients reporting treatment-related adverse events; ratio of CD8/Treg TILs; plasma L-Kyn/L-Trp ratio
	NCT01604889	I, II	Metastatic melanoma	Ongoing	Ipilimumab	Safety and tolerability, overall survival.
	NCT02959437	I, II	Advanced solid tumors	Recruiting	Azacitidine Pembrolizumab	Frequency, duration, and severity of adverse events; objective response rate
	NCT02559492	I	Advanced solid tumors	Recruiting	JAK inhibitor (INCB039110) PI3K-delta inhibitor (INCB050465)	Dose-limiting toxicities
	NCT01195311	I	Advanced malignancies	Completed		Safety and tolerability.
	NCT03006302	II	Metastatic pancreatic adenocarcinoma	Not yet recruiting	Pembrolizumab, cyclophosphamide, CRS-207, GVAX	Recommended dose of Epacadostat and 6 month survival.
	NCT02298153	I	Non-small Cell Lung Cancer Urothelial Carcinoma	Recruiting	Atezolizumab	Incidence of adverse events and incidence of dose-limiting toxicities
	NCT02318277	I, II	Selected advanced solid tumors	Recruiting	Durvalumab (MEDI4736)	Dose-limiting toxicities and frequency, duration, and severity of adverse events; objective response rate
	NCT02178722	I, II	Selected solid tumors	Recruiting	Pembrolizumab	Dose limiting toxicities and objective response rate
	NCT02327078	I, II	Selected solid tumors	Recruiting	Nivolumab	Safety and tolerability (I) and overall objective response rate, overall survival (II)
	NCT02862457	I	Advanced solid tumors	Recruiting	Pembrolizumab	Dose-limiting toxicities and adverse events
	NCT02752074	III	Melanoma	Recruiting	Pembrolizumab	Progression-free and overall survival
	NCT01982487	I, II	Reproductive tract tumors	Withdrawn	ALVAC(2)-NY-ESO-1 (M)/ TRICOM vaccine	Maximum tolerated dose of IDO1 inhibitor
	NCT03085914	I, II	Advanced and metastatic solid tumors	Not yet recruiting	Nivolumab, Pembrolizumab and chemotherapy	Safety and tolerability (I) and overall objective response rate, overall survival (II)

Table 3 (*Continued*)

Enzyme inhibitor	Trial number	Phase	Cancer	Status	Combined treatment	Primary outcome
1-methyl-D-tryptophan (Indoximod)	NCT02460367	I, II	Non-small Cell Lung Cancer	Recruiting	Docetaxel Tergenpumatucel-L	Limiting Toxicity and progression free survival
	NCT02502708	I	Malignant brain tumor	Recruiting	Temozolomide, radiation	Limiting Toxicity and objective response rate
	NCT01792050	II	Metastatic breast cancer	Ongoing	Docetaxel, Paclitaxel	Progression free survival
	NCT02073123	I, II	Metastatic melanoma	Recruiting	Ipilimumab, Nivolumab, Pembrolizumab	Safety and tolerability (I), overall response rate (II)
	NCT02052648	I, II	Malignant brain tumor	Recruiting	Temozolomide Bevacizumab Radiation	Determine phase 2 dosing (I); efficacy as 6 month progression-free survival (II)
	NCT02077881	I, II	Metastatic pancreatic cancer	Recruiting	Gemcitabine Paclitaxel	Dosing, limiting toxicity, survival
	NCT00567931	I	Adult solid tumor	Completed		Incidence of adverse events and definition of maximum tolerated dose
	NCT02835729	I, II	Acute myeloid leukemia	Recruiting	Cytarabine, Idarubicin	Safety and tolerability (I), overall survival (II)
	NCT00739609	I	Selected solid tumors	Terminated		Safety and efficacy of administration of D-1MT
	NCT01560923	II	Metastatic prostate cancer	Ongoing	Sipuleucel-T	Immune response to Sipuleucel-T
	NCT01042535	I, II	Metastatic breast cancer	Ongoing	Adenovirus-p53 transduced dendritic cell vaccine	Maximum tolerated dose and number of participants with stable disease in response to study therapy
	NCT01302821	Pilot study	Breast cancer	Withdrawn	Adenovirus-p53 transduced dendritic cell vaccine	Occurrence of Detected Changes in Regions of Interest
	NCT01191216	I	Adult solid tumor	Completed	Docetaxel	Maximum tolerated dose
GDC-0919 (IDO inhibitor)	NCT02048709	I	Solid tumor	Completed		Limiting Toxicity
	NCT02471846	I	Metastatic incurable solid tumor	Recruiting	Atezolizumab	Dose-limiting toxicities and adverse events
PF-06840003 (IDO inhibitor)	NCT02764151	I	Oligodendroglioma Astrocytoma Malignant Glioma	Recruiting		Dose-limiting toxicities and disease control
IDO peptide vaccine	NCT03047928	I, II	Metastatic melanoma	Ongoing, Not yet recruiting	Nivolumab PD-L1/IDO peptide	Safety and tolerability
	NCT01219348	I	Lung cancer	Completed		Safety and tolerability
	NCT01543464	II	Malignant melanoma	Terminated	GM-CSF, Imiquimod and Temozolomide	Clinical benefit rate
	NCT02077114	I	Malignant melanoma with metastasis	Completed	Ipilimumab, Vemurafenib	Safety and tolerability

In many cancers, such as breast [17], gastric [18], prostate [19], neuroblastoma [20] and acute myeloid leukemia [21] the ARG activity in tumor environments is enhanced, generating an inhospitable milieu for T cell fitness. Local immune suppression can be driven by ARG2-expressing cancer-associated fibroblasts [22] but, overall, the most common observation is the expansion and accumulation of ARG1-producing tumor-infiltrating myeloid cells, such as tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs) and DCs. In these cells, ARG1 is turned on in response to several tumor-derived soluble factors and metabolites, such as interleukin (IL)-4, IL-13, IL-6, macrophage and granulocyte macrophage colony-stimulating factor (M-CSF and GM-CSF), transforming-growth factor (TGF)-β, lactate and cyclic adenosine monophosphate (cAMP), but also by molecular pathways controlled by the hypoxia inducible factor-1 alpha (HIF-1α) (reviewed in Refs. [23,24]).

The ARG1 expression is finely tuned by cell-specific transcription factors and nuclear receptors. Indeed, the peroxisome proliferator-activated receptors (PPARγ and PPARδ), members of signal transducer and activator of transcription family (STAT6, STAT3), the CCAAT-enhancer-binding proteins (c/EBPβ) and the interferon regulator factor 8 (IRF8), as well as CHOP, PU.1, Kruppel-like factor 4 (KLF4) and activator-protein 1 (AP-1) transcriptional factors promote ARG1 expression binding directly to distinct region of the *ARG1* gene promoter (reviewed in Refs. [23,25]).

Since many of these tumor-associated myeloid cells also trigger nitric oxide synthase enzymes (iNOS or NOS2), which normally produce NO from L-arginine and oxygen, the low level of L-arginine induced by the forced ARG1 activity promotes NOS uncoupling and consequent production of superoxide anion (O_2^-). Under pathological contexts, such as cancer, NO and O_2^- levels raise and combine to generate a variety of reactive nitrogen species (RNS) such as peroxinitrites ($ONOO^-$), which compromise both the activity and migration of T cells in tumor site [26–28].

However, we recently demonstrated that ARG1 has a hierarchical negative role when compared to NOS2 in creating a tumor-derived immunosuppressive environment since NOS2-expressing myeloid cells (such as TipDCs) can mediate a strong anti-tumor effect collaborating with adoptively transferred T cells in tumor debulking [29••]. It is still not clear whether ARG1 activity in patients negatively affects the anti-tumor immune response. However, the prompt reduction in $ARG1^+$ myeloid cells in melanoma patients treated with Ipilimumab suggests that the anti-tumor activity of this checkpoint inhibitor might involve, in part, ARG1 systemic modulation [30]. It remains to be determined whether the drop in $ARG1^+$ myeloid cells is predictive of a better clinical outcome following different immunotherapy approaches.

IDO1: not just catabolism of tryptophan

An important mechanism of immune resistance in tumors involves the metabolism of L-tryptophan (Trp), the rarest essential amino acid found in food. More than 90% of Trp entered with diet is catabolized via the kynurenine pathway, a cascade of enzymatic steps that produces several biologically active molecules, collectively known as kynurenines, before finally producing the essential pyridine nucleotide, NAD^+ (nicotinamide adenine dinucleotide) [2,31–33]. NAD^+ is a fundamental coenzyme for various physiological processes including DNA repair, cell growth and energy metabolism [34]. The most interesting enzymes along the pathway are indoleamine 2,3-dioxygenase 1 (IDO1) and tryptophan dioxygenase (TDO), which mediate the first rate-limiting step by promoting the oxidative breakdown of the Trp indole group. Though catalyzing the same enzymatic reaction, IDO1 and TDO show quite distinct structural features. In fact, the definition of the crystal structure of human IDO1, a monomeric and cytoplasmic enzyme, has revealed a folding into a catalytic large C-terminal domain, a noncatalytic small N-terminal domain, and a long loop connecting the two domains. In contrast, TDO has a homotetrameric structure, in which each monomer, when aligned to IDO1, appears to contain the large catalytic but not the small domain. IDO1 and TDO are also endowed with a distinct tissue expression, being IDO1 highly inducible in immune cells and TDO constitutive and mainly confined in the liver. Nevertheless, both of them have been found to be also expressed in several types of tumors and to play a major role in dampening anti-tumor immunity. Although indicated for years as the third immunoregulatory Trp catabolizing enzyme, the IDO1 paralogue IDO2 seems rather to be pro-inflammatory and to catalyze a distinct reaction [35].

IDO1- and TDO-mediated immunoregulatory effects involve Trp deprivation but also production of kynurenines. As a result, the catalytic activity of these enzymes mediates multiple effects on T lymphocytes, including inhibition of proliferation, apoptosis, and differentiation towards a regulatory phenotype [33]. Whereas Trp deprivation mainly downregulates the expression of CD3ζ in T lymphocytes similarly to ARG1 [36], L-kynurenine, the product of both IDO1 and TDO, is an endogenous agonist of the arylhydrocarbon receptor (AhR), a ligand-activated transcription factor in both T cells and DCs, the most professional antigen presenting cells. As a result, AhR activation promotes conversion of effector T lymphocytes into regulatory T (Treg) cells and upregulates IDO1 expression in DCs, further amplifying immunoregulatory effects and blocking anti-tumor immunity [37–39].

Few years ago, a completely new perspective was provided for IDO1 but not TDO or IDO2 biology [39,40]. IDO1 does not merely degrade Trp and produce kynurenines, but it also acts as a signal-transducing molecule, an effect that leads to long-term expression of IDO1 in DCs and immune tolerance *in vivo* and is mostly independent of IDO1's enzymic activity. IDO1's signaling function relies on the presence of two immunoreceptor tyrosine-based inhibitory motifs (ITIMs) in the noncatalytic, small domain of IDO1. Interestingly, IDO2 contains only one functional ITIM and does not transduce signals.

IDO1 nonenzymatic activity is triggered in DCs by the immunosuppressive cytokine TGF-β, which promotes IDO1 phosphorylation by kinases of the Src family and consequent direct interaction of the phosphorylated enzyme with tyrosine phosphatases SHP-1 and SHP-2. In contrast, pro-inflammatory IL-6 shortens IDO1's half-life driving direct interaction with suppressor of cytokine signalling 3 (SOCS3) [41•,42]. In fact, SOCS3, upon binding the same phosphorylated ITIMs bindable by SHPs, leads to ubiquitination and subsequent proteasomal degradation of IDO1 by recruiting members of the E3 ubiquitin ligase complex. Thus, depending on environmental conditions, IDO1 can bind distinct molecular partners, which can either prolong IDO1's half-life and promote long-term immunoregulatory effects or reduce IDO1's half-life and favor inflammatory responses. Very recently, by means of IDO1 mutants, we were able to ascertain that each phosphorylated IDO1 ITIM would favor binding of SHPs (ITIM1) or SOCS3 (ITIM2) [43].

As a whole, these observations would indicate that the noncatalytic portion of IDO1 is a regulatory domain, which can act either positively or negatively for IDO1 expression depending on the cellular microenvironment and, in addition to the catalytic site, may represent a novel drug target.

TGF-β, Arg1, and IDO1: an immunosuppressive triad at work in dendritic cells

As a whole, data available on IDO1 and ARG1 suggest that the two enzymes work in different cells, either through pathways that deplete the amino acids or via the combined effects of immunoregulatory metabolites and signaling activity. While IL-4 and IFN-γ are the main inducers of ARG1 [24] and IDO1 [31] in myeloid cells, respectively, the cytokine TGF-β can affect both enzymes, although apparently in different cell types. TGF-β is indeed able to confer both IDO1 competence and tolerogenic activity on otherwise immunogenic $CD8^-$ DCs [44] and enhance the ARG1 activity in rat peritoneal macrophages [45].

Recently, we proposed a new 'relay' pathway in mature conventional DCs based on the sequential actions of TGF-β, ARG1, and IDO1 [46••]. We found that TGF-β induces both ARG1 and IDO1 in DCs, with ARG1 being upregulated much more rapidly than IDO1. The sequence between the two enzymes also implies the need of ARG1 activity to increase IDO1 expression. As opposed to IDO1, which is endowed with signaling activity [47], ARG1 was found to affect cellular functions through regulation of L-arginine and L-ornithine availability. L-ornithine is the substrate for both ornithine decarboxylase (ODC) to produce polyamines, involved in the control of inflammation, and ornithine aminotransferase (OAT) to generate proline, fundamental for the synthesis of collagen and thus for tissue repair. As a consequence of ARG1 activation by TGF-β, DCs start to produce L-ornithine, which, in turn, favors the phosphorylation of IDO1 and the consequent activation of long-term immunoregulatory signaling. However, this activity is strictly dependent on the decarboxylation of ornithine into polyamines, since it is blunted in the presence of a pharmacological inhibitor of ODC.

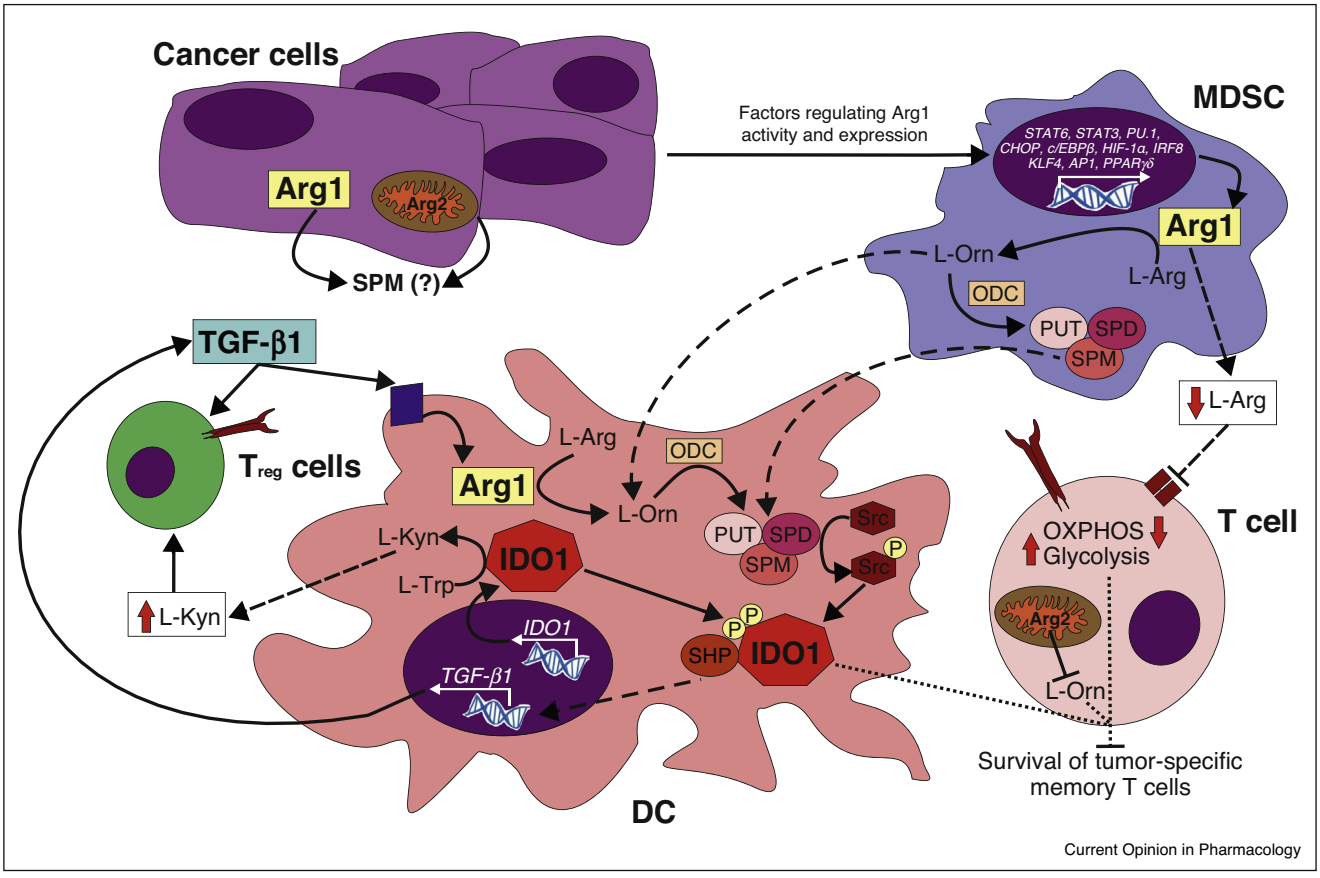
Polyamines are bioactive polycations extensively studied in tumors, where they promote cancer cell proliferation via the activation of different kinases, such as MAPK and Src kinase [48]. Our data thus unveiled a new role for the polyamine spermidine that, through the activation of the Src kinase, promotes IDO1 phosphorylation and signaling events in DCs. This circuitry, turned on by TGF-β, can occur not only between DCs, but also between DCs and MDSCs. MDSCs, being an abundant source of TGF-β and expressing high levels of ARG1, can educate DCs to express an IDO1-dependent tolerogenic phenotype, via the arginine metabolites (Figure 1).

The network established by TGF-β, ARG1, and IDO1 could be very important in the context of cancer. In order to propel their progression and invasion, tumors are very apt to co-opt metabolic and immunosuppressive networks, such as the one established between IDO1 and ARG1 (Figure 1). In addition to direct amino acid degradation by cancer cells themselves [49,50], tumors could recruit immunoregulatory cells expressing ARG1 and IDO1 and secrete immunosuppressive cytokines, such as TGF-β and IL-10 [51,52]. Therefore, the simultaneous inhibition of the ARG1 and IDO1 activities, and/or TGF-β signaling, may provide a powerful strategy in tumor immunotherapy.

Pharmacological control

The anti-tumor effect of ARG inhibitors is suggested by *in vitro* and *in vivo* preclinical data: NG-hydroxy-L-arginine [NOHA] inhibits ARG activity restores tumor-infiltrating T lymphocyte responsiveness to stimulation in human prostate carcinoma organ cultures [19]; N^{ω}-hydroxy-nor-Arginine [nor-NOHA] abrogates T cell proliferative arrest, favoring the immune attack of cancer cells [53]; analogously, the S-(2-Boronoethyl)-L-cysteine hydrochloride [BEC hydrochloride] administration in

Figure 1



Scheme of the relay pathway between L-arginine and L-tryptophan catabolism.
Cancer cells produce different cytokins such as IL-6, IL-4, IL-10, GM-CSF, M-CSF, TGF-β that collectively activate several arginase 1 (ARG1)-regulating transcriptional factors on myeloid-derived suppressor cells (MDSCs). The up-regulation of ARG1 activity produces a consumption of extracellular L-arginine (L-Arg) since it is converted into L-ornithine (L-Orn). Low levels of extracellular L-Arg blocks T cell fitness by favoring a glycolysis metabolism. MDSCs convert L-Orn into polyamines such as putrescine (PUT), spermidine (SPD), and spermine (SPM) by the activation of the enzyme ornithine decarboxylase (ODC). PUT, SPM and SPD can be released in the extracellular environment by MDSCs and condition dendritic cells (DCs) to trigger IDO1. High concentrations of polyamines favor the activation of the Src kinase that promotes IDO1 phosphorylation and SHP-related signaling events prolonging IDO1 expression and sustaining TGF-β production. This activated state is maintained by a positive autocrine loop mediated by the release/uptake of TGF-β. After TGF-β binding to its receptor, DCs switch on the ARG1-dependent metabolism that preserves their intracellular high concentration of both L-Orn and polyamines driving IDO1-related immunosuppression. Immunosuppressive functions are based on either the TGF-β release that supports the clonal expansion of antigen-specific natural regulatory T (Treg) cells and induces the conversion of naïve $CD4^+$ T cells into induced Treg cells or the catabolism of L-tryptophan (L-Trp) into L-kynurenine (L-Kyn) that blocks T cell proliferation and sustain Treg differentiation.

association with immunotherapy limits cancer progression in tumor-bearing mice [54]. The *in vivo* injection of drugs with multiple actions, including ARG transcription inhibition, such as AT38 ([3-(aminocarbonyl) furoxan-4-yl] methyl salicylate) [27] and NCX 4016 (nitroaspirine) [55], restores the anti-cancer immune response favoring immunotherapy success. Finally, the administration of phosphodiesterase 5 inhibitors, another potential ARG transcription inhibitor, increases the therapeutic impact of adoptive T cell therapy [56] and one of such drugs is being tested in a phase II clinical trial.

An impact on controlling tumor progression in several preclinical cancer settings has been recently described with a novel ARG-inhibitor, CB-1158 [57] (Table 1). A phase I clinical trial is now investigating the safety profile of CB-1158 in advanced solid tumors in a combination therapy with the immune checkpoint inhibitor Nivolumab (Table 2. Several compounds targeting IDO1 catalytic activity have demonstrated anti-tumor effects (Table 1). Among these, 1-methyl-DL-tryptophan (1-MT) has been the most widely studied. 1-MT is a tryptophan analogue with an *N*-methyl substitution at the indolic ring. Biochemical and cellular assays have revealed that 1-MT isomers have a different activity; in particular, the *S*-stereoisomer (L-1MT) is more active than the *R*-stereoisomer (D-1MT) [58]. However, despite these *in vitro* evidences, D-1MT (indoximod) has proven

to be more effective that L-1MT in delaying tumor growth *in vivo* [59,60] and is actually in Phase I/II clinical trials in combination with chemotherapeutic drugs (Table 3). From a library of natural products, novel modulators of IDO1 have been discovered, including brassinin, exiguamine A, annulin B and NSC401366. Although most of these natural compounds have been used for lead optimization studies, none of them has reached clinical trials. On the other hand, the screening of synthetic small molecule libraries has led to the identification of potent and selective IDO1 ligands. One of the most promising candidates is Epacadostat (also known as INCB024360), a nanomolar inhibitor of IDO1 developed by the Incyte Corporation. Epacadostat is an orally administered, hydroxyamide small molecule inhibitor of IDO1 that not only demonstrated an impact in restoring NK and T cell function and reducing the T cell conversion in Treg-like lymphocytes *in vitro* but also suppressed *in vivo* kynurenine generation and tumor growth in immunocompetent, but not immunodeficient, mice [61]. Moreover, in association with Ipilimumab, this oral inhibitor promotes both a favourable objective response rate and an increase control disease rate in patients with advanced cancer [62]. Interestingly, CTLA-4, an inhibitory receptor expressed by T cells, can activate a 'reverse signalling' via B7 molecules in DCs and upregulate IDO1 [63], thus suggesting that Ipilimumab may indirectly also inhibit the IDO1 pathway. Recently, New Link Genetics developed the inhibitor GDG-0919, a molecule active in nanomolar range with good oral bioavailability. A phase I clinical trial is now investigating the toxicity profile of this IDO1-inhibitor as single therapy for advanced solid tumors. Finally a new inhibitor designed by Pfizer and Iteos Therapeutic is being tested in a phase I clinical trial [64] (Table 3).

In conclusion, achieving a better understanding of the L-arginine and L-tryptophan metabolism in cancer, the complexity of amino acid catabolism-sensing pathways and immune response to tumor is warranted for providing new strategies for cancer immunotherapy.

Conflict of interest

None.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Brahmer J *et al.*: **Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer**. *N. Engl. J. Med.* 2015, **373**:123-135.
2. Grohmann U, Bronte V: **Control of immune response by amino acid metabolism**. *Immunol. Rev.* 2010, **236**:243-264.
3. Hanahan D, Weinberg RA: **Hallmarks of cancer: the next generation**. *Cell* 2011, **144**:646-674.
4. Vander Heiden MG, Cantley LC, Thompson CB: **Understanding the Warburg effect: the metabolic requirements of cell proliferation**. *Science* 2009, **324**:1029-1033.
5. •• Geiger R *et al.*: **L-arginine modulates T. cell metabolism and enhances survival and anti-tumor activity**. *Cell* 2016, **167** p. 829–842.e13.

This is the first work demonstrating that L-arginine plays a crucial control of metabolic fitness, survival capacity, and anti-tumor activity of central memory T cells.

6. Rodriguez PC *et al.*: **L-arginine consumption by macrophages modulates the expression of CD3 zeta chain in T lymphocytes**. *J. Immunol.* 2003, **171**:1232-1239.
7. Rodriguez PC, Quiceno DG, Ochoa AC: **L-arginine availability regulates T-lymphocyte cell-cycle progression**. *Blood* 2007, **109**:1568-1573.
8. Jenkinson CP, Grody WW, Cederbaum SD: **Comparative properties of arginases**. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 1996, **114**:107-132.
9. Morris SM Jr: **Regulation of enzymes of the urea cycle and arginine metabolism**. *Annu. Rev. Nutr.* 2002, **22**:87-105.
10. Kim PS *et al.*: **Expression of the liver form of arginase in erythrocytes**. *Mol. Genet. Metab.* 2002, **76**:100-110.
11. Jacobsen LC *et al.*: **Arginase 1 is expressed in myelocytes/metamyelocytes and localized in gelatinase granules of human neutrophils**. *Blood* 2007, **109**:3084-3087.
12. Rotondo R *et al.*: **Exocytosis of azurophil and arginase 1-containing granules by activated polymorphonuclear neutrophils is required to inhibit T lymphocyte proliferation**. *J. Leukoc. Biol.* 2011, **89**:721-727.
13. Monticelli LA *et al.*: **Arginase 1 is an innate lymphoid-cell-intrinsic metabolic checkpoint controlling type 2 inflammation**. *Nat. Immunol.* 2016, **17**:656-665.
14. Dzik JM: **Evolutionary roots of arginase expression and regulation**. *Front. Immunol.* 2014, **5**:544.
15. Hackett CS *et al.*: **Expression quantitative trait loci and receptor pharmacology implicate Arg1 and the GABA-A receptor as therapeutic targets in neuroblastoma**. *Cell Rep.* 2014, **9**:1034-1046.
16. Elahi S *et al.*: **Immunosuppressive CD71+ erythroid cells compromise neonatal host defence against infection**. *Nature* 2013, **504**:158-162.
17. Porembska Z *et al.*: **Arginase in patients with breast cancer**. *Clin. Chim. Acta* 2003, **328**:105-111.
18. Wu CW *et al.*: **Serum arginase level in patients with gastric cancer**. *J. Clin. Gastroenterol.* 1994, **18**:84-85.
19. Bronte V *et al.*: **Boosting antitumor responses of T lymphocytes infiltrating human prostate cancers**. *J. Exp. Med.* 2005, **201**:1257-1268.
20. Mussai F *et al.*: **Neuroblastoma arginase activity creates an immunosuppressive microenvironment that impairs autologous and engineered immunity**. *Cancer Res.* 2015, **75**:3043-3053.
21. Mussai F *et al.*: **Acute myeloid leukemia creates an arginase-dependent immunosuppressive microenvironment**. *Blood* 2013, **122**:749-758.
22. Ino Y *et al.*: **Arginase II. expressed in cancer-associated fibroblasts indicates tissue hypoxia and predicts poor outcome in patients with pancreatic cancer**. *PLoS One* 2013, **8**:e55146.
23. Ugel S *et al.*: **Tumor-induced myeloid deviation: when myeloid-derived suppressor cells meet tumor-associated macrophages**. *J. Clin. Invest.* 2015, **125**:3365-3376.
24. Bronte V, Zanovello P: **Regulation of immune responses by L-arginine metabolism**. *Nat. Rev. Immunol.* 2005, **5**:641-654.

25. Marvel D, Gabrilovich DI: **Myeloid-derived suppressor cells in the tumor microenvironment: expect the unexpected**. *J. Clin. Invest.* 2015, **125**:3356-3364.

26. Nagaraj S *et al.*: **Mechanism of T cell tolerance induced by myeloid-derived suppressor cells**. *J. Immunol.* 2010, **184**:3106-3116.

27. Molon B *et al.*: **Chemokine nitration prevents intratumoral infiltration of antigen-specific T cells**. *J. Exp. Med.* 2011, **208**:1949-1962.

28. De Sanctis F *et al.*: **The emerging immunological role of post-translational modifications by reactive nitrogen species in cancer microenvironment**. *Front. Immunol.* 2014, **5**:69.

29. •• Marigo I *et al.*: **T cell cancer therapy requires CD40-CD40L activation of tumor necrosis factor and inducible nitric-oxide-synthase-producing dendritic cells**. *Cancer Cell* 2016, **30**:377-390.

This work highlights, for the first time, ARG1 has a hierarchical negative role when compared to NOS2 in creating a tumor-derived immunosuppressive environment.

30. Pico de Coana Y *et al.*: **Ipilimumab treatment results in an early decrease in the frequency of circulating granulocytic myeloid-derived suppressor cells as well as their Arginase1 production**. *Cancer Immunol. Res.* 2013, **1**:158-162.

31. Grohmann U, Fallarino F, Puccetti P: **Tolerance, DCs and tryptophan: much ado about IDO**. *Trends Immunol.* 2003, **24**:242-248.

32. Puccetti P, Grohmann U: **IDO and regulatory T cells: a role for reverse signalling and non-canonical NF-kappaB activation**. *Nat. Rev. Immunol.* 2007, **7**:817-823.

33. van Baren N, Van den Eynde BJ: **Tryptophan-degrading enzymes in tumoral immune resistance**. *Front. Immunol.* 2015, **6**:34.

34. Kennedy BE *et al.*: **NAD+ salvage pathway in cancer metabolism and therapy**. *Pharmacol. Res.* 2016, **114**:274-283.

35. Merlo LM, Mandik-Nayak L: **IDO2: a pathogenic mediator of inflammatory autoimmunity**. *Clin. Med. Insights Pathol.* 2016, **9**:21-28.

36. Fallarino F *et al.*: **The combined effects of tryptophan starvation and tryptophan catabolites down-regulate T cell receptor zeta-chain and induce a regulatory phenotype in naive T cells**. *J. Immunol.* 2006, **176**:6752-6761.

37. Platten M, Wick W, Van den Eynde BJ: **Tryptophan catabolism in cancer: beyond IDO and tryptophan depletion**. *Cancer Res.* 2012, **72**:5435-5440.

38. Bessede A *et al.*: **Aryl hydrocarbon receptor control of a disease tolerance defence pathway**. *Nature* 2014, **511**:184-190.

39. Grohmann U, Puccetti P: **The coevolution of IDO1 and AhR in the emergence of regulatory T-cells in mammals**. *Front. Immunol.* 2015, **6**:58.

40. Orabona C, Pallotta MT, Grohmann U: **Different partners, opposite outcomes: a new perspective of the immunobiology of indoleamine 2,3-dioxygenase**. *Mol. Med.* 2012, **18**:834-842.

41. • Orabona C *et al.*: **SOCS3 drives proteasomal degradation of indoleamine 2,3-dioxygenase (IDO) and antagonizes IDO-dependent tolerogenesis**. *Proc. Natl. Acad. Sci. U. S. A.* 2008, **105**:20828-20833.

This work demonstrated the occurrence of regulatory proteloysis occurring to IDO1 in DCs exposed to inflammatory signals.

42. Orabona C, Grohmann U: **Indoleamine 2,3-dioxygenase and regulatory function: tryptophan starvation and beyond**. *Methods Mol. Biol.* 2011, **677**:269-280.

43. Albini E *et al.*: **Distinct roles of immunoreceptor tyrosine-based motifs in immunosuppressive indoleamine 2,3-dioxygenase 1**. *J. Cell Mol. Med.* 2017, **21**:165-176.

44. Belladonna ML *et al.*: **Cutting edge: autocrine TGF-beta sustains default tolerogenesis by IDO-competent dendritic cells**. *J. Immunol.* 2008, **181**:5194-5198.

45. Boutard V *et al.*: **Transforming growth factor-beta stimulates arginase activity in macrophages: implications for the regulation of macrophage cytotoxicity**. *J. Immunol.* 1995, **155**:2077-2084.

46. •• Mondanelli G *et al.*: **A relay pathway between arginine and tryptophan metabolism confers immunosuppressive properties on dendritic cells**. *Immunity* 2017, **46**:233-244.

This work highlights, for the first time, an immunosuppressive, entwined pathway between TGF-β, Arg1, and iDO1.

47. Pallotta MT *et al.*: **Indoleamine 2,3-dioxygenase is a signaling protein in long-term tolerance by dendritic cells**. *Nat. Immunol.* 2011, **12**:870-878.

48. Holtta E, Auvinen M, Andersson LC: **Polyamines are essential for cell transformation by pp60v-src: delineation of molecular events relevant for the transformed phenotype**. *J. Cell Biol.* 1993, **122**:903-914.

49. Godin-Ethier J *et al.*: **Indoleamine 2,3-dioxygenase expression in human cancers: clinical and immunologic perspectives**. *Clin. Cancer Res.* 2011, **17**:6985-6991.

50. Gannon PO *et al.*: **Androgen-regulated expression of arginase 1, arginase 2 and interleukin-8 in human prostate cancer**. *PLoS One* 2010, **5**:e12107.

51. Bronte V *et al.*: **IL-4-induced arginase 1 suppresses alloreactive T cells in tumor-bearing mice**. *J. Immunol.* 2003, **170**:270-278.

52. Uyttenhove C *et al.*: **Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase**. *Nat. Med.* 2003, **9**:1269-1274.

53. Rodriguez PC *et al.*: **Arginase I production in the tumor microenvironment by mature myeloid cells inhibits T-cell receptor expression and antigen-specific T-cell responses**. *Cancer Res.* 2004, **64**:5839-5849.

54. Lorvik KB *et al.*: **Adoptive transfer of tumor-specific Th2 cells eradicates tumors by triggering an in situ inflammatory immune response**. *Cancer Res.* 2016, **76**:6864-6876.

55. De Santo C *et al.*: **Nitroaspirin corrects immune dysfunction in tumor-bearing hosts and promotes tumor eradication by cancer vaccination**. *Proc. Natl. Acad. Sci. U. S. A.* 2005, **102**:4185-4190.

56. Serafini P *et al.*: **Phosphodiesterase-5 inhibition augments endogenous antitumor immunity by reducing myeloid-derived suppressor cell function**. *J. Exp. Med.* 2006, **203**:2691-2702.

57. Works M *et al.*: **Immuno-oncology agent CB-1158 is a potent and selective arginase inhibitor and causes an immune-mediated anti-tumor response**. *Cancer Res.* 2016, **76**.

58. Lob S *et al.*: **Levo- but not dextro-1-methyl tryptophan abrogates the IDO activity of human dendritic cells**. *Blood* 2008, **111**:2152-2154.

59. Hou DY *et al.*: **Inhibition of indoleamine 2,3-dioxygenase in dendritic cells by stereoisomers of 1-methyl-tryptophan correlates with antitumor responses**. *Cancer Res.* 2007, **67**:792-801.

60. Jochems C *et al.*: **The IDO1 selective inhibitor epacadostat enhances dendritic cell immunogenicity and lytic ability of tumor antigen-specific T cells**. *Oncotarget* 2016, **7**:37762-37772.

61. Liu X *et al.*: **Selective inhibition of IDO1 effectively regulates mediators of antitumor immunity**. *Blood* 2010, **115**:3520-3530.

62. Gibney G *et al.*: **Updated results from a phase 1/2 study of epacadostat (INCB024360) in combination with ipilimumab in patients with metastatic melanoma**. *Eur. J. Cancer* 2015, **51**: S106-S107.

63. Grohmann U *et al.*: **CTLA-4-Ig regulates tryptophan catabolism in vivo**. *Nat. Immunol.* 2002, **3**:1097-1101.

64. Tumang J *et al.*: **Abstract 4863: PF-06840003: a highly selective IDO-1 inhibitor that shows good in vivo efficacy in combination with immune checkpoint inhibitors**. *Cancer Res.* 2016, **76**:4863.