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Nicolas Curdy, Olivia Lanvin, Camille Laurent, Jean-Jacques Fournié,  
Don-Marc Franchini

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1 **Regulatory Mechanisms of Inhibitory Immune Checkpoint Receptors**

2

3 Nicolas Curdy<sup>1-3</sup>, Olivia Lanvin<sup>1-3</sup>, Camille Laurent<sup>1-3</sup>, Jean-Jacques Fournié<sup>1-3</sup>, Don-Marc  
4 Franchini<sup>1-3\*</sup>

5

6 <sup>1</sup>Cancer Research Center of Toulouse (CRCT), INSERM UMR 1037, CNRS ERL 5294, 31037  
7 Toulouse, France

8 <sup>2</sup>Université Toulouse III Paul Sabatier, 31330 Toulouse, France

9 <sup>3</sup>Institut Universitaire du Cancer de Toulouse-Oncopole, 31100 Toulouse, France

10

11 \*Correspondence: [don-marc.franchini@inserm.fr](mailto:don-marc.franchini@inserm.fr) (D.M. Franchini)

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22 **Abstract**

23 T cells responding to persistent tumor or viral antigens progressively lose their functional  
24 properties, a feature known as exhaustion. This state is also characterized by cell surface  
25 expression of multiple inhibitory immune checkpoint receptors. Cancer immunotherapies  
26 targeting immune checkpoints have shown impressive clinical outcomes, but still require  
27 substantial improvements due to the limited number of patients who benefit from it. Targeting  
28 the mechanisms controlling immune checkpoints expression could represent a step towards this  
29 aim. Accumulating data indicate that this strategy limits immune checkpoint expression, with  
30 in some instances the simultaneous inhibition of several immune checkpoints. In this review,  
31 we discuss various mechanisms through which immune checkpoint receptors are activated or  
32 regulated, and discuss ways these mechanisms could be exploited to develop more effective  
33 future immunotherapies.

34

35 **Physiological and pathological function of Inhibitory Immune Checkpoints expression**

36

37 T cell activation triggers a transcriptional reprogramming allowing T cell effector or memory  
38 functions, but also the expression of inhibitory immune checkpoint receptors (IRs) meant for  
39 modulating these functions, such as programmed cell death 1 (PD-1) and cytotoxic T  
40 lymphocyte antigen 4 (CTLA-4). In a physiological context, IRs expression is required to  
41 maintain immune homeostasis and self-tolerance, and therefore to avoid autoimmune reaction  
42 [1]. In cancer and in chronic viral infection, however, persistent stimulation increases the  
43 number and the level of expression of these IRs at the T cell surface, leading to a gradual loss  
44 of T cell effector functions, a phenomenon called exhaustion [2,3].

45 The immune checkpoint blockade (ICB) targeting CTLA-4, PD-1 or its ligand PD-L1 with  
46 antibodies gave unprecedented results in patients with previously incurable cancers [4]. Durable  
47 benefits, however, are only observed in a subset of patients. To improve this response rate and  
48 the number of responding cancer types, combination therapies, by targeting other IRs for  
49 instance, are now undertaken.

50 Tumor cells often overexpress several ligands of various IRs, such as PD-L1, to synergistically  
51 use the co-expression of IRs at the T cell surface as an immune escape mechanism.  
52 Consistently, tremendous efforts have been made to accumulate insights about PD-L1  
53 regulation in the aim of improving current immunotherapies [5,6]. Along the same line, a better  
54 understanding of the mechanisms that control IRs is needed to be able to modulate their  
55 expression and counter tumor resistance. Here, we describe the various facets of IRs regulation,  
56 and highlight how these regulatory mechanisms are, or could be, exploited to optimize current  
57 immunotherapies.

58

59

## 60 **Receptors and Signaling Activating IRs Expression**

61

62 T cell receptor (TCR) stimulation and signaling are the primary drivers of IRs expression, but  
63 additional pathways, which, for instance, can be stimulated by factors secreted within the tumor  
64 microenvironment (TME), synergize to sustain and increase their expression (**Figure 1**).

65

### 66 *TCR-Dependent Signaling*

67 Initial studies pointed the TCR and its co-receptor CD28 as the primary trigger for IRs  
68 expression. Taylor *et al.* showed that upon T cell activation, the glycogen synthase kinase  
69 (GSK)  $3\alpha/\beta$  is inactivated by phosphorylation induced from the TCR/CD28 signaling involving  
70 the PI3K/AKT pathway [7]. GSK- $3\alpha/\beta$  is a serine-threonine kinase that controls *Tbx21*  
71 transcription (encoding for T-bet), which in turn decreases transcription of the PD-1-encoding  
72 *PDCD1* gene. Consequently, inhibition of GSK- $3\alpha/\beta$  induces T-bet expression and PD-1  
73 repression, which increases cytotoxic T lymphocytes activity and limits infection from viruses  
74 such as herpes virus and lymphocytic choriomeningitis virus (LCMV) in mice models [7].

75 In another study, Balkhi *et al.* showed that TCR/CD28 stimulation phosphorylates and activates  
76 the p38MAPK/JNK pathway, which leads to cJun/ATF2 complex activation [8]. A downstream  
77 target of this complex is the transcription factor Yin-Yang 1 (YY1), which regulates the  
78 expression of PD-1, lymphocyte-activation gene-3 (LAG-3) and T cell immunoglobulin and  
79 mucin domain-3 (TIM-3). YY1 binds directly to the PD-1 and LAG-3 promoter regions, but  
80 requires GATA3 for TIM-3 promoter binding due to the absence of YY1 consensus binding  
81 site. Interestingly, YY1 expression was elevated in exhausted T cells from patients with either  
82 melanoma or HIV infection [8].

83

### 84 *Cytokine Receptors*

85 The common  $\gamma$ -chain cytokines IL-2, IL-7, IL-15 and IL-21, which trigger Janus kinase 1  
86 (JAK1), JAK3 and the signal transducer and activator of transcription (STAT) dependent  
87 signaling, increase PD-1 [9] and TIM-3 [10] expression in T lymphocytes, **but are not**  
88 **necessarily associated with T cell exhaustion. In this regard, it is noteworthy that inhibitory**  
89 **function of TIM-3 is somehow controversial [11].**

90 IL-15 secreted by gastric cancer mesenchymal stem cells activates STAT5 signaling in CD4<sup>+</sup>  
91 T cells, which induces further regulatory T cells (Treg) enrichment and PD-1 expression, and  
92 eventually promotes tumor progression [12]. Similar results were observed with low-dose of  
93 IL-2 in chronic graft-versus-host-disease [13]. In CD8<sup>+</sup> tumor-infiltrating lymphocytes (TILs)  
94 from breast cancer, IL-15 induced TIM-3 expression, but without enhancing PD-1 expression  
95 [14].

96 In triple negative breast cancer, tumor-derived IL-18 induces PD-1 expression on immature,  
97 non-cytotoxic NK cells, and is associated with poor prognosis [15]. In patients with follicular  
98 lymphoma, IL-12 expressed in the TME favors T cell exhaustion by a STAT4-dependent TIM-  
99 3 upregulation [16]. Together, IL-12 and IL-18 act to promote TIM-3 on  $\gamma\delta$  T cells following  
100 malaria infection, and decrease their cytotoxic functions [17].

101 Another member of the IL-12 family cytokine, IL-27, was identified as crucial for TIM-3  
102 expression [18]. Mechanistically, through STAT1 and STAT3 signaling, IL-27 induces the  
103 expression of the transcription factors NFIL3 and T-bet, which then promote chromatin  
104 remodeling at the TIM-3 locus and TIM-3 expression. IL-27 and NFIL3 are required to suppress  
105 T helper (Th) 1 cell-mediated colitis, and IL-27 receptor deficiency reduces tumor burden in  
106 B16F10 melanoma and Lewis Lung carcinoma-bearing mouse models [18]. In addition to TIM-  
107 3, IL-27 controls a wider range of IRs including T cell immunoglobulin and ITIM domain  
108 (TIGIT), LAG-3, CTLA-4 and PD-1 and the newly identified IRs PDPN and PROCR [19–22].  
109 This control relies on both STAT1 activity [21] and on the transcription factors PRDM1 (also

110 known as BLIMP1) and c-MAF [22]. Deletion of both transcription factors results in better  
111 tumor growth control in B16F10 melanoma-bearing mice [22].

112 IL-6 also induces c-MAF in a STAT3-dependent signaling, which alters tumor-specific Th1  
113 functions and favors tumor progression [23,24]. This effect can be counteracted by combining  
114 IL-6 blockade with ICB [25]. In CD8 T cells, IL-6/c-MAF axis is associated with exhaustion  
115 [26], and in patients with rheumatoid arthritis, the progression of disease is associated with  
116 enhanced IL-6/STAT3 signaling which positively correlates with PD-1 expression [27].

117

118 The immunosuppressive cytokine transforming growth factor  $\beta$  (TGF $\beta$ ) is generally abundant  
119 in the TME. Concurrently to T cell stimulation, TGF $\beta$  signaling enhances PD-1 transcription  
120 by activating SMAD2/3 transcription factors [28]. However, only SMAD3 appeared essential  
121 for PD-1 expression. Furthermore, SMAD3 deficiency decreases tumor growth and is  
122 associated with a diminished expression of PD-1 at the CD8<sup>+</sup> TILs surface in melanoma and  
123 colon cancer mouse models [28]. In addition, in a chronic LCMV infection mouse model,  
124 SMAD4, which forms heterodimers with SMAD2/3, was required for optimal CD4<sup>+</sup> T cell  
125 function and PD-1 expression [29]. Moreover, in a melanoma mouse model, TGF $\beta$  induces c-  
126 MAF expression [26], a transcription factor involved in multiple IRs expression [22]. TGF $\beta$   
127 enhances PD-1 and LAG-3 expression and eventually contributes to CD8 TILs exhaustion [26].

128 The genome organizer SATB1 maintains a repressive chromatin state at the *PDCDI* locus by  
129 recruiting the chromatin remodeling/histone deacetylase-containing NuRD repressor complex  
130 [30]. Tumor-derived TGF $\beta$  signaling reduces the expression of SATB1, thereby increasing  
131 histone 3 acetylation (H3ac) at the *PDCDI* locus and subsequent SMAD3-dependent *PDCDI*  
132 transcription. Accordingly, CD8<sup>+</sup> TILs from ovarian cancer patients present a negative  
133 correlation between SATB1 and PD-1 expression, and T cells deficient in SATB1 no longer  
134 control tumor growth in ovarian and lung carcinoma mouse models [30].

135

136 Interferon (IFN)-mediated inflammatory response also induces IRs expression. IFN $\alpha$  promotes  
137 and sustains *PDCDI* transcription through direct binding of IFN-responsive factor (IRF) 9 to  
138 the IFN-stimulated response element located in the promoter region [31]. On the other hand,  
139 IFN $\beta$  upregulates TIM-3 expression on Th1 cells, increasing the number of PD-1<sup>+</sup>TIM-3<sup>+</sup> cells,  
140 which results in less severe experimental autoimmune encephalomyelitis in mice [32].

141 Within the TME, IFN $\gamma$  released by CD8<sup>+</sup> T cells induces the production of kynurenine (Kyn)  
142 by adjacent tumor cells [33]. Kyn uptake by TILs, performed by the amino acid transporters  
143 SLC7A8 and PAT4, then induces and activates the aryl hydrocarbon receptor (AhR), which  
144 translocates to the nucleus to upregulate PD-1 expression. Consistently, expression of the  
145 Kyn/AhR pathway positively correlates with PD-1 expression on TILs from breast and colon  
146 cancer patients. Additionally, blockade of the Kyn/AhR pathways in melanoma-bearing mice  
147 results in decreased PD-1 expression on TILs along with controlled tumor growth and better  
148 survival of mice [33].

149 Besides immune cells, cytokine-dependent IRs expression has been evidenced on tumors cells.  
150 In human melanoma cells, IFN $\gamma$ -mediated signaling with STAT1 activation recruits CREB-  
151 binding protein (CBP)/p300 and induces histone acetylation at the CTLA-4 promoter to  
152 promote its expression [34]. In melanoma patients, benefits of anti-CTLA-4 antibody treatment  
153 were associated with high expression of CTLA-4. Interestingly, expression of CTLA-4  
154 positively correlates with expression of PD-L1, TIM-3 and LAG-3 [34]. **In relapsing tumors,**  
155 **sustained IFN signaling increases immunosuppressive mechanisms through the induction of a**  
156 **multigenic program [35]. Among these genes, multiple ligands for IRs are expressed,**  
157 **suggesting a link with IR-based immune escape mechanisms. While ICB alone partially restores**  
158 **the antitumor response, this effect is greatly improved when IFN signaling is compromised.**  
159 **Interestingly, the authors showed that to be effective, ICB must precede IFN signaling**



160 inhibition [35]. Consistently, IFN signaling pathway is often mutated in patients with melanoma  
161 unresponsive to ICB [36–38]. Along with the fact that PD-1 expression by melanoma cells  
162 promotes tumor growth [39], these studies emphasized the importance of understanding the  
163 mechanistic regulation of IRs expression in tumor cells to target immunosuppressive  
164 mechanisms and thwart tumorigenesis.

165

#### 166 *Non-Cytokine Receptors*

167 Additional receptors and signaling have been described for regulating PD-1 expression. *In vitro*  
168 activation of Notch receptor triggers RBPJk and Notch intracellular domain occupancy at the  
169 *PDCDI* promoter region to promote *PDCDI* transcription [40]. In early-stage septic shock  
170 patients, upregulation of this Notch pathway correlates with PD-1 expression [41].

171 In addition, signaling through glucocorticoid (GC) receptor expressed on NK cells induces PD-  
172 1 expression after stimulation by GC produced upon mouse cytomegalovirus infection [42]. As  
173 a result, IFN $\gamma$  overproduction is decreased, preserving from its associated immunopathology  
174 but without impairing viral clearance, revealing a fine-tuning by the neuroendocrine system  
175 [42].

176 Tumor cells generally secrete immunosuppressive factors into TME. Notably, activation of the  
177 cyclic adenosine monophosphate (cAMP) signaling pathway in human and mouse CD4<sup>+</sup> T cells  
178 increases CTLA-4 expression [43,44]. Moreover, activation of the cAMP signaling pathway by  
179 the prostaglandin E2 (PGE2) secreted by tumor cells upregulates TIM-3 expression in Jurkat  
180 cell line [45].

181 In addition, tumor cells can express high level of vascular endothelial growth factor A (VEGF-  
182 A), an immunosuppressive factor, within the TME. Upon activation, CD8<sup>+</sup> T cells upregulate  
183 VEGF receptors, which are activated by VEGF-A [46]. The resulting signaling entails the  
184 PLC $\gamma$ -calcineurin-NFAT pathway, which enhances the simultaneous cell surface expression of

185 PD-1, CTLA-4, TIM-3 and LAG-3. Concurrently, the transcription of IRs 2B4, CD160,  
186 KLRG1 and BTLA is also triggered. While anti-PD-1 antibody has minimal impact on the  
187 tumor in a colorectal mouse model, VEGF-A blockade decreases the proportion of TILs co-  
188 expressing IRs and reduces tumor growth, an effect even increased upon association with anti-  
189 PD-1 antibody [46]. Similar observations were obtained from small-cell lung cancer mice  
190 where anti-PD-L1 resistant tumor-bearing mice developed more exhausted lymphocytes  
191 expressing PD-1, TIM-3 and LAG-3 [47]. Addition of anti-VEGF antibody causes IRs  
192 downregulation and better survival [47].

193

### 194 **Epigenetic Regulation of IRs**

195

196 Differentiation of T lymphocytes in response to T cell stimulation by viral infection or tumor  
197 cells is accompanied by global changes in chromatin accessibility [48–50]. IRs are among the  
198 genes for whose chromatin becomes accessible, correlating with their increased expression  
199 levels.

200 These changes stem from DNA methylation and histone modifications that influence the  
201 chromatin state (**Figure 2**). Early studies identified *cis*-regulatory elements at the *PDCDI* locus  
202 regulated by DNA methylation and histone modifications [51]. Typically, PD-1 expression  
203 inversely correlates to the level of DNA methylation, and is associated with an enrichment in  
204 the permissive histone marks H3K9ac, H3K27ac or H3K4me1 [51]. In human breast and  
205 colorectal cancer, expression of PD-1, CTLA-4, TIM-3, LAG-3 and TIGIT was associated  
206 likewise with a decreased in both DNA methylation and repressive histone marks H3K9me3  
207 and H3K27me3 [52,53].

208 The plasticity of epigenetic marks allows cells to constantly evolve and adapt to environmental  
209 stimuli. Progression to exhaustion status, however, seems to set a more definitive epigenetic

210 program [48–50,54,55]. Although IRs are associated with accessible chromatin in exhausted T  
211 cells, it is unlikely to be a specific feature, as all changes are acquired and do not evolve further  
212 from the effector state. An exception is found at the *PDCDI* locus for a region located at -23  
213 kb away from the transcription start site, corresponding to the exhaustion-associated *PDCDI*  
214 enhancer that becomes specifically accessible in exhausted T cells [48–50,54,55].

215 After chronic LCMV infection, the challenged CD8<sup>+</sup> T cells keep the *PDCDI* promoter stably  
216 unmethylated, along with an impaired clonal expansion long after cells were transferred into  
217 uninfected mice, therefore preserving this exhaustion [56]. Nevertheless, the global DNA  
218 methylation changes that occur during T cell exhaustion preferentially target both effector and  
219 proliferation programs [57]. Instead, IRs genes undergo a stable DNA demethylation preserved  
220 from effector to exhausted states. Consequently, the reinvigoration of exhausted T cells by  
221 DNA demethylating agents principally occurs through re-induction of these effector and  
222 proliferation programs but without affecting IRs expression [57].

223

## 224 **Transcriptional Factors Controlling IRs Expression**

225

226 Chromatin accessibility and epigenetic changes are in close relationship with transcription  
227 factors activity (**Figure 2**). The *cis*-regulatory elements at the *PDCDI* gene are recognized by  
228 transcription factors such as nuclear factor of activated T cells 2 (NFAT2), activator protein 1  
229 (AP1) subunit c-Fos, forkhead box protein O1 (FoxO1), nuclear factor-κB (NF-κB), B  
230 lymphocyte-induced maturation protein 1 (BLIMP-1) and T-bet [51].

231 NFAT is a key regulator of T cell differentiation, able to balance towards effector or exhaustion  
232 programs, according to its association with AP-1. Without AP-1, NFAT1 is enriched at the  
233 promoter regions of genes encoding for the IRs PD-1, CTLA-4, TIM-3 and LAG-3.  
234 Consequently, NFAT1 binding enhances IRs expression, which is associated with a diminished

235 control of viral infection and tumor growth [58]. Importantly, all members of the NFAT family  
236 cooperate to control IRs expression [58,59].

237 Analysis of the transcriptional programs triggered by NFAT1 revealed that genes controlled by  
238 NFAT1 overlap with genes involved in exhaustion and anergy [58]. Moreover, these genes  
239 match the NFAT-associated chromatin accessibility regions found in exhausted CD8<sup>+</sup> T cells  
240 from mouse models of chronic infection by LCMV and of B16 melanoma tumor growth  
241 [48,49]. Among these genes, IRF4, PRDM1 (BLIMP1) and NR4A-family transcription factors  
242 were identified.

243 IRF4 is a transcription factor required for effector CD8<sup>+</sup> T cell differentiation and expansion.  
244 RNA-sequencing analysis from LCMV-mediated exhausted T cells specifically identified  
245 IRF4, along with genes encoding transcription factors such as PRDM1, BATF and NFAT2, and  
246 IRs PD-1, LAG-3, TIM-3, CTLA-4, CD160, 2B4 and TIGIT [60]. Reduction of IRF4  
247 expression restores T cells functional properties, and downregulates the expression of the  
248 aforementioned IRs. Similar observations were obtained with T cells deficient in BATF. In  
249 addition, genome-wide DNA binding analysis revealed enrichment of the transcription factors  
250 IRF4, BATF and NFAT at genes encoding these IRs, suggesting a cooperative regulation of  
251 such genes [60].

252 As aforesaid, PRDM1 was described as a regulator of multiple IRs by cooperating with c-MAF  
253 in an IL-27-dependent manner [22]. Moreover, in a study using toxoplasma encephalitis-  
254 susceptible model, toxoplasma-specific CD4<sup>+</sup> T cells display exhaustion features, associated  
255 with elevated expression of PD-1, CTLA-4, LAG-3 and 2B4 [61]. These cells also highly  
256 express BLIMP1, which when deleted, improved pathogen control. While BLIMP1-deficient  
257 CD4<sup>+</sup> T cells display a reduced expression of IRs that is associated with improved functions,  
258 they also reduce IRs expression (PD-1, CD160 and LAG-3) and improve functionality of CD8<sup>+</sup>  
259 T cells [61]. Further support for this observation was obtained from patients with acute myeloid

260 leukemia [62]. T lymphocytes from such patients upregulate BLIMP1 expression, which is  
261 correlated with the expression of PD-1, TIGIT, TIM-3, 2B4 and CD160 in CD4<sup>+</sup> T cells and  
262 with PD-1 and TIGIT in CD8<sup>+</sup> T cells, and finally associated with reduced T cells functions.  
263 Importantly, BLIMP1 directly binds PD-1 and TIGIT promoter and positively regulates their  
264 expression [62].

265 NR4A transcription factors family comprises three members, NR4A1, NR4A2 and NR4A3,  
266 initially revealed as critical for Treg development [63]. Recently, NR4A factors were identified  
267 as essential in mediating T cell exhaustion [64,65]. In a melanoma mouse model, NR4A factors  
268 were upregulated in CD8<sup>+</sup> T cells with an exhaustion phenotype PD-1<sup>hi</sup>TIM-3<sup>hi</sup>, and motifs for  
269 NR4A binding were enriched in accessible chromatin of these cells [64]. Additionally, CD8<sup>+</sup> T  
270 cells transduced with either NR4A factors have higher expression of PD-1, TIM-3, LAG-3,  
271 TIGIT and 2B4 IRs. Consequently, all NRA4 cooperatively impair antitumor immunity in  
272 melanoma and colorectal mouse models [64]. Similarly, in tolerant CD4<sup>+</sup> T cells, transcriptomic  
273 and epigenetic analysis revealed homologies with exhausted (virus-induced) and anergic  
274 (NFAT-mediated) states [65]. Notably, NR4A1 was substantially expressed in tolerant cells,  
275 where NR4A1 deficiency exacerbates CD4<sup>+</sup> T cells-mediated colitis. The authors further  
276 assessed the role of NR4A1 in exhaustion and found that accordingly, NR4A1 deficiency  
277 enhances CD8<sup>+</sup> T cells-mediated tumor eradication and virus-specific CD8<sup>+</sup> T cells activity.  
278 Importantly, the expression of PD-1 and TIM-3 was significantly reduced in both instances,  
279 consistent with the fact that NR4A is recruited at the regulatory regions of these genes [65].  
280 Altogether, these studies indicate that NR4A induce a transcriptional circuit enforcing  
281 exhaustion program throughout IRs expression, among others.

282 Another transcription factor included in the NFAT/IRF4-dependent reprogramming is the  
283 hypoxia-inducible factor (HIF) 1 $\alpha$  [58,60]. HIF pathway is typically induced during hypoxia, a  
284 feature of the microenvironment established by tumors or during viral infection. In CD8<sup>+</sup> T

285 cells experiencing LCMV infection, upregulation of the HIF pathway was associated with the  
286 simultaneous expression of IRs CTLA-4, TIM-3, LAG-3 and 2B4 [66].  
287 Interestingly, besides the viral and tumor context, inflammation caused by hypoxia during  
288 cerebral ischemia can lead to severe neurological deficits. Using a mouse model of hypoxia-  
289 ischemia revealed a HIF1 $\alpha$ -dependent TIM-3 expression by glial cells [67]. Consequently,  
290 blockade of TIM-3, as observed with HIF1 $\alpha$  deficiency, limits expression of the  
291 chemoattractants IL-1 $\beta$  and CXCL1 by glial cells, thereby reducing neutrophils infiltration and  
292 eventually brain damage [67]. In a similar model, the HIF1 $\alpha$ /TIM-3 axis has been shown to be  
293 negatively controlled by the cannabinoid type 2 receptors (CB2R), which are mainly expressed  
294 on immune cells such as microglia, as CB2R deficiency was associated with TIM-3  
295 upregulation and larger brain lesions [68].

296

297

## 298 **Post-Transcriptional Regulation of IRs Expression**

299

### 300 *Regulation of IRs Expression by MicroRNAs*

301 MicroRNAs (miR) are short non-coding RNAs that negatively regulate mRNAs at the post-  
302 transcriptional level by targeting their 3' untranslated region (UTR) [69]. They are implicated  
303 in many aspects of immunity, and play an important role in tumor development and  
304 autoimmunity [70], and represent potential targets to improve current ICB therapies [71]  
305 **(Figure 3)**.

306 MiR-155, described as a central regulator of immune response, directly binds to the 3'UTR of  
307 *CTLA-4* and *BTLA* and decreases their protein expression [72,73]. Accordingly, miR-155  
308 deficiency reduces CD8<sup>+</sup> T cells effector functions and decrease their ability to control viral  
309 infection and tumor growth [74,75]. However, these results might not be directly linked to IRs

310 expression, as miR-155 is expressed in exhausted T cells from melanoma-bearing mice [76],  
311 and is associated with T cell exhaustion in a chronic viral infection mouse model and correlates  
312 positively with several IRs expression (PD-1, CD160, 2B4) [77].

313 In melanoma-bearing mice, CD4<sup>+</sup> T cells expressing PD-1 downregulate the expression of miR-  
314 28 as compared to the PD-1 negative cells [76]. MiR-28 binds to the 3'UTR of *PDCDI*, *TIM-*  
315 *3* and *BTLA*, whose expression consistently increase upon miR-28 silencing.

316 MiR-138 binds to the 3'UTR of *PDCDI* and *CTLA-4* mRNAs, and inhibits their protein  
317 expression [78]. Moreover, the administration of miR-138 in mice with glioma induces a  
318 reduction of tumor burden, and a decrease in PD-1 and CTLA-4 expression on T cells in the  
319 TME. Using the same model, Yang *et al.* showed that miR-15a/16 deficiency correlates with a  
320 lower expression of PD-1, TIM-3 and LAG-3 [79].

321 Several other miRs have been described to independently modulate IRs expression. MiR-4717  
322 [80] and miR-374b [81] bind the *PDCDI* 3'UTR and inhibit PD-1 expression, while EBV-miR-  
323 BART cluster correlates with PD-1 expression [82]. CTLA-4 is a potential target for miR-487a-  
324 3p, which can bind the *CTLA-4* 3'UTR [83], and for miR-181a [84]. MiR-584-5p binds to the  
325 3'UTR of *KLRG1* and decreases its expression [85], while miR-330-5p and miR-498 expression  
326 correlate with TIM-3 downregulation [86,87]. MiR-23a, a downstream target of the long-  
327 noncoding RNA MEG3, modulates TIGIT expression by binding to the 3'UTR of its mRNA  
328 [88]. In CD4<sup>+</sup> T cells derived from patients with autoimmune aplastic anemia, MEG3 is  
329 downregulated, allowing the overexpression of miR-23a and inhibition of TIGIT expression  
330 [88].

331

### 332 *Control of IRs mRNA Translation*

333 Recently, we identified a post-transcriptional mechanism that simultaneously modulate the  
334 expression of several IRs [89] (**Figure 3**). Following T lymphocytes stimulation, the newly

335 induced mRNAs of IRs associate with stress granule-specific factors, such as G3BP1. These  
336 ribonucleoprotein complexes are then transported by the kinesin 1, a molecular motor  
337 composed of KIF5B and KLC1, along the microtubules to form stress granules [89]. Without  
338 this molecular motor or without the microtubules, the maturation of the stress granules remains  
339 locked in a state that does not allow their disassembly. Hence, this prevents the transport and  
340 delivery of these mRNAs to the polysomes for their translation. Most of the IRs (PD-1, CTLA-  
341 4, LAG-3, TIM-3, BTLA and TIGIT) are regulated by these stress granules and the microtubule  
342 network, whereas stimulatory receptors (OX40, GITR, 4 -1BB) are not [89]. An essential  
343 feature to follow stress granules regulation seems to lie within the higher length of the 3'UTR  
344 mRNAs. In IRs, their mean length is similar to that of neuronal mRNAs displaying microtubule  
345 transport for synaptic translation. Consequently, microtubule-targeting agents decrease IRs  
346 expression on TILs of patients with Hodgkin's lymphoma, and display a significantly increased  
347 risk for immune-related adverse events, as observed with ICB antibodies [89].

348

#### 349 **Control of IRs at the Protein Level**

350

351 Protein stability, transport and turnover are crucial to maintain the proper homeostasis within  
352 the cells and the surrounding environment. These different processes are undertaken by  
353 conjugating partners or through specific post-translational modifications (PTM).

354 Huang *et al.* showed that co-expression of CEACAM1, a TIM-3 ligand also expressed by  
355 lymphocytes, is required for TIM-3 stabilization and cell surface expression [90]. Indeed,  
356 CEACAM1 deficiency causes TIM-3 hypoglycosylation and intracellular retention. In mice,  
357 CEACAM1 deficiency enhances mucosa-associated inflammation owing to TIM-3 lower  
358 expression at the CD4<sup>+</sup> T cell surface. On the other hand, in a colorectal cancer mouse model,



359 CEACAM1-deficient mice exhibited lower tumor burden and decreased TIM-3 expression by  
360 the CD8<sup>+</sup> TILs [90].  
361 CTLA-4 cell surface retention also depends on N-glycan branching [91], a hallmark altered in  
362 the multiple sclerosis autoimmune disease [92]. Moreover, Treg suppressive functions depend  
363 on glycosylation, whose level correlates with cell surface marker PD-1 and CTLA-4 [93].  
364 CRISPR-Cas9-based screening identified core fucosylation, catalyzed by FUT8, as required for  
365 PD-1 cell surface expression [94]. Impeding this modification causes intracellular retention of  
366 PD-1 and enhances T cell activity. Consequently, B16 melanoma-bearing mice treated with  
367 fucosylation inhibitors present a decreased tumor growth associated with increased rates of  
368 TILs [94]. Moreover, the dynamics of PD-1 expression at the T cell surface is regulated by  
369 ubiquitination-induced degradation. This reaction is mediated by the F-box protein FBXO38,  
370 an E3 ubiquitin ligase member of the SKP1-CUL1-F-box protein family [95]. In B16F10  
371 melanoma and MC38 colorectal carcinoma mouse models deficient in FBXO38, anti-tumor  
372 immunity is compromised due to the higher expression of PD-1 at the surface of TILs. Anti-  
373 PD-1 immunotherapy counteracts the FBXO38 deficiency, while IL-2 treatment could enhance  
374 FBXO38 gene expression [95].

375

## 376 **Concluding Remarks and future Perspectives**

377

378 IRs targeting-based immunotherapies have shown unprecedented clinical benefits and durable  
379 responses. Yet, despite these progresses, biomarkers identification, efficacy, and drug  
380 resistance still pose significant therapeutic challenges. Because immune escape is partly due to  
381 simultaneous expression of IRs, increasing the knowledge underlying their expression  
382 represents a major concern (see **Outstanding Questions**).

383 Many researches revealed one layer of the regulatory mechanisms for one specific IR, and in  
384 some instances, mechanisms controlling several IRs simultaneously have been identified.  
385 Because the expression of IRs is not restricted to T lymphocytes but is found in basically all  
386 immune cell types, it will be important to verify and validate the regulatory mechanisms in  
387 these cells. Moreover, pathological context influences IRs expression on immune cells, for  
388 instance through secreting factors. This cell- and context-dependent regulation emphasizes the  
389 need to further improve the characterization of IRs regulation. This fundamental knowledge has  
390 the potential to facilitate the development of alternative strategies able to improve therapeutic  
391 outcomes unrestrictedly in cancer (**Box 1**) as well as in viral infection, autoimmune diseases  
392 including neurological disorders.

393 Besides IRs, targeting of PD-L1 has met clear-cut clinical success. As for IRs, deciphering the  
394 mechanisms regulating PD-L1 expression is indispensable to counteract immune escape  
395 mechanisms, and tremendous efforts have been made to achieve this objective [5,6].  
396 Interestingly, looking at the mechanisms regulating on one hand the IRs and on the other hand  
397 PD-L1 reveals striking overlaps (**Box 2**). Future research will evaluate the targeting of such  
398 regulatory pathways for enhancing antitumor immunity, and will inspire the design of new  
399 therapeutic strategies in the upcoming years.

400

401 **Box 1. Potential of Targeting IRs Regulatory Mechanism for Immunotherapies.**

402

403 The different regulatory levels controlling IRs expression offer a multitude of theoretical targets  
404 to modulate IR mRNA or protein levels and to enhance antitumor immunity (Figure I).

405 Conceivably, epigenetic drugs such as 5-azacytidine currently used for treating myelodysplastic  
406 syndromes and acute myeloid leukemia might owe their efficacy to both impairing DNA  
407 methylation of the cancer cells while reinvigorating cytolytic lymphocytes. Thus, associations  
408 of epigenetic therapy and immunotherapy can target the epigenetic control of immune cells [96]  
409 as well as the IR expression profiles. Ongoing phase II trial with azacitidine, entinostat, and  
410 nivolumab *versus* nivolumab alone in patients with recurrent metastatic non-small cell lung  
411 cancer (<https://clinicaltrials.gov/>) will determine the efficacy of such combinations, and  
412 validate their ability to simultaneously hit multiple IR targets.

413 The therapeutic targeting of transcription factors in cancer was clinically validated with drugs  
414 targeting nuclear hormone receptors [97]. Despite their potential, no inhibitor of HIF1/2 has  
415 met both safety and clinical efficacy so far [98]. On the other hand, cannabinoid agonists of  
416 CB2R might reinvigorate cytolytic lymphocytes, and could be tested in combination with ICB  
417 of cancers such as neuroblastoma, hepatocellular carcinoma or pancreatic ductal  
418 adenocarcinoma.

419 In addition, drugs selectively inhibiting either stress granules assembly or mRNA transport,  
420 such as integrated stress response inhibitor (ISRIB) or microtubule inhibitors, respectively, can  
421 impair both the expression and downstream function of IRs. In lymphocytes stimulated within  
422 tumors, these agents inhibit simultaneously the translation of several IRs. These drugs now  
423 warrant clinical investigation, notably in combination with nivolumab in cancer patients  
424 relapsed after first-line ICB.

425 PTM have a pivotal role in immune function but also in cancer formation and progression. In  
426 particular, as observed with the regulation of PD-1 expression by FBXO38 [95], F-box-  
427 containing E3 ligases might represent future targets to selectively modulate IRs expression [99],  
428 and inhibitors of cullin neddylation pevonedistat could potentiate immunotherapy [100].  
429 Moreover, targeting glycosylation could bring benefits in autoimmunity or tumor control [101].  
430 Indeed, in melanoma mouse model, fucosylation inhibition can not only act on tumor  
431 aggressiveness [102] but also reduce PD-1 expression on T lymphocytes and enhance their  
432 effector functions, which is even strengthened with anti-PD-1 immunotherapy [94]. Benefits of  
433 such an inhibitor, the 2-fluorofucose, combined with immunotherapy will be determined by  
434 ongoing clinical trial (NCT02952989).

435 Altogether, identification of key regulatory elements able to modulate IRs expression will allow  
436 the design of new therapeutic approaches or molecules eventually effective for human cancer  
437 treatment.

438

439 **Box 2. Similarities between PD-L1 and IRs Expression Regulation.**

440

441 Among all immune checkpoints, the PD-1/PD-L1 axis is the best characterized. The regulation  
442 underlying PD-L1 expression has been the focus of remarkable research, and expanding the  
443 knowledge concerning PD-L1 regulation has shown great promises for improving current  
444 immunotherapies or by suggesting alternative strategies [5,6]. These have revealed rather  
445 complex multifactorial regulatory mechanisms that occur from triggering receptors to PTM.  
446 Interestingly, some of these mechanisms stand out by the fact that they are also described to  
447 regulate IRs. Therefore, future therapeutics targeting these shared pathways could be designed  
448 to inhibit both receptors and ligands, which could further alleviate immune escape mechanisms.  
449 For instance, IL-27 regulates the expression of multiple IRs on T cells [21,22]. Similarly, PD-  
450 L1 expression on T cells was shown to require IL-27/STAT1 signaling [21,103], being involved  
451 in suppressing Th17 differentiation [103]. In addition, IL-27 exerts immune regulatory  
452 properties by inducing the expression of PD-L1 on dendritic cells and human epithelial ovarian  
453 cancer cells [5].

454 Furthermore, several miR involved in IRs expression have also been shown to interact with the  
455 3'UTR of *PD-L1* mRNA and suppress its translation [5,71]. Reduction of PD-L1 was observed  
456 on colorectal cancer cell lines by miR-138, on primary human dermal lymphatic endothelial  
457 cells by miR-155, and on malignant pleural mesothelioma cells by miR-15/16 [5,71].

458 Whole genome and RNA sequencing analyses of tumor cells overexpressing PD-L1 revealed  
459 chromosomal aberration at the PD-L1 locus consisting in 3'UTR disruption [104]. In T cells, it  
460 was suggested that stress granules-dependent IRs expression relies on the 3'UTR of IRs  
461 mRNAs [89]. These data suggest that the 3'UTR of mRNAs restrain PD-L1 and IRs expression.  
462 It is therefore tempting to speculate that stabilization of these interactions could reduce both  
463 PD-L1 and IRs expression. Along the same line, inhibition of IL-27 expression or signaling, as

464 well as use of miR represent challenging approaches to consider for harnessing the potential of  
465 immunotherapies.

466

467 **Disclaimer Statement**

468 N.C. and C.L. declare no conflict of interest. O.L., J.J.F and D.M.F. co-authored two INSERM  
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470

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477

478 **References**

- 479 1 Zhang, Q. and Vignali, D.A.A. (2016) Co-stimulatory and Co-inhibitory Pathways in  
480 Autoimmunity. *Immunity* 44, 1034–1051
- 481 2 Thommen, D.S. and Schumacher, T.N. (2018) T Cell Dysfunction in Cancer. *Cancer Cell*  
482 33, 547–562
- 483 3 McLane, L.M. *et al.* (2019) CD8 T Cell Exhaustion During Chronic Viral Infection and  
484 Cancer. *Annual Review of Immunology* 37,
- 485 4 Ribas, A. and Wolchok, J.D. (2018) Cancer immunotherapy using checkpoint blockade.  
486 *Science* 359, 1350–1355
- 487 5 Sun, C. *et al.* (2018) Regulation and Function of the PD-L1 Checkpoint. *Immunity* 48, 434–  
488 452
- 489 6 Zhang, J. *et al.* (2018) Biochemical Aspects of PD-L1 Regulation in Cancer  
490 Immunotherapy. *Trends in Biochemical Sciences* 43, 1014–1032
- 491 7 Taylor, A. *et al.* (2016) Glycogen Synthase Kinase 3 Inactivation Drives T-bet-Mediated  
492 Downregulation of Co-receptor PD-1 to Enhance CD8+ Cytolytic T Cell Responses.  
493 *Immunity* 44, 274–286
- 494 8 Balkhi, M.Y. *et al.* (2018) YY1 Upregulates Checkpoint Receptors and Downregulates  
495 Type I Cytokines in Exhausted, Chronically Stimulated Human T Cells. *iScience* 2, 105–  
496 122
- 497 9 Kinter, A.L. *et al.* (2008) The common  $\gamma$ -chain cytokines IL-2, IL-7, IL-15, and IL-21  
498 induce the expression of programmed death-1 and its ligands. *The Journal of Immunology*  
499 181, 6738–6746
- 500 10 Mujib, S. *et al.* (2012) Antigen-Independent Induction of Tim-3 Expression on Human  
501 T Cells by the Common  $\gamma$ -Chain Cytokines IL-2, IL-7, IL-15, and IL-21 Is Associated with  
502 Proliferation and Is Dependent on the Phosphoinositide 3-Kinase Pathway. *The Journal of*  
503 *Immunology* 188, 3745–3756
- 504 11 Avery, L. *et al.* (2018) Tim-3 co-stimulation promotes short-lived effector T cells,  
505 restricts memory precursors, and is dispensable for T cell exhaustion. *Proceedings of the*  
506 *National Academy of Sciences* 115, 2455–2460
- 507 12 Sun, L. *et al.* (2018) Human Gastric Cancer Mesenchymal Stem Cell-Derived IL15  
508 Contributes to Tumor Cell Epithelial-Mesenchymal Transition via Upregulation Tregs  
509 Ratio and PD-1 Expression in CD4<sup>+</sup> T Cell. *Stem Cells and Development* 27, 1203–1214



510 13 Asano, T. *et al.* (2017) PD-1 modulates regulatory T-cell homeostasis during low-dose  
511 interleukin-2 therapy. *Blood* 129, 2186–2197

512 14 Heon, E.K. *et al.* (2015) IL-15 induces strong but short-lived tumor-infiltrating CD8 T  
513 cell responses through the regulation of Tim-3 in breast cancer. *Biochemical and*  
514 *Biophysical Research Communications* 464, 360–366

515 15 Park, I.H. *et al.* (2017) Tumor-derived IL-18 induces PD-1 expression on  
516 immunosuppressive NK cells in triple-negative breast cancer. *Oncotarget* 8, 32722-32730

517 16 Yang, Z.-Z. *et al.* (2012) IL-12 upregulates TIM-3 expression and induces T cell  
518 exhaustion in patients with follicular B cell non-Hodgkin lymphoma. *Journal of Clinical*  
519 *Investigation* 122, 1271–1282

520 17 Schofield, L. *et al.* (2017) Synergistic effect of IL-12 and IL-18 induces TIM3  
521 regulation of  $\gamma\delta$  T cell function and decreases the risk of clinical malaria in children living  
522 in Papua New Guinea. *BMC Medicine* 15,

523 18 Zhu, C. *et al.* (2015) An IL-27/NFIL3 signalling axis drives Tim-3 and IL-10  
524 expression and T-cell dysfunction. *Nature Communications* 6, 6072

525 19 Do, J. *et al.* (2016) An IL-27/Lag3 axis enhances Foxp3+ regulatory T cell–  
526 suppressive function and therapeutic efficacy. *Mucosal Immunology* 9, 137–145

527 20 Ma, Q.-Y. *et al.* (2017) Function and regulation of LAG3 on CD4 + CD25 - T cells in  
528 non-small cell lung cancer. *Experimental Cell Research* 360, 358–364

529 21 DeLong, J.H. *et al.* (2019) IL-27 and TCR Stimulation Promote T Cell Expression of  
530 Multiple Inhibitory Receptors. *ImmunoHorizons* 3, 13–25

531 22 Chihara, N. *et al.* (2018) Induction and transcriptional regulation of the co-inhibitory  
532 gene module in T cells. *Nature* 558, 454–459

533 23 Tsukamoto, H. *et al.* (2015) IL-6-mediated environmental conditioning of defective  
534 Th1 differentiation dampens antitumour immune responses in old age. *Nat Commun* 6,  
535 6702

536 24 Tsukamoto, H. *et al.* (2017) Soluble IL6R Expressed by Myeloid Cells Reduces  
537 Tumor-Specific Th1 Differentiation and Drives Tumor Progression. *Cancer Res* 77, 2279–  
538 2291

539 25 Tsukamoto, H. *et al.* (2018) Combined Blockade of IL6 and PD-1/PD-L1 Signaling  
540 Abrogates Mutual Regulation of Their Immunosuppressive Effects in the Tumor  
541 Microenvironment. *Cancer Res* 78, 5011–5022

542 26 Giordano, M. *et al.* (2015) Molecular profiling of CD8 T cells in autochthonous  
543 melanoma identifies Maf as driver of exhaustion. *The EMBO Journal* 34, 2042–2058

544 27 Niu, Q. *et al.* (2018) Enhanced IL-6/phosphorylated STAT3 signaling is related to the  
545 imbalance of circulating T follicular helper/T follicular regulatory cells in patients with  
546 rheumatoid arthritis. *Arthritis Res Ther* 20, 200

547 28 Park, B.V. *et al.* (2016) TGFbeta1-Mediated SMAD3 Enhances PD-1 Expression on  
548 Antigen-Specific T Cells in Cancer. *Cancer Discovery* 6, 1366–1381

549 29 Lewis, G.M. *et al.* (2016) TGF- $\beta$  receptor maintains CD4 T helper cell identity during  
550 chronic viral infections. *Journal of Clinical Investigation* 126, 3799–3813

551 30 Stephen, T.L. *et al.* (2017) SATB1 Expression Governs Epigenetic Repression of PD-  
552 1 in Tumor-Reactive T Cells. *Immunity* 46, 51–64

553 31 Terawaki, S. *et al.* (2011) IFN- $\alpha$  Directly Promotes Programmed Cell Death-1  
554 Transcription and Limits the Duration of T Cell-Mediated Immunity. *The Journal of*  
555 *Immunology* 186, 2772–2779

556 32 Boivin, N. *et al.* (2015) Interferon- $\beta$  Suppresses Murine Th1 Cell Function in the  
557 Absence of Antigen-Presenting Cells. *PLoS ONE* 10, e0124802

558 33 Liu, Y. *et al.* (2018) Tumor-Repopulating Cells Induce PD-1 Expression in CD8 + T  
559 Cells by Transferring Kynurenine and AhR Activation. *Cancer Cell* 33, 480-494

560 34 Mo, X. *et al.* (2018) Interferon- $\gamma$  Signaling in Melanocytes and Melanoma Cells  
561 Regulates Expression of CTLA-4. *Cancer Research* 78, 436–450

562 35 Benci, J.L. *et al.* (2016) Tumor Interferon Signaling Regulates a Multigenic  
563 Resistance Program to Immune Checkpoint Blockade. *Cell* 167, 1540-1554

564 36 Zaretsky, J.M. *et al.* (2016) Mutations Associated with Acquired Resistance to PD-1  
565 Blockade in Melanoma. *New England Journal of Medicine* 375, 819–829

566 37 Gao, J. *et al.* (2016) Loss of IFN- $\gamma$  Pathway Genes in Tumor Cells as a Mechanism of  
567 Resistance to Anti-CTLA-4 Therapy. *Cell* 167, 397-404

568 38 Shin, D.S. *et al.* (2017) Primary Resistance to PD-1 Blockade Mediated by *JAK1/2*  
569 Mutations. *Cancer Discov* 7, 188–201

570 39 Kleffel, S. *et al.* (2015) Melanoma Cell-Intrinsic PD-1 Receptor Functions Promote  
571 Tumor Growth. *Cell* 162, 1242–1256

572 40 Mathieu, M. *et al.* (2013) Notch signaling regulates PD-1 expression during CD8 (+)  
573 T-cell activation. *Immunology and Cell Biology* 91, 82–88

574 41 Pan, T. *et al.* (2015) Notch Signaling Pathway Was Involved in Regulating  
575 Programmed Cell Death 1 Expression during Sepsis-Induced Immunosuppression.  
576 *Mediators of Inflammation* 2015, 1–9

577 42 Quatrini, L. *et al.* (2018) Endogenous glucocorticoids control host resistance to viral  
578 infection through the tissue-specific regulation of PD-1 expression on NK cells. *Nature*  
579 *Immunology* 19, 954–962

580 43 Vendetti, S. *et al.* (2002) Cyclic Adenosine 5'-Monophosphate and Calcium Induce  
581 CD152 (CTLA-4) Up-Regulation in Resting CD4+ T Lymphocytes. *The Journal of*  
582 *Immunology* 169, 6231–6235

583 44 Li, J. *et al.* (2013) Regulation of Cytotoxic T Lymphocyte Antigen 4 by Cyclic AMP.  
584 *American Journal of Respiratory Cell and Molecular Biology* 48, 63–70

585 45 Yun, S.J. *et al.* (2019) Regulation of TIM-3 expression in a human T cell line by  
586 tumor-conditioned media and cyclic AMP-dependent signaling. *Molecular Immunology*  
587 105, 224–232

588 46 Voron, T. *et al.* (2015) VEGF-A modulates expression of inhibitory checkpoints on  
589 CD8+ T cells in tumors. *The Journal of Experimental Medicine* 212, 139–148

590 47 Meder, L. *et al.* (2018) Combined VEGF and PD-L1 Blockade Displays Synergistic  
591 Treatment Effects in an Autochthonous Mouse Model of Small Cell Lung Cancer. *Cancer*  
592 *Res* 78, 4270–4281

593 48 Scott-Browne, J.P. *et al.* (2016) Dynamic Changes in Chromatin Accessibility Occur  
594 in CD8 + T Cells Responding to Viral Infection. *Immunity* 45, 1327–1340

595 49 Mognol, G.P. *et al.* (2017) Exhaustion-associated regulatory regions in CD8+ tumor-  
596 infiltrating T cells. *Proceedings of the National Academy of Sciences* 114, E2776–E2785

597 50 Philip, M. *et al.* (2017) Chromatin states define tumour-specific T cell dysfunction and  
598 reprogramming. *Nature* 545, 452–456

599 51 Bally, A.P.R. *et al.* (2016) Genetic and Epigenetic Regulation of PD-1 Expression.  
600 *The Journal of Immunology* 196, 2431–2437

601 52 Sasidharan Nair, V. *et al.* (2018) DNA methylation and repressive H3K9 and H3K27  
602 trimethylation in the promoter regions of PD-1, CTLA-4, TIM-3, LAG-3, TIGIT, and PD-  
603 L1 genes in human primary breast cancer. *Clinical Epigenetics* 10, 78

604 53 Sasidharan Nair, V. *et al.* (2018) DNA methylation and repressive histones in the  
605 promoters of PD-1, CTLA-4, TIM-3, LAG-3, TIGIT, PD-L1, and galectin-9 genes in  
606 human colorectal cancer. *Clinical Epigenetics* 10, 104

607 54 Pauken, K.E. *et al.* (2016) Epigenetic stability of exhausted T cells limits durability of  
608 reinvigoration by PD-1 blockade. *Science* 354, 1160–1165

609 55 Sen, D.R. *et al.* (2016) The epigenetic landscape of T cell exhaustion. *Science* 354,  
610 1165–1169

611 56 Ahn, E. *et al.* (2016) Demethylation of the PD-1 Promoter Is Imprinted during the  
612 Effector Phase of CD8 T Cell Exhaustion. *J. Virol.* 90, 8934–8946

613 57 Ghoneim, H.E. *et al.* (2017) De Novo Epigenetic Programs Inhibit PD-1 Blockade-  
614 Mediated T Cell Rejuvenation. *Cell* 170, 142-157

615 58 Martinez, G.J. *et al.* (2015) The Transcription Factor NFAT Promotes Exhaustion of  
616 Activated CD8 + T Cells. *Immunity* 42, 265–278

617 59 Martinez, G.J. *et al.* (2016) Cutting Edge: NFAT Transcription Factors Promote the  
618 Generation of Follicular Helper T Cells in Response to Acute Viral Infection. *The Journal*  
619 *of Immunology* 196, 2015–2019

620 60 Man, K. *et al.* (2017) Transcription Factor IRF4 Promotes CD8+ T Cell Exhaustion  
621 and Limits the Development of Memory-like T Cells during Chronic Infection. *Immunity*  
622 47, 1129-1141

623 61 Hwang, S. *et al.* (2016) Blimp-1–mediated CD4 T cell exhaustion causes CD8 T cell  
624 dysfunction during chronic toxoplasmosis. *J. Exp. Med.* 213, 1799–1818

625 62 Zhu, L. *et al.* (2017) Blimp-1 impairs T cell function via upregulation of TIGIT and  
626 PD-1 in patients with acute myeloid leukemia. *Journal of Hematology & Oncology* 10,

627 63 Sekiya, T. *et al.* (2013) Nr4a receptors are essential for thymic regulatory T cell  
628 development and immune homeostasis. *Nat Immunol* 14, 230–237

629 64 Chen, J. *et al.* (2019) NR4A transcription factors limit CAR T cell function in solid  
630 tumours. *Nature* 567, 530–534

631 65 Liu, X. *et al.* (2019) Genome-wide analysis identifies NR4A1 as a key mediator of T  
632 cell dysfunction. *Nature* 567, 525–529

633 66 Doedens, A.L. *et al.* (2013) Hypoxia-inducible factors enhance the effector responses  
634 of CD8+ T cells to persistent antigen. *Nat Immunol* 14, 1173–1182

635 67 Koh, H.S. *et al.* (2015) The HIF-1/glial TIM-3 axis controls inflammation-associated  
636 brain damage under hypoxia. *Nature Communications* 6, 6340

637 68 Kossatz, E. *et al.* (2016) CB2 cannabinoid receptors modulate HIF-1 $\alpha$  and TIM-3  
638 expression in a hypoxia-ischemia mouse model. *European Neuropsychopharmacology* 26,  
639 1972–1988

640 69 Bartel, D.P. (2018) Metazoan MicroRNAs. *Cell* 173, 20–51

641 70 Mehta, A. and Baltimore, D. (2016) MicroRNAs as regulatory elements in immune  
642 system logic. *Nature Reviews Immunology* 16, 279–294

643 71 Dragomir, M. *et al.* (2018) Key questions about the checkpoint blockade-are  
644 microRNAs an answer? *Cancer Biology & Medicine* 15, 103

645 72 Zhang, Y. *et al.* (2017) miR-155 contributes to Df1-induced asthma by increasing the  
646 proliferative response of Th cells via CTLA-4 downregulation. *Cellular Immunology* 314,  
647 1–9

648 73 Liu, Y. *et al.* (2016) B and T Lymphocyte Attenuator is a Target of miR-155 during  
649 Naive CD4+ T Cell Activation. *Iran J Immunol* 13, 89–99

650 74 Dudda, J.C. *et al.* (2013) MicroRNA-155 Is Required for Effector CD8+ T Cell  
651 Responses to Virus Infection and Cancer. *Immunity* 38, 742–753

652 75 Huffaker, T.B. *et al.* (2017) Antitumor immunity is defective in T cell-specific  
653 microRNA-155-deficient mice and is rescued by immune checkpoint blockade. *Journal of*  
654 *Biological Chemistry* 292, 18530–18541

655 76 Li, Q. *et al.* (2016) miR-28 modulates exhaustive differentiation of T cells through  
656 silencing programmed cell death-1 and regulating cytokine secretion. *Oncotarget* 7,

657 77 Stelekati, E. *et al.* (2018) Long-Term Persistence of Exhausted CD8 T Cells in  
658 Chronic Infection Is Regulated by MicroRNA-155. *Cell Reports* 23, 2142–2156

659 78 Wei, J. *et al.* (2016) MiR-138 exerts anti-glioma efficacy by targeting immune  
660 checkpoints. *Neuro-Oncology* 18, 639–648

661 79 Yang, J. *et al.* (2017) MiR-15a/16 deficiency enhances anti-tumor immunity of  
662 glioma-infiltrating CD8+ T cells through targeting mTOR: MiR-15a/16 regulates glioma-  
663 infiltrating CD8+ T cells activation. *International Journal of Cancer* 141, 2082–2092

664 80 Zhang, G. *et al.* (2015) microRNA-4717 differentially interacts with its polymorphic  
665 target in the *PDI* 3' untranslated region: A mechanism for regulating PD-1 expression and  
666 function in HBV-associated liver diseases. *Oncotarget* 6,

667 81 Huang, F. *et al.* (2018) MicroRNA-374b inhibits liver cancer progression via down  
668 regulating programmed cell death-1 expression on cytokine-induced killer cells. *Oncol Lett*  
669 DOI: 10.3892/ol.2018.7951

670 82 Pandya, D. *et al.* (2015) Epstein-Barr Virus MicroRNA Expression Increases  
671 Aggressiveness of Solid Malignancies. *PLOS ONE* 10, e0136058

672 83 Zurawek, M. *et al.* (2018) miR-487a-3p upregulated in type 1 diabetes targets CTLA4  
673 and FOXO3. *Diabetes Research and Clinical Practice* 142, 146–153

674 84 Serr, I. *et al.* (2018) A miRNA181a/NFAT5 axis links impaired T cell tolerance  
675 induction with autoimmune type 1 diabetes. *Science Translational Medicine* 10, eaag1782

676 85 Cipolla, G.A. *et al.* (2016) A 3'UTR polymorphism marks differential KLRG1 mRNA  
677 levels through disruption of a miR-584-5p binding site and associates with pemphigus

678 foliaceous susceptibility. *Biochimica et Biophysica Acta (BBA) - Gene Regulatory*  
679 *Mechanisms* 1859, 1306–1313

680 86 Fooladinezhad, H. *et al.* (2016) Negative regulation of TIM-3 expression in AML cell  
681 line (HL-60) using miR-330-5p. *British Journal of Biomedical Science* 73, 129–133

682 87 Moghaddam, Y. *et al.* (2018) Evaluation of the effect of TIM-3 suppression by miR-  
683 498 and its effect on apoptosis and proliferation rate of HL-60 cell line. *Pathology -*  
684 *Research and Practice* 214, 1482–1488

685 88 Wang, J. *et al.* (2019) MEG3 modulates TIGIT expression and CD4 + T cell activation  
686 through absorbing miR-23a. *Molecular and Cellular Biochemistry* 454, 67–76

687 89 Franchini, D.-M. *et al.* (2019) Microtubule-Driven Stress Granule Dynamics Regulate  
688 Inhibitory Immune Checkpoint Expression in T Cells. *Cell Reports* 26, 94–107

689 90 Huang, Y.-H. *et al.* (2015) CEACAM1 regulates TIM-3-mediated tolerance and  
690 exhaustion. *Nature* 517, 386–390

691 91 Lau, K.S. *et al.* (2007) Complex N-Glycan Number and Degree of Branching  
692 Cooperate to Regulate Cell Proliferation and Differentiation. *Cell* 129, 123–134

693 92 Mkhikian, H. *et al.* (2011) Genetics and the environment converge to dysregulate N-  
694 glycosylation in multiple sclerosis. *Nature Communications* 2,

695 93 Cabral, J. *et al.* (2017) Distinctive Surface Glycosylation Patterns Associated With  
696 Mouse and Human CD4+ Regulatory T Cells and Their Suppressive Function. *Frontiers in*  
697 *Immunology* 8,

698 94 Okada, M. *et al.* (2017) Blockage of Core Fucosylation Reduces Cell-Surface  
699 Expression of PD-1 and Promotes Anti-tumor Immune Responses of T Cells. *Cell Reports*  
700 20, 1017–1028

701 95 Meng, X. *et al.* (2018) FBXO38 mediates PD-1 ubiquitination and regulates anti-  
702 tumour immunity of T cells. *Nature* 564, 130–135

703 96 Mohammad, H.P. *et al.* (2019) Targeting epigenetic modifications in cancer therapy:  
704 erasing the roadmap to cancer. *Nature Medicine* 25, 403–418

705 97 Bhagwat, A.S. and Vakoc, C.R. (2015) Targeting Transcription Factors in Cancer.  
706 *Trends in Cancer* 1, 53–65

707 98 Yu, T. *et al.* (2017) Development of Inhibitors Targeting Hypoxia-Inducible Factor 1  
708 and 2 for Cancer Therapy. *Yonsei Med J* 58, 489

709 99 Fujita, Y. *et al.* (2019) Ubiquitin Ligases in Cancer Immunotherapy – Balancing  
710 Antitumor and Autoimmunity. *Trends in Molecular Medicine* 25, 428–443

711 100 Filippova, N. *et al.* (2018) Blocking PD1/PDL1 Interactions Together with MLN4924  
712 Therapy is a Potential Strategy for Glioma Treatment. *J Cancer Sci Ther* 10,  
713 101 Li, J. *et al.* (2018) Unmasking Fucosylation: from Cell Adhesion to Immune System  
714 Regulation and Diseases. *Cell Chemical Biology* 25, 499–512  
715 102 Agrawal, P. *et al.* (2017) A Systems Biology Approach Identifies FUT8 as a Driver of  
716 Melanoma Metastasis. *Cancer Cell* 31, 804-819  
717 103 Hirahara, K. *et al.* (2012) Interleukin-27 Priming of T Cells Controls IL-17 Production  
718 In trans via Induction of the Ligand PD-L1. *Immunity* 36, 1017–1030  
719 104 Kataoka, K. *et al.* (2016) Aberrant PD-L1 expression through 3'-UTR disruption in  
720 multiple cancers. *Nature* 534, 402–406  
721

722 **Figure Legends**

723

724 **Figure 1. Overview of the Receptors and the Underlying Signaling Pathways Leading to**  
725 **IRs Expression.**

726 In T cells, IRs expression is induced by several receptors following their stimulation, and  
727 involves different signaling pathways. Kinases are illustrated in blue and transcription factors  
728 in yellow. In green are represented PLC $\gamma$  (phospholipase C  $\gamma$ ), DAG (diacylglycerol) and CaN  
729 (calcineurin) that do not belong to the kinases or transcription factors. The endoplasmic  
730 reticulum (ER) is represented in purple. The IRs regulated by a signaling pathway are indicated  
731 in green boxes at the bottom of each corresponding pathway.

732

733 **Figure 2. Epigenetic and Transcriptional Regulation of IRs.**

734 Upper panel: accessible chromatin that possesses unmethylated cytosines and permissive  
735 histone marks such as H3K4me1, H3K9ac and H3K27ac. Transcription factors can then bind  
736 to the regulatory regions (i.e. promoter) to allow transcription. Main transcription factors  
737 involved in the control of multiple IRs are represented. Lower panel: within closed chromatin,  
738 constituted of methylated cytosines and repressive histone marks such as H3K9me3 and  
739 H3K27me3, transcription factors binding to the regulatory regions is precluded, thereby  
740 preventing transcription of IRs.

741

742 **Figure 3. Post-Transcriptional and Post-Translational Regulations of IRs.**

743 The mRNAs-encoding IRs that exit from the nucleus undergo post-translational regulatory  
744 controls. These regulations operate through miR and stress granules targeting, and control  
745 translation of multiple IRs. For miR regulation, mRNAs are depicted as “naked” for  
746 simplification. Once translated, a further control step occurs through post-translational



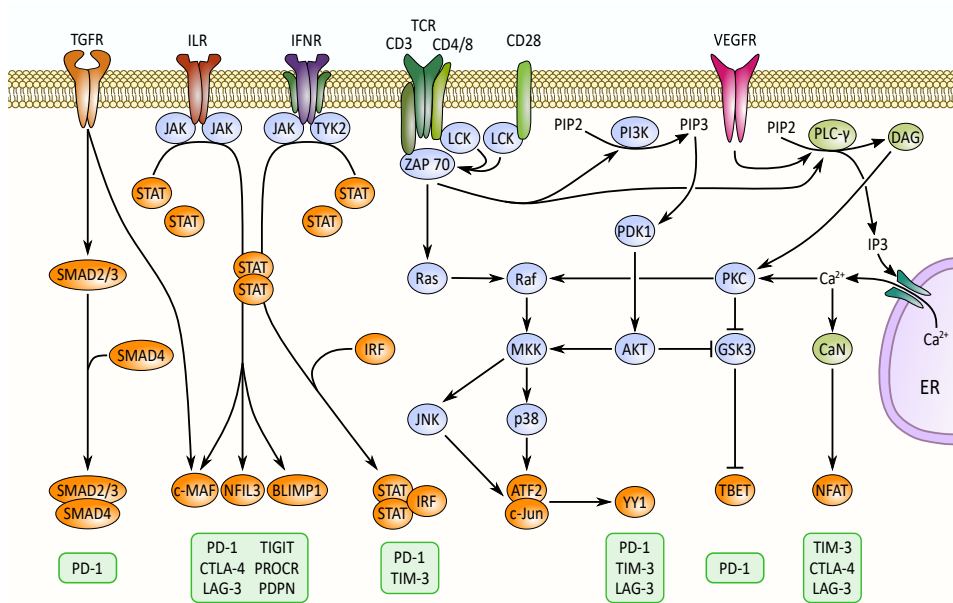
747 modifications such as fucosylation and ubiquitination (Ub), which finalize the regulation of  
748 PD-1 cell surface expression.

749

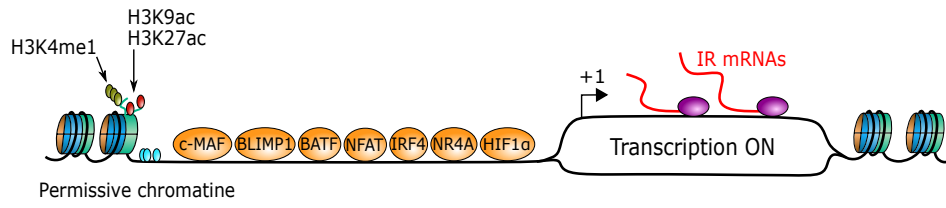
750 **Figure I. Targeting regulatory mechanisms of IRs expression along with ICB to potentiate**  
751 **antitumor immunity.**

752 The biological processes leading to IR expression and eventually to T cell functions inhibition  
753 are indicated in the blue boxes. Various small molecule inhibitors (written in red) can modulate  
754 IR expression at the chromatin, RNA or protein levels to limit IRs cell surface expression. In  
755 contrast, ICB (in green) target the function of IRs expressed at the cell surface. Combination of  
756 both types of targeting can synergize to enhance antitumor immunity. Targeting agents are not  
757 exhaustively listed, and new therapeutic agents could be developed at all targeting levels. The  
758 graphic at the right-hand side represents an immune reaction of T cells before (blue) and after  
759 (red) treatment with targeting agents, against a tumor cell. Expression of IR at the T cell surface  
760 (blue cell) and interaction with its corresponding IR-ligand, expressed at the tumor cell surface,  
761 lead to T cell functions inhibition and dampen antitumor immunity. Treatment with small  
762 molecule inhibitors and ICB reduces IR expression and function, respectively, on T cell (in red)  
763 to eventually improve antitumor immunity. DNMTi, DNA methyl transferase inhibitor;  
764 HDACi, histone deacetylase inhibitor; MTD, microtubule-targeting drugs.

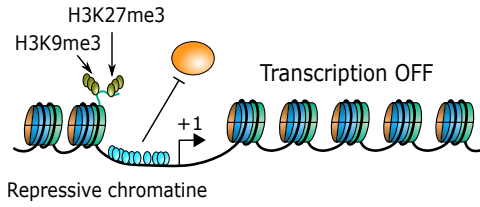
765








Expression of Inhibitory Immune Checkpoint Receptors



Repression of Inhibitory Immune Checkpoint Receptors



-  Transcription factor
-  RNA polymerase II
-  Acetylation
-  Methylation
-  Methylcytosine

