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1 ICOS-L as a Potential Therapeutic Target for Cancer

2 Immunotherapy

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15 Graphical Abstract

16 Abstract

17 *Background:* The co-stimulatory B7 family members are cell-surface protein ligands, binding to receptors on lymphocytes
18 to regulate immune responses. One of them is the inducible co-stimulatory molecule ligand (ICOS-L). This is expressed
19 on professional antigen-presenting cells (APCs), including B cells, macrophages, and dendritic cells (DCs), but it can also
20 be expressed by endothelial cells, lung epithelium and in tumour microenvironment cells. ICOS-L is important for
21 memory and effector T cells during the specific humoral immune responses, but its role in cancer is not yet understood.

22 *Objective:* To discuss the role of ICOS/ICOS-L in cancer, given importance of identifying selective targets for cancer
23 treatment and knowing the mechanism of immune evasion by tumour

24 *Main findings:* ICOS/ICOS-L signal has opposite effects on the T-cell response. ICOS-L is activated in several types of
25 cancers to maintain immunosuppressive CD4⁺ T cell subsets, such as regulatory T cells (Rs). ICOS-L over-expression is
26 associated with tumour progression and poor overall survival. In colon cancer, activation of this co-stimulatory signal is
27 associated with improved survival suggesting a dualistic effect of the ICOS/ICOS-L signal pathway. Interestingly,
28 following anti-cancer vaccine or anti-CTLA-4 treatment, ICOS⁺ T cells increased significantly in both the CD4⁺ and
29 CD8⁺ population and the ratio Teff/Treg increased in tumour microenvironment. Thus suggesting a potential role of
30 ICOS/ICOS-L in improving effectiveness of cancer therapy.

31
32 *Conclusion:* ICOS/ICOS-L signal pathway has the potential to improve cancer treatment. However, future studies in other
33 models are needed to understand whether inhibition of ICOS expression or the blockage of its co-stimulation could be a
34 potential therapeutic target or adjuvant treatment for immunotherapy.

35
36 **Keywords:** ICOS-L, ICOS, CD275, CD278, B7, Tregs, cancer

37

38 **1.1 ICOS-L, a member of co-stimulatory B7 family.**

39 The co-stimulatory B7 family members are cell-surface protein ligands, binding to receptors expressed on lymphocytes
40 and involved in regulating immune responses. This system not only provides positive signals to stimulate T-cell
41 activation, but also it delivers negative signals to inhibit T-cell responses. The inducible co-stimulatory molecule ligand
42 (ICOS-L), also known as B7RP-1, B7-H2, LICOS, GL50, B7h and CD275, is a member of B7 family. It is expressed on
43 professional antigen-presenting cells (APCs), including B cells, macrophages and dendritic cells (DCs), but also in certain
44 endothelial cells and lung epithelium [1-3]. Despite limited evidence, ICOS-L is known to act as a co-stimulatory signal
45 for T-cell proliferation and cytokines secretion and to induce B-cell proliferation and differentiation into plasma cells.
46 ICOS-L could also play an important role in mediating local tissue responses to inflammatory conditions and in
47 modulating the secondary immune response by co-stimulating memory T-cell function [2, 3].

48 **1.1.1. ICOS-L: gene, transcripts and proteins.**

49 The gene encoding the inducible co-stimulator ligand (ICOS-L), is located in the 21q22.3, is 24,477 bases in
50 length and contains 10 exons [4]. At transcriptional level, four transcript variants (Var) have been cloned. Var_a (the
51 canonical form) represents the longest transcript (3320 nts) and encodes the isoform *a*, Var_b (1188 nt) uses an alternative
52 3' terminal exon and it thus differs in the 3' coding region and 3' UTR, compared to Var_a. Var_c (2969 nt) lacks an alternate
53 in-frame exon in the 5' coding region, compared to Var_a, resulting in an isoform *c* that is shorter than isoform *a*. Var_d
54 (3168 nt) uses an alternate splice site in the 5' region, resulting in translation initiation at a downstream in-frame start
55 codon, compared to Var_a. The encoded isoform *d* is shorter at the N-terminus, compared to isoform *a* (Fig.1,
56 Supplementary 1). Isoform *a* is widely expressed (brain, heart, kidney, liver, lung, pancreas, placenta, skeletal muscle,
57 bone marrow, colon, ovary, prostate, testis, lymph nodes, leukocytes, spleen, thymus and tonsil), while isoform *b* is
58 detected only in lymph nodes, leukocytes, spleen and it is expressed on activated monocytes and dendritic cells [5]. Tissue
59 and organ expression of Var_c and Var_d are yet to be studied.

60 **1.2 Immunological role of ICOS-L**

61 ICOS-L specifically binds the T-cell inducible co-stimulatory molecule (ICOS), also named CD278 [3], which
62 is expressed at high levels in germinal center T cells or TFH (follicular helper) cells. Activation of ICOS receptor induces
63 recruitment of class IA phosphatidylinositol 3-kinase (PI3K), a signalling molecule that leads to the production of
64 membrane-bound phosphatidylinositol 3,4,5-trisphosphate (PIP3). This culminates in the activation of Akt, a kinase that
65 promotes cellular proliferation and survival. ICOS activation also recruits the p50 α and p85 α regulatory subunits and
66 p110 δ catalytic subunit of PI3K. *In vivo* and *in vitro* experiments indicate that ICOS/ICOS-L pathway activation
67 contributes to the production of cytokines, such as IL-5, IL-4, IL-10 and IL-13. ICOS/ICOS-L co-stimulatory signal is
68 important for memory and T helper cell effector functions. This is particularly important for T helper-2 differentiation
69 and antibodies response during the specific humoral immune responses against pathogens such as bacteria, parasites, and
70 viruses [1,2]. Moreover, ICOS-L seem to alternatively promote or repress T helper-1 responses under different infection
71 conditions and these divergent phenotypes may in part be explained by ICOS-dependent regulatory T cells (Tregs)
72 induction (**Fig. 2**). Thus, the ability of ICOS-L to influence Tregs induction points to a complex role in regulating CD4⁺
73 T cell differentiation, and suggests that ICOS-L signalling may be mutually important in preventing immune-mediated
74 pathology, as well as inducing pro-inflammatory CD4⁺ T cells.

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78 **1.3 ICOS-L in tumour infiltrate lymphocytes**

79 Malignant cells may adopt several mechanisms to interfere with the effector immune response or with regulatory
80 cells in tumour microenvironment, in order to escape from immune surveillance. Regulatory T cells (Tregs) constitute
81 5% to 10% of all peripheral CD4⁺ T cells and play an important role in maintaining tolerance to both auto-immune and
82 cancer cells. Starting from the identification of Forkhead box protein 3 (Foxp3) as a critical transcriptional factor for
83 Tregs, Foxp3⁺CD4⁺ T cells have been regarded as Tregs with immunosuppressive functions and divided in three
84 subpopulations: effector Tregs (eTregs), naive Tregs, and non-Tregs [6]. Tregs, like other T cells, respond to TCR
85 stimulation. They also express co-stimulatory receptors, such as CTLA-4, PD-1, ICOS and CD28 that further promote
86 their activation, proliferation and survival(ref). A large body of evidence confirms that activation of ICOS/ICOS-L
87 pathway is involved in maintenance of this subtype of T cells in tumour microenvironment, which usually is associated
88 with a poor prognosis of patient [6].

89

90 **1.3.1. ICOS-L in cancer**

91 ICOS/ICOS-L pathway was reportedly activated in melanoma [7], myeloma [8,9], breast [10], ovarian [11],
92 gastric [6], liver [12] and colorectal cancers [13]. Furthermore, ICOS-L was also found in classical Hodgking lymphoma
93 [14], B lymphoma [2], leukemia [15], glioblastoma [16] and rhabdomyosarcoma [17], but its role in these cancers is yet
94 to be completely elucidated. In tumour microenvironment, Treg cells contribute to tumour growth by inhibiting tumour-
95 specific immunity through not yet characterized mechanisms. It is hypothesised that this mechanism may involve FAS-
96 L, CD39/Adenosine, perforins, CTLA-4, or PD-1 and the generation of high levels of IL-10, which mediate a suppressive
97 capability, especially against dendritic cell functions. Finally, it was demonstrated that ICOS/ICOS-L pathway could
98 modulate the efficacy of therapy in patients with prostate cancer [18], pancreatic cancer [19], bladder cancer [20], multiple
99 myeloma [21] and melanoma [22,23].

100

101 **1.3.2 ICOS-L in melanoma**

102 Melanoma tumour biopsies analysis showed an estimated 25% of the CD4⁺ T cells found were Foxp3⁺, more
103 than 3 times the abundance found in the peripheral blood of the same patients (7%).This indicates that ICOS-L expressed
104 by melanoma cells could co-stimulate Tregs to induce high levels of Foxp3, CD25, ICOS and also production of IL-10.
105 This evidence suggest that tumour cells may act themselves as direct APC, since they can express HLA class II and
106 provide self-antigen presentation and co-stimulation through ICOS-L, allowing a tumour self-tolerance [7].

107

108 **1.3.3. ICOS-L in haematological tumors**

109 In several haematological malignancies, ICOS-L was expressed in cancer cells. Malignant Hodgkin Reed
110 Sternberg cells derived from a germinal center B cells over-expressed different surface molecules, including CD70,
111 CD80/CD86, CD30, CD40, OX40-L/CD252, and ICOS-L. Their respective receptors were often present on typical
112 Hocking lymphoma microenvironment immune cells and was considered to be up-regulated on activated T Helper cells
113 (ref). Evidence suggests that malignant tumours were capable of sequestering a substantial number of activated functional
114 T cells, contributing to a profound systemic immune deficit in advanced disease [14]. Additionally, it was shown that
115 leukemic cells, obtained from patients with acute myeloid leukemia, express ICOS-L and this it was associated with a
116 poor prognosis(ref). Thus suggesting that expression of ICOS-L in leukemic cells might contribute to their proliferation
117 by helping them to evade antitumor immune responses [15]. Although ICOS-L expression has been evaluated on B
118 lymphoma tissues, its biological functions in regulating anticancer immune response is not completely understood [2].

119 Immunohistochemical staining showed that ICOS-L was expressed in thyroid B-cell lymphoma but not in thyroid
120 adenoma tissue or healthy thyroid tissue, suggesting that ICOS-L molecules may be involved in the development of
121 malignant B lymphoma [2]. In an *in vitro* model of myeloma, tumour cells express ICOS-L [8,9] which directly induces
122 not only Tregs expansion but also Treg cells generation in a contact-dependent manner and in absence of APC cells. The
123 induction of Treg cells mediated by cell-to-cell contact with the ICOS/ICOS-L system supports the hypothesis that this
124 pathway may play a central role in the immune suppression response [8].

125

126 **1.3.4 ICOS-L in ovarian and breast cancer**

127 While previously described evidence indicated ICOS-L expression in tumour cells, recent investigations in
128 ovarian cancer and breast cancer are in contrast with this theory. In ovarian and breast cancer, ICOS-L was not expressed
129 in cancer cells indicating that Tregs expansion was activated by other cells expressing ICOS [10,11]. In ovarian cancer
130 many Foxp3⁺ Treg cells were found to infiltrate the tumour microenvironment, a phenomenon that strongly predicts
131 disease progression. These Tregs constitutively expressed ICOS and were frequently detected in the tumour of patients
132 with epithelial ovarian cancer. Survival, proliferation and functions of ICOS⁺Foxp3⁺Treg cells was strictly dependent to
133 the presence of tumour-infiltrating plasmacytoid dendritic cells (pDCs) [11]. Human DCs consist of two subsets, myeloid
134 dendritic cells (mDCs) and plasmacytoid dendritic cells (pDCs). The first derive from TLR2-6, 8 and induce T helper 1
135 effector cells, while pDCs mature from TLR7 and 9 and have been reported to exist in most human solid tumours. pDCs
136 are associated with induction and maintenance of immunosuppressive conditions by the secretion of indoleamine 2,3-
137 dioxygenase, IL-3, expression of ICOS-L, CD40-L and through Treg cells activation. There is substantial evidence
138 suggesting that pDCs have a specialized role in the induction of peripheral tolerance by inducing IL-10-production
139 through Tregs-ICOS-L/ICOS signalling [6,24]. In summary, in epithelial ovarian cancer the ICOS/ICOS-L pathway
140 activation involved a tumor-infiltrating pDCs that express high levels of ICOS-L, while cancer cells did not express
141 significant levels of this protein. This evidence supports the hypothesis that tumor-infiltrating pDCs were directly
142 involved in creating an immunosuppressive tumour microenvironment through the expansion of the ICOS⁺Foxp3⁺ Treg
143 cell subset [11].

144 Likewise, infiltration of pDS indirectly induced by ICOS was associated with poor prognosis of breast cancer
145 patients [10]. While ICOS-L was not expressed in neither primary breast tumour cells or in breast tumour cell lines, ICOS
146 engagement was described as a major contributing pathway to ICOS⁺Tregs local accumulation. Tumour-associated
147 ICOS⁺Tregs directly interacted with pDCs *in situ*, as confirmed by immunohistochemistry on primary breast tumour
148 frozen sections. Upstream of this cascade, CCL22 was produced by tumor cells to recruit CCR4⁺Treg cells from the
149 bloodstream to breast tumour microenvironment. This resulted in CCR4⁺Treg cells local expansion through ICOS/ICOS-
150 L interaction with pDC in breast tumour environment [10].

151

152 **1.3.5. ICOS-L in gastric and liver cancers**

153 In gastric cancer, a high number of ICOS⁺ cells were found in gastric tissue of late-stage gastric cancer patients.
154 In this patients, the expression level of ICOS-L was high in pDCs while high ICOS expression in Foxp3⁺ infiltrating T
155 lymphocyte was associated with a poor prognosis. Immunohistochemistry results showed a coexistence of ICOS⁺Foxp3⁺
156 cells and pDCs in gastric cancer tissues suggesting some functional relationship between these cells, as seen in other
157 tumour types [6]. Additionally, this ICOS/ICOS-L pathway was not only involved in regulating the Treg cell subset but
158 also other types of inhibitory T cells potentially involved in local immunosuppression. Among these, T regulatory type
159 1 cells (Treg1), initially considered important in promoting and maintaining tolerance in autoimmunity, allergy and

160 transplantation, also demonstrated to be involved in promoting tumour escape from immune surveillance [12]. In patients
161 with primary and secondary liver cancer, tumour infiltrating Treg1 cells contributed to local immune suppression via
162 ICOS/ICOS-L signalling and ICOS-L⁺pDCs stimulated IL-10 production in the tumour microenvironment [12].

163

164 **1.3.6. ICOS-L in others solid cancers.**

165 ICOS-L was found expressed in human glioma cells, both *in vitro* and *in vivo* but not in normal central nervous
166 system tissues adjacent to the neoplastic cells. Whether ICOS-L plays a role in supporting these malignancy is yet to be
167 investigated [16]. In rhabdomyosarcoma (RMS), the most common paediatric soft tissue malignancy, ICOS-L **was**
168 expressed in FLOH1, RH41, RD6, and TE671 RMS cell lines and its expression levels marginally increased in presence
169 of TNF α [17]. Even if the involvement of ICOS/ICOS-L pathway supported cancer progression and worsen prognosis in
170 several cancer models, in colorectal cancer the expression of ICOS was associated with a good prognosis [13]. Although
171 limited data is available on ICOS-L expression in colorectal cancer, ICOS is known to be mainly expressed on CD4⁺T
172 cells in patients' tumour tissues or peripheral blood cells [13]. Compared to ICOS⁻T cells, ICOS⁺T cells produce more
173 INF γ and less IL-4 and show up-regulated expression of transcriptional factor of Th1 cells (T-bet). This suggests that
174 ICOS promotes Th1 effector response in patients with colon cancer. It is known that Th1 cells inhibit tumour cell invasion
175 and metastasis by communicating with tumour-associated myeloid cells (i.e. tumour-associated macrophages and
176 myeloid-derived suppressor cells) contributing to an improvement of survival. Studies showed a significant correlation
177 between high ICOS expression and good prognosis, in relation to tumour size, Carcinoembryonic antigen (CEA) levels,
178 tumor staging, lymphatic metastasis and distant metastasis (TNM classifications). Furthermore, ICOS expression was
179 correlated with the expression of checkpoint inhibitors CTLA-4 and PD-1 on T cells, indicating that ICOS could also be
180 a useful marker for a selection of anti-CTLA-4 or PD-1 therapy in colorectal cancer [13].

181

182 **1.4 ICOS/ICOS-L pathway in cancer immunotherapy**

183 The opposite effects of ICOS/ICOS-L pathway on the T-cell response may explain the lack of antitumor efficacy
184 of ICOS-L blockade in mono-therapy [25]. Instead, studies blocking the ICOS/ICOS-L pathway in combination with
185 other therapy showed different results. For example, therapy with GM-CSF-modified cancer cell vaccine combined with
186 anti-ICOS monoclonal antibody induced more powerful anti-tumour immunity [18]. Indeed, Mo *et al.* demonstrated in
187 murine models of prostate cancer how tumour-infiltrated T lymphocyte increased after treatment with GM-CSF-modified
188 cancer cell vaccine and how combination therapy induced higher infiltration compared with vaccine alone. Furthermore,
189 ICOS⁺Foxp3⁺T cells infiltration into tumour tissues were higher after vaccine therapy, while the proportion of these cells
190 decrease in combination treatment compared with control condition. Although the vaccine induced an efficient antitumor
191 immunity inhibiting tumour growth, it also induced an increase of Tregs tumour infiltration that could challenge the
192 effectiveness of this therapy. In this case, combination with ICOS blocking could deplete infiltrated Tregs with the
193 possibility to enhance the vaccine-induced immunity [18].

194

195 Further supporting this evidence, treatment of pancreatic cancer targeting mesothelin (MSLN), a potential immune-
196 therapeutic target, showed similar results. MSLN-virus-like particles (mMSLN-VLPs) immunization was able to break
197 tolerance to intrinsic MSLN, resulting in reduced frequency of CD4⁺Foxp3⁺ICOS⁻Treg cells and stimulation of cytotoxic
198 CD8⁺ T cells antitumor activity. However, because mMSLN-VLP induces IL-6 production, increasing ICOS-L expression
199 on pDC-like cells and proliferation of immunosuppressive CD4⁺Foxp3⁺ICOS⁺Treg cells, combination therapies with
200 ICOS blocking remain necessary [19]. In an *in vitro* model of multiple myeloma, lenalidomide pre-treatment of MM cell

201 lines reduced Treg generation and the Treg/TEff ratio, probably due to a reduced ICOS-L gene transcription. Combined
202 treatment with lenalidomide and dexamethasone significantly reduced both Treg induction and the Treg/TEff cell ratio
203 [21]. Additionally, depletion of Tregs in conjunction with ICOS agonist could remove the potential immunosuppressive
204 effects of ICOS signalling and allow ICOS agonist to act solely in promoting activity of CD4⁺ Teff. In fact, ICOS is
205 highly expressed in tumor Tregs, and thus a single ICOS agonist with strong Fc engagement may be sufficient to induce
206 a simultaneous ADCC-mediated depletion of Tregs and agonist-based enhancement of anti-tumour responses [25].
207

208 Nevertheless, blocking of ICOS/ICOS-L system not always demonstrated to be good strategy. Results in *in vivo* models
209 of prostate, melanoma [22] and bladder cancer [20], showed anti-CTLA-4 therapy led to an increase in the frequency
210 of CD4⁺ICOS⁺ and CD8⁺ICOS⁺ T cells producing IL-2 and IFN- γ . Here, ICOS seemed to play an important role in the
211 activation/development of functional antitumor CD8 T cells [23]. In mouse syngeneic tumours, ICOS was found highly
212 expressed by Tregs, but it was also found up-regulated across CD8⁺ and CD4⁺ effector populations, suggesting that ICOS
213 expression was associated with opposite functions. According to this evidence, ICOS/ICOS-L pathway could be another
214 therapeutic target and it may have implications in the development of novel combined cancer immunotherapy strategies
215 [22,23]. Indeed, in murine models of prostate cancer, CTLA-4 blockade enhanced activation of tumour-reactive T cells
216 with concomitant up-regulation of ICOS. In this context, IVAX (vaccine of ICOSL-positive tumour cells) triggered the
217 ICOS pathway, leading to a higher density of Teff cells inside the tumour, as indicated by an increase in the Teff/Treg
218 cell ratio [22]. Likewise, after anti-CTLA-4 treatment ICOS⁺ T cells increased significantly in both the CD4⁺ and CD8⁺
219 populations. In this case, anti-CTLA-4 therapy increased a population of Foxp3⁻ cells and then stimulated an expansion
220 of ICOS⁺ T effector cells over Treg cells. This results suggest that ICOS⁺ T cells may represent a population of T effector
221 cells that play an important role in antitumor immune responses induced by anti-CTLA-4 therapy. Further, ICOS⁺ T cells
222 produced Th1 cytokine IFN- γ and cytokine IL-2 necessary for effective antitumor responses, suggesting that ICOS⁺ T
223 cells might play a functional role in improving the effectiveness of combinatorial immunotherapy. In a recent clinical
224 trial, treatment of patients with bladder cancer were with a blocking anti-CTLA-4 mAb resulted in increased percentage
225 of CD4⁺T cells in peripheral blood and tumour tissues, thus potential higher levels of ICOS. Upon re-stimulation,
226 ICOS⁺CD4⁺ T cells produced greater levels of IFN- γ compared to ICOS⁻CD4⁺ T cells, suggesting that T cells with higher
227 levels of ICOS, have elevated effector functions in antitumor activity, as seen in melanoma models [20].
228

229 2. CONCLUSION

230 The pathway ICOS/ICOS-L is involved in several processes in cancer, ranging from support of tumour growth but, at the
231 same time, improvement of immune-stimulating therapy efficacy. Data suggest that inhibition of ICOS/ICOS-L system
232 alone may not particularly effective in treatment of cancer because this pathway showed opposing effects in regulating
233 T-cell response. The importance of ICOS/ICOS-L blockade was attributable to its role as adjuvant in combined cancer
234 immunotherapy strategies. ICOS expression was positively correlated with the expression of other important immune
235 checkpoints (CTLA-4 and PD-1 on T cells) in colon cancer patients, with antibodies anti-CTLA-4 and anti PD-1 currently
236 being used for the treatment of several types of cancer. This suggest that ICOS could be a marker in therapy selection,
237 with effectiveness depending on CTLA-4 or PD-1 expression correlated with ICOS expression. While increasing of
238 Tregs in tumour microenvironment could reduce vaccine efficacy, a combination with anti-ICOS antibody might be a
239 more effective therapy. This could increase immune response, as it would reduce the population of tumour infiltrated
240 Tregs. In summary, ICOS/ICOS-L pathway could be considered as a novel immune target and its role should be
241 investigated in other cancers, taking into account that this pathway shows a dualistic behaviour.

242

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245 A.C and S.G. contributed to revision.

246

247 **Legends**

248

249 **Figure 1.** Alignment analysis of ICOS-L transcripts. NM_015259.5 (Vara, 3320 nt), NM_001283050.1 (Varb, 1188 nt),
250 NM_001283051.1 (Varc, 2969 nt), NM_001283052.1 (Vard, 3168 nt). The analysis was performed by Clustal Omega
251 software. * (Asterisk) indicates positions that have a single, fully conserved residue.

252

253 **Figure 2.** Sequence alignment analysis of ICOS-L protein variants. Vara (NP_056074.1, 302 aa), Varb
254 (NP_001269979.1, 309 aa), Varc (NP_001269980.1, 185 aa), Vard (NP_001269981.1, 217 aa). * (asterisk) indicates
255 positions which have a single, fully conserved residue. : (colon) indicates conservation between groups of strongly similar
256 properties..(period) indicates conservation between groups of weakly similar properties. In Bold: trans-membrane
257 domain, Clear: cytoplasmatic domain, Underline and clear: extracellular domain. Alignment analysis was performed by
258 Clustar Omega software.

259

260 **Figure 3. ICOS-ICOS-L pathways.** ICOS-L is a member of B7 family expressed on professional antigen-presenting
261 cells (APCs) and it binds ICOS receptor expressed on T cells. Activation of ICOS receptor induces the recruitment PI3K,
262 a signalling molecule that leads to the activation of Akt, a kinase that promote cellular proliferation and survival.
263 ICOS/ICOS-L pathway activation contributes to the production of cytokines, such as IL-5, IL-4, IL-13 and IL-10. It is
264 suggested that ICOS/ICOS-L co-stimulatory signal is very important for memory and T helper cell effector functions but,
265 ICOS is seemingly capable of alternatively promoting or repressing T helper-1 responses by ICOS-dependent regulatory
266 T cells (Tregs) induction.

267

268 Tab. 1 Differential role of ICOS-L/ICOS activation in different tumours. ICOS/ICOS-L pathway is activated in different
269 types of cancer and it plays a central role in immune suppression. ICOS-L is present in tumour microenvironment because
270 it is expressed by cancer cells or tumour-infiltrating plasmacytoid dendritic cells (pDCs).

271

272

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