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## The twin cytokines interleukin-34 and CSF-1: masterful conductors of macrophage homeostasis

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1                                   **The twin cytokines Interleukin-34 and M-CSF:**  
2                                   **masterful conductors of macrophage homeostasis**

3

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14

15          **Short title for the running head:** IL-34 and M-CSF in the biology of macrophages

16

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28 **Abstract**

29 Macrophages are specialised cells that control tissue homeostasis. They include non-resident  
30 and tissue-resident macrophage populations which are characterised by the expression of  
31 particular cell surface markers and the secretion of molecules with a wide range of biological  
32 functions. The differentiation and polarisation of macrophages relies on specific growth  
33 factors and their receptors. The macrophage-colony stimulating factor (M-CSF) and  
34 interleukine-34 (IL-34), also known as “twin” cytokines, are part of this regulatory landscape.  
35 M-CSF and IL-34 share a common receptor, the macrophage-colony stimulating factor  
36 receptor (CD115), which is activated in a similar way by both factors and turns on identical  
37 signalling pathways. However, there is some discrete differential activation leading to specific  
38 activities. In this review, we discuss recent advances in understanding the role of the twin  
39 cytokines in macrophage differentiation, from their interaction with CD115 and the activation  
40 of signalling pathways, to their implication in macrophage polarisation of non-resident and  
41 tissue-resident macrophages, with special focus on IL-34 and its involvement in healthy and  
42 pathogenic contexts.

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## 63 **Introduction**

64

65 In 1883, Eli Metchnikoff discovered a crucial biological process involved in cellular and  
66 tissue homeostasis: phagocytosis. This term describes the ability of some cells to engulf a  
67 variety of particles, from viruses to bacteria, fungi, dead cells and other solid materials  
68 (Jaumouillé & Grinstein, 2016). Specialised phagocytosis cells include granulocytes, dendritic  
69 cells and macrophages, and they are part of the innate immune system (Biron, 2016).  
70 Macrophages play a central role in maintaining general tissue homeostasis and are also active  
71 actors during inflammation, auto-immunity, infection, and cancer (Vannella & Wynn, 2017;  
72 Wynn, et al., 2013).

73

74 Following the initial classification established by van Furth and Cohn in 1968, macrophages  
75 were considered to be part of the mononuclear phagocyte system, originating from  
76 haematopoietic stem cells located in the bone marrow (van Furth & Cohn, 1968). Although  
77 this classification is still used, studies working on specific tissue macrophages in mice over  
78 the last few years have suggested an ontogeny dichotomy in macrophages (Davies & Taylor,  
79 2015; Perdiguero & Geissmann, 2016; Franken, et al., 2016). According to these studies, one  
80 pool of macrophages originates in the haematopoietic stem cell lineage in the bone marrow  
81 (Figure 1). These macrophages, known as “non-resident” macrophages, are some of the  
82 circulating monocytes that can extravasate from blood to tissues and enrich the local  
83 population of macrophages. The other pool of macrophages, known as “tissue-resident”  
84 macrophages originate from the yolk sac and foetal liver during embryonic development  
85 (Figure 1) (Hoeffel & Ginhoux, 2018; Stremmel, et al., 2018). Tissue-resident macrophages  
86 include specialised macrophages such as the microglia in the neural system, Kupffer cells in  
87 the liver, or Langerhans cells in the skin. They are responsible for the homeostasis,  
88 development and maintenance of each specific tissue (Okabe, 2018). In adult tissues, the local  
89 population of macrophages is maintained by autonomous proliferation and can be reinforced  
90 by macrophages migrating from the bone marrow (Hashimoto, et al., 2013). Independently of  
91 their origin, the plasticity of macrophages allows them to express conventional surface  
92 markers as well as tissue specific markers, adding an additional layer to the complexity of  
93 classifying them. As a result, their classification varies from one author to another (Hoeffel &  
94 Ginhoux, 2018; Shapouri-Moghaddam, et al., 2018; Hume, et al., 2019).

95



96 Regardless of their origin, the proliferation and differentiation of monocytes/macrophages  
97 rely on the interaction of specific growth factors such as Macrophage Colony Stimulating  
98 Factor (M-CSF, CSF1), Granulocyte Colony Stimulating Factor (G-CSF), Granulocyte-  
99 Macrophage Colony Stimulating Factor (GM-CSF), Interleukin(IL)-6, IL-34 and their  
100 particular receptors. The absence of a ligand or receptor either compromises the proliferation  
101 of macrophage populations or impacts their differentiation (Dai, et al., 2002; Sakagami, et al.,  
102 2009; Wang, et al., 2012; Meshkibaf, et al., 2014). In the present review, we will focus on the  
103 role of the IL-34 cytokine which has strongly modified our vision of macrophage biology in  
104 the last decade. The contribution of IL-34 to the proliferation, differentiation and polarisation  
105 of “non-resident” and “tissue-resident” macrophages in healthy conditions and during  
106 pathologic situations will be described and discussed, together with their therapeutic value.

107

## 108 **1. Macrophage differentiation and growth factors**

109 Circulating monocytes originate in CFU-M precursors and can extravasate from blood to  
110 tissues to become the mononuclear phagocyte lineage. Their terminal differentiation into  
111 macrophages is regulated by specific growth factors such as M-CSF and IL-34 (Stanley, et al.,  
112 1978; Lin, et al., 2008). However, other growth factors, such as GM-CSF and IL-6, can also  
113 modulate macrophage differentiation. For instance, IL-6 is able to induce osteoclasts to shift  
114 into macrophages via differential phosphorylation of the signal transducer and activator of  
115 transcription-3 (STAT3) (Duplomb, et al., 2008). In the case of GM-CSF, Alothaimen *et al.*  
116 showed more recently that GM-CSF specifically promoted differentiation of macrophage  
117 subpopulations characterised by high induction of the antigen-specific CD8<sup>+</sup> T cell type  
118 during lymphocytic choriomeningitis virus infections (Alothaimen, et al., "in press").

119

### 120 **1.1 M-CSF/ CD115/ IL-34: more than a “ménage à trois”**

121 For decades, M-CSF was considered to be the main driver in the differentiation of myeloid  
122 precursors toward monocytic lineage and into macrophages. Macrophage differentiation  
123 requires the interaction between M-CSF and its unique receptor, the M-CSF receptor (also  
124 known as CSF-1R, c-fms or CD115). This interaction triggers a cascade of signalling  
125 pathways that promote macrophage differentiation, proliferation, survival and proper  
126 functioning (Guilbert & Stanley, 1980; Yeung, et al., 1987; Stanley & Chitu, 2014).  
127 Nevertheless, in 2002, studies using knock-out mice for CD115 (Csf1<sup>-/-</sup>) demonstrated that  
128 CD115-KO mice exhibited an osteopetrotic phenotype and a more severe depletion of the  
129 macrophage pool than M-CSF deficient mice (Csf1<sup>op/op</sup>). Interestingly, Csf1<sup>op/op</sup> mice showed

130 a slight alteration in microglia and Langerhans cells (Dai, et al., 2002). Overall, these  
131 observations suggested the existence of additional mechanisms capable of compensating for  
132 the absence of M-CSF. In 2008, a screening study analyzing the interactions between secreted  
133 proteins and receptors in cell-cell signalling models identified a new cytokine that promoted  
134 monocyte survival in a CD115 dependent manner (Lin, et al., 2008). This protein was named  
135 IL-34 and became the twin cytokine to M-CSF, sharing a common receptor. Later on, a series  
136 of publications described the essential role of the IL-34/CD115 interaction in the development  
137 and maintenance of osteoclast precursors, microglia and Langerhans cells (Wang, et al., 2012;  
138 Greter, et al., 2012; Nakamichi, et al., 2012). CD115 is the only haematopoietic receptor with  
139 two different ligands (M-CSF and IL-34).

140

141 The interactions between CD115 and its two ligands, M-CSF and IL-34, has been described in  
142 (Stanley & Chitu, 2014). Both ligands are present in a homodimeric form that also activates  
143 and promotes the dimerisation of CD115 (Figure 2A). Attending the species specificity of M-  
144 CSF/CD115 and IL-34/CD115, Gow *et al.* demonstrated that M-CSF from pigs shared the  
145 same activity as human M-CSF. Porcine M-CSF was able to activate mouse, cat, dog and  
146 human CD115. However, no cross-specificity was observed between human and mouse M-  
147 CFS/CD115. In the case of IL-34, human and mouse IL-34 activated porcine CD115, but both  
148 cytokines presented partial cross-reactivity (Gow, et al., 2012). The cytokine M-CSF binds to  
149 CD115 in a hydrophilic manner, whereas IL-34 binds to CD115 in a hydrophobic manner  
150 (Liu, et al., 2012) (Table 1). The nature of these molecular interactions established that each  
151 cytokine presented specific kinetics of association to CD115 (Figure 2B). The M-CSF/CD115  
152 complex was characterised by quick dissociation kinetics compared to the IL-34/CD115  
153 complex. Futhermore, *in vitro* experiments showed the lower dissociation kinetics of the IL-  
154 34/CD115 complex, which may be associated with a longer span of cell signalling pathway  
155 activation and differential biological functions (Stanley, et al., 1978; Lin, et al., 2008; Liu, et  
156 al., 2012; Ma, et al., 2012). The binding of IL-34 to CD115 results in the activation of  
157 multiple signalling pathways including Extracellular signal Regulated protein Kinases 1 and 2  
158 (ERK1/2), Focal Adhesion Kinase (FAK), Janus Kinase (JAK); c-JUN N-terminal Kinase  
159 (JNK), p38 mitogen-activated kinase, PhosphoInositide 3-Kinase (PI3K)/AKT, transcription  
160 factor Nuclear Factor kappa Beta (NFκB), Signal Transducer and Activator of Transcription 3  
161 (STAT3) and the Src kinase family (Figure 3) (Chihara, et al., 2010; Eda, et al., 2011;  
162 Baud'Huin, et al., 2010; Chen, et al., 2014; Wei, et al., 2010; Yu, et al., 2014; Lin, et al., 2019;  
163 Zhou, et al., 2018; Zins, et al., 2018; Truong, et al., 2018). Chiara *et al.* showed that the

164 CD115 receptor bound to its ligands by different domains, inducing differential activation of  
165 the receptor and subsequential bioactivities. Compared to M-CSF, IL-34 induced a strong but  
166 transitory phosphorylation of CD115 tyrosines and downstream proteins, with rapid  
167 downregulation of the receptor (Chihara, et al., 2010). The differential binding properties of  
168 M-CSF and IL-34 to CD115 may explain the variety and degree of activation of the different  
169 signalling pathways and their biological outcomes. Segaliny *et al.* demonstrated that both  
170 cytokines could work in a dual manner, showing additive and competitive biological  
171 properties. Moreover, M-CSF and IL-34 were able to generate a heterodimer that may play  
172 specific roles during CD115 signalling (Ségaliny, et al., 2015). The complexity, diversity and  
173 regulation of cell signalling events can be explained by the formation of this kind of  
174 heterodimer, like IL-12 or IL-17 (Detry, et al., 2019; Gorczynski, 2020). These data suggest  
175 that the interaction of CD115 and its ligands relies on a more complex relation than the  
176 proposed “ménage à trois” (Droin & Solary, 2010), and that the formation of new  
177 heterodimers between IL-34 and other cytokines should be not excluded.

178

## 179 **1.2 IL-34, a promiscuous cytokine with therapeutic potential**

180 CD115 is not the exclusive receptor of IL-34. Two additional receptors have been proposed:  
181 the receptor type Protein-Tyrosine Phosphatase-zeta (PTP-  $\zeta$ ) and the transmembrane heparin  
182 sulfate proteoglycan syndecan-1 (or CD138) (Nandi, et al., 2013; Segaliny, et al., 2015). The  
183 high expression of IL-34 in different areas of the adult brain where CD115 was absent suggest  
184 the existence of an additional receptor for IL-34 (Nandi, et al., 2012). By using a CD115  
185 depleted U251 glioblastoma cell line and affinity chromatography, Nandi *et al.* demonstrated  
186 that IL-34 bound specifically to PTP- $\zeta$  in a chondroitin sulfate-dependent manner. The  
187 interaction between IL-34 and PTP-  $\zeta$  induced tyrosine phosphorylations of the FAK and  
188 paxilin proteins, impairing the cell proliferation and motility of U251 glioblastoma cells.  
189 These results suggest that the IL-34/PTP- $\zeta$  complex acts as a tumorigenic suppressor in  
190 glioblastoma (Nandi, et al., 2013). PTP-  $\zeta$  was also expressed in the intestinal tissue of  
191 healthy people, mainly in the colon, whereas IL-34 was mainly expressed in the ileum.  
192 However, in inflammatory bowel diseases, IL-34 was coexpressed in the same regions as  
193 PTP-  $\zeta$  and CD115 (Zwicker, et al., 2016). Franzé *et al.* showed that IL-34 was over-  
194 expressed in colorectal cancer (CRC) tissues and PTP-  $\zeta$  was also expressed in tumoral and  
195 non-tumoral areas of CRC samples. However, an increase in CD115 expression alone, and not  
196 PTP-  $\zeta$ , was observed in CRC cells (Franzè, et al., 2018). Additional studies are needed to  
197 decipher the role of the IL-34/PTP-  $\zeta$  complex in these tissues.

198

199 IL-34 can also bind to syndecan-1 in a low affinity manner (Segaliny, et al., 2015). Segaliny  
200 *et al.* showed that syndecan-1 modulated the phosphorylation of CD115 induced by IL-34,  
201 proposing that syndecan-1 could act as a regulator of IL-34 bioavailability. Moreover,  
202 syndecan-1 controlled the macrophage migration induced *in vitro* by IL-34 (Segaliny, et al.,  
203 2015).

204

205 The cytokine M-CSF can be expressed in three different isoforms: a secreted glycoprotein, a  
206 secreted proteoglycan and a membrane-spanning cell surface glycoprotein (Pixley & Stanley,  
207 2004). Recently, Ogawa *et al.* showed the existence of a cell surface isoform of IL-34  
208 (Ogawa, et al., 2019). In secondary lymphoid tissue, follicular dendritic cells (FDC) were able  
209 to express IL-34 that induced, via CD115, the differentiation of a novel class of monocytes,  
210 named FDC-induced monocytic cells. To induce differentiation, IL-34 required the  
211 participation of the molecular chaperone 78-KDa glucose-regulated protein (GRP78) (Ogawa,  
212 et al., 2019). How the cell-surface IL-34 variant induces the differentiation of monocytes  
213 remains unclear. All these data are evidence of new potential targets that need to be taken into  
214 account when developing further therapies against IL-34.

215

## 216 **2. From monocytes to “non-resident” macrophages**

### 217 **2.1 Regulation of M1 and M2 differentiation by IL-34**

218 In healthy conditions, M-CSF and IL-34 act identically to promote macrophage differentiation  
219 and survival via their common receptor, CD115. In human monocytes, M-CSF and IL-34  
220 activate similar signalling pathways (STAT, AKT, ERK1/2) that trigger the proliferation of  
221 circulating monocytes and their differentiation into macrophages. Moreover, both cytokines  
222 induce the capacity for autophagy by activating AMPK and ULK1 pathways and caspase  
223 activities (caspases 3 and 8), two essential properties of macrophages (Boulakirba, et al.,  
224 2018). Depending on their microenvironments, naive-circulating monocytes can differentiate  
225 into two types of macrophage: M1 and M2. “Pro-inflammatory” M1 macrophages respond to  
226 pro-inflammatory molecules, such as interferon gamma (IFN- $\gamma$ ) and lipopolysaccharides  
227 (LPS), by upregulating IL-6, IL-12 and TNF- $\alpha$  and promoting activation of the immune  
228 response via Th1. “Anti-inflammatory” M2 macrophages respond to IL-4 stimulation by  
229 upregulating IL-10 and promoting activation of Th2 (Locati, et al., 2013; Murray, et al., 2014;  
230 Locati, et al., 2020). Treating circulating monocytes with IL-34 induced macrophage  
231 differentiation into the M2 type (CD14<sup>+</sup> CD163<sup>+</sup>), with high production of IL-10 and low

232 expression of IL-12 (Figure 4). This differentiation could be reversed to the M1 type by GM-  
233 CSF and INF- $\gamma$  treatments (Foucher, et al., 2013). In agreement with these results, Lindau *et*  
234 *al.* showed that IL-34 expressed at the foetal-maternal interface also induced polarisation of  
235 macrophages into CD14<sup>+</sup> CD163<sup>+</sup> with production of IL-10 that may contribute to a local  
236 immune-tolerant environment (Lindau, et al., 2018). In addition, M-CSF and IL-34 activated  
237 macrophages show different polarisation potential in the immune response (Boulakirba, et al.,  
238 2018). M-CSF-differentiated M1 macrophages enhanced naive T lymphocyte polarisation into  
239 Th1 better than IL-34-differentiated M1 macrophages. However, no differences between  
240 either cytokine-differentiated macrophages were observed with respect to Th2 cell  
241 polarisation (Boulakirba, et al., 2018). Moreover, using a human leukaemia model, IL-34  
242 enhanced the differentiation of leukaemia cells into differentiated macrophages by means of  
243 an increase in CD14 or CD68 and a decrease in CD71, a cell surface marker for immature  
244 myeloid cells. This suggests that IL-34 is able to reprogramme leukaemia cells from a naive  
245 state to mature and functional monocytes (Booker, et al., 2015).

246

247 This dichotomy in macrophage differentiation between M-CSF and IL-34 is also observed in  
248 species other than humans and rodents. In birds, Troung *et al.* demonstrated that chicken IL-  
249 34 interacted with CD115 triggering the activation of multiple signalling pathways (JAK,  
250 STAT 1/3, NF $\kappa$ B, TYK2, MAPK) and the induction of a specific pro-inflammatory response  
251 by upregulating the secretion of Th1 and Th17 cytokines (Truong, et al., 2018). In frogs, IL-  
252 34 and not M-CSF-differentiated macrophages showed an ability to resist bacterial infections  
253 (Popovic, et al., 2019). In fish, rainbow trout IL-34 was expressed with relatively high levels  
254 along the tissues compared to the two M-CSF cytokines that showed variable expression  
255 levels and that presented in this fish (Wang, et al., 2013). Moreover, IL-34 expression, but not  
256 M-CSF, increased significantly during the inflammatory process and induced macrophage  
257 proliferation (Wang, et al., 2013). In grass carp, IL-34 showed a similar capacity with regard  
258 to macrophage differentiation in an inflammatory context (Xue, et al., 2019). Shen *et al.*  
259 identified the homologue cytokine IL-34 in the mudskipper fish, where IL-34 levels increased  
260 after bacterial infection and induced macrophage differentiation with high phagocytic activity  
261 in a CD115-dependent manner (Shen, et al., 2020). Hoang *et al.* showed that IL-34 could be  
262 used as an adjuvant to DNA vaccine treatment against nocardiosis infection in fish (Hoang, et  
263 al., 2020). Finally, zebrafish have been suggested as a good model for studying the role of IL-  
264 34 during brain development or in brain disorders and liver or skin diseases (Wu, et al., 2018;  
265 Jiang, et al., 2019; Kuil, et al., 2019).

266

## 267 **2.2 IL-34-differentiated macrophages in viral infections**

268 During viral infection, macrophages play an essential role, detecting virus particles and  
269 triggering an anti-viral immune response by producing a variety of cytokines.

270

271 Infection by the human immunodeficiency virus-1 (HIV-1) is characterised by the loss of T  
272 lymphocytes in a progressive manner and susceptibility to opportunistic infections (Sattentau  
273 & Stevenson, 2016). IL-34-induced macrophages were characterised by better resistance to  
274 HIV-1 infection than MCSF-differentiated cells (Paquin-Proulx, et al., 2018). The HIV-1  
275 resistance of IL-34-macrophages lay in the specific expression of restriction factor genes  
276 APOBEC, IFITM and SAMHD, blocking the replication progress of the virus (Paquin-Proulx,  
277 et al., 2018). However, even if the immune system tries to slow down the progression of HIV-  
278 1, the virus can invade the central nervous system (CNS) in the early stages of infection,  
279 inducing severe neurotoxic effects (Valcour, et al., 2012). Mathews *et al.* engineered a  
280 humanised mouse model that produces human microglia and mimics viral infections  
281 (Mathews, et al., 2019). The authors demonstrated that IL-34 was responsible for microglia  
282 proliferation in the mouse's brain. The IL-34 microglia favoured HIV-1 infection, induced  
283 inflammation and a neurotoxic response, and formed an important reservoir for virus particles  
284 (Mathews, et al., 2019).

285

286 Influenza viruses are characterised by the production of seasonal epidemic disease that can  
287 occasionally generate global pandemics (Krammer, et al., 2018). Yu *et al.* observed that  
288 patients infected with influenza A virus (IAV) secreted high levels of IL-34 in blood serum.  
289 The authors showed that IAV infection stimulated the production of IL-22, which induced the  
290 expression of IL-34 which, in a negative feedback loop, regulated the activity of IL-22. These  
291 results suggest that IL-34 promoted the activation of an inflammatory response in influenza  
292 virus infection (Yu, et al., 2015 ).

293

294 Infection by hepatitis C virus (HCV) is associated with the formation of chronic liver diseases  
295 such as liver fibrosis, and IL-34 may contribute to this pathogenesis. Patients with HCV  
296 presented high levels of M-CSF and IL-34 in their blood serum (Preisser, et al., 2014). HCV  
297 infection induced the production of both cytokines by hepatocyte cells and this increased  
298 macrophage proliferation and differentiation with profibrogenic properties. In turn,  
299 macrophages negatively regulated NK cells, promoting the survival and activation of stellate

300 cells, which secreted type I collagen. Moreover, the production of IL-13 during liver fibrosis  
301 enhanced the synthesis of type I collagen by decreasing expression of the collagenase MMP-1  
302 (Preisser, et al., 2014). As with HCV, hepatitis B virus (HBV) infections could lead to hepatic  
303 fibrosis and chronic inflammation in which IL-34 was involved. In a rat pre-clinical model,  
304 IL-34 inhibited the replication of HBV, and HBV patients presented significantly lower levels  
305 of IL-34 in their blood serum compared to healthy donors (Cheng, et al., 2017). Interestingly,  
306 the level of IL-34 detected in blood plasma differed according to the phases of chronic HBV  
307 infection and correlated with progression of liver fibrosis and poor prognosis (Wang, et al.,  
308 2018). The discrepancy between the two studies can be explained by the chronicity of the  
309 infected patients, or by the accuracy of the different methods selected for IL-34 quantification  
310 in serum. As observed in HCV infections (Preisser, et al., 2014), IL-34 levels correlated with  
311 the chronicity of the HBV infection. However further studies are needed to clarify the  
312 functional relationship between IL-34 and HBV infection. HBV is also considered to be a  
313 major factor associated with the development of hepatocellular carcinoma (HCC)  
314 development. Expression of the HBX viral particle in HCC-infected cells induced expression  
315 of IL-34, which promoted cancer cell proliferation and migration via CD115 and syndecan-1  
316 receptors in an ERK- and STAT3-dependent manner (Kong, et al., 2019).

317

318 Infection by the Hantaan virus causes Haemorrhagic Fever Renal Syndrome (HFRS). Patients  
319 with HFRS show high levels of IL-34 in their blood plasma which correlates with an increase  
320 in phagocytic (CD14<sup>+</sup>CD16<sup>-</sup>) and inflammatory (CD14<sup>+</sup>CD16<sup>+</sup>) monocytes. Moreover, the  
321 increase in IL-34 may contribute to the virus' expansion (Tang, et al., 2019).

322

323 IL-34 plays an important role during viral infections in other species. In fish, Xue *et al.*  
324 showed that in the grass carp, infection by grass carp reovirus II induced the expression of IL-  
325 34 and generated a pro-inflammatory response by producing IL-1 $\beta$ , IL-6 and IL-8, and  
326 inhibiting anti-inflammatory factors such as IL-10 and the transforming growth factor  $\beta$ 1  
327 (TGF- $\beta$ 1) (Xue, et al., 2019). Amphibian populations are dramatically affected by the frog  
328 virus 3 (FV3) ranavirus (Grayfer & Robert, 2016). In *Xenopus laevis* M-CSF and IL-34  
329 polarised macrophage differentiation with distinct functionalities (Grayfer & Robert, 2016).  
330 Both subtypes of macrophage expressed specific pattern recognition receptors (PRRs) that  
331 were essential for pathogen recognition. IL-34-activated macrophages highly expressed  
332 antiviral interferon genes, showing better anti-FV3 properties than M-CSF-activated

333 macrophages. Similar to HIV-1 virus infection, FV3 infection induced the expression of  
334 antiviral restriction factors IFN $\lambda$ , INOS and APOBEC in IL-34-activated macrophages  
335 (Yaparla, et al., 2018). Consequently, an increase in toll-like receptor 2 and 4 transcripts was  
336 detected in macrophages implicated in the recognition of bacterial cell wall  
337 lipopolysaccharide (LPS), as well as in the secretion of antiviral interferon IFN $\gamma$  and TNF- $\alpha$ .  
338 Overall, these data suggest that IL-34 induces differential transcription programmes and  
339 functions compared to M-CSF during viral infection in high vertebrates (Yaparla, et al.,  
340 2019).

341

### 342 **2.3 Effects of IL-34 in tumour-associated macrophage polarisation**

343 One characteristic of tumorigenesis is the infiltration of macrophages into the affected tissue  
344 or organs. These macrophages are known as tumour-associated macrophages (TAMs). TAMs  
345 are considered to be heterogenous populations that originate in adult circulating myeloid  
346 precursors and “tissue-resident” macrophage precursors (Locati, et al., 2020). TAMs are  
347 responsible for driving pro- or anti-inflammatory responses by controlling immunocompetent,  
348 stroma and vascular cells according to the tumour microenvironment (TEM), (Mantovani, et  
349 al., 2017). TAMs can be classified into two subtypes: M2-like macrophages, which favour  
350 tumour progression, angiogenesis and metastasis, and M1 macrophages, which facilitate local  
351 inflammation leading to the anti-tumour response (Williams, et al., 2016). However,  
352 depending on the surrounding TEM, TAMs can acquire M1, M2 or dual polarisation (Wang,  
353 et al., 2019). Recent advances in TAM functions and their involvement in cancer and  
354 inflammatory diseases have been reviewed in (Wang, et al., 2019; Kumar, et al., 2020; Zhou,  
355 et al., 2020).

356

357 TAM differentiation and polarisation are driven differently by M-CSF and IL-34 (Jeannin, et  
358 al., 2018). Several publications have demonstrated the role of IL-34 in TAM polarisation and  
359 the pro-tumorigenic effect of IL-34-derived TAMs. Segaliny *et al.* demonstrated that IL-34  
360 macrophage extravasation and polarisation to the M2 phenotype promoted osteosarcoma  
361 proliferation and metastasis expansion (Ségaly, et al., 2015). In lung cancer, tumour cells  
362 were able to express IL-34, which induced TAM polarisation into an M2 pro-tumorigenic  
363 phenotype with properties of chemoresistance to tumour cells through Akt signalling pathway  
364 activation (Baghdadi, et al., 2016). Moreover, the co-expression of both cytokines, M-CSF  
365 and IL-34, was expressed by lung cancer cells, and correlated with an increase in the TAM  
366 population and poor prognosis compared to weaker or no expression of single ligands. This



367 suggests that, in particular conditions, both cytokines may create a new heterodimer cytokine  
368 that may bind to CD115 (Ségaliny, et al., 2015). This new cytokine may result in specific  
369 functions of myeloid and cancer cells during lung cancer development (Baghdadi, et al.,  
370 2018). Another study showed that nitric oxide therapy in tumorigenic castration-resistant  
371 prostate cancer xenografts reduced tumour progression and correlates with a decrease in the  
372 IL-34-derivated TAM population (Arora, et al., 2018). Taken together, these observations  
373 suggest that IL-34 may play an essential role in both the pro-tumorigenic polarisation of  
374 TAMs and tumour progression, as revealed in various other cancers. In colorectal cancer, high  
375 levels of IL-34 correlated with TAM infiltration and a poor prognosis for the affected patients  
376 (Kobayashi, et al., 2019). In ovarian cancer, cytotoxic chemotherapy enhanced the expression  
377 of IL-34, CD115 and associated TAMs, correlating with poor patient survival (Endo, et al.,  
378 2019). Similar observations were obtained in hepatocellular carcinoma (Zhou, et al., 2016;  
379 Noda, et al., 2019). However, the results were more contrasted in breast cancer in which IL-  
380 34 displayed different prognosis properties depending on the cancer subtype. High levels of  
381 IL-34 were correlated to better survival and good prognosis in the luminal B and HER2  
382 subtypes, whereas IL-34 was associated with poor prognosis in basal breast tumours (Zins, et  
383 al., 2018).

384

### 385 **3. Tissue-resident macrophages and IL-34**

386 In healthy tissues, the local population of macrophages is sustained by autonomous  
387 proliferation (Hashimoto, et al., 2013). During pathologic episodes, the death of local  
388 macrophages induces the infiltration of circulating monocytes to reinforce the local  
389 population until proliferation of the remaining resident macrophages. These circulating  
390 monocytes end up acquiring tissue-resident macrophage features, indicating the presence of  
391 local signals that confer tissue-specific identities (van de Laar, et al., 2016). Several authors  
392 have suggested that the autonomous proliferation of local macrophages is regulated by the  
393 existence of a tissue “niche”. Each tissue niche consists in cell-cell circuits based on the  
394 mutual benefits of stroma cells and tissue-resident immune cells by means of the secretion of  
395 specific factors (Guilliams, et al., 2020). The following sections will be dedicated to the role  
396 of IL-34 in the differentiation of tissue-resident macrophages.

397

#### 398 **3.1 IL-34 in osteoclast differentiation and associated bone diseases**

399 Alterations in bone homeostasis can be translated into an imbalance between osteoblast and  
400 osteoclast populations, which leads to bone malformations and diseases such as osteopetrosis

401 or osteoporosis. As part of the tissue-specific mononuclear phagocyte lineage, osteoclasts play  
402 an essential role in maintaining bone homeostasis. A key factor in the differentiation of  
403 osteoclast progenitors is the Receptor Activator of Nuclear Factor kappa-B ligand (RANKL)  
404 (Liu & Zhang, 2015; Ono & Nakashima, 2018). In addition, other factors, such as IL-6 and  
405 M-CSF, act as enhancers for osteoclast generation (Heymann, et al., 1998; Heymann &  
406 Rousselle, 2000; Yamashita, et al., 2012). As already mentioned, in studies using knockout  
407 mice for M-CSF and CD115, IL-34 circumvented the observed osteopetrosis phenotype,  
408 suggesting that IL-34 may play a role in osteoclast generation (Nakamichi, et al., 2012; Wei,  
409 et al., 2010). In 2010 Baud'Huin *et al.* demonstrated that a combined treatment with IL-34  
410 and RANKL induced osteoclastogenesis in humans and mice, and that IL-34 could replace M-  
411 CSF in osteoclastogenesis (Baud'Huin, et al., 2010). Both cytokines, M-CSF and IL-34, were  
412 able to regulate the adhesion, differentiation and proliferation of osteoclast precursors but not  
413 osteoclast survival in the bone marrow (Baud'Huin, et al., 2010). Furthermore, human and  
414 mouse IL-34/RANKL differentiated osteoclasts presented bone reabsorbing activities (Chen,  
415 et al., 2011). In the bone marrow, TNF- $\alpha$  stimulates the expression of IL-34 and M-CSF in  
416 osteoblast via the NF $\kappa$ B pathway (Yu, et al., 2014). In pathologic situations such as  
417 osteopetrosis, the spleen acts as a reservoir for osteoclast progenitors. In a vitamin D-  
418 dependent manner, vascular endothelial cells from the spleen expressed the IL-34 that was  
419 needed to maintain and conscribe osteoclast progenitors from the spleen to the bone tissue  
420 (Nakamichi, et al., 2012). Recently, a study using bone marrow macrophages from mice  
421 reported the promoting function of IL-34 and RANKL in osteoclast differentiation via  
422 activation of the JAK2/STAT3 signalling pathway. This activation was reversed in the  
423 presence of the protease inhibitor AG490, which also favoured the expression of SMAD7, an  
424 antagonist TGF- $\beta$  protein for the STAT pathway (Cheng, et al., 2017). Segaliny *et al.*  
425 demonstrated that osteosarcoma cells expressed IL-34 and that this expression was regulated  
426 by TNF- $\alpha$  and IL1- $\beta$  (Ségallyny, et al., 2015). In this context, IL-34 appeared to be a cancer  
427 proliferation and metastatic factor in osteosarcoma, with a potential role in angiogenesis via  
428 glycosaminoglycan during tumour development. In relation with this pro-angiogenic effect,  
429 IL-34 promoted macrophage extravasation and polarisation to an M2 phenotype (Ségallyny, et  
430 al., 2015).

431

432 Additionally, IL-34 plays a regulatory role in inflammatory diseases. In the past few years, an  
433 increasing number of publications have associated IL-34 with a bad prognosis in rheumatoid

434 arthritis (RA) (Chemel, et al., 2012; Galligan & Fish, 2017; Wang, et al., 2018; Elkhider, et  
435 al., "in press"). Chemel *et al.* was the first to describe the correlation between IL-34 and  
436 inflammation levels in RA and found that IL-34 expression was upmodulated by IL-1 $\beta$  and  
437 TNF- $\alpha$  stimulation in synoviocytes (Chemel, et al., 2012). In contrast, two members of the  
438 transforming growth factor  $\beta$  family, BMP-2 and TGF- $\beta$ 1, were described as inhibiting  
439 expression of IL-34, acting as regulators of inflammation during RA (Chemel, et al., 2017).  
440 While macrophages are potential targets of IL-34, fibroblast-like synoviocytes were identified  
441 as new targets expressing CD115. The binding of IL-34 to its receptor activated the  
442 expression and secretion of IL-6 (via the JNK/P38/NF- $\kappa$ B signalling pathway), which in turn  
443 induced polarisation of naive T lymphocytes into a Th17 population (Wang, et al., 2018;  
444 Wang, et al., 2017). Moreover, IL-34 activated/inhibited the expression of RANKL/OPG by  
445 fibroblast-like synoviocytes and circulating monocytes promoting cartilage and bone  
446 destruction in an IL-17-dependent manner, which was consistent with the correlation  
447 observed between increased IL-34 and RANKL levels in patients with RA (Cui, et al., 2019;  
448 Li, et al., 2020). IL-34 is suspected of playing a role in various other autoimmune diseases  
449 (Table 2). Systemic sclerosis (SSc) affects the connective tissue of the skin, lung, bowels and  
450 other internal organs. IL-34 levels were increased in the serum of patients with SSc and  
451 correlated with poor prognosis. Moreover, the increase in IL-34 also correlated with the  
452 development of interstitial lung disease (ILD) (Kuzumi, et al., 2018). As SSc patients also  
453 presented an increase of Th17 cells (Rolla, et al., 2016), authors have suggested that IL-34  
454 may enhance the proliferation of Th17 cells, contributing to the development of ILD  
455 (Kuzumi, et al., 2018). Systemic lupus erythematosus (SLE) is characterised by an acute  
456 nephritis process. Wada *et al.* demonstrated that IL-34 and its two receptors, CD115 and PTP-  
457  $\zeta$ , were highly expressed in patients with lupus nephritis. IL-34 induced circulating  
458 monocytes and intrarenal macrophage proliferation and accumulation, together with B and T  
459 lymphocyte enrichment in kidney areas, promoting the inflammation process and tubular  
460 epithelial cell apoptosis (Wada, et al., 2019). In Sjogren's syndrome, Ciccia *et al.* showed  
461 that IL-34 expression increased in the ductal epithelial cells of inflamed salivary glands. The  
462 increase in IL-34 correlated with the expansion of pro-inflammatory monocytes and  
463 expression of IL-23 and IL-17, suggesting that IL-34 plays an important role during the  
464 inflammatory process in Sjogren's syndrome (Ciccia, et al., 2013).

465

466 Periodontitis is another type of bone-degenerative illness associated with osteoclastogenesis.  
467 A few studies have showed that TNF- $\alpha$  and IL1- $\beta$  upregulated IL-34 expression by human  
468 gingival fibroblasts, which promoted osteoclastogenesis in combination with RANKL  
469 (Boström & Lundberg, 2013; Kawabe, et al., 2015). Clinically, a positive correlation between  
470 levels of IL-34 in gingival crevicular fluids and the aggressiveness of periodontitis was  
471 observed (Batra, et al., 2019).

472  
473 In relation with myeloid differentiation, multiple myeloma (MM) is a haematological disease  
474 that affects the axial skeleton of affected patients in terms of bone fragility. MM murine cells  
475 expressed IL-34 *in vitro* which was upmodulated by inflammatory factors such as IL1- $\beta$ , IL-6,  
476 TNF- $\alpha$  and surprisingly TGF- $\beta$  (Chemel, et al., 2017). IL-34 was also detected in bone  
477 marrow fluids from MM patients. In pathological context of MM, IL-34 promoted CD14<sup>+</sup>  
478 monocyte differentiation into osteoclasts by upregulating the expression levels of  
479 osteoclastogenesis-related genes such as DC-STAMP and OC-STAPM. IL-34 did not appear  
480 essential for osteoclast differentiation in MM but IL-34 activation accelerated the osteolytic  
481 process and bone lesions in patients (Baghdadi, et al., 2019).

482

### 483 **3.2 IL-34 in microglia differentiation and neural disorders**

484 Microglia are the resident macrophage population of the central neural system (CNS) and  
485 present neurotoxic and neuroprotective activities (Nakajima & Kohsaka, 2001). Microglia  
486 development depends on CD115 activity and, by consequence, its ligands M-CSF and IL-34  
487 (Dai, et al., 2002). IL-34 is basically expressed by neurons whereas M-CSF is expressed by  
488 astrocytes, microglia and oligodendrocytes (Cahoy, et al., 2008; Mizuno, et al., 2011). M-CSF  
489 or IL-34 knockout mice highlighted the harmful impact of one of these two cytokines on the  
490 survival/differentiation of microglia populations in different regions of the CNS (Wang, et al.,  
491 2012; Greter, et al., 2012; Kondo & Duncan, 2009). IL-34 was not essential for the  
492 development of embryonic microglia cells but appeared crucial for their maintenance during  
493 adulthood (Greter, et al., 2012). In agreement with this, Easley-Neal *et al.* demonstrated the  
494 essential role played by IL-34 in microglia support during postnatal life using specific  
495 function-blocking antibodies against each CD115 ligands (Easley-Neal, et al., 2019). Only M-  
496 CSF seemed to be required to establish microglia in the embryonic brain. In adult mice, IL-34  
497 was necessary for proper development of the grey matter while, in contrast, M-CSF was  
498 necessary for white matter development. The regional localisation of each cytokine correlated

499 with the affected regions. Interestingly, regions of the brain with a mix of grey and white  
500 matters, such as the cerebellum and the dentate gyrus, showed differential responses to anti-  
501 M-CSF antibodies with no effect in the dentate gyrus and partial depletion of the microglia  
502 in the cerebellum, whereas no effect was observed for anti-IL-34 antibodies. However,  
503 treatment with both antibodies showed depletion of the microglia in both areas, suggesting the  
504 presence of a compensation mechanism between the two cytokines (Easley-Neal, et al., 2019).  
505 However, by using a zebrafish model, two independent research teams recently demonstrated  
506 that IL-34 was required for proper colonisation of microglia progenitors from the yolk sac to  
507 the head region in the early stages of embryo development (Wu, et al., 2018; Kuil, et al.,  
508 2019). Afterwards, IL-34/CD115 signalling and neural apoptosis determined microglia  
509 development in the different regions of the CNS (Wu, et al., 2018). Additionally, IL-34 was  
510 also responsible for the distribution of tissue-resident macrophages from the yolk sac to other  
511 parts of the embryo, such as the epidermis (Kuil, et al., 2019). In the retina, IL-34 was mainly  
512 expressed by the retinal ganglion cells and was responsible for maintaining the retinal  
513 microglia population localised at the inner plexiform layer of the neural parenchyma. This  
514 specific microglia subtype is implicated in the feedback regulation of cone bipolar cell axons  
515 (O'Koren, et al., 2019).

516

517 As observed in other tissues, IL-34 plays an important role in pathogenic situations (Wright-  
518 Jin & Gutmann, 2019). Depending on the disease, IL-34 is considered a neuroprotective or a  
519 neurotoxic agent. In neurodegenerative diseases, IL-34 primarily produced by neurons  
520 promoted microglia differentiation and proliferation via the CD115 receptor in an Alzheimer  
521 disease (AD) mouse model (Mizuno, et al., 2011). AD was characterised by the production  
522 and accumulation of neurotoxic oligomeric  $\beta$ -amyloid peptides. IL-34-differentiated microglia  
523 were able to abolish the neurotoxic effects of  $\beta$ -amyloid peptides and derivatives by the  
524 production of the insulin degrading enzyme (IDE), haeme oxygenase-1 (HO-1) and TGF- $\beta$   
525 (Mizuno, et al., 2011). These results suggest that IL-34 may act as a neuroprotector agent  
526 and that the mechanism of action of IL-34-differentiated microglia may differ from that of M-  
527 CSF-differentiated cells (Mizuno, et al., 2011; Ma, et al., 2012). A recent report revealed that  
528 IL-34 and M-CSF cytokines induced the differentiation of microglia into a CD11c<sup>+</sup> type  
529 (Wlodarczyk, et al., 2019). The CD11c<sup>+</sup> microglia is characterised by the expression of  
530 insulin-like growth factor-1, which is important for neural myelination and survival  
531 (Wlodarczyk, et al., 2017). Walker *et al.* reported that both cytokines induced pro-

532 inflammatory rather than anti-inflammatory activation of microglia with a major induction of  
533 the IL-1 $\beta$  factor (Walker, et al., 2017). Another example of IL-34 neuroprotection occurs in  
534 retinal diseases such as photoreceptor degeneration disease. Specific IL-34 retinal microglia  
535 populations massively migrate to the subretinal space during photoretinial damage to  
536 cooperate in the protection of the retinal pigment epithelium (O'Koren, et al., 2019). On the  
537 other hand, IL-34 may display some neurotoxic effects in Huntington's disease, characterised  
538 by high expression of the amyloidogenic fragment of the Huntington protein (mHTTx1). This  
539 accumulation, which was mediated by Neural I $\kappa$ B Kinase (IKK)/NKKbeta, induced high  
540 expression of IL-34 by neurons, activating the local microglia and inducing the production of  
541 neurotoxic pro-inflammatory molecules (Khoshnan, et al., 2017).

542  
543 Multiple sclerosis (MS) is characterised by strong demyelination of the CNS, as well as low  
544 levels of vitamin D (Lemus, et al., 2018) (Table 2). Recently, Lee *et al.* demonstrated that  
545 vitamin D induced moderate expression of IL-34 by neurons that led to microglia  
546 differentiation with partial anti-inflammatory activity. However, *in vitro* stimulation of  
547 microglia cells with IL-34 reduced the secretion of inflammatory mediators and promoted the  
548 expression of anti-inflammatory mediators, suggesting a potential role for IL-34 in the  
549 prevention of neuron demyelination (Lee, et al., 2020).

550  
551 Several articles support the evidence that IL-34-derived microglia are necessary for viral and  
552 prion protection in the CNS. As previously mentioned, HIV-1 invades the CNS, generating  
553 severe neurotoxic effects (Valcour, et al., 2012). In humanised mouse models, IL-34-  
554 differentiated microglia acted as a reservoir for virus particles, promoting the persistence of  
555 HIV-1 infection (Mathews, et al., 2019). West Nile virus (WNV) affects neuronal synapses  
556 within the hippocampus region. IL-34-deficient mice were resistant to WNV and seemed  
557 protected against the infection as shown by their minor loss of neural synapses. However, the  
558 main role of IL-34 in this infection remains unknown (Vasek, et al., 2016).

559  
560 Prion infections are another type of infection that generate neural degenerative diseases. Zhu  
561 *et al.* demonstrated that microglia play an essential role as a neuroprotective agent in prion  
562 infections and that the ablation of microglia resulted in an increase in prion malignancy (Zhu,  
563 et al., 2016). IL-34 secreted by neurons sustained microglia proliferation and consequently  
564 played a crucial role in the CNS (Wang, et al., 2012). Conditional mice for IL-34 experienced

565 an acceleration of prion infection compared to healthy mice, suggesting that microglia play a  
566 neuroprotective role in prion pathogenesis (Zhu, et al., 2016).

567

568

### 569 **3.3 Role of IL-34 in the differentiation of Langerhans cells and melanomas**

570 Langerhans cells (LCs), together with microglia, belong to the most specialised tissue-resident  
571 macrophages. LCs are the first immune barrier of the skin and contribute to its homeostasis.  
572 As with microglia, LCs autonomously maintain their own population with minimal  
573 contribution from circulating monocytes from the bone marrow and spleen (Ginhoux, et al.,  
574 2006). Like all monocytic cells, LCs express CD115, which is essential for their development,  
575 proliferation and survival (Greter, et al., 2012; Ginhoux, et al., 2006). M-CSF or IL-34  
576 knockout mice revealed a differential functional impact of both cytokines on LCs. The  
577 absence of IL-34, but not M-CSF, compromised the presence of the LC population in the skin  
578 (Wang, et al., 2012; Ginhoux, et al., 2006). During development, LC precursors derived  
579 mainly from the foetal liver, with a reduced population originating from the yolk sac (Hoeffel,  
580 et al., 2012). In contrast to microglia, IL-34 was essential for LC development in the skin  
581 rudiment during embryogenesis in mice, and for cell maintenance in adult mice (Greter, et al.,  
582 2012; Wang, et al., 2016). During skin maturation, keratinocytes were the main producers of  
583 IL-34, which enhanced LC differentiation and proliferation in the skin dermis (Wang, et al.,  
584 2016). M-CSF and IL-34 exhibited differential activities in LC renewal depending on the  
585 pathophysiological context. Neutrophil-derived M-CSFs drove the renewal of LCs in the  
586 course of skin injury, while IL-34-derived keratinocytes were the main drivers of LC renewal  
587 in intact skin (Greter, et al., 2012; Wang, et al., 2016). Reinforcing this observation, it has  
588 been shown that M-CSF - but not IL-34 - was responsible for LC differentiation and pro-  
589 inflammatory activity in Langerhans cell histiocytosis (Lonardi, et al., "in press"). Overall,  
590 these observations suggest that both CD115 ligands show non-redundant roles in the lifespan  
591 of LCs (Wang, et al., 2016; Lonardi, et al., "in press").

592

593 IL-34 is highly expressed by tumour tissue in patients with melanoma, and its levels appear to  
594 depend on clinical status. An increase in IL-34 expression was observed in refractory  
595 melanomas that correlated with the expansion of CD163<sup>+</sup> differentiated M2 macrophages  
596 (Han, et al., 2018). Similarly, the RUNX1/CD115/IL-34 axis was responsible for the tumour  
597 rebound in BRAF inhibitor resistant melanoma. The emergence of a population resistant to  
598 the BRAF inhibitor was associated with an increase in CD115 and IL-34 expression and

599 activation of ERK1/2 and AKT signalling pathways to promote tumour survival and  
600 proliferation (Giricz, et al., 2018).

601

602

603

### 604 **3.4 IL-34 in gut macrophage differentiation and intestinal disorders**

605 Macrophages are essential in the maintenance of healthy tissue homeostasis as well as in a  
606 pathogenic context. Gut macrophages originate in bone marrow. Some authors reported the  
607 existence of a heterogeneous population of macrophages in the intestine, divided into two  
608 major populations: resident macrophages and inflammatory macrophages (Platt, et al., 2010;  
609 Geissmann, et al., 2010; Guilliams, et al., 2018). By using multi-parameter flow cytometry  
610 and lineage tracking techniques, Bain *et al.* investigated the heterogeneity of macrophage  
611 populations localised in the human and mouse intestines (Bain, et al., 2013). In healthy  
612 conditions, gut macrophages underwent a continuum of differentiation from immature to fully  
613 mature subsets. Initially, macrophages were characterised by high expression of Ly6C. Then,  
614 during the maturation process, the macrophage population changed from high to low Ly6C  
615 expression, accompanied by high expression of CX3CR1, F4/80, CD64, CD11c, CD163 and  
616 CD206. In addition, mature macrophages acquired an anti-inflammatory state by presenting  
617 phagocyte activity as well as production of IL-10 (Bain, et al., 2013). On the other hand, in  
618 inflammatory bowel diseases (IBD), macrophage populations quickly turned to pro-  
619 inflammatory subsets characterised by high expression of Ly6C and CD14 (Bain, et al.,  
620 2013).

621

622 Intestine macrophage differentiation depends on activation of CD115 by its ligands, M-CSF  
623 and IL-34, which are differently distributed and expressed along the intestine. M-CSF was  
624 mainly expressed in the colon, whereas IL-34 was principally expressed in the ileum  
625 (Zwicker, et al., 2015). Both cytokines were produced by the intestinal epithelium in response  
626 to TNF- $\alpha$  via the NF $\kappa$ B pathway. Inflammation of the gut in IBD patients was characterised  
627 by an increase in IL-34 levels produced by mononuclear cells in the lamina propria. This  
628 increase in IL-34 induced macrophage differentiation and the expression of pro-inflammatory  
629 factors TNF- $\alpha$  and IL-6 (Franzè, et al., 2015). Franzè *et al.* recently demonstrated that IL-34  
630 induced, by specific activation of the p38 MAP pathway, the secretion of collagen leading to  
631 mucosal inflammation in CD patients (Franzè, et al., "in press").

632



633 Colorectal carcinoma cells express CD115 and can be considered as potential targets of  
634 CD115 ligands (Franzè, et al., 2016). IL-34 induced the expression of CCL20 in a DLD1  
635 colorectal adenoma cell line via activation of the ERK1/2 pathway and stimulation of the  
636 inflammatory response. This suggests that IL-34 participates in the crosstalk between the  
637 epithelium and immune system in inflammatory bowel diseases (Franzè, et al., 2016). A  
638 recent study demonstrated that IL-34 was expressed in colorectal carcinoma patient samples  
639 and induced the proliferation and invasion of colorectal cancer cells. Furthermore, HT29 cell  
640 proliferation implied activation of the ERK1/2 signalling pathway by IL-34 through CD115,  
641 and not by the M-CSF cytokine. In fact, inhibition of IL-34 increased the sensitivity of colon  
642 tumour cells to oxaliplatin, suggesting that IL-34 may act as a pro-inflammatory and pro-  
643 tumorigenic factor in IBD disease and colorectal carcinomas (Franzè, et al., 2018).

644

### 645 **3.5 Involvement of IL-34 in the differentiation of Kupffer cells and liver diseases**

646 Kupffer cells (KCs) are tissue-resident macrophages in the liver. KCs are responsible for the  
647 homeostasis of this organ in both healthy and pathological situations (Li, et al., 2017). Like  
648 LCs, KCs originate in the foetal liver and yolk sac. In the adult liver, the FC population is  
649 maintained by self-renewal and by bone marrow circulating monocytes (Hashimoto, et al.,  
650 2013). Depending on the microenvironment, KCs can be polarised into an M1 pro-  
651 inflammatory subtype leading to a Th1 response, or into an M2 anti-inflammatory subtype  
652 leading to a Th2 response. The latter is associated with inducing and maintaining immune  
653 tolerance during liver transplantation (Atif, et al., 2020). Rat and human regulatory T (Treg)  
654 cells FOXP3<sup>+</sup> CD4<sup>+</sup> or CD8<sup>+</sup> cells expressed IL-34 (Bézie, et al., 2015). Treg FOXP3<sup>+</sup> cells,  
655 the main players in immune tolerance, seemed sensitive to IL-34 (Atif, et al., 2020). IL-34  
656 treatment effectively seemed to induce Treg FOXP3<sup>+</sup> cell proliferation by polarising CD14<sup>+</sup>  
657 macrophages that over-expressed arginase-1 and inducible NO synthase, both implicated in  
658 the inhibition of T lymphocyte proliferation (Bézie, et al., 2015). These data suggest the  
659 presence of a positive feedback loop between IL-34 expression and FOXP3<sup>+</sup> Tregs.  
660 Interestingly, the fact that IL-34 targeted FOXP3<sup>+</sup> Tregs, which are essential for inhibiting the  
661 anti-donor immune response during allografts, suggests that IL-34 plays a potential role in  
662 transplantation therapy (Bézie, et al., 2015). In agreement with these data, during pregnancy;  
663 IL-34 induced macrophage polarisation into an M2 state, suggesting that IL-34 may play a  
664 role in maintaining tolerance during the gestation process (Lindau, et al., 2018). Reinforcing  
665 the role of IL-34 in immune tolerance, Zhao *et al.* showed that IL-34 treatment inhibited acute  
666 rejection in liver transplant in rats. The authors observed that IL-34 specifically induced the

667 polarisation of KCs from an M1 to an M2 status. This function was mediated by activation of  
668 the PI3K/AKT/mTOR pathway and was inhibited by rapamycin, an inhibitor of the mTOR  
669 pathways (Baek, et al., 2015). Recently, Jiant *et al.* reported that in zebrafish, *in vivo* ectopic  
670 expression of IL-34 in the liver or skin induced migration of macrophages to both regions  
671 (Jiang, et al., 2019). Consistent with previous studies, IL-34 may have a protective effect by  
672 mobilising and polarising macrophages in the liver (Jiang, et al., 2019).

673

674 In non-alcoholic fatty liver disease (NAFLD), a positive correlation between IL-34 levels in  
675 serum and the severity of NAFLD has been described (Shoji, et al., 2016). IL-34 production  
676 by liver fibroblasts was enhanced by TNF- $\alpha$  (Shoji, et al., 2016). In addition, IL-34 induced  
677 MIP3 $\alpha^+$ /CCL20 $^+$  macrophage differentiation, which promoted the production of collagen by  
678 hepatic stellate cells and subsequently development of fibrosis (Preisser, et al., 2014; Shoji, et  
679 al., 2016). The role of IL-34 in fibrosis was recently confirmed. Soluble egg antigen (SEA)  
680 produced by schistosome egg impairs NF- $\kappa$ B activation in hepatic stellate cells. This inhibits  
681 TNF- $\alpha$ , blocking the IL-34-associated liver fibrosis (Chen, et al., 2019). Consequently, IL-34  
682 may be used as an independent marker for liver fibrosis in NAFLD (Shoji, et al., 2016).

683

## 684 **Conclusion**

685 Final macrophage differentiation relies on the action of specific growth factors and their  
686 specific receptors. The studies discussed in this review have shown that of these factors, the  
687 “twin” cytokines M-CSF and IL-34 play an essential role in the differentiation and  
688 proliferation of non-resident and tissue-resident macrophages (Stanley, et al., 1978; Lin, et al.,  
689 2008). Both cytokines share a common receptor, CD115, and their binding to the receptor  
690 activates the same signalling pathways. However, the similarities between M-CSF and IL-34  
691 ended there. Each cytokine presented specific domains of interaction with CD115 and the  
692 nature of these interactions generated differential activation of the receptor (Liu, et al., 2012).  
693 IL-34 induced a strong but transitory phosphorylation of CD115 tyrosines, followed by rapid  
694 downregulation of the receptor (Chihara, et al., 2010). These differences in CD115 activation  
695 generated a variety of downstream signalling pathway activations and, by consequence, a  
696 wide range of biological functions in macrophages and other cell types. Adding another layer  
697 of complexity, M-CSF and IL-34 were able to generate a functional heterodimer (Ségaly, et  
698 al., 2015), and both cytokines could be present in different isoforms (Pixley & Stanley, 2004;  
699 Ogawa, et al., 2019). Moreover, IL-34 bound to PTP- $\zeta$  and syndecan-1 receptors with

700 particular bioactivities (Nandi, et al., 2013; Segaliny, et al., 2015). Overall, the studies  
701 discussed in this review suggest that activation of CD115 by its ligands, together with their  
702 biological implications, involves a complex network of specific interactions. New studies and  
703 approaches will be needed to decipher how each particular cytokine regulates the different  
704 signalling pathways and their roles in macrophage differentiation. Moreover, the existence of  
705 new heterodimers, isoforms or receptors cannot be excluded.

706

707 Under normal physiology conditions, and depending on their microenvironment, circulating  
708 monocytes could be differentiated by M-CSF and IL-34 into specific non-resident  
709 macrophages with a “pro-inflammatory” M1 phenotype or in an “anti-inflammatory” M2  
710 phenotype. Treating monocytes with IL-34 primarily induced macrophage differentiation into  
711 an M2 phenotype (Foucher, et al., 2013). The ability of each cytokine to induce different  
712 macrophage differentiation was also observed in other species, such as birds (Truong, et al.,  
713 2018), fish (Wang, et al., 2013; Xue, et al., 2019; Shen, et al., 2020; Jiang, et al., 2019) and  
714 frogs (Popovic, et al., 2019), contributing to and offering new potential animal models for  
715 advancement in knowledge of both cytokines and macrophage differentiation.

716

717 M-CSF and IL-34 are also important for the development, differentiation and proliferation of  
718 tissue-resident macrophages. In bone, IL-34 is able to regulate the adhesion, differentiation  
719 and proliferation of osteoclast precursors, and may replace M-CSF during osteoclastogenesis  
720 (Yu, et al., 2014). In the CNS, IL-34 is necessary for maintaining microglia during adulthood  
721 (Greter, et al., 2012), as well as for the proper development of the grey matter (Easley-Neal, et  
722 al., 2019) and the retinal microglia population (O’Koren, et al., 2019). In the skin, IL-34 has  
723 shown that it is essential for the development of LCs in the skin rudiment, the  
724 differentiation/proliferation of LCs in the dermis, and its absence compromised the presence  
725 of LCs in the skin. Moreover, IL-34 is essential for the renewal of LCs in intact skin (Wang,  
726 et al., 2012; Greter, et al., 2012; Ginhoux, et al., 2006; Wang, et al., 2016). IL-34 was also  
727 important for macrophage differentiation in other organs such as the gut (Zwicker, et al.,  
728 2015), kidneys (Baek, et al., 2015), spleen (Nakamichi, et al., 2012; Xue, et al., 2019) and  
729 liver (Atif, et al., 2020). The specific functions of IL-34 in most of these tissues remains  
730 unclear and recent advances in single cell omics and organoid techniques may be helpful tools  
731 for better understanding the molecular and physiological cytokine-specificity of each subset  
732 of IL-34-derived macrophages, from non-resident to tissue-resident populations.

733

734 Depending on the tissue and microenvironment, the cytokine IL-34 has a positive or negative  
735 role and can be considered the Dr. Jekyll and Mr. Hyde cytokine. On the one hand, IL-34 acts  
736 a beneficial factor with high potential as a therapeutic molecule. In the CNS, IL-34-derived  
737 microglia have a neuroprotective function by favoring neural myelination and survival in AD  
738 (Mizuno, et al., 2011; Wlodarczyk, et al., 2017). In retinal disease, IL-34 retinal microglia  
739 protect the retinal pigment epithelium from damage (O'Koren, et al., 2019). Secretion of IL-  
740 34 by neurons in prion infections helps microglia proliferation and neuroprotection (Wang, et  
741 al., 2012). Moreover, IL-34 induces microglia resistance to West Nile virus infection (Vasek,  
742 et al., 2016), and the same effects have been observed in other viral infections, such as HIV-1  
743 (Paquin-Proulx, et al., 2018) or FV3 (Yaparla, et al., 2018). In addition, IL-34 is associated  
744 with inducing and maintaining immune tolerance in liver transplantations (Atif, et al., 2020;  
745 Bézie, et al., 2015) as well as during pregnancy (Lindau, et al., 2018). These data suggest that  
746 IL-34 can be used as a powerful tool for improving graft generation and allograft tolerance.

747

748 On the other hand, as a Mr. Hyde cytokine, IL-34 can be considered a good therapeutic  
749 target against a wide range of diseases, from virus infections such as HCV or HBV (Preisser,  
750 et al., 2014; Wang, et al., 2018), to autoimmune diseases such as RA (Galligan & Fish, 2017;  
751 Wang, et al., 2018; Wang, et al., 2017), systemic sclerosis (Kuzumi, et al., 2018), systemic  
752 lupus erythematosus (Wada, et al., 2019), Sjogren's syndrome (Ciccia, et al., 2013) and  
753 inflammatory bowel diseases (Franzé, et al., 2015; Franzè, et al., "in press"), to cancer-like  
754 osteosarcoma (Ségaligny, et al., 2015), lung cancers (Baghdadi, et al., 2016), melanoma (Han,  
755 et al., 2018), colorectal cancer (Franzè, et al., 2018; Kobayashi, et al., 2019), ovarian tumours  
756 (Endo, et al., 2019), prostate cancer (Arora, et al., 2018) and hepatocellular carcinoma (Zhou,  
757 et al., 2016; Noda, et al., 2019). In the majority of these conditions, authors have reported that  
758 high levels of IL-34 in serum or tissue fluids correlated with poor prognosis and survival, and  
759 suggested using IL-34 as an indicator of disease grade (Udomsinprasert, et al., 2019; Ge, et  
760 al., 2019). Furthermore, in cancer, IL-34 has been described as a pro-tumorigenic factor in a  
761 variety of tumours. IL-34 favoured tumour cell proliferation and metastasis by promoting IL-  
762 34-derived macrophage extravasation and polarisation to a TAM M2 phenotype. In most of  
763 the tumours, high levels of IL-34 and TAMs correlated with poor prognosis and survival.  
764 However, there were some exceptions, such as in the breast luminal B and HER2 tumour  
765 subtypes, where high levels of IL-34 were associated with better survival and a good  
766 prognosis. Therapies against TAMs have been developed in recent years. Reducing and

767 repolarising TAM macrophages has been shown to be a promising therapy (Arora, et al.,  
768 2018; Cassetta & Pollard, 2018; Xun, et al., "in press").

769

770 In the past few years, most strategies against the pathogenic effects of M-CSF/CD115 and IL-  
771 34/CD115 have focused on CD115. However, due to CD115 being involved in multiple  
772 biological processes, specific tumour therapies against CD115 have generated undesirable  
773 side effects (Kumari, et al., 2018). This has brought other actors in the complex to the  
774 attention of the scientific community, notably its ligands M-CSF and IL-34 (Cannarile, et al.,  
775 2017; Xu, et al., 2019; Yin, et al., 2020). In the present review we have discussed the role of  
776 IL-34 in macrophage differentiation and its positive or negative effects in healthy and  
777 pathogenic situations. All these observations indicate that IL-34 should be considered as a  
778 potential therapeutic target as well as an interesting therapeutic tool in health issues. Further  
779 studies will be needed to evaluate the effects and implications of these new IL-34 therapies.

780

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782

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### 1163 **Figure legends**

1164 **Figure 1. Macrophage ontogeny and implications of IL-34 during macrophage**  
1165 **differentiation.** Depending on their origin, macrophages are divided into two different  
1166 populations: tissue-resident macrophages and non-resident macrophages. Tissue-resident  
1167 macrophages originate in the embryonic yolk sac, foetal liver and the bone marrow. Tissue-  
1168 resident macrophages are capable of self-renewal of their own population (round arrows).  
1169 However, in pathogenic situations, non-resident macrophages can migrate into the affected  
1170 tissues and replenish the local populations by acquiring tissue specificities. Depending on the  
1171 tissue, IL-34 drives macrophage differentiation, proliferation, maintenance, migration, and  
1172 adhesion. Non-resident macrophages originate in the bone marrow and spleen. Circulating  
1173 monocytes can extravasate and migrate to different tissues where, through the actions of  
1174 different growth factors, they induce their polarisation into M1 or M2 subtypes. M1  
1175 macrophages detect pathogenic particles or inflammatory molecules such as LPS or INT- $\gamma$   
1176 and display pro-inflammatory functions by secretion of pro-inflammatory factors such as  
1177 TNF- $\alpha$ , IL-6 and Il-12. M2 macrophages are sensitive to molecules such as IL-4 or IL-13 and  
1178 display an anti-inflammatory profile by producing soluble factors such as IL-10. IL-34 mainly  
1179 induces the polarisation of monocytes into an M2 subset. In pathological situations such as  
1180 bacterial, viral infection or inflammation, IL-34 can act as a pro- or anti-viral/inflammatory  
1181 agent. In cancer, IL-34 behaves in a pro- or anti-tumour manner. IL-34 also induces

**Table1. Kinetic properties of M-CSF and IL-34 binding to CD115**

<b>Proteins</b>	<b>Binding characteristic</b>	<b>K<sub>D</sub></b>	<b>K<sub>on</sub></b>	<b>K<sub>off</sub></b>
<b>M-CSF / CD115</b>	<b>hydrophilic</b>	<b>1 pM</b>	<b>6.29 x 10<sup>7</sup> s<sup>-1</sup> M<sup>-1</sup></b>	<b>6.55 x 10<sup>-5</sup> s<sup>-1</sup></b>
<b>IL-34 / CD115</b>	<b>hydrophobic</b>	<b>34 pM</b>	<b>1.7 x 10<sup>7</sup> s<sup>-1</sup> M<sup>-1</sup></b>	<b>6.03 x 10<sup>-4</sup> s<sup>-1</sup></b>

K<sub>D</sub>: equilibrium dissociation constant; K<sub>on</sub>: association rate constant; K<sub>off</sub>: dissociation rate constant. Data from (10.1126/science.1154370).



**Table 2: IL-34 and autoimmune diseases**

Diseases	Major finding related to IL-34	References
<p><b>Bowel Autoimmune Diseases</b></p>	<p>High IL-34 expression levels in lamina propria compartments in Crohn’s disease (CD) and Ulcerative colitis (UC). IL-34 enhances inflammatory response by up-regulation of TNF-<math>\alpha</math> and IL-6 factors.</p>	<p>Franzè et al. (2015)</p>
	<p>High expression levels of IL-34 in CD than in UC, with IL-34 mainly expressed in the Ileum. TNF-<math>\alpha</math> induces IL-34 production by epithelial cells.</p>	<p>Zwicker et al. (2015)</p>
	<p>IL-34 participates in the cross talk between epithelial and immune cells by induction of CCL20 chemokine expression.</p>	<p>Franzè et al. (2016)</p>
	<p>IL-34 is highly produced in the fibrotic gut of CD patients and contributes to collagen production by enhancing COL1A1 and COL3A1 expression in a p38MAP kinase-depending mechanism.</p>	<p>Franzè et al. (2020)</p>
<p><b>Multiple Sclerosis (MS)</b></p>	<p>IL-34 acts as a neuroprotective factor by inducing microglia differentiation with anti-inflammatory properties.</p>	<p>Mizuno (2011)</p>
	<p>IL-34 expression by neurons contributes to the re-establishment of tight junctions and blood brain barrier by epithelial cells. IL-34 induces microglia differentiation with anti-inflammatory properties.</p>	<p>Jin et al. (2014)</p>
	<p>IL-34 levels do not change in relapsing-remitting MS.</p>	<p>Abdel-Dayen et al. (2019)</p>
	<p>IL-34 induces neuroprotection by expansion of CD11c<sup>+</sup> microglia population via CD115.</p>	<p>Wlodarczyk et al. (2019)</p>
	<p>IL-34 expression is down regulated in cerebrospinal fluids in MS affected patients.</p>	<p>Safari-Alighiarloo et al. (2020)</p>
	<p>In children, Vitamin D partially induces IL-34 expression by neurons conferring neuroprotection against MS.</p>	<p>Lee et al. (2020)</p>

<b>Psoriasis</b>	High levels of IL-34 in serum of patients affected by psoriatic Arthritis correlate with high levels of osteoclast precursors and poor prognosis.	Li et al. (2017)
<b>Rheumatoid Arthritis (RA)</b>	IL-34 is highly expressed by synovial fibroblast of RA affected patients. IL-34 expression is induced by pro-inflammatory factors IL-1 $\beta$ and TNF- $\alpha$ . High levels of IL-34 correlate with severity and poor prognosis of RA.	Chemel et al. (2012)
	High levels of IL-34 in synovial fluids of RA patients. IL-34 induces osteoclast differentiation in RA. TNF- $\alpha$ induces IL-34 expression by synovial fibroblast via NF $\kappa$ B and JNK pathways.	Hwang et al. (2012)
	IL-34 high levels in serum and synovial fluid of RA patients. IL-34 induces the expression of pro-inflammatory factor IL-17 by circulating mononuclear cells.	Tian et al. (2013)
	High levels of IL-34 in serum of RA patients positively correlate with IL-6, RANKL and anti-cyclic citrullinated peptide (CCP) antibody levels.	Moon et al. (2013)
	High levels of IL-34 in serum and synovial fluids positively correlate with rheumatoid factors (RF), current smoking, erythrocyte sedimentation rate (ESR) and C-reactive protein levels. IL-34 as an independent risk factor for radiographic progression of RA.	Chang et al. (2014)
	High levels of IL-34 in serum of RA patients in stage III of hand R-ray score. IL-34 levels positively correlate with increase of pro-inflammatory factors IL-6, IL-8, MMP-3 and C-reactive protein.	Zhang et al. (2015)
	Treatment with TNF- $\alpha$ antagonist reduces levels of IL-34 after 3 months of treatment and correlates with good prognosis of RA.	Ding et al. (2015)
	Simultaneous inhibition of M-CSF and IL-34 cytokines decrease pathology symptoms in RA mouse models and humans.	Garcia et al. (2016)
	High levels of IL-34 in serum and synovial fluids of RA patients. IL-34 enhances synovial fibroblast apoptosis resistance by production of miR-21 via STAT3 signaling pathway activation.	Yang et al. (2016)

	BMP2 and TGF- $\beta$ acts as controllers of inflammatory process in RA by inhibition of IL-34 expression in synovial fibroblast.	Chemel et al. (2017)
	High levels of IL34 in serum of RA patients positively correlate with C-reactive protein, ESR, RF and anti-CCP antibody. IL-34 induces the expression of IL-6 cytokine and subsequently promotes Th17 production.	Wang et al. (2017a)
	IL-34 plays an essential role in the immune cell cross talk during RA. IL-34/CD115 complex stimulates the expression of ROS in THP-1 cells, inducing IL-6 secretion and Th17 production.	Wang et al. (2017b)
	IL-34 participates in the establishment of RA in mice by induction of proliferation, migration and transformation of circulating fibrocytes in fibroblast-like synovial cells in affected joints.	Galligan et al. (2017)
	High levels of IL-34 in serum of RA patients positively correlate with RANKL, DAS28-ERS, C-reactive protein, RF and bone erosion score. IL-34 levels can be used as a predictor of bone erosion.	Li et al. (2019)
	IL-34 participates in local joint destruction and osteoporosis during RA by induction of RANKL expression and inhibition of OPG, partially mediate by IL-17, in sinoviocytes fibroblast and circulating monocytes.	Cui et al. (2019)
	IL-34 may participate indirectly in angiogenesis process in RA by induction of VEGF and HIF-1 $\alpha$ factors secretion in RA circulating monocytes.	Ding et al. (2019)
	IL-34 modulates the proliferation and migration of synoviocytes fibroblast in RA.	Elkhider et al. (2020)
<b>Sjogren Syndrome (pSS)</b>	IL-34 expression correlates with expansion of pro-inflammatory CD14 <sup>bright</sup> CD16 <sup>+</sup> monocytes in salivary glands. IL-34 acts as a pathogenic factor in pSS.	Ciccia et al. (2013)
	High levels of IL-34 in serum of pSS patients are positively associated with levels of RF, IgG and $\gamma$ -globulin. IL-34 induces hyper-activation of B cells and antibodies production.	Liu et al. (2019)

<b>Systemic Lupus Erythematosus (SLE)</b>	High levels of IL-34 in serum of children with SLE correlate with high SLE Disease Activity Index (SLEDAI), anti-double-stranded DNA antibody (anti-sdDNA) and C-reactive protein.	Wang et al. (2016)
	IL-34 levels are detectable in serum of SLE affected patients and correlate with SLEDAI and high IgG. IL-34 as a potential disease activity marker for SLE.	Xie et al. (2018)
	High levels of IL-34 in serum and urine correlate with poor prognosis of SLE patients. IL-34 expression is associated with high expression of CD115 and PTP- $\zeta$ and induces differentiation and accumulation of intrarenal macrophages that favors tubular epithelial cell apoptosis.	Wada et al. (2019)
	High levels of IL-34 in serum of children with SLE correlate with high SLEDAI, anti-sdDNA and C-reactive protein, with a more aggressive effect than adult SLE.	El-Banna et al. (2020)
	High levels of IL-34 in serum of patients affected by lupus nephritis and correlate with SLEDAI, anti-sdDNA and C-reactive protein. IL-34 can be used as surrogate marker for early detection of lupus nephritis diseases.	Abdel-Rehim et al. (2020)
<b>Systemic Sclerosis (SS)</b>	High levels of IL-34 in serum of SS patients correlate with expansion of M2 and Th17 macrophages and severity of interstitial lung disease.	Kuzumi et al. (2018)

1182 macrophage differentiation into tumour-associated macrophages (TAMs), which are  
1183 characterised by an M2-like phenotype that promotes tumour proliferation, angiogenesis and  
1184 metastasis. The capacity of IL-34 to act in a positive or negative direction is tissue- and  
1185 microenvironment-dependent.

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1187 **Figure 2. Molecular modelling of CD115 binding to its ligands.** A) Representation of the  
1188 three-dimensional crystal structure of the M-CSF/CD115 complex. In red and orange:  
1189 monomers of M-CSF; and in blue and purple: monomers of CD115. B) Representation of the  
1190 three-dimensional crystal structure of the IL-34/CD115 complex. In green and light green:  
1191 monomers of IL-34; and in blue and purple: monomers of CD115.

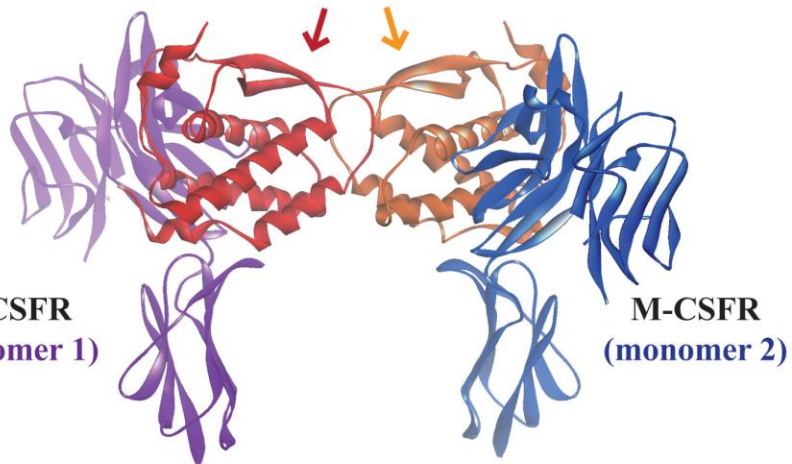
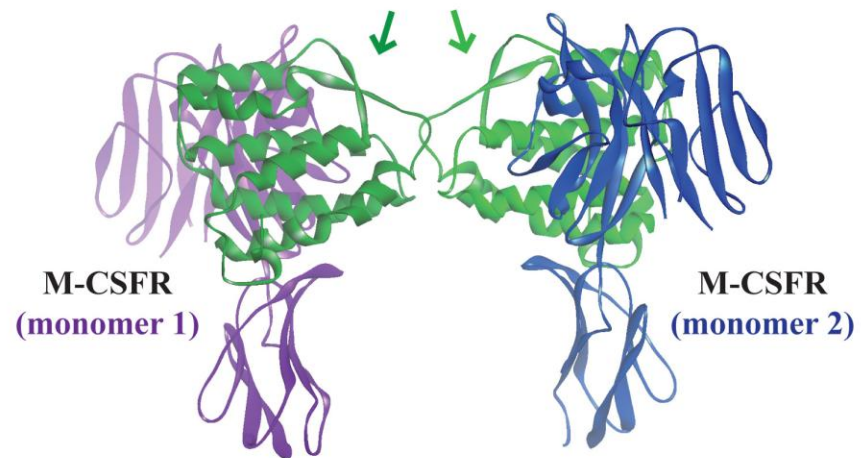
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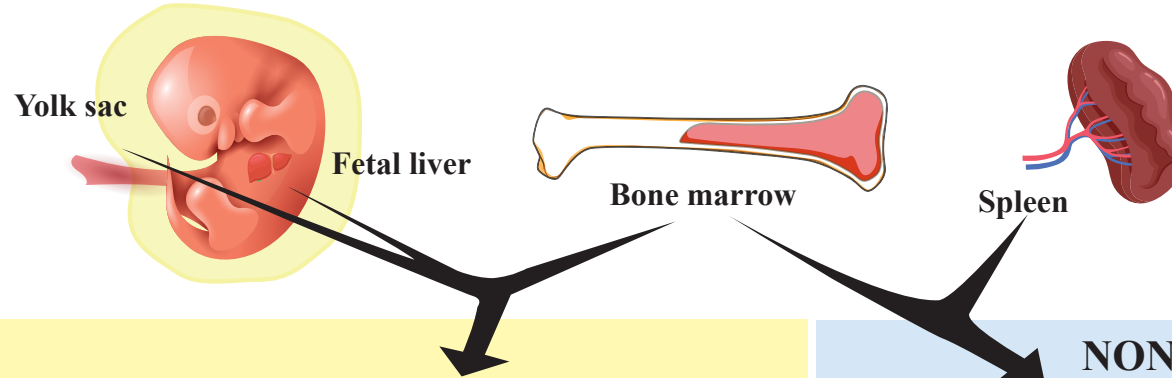
1193 **Figure 3. IL-34 signalling pathways involved in macrophage differentiation and non-**  
1194 **monocyte cells.** A) Various stimuli, such as bacterial or viral infections, pro-inflammatory  
1195 cytokines, DNA damage, or chemical molecules modulate IL-34 expression. IL-34 binds to  
1196 CD115 or to syndecan-1 receptors expressed at the cell surface of monocytes/macrophages.  
1197 The binding of IL-34 to CD115 induces activation of CD115 through auto-phosphorylation of  
1198 the different tyrosines present in the cytosolic domain of CD115. Compared to M-CSF, IL-34  
1199 induces strong and transient activation of CD115, as well as rapid downregulation of CD115.  
1200 These differences between the two cytokines imply differential activation of downstream  
1201 signalling pathways that result in a diversity of macrophage biological processes such as  
1202 differentiation, proliferation, survival or migration. The binding of IL-34 to the chondroitin  
1203 chains of syndecan-1 results in *in vitro* phosphorylations of the tyrosines Y708 and Y723 of  
1204 CD115, suggesting that the complex IL-34/syndecan-1 can act as a regulator of CD115  
1205 activity. Moreover, IL-34/syndecan-1 interaction regulates macrophage migration. B) IL-34  
1206 expression in the microenvironment of epithelial cells, fibroblasts and tumour cells can  
1207 induce, via CD155, activation of the different signalling pathways implicated in biological  
1208 functions such as cell proliferation, migration, survival and cytokines. IL-34 also binds to  
1209 PTP- $\zeta$  and controls inhibition of migration and proliferation of tumour cells lines such as  
1210 glioblastoma U251.

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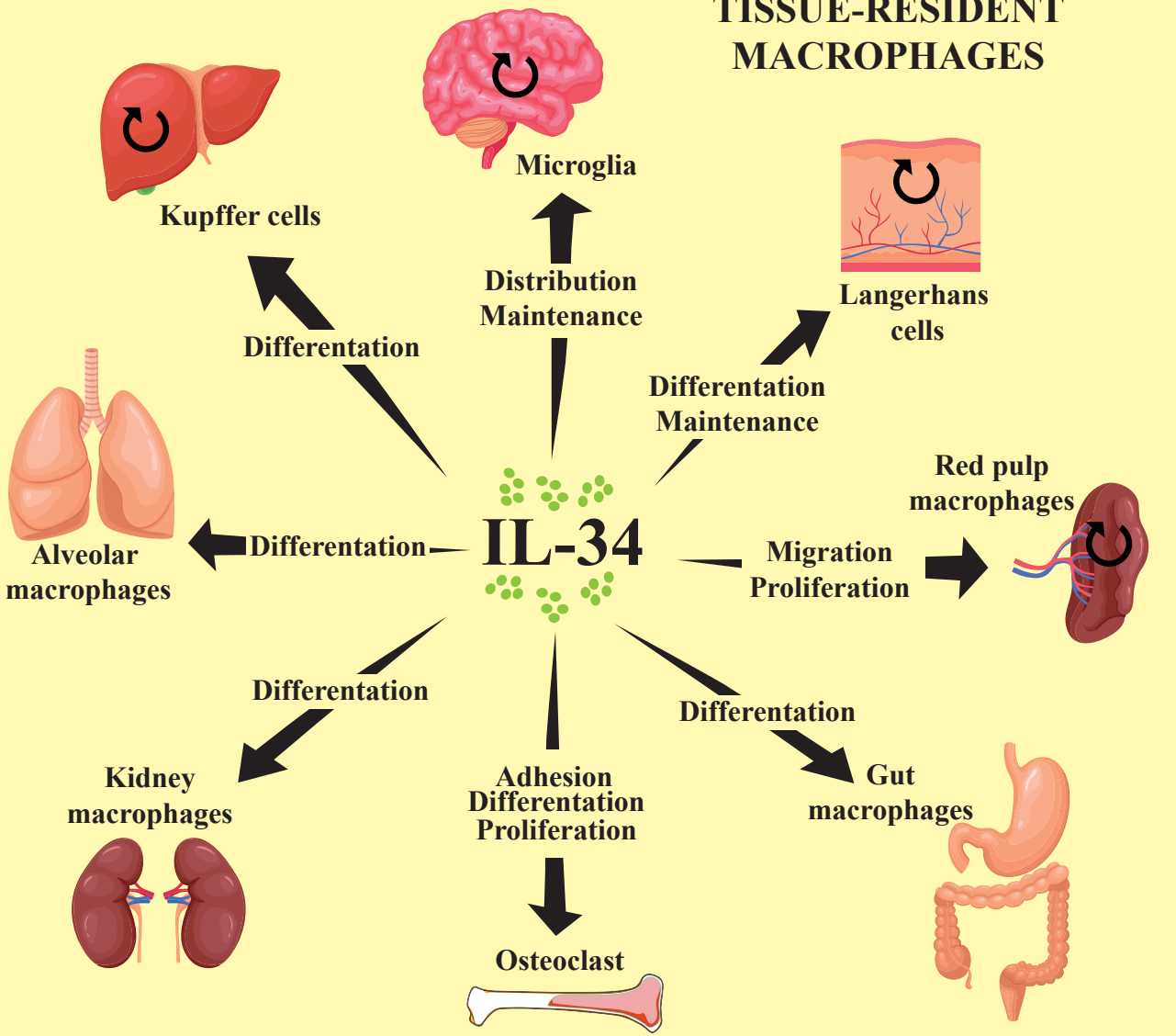
1212 **Figure 4: IL-34 is a pro-M2 macrophage differentiation factor.** Macrophage isolation and  
1213 treatment were performed as described in (Guihard, et al., 2012). A) IL-34 treatment induced  
1214 macrophage differentiation with an M2 phenotype, alone or in combination with IL-4 and IL-  
1215 10. Macrophages were treated with IL-34 (50 ng/ml) or in combination with IFN- $\gamma$  (50 ng/ml;

1216 pro M1), IL-4 (50 ng/ml; pro M2a), and IL-10 (50 ng/ml; pro M2c), for 2 days and cells were  
1217 analysed by means of flow cytometry using specific antibodies for both M1-like macrophages  
1218 (CD14, CD86 and CD64) and M2-like macrophages (CD163, CD200R and CD206). **B)**  
1219 Comparison of macrophage differentiation after treatment with the cytokines GM-CSF (20  
1220 ng/ml), M-CSF (50 ng/ml) and IL-34 (50 ng/ml) alone or in combination with IFN- $\gamma$ , IL-4 or  
1221 IL-10 as performed in A. The three cytokines in combination with IFN- $\gamma$  were able to induce  
1222 M1 macrophage differentiation as shown by the increase in CD64, an M1 marker. IL-34  
1223 modulates M2 markers (CD163, CD200R and CD206) alone or in combination with IL-4 and  
1224 IL-10. No effect of IL-34 was observed in CD14 expression. Overall, IL-34 was able to  
1225 induce M1 and M2 macrophage differentiation with a specific increase in CD163, an M2  
1226 marker.  
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1228

**A****M-CSF  
(homodimer)****B****IL-34  
(homodimer)**



**TISSUE-RESIDENT MACROPHAGES**



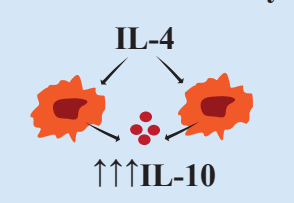
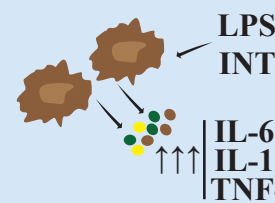
**NON-RESIDENT MACROPHAGES**

Monocytes

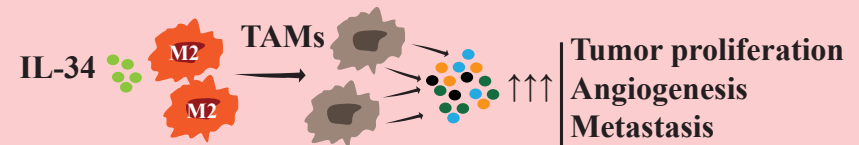
**IL-34**

**M1**  
Pro-inflammatory

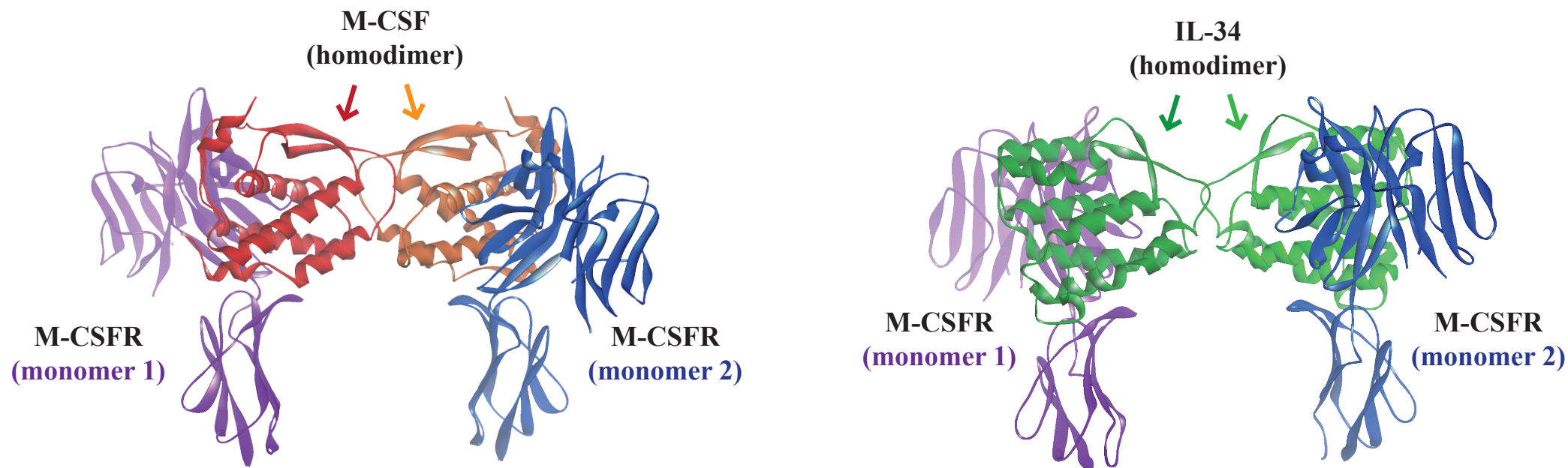
**M2**  
Anti-inflammatory



- IL-34**
- **INFECTION**  
Macrophage resistance to bacterial & virus  
Favor viral expansion  
Pro-inflammatory activity
  - **INFLAMMATION**  
Pro- or anti-inflammatory activity  
Neuroprotective or neurotoxic activity  
Immunotolerance
  - **CANCER**  
Pro- or Anti-tumorigenic activity  
Activation and proliferation of TAMs

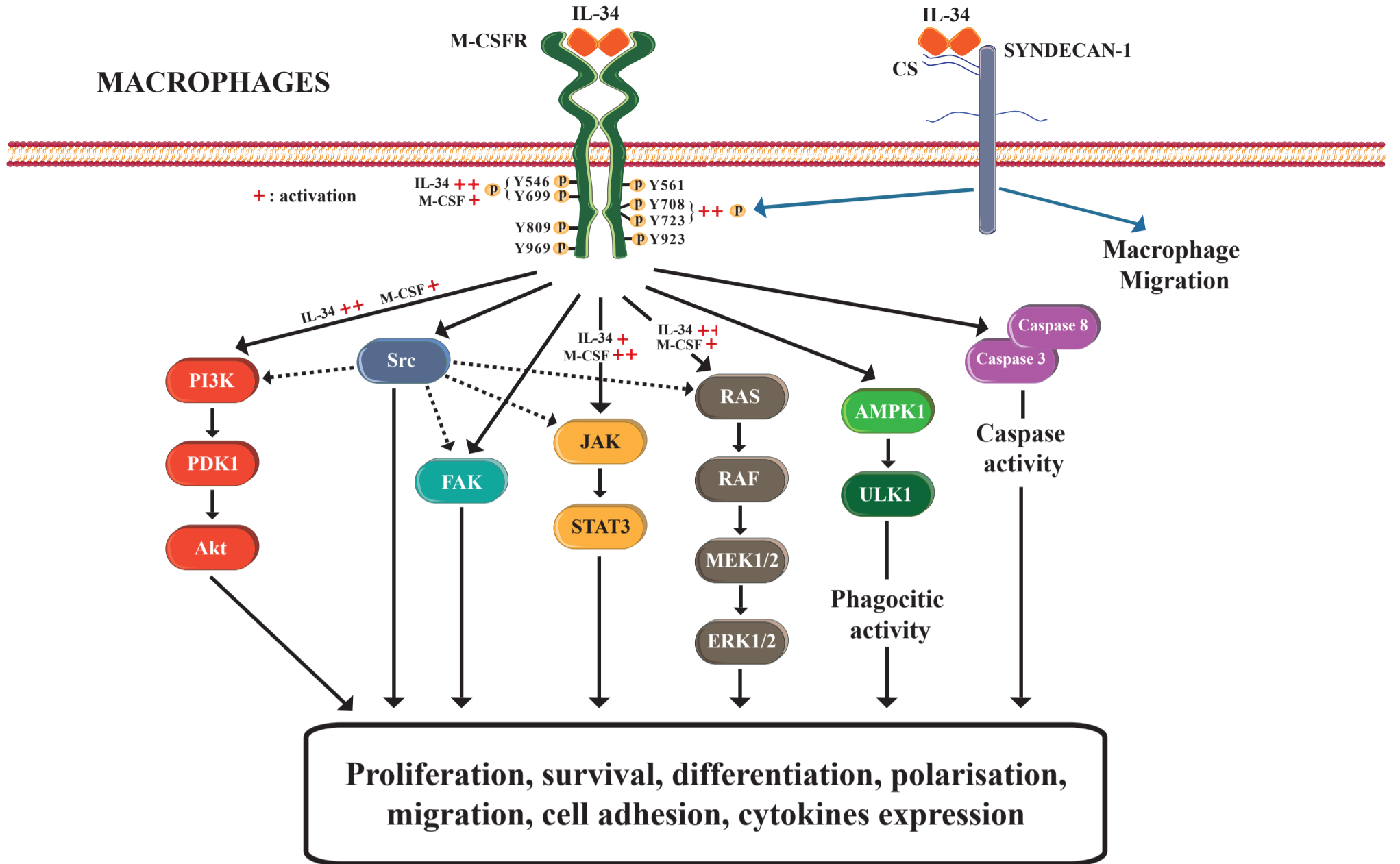




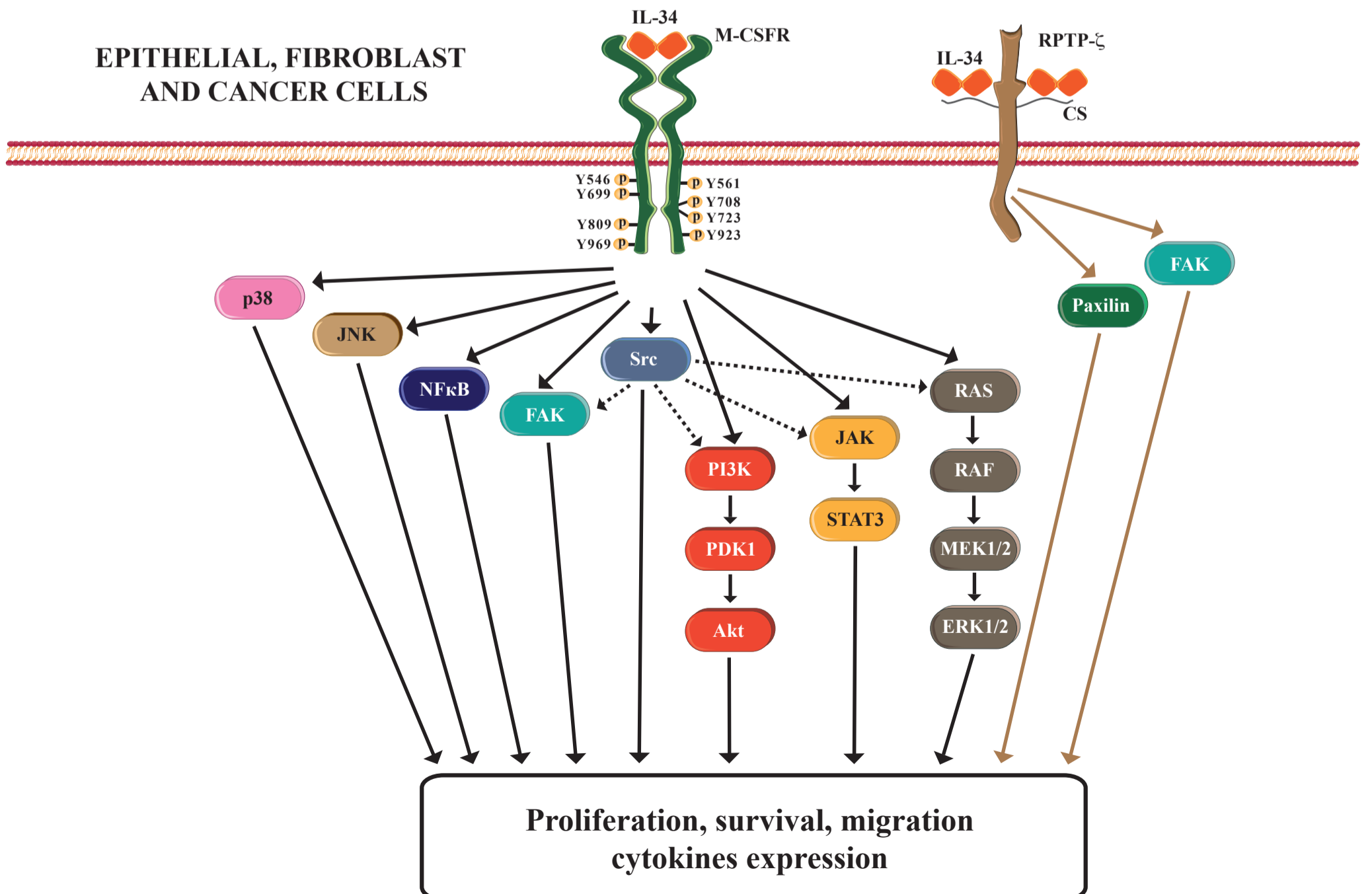
**A****Table 1**

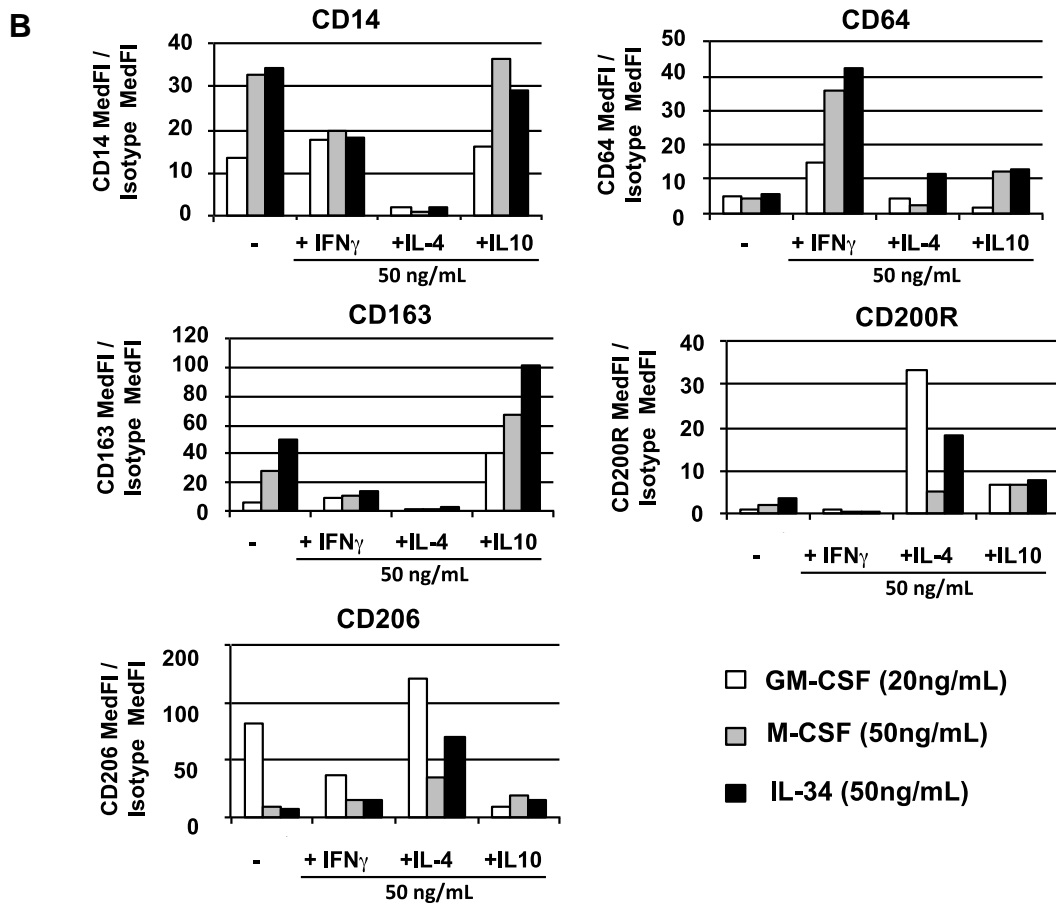
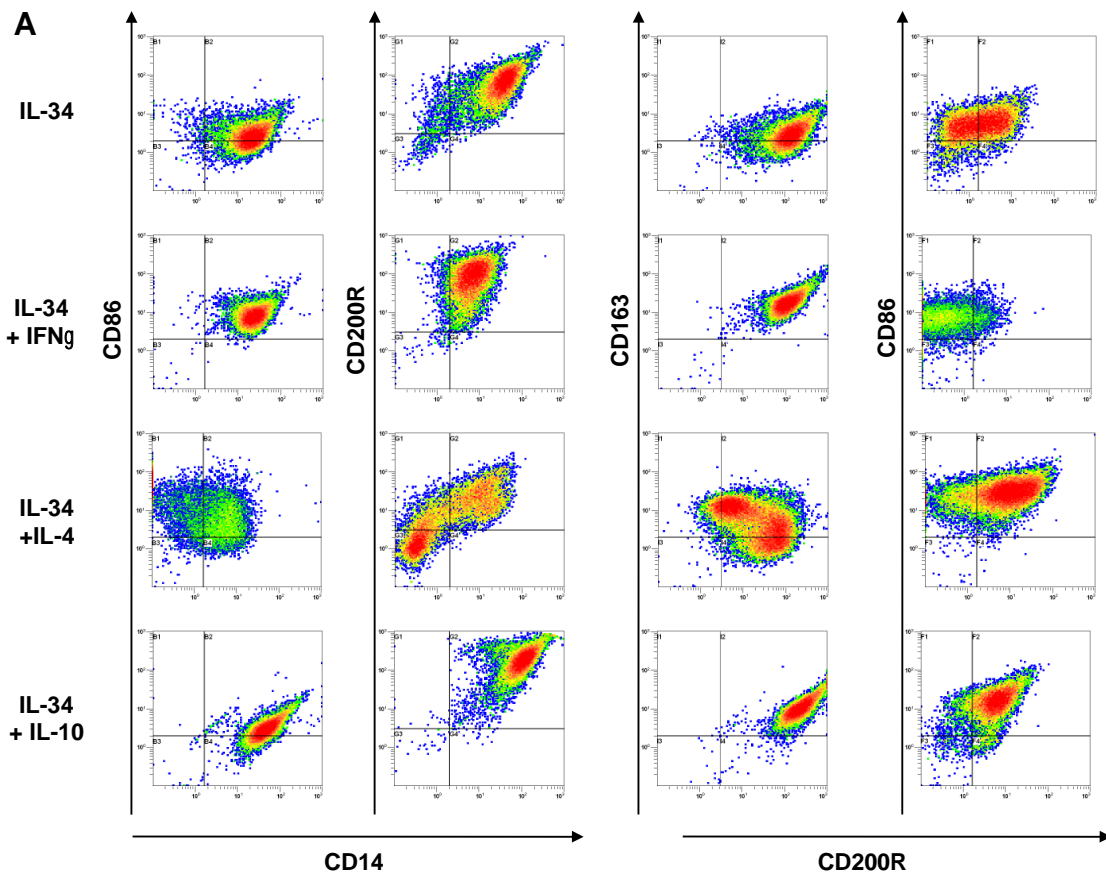
	Bound nature	$K_D$	$K_{on}$	$K_{off}$
M-CSF : M-CSFR	hydrophilic	1 pM	$6.29 \times 10^7 \text{ s}^{-1} \text{ M}^{-1}$	$6.55 \times 10^{-5} \text{ s}^{-1}$
IL-34 : M-CSFR	hydrofobic	34 pM	$1.7 \times 10^7 \text{ s}^{-1} \text{ M}^{-1}$	$6.03 \times 10^{-4} \text{ s}^{-1}$

A

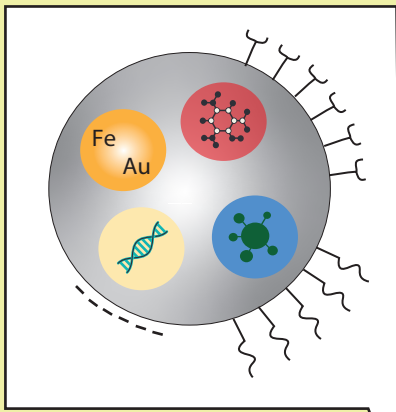


B

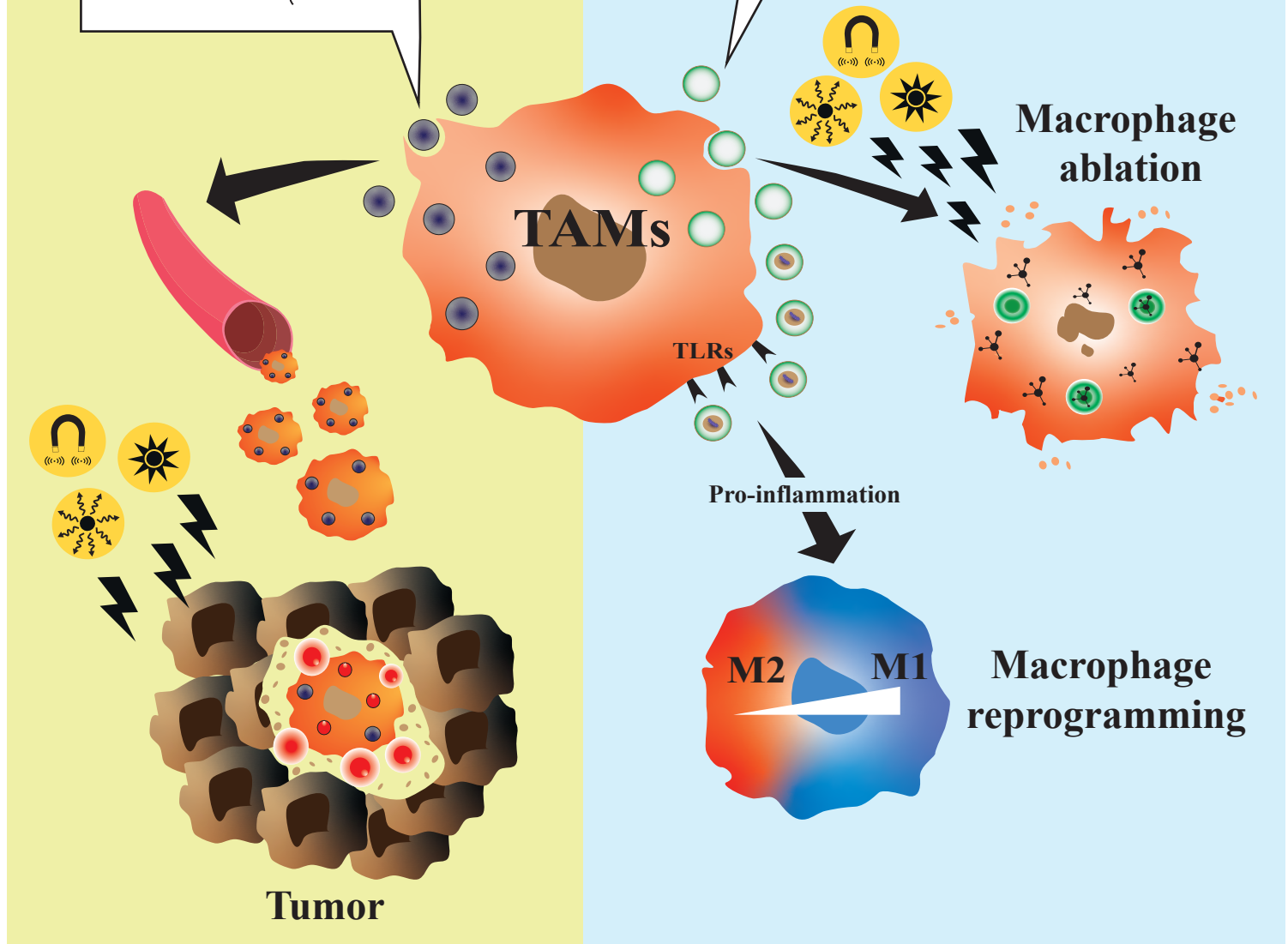
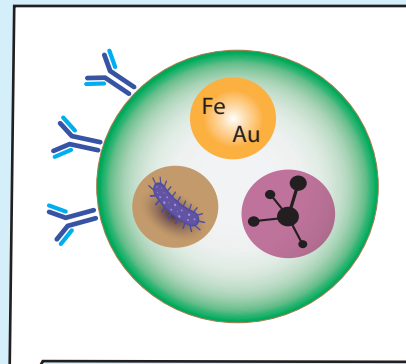














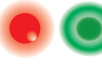





# Drug delivery system



# Macrophage targeting



- |   |                               |   |                                  |   |                             |
|---|-------------------------------|---|----------------------------------|---|-----------------------------|
|  | <b>Tumour Cells</b>           |  | <b>Mechanic element</b>          |  | <b>Bacto therapy</b>        |
|  | <b>M2-like macrophage</b>     |  | <b>siRNA therapy</b>             |  | <b>Viral therapy</b>        |
|  | <b>M1-like macrophage</b>     |  | <b>Chemo therapy</b>             |  | <b>Blocking Antibodies</b>  |
|  | <b>Nanoparticle</b>           |  | <b>ROS</b>                       |  | <b>TLR receptors</b>        |
|  | <b>Activated nanoparticle</b> |  | <b>O<sub>2</sub> Radical</b>     |  | <b>Coating modification</b> |
|   |                               |  | <b>Electromagnetic radiation</b> |   |                             |