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1 What role for AHR activation in IL4I1-mediated immunosuppression ?

2
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13 microenvironment, immune escape

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24 The authors have no conflict of interest to declare.

25 26 27 28 29 **Abstract**

30 The amino-acid catabolizing enzyme Interleukin-4 induced gene 1 (IL4I1) remains poorly
31 characterized despite it is emerging as a pertinent therapeutic target for cancer. IL4I1 is secreted in
32 the synaptic cleft by antigen-presenting cells. It inhibits TCR signaling, modulates naïve T cell
33 differentiation and limits effector T cell proliferation. IL4I1 expression in tumors shapes the tumor
34 microenvironment and impairs the antitumor cytotoxic T cell response, thereby facilitating cancer
35 immune escape. Several mechanisms participate in these effects. Recent data suggest a role of new
36 IL4I1 metabolites in activation of the aryl-hydrocarbon receptor (AHR). Here, we observe that
37 expression of IL4I1 is poorly correlated with that of validated targets of AHR in human cancers.
38 Moreover, dendritic cells do not upregulate AHR target genes in relation with IL4I1 expression *in*
39 *vivo*. Finally, IL4I1 activity towards tryptophan leading to production of AHR-activating products is
40 very low, and should be negligible when tryptophan-degrading enzymes of higher affinity compete
41 for the substrate. We recently showed that IL4I1 expression by dendritic cells directly regulates
42 immune synapse formation and modulates the repertoire and memory differentiation of responding
43 CD8 T cells after viral infection. Thus, IL4I1 may restrain tumor control through regulating the priming
44 of tumor-specific CD8 T cells, independently of AHR activation.

49 Introduction

50 Amino-acid catabolizing enzymes, responsible for the diminution of T-cell activation, proliferation,
51 and/or function, have gained growing importance in immunology over the last 20 years (1). Several
52 are now clearly perceived to be central actors of tumor escape from immunosurveillance. Illustrating
53 this interest, indoleamine 2,3-dioxygenase (IDO1) has been proposed as a possible target for the
54 treatment of cancer and small-molecule inhibitors have been tested in preclinical models and Phase
55 I/II trials, with promising results. However, in a Phase III clinical trial in melanoma patients, the
56 absence of any additional clinical benefit of the most advanced molecule, epacadostat, in
57 combination with pembrolizumab has prompted rethinking of this strategy (2). Other
58 immunosuppressive enzymes represent possible candidates for chemical inhibition.

59 Since the first study to identify human IL4I1 as a phenylalanine (Phe) oxidase secreted by antigen-
60 presenting cells (dendritic cells [DC], monocytes, macrophages, and B-cells) in 2007, it has also been
61 demonstrated to inhibit human T-cell proliferation *in vitro* (3, 4). Both CD8⁺ and CD4⁺ T cells were
62 equally inhibited, but CD45RO⁺ (memory) cells were more sensitive than CD45RA⁺ (naïve) cells. IL4I1
63 also facilitated the differentiation of FoxP3⁺ regulatory T cells from naïve CD4⁺ T cells (5). IL4I1 is
64 produced by the macrophages that infiltrate most human solid tumors and by cancerous cells in the
65 case of certain B-cell lymphoma types (6). Recent single cell analyses have also identified IL4I1
66 expression in tolerogenic DC subpopulations of the tumor microenvironment, which can express
67 *IDO1* (7, 8).

68 The role of IL4I1 has been characterized in mouse models of transplanted and spontaneous
69 melanoma, both in WT and IL4I1 deficient backgrounds, clearly showing that it facilitates tumor
70 growth by inhibiting the antitumor cytotoxic T-cell response and remodeling the tumor immune
71 microenvironment (9, 10). These observations have been recently extended to a model of chronic
72 lymphocytic leukemia (11). In line with this, clinical correlations have been reported between IL4I1
73 expression by stromal cells and invasion of the sentinel lymph nodes, a higher melanoma stage, and
74 rapid relapse in human primary cutaneous melanomas, in which IL4I1 expression was analyzed by
75 immunohistochemistry (12). Most interestingly, zones in which IL4I1 expression was concentrated
76 were depleted of cytotoxic CD8⁺ T cells and enriched with regulatory FoxP3⁺ T cells. Moreover, *IL4I1*
77 was overexpressed in melanoma patients with progressive disease under treatment with the anti-PD-
78 1 antibody nivolumab, suggesting a relation between IL4I1 expression and resistance to immune-
79 checkpoint blockade (11).

80 We initially proposed IL4I1 as a prognostic biomarker and therapeutic target in cancer in 2009 (6).
81 Transcriptomic data from several groups also indicated that IL4I1 is associated with poor outcome in
82 certain human carcinomas (breast, renal, and colon carcinoma and glioma) (13 and reviewed in 14).
83 Sadik *et al* recently showed IL4I1 expression in tumor cells of low-grade gliomas and glioblastomas
84 and observed correlation with diminished patient survival (11). Glioblastoma is particular in that it is
85 suspected to be derived from neural stem cells (15) and isoform 2 of IL4I1 is expressed in several
86 neural cell types (16), with a still undefined function that may involve control of the level of
87 neurotransmitter amines (17, 18). In the same work, they demonstrate for the first time that IL4I1
88 promotes glioblastoma motility *in vitro*, an effect that may be highly relevant for metastatic
89 processes. Finally, their work brings new insights by proposing that the immunosuppressive effect of
90 IL4I1 in tumors is mediated by activation of the ligand-activated transcription factor aryl hydrocarbon
91 receptor (AHR). AHR is widely distributed in tissues and displays very diverse activities, depending on
92 the cellular and molecular context. Its role in cancer is complex and incompletely deciphered,
93 involving both pro and anti-tumorigenic effects that affect directly tumor cells or the tumor
94 microenvironment (19). Here, we examine the hypothesis of IL4I1-mediated AHR activation and
95 propose a model of IL4I1 action during cancer development.

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97

98 **Material and methods**

99 **Transcriptomic analysis of human primary tumors and mouse DCs**

100 Data from human primary tumors: breast, colon, and ovarian cancer, melanoma, mesothelioma, and
101 glioblastoma were retrieved from The Cancer Genome Atlas (TCGA) using the Xena platform. Among
102 genes used in the Sadik *et al.* study (11), we defined 45 experimentally-confirmed AHR target genes
103 according to the Harmonizome platform (20), which report genes identified by Lo and Mathews (21).
104 Spearman's rho were calculated using GraphPad Prism 8.

105 Genes modulated by 2,3,7,8-tétrachlorodibenzo-p-dioxine (TCDD) in human cells (hepatocytes,
106 CD34⁺ hematopoietic stem cells, MCF7 and HL60 cell lines) were retrieved from GSE7765, GSE14553,
107 GSE16160, GSE24193, GSE46874, GSE122518 (NCBI GEO site), based on metaanalysis by
108 Oshchepkova et al (22).

109 DCs were obtained by negative cell sorting from spleens of WT and IL411^{-/-} mice at steady state and at
110 24h of infection with lymphocytic choriomeningitis virus (LMCV) strain WE2.2, as described in Puiffe
111 et al. (23). Mouse experiments were approved by the local Ethical Committee for Animal
112 Experimentation (Cometh Anses/ENVA/UPEC) and the French Research ministry under the number
113 05338.03.

114 Total RNA was isolated from purified DCs using RLT buffer and RNeasy columns (Qiagen). Libraries of
115 polyA mRNA were generated using the TruSeq[®] Stranded mRNA Library Prep kit (Illumina) with
116 double indexing using TruSeq RNA UD Illumina Indexes. RNA was reverse transcribed using
117 SuperScript[™] II (Invitrogen). Next-generation sequencing was performed by 75 bp single reading with
118 the NextSeq 500/550 High Output Kit v2.5 (75 Cycles) on a NextSeq 500 analyzer (all from Illumina).

119 The twelve samples (40,675,782 ± 2,630,770 reads/sample) were quality-checked using the software
120 FastQC (version 0.11.8). We checked that rRNA depletion had the expected quality (less than 2.32 ±
121 0.88 of total RNA, no prokaryotic contamination) and that more than 93% of the reads mapped to
122 the mouse genome (GRCm38) using SortMeRNA (version 2.1b), FastQScreen (version 0.13) and
123 Kraken2 (version 2.0.9 / default database). Trimmomatic (version 0.39) was used to filter reads using
124 a quality 20 (sliding window of 5 reads) and minimal length of 50pb, which led to more than 96% of
125 surviving reads. Filtered reads (39,151,723 ± 2,545,395) were aligned to the mouse genome
126 (GRCm38) using STAR (version 2.6.1d). The mapping of the reads for the different regions of the
127 genome and the level of gene expression was calculated using RSEM (version 1.3.2). The level of
128 gene expression was normalized in CPM (counts per million). A heatmap was generated using
129 GraphPad Prism 8 for the selected AHR-dependent genes and IL411, showing the mean CPM from
130 three mice per condition.

131

132

133 **IL411 enzymatic activity**

134 Phe and Trp were dissolved in 0.1 N HCl and a 100 ng/ml solution of human recombinant IL411 (R&D)
135 was used. Hydrogen peroxide production from IL411 activity was measured at 30 minutes according
136 to Carbonnelle-Puscian et al. (6) with serial dilutions (1250 to 78 μM) of each amino acid. The Km and
137 Vmax were calculated using GraphPad Prism 8. IL411 activity was also measured on the inducible
138 recombinant HEK cell line expressing IL411 (described in Boulland et al (3). Lysates in Phosphate
139 Buffer Saline containing protease inhibitors (Complete mini, Roche, Meylan, France) were obtained
140 by 3 freeze-thaw cycles. The enzymatic test was performed as described above, on 40 μL cell lysates
141 corresponding to 5x10⁵ cells. Activity was calculated as pmol H₂O₂ produced per min and per mL.

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145 **Results and discussion**

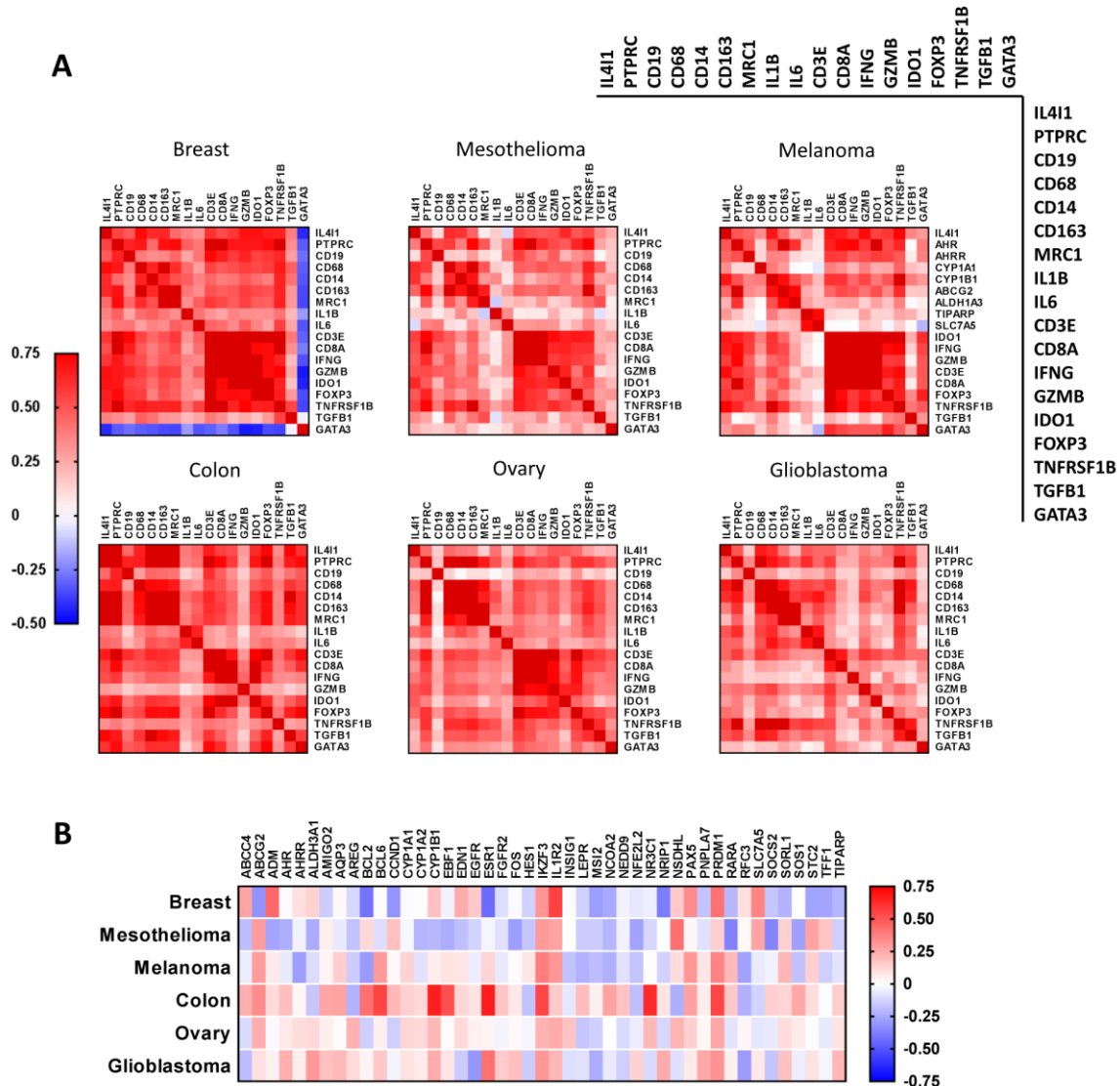
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147 **Relation between IL411 expression and activation of the transcription factor AHR.**

148 T-cell inhibition by IL411 has been mainly attributed to its enzymatic activity via the metabolic
149 consequences of Phe consumption and the effect of hydrogen peroxide (3, 4), although inhibition of
150 early TCR signaling by IL411 does not involve amino acid deficiency, nor the presence of Phe
151 catabolites (24). Sadik *et al.* establish a correlation between the expression of IL411 and of the pan-
152 tissue AHR signature they generated. We examined the list of genes used for this AHR signature (11)
153 and were puzzled by the fact that it contains several genes, of which the expression may reflect the
154 immune infiltrate of the tumor, in particular, *IFNG*, *CD3E*, and *CD8A* (Table I). Indeed, the expression
155 of *IL411* in primary tumors from TCGA correlates very strongly with that of T-cell related genes, as
156 well as others, such as *IDO1*, which is indicative of the activation of a Th1 and cytotoxic T-cell
157 response in the tumor bed ($CD3E = 0.57 \pm 0.11$, $CD8A = 0.49 \pm 0.13$, $IFNG = 0.47 \pm 0.13$, $IDO1 =$
158 0.55 ± 0.15 ; mean rho \pm SD on the six tumor types analyzed; **Fig. 1A**). This is in accordance with
159 previous results in human and mouse melanomas (10, 12) and with the known regulation of IL411
160 expression by interferons (4). This is also in line with the reanalysis by Sadik *et al.* of data from Riaz *et*
161 *al.* (25), indicating concomitant induction of *IL411* and *IDO1* in patients with advanced melanoma
162 treated with anti-PD-1. This induction may result from restored local production of IFN γ by
163 reinvigorated T cells. As IL411 is produced by tumor-associated macrophages in most solid tumors (6),
164 we also observed a strong correlation between IL411 and various genes attesting the presence of
165 macrophages in the tumor bed ($CD68 = 0.57 \pm 0.15$; $CD14 = 0.63 \pm 0.15$; $CD163 = 0.51 \pm 0.17$; mean
166 rho \pm SD on the six tumor types analyzed).

ABCC4	C21orf33	CYP3A4	FOXQ1	IL1B	MSI2	PCK2	SERPINE1	TNFSF9
ABCG2	CARD11	DKK3	FPR2	IL1R2	MYC	PDE2A	SESN2	TXNRD1
ACTA2	CAV1	DLX3	GATA3	IL2	NANOG	PDS5B	SH3KBP1	UGT1A6
ADM	CCL5	DUOX2	GF11	IL6	NCOA2	PER1	SLC10A1	VAV3
AHR	CCND1	EBF1	GHR	INSIG1	NCOR2	PHGDH	SLC3A2	XDH
AHRR	CD36	EDN1	GNA13	IRF8	NDRG1	PIWIL1	SLC7A5	ZIC3
ALDH3A1	CD3E	EGFR	GSTA2	JAG1	NEDD9	PIWIL2	SMAD3	
AMIGO2	CD8A	EGR1	GSTM1	JUP	NFE2L2	PLA2G4A	SMAD7	
AQP3	CDK4	EPGN	HES1	KDM1A	NOS1	PNPLA7	SOCS2	
AREG	CDKN1A	EREG	HIF1A	KIAA1549	NOS3	POLK	SORL1	
ARG2	CFTR	ESR1	HLA-DRB4	KIT	NPTX1	PPARGC1A	SOS1	
ATP12A	CRH	F3	HMOX1	KMO	NQO1	PRDM1	SPRR2D	
ATP5IF1	CXCL2	FAS	HSD17B4	LEPR	NR1H3	PTGS2	STC2	
ATP5ME	CYBB	FAT1	HSPB2	LHCGR	NR1H4	RARA	TFF1	
ATP6AP2	CYP19A1	FBXO32	ID1	LIFR	NR3C1	REL	TGFBI	
BCL11B	CYP1A1	FGFR2	ID2	LPL	NRIP1	RFC3	TGM1	
BCL2	CYP1A2	FIG4	IFNG	LTBP1	NSDHL	RSPO3	TH	
BCL6	CYP1B1	FLG	IGF2	LYN	OVOL1	SCARB1	THBS1	
BLNK	CYP2B6	FOS	IGFBP1	MID1	PAX5	SCIN	TIPARP	
BRCA1	CYP2E1	FOXA1	IKZF3	MMP1	PCK1	SERPINB2	TJP1	

167 **Table I.** Genes of the AHR signature in Sadik *et al.* (n = 166). Forty-five genes identified as AHR targets by CHIP-
168 Seq are in bold (21). Blue and red boxes indicate genes that are upregulated and downregulated, respectively,
169 by TCDD in GSE7765, GSE14553, GSE16160, GSE24193, GSE46874, GSE122518. HLA-DRB4 is upregulated in
170 CD34⁺ cells, but downregulated in hepatocytes. The most robustly AHR-dependent genes in this list are *AHRR*,
171 *CYP1A1*, *CYP1B1*, *NFE2L2*, *SLC7A5*, *TIPARP* (all upregulated) according to the metaanalysis of Oshchepkova *et al.*
172 (22).



173
 174 **Fig.1. Correlation between the expression of IL4I1, immune-response genes and AHR-regulated genes in**
 175 **primary tumors. (A) Correlation between IL4I1 and PTPRC (coding the panleukocyte marker CD45), CD19 (B**
 176 **cells), CD68, CD14, CD163, MRC1, IL1B, IL6 (macrophages and inflammation), CD3E, CD8A, IFNG, GZMB, IDO1,**
 177 **FOXP3, TGFB1, TNFRSF1B and GATA3 (T-cell infiltration, Th1 response, regulatory T-cells, Th2 cells). (B)**
 178 **Correlation between IL4I1 and AHR-dependent genes. Each line shows results for one of the six primary cancer**
 179 **types analyzed. Values above 0.5 are considered highly significant correlations.**
 180

181 A comparison of the AHR signature reported by Sadik *et al.* to validated AHR targets identified by
 182 CHIP-seq in the study of Lo and Matthews (21), retrieved 45 genes common to both lists, which did
 183 not include IFNG, CD3E, or CD8A. We analyzed these 45 genes for correlations with IL4I1 expression
 184 in primary tumors from the TCGA database using the Xena platform (26) (Fig. 1B). With few
 185 exceptions, Spearman's rho was consistently inferior to 0.25; depending on tumor type, 1 to 15
 186 genes had rho > 0.25 and 0 to 9 had rho < -0.25 (total, 1 to 15 genes modulated). Colon carcinoma
 187 displayed the highest correlation between IL4I1 and AHR-regulated genes (15 genes upregulated
 188 with rho > 0.25, 7 with rho > 0.5, none downregulated with rho < -0.25). Three of these genes (CYP1B1,
 189 ESR1, ALDH3A1) are known to be upregulated by TCDD *in vitro* (Table I). However, in colon cancer,
 190 the diet and/or the microbiota can provide numerous AHR ligands.

191 To confirm these observations on murine cells, we infected WT and IL4I1-deficient (IL4I1^{-/-}) mice with
 192 LCMV and sorted splenic DCs both at steady state and after infection (Fig. 2). Transcriptomic analysis

193 did not show any relationship between IL4I1 expression and activation of the 45 AHR target genes.
 194 Despite vigorous induction of IL4I1 expression in response to LCMV, AHR-related genes were not
 195 significantly upregulated compared to the steady state. In addition, these genes were similarly
 196 expressed in DCs from both mouse strains. Thus, the relationship between IL4I1 expression and
 197 induction of the AHR pathway may be only indirect, implying that targeting of the AHR pathway and
 198 IL4I1 for cancer treatment may be independent, and,
 199 potentially synergistic.

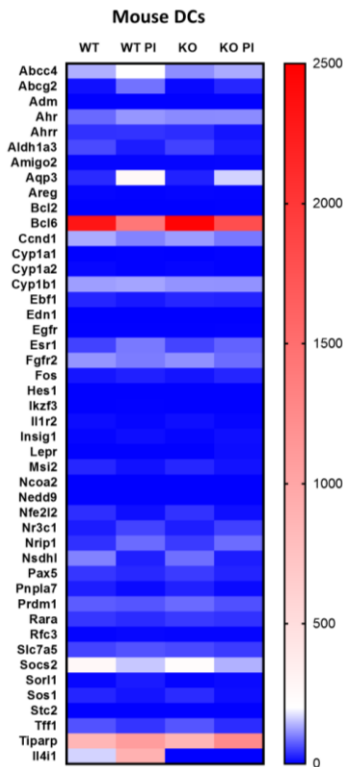


Fig. 2. Transcriptomic analysis of purified DCs from WT and IL4I1^{-/-} mice at steady state and after 24 h of LCMV infection. The heat map shows counts per million for the selected AHR-dependent genes and IL4I1 (mean from three mice per group). A previous analysis of the cDNA library at steady state has been published in Puiffe et al. (23). PI: post-infection; KO: IL4I1^{-/-}.

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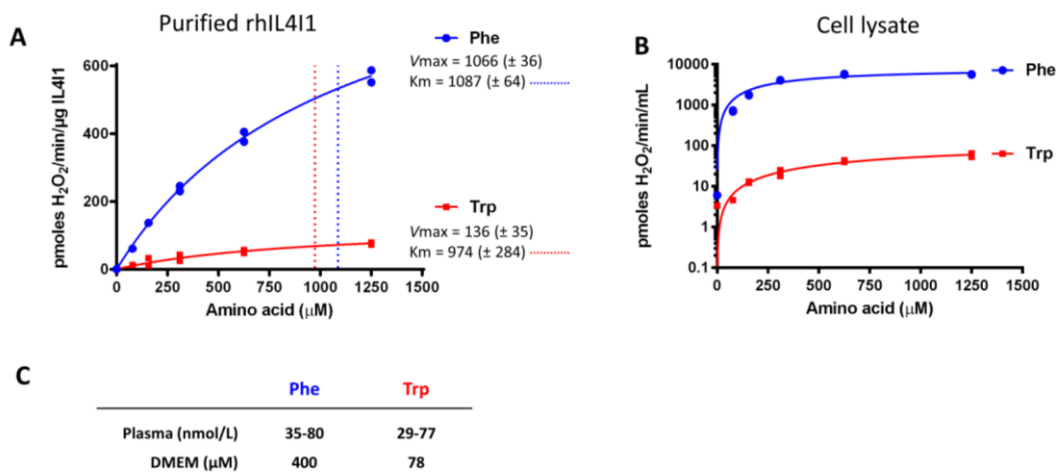
223 IL4I1 degradation of tryptophan (Trp)

224 Sadik *et al.* demonstrate that IL4I1 degrades the essential amino acid Trp into indole-3-pyruvate
 225 (I3P), which is immediately converted *in vitro* to kynurenic acid (KynA) and other derivatives. I3P,
 226 KynA, and indole-3-aldehyde all activate AHR (11). A study contemporaneously published by Zhang *et al.*
 227 also provides arguments for I3P production by IL4I1 activity (27). However, IL4I1 has a strong
 228 preference towards Phe. A greater than five-fold higher Phe-degrading than Trp-degrading activity
 229 was initially observed using a relatively insensitive colorimetric test (3). Indeed, although
 230 measurement of the Km using an optimized technique shows similar values for the two amino acids,
 231 the Vmax of IL4I1 towards Phe is almost 10-fold higher than that towards Trp (Fig. 3A). We validated
 232 these data on human cell lysates obtained from recombinant HEK cells expressing IL4I1 (Fig. 3B). The
 233 difference between Phe and Trp catabolism was even greater than with the purified enzyme. This might
 234 be due to different glycosylation, as commercial IL4I1 is produced in Chinese hamster ovary cells.
 235 Moreover, in the human body, the concentration of Phe is equal to or slightly higher than that of Trp
 236 and the concentration of Phe is substantially higher in classical culture media than that of Trp (Fig.
 237 3C). Thus, the *in vitro* and *in vivo* catabolism of Trp by IL4I1 should be less than that of Phe and the
 238 production of phenylpyruvate should overtake that of I3P. This also suggests that IDO1, whose
 239 affinity for the Trp substrate is around 50 folds higher than IL4I1 (Trp Km~20 μM for IDO (28) versus
 240 974 μM for IL4I1) and which is often expressed concomitantly with IL4I1, as stated above, may
 241 degrade Trp more efficiently than IL4I1. In accordance, a metabolomic study demonstrates a
 242 significant increase in phenylpyruvate and phenyllactate in ovarian cancer in comparison to normal

243 ovary, but does not detect variations in indole compounds (29). Another indirect argument may
 244 come from a study in an orthotopic model of mouse pancreatic cancer. In this model, tumor
 245 regression after treatment with metformin and torin 2 (mTORC1 and mTORC2 inhibitors) was
 246 associated with a 4-fold increase of plasmatic phenylalanine (30). Finally, the use of a specific IL4I1
 247 inhibitor in mouse with B16 melanoma led to significant decrease of phenylpyruvate levels in the
 248 tumors (31).

249 The I3P produced by IL4I1 in the presence of H₂O₂ (which is a byproduct of the enzymatic reaction)
 250 would spontaneously be degraded to KynA the main mediator of AHR activation (11). However, KynA
 251 could be produced independently of IL4I1 activity and experiments showing AHR activation *in vitro*
 252 required largely supraphysiological concentrations of the products (3.13 to 40 μM I3P) and Trp
 253 consumption required five days of culture to be measured.

254 Overall, these observations raise questions about the *in vivo* relevance of Trp degradation by IL4I1, in
 255 particular, in comparison to the effect of IDO1 on the same substrate. In any case, treatment with
 256 IL4I1 inhibitors should block the enzyme, regardless of the amino-acid substrate, Phe, Trp, or even
 257 arginine, which has also been demonstrated to be a minor IL4I1 substrate (14).
 258



259

260 **Fig. 3. IL4I1 activity towards phenylalanine (Phe) and tryptophan (Trp).** (A) Hydrogen peroxide production
 261 from the activity of recombinant human IL4I1 was measured in two independent experiments performed in
 262 duplicate. Values for K_m and V_{max} (\pm SD) were determined. The blue and red dotted lines indicate K_m for Phe
 263 and Trp, respectively. (B) IL4I1 activity was measured as in A on cell lysates from recombinant HEK cells
 264 expressing human IL4I1 (one experiment performed in duplicate). (C) Concentration of Phe and Trp in human
 265 plasma (reference values from the Mayo clinic) and in DMEM.

266

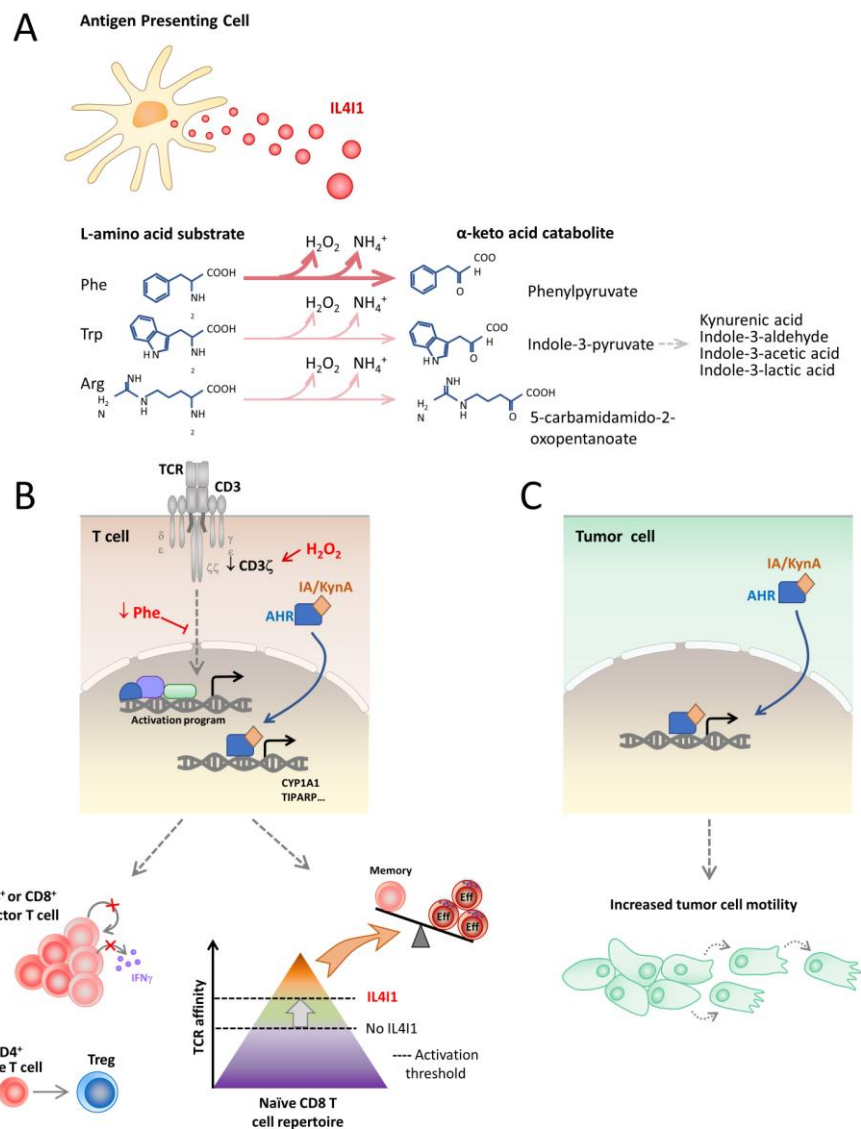
267 Implications for cancer of IL4I1 modulation of CD8 T cell priming

268 We recently dissected the effect of IL4I1 during the priming of naïve CD8 T cells using a model of
 269 acute LCMV infection in WT and IL4I1^{-/-} mice (23). This work revealed unsuspected aspects of IL4I1
 270 action. Somewhat counterintuitively, the effector CD8 T cell response was stronger in WT (IL4I1⁺)
 271 mice than in IL4I1^{-/-} mice. Indeed, the genetic inactivation of *IL4I1* was associated with delayed and
 272 slightly diminished expansion of functional short lived-effector CD8 T cells, but enhanced memory T
 273 cell differentiation. These observations were not related to intrinsic differences of CD8 T cells
 274 between WT and IL4I1^{-/-} mice, but resulted from modulation of immune synapse formation and early
 275 activation events by IL4I1-expressing DCs. Indeed, IL4I1 enhanced the T cell activation threshold,
 276 thereby favoring the priming of high-affinity clones, restriction of the response to the most
 277 immunodominant peptides and rapid acquisition of effector differentiation.

278 These effects, which are unrelated to AHR activation (**Fig. 2**), are relevant for the T-cell response
279 against cancer. Indeed, IL4I1 expression not only inhibits the proliferation and function of anti-tumor
280 effector T cells in the tumor microenvironment, as previously shown, but its presence in secondary
281 lymphoid organs may restrain the repertoire of primed anti-tumor CD8 T-cells, limiting control of
282 mutant tumor subclones. Moreover, as IL4I1 is associated with diminished differentiation of memory
283 CD8 T cells, it may limit the capacity to durably regenerate anti-tumor effector cells. Thus, inhibition
284 of IL4I1 should both improve the antitumor effector response and allow long-lasting revitalized
285 immunity against cancer.

286
287 Overall, it has been clearly demonstrated in the literature that IL4I1 alters the antitumor CD8 T-cell
288 response and that IL4I1-mediated immunoregulation facilitates cancer growth, affecting patient
289 survival and potentially fostering resistance to immune checkpoint inhibitors. Despite these new
290 advances, there are still uncertainties concerning the mechanism of the action of IL4I1 on the T-cell
291 response at the molecular and cellular levels. Recent data suggest that IL4I1 affects directly both CD8
292 T cells and tumor cells (**Fig. 4**). Indeed, IL4I1-dependent modulation of the affinity and functional
293 properties of the CD8 T-cell repertoire elicited at priming may interfere with its capacity to combat
294 tumor cells (23). In this case, IL4I1 effects on early T cell signaling and immune synapse formation are
295 too rapid to involve gene regulation. In particular contexts where sufficient Trp catabolites are
296 generated, IL4I1-mediated AHR activation may play a role in enhancing tumor-cell migration and
297 metastasis (11), or tumor cell survival, as I3P has also been recently proposed to elicit a cell
298 protective gene expression program (32). Pharmacological blockade of IL4I1 is therefore of high
299 interest.

300 In contrast to other immunoregulatory enzymes, IL4I1 is a secreted protein and is therefore easily
301 accessible to pharmacological blockade (33). An orally-available compound with potent IL4I1
302 inhibitory activity *in vitro* has been recently developed (31). This compound limits the growth of
303 several tumors in mouse models, without significant toxicity, opening the way to IL4I1 targeting in
304 human cancer. Thus, a complete understanding of the actions of IL4I1 will be fundamental to
305 facilitating clinical development and avoiding the failure that occurred with epacadostat (2).



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Fig. 4. Mechanism of action of IL411 in the context of cancer. (A) IL411 is secreted into the synaptic cleft between an antigen-presenting cell and a T cell. L-amino acid oxidation by IL411 produces an α -keto acid, while liberating hydrogen peroxide (H_2O_2) and ammonia (NH_4^+). Phe is the most rapidly degraded amino acid but Trp and arginine (Arg) are also IL411 substrates. Sadik *et al.* showed that indole-3-pyruvate produced by Trp degradation is not detectable, as it is rapidly converted into non AHR-stimulating indole derivatives and kynurenic acid (KynA) and indole-3-aldehyde (IA), which are AHR agonists. (B) IL411 enzymatic activity is responsible for the consumption of Phe, which deprives T cells of this essential amino acid, and potentially participates in limiting the availability of Trp and Arg. This leads to decreased activation of the mTORC1 pathway downstream of the TCR and costimulatory receptors. T-cell signaling is also sensitive to hydrogen peroxide, which reduces membrane exposure of the CD3 ζ chain. The mechanism of inhibition of early TCR signaling (ZAP70 activation) is still unresolved. Phe depletion and IL411-produced second messengers limit effector T-cell proliferation and favor regulatory T-cell differentiation from naïve CD4 T cells, individually or in combination. As shown by Puiffe *et al.*, IL411 also increases the threshold of CD8 T-cell activation, thus restraining the primed repertoire to high-affinity clones and favoring the differentiation of effector cells at the expense of memory cells. Cancer development is favored by the diminution of the quality and intensity of the T-cell response. (C) Metastasis may also be promoted by increased tumor-cell motility induced by KynA and IA stimulation of the AHR pathway in tumor cells.

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Bibliography

1. P. J. Murray, Amino acid auxotrophy as a system of immunological control nodes. *Nat. Immunol.* **17**, 132–139 (2016).
2. B. J. Van den Eynde, N. van Baren, J.-F. Baurain, Is There a Clinical Future for IDO1 Inhibitors After the Failure of Epacadostat in Melanoma? *Annu. Rev. Cancer Biol.* **4**, 241–256 (2020).
3. M.-L. Boulland, J. Marquet, V. Molinier-Frenkel, P. Möller, C. Guiter, F. Lasoudris, C. Copie-Bergman, M. Baia, P. Gaulard, K. Leroy, F. Castellano, Human IL4I1 is a secreted L-phenylalanine oxidase expressed by mature dendritic cells that inhibits T-lymphocyte proliferation. *Blood.* **110**, 220–227 (2007).
4. J. Marquet, F. Lasoudris, C. Cousin, M.-L. Puiffe, N. Martin-Garcia, V. Baud, F. Chereau, J.-P. Farcet, V. Molinier-Frenkel, F. Castellano, Dichotomy between factors inducing the immunosuppressive enzyme IL-4-induced gene 1 (IL4I1) in B lymphocytes and mononuclear phagocytes. *Eur. J. Immunol.* **40**, 2557–2568 (2010).
5. C. Cousin, A. Aubatin, S. Le Gouvello, L. Apetoh, F. Castellano, V. Molinier-Frenkel, The immunosuppressive enzyme IL4I1 promotes FoxP3(+) regulatory T lymphocyte differentiation. *Eur. J. Immunol.* **45**, 1772–1782 (2015).
6. A. Carbonnelle-Puscian, C. Copie-Bergman, M. Baia, N. Martin-Garcia, Y. Allory, C. Haioun, A. Crémades, I. Abd-Alsamad, J.-P. Farcet, P. Gaulard, F. Castellano, V. Molinier-Frenkel, The novel immunosuppressive enzyme IL4I1 is expressed by neoplastic cells of several B-cell lymphomas and by tumor-associated macrophages. *Leukemia.* **23**, 952–960 (2009).
7. B. Maier, A. M. Leader, S. T. Chen, N. Tung, C. Chang, J. LeBerichel, A. Chudnovskiy, S. Maskey, L. Walker, J. P. Finnigan, M. E. Kirkling, B. Reizis, S. Ghosh, N. R. D’Amore, N. Bhardwaj, C. V. Rothlin, A. Wolf, R. Flores, T. Marron, A. H. Rahman, E. Kenigsberg, B. D. Brown, M. Merad, A conserved dendritic-cell regulatory program limits antitumor immunity. *Nature.* **580**, 257–262 (2020).
8. Y. Liu, S. He, X.-L. Wang, W. Peng, Q.-Y. Chen, D.-M. Chi, J.-R. Chen, B.-W. Han, G.-W. Lin, Y.-Q. Li, Q.-Y. Wang, R.-J. Peng, P.-P. Wei, X. Guo, B. Li, X. Xia, H.-Q. Mai, X.-D. Hu, Z. Zhang, Y.-X. Zeng, J.-X. Bei, Tumour heterogeneity and intercellular networks of nasopharyngeal carcinoma at single cell resolution. *Nat. Commun.* **12**, 741 (2021).
9. F. Lasoudris, C. Cousin, A. Prevost-Blondel, N. Martin-Garcia, I. Abd-Alsamad, N. Ortonne, J.-P. Farcet, F. Castellano, V. Molinier-Frenkel, IL4I1: an inhibitor of the CD8+ antitumor T-cell response in vivo. *Eur. J. Immunol.* **41**, 1629–1638 (2011).
10. L. Bod, R. Lengagne, L. Wrobel, J. P. Ramspott, M. Kato, M.-F. Avril, F. Castellano, V. Molinier-Frenkel, A. Prévost-Blondel, IL4-induced gene 1 promotes tumor growth by shaping the immune microenvironment in melanoma. *Oncoimmunology.* **6**, e1278331 (2017).
11. A. Sadik, L. F. Somarribas Patterson, S. Öztürk, S. R. Mohapatra, V. Panitz, P. F. Secker, P. Pfänder, S. Loth, H. Salem, M. T. Prentzell, B. Berdel, M. Iskar, E. Faessler, F. Reuter, I. Kirst, V. Kalter, K. I. Foerster, E. Jäger, C. R. Guevara, M. Sobeh, T. Hielscher, G. Poschet, A. Reinhardt, J. C. Hassel, M. Zapatka, U. Hahn, A. von Deimling, C. Hopf, R. Schlichting, B. I. Escher, J. Burhenne, W. E. Haefeli, N. Ishaque, A. Böhme, S. Schäuble, K. Thedieck, S. Trump, M. Seiffert, C. A. Opitz, IL4I1 Is a Metabolic Immune Checkpoint that Activates the AHR and Promotes Tumor Progression. *Cell*, S0092867420309466 (2020).
12. J. P. Ramspott, F. Bekkat, L. Bod, M. Favier, B. Terris, A. Salomon, L. Djerroudi, K. S. Zaenker, Y. Richard, V. Molinier-Frenkel, F. Castellano, M.-F. Avril, A. Prévost-Blondel, Emerging Role of IL-4-Induced Gene 1 as a Prognostic Biomarker Affecting the Local T-Cell Response in Human Cutaneous Melanoma. *J. Invest. Dermatol.* **138**, 2625–2634 (2018).
13. M. Liu, Q. Pan, R. Xiao, Y. Yu, W. Lu, L. Wang, A cluster of metabolism-related genes predict prognosis and progression of clear cell renal cell carcinoma. *Sci. Rep.* **10**, 12949 (2020).
14. V. Molinier-Frenkel, A. Prévost-Blondel, F. Castellano, The IL4I1 Enzyme: A New Player in the Immunosuppressive Tumor Microenvironment. *Cells.* **8** (2019), doi:10.3390/cells8070757.
15. J. H. Lee, J. E. Lee, J. Y. Kahng, S. H. Kim, J. S. Park, S. J. Yoon, J.-Y. Um, W. K. Kim, J.-K. Lee, J. Park, E. H.

382 Kim, J.-H. Lee, J.-H. Lee, W.-S. Chung, Y. S. Ju, S.-H. Park, J. H. Chang, S.-G. Kang, J. H. Lee, Human glioblastoma
383 arises from subventricular zone cells with low-level driver mutations. *Nature*. **560**, 243–247 (2018).

384 16. S. Wiemann, A. Kolb-Kokocinski, A. Poustka, Alternative pre-mRNA processing regulates cell-type
385 specific expression of the IL4I1 and NUP62 genes. *BMC Biol.* **3**, 16 (2005).

386 17. Y. Gao, J. Mu, T. Xu, T. Linghu, H. Zhao, J. Tian, X. Qin, Metabolomic analysis of the hippocampus in a
387 rat model of chronic mild unpredictable stress-induced depression based on a pathway crosstalk and network
388 module approach. *J. Pharm. Biomed. Anal.* **193**, 113755 (2021).

389 18. F. Castellano, V. Molinier-Frenkel, Control of T-cell activation and signaling by amino-acid catabolizing
390 enzymes. *Front. Cell Dev. Biol.*

391 19. A. Paris, N. Tardif, M.-D. Galibert, S. Corre, AhR and Cancer: From Gene Profiling to Targeted Therapy.
392 *Int. J. Mol. Sci.* **22**, 752 (2021).

393 20. A. D. Rouillard, G. W. Gundersen, N. F. Fernandez, Z. Wang, C. D. Monteiro, M. G. McDermott, A.
394 Ma'ayan, The harmonizome: a collection of processed datasets gathered to serve and mine knowledge about
395 genes and proteins. *Database*. **2016**, baw100 (2016).

396 21. R. Lo, J. Matthews, High-resolution genome-wide mapping of AHR and ARNT binding sites by CHIP-Seq.
397 *Toxicol. Sci. Off. J. Soc. Toxicol.* **130**, 349–361 (2012).

398 22. E. Oshchepkova, Y. Sizentsova, D. Wiebe, V. Mironova, N. Kolchanov, Meta-Analysis of Transcriptome
399 Data Detected New Potential Players in Response to Dioxin Exposure in Humans. *Int. J. Mol. Sci.* **21** (2020),
400 doi:10.3390/ijms21217858.

401 23. M.-L. Puiffe, A. Dupont, N. Sako, J. Gatineau, J. L. Cohen, D. Mestivier, A. Lebon, A. Prévost-Blondel, F.
402 Castellano, V. Molinier-Frenkel, IL4I1 Accelerates the Expansion of Effector CD8+ T Cells at the Expense of
403 Memory Precursors by Increasing the Threshold of T-Cell Activation. *Front. Immunol.* **11**, 600012 (2020).

404 24. A. Aubatin, N. Sako, X. Decrouy, E. Donnadieu, V. Molinier-Frenkel, F. Castellano, IL4-induced gene 1 is
405 secreted at the immune synapse and modulates TCR activation independently of its enzymatic activity. *Eur. J.*
406 *Immunol.* **48**, 106–119 (2018).

407 25. N. Riaz, J. J. Havel, V. Makarov, A. Desrichard, W. J. Urba, J. S. Sims, F. S. Hodi, S. Martín-Algarra, R.
408 Mandal, W. H. Sharfman, S. Bhatia, W.-J. Hwu, T. F. Gajewski, C. L. Slingluff, D. Chowell, S. M. Kendall, H. Chang,
409 R. Shah, F. Kuo, L. G. T. Morris, J.-W. Sidhom, J. P. Schneck, C. E. Horak, N. Weinhold, T. A. Chan, Tumor and
410 Microenvironment Evolution during Immunotherapy with Nivolumab. *Cell*. **171**, 934-949.e16 (2017).

411 26. M. J. Goldman, B. Craft, M. Hastie, K. Repečka, F. McDade, A. Kamath, A. Banerjee, Y. Luo, D. Rogers,
412 A. N. Brooks, J. Zhu, D. Haussler, Visualizing and interpreting cancer genomics data via the Xena platform. *Nat.*
413 *Biotechnol.* **38**, 675–678 (2020).

414 27. X. Zhang, M. Gan, J. Li, H. Li, M. Su, D. Tan, S. Wang, M. Jia, L. Zhang, G. Chen, *J. Agric. Food Chem.*, in
415 press, doi:10.1021/acs.jafc.0c03735.

416 28. L. Zhai, E. Ladomersky, A. Lenzen, B. Nguyen, R. Patel, K. L. Lauing, M. Wu, D. A. Wainwright, IDO1 in
417 cancer: a Gemini of immune checkpoints. *Cell. Mol. Immunol.* **15**, 447–457 (2018).

418 29. M. Y. Fong, J. McDunn, S. S. Kakar, Identification of metabolites in the normal ovary and their
419 transformation in primary and metastatic ovarian cancer. *PLoS One*. **6**, e19963 (2011).

420 30. G. A. Soliman, S. M. Steenson, A. H. Eteko, Effects of Metformin and a Mammalian Target of
421 Rapamycin (mTOR) ATP-Competitive Inhibitor on Targeted Metabolomics in Pancreatic Cancer Cell Line.
422 *Metabolomics Open Access*. **6** (2016), doi:10.4172/2153-0769.1000183.

423 31. A. MacKinnon, D. Bhupathi, J. Chen, T. Huang, W. Li, Y. Ma, N. Sotirovska, S. Steggerda, W. Zhang, F.
424 Parlati, 705 Anti-tumor activity of CB-668, a potent, selective and orally bioavailable small-molecule inhibitor of
425 the immuno-suppressive enzyme Interleukin 4 (IL-4)-Induced Gene 1 (IL4I1). *J. Immunother. Cancer*. **8**, A747–
426 A747 (2020).

427 32. L. Zeitler, A. Fiore, C. Meyer, M. Russier, G. Zanella, S. Suppmann, M. Gargaro, S. S. Sidhu, S. Seshagiri,
428 C. Ohnmacht, T. Köcher, F. Fallarino, A. Linkermann, P. J. Murray, Anti-ferroptotic mechanism of IL4i1-mediated
429 amino acid metabolism. *eLife*. **10** (2021), doi:10.7554/eLife.64806.

430 33. M. Presset, D. Djordjevic, A. Dupont, E. Le Gall, V. Molinier-Frenkel, F. Castellano, Identification of
431 inhibitors of the immunosuppressive enzyme IL4I1. *Bioorganic Chem.* **94**, 103463 (2020).

432