

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

Javier Muñoz-Garcia, Denis Cochonneau, Stéphane Télétchéa, Emilie Moranton, Didier Lanoe, Régis Brion, Frédéric Lézot, Marie-Françoise Heymann, Dominique Heymann

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| 1 | The twin cytokines Interleukin-34 and M-CSF: |
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| 4 5 6 | Javier Muñoz-Garcia ^{1,2,*} , Denis Cochonneau ¹ , Emilie Moranton ¹ , Régis Brion ³ , Stéphane Télétchéa ⁴ , Didier Lanoe ¹ , Frédéric Lézot ³ , Marie-Françoise Heymann ¹ , Dominique Heymann ^{1,5,*} |
| U | 1 |
| 7 8 | ¹ Université de Nantes, INSERM, U1232, CRCiNA, Institut de Cancérologie de l'Ouest, Saint-Herblain, F-44805, France; |
| 9 | ² SATT Ouest Valorisation, Nantes, France; |
| 10 | ³ Université de Nantes, INSERM, U1238, PhyOs, Nantes, France |
| 11 | ⁴ UFIP, Université de Nantes, CNRS, UMR 6286, Nantes, France |
| 12 | ⁵ Department of Oncology and Metabolism, Medical School, University of Sheffield, |
| 13 | Sheffield, UK |
| 14 | |
| 15 | Short title for the running head: IL-34 and M-CSF in the biology of macrophages |
| 16 | |
| 17 | Corresponding Authors: |
| 18 | Dr Javier Muñoz-Garcia |
| 19 | Institut de Cancérologie de l'Ouest, Inserm U1232 |
| 20 | Blvd Jacques Monod, 44805 Saint-Herblain, France |
| 21 | Email: javier.munoz@ouest-valorisation.fr |
| 22 | |
| 23 | Prof. Dominique Heymann |
| 24 | Institut de Cancérologie de l'Ouest, Inserm U1232 |
| 25 | Blvd Jacques Monod, 44805 Saint-Herblain, France |
| 26 | Email: dominique.heymann@univ-nantes.fr |
| 27 | Tel: +33 (0) 240 679 841 |

28 Abstract

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

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63 Introduction

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

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

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

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

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

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120 1.1 M-CSF/ CD115/ IL-34: more than a "ménage à trois"

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

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

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

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

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179 **1.2 IL-34**, a promiscouos 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- ζ) and the transmembrane heparin sulfate proteoglycan syndecan-1 (or CD138) (Nandi, et al., 2013; Segaliny, et al., 2015). The 182 high expression of IL-34 in different areas of the adult brain where CD115 was absent suggest 183 the existence of an additional receptor for IL-34 (Nandi, et al., 2012). By using a CD115 184 depleted U251 glioblastoma cell line and affinity chromatography, Nandi et al. demonstrated 185 that IL-34 bound specifically to PTP- ζ in a chondroitin sulfate-dependent manner. The 186 interaction between IL-34 and PTP- ζ induced tyrosine phosphorylations of the FAK and 187 paxilin proteins, impairing the cell proliferation and motility of U251 glioblastoma cells. 188 These results suggest that the IL-34/PTP- ζ complex acts as a tumurigenic suppressor in 189 glioblastoma (Nandi, et al., 2013). PTP- ζ was also expressed in the instestinal tissue of 190 healthy people, mainly in the colon, whereas IL-34 was mainly expressed in the ileum. 191 However, in inflammatory bowel diseases, IL-34 was coexpressed in the same regions as 192 PTP- ζ and CD115 (Zwicker, et al., 2016). Franzé et al. showed that IL-34 was over-193 expressed in colorectal cancer (CRC) tissues and PTP- ζ was also expressed in tumoral and 194 non-tumoral areas of CRC samples. However, an increase in CD115 expression alone, and not 195 PTP- ζ , was observed in CRC cells (Franzè, et al., 2018). Additional studies are needed to 196 decipher the role of the IL-34/PTP- ζ complex in these tissues. 197

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

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The cytokine M-CSF can be expressed in three different isoforms: a secreted glycoprotein, a 205 206 secreted proteoglycan and a membrane-spanning cell surface glycoprotein (Pixley & Stanley, 2004). Recently, Ogawa et al. showed the existence of a cell surface isoform of IL-34 207 208 (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 participation of the molecular chaperone 78-KDa glucose-regulated protein (GRP78) (Ogawa, 211 212 et al., 2019). How the cell-surface IL-34 variant induces the differentiation of monocytes remains unclear. All these data are evidence of new potential targets that need to be taken into 213 214 account when developing further therapies against IL-34.

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216 **2. From monocytes to "non-resident" macrophages**

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

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

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

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

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267 2.2 IL-34-differentiated macrophages in viral infections

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

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Infection by the human immunodeficiency virus-1 (HIV-1) is characterised by the loss of T 271 lymphocytes in a progressive manner and susceptibility to opportunistic infections (Sattentau 272 & Stevenson, 2016). IL-34-induced macrophages were characterised by better resistance to 273 274 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, et al., 2018). However, even if the immune system tries to slow down the progression of HIV-277 278 1, the virus can invade the central nervous system (CNS) in the early stages of infection, inducing severe neurotoxic effects (Valcour, et al., 2012). Mathews et al. engineered a 279 280 humanised mouse model that produces human microglia and mimics viral infections (Mathews, et al., 2019). The authors demonstrated that IL-34 was responsible for microglia 281 282 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 (Mathews, et al., 2019). 284

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

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

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

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

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

macrophages. Similar to HIV-1 virus infection, FV3 infection induced the expression of 333 antiviral restriction factors IFNX, INOS and APOBEC in IL-34-activated macrophages 334 (Yaparla, et al., 2018). Consequently, an increase in toll-like receptor 2 and 4 transcripts was 335 detected in macrophages implicated in the recognition of bacterial cell wall 336 lipopolysaccharide (LPS), as well as in the secretion of antiviral interferon IFN7 and TNF-α. 337 Overall, these data suggest that IL-34 induces differential transcription programmes and 338 functions compared to M-CSF during viral infection in high vertebrates (Yaparla, et al., 339 340 2019).

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

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

suggests that, in particular conditions, both cytokines may create a new heterodimer cytokine 367 that may bind to CD115 (Ségaliny, et al., 2015). This new cytokine may result in specific 368 functions of myeloid and cancer cells during lung cancer development (Baghdadi, et al., 369 370 2018). Another study showed that nitric oxide therapy in tumorigenic castration-resistant prostate cancer xenografts reduced tumour progression and correlates with a decrease in the 371 IL-34-derivated TAM population (Arora, et al., 2018). Taken together, these observations 372 suggest that IL-34 may play an essential role in both the pro-tumorigenic polarisation of 373 TAMs and tumour progression, as revealed in various other cancers. In colorectal cancer, high 374 375 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., 2019). Similar observations were obtained in hepatocellular carcinoma (Zhou, et al., 2016; 378 379 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 subtypes, whereas IL-34 was associated with poor prognosis in basal breast tumours (Zins, et 382 383 al., 2018).

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

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

397

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

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

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

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

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

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

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In relation with myeloid differentiation, multiple myeloma (MM) is a haematological disease 473 474 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- β , IL- β , TNF- α and surprisingly TGF- β (Chemel, et al., 2017). IL-34 was also detected in bone 476 marrow fluids from MM patients. In pathological context of MM, IL-34 promoted CD14⁺ 477 monocyte differentiation into osteoclasts by upregulating the expression levels of 478 osteoclastogenesis-related genes such us DC-STAMP and OC-STAPM. IL-34 did not appear 479 480 essential for osteoclast differentiation in MM but IL-34 activation accelerated the osteolytic process and bone lesions in patients (Baghdadi, et al., 2019). 481

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483 **3.2 IL-34 in microglia differentiaton and neural disorders**

Microglia are the resident macrophage population of the central neural system (CNS) and 484 485 present neurotoxic and neuroprotective activities (Nakajima & Kohsaka, 2001). Microglia development depends on CD115 activity and, by consequence, its ligands M-CSF and IL-34 486 (Dai, et al., 2002). IL-34 is basically expressed by neurons whereas M-CSF is expressed by 487 astrocytes, microglia and oligodendrocytes (Cahoy, et al., 2008; Mizuno, et al., 2011). M-CSF 488 or IL-34 knockout mice highlighted the harmful impact of one of these two cytokines on the 489 490 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 development of embryonic microglia cells but appeared crucial for their maintenance during 492 adulthood (Greter, et al., 2012). In agreement with this, Easley-Neal et al. demonstrated the 493 essential role played by IL-34 in microglia support during postnatal life using specific 494 495 function-blocking antibodies against each CD115 ligands (Easley-Neal, et al., 2019). Only M-CSF seemed to be required to establish microglia in the embryonic brain. In adult mice, IL-34 496 497 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

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

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

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

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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).

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551 Several articles support the evidence that IL-34-derived microglia are necessary for viral and prion protection in the CNS. As previously mentioned, HIV-1 invades the CNS, generating 552 553 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 within the hippocampus region. IL-34-deficient mice were resistant to WNV and seemed 556 557 protected against the infection as shown by their minor loss of neural synapses. However, the main role of IL-34 in this infection remains unknown (Vasek, et al., 2016). 558

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

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

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

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

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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 pathogenic context. Gut macrophages originate in bone marrow. Some authors reported the 606 607 existence of a heterogeneous population of macrophages in the intestine, divided into two major populations: resident macrophages and inflammatory macrophages (Platt, et al., 2010; 608 609 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 conditions, gut macrophages underwent a continuum of differentiation from immature to fully 612 613 mature subsets. Initially, macrophages were characterised by high expression of Ly6C. Then, during the maturation process, the macrophage population changed from high to low Ly6C 614 615 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 phagocyte activity as well as production of IL-10 (Bain, et al., 2013). On the other hand, in 617 inflammatory bowel diseases (IBD), macrophage populations quickly turned to pro-618 inflammatory subsets characterised by high expression of Ly6C and CD14 (Bain, et al., 619 620 2013).

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

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

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

Kupffer cells (KCs) are tissue-resident macrophages in the liver. KCs are responsible for the 646 647 homeostasis of this organ in both healthy and pathological situations (Li, et al., 2017). Like LCs, KCs originate in the foetal liver and yolk sac. In the adult liver, the FC population is 648 649 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 proinflammatory subtype leading to a Th1 response, or into an M2 anti-inflammatory subtype 651 leading to a Th2 response. The latter is associated with inducing and maintaining immune 652 tolerance during liver transplantation (Atif, et al., 2020). Rat and human regulatory T (Treg) 653 cells FOXP3⁺ CD4⁺ or CD8⁺ cells expressed IL-34 (Bézie, et al., 2015). Treg FOXP3⁺ cells, 654 the main players in immune tolerance, seemed sensitive to IL-34 (Atif, et al., 2020). IL-34 655 treatment effectively seemed to induce Treg FOXP3⁺ cell proliferation by polarising CD14⁺ 656 macrophages that over-expressed arginase-1 and inducible NO synthase, both implicated in 657 the inhibition of T lymphocyte proliferation (Bézie, et al., 2015). These data suggest the 658 presence of a positive feedback loop between IL-34 expression and FOXP3⁺ Tregs. 659 Interestingly, the fact that IL-34 targeted FOXP3⁺ Tregs, which are essential for inhibiting the 660 anti-donor immune response during allografts, suggests that IL-34 plays a potential role in 661 transplantation therapy (Bézie, et al., 2015). In agreement with these data, during pregnancy; 662 IL-34 induced macrophage polarisation into an M2 state, suggesting that IL-34 may play a 663 664 role in maintaining tolerance during the gestation process (Lindau, et al., 2018). Reinforcing the role of IL-34 in immune tolerance, Zhao et al. showed that IL-34 treatment inhibited acute 665 666 rejection in liver transplant in rats. The authors observed that IL-34 specifically induced the polarisation of KCs from an M1 to an M2 status. This function was mediated by activation of the PI3K/AKT/mTOR pathway and was inhibited by rapamycine, an inhibitor of the mTOR pathways (Baek, et al., 2015). Recently, Jiant *et al.* reported that in zebrafish, *in vivo* ectopic expression of IL-34 in the liver or skin induced migration of macrophages to both regions (Jiang, et al., 2019). Consistent with previous studies, IL-34 may have a protective effect by mobilising and polarising macrophages in the liver (Jiang, et al., 2019).

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In non-alcoholic fatty liver disease (NAFLD), a positive correlation between IL-34 levels in 674 675 serum and the severity of NAFLD has been described (Shoji, et al., 2016). IL-34 production by liver fibroblasts was enhanced by TNF-α (Shoji, et al., 2016). In addition, IL-34 induced 676 MIP $3\alpha^+$ /CCL 20^+ macrophage differentiation, which promoted the production of collagen by 677 hepatic stellate cells and subsequently development of fibrosis (Preisser, et al., 2014; Shoji, et 678 al., 2016). The role of IL-34 in fibrosis was recently confirmed. Soluble egg antigen (SEA) 679 produced by schistosome egg impairs NF-kB activation in hepatic stellate cells. This inhibits 680 TNF-α, blocking the IL-34-associated liver fibrosis (Chen, et al., 2019). Consequently, IL-34 681 682 may be used as an independent marker for liver fibrosis in NAFLD (Shoji, et al., 2016).

683

684 Conclusion

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

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

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

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

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

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

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

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781 **References**

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- Alothaimeen, T. et al., "in press". Granulocyte/Macrophage Colony-Stimulating Factor-Derived
 Macrophages Exhibit Distinctive Early Immune Response to Lymphocytic Choriomeningitis Virus
 Infection. *Viral Immunol.*
- Arora, H. et al., 2018. Alterations of tumor microenvironment by nitric oxide impedes castrationresistant prostate cancer growth. *Proceedings of the National Academy of Sciences of the United States of America*, 30 10, 115(44), pp. 11298-11303.
- Atif, M. et al., 2020. Regulatory T cells in solid organ transplantation. *Clinical & Translational Immunology*, 9(2), p. e01099.
- Baek, J. H. et al., 2015. IL-34 mediates acute kidney injury and worsens subsequent chronic kidney
 disease. *Journal of Clinical Investigation*, 3 8, 125(8), pp. 3198-3214.
- Baghdadi, M. et al., 2018. High co-expression of IL-34 and M-CSF correlates with tumor progression
 and poor survival in lung cancers. *Scientific Reports*, 1 12, 8(1), p. 418.
- Baghdadi, M. et al., 2019. A role for IL-34 in osteolytic disease of multiple myeloma. *Blood Advances*,
 26 2, 3(4), pp. 541-551.
- 797 Baghdadi, M. et al., 2016. Chemotherapy-induced IL34 enhances immunosuppression by tumor-
- associated macrophages and mediates survival of chemoresistant lung cancer cells. *Cancer Research,* 15 10, 76(20), pp. 6030-6042.

- 800 Bain, C. C. et al., 2013. Resident and pro-inflammatory macrophages in the colon represent
- alternative context-dependent fates of the same Ly6C hi monocyte precursors. *Mucosal Immunology*,
 5, 6(3), pp. 498-510.
- 803 Batra, P., Das, S. & Patel, P., 2019. Comparative evaluation of Gingival Crevicular Fluid (GCF) levels of
- 804 Interleukin-34 levels in periodontally healthy and in patients with chronic and aggressive
- periodontitis- A cross-sectional study. *Saudi Dental Journal*, 17, 31(3), pp. 316-321.
- Baud'Huin, M. et al., 2010. Interleukin-34 is expressed by giant cell tumours of bone and plays a key
 role in RANKL-induced osteoclastogenesis. *Journal of Pathology*, 221(1), pp. 77-86.
- Bézie, S. et al., 2015. IL-34 is a Treg-specific cytokine and mediates transplant tolerance. *Journal of Clinical Investigation*, 1 10, 125(10), pp. 3952-3964.
- 810 Biron, C. A., 2016. Innate Immunity: Recognizing and Responding to Foreign Invaders-No Training
- Needed. In: *Viral Pathogenesis: From Basics to Systems Biology.* : Third Edition ed. s.l.:Elsevier Inc.,
 pp. 41-55.
- Booker, B. E., Clark, R. S., Pellom, S. T. & Adunyah, S. E., 2015. Interleukin-34 induces monocytic-like
 differentiation in leukemia cell lines. *Int J Biochem Mol Biol*, 6(1), pp. 1-16.
- 815 Boström, E. A. & Lundberg, P., 2013. The newly discovered cytokine IL-34 is expressed in gingival
- 816 fibroblasts, shows enhanced expression by pro-inflammatory cytokines, and stimulates osteoclast
- 817 differentiation. *PLoS ONE*, 10 12, 8(12), p. e81665.
- Boulakirba, S. et al., 2018. IL-34 and CSF-1 display an equivalent macrophage differentiation ability
 but a different polarization potential. *Scientific Reports*, 1 12, 8(1), p. 256.
- 820 Cahoy, J. D. et al., 2008. A transcriptome database for astrocytes, neurons, and oligodendrocytes: A
- new resource for understanding brain development and function. *Journal of Neuroscience*, 2 1, 28(1),
 pp. 264-278.
- Cannarile, M. A. et al., 2017. Colony-stimulating factor 1 receptor (CSF1R) inhibitors in cancer
 therapy. *Journal for immunotherapy of cancer*, 18 7, 5(1), p. 53.
- 825 Cassetta, L. & Pollard, J. W., 2018. *Targeting macrophages: Therapeutic approaches in cancer.*826 s.l.:Nature Publishing Group.
- 827 Chemel, M. et al., 2017. Bone Morphogenetic Protein 2 and Transforming Growth Factor β1 Inhibit
- the Expression of the Proinflammatory Cytokine IL-34 in Rheumatoid Arthritis Synovial Fibroblasts. *American Journal of Pathology*, 1 1, 187(1), pp. 156-162.
- Chemel, M. et al., 2012. Interleukin 34 expression is associated with synovitis severity in rheumatoid
 arthritis patients. *Annals of the Rheumatic Diseases*, 1, 71(1), pp. 150-154.
- Cheng, S.-T.et al., 2017. Interleukin-34 inhibits hepatitis B virus replication in vitro and in vivo. *PLoS ONE*, 12(6), p. e0179605.
- Cheng, X., Wan, Q. L. & Li, Z. B., 2017. AG490 suppresses interleukin-34-mediated osteoclastogenesis
 in mice bone marrow macrophages. *Cell Biology International*, 1 6, 41(6), pp. 659-668.
- 836 Chen, L. et al., 2019. Schistosoma japonicum soluble egg antigen inhibits TNF-α-induced IL-34
- expression in hepatic stellate cells. *Parasitology Research*, 14 2, 118(2), pp. 551-557.

- Chen, T. et al., 2014. Embryonic stem cells promoting macrophage survival and function are crucial
 for teratoma development. *Frontiers in Immunology*, 5(JUL), p. 275.
- 840 Chen, Z. et al., 2011. The critical role of IL-34 in osteoclastogenesis. *PLoS ONE,* 6(4), p. e18689.

Chihara, T. et al., 2010. IL-34 and M-CSF share the receptor Fms but are not identical in biological
activity and signal activation. *Cell Death and Differentiation*, 12, 17(12), pp. 1917-1927.

- 843 Ciccia, F. et al., 2013. IL-34 is overexpressed in the inflamed salivary glands of patients with Sjögren's
- syndrome and is associated with the local expansion of pro-inflammatory CD14brightCD16+
 monocytes. *Rheumatology (Oxford, England),* 6, 52(6), pp. 1009-1017.
- Cui, M. y. et al., 2019. Effects of IL-34 on the secretion of RANKL/OPG by fibroblast-like synoviocytes
 and peripheral blood mononuclear cells in rheumatoid arthritis. *European Cytokine Network*, 16,
- 848 30(2), pp. 67-73.
- Dai, X.-M.et al., 2002. Targeted disruption of the mouse colony-stimulating factor 1 receptor gene
- results in osteopetrosis, mononuclear phagocyte deficiency, increased primitive progenitor cell
 frequencies, and reproductive defects. *Blood*, 99(1), pp. 111-20.
- Davies, L. C. & Taylor, P. R., 2015. Tissue-resident macrophages: Then and now. *Immunology*, 1 4,
 144(4), pp. 541-548.
- Detry, S. et al., 2019. Revisiting the combinatorial potential of cytokine subunits in the IL-12 family. *Biochemical Pharmacology*, 1 7, Volume 165, pp. 240-248.
- Droin, N. & Solary, E., 2010. Editorial: CSF1R, CSF-1, and IL-34, a "ménage à trois" conserved across
 vertebrates. *Journal of Leukocyte Biology*, *5*, 87(5), pp. 745-747.
- 858 Duplomb, L. et al., 2008. Interleukin-6 Inhibits Receptor Activator of Nuclear Factor κB Ligand-
- 859 Induced Osteoclastogenesis by Diverting Cells into the Macrophage Lineage: Key Role of Serine727
- 860 Phosphorylation of Signal Transducer and Activator of Transcription 3. *Endocrinology*, 149(7), p.
- 861 3688–3697.
 - 862 Easley-Neal, C. et al., 2019. CSF1R Ligands IL-34 and CSF1 Are Differentially Required for Microglia
 - B63 Development and Maintenance in White and Gray Matter Brain Regions. *Frontiers in Immunology*, 20B64 9, Volume 10, p. 2199.
- Eda, H., Shimada, H., Beidler, D. R. & Monahan, J. B., 2011. Proinflammatory cytokines, IL-1β and
- TNF-α, induce expression of interleukin-34 mRNA via JNK- and p44/42 MAPK-NF-κB pathway but not
 p38 pathway in osteoblasts. *Rheumatology International*, 11, 31(11), pp. 1525-1530.
- 868 Elkhider, A. et al., "in press". IL-34 Modulates Rheumatoid Synovial Fibroblasts Proliferation and
 869 Migration via ERK/AKT Signalling Pathway. *Clinical and experimental rheumatology.*
- Endo, H. et al., 2019. Interleukin-34 expression in ovarian cancer: A possible correlation with disease
 progression. *International Immunology*, 3 12, 32(3), pp. 175-186.
- 872 Foucher, E. D. et al., 2013. IL-34 Induces the Differentiation of Human Monocytes into
- 873 Immunosuppressive Macrophages. Antagonistic Effects of GM-CSF and IFNγ. *PLoS ONE*, 8 2, 8(2), p.
 874 e56045.
- Franken, L., Schiwon, M. & Kurts, C., 2016. Macrophages: Sentinels and regulators of the immune
 system. *Cellular Microbiology*, 1 4, 18(4), pp. 475-487.

- Franzè, E. et al., "in press". Interleukin-34 Stimulates Gut Fibroblasts to Produce Collagen Synthesis. *Journal of Crohn's and Colitis*, 9 4.
- Franzè, E. et al., 2018. Interleukin-34 sustains pro-tumorigenic signals in colon cancer tissue. *Oncotarget*, 9(3), pp. 3432-3445.
- Franzè, E. et al., 2016. Interleukin-34 induces Cc-chemokine ligand 20 in gut epithelial cells. *Journal of Crohn's and Colitis*, 1 1, 10(1), pp. 87-94.
- Franzé, E. et al., 2015. Interleukin-34 sustains inflammatory pathways in the gut. *Clinical Science*,
 129(3), pp. 271-280.
- Galligan, C. L. & Fish, E. N., 2017. Interleukin-34 Promotes Fibrocyte Proliferation. *Journal of Interferon and Cytokine Research*, 1 10, 37(10), pp. 440-448.
- Geissmann, F. et al., 2010. Development of Monocytes, Macrophages, and Dendritic Cells. *Science*,
 327(5966), pp. 656-61.
- 889 Ge, Y., Huang, M. & Yao, Y. M., 2019. Immunomodulation of interleukin-34 and its potential
- significance as a disease biomarker and therapeutic target. *International journal of biological sciences*, 15(9), pp. 1835-1845.
- Ginhoux, F. et al., 2006. Langerhans cells arise from monocytes in vivo. *Nature Immunology*, 3, 7(3),
 pp. 265-273.
- 6 Giricz, O. et al., 2018. The RUNX1/IL-34/CSF-1R axis is an autocrinally regulated modulator of resistance to BRAF-V600E inhibition in melanoma. *JCI insight*, 26 7, 3(14), p. e120422.
- Gorczynski, R. M., 2020. IL-17 Signaling in the Tumor Microenvironment. *Adv Exp Med Biol*, Volume
 1240, pp. 47-58.
- Gow, D. J. et al., 2012. Cloning and expression of porcine Colony Stimulating Factor-1 (CSF-1) and
 Colony Stimulating Factor-1 Receptor (CSF-1R) and analysis of the species specificity of stimulation by
- 900 CSF-1 and Interleukin 34. *Cytokine*, 60(3), pp. 793-805.
- Grayfer, L. & Robert, J., 2016. Amphibian macrophage development and antiviral defenses.
 Developmental and Comparative Immunology, 1 5, Volume 58, pp. 60-67.
- Greter, M. et al., 2012. Stroma-Derived Interleukin-34 Controls the Development and Maintenance of
 Langerhans Cells and the Maintenance of Microglia. *Immunity*, 14 12, 37(6), pp. 1050-1060.
- Guihard, P. et al., 2012. Induction of osteogenesis in mesenchymal stem cells by activated
 monocytes/macrophages depends on oncostatin M signaling. *Stem Cells*, 4, 30(4), pp. 762-772.
- Guilbert, L. J. & Stanley, E. R., 1980. Specific Interaction of Murine Colony-Stimulating Factor With
 Mononuclear Phagocytic Cells. *J Cell Biol*, 85(1), pp. 153-9.
- Guilliams, M., Mildner, A. & Yona, S., 2018. Developmental and Functional Heterogeneity of
 Monocytes. *Immunity*, 16 10, 49(4), pp. 595-613.
- Guilliams, M., Thierry, G. R., Bonnardel, J. & Bajenoff, M., 2020. Establishment and Maintenance of
 the Macrophage Niche. *Immunity*, 17 3, 52(3), pp. 434-451.
- 913 Han, N. et al., 2018. Enhanced IL-34 expression in Nivolumab-resistant metastatic melanoma.
- 914 Inflammation and Regeneration, 12, 38(1), p. 3.

- Hashimoto, D. et al., 2013. Tissue-resident macrophages self-maintain locally throughout adult life
 with minimal contribution from circulating monocytes. *Immunity*, 18 4, 38(4), pp. 792-804.
- 917 Heymann, D. et al., 1998. Cytokines, Growth Factors and Osteoclasts. *Cytokine*, 10(3), pp. 155-68.
- Heymann, D. & Rousselle, A. V., 2000. gp130 cytokine family and bone cells. *Cytokine*, 12(10), pp.
 1455-1468.
- Hoang, H. H., Wang, P. C. & Chen, S. C., 2020. Interleukin 34 serves as a novel molecular adjuvant
- against nocardia seriolae infection in largemouth bass (Micropterus salmoides). *Vaccines*, 1 4, 8(2), p.
 E151.
- Hoeffel, G. & Ginhoux, F., 2018. Fetal monocytes and the origins of tissue-resident macrophages. *Cellular Immunology*, 1 8, Volume 330, pp. 5-15.
- 925 Hoeffel, G. et al., 2012. Adult Langerhans cells derive predominantly from embryonic fetal liver
- 926 monocytes with a minor contribution of yolk sac-derived macrophages. *Journal of Experimental*927 *Medicine*, 4 6, 209(6), pp. 1167-1181.
- Hume, D. A., Irvine, K. M. & Pridans, C., 2019. The Mononuclear Phagocyte System: The Relationship
 between Monocytes and Macrophages. *Trends in Immunology*, 1 2, 40(2), pp. 98-112.
- Jaumouillé, V. & Grinstein, S., 2016. Molecular Mechanisms of Phagosome Formation. *Microbiology Spectrum*, 2 6.4(3).
- Jeannin, P., Paolini, L., Adam, C. & Delneste, Y., 2018. The roles of CSFs on the functional polarization
 of tumor-associated macrophages. *FEBS J.*, 1 2, 285(4), pp. 680-699.
- Jiang, Y., Chen, J., Yen, K. & Xu, J., 2019. Ectopically Expressed IL-34 Can Efficiently Induce
 Macrophage Migration to the Liver in Zebrafish. *Zebrafish*, 1 4, 16(2), pp. 165-170.
- 836 Kawabe, M. et al., 2015. Expression of interleukin-34 and colony stimulating factor-1 in the
- stimulated periodontal ligament cells with tumor necrosis factor-α. *Medical Molecular Morphology,*14 9, 48(3), pp. 169-176.
- Shoshnan, A. et al., 2017. IKK β and mutant huntingtin interactions regulate the expression of IL-34:
 Implications for microglialmediated neurodegeneration in HD. *Human Molecular Genetics*, 26(21),
 pp. 4267-4277.
- Kobayashi, T. et al., 2019. Prognostic value of IL-34 in colorectal cancer patients. *Immunological Medicine*, 2 10, 42(4), pp. 169-175.
- Kondo, Y. & Duncan, I. D., 2009. Selective reduction in microglia density and function in the white
 matter of colony-stimulating factor-1-deficient mice. *Journal of Neuroscience Research*, 9, 87(12), pp.
 2686-2695.
- 947 Kong, F. et al., 2019. Interleukin-34 mediated by hepatitis B virus X protein via
- 948 CCAAT/enhancer-binding protein α contributes to the proliferation and migration of hepatoma cells.
- 949 *Cell Proliferation*, 52(6), p. e12703.
- 950 Krammer, F. et al., 2018. Influenza. *Nat Rev Dis Primers*, 1(4), p. 3.
- 951 Kuil, L. E. et al., 2019. Reverse genetic screen reveals that II34 facilitates yolk sac macrophage
- 952 distribution and seeding of the brain. *DMM Disease Models and Mechanisms*, 1 3, 12(3), p.

953 dmm037762.

- 954 Kumari, A., Silakari, O. & Singh, R. K., 2018. Recent advances in colony stimulating factor-1
- 955 receptor/c-FMS as an emerging target for various therapeutic implications. *Biomedicine &*956 *Pharmacotherapy*, 1 7, Volume 103, pp. 662-679.
- Kumar, S., Ramesh, A. & Kulkarni, A., 2020. Targeting macrophages: a novel avenue for cancer drug
 discovery. *Expert Opin Drug Discov*, 3 5, 15(5), pp. 561-574.
- Kuzumi, A. et al., 2018. Serum interleukin-34 levels in patients with systemic sclerosis: Clinical
 association with interstitial lung disease. *Journal of Dermatology*, 1 10, 45(10), pp. 1216-1220.
- Lee, P. W. et al., 2020. Neuron-Specific Vitamin D Signaling Attenuates Microglia Activation and CNS
 Autoimmunity. *Frontiers in Neurology*, 31 1, Volume 11, p. 19.
- Lemus, H. N., Warrington, A. E. & Rodriguez, M., 2018. Multiple Sclerosis: Mechanisms of Disease and
 Strategies for Myelin and Axonal Repair. *Neurologic Clinics*, 1 2, 36(1), pp. 1-11.
- 265 Lindau, R. et al., 2018. Interleukin-34 is present at the fetal-maternal interface and induces
- 966 immunoregulatory macrophages of a decidual phenotype in vitro. *Human Reproduction*, 1 4, 33(4),967 pp. 588-599.
- Lin, H. et al., 2008. Discovery of a cytokine and its receptor by functional screening of the
 extracellular proteome. *Science*, 320(5877), pp. 807-11.
- Li, N. et al., 2020. The correlation between interleukin-34 and bone erosion under ultrasound in
 rheumatoid arthritis. *Modern Rheumatology*, 3 3, 30(2), pp. 269-275.
- 272 Lin, K. et al., 2019. Autocrine Production of Interleukin-34 Promotes the Development of
- 973 Endometriosis through CSF1R/JAK3/STAT6 signaling. *Scientific Reports*, 1 12, 9(1), p. 16781.
- Li, P. et al., 2017. The role of Kupffer cells in hepatic diseases. *Molecular Immunology*, 1 5, Volume
 85, pp. 222-229.
- Liu, H. et al., 2012. The mechanism of shared but distinct CSF-1R signaling by the non-homologous
 cytokines IL-34 and CSF-1. *Biochimica et Biophysica Acta Proteins and Proteomics*, 7, 1824(7), pp.
 938-945.
- 979 Liu, W. & Zhang, X., 2015. Receptor activator of nuclear factor-κB ligand
- 980 (RANKL)/RANK/osteoprotegerin system in bone and other tissues (Review). *Molecular Medicine* 981 *Reports*, 1 5, 11(5), pp. 3212-3218.
- Locati, M., Curtale, G. & Mantovani, A., 2020. Diversity, Mechanisms, and Significance of Macrophage
 Plasticity. *Annu Rev Pathol*, Volume 15, pp. 123-147.
- Locati, M., Mantovani, A. & Sica, A., 2013. Macrophage Activation and Polarization as an Adaptive
 Component of Innate Immunity. *Adv Immunol*, Volume 120, pp. 163-184.
- Lonardi, S. et al., "in press". CSF1R is required for differentiation and migration of Langerhans cells
 and Langerhans cell histiocytosis. *Cancer Immunology Research*, 1 4.p. canimm.0232.2019.
- Ma, D. et al., 2012. TGF-β induced by interleukin-34-stimulated microglia regulates microglial
 proliferation and attenuates oligomeric amyloid β neurotoxicity. *Neuroscience Letters*, 31 10, 529(1),
 pp. 86-91.
- Mantovani, A. et al., 2017. Tumour-associated macrophages as treatment targets in oncology. *Nat Rev Clin Oncol*, 17, 14(7), pp. 399-416.

- Mathews, S. et al., 2019. Human Interleukin-34 facilitates microglia-like cell differentiation and
 persistent HIV-1 infection in humanized mice. *Molecular Neurodegeneration*, 5 3, 14(1), p. 12.
- Ma, X. et al., 2012. Structural basis for the dual recognition of helical cytokines IL-34 and CSF-1 by
 CSF-1R. *Structure*, 4 4, 20(4), pp. 676-687.

Meshkibaf, S., William Gower, M., Dekaban, G. A. & Ouk Kim, S., 2014. G-CSF preferentially supports
the generation of gut-homing Gr-1 high macrophages in M-CSF-treated bone marrow cells. *Journal of Leukocyte Biology*, 10, 96(4), pp. 549-561.

- Mizuno, T. et al., 2011. Interleukin-34 selectively enhances the neuroprotective effects of microglia
 to attenuate oligomeric amyloid-β neurotoxicity. *American Journal of Pathology*, 10, 179(4), pp.
 2016-2027.
- Murray, P. J. et al., 2014. Macrophage Activation and Polarization: Nomenclature and Experimental
 Guidelines. *Immunity*, 17 7, 41(1), pp. 14-20.
- Nakajima, K. & Kohsaka, S., 2001. Microglia: Activation and Their Significance in the Central Nervous
 System. *The Journal of Biochemistry*, 130(2), pp. 169-175.
- Nakamichi, Y. et al., 2012. Spleen serves as a reservoir of osteoclast precursors through vitamin D induced IL-34 expression in osteopetrotic op/op mice. *Proceedings of the National Academy of Sciences of the United States of America*, 19 6, 109(25), pp. 10006-10011.
- Nandi, S. et al., 2013. Receptor-type protein-tyrosine phosphatase ζ is a functional receptor for
 interleukin-34. *Journal of Biological Chemistry*, 26 7, 288(30), pp. 21972-21986.
- 1012 Nandi, S. et al., 2012. The CSF-1 receptor ligands IL-34 and CSF-1 exhibit distinct developmental brain
- 1013 expression patterns and regulate neural progenitor cell maintenance and maturation. *Developmental*
- 1014 *Biology,* 15 7, 367(2), pp. 100-113.
- Noda, Y. et al., 2019. High serum interleukin-34 level is a predictor of poor prognosis in patients with
 non-viral hepatocellular carcinoma. *Hepatology Research*, 1 9, 49(9), pp. 1046-1053.
- Ogawa, S. et al., 2019. Interleukin 34 (IL-34) cell-surface localization regulated by the molecular
 chaperone 78-kDa glucose-regulated protein facilitates the differentiation of monocytic cells. *Journal*
- 1019 *of Biological Chemistry*, 15 2, 294(7), pp. 2386-2396.
- Okabe, Y., 2018. Molecular control of the identity of tissue-resident macrophages. *International Immunology*, 29 10, 30(11), pp. 485-491.
- 1022 O'Koren, E. G. et al., 2019. Microglial Function Is Distinct in Different Anatomical Locations during
 1023 Retinal Homeostasis and Degeneration. *Immunity*, 19 3, 50(3), pp. 723-737.e7.
- 1024 Ono, T. & Nakashima, T., 2018. Recent advances in osteoclast biology. *Histochemistry and Cell*1025 *Biology*, 1 4, 149(4), pp. 325-341.
- Paquin-Proulx, D. et al., 2018. Human interleukin-34-derived macrophages have increased resistant
 to HIV-1 infection. *Cytokine*, 1 11, Volume 111, pp. 272-277.
- Perdiguero, E. G. & Geissmann, F., 2016. The development and maintenance of resident
 macrophages. *Nat Immunol*, 1 1, 17(1), pp. 2-8.
- Pixley, F. J. & Stanley, E. R., 2004. CSF-1 regulation of the wandering macrophage: Complexity in
 action. *Trends Cell Biol*, 11, 14(11), pp. 628-638.

- Platt, A. M. et al., 2010. An Independent Subset of TLR Expressing CCR2-Dependent Macrophages
 Promotes Colonic Inflammation. *The Journal of Immunology*, 15 6, 184(12), pp. 6843-6854.
- Popovic, M. et al., 2019. Colony-stimulating factor-1- and interleukin-34-derived macrophages differ
 in their susceptibility to Mycobacterium marinum. *Journal of Leukocyte Biology*, 1 12, 106(6), pp.
 1257-1269.
- Preisser, L. et al., 2014. IL-34 and Macrophage Colony-Stimulating Factor Are Overexpressed in
 Hepatitis C Virus Fibrosis and Induce Profibrotic Macrophages That Promote Collagen Synthesis by
 Hepatic Stellate Cells. *HEPATOLOGY*, 60(6), pp. 1879-1890.
- Rolla, G. et al., 2016. Th-17 cytokines and interstitial lung involvement in systemic sclerosis. *Journal of Breath Research*, Volume 10, p. 046013.
- Sakagami, T. et al., 2009. Human GM-CSF Autoantibodies and Reproduction of Pulmonary Alveolar
 Proteinosis. *N Engl J Med*, 27(361), pp. 2679-81.
- Sattentau, Q. J. & Stevenson, M., 2016. Macrophages and HIV-1: An Unhealthy Constellation. *Cell Host Microbe*, 9 3, 19(3), pp. 304-310.
- Ségaliny, A. I. et al., 2015. IL-34 and M-CSF form a novel heteromeric cytokine and regulate the M CSF receptor activation and localization. *Cytokine*, 1 12, 76(2), pp. 170-181.
- Segaliny, A. I. et al., 2015. Syndecan-1 regulates the biological activities of interleukin-34. *Biochimica et Biophysica Acta Molecular Cell Research*, 1 5, 1853(5), pp. 1010-1021.
- Ségaliny, A. I. et al., 2015. Interleukin-34 promotes tumor progression and metastatic process in
 osteosarcoma through induction of angiogenesis and macrophage recruitment. *International Journal*of *Cancer*, 1 7, 137(1), pp. 73-85.
- Shapouri-Moghaddam, A. et al., 2018. Macrophage plasticity, polarization, and function in health and
 disease. *J Cell Physiol.*, 1 9, 233(9), pp. 6425-6440.
- 1055 Shen, H. Y. et al., 2020. Mudskipper interleukin-34 modulates the functions of
- monocytes/macrophages via the colony-stimulating factor-1 receptor 1. *Zoological research*, 18 3,
 41(2), pp. 123-137.
- 1058 Shoji, H. et al., 2016. Interleukin-34 as a fibroblast-derived marker of liver fibrosis in patients with 1059 non-alcoholic fatty liver disease. *Scientific Reports*, 17, Volume 6, p. 28814.
- Stanley, E. R., Chen, D. M. & Lin, H. S., 1978. Induction of macrophage production and proliferation
 by a purified colony stimulating factor. *Nature*, 274(5667), pp. 168-70.
- Stanley, E. R. & Chitu, V., 2014. CSF-1 receptor signaling in myeloid cells. *Cold Spring Harb Perspect Biol*, 6(6), p. a021857.
- Stremmel, C. et al., 2018. Yolk sac macrophage progenitors traffic to the embryo during defined
 stages of development. *Nature Communications*, 1 12.9(1).
- Tang, K. et al., 2019. Elevated plasma interleukin 34 levels correlate with disease severity-reflecting
 parameters of patients with haemorrhagic fever with renal syndrome. *Infectious Diseases*, 2 12,
 51(11-12), pp. 847-853.

- 1069 Truong, A. D. et al., 2018. Interleukin-34 regulates Th1 and Th17 cytokine production by activating 1070 multiple signaling pathways through CSF-1R in chicken cell lines. *International Journal of Molecular*
- 1071 *Sciences,* 1 6, 19(6), p. 1665.
- 1072 Udomsinprasert, W., Jittikoon, J. & Honsawek, S., 2019. Interleukin-34 as a promising clinical
- biomarker and therapeutic target for inflammatory arthritis. *Cytokine & Growth Factor Reviews,* 1 6,
 Volume 47, pp. 43-53.
- 1075 Valcour, V. et al., 2012. Central Nervous System Viral Invasion and Inflammation During Acute HIV
 1076 Infection. *J Infect Dis*, 2(206), pp. 275-82.
- 1077 van de Laar, L. et al., 2016. Yolk Sac Macrophages, Fetal Liver, and Adult Monocytes Can Colonize an
 1078 Empty Niche and Develop into Functional Tissue-Resident Macrophages. *Immunity*, 19 4, 44(4), pp.
 1079 755-768.
- 1080 van Furth, R. & Cohn, Z. A., 1968. The Origin and Kinetics of Mononuclear Phagocytes. 128(3), pp.1081 415-35.
- Vannella, K. M. & Wynn, T. A., 2017. Mechanisms of Organ Injury and Repair by Macrophages.
 Annual Review of Physiology, 10 2, 79(1), pp. 593-617.
- Vasek, M. J. et al., 2016. A complement-microglial axis drives synapse loss during virus-induced
 memory impairment. *Nature*, 22 6, 534(7608), pp. 538-543.
- Wada, Y. et al., 2019. IL-34-Dependent intrarenal and systemic mechanisms promote lupus nephritis
 in MRL-Faslpr mice. *Journal of the American Society of Nephrology*, 1 2, 30(2), pp. 244-259.
- Walker, D. G., Tang, T. M. & Lue, L. F., 2017. Studies on colony stimulating factor receptor-1 and
 ligands colony stimulating factor-1 and interleukin-34 in Alzheimer's disease brains and human
 microglia. *Frontiers in Aging Neuroscience*, 9 8, 9(9), p. 244.
- Wang, B. et al., 2017. IL-34 upregulated Th17 production through increased IL-6 expression by
 rheumatoid fibroblast-like synoviocytes. *Mediators of Inflammation*, Volume 2017, p. 1567120.
- Wang, B. et al., 2018. Increased IL-6 expression on THP-1 by IL-34 stimulation up-regulated
 rheumatoid arthritis Th17 cells. *Clinical rheumatology*, 1 1, 37(1), pp. 127-137.
- Wang, J., Li, D., Cang, H. & Guo, B., 2019. Crosstalk between cancer and immune cells: Role of tumorassociated macrophages in the tumor microenvironment. *Cancer Med.*, 8(10), pp. 4709-4721.
- Wang, T. et al., 2013. Identification of IL-34 in teleost fish: Differential expression of rainbow trout IL34, MCSF1 and MCSF2, ligands of the MCSF receptor. *Molecular Immunology*, 4, 53(4), pp. 398-409.
- Wang, Y. et al., 2016. Nonredundant roles of keratinocyte-derived IL-34 and neutrophil-derived CSF1
 in Langerhans cell renewal in the steady state and during inflammation. *European Journal of Immunology*, 1 3, 46(3), pp. 552-559.
- Wang, Y.-Q.et al., 2018. Serum interleukin-34 Level Can Be an Indicator of Liver Fibrosis in Patients
 With Chronic Hepatitis B Virus Infection. *World J Gastroenterol*, 24(12), pp. 1312-1320.
- Wang, Y. et al., 2012. IL-34 is a tissue-restricted ligand of CSF1R required for the development of
 Langerhans cells and microglia. *Nature Immunology*, 8, 13(8), pp. 753-760.
- Wei, S. et al., 2010. Functional overlap but differential expression of CSF-1 and IL-34 in their CSF-1
 receptor-mediated regulation of myeloid cells. *Journal of Leukocyte Biology*, 9, 88(3), pp. 495-505.

- Williams, C. B., Yeh, E. S. & Soloff, A. C., 2016. Tumor-associated macrophages: Unwitting
 accomplices in breast cancer malignancy. *NPJ Breast Cancer*, 14 12, 2(1), p. 15025.
- 1110 Wlodarczyk, A. et al., 2019. CSF1R stimulation promotes increased neuroprotection by CD11c+
- 1111 microglia in EAE. *Frontiers in Cellular Neuroscience*, 10 1, Volume 12, p. 523.
- Wlodarczyk, A. et al., 2017. A novel microglial subset plays a key role in myelinogenesis in developing
 brain. *The EMBO Journal*, 15 11, 36(22), pp. 3292-3308.
- Wright-Jin, E. C. & Gutmann, D. H., 2019. Microglia as Dynamic Cellular Mediators of Brain Function. *Trends in Molecular Medicine*, 1 11, 25(11), pp. 967-979.
- Wu, S. et al., 2018. II34-Csf1r Pathway Regulates the Migration and Colonization of Microglial
 Precursors. *Developmental Cell*, 10 9, 46(5), pp. 552-563.e4.
- Wynn, T. A., Chawla, A. & Pollard, J. W., 2013. Macrophage biology in development, homeostasis anddisease. 25 4, 496(7446), pp. 445-455.
- 1120 Xue, Y. et al., 2019. Functional characterisation of interleukin 34 in grass carp Ctenopharyngodon 1121 idella. *Fish and Shellfish Immunology*, 1 9, Volume 92, pp. 91-100.
- 1122 Xun, Q. et al., "in press". Small-Molecule CSF1R Inhibitors as Anticancer Agents. *Current Medicinal* 1123 *Chemistry.*
- 1124 Xu, W. D. et al., 2019. Targeting IL-34 in inflammatory autoimmune diseases. *Journal of cellular* 1125 *physiology*, 234(12), pp. 21810-21816.
- Yamashita, T., Takahashi, N. & Udagawa, N., 2012. New roles of osteoblasts involved in osteoclast
 differentiation. *World Journal of Orthopaedics*, 3(11), pp. 175-181.
- 1128 Yaparla, A. et al., 2019. The amphibian (Xenopus laevis) colony-stimulating factor-1 and interleukin-
- 1129 34-derived macrophages possess disparate pathogen recognition capacities. *Developmental and*
- 1130 *Comparative Immunology,* 1 9, Volume 98, pp. 89-97.
- Yaparla, A., Popovic, M. & Grayfer, L., 2018. Differentiation-dependent antiviral capacities of
 amphibian (Xenopus laevis) macrophages. *Journal of Biological Chemistry*, 2 2, 293(5), pp. 1736-1744.
- 1133 Yeung, Y. G. et al., 1987. Purification of the colony-stimulating factor 1 receptor and demonstration
- 1134 of its tyrosine kinase activity (macrophages/CSF-1/c-fms protooncogene/growth
- 1135 factors/phosphorylation). *Proc Natl Acad Sci U S A*, 84(5), pp. 1268-1271.
- Yin, M. et al., 2020. Potent BRD4 inhibitor suppresses cancer cell-macrophage interaction. *Nature Communications*, 12, 11(1), p. 1833.
- Yu, G. et al., 2015 . Activation of the Interleukin-34 Inflammatory Pathway in Response to Influenza A
 Virus Infection. *The American Journal of the Medical Sciences*, 349(2), pp. 145-50.
- Yu, Y. et al., 2014. Tumor necrosis factor-α induces interleukin-34 expression through nuclear factorκB activation in MC3T3-E1 osteoblastic cells. *Molecular Medicine Reports*, 10(3), pp. 1371-1376.
- 1142 Zhou, J. et al., 2018. IL-34 regulates IL-6 and IL-8 production in human lung fibroblasts via MAPK,
- PI3K-Akt, JAK and NF-κB signaling pathways. *International Immunopharmacology*, 1 8, Volume 61, pp.
 1144 119-125.
- 1145 Zhou, J. et al., 2020. Tumor-Associated Macrophages: Recent Insights and Therapies. *Front Oncol.*, 251146 2, Volume 10, p. 188.

- 1147 Zhou, S.-L.et al., 2016. miR-28-5p-IL-34-macrophage Feedback Loop Modulates Hepatocellular
 1148 Carcinoma Metastasis. *HEPATOLOGY*, 63(5), pp. 1560-75.
- Zhu, C. et al., 2016. A neuroprotective role for microglia in prion diseases. *Journal of Experimental Medicine*, 1 5, 213(6), pp. 1047-1059.
- Zins, K. et al., 2018. Differential prognostic impact of interleukin-34 mRNA expression and infiltrating
 immune cell composition in intrinsic breast cancer subtypes. *Oncotarget*, 9(33), pp. 23126-23148.
- Zwicker, S. et al., 2016. Receptor-type protein-tyrosine phosphatase ζ and colony stimulating factor-1
 receptor in the intestine: Cellular expression and cytokine- and chemokine responses by interleukin34 and colony stimulating factor-1. *PLoS ONE*, 1 11, 11(11), p. e0167324.
- Zwicker, S. et al., 2015. Interleukin 34: A new modulator of human and experimental inflammatory
 bowel disease. *Clinical Science*, 129(3), pp. 281-290.
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- 1163 **Figure legends**

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

| Proteins | Binding characteristic | KD | Kon | K _{off} |
|---------------|---------------------------|-------|------------------------------------------------------|-----------------------------------------|
| M-CSF / CD115 | hydrophilic | 1 pM | $6.29 \text{ x } 10^7 \text{ s}^{-1} \text{ M}^{-1}$ | 6.55 x 10 ⁻⁵ s ⁻¹ |
| IL-34 / CD115 | hydrophobic | 34 pM | 1.7 x 107 s-1 M ⁻¹ | 6.03 x 10 ⁻⁴ s ⁻¹ |

 $\overline{K_D}$: equilibrium dissociation constant; K_{on} : association rate constant; K_{off} : dissociation rate constant. Data from (10.1126/science.1154370).

| 1 abic 2. 11-37 and autommune discases | Table | 2: IL-3 | 34 and | autoimmune | diseases |
|----------------------------------------|--------------|---------|--------|------------|----------|
|----------------------------------------|--------------|---------|--------|------------|----------|

| Diseases | Major finding related to IL-34 | References |
|------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|
| Bowel Autoimmune Diseases | 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-α and IL-6 factors. | Franzè et al. (2015) |
| | High expression levels of IL-34 in CD than in UC, with IL-34 mainly expressed in the lleum. TNF- α induces IL-34 production by epithelial cells. | Zwicker et al. (2015) |
| | IL-34 participates in the cross talk between epithelial and immune cells by induction of CCL20 chemokine expression. | Franzè et al. (2016) |
| | 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. | Franzè et al. (2020) |
| Multiple Sclerosis (MS) | IL-34 acts as a neuroprotective factor by inducing microglia differentiation with anti-inflammatory properties. | Mizuno (2011) |
| | 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. | Jin et al. (2014) |
| | IL-34 levels do not change in relapsing-remitting MS. | Abdel-Dayen et al. (2019) |
| | IL-34 induces neuroprotection by expansion of CD11c ⁺ microglia population via CD115. | Wlodarczyk et al. (2019) |
| | IL-34 expression is down regulated in cerebrospinal fluids in MS affected patients. | Safari-Alighiarloo et al. (2020) |
| | In children, Vitamin D partially induces IL-34 expression by neurons conferring neuroprotection against MS. | Lee et al. (2020) |

| Psoriasis | 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) |
|------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|
| Rheumatoid Arthritis (RA) | IL-34 is highly expressed by synovial fibroblast of RA affected patients. IL-34 expression is induced by pro-inflammatory factors IL-1β and TNF- α . 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- α induces IL-34 expression by synovial fibroblast via NF κ 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- α 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- β 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 α 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) |
| Sjogren Syndrome (pSS) | IL-34 expression correlates with expansion of pro-inflammatory CD14 ^{bright} CD16 ⁺ 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 γ -globulin. IL-34 induces hyper-activation of B cells and antibodies production. | Liu et al. (2019) |

| Systemic Lupus Erythematosus (SLE) | 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- ζ 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 that 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 use as surrogate marker for early detection of lupus nephritis diseases. | Abdel-Rehim et al. (2020) |
| Systemic Sclerosis (SS) | 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.

1186

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

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Figure 3. IL-34 signalling pathways involved in macrophage differentiation and non-1193 1194 monocyte cells. A) Various stimuli, such as bacterial or viral infections, pro-inflammatory cytokines, DNA damage, or chemical molecules modulate IL-34 expression. IL-34 binds to 1195 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 signalling pathways that result in a diversity of macrophage biological processes such as 1201 differentiation, proliferation, survival or migration. The binding of IL-34 to the chondroitin 1202 chains of syndecan-1 results in *in vitro* phosphorylations of the tyrosines Y708 and Y723 of 1203 CD115, suggesting that the complex IL-34/syndecan-1 can act as a regulator of CD115 1204 activity. Moreover, IL-34/syndecan-1 interaction regulates macrophage migration. B) IL-34 1205 expression in the microenvironment of epithelial cells, fibroblasts and tumour cells can 1206 1207 induce, via CD155, activation of the different signalling pathways implicated in biological functions such as cell proliferation, migration, survival and cytokines. IL-34 also binds to 1208 1209 PTP- ζ and controls inhibition of migration and proliferation of tumour cells lines such as glioblastoma U251. 1210

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Figure 4: IL-34 is a pro-M2 macrophage differentiation factor. Macrophage isolation and
treatment were performed as described in (Guihard, et al., 2012). A) IL-34 treatment induced
macrophage differentiation with an M2 phenotype, alone or in combination with IL-4 and ILMacrophages were treated with IL-34 (50 ng/ml) or in combination with IFN-γ (50 ng/ml;

- pro M1), IL-4 (50 ng/ml; pro M2a), and IL-10 (50 ng/ml; pro M2c), for 2 days and cells were 1216 analysed by means of flow cytometry using specific antibodies for both M1-like macrophages 1217 (CD14, CD86 and CD64) and M2-like macrophages (CD163, CD200R and CD206). B) 1218 Comparison of macrophage differentiation after treatment with the cytokines GM-CSF (20 1219 1220 ng/ml), M-CSF (50 ng/ml) and IL-34 (50 ng/ml) alone or in combination with IFN-y, IL-4 or IL-10 as performed in A. The three cytokines in combination with IFN- γ were able to induce 1221 1222 M1 macrophage differentiation as shown by the increase in CD64, an M1 marker. IL-34 modulates M2 markers (CD163, CD200R and CD206) alone or in combination with IL-4 and 1223 IL-10. No effect of IL-34 was observed in CD14 expression. Overall, IL-34 was able to 1224 induce M1 and M2 macrophage differentiation with a specific increase in CD163, an M2 1225 1226 marker.
- 1227
- 1228



M-CSFR

(monomer 2)





Table 1

| | Bound nature | KD | Kon | K _{off} |
|----------------|--------------|-------|-------------------------------------------------------|----------------------------------------|
| M-CSF : M-CSFR | hydrophilic | 1 pM | 6.29 x10 ⁷ s ⁻¹ M ⁻¹ | 6.55 x10 ⁻⁵ s ⁻¹ |
| IL-34 : M-CSFR | hydrofobic | 34 pM | 1.7 x10 ⁷ s ⁻¹ M ⁻¹ | 6.03 x10 ⁻⁴ s ⁻¹ |







CD14

CD200R



В

