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

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

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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.

Introduction

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 variety of particles, from viruses to bacteria, fungi, dead cells and other solid materials (Jaumouillé & Grinstein, 2016). Specialised phagocytosis cells include granulocytes, dendritic cells and macrophages, and they are part of the innate immune system (Biron, 2016). 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; Wynn, et al., 2013).

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 haematopoietic stem cells located in the bone marrow (van Furth & Cohn, 1968). Although this classification is still used, studies working on specific tissue macrophages in mice over the last few years have suggested an ontogeny dichotomy in macrophages (Davies & Taylor, 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 (Figure 1). These macrophages, known as "non-resident" macrophages, are some of the circulating monocytes that can extravasate from blood to tissues and enrich the local population of macrophages. The other pool of macrophages, known as "tissue-resident" macrophages originate from the yolk sac and foetal liver during embryonic development (Figure 1) (Hoeffel & Ginhoux, 2018; Stremmel, et al., 2018). Tissue-resident macrophages include specialised macrophages such as the microglia in the neural system, Kupffer cells in 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 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 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 classifying them. As a result, their classification varies from one author to another (Hoeffel & Ginhoux, 2018; Shapouri-Moghaddam, et al., 2018; Hume, et al., 2019).

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

1. Macrophage differentiation and growth factors

Circulating monocytes originate in CFU-M precursors and can extravasate from blood to 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., 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 into macrophages via differential phosphorylation of the signal transducer and activator of transcription-3 (STAT3) (Duplomb, et al., 2008). In the case of GM-CSF, Alothaimeen *et al.* showed more recently that GM-CSF specifically promoted differentiation of macrophage subpopulations characterised by high induction of the antigen-specific $CD8^+$ T cell type during lymphocytic choriomeningitis virus infections (Alothaimeen, et al., "in press").

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 precursors toward monocytic lineage and into macrophages. Macrophage differentiation 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 pathways that promote macrophage differentiation, proliferation, survival and proper functioning (Guilbert & Stanley, 1980; Yeung, et al., 1987; Stanley & Chitu, 2014). Nevertheless, in 2002, studies using knock-out mice for CD115 ($Csfr^{-/-}$) demonstrated that CD115-KO mice exhibited an osteopetrotic phenotype and a more severe depletion of the macrophage pool than M-CSF deficient mice ($Csf1^{op/op}$). Interestingly, $Csf1^{op/op}$ mice showed

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

The interactions between CD115 and its two ligands, M-CSF and IL-34, has been described in (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-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 human CD115. However, no cross-specifity was observed between human and mouse M-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 CD115 in a hydrophilic manner, whereas IL-34 binds to CD115 in a hydrophobic manner (Liu, et al., 2012) (Table 1). The nature of these molecular interactions established that each cytokine presented specific kinetics of association to CD115 (Figure 2B). The M-CSF/CD115 complex was characterised by quick dissociation kinetics compared to the IL-34/CD115 complex. Futhermore, *in vitro* experiments showed the lower dissociation kinectics of the IL-34/CD115 complex, which may be associated with a longer span of cell signalling pathway activation and differential biological functions (Stanley, et al., 1978; Lin, et al., 2008; Liu, et al., 2012; Ma, et al., 2012). The binding of IL-34 to CD115 results in the activation of 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 (JNK), p38 mitogen-activated kinase, PhosphoInositide 3-Kinase (PI3K)/AKT, transcription factor Nuclear Factor kappa Beta (NFκB), Signal Transducer and Activator of Transcription 3 (STAT3) and the Scr kinase family (Figure 3) (Chihara, et al., 2010; Eda, et al., 2011; Baud'Huin, et al., 2010; Chen, et al., 2014; Wei, et al., 2010; Yu, et al., 2014; Lin, et al., 2019; 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 the receptor and subsequential bioactivities. Compared to M-CSF, IL-34 induced a strong but transitory phosphorylation of CD115 tyrosines and downstream proteins, with rapid downregulation of the receptor (Chihara, et al., 2010). The differential binding properties of M-CSF and IL-34 to CD115 may explain the variety and degree of activation of the different signalling pathways and their biological outcomes. Segaliny *et al.* demonstrated that both cytokines could work in a dual manner, showing additive and competitive biological properties. Moreover, M-CSF and IL-34 were able to generate a heterodimer that may play 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 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 proposed "ménage à trois" (Droin & Solary, 2010), and that the formation of new heterodimers between IL-34 and other cytokines should be not excluded.

1.2 IL-34, a promiscouos cytokine with therapeutic potential

CD115 is not the exclusive receptor of IL-34. Two additional receptors have been proposed: 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 high expression of IL-34 in different areas of the adult brain where CD115 was absent suggest the existence of an additional receptor for IL-34 (Nandi, et al., 2012). By using a CD115 depleted U251 glioblastoma cell line and affinity chromatography, Nandi *et al.* demonstrated that IL-34 bound specifically to PTP-ζ in a chondroitin sulfate-dependent manner. The interaction between IL-34 and PTP-ζ induced tyrosine phosphorylations of the FAK and paxilin proteins, impairing the cell proliferation and motility of U251 glioblastoma cells. These results suggest that the IL-34/PTP-ζ complex acts as a tumurigenic suppressor in glioblastoma (Nandi, et al., 2013). PTP-ζ was also expressed in the instestinal tissue of healthy people, mainly in the colon, whereas IL-34 was mainly expressed in the ileum. However, in inflammatory bowel diseases, IL-34 was coexpressed in the same regions as PTP-ζ and CD115 (Zwicker, et al., 2016). Franzé *et al*. showed that IL-34 was over-expressed in colorectal cancer (CRC) tissues and PTP-ζ was also expressed in tumoral and non-tumoral areas of CRC samples. However, an increase in CD115 expression alone, and not PTP-ζ, was observed in CRC cells (Franzè, et al., 2018). Additional studies are needed to decipher the role of the IL-34/PTP-ζ complex in these tissues.

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., 2015).

The cytokine M-CSF can be expressed in three different isoforms: a secreted glycoprotein, a 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 (Ogawa, et al., 2019). In secondary lymphoid tissue, follicular dendritic cells (FDC) were able to express IL-34 that induced, via CD115, the differentiation of a novel class of monocytes, named FDC-induced monocytic cells. To induce differentiation, IL-34 required the participation of the molecular chaperone 78-KDa glucose-regulated protein (GRP78) (Ogawa, 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 account when developing further therapies against IL-34.

2. From monocytes to "non-resident" macrophages

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

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

This dichotomy in macrophage differentiation between M-CSF and IL-34 is also observed in species other than humans and rodents. In birds, Troung *et al.* demonstrated that chicken IL-34 interacted with CD115 triggering the activation of multiple signalling pathways (JAK, STAT 1/3, NFκB, TYK2, MAPK) and the induction of a specific pro-inflammatory response 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 (Popovic, et al., 2019). In fish, rainbow trout IL-34 was expressed with relatively high levels along the tissues compared to the two M-CSF cytokines that showed variable expression 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 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.* identified the homologue cytokine IL-34 in the mudskipper fish, where IL-34 levels increased after bacterial infection and induced macrophage differentiation with high phagocytic activity in a CD115-dependent manner (Shen, et al., 2020). Hoang *et al.* showed that IL-34 could be used as an adjuvant to DNA vaccine treatment against nocardiosis infection in fish (Hoang, et al., 2020). Finally, zebrafish have been suggested as a good model for studying the role of IL-34 during brain development or in brain disorders and liver or skin diseases (Wu, et al., 2018; Jiang, et al., 2019; Kuil, et al., 2019).

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.

Infection by the human immunodeficiency virus-1 (HIV-1) is characterised by the loss of T lymphocytes in a progressive manner and susceptibility to opportunistic infections (Sattentau & Stevenson, 2016). IL-34-induced macrophages were characterised by better resistance to HIV-1 infection than MCSF-differentiated cells (Paquin-Proulx, et al., 2018). The HIV-1 resistance of IL-34-macrophages lay in the specific expression of restriction factor genes 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-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 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 proliferation in the mouse's brain. The IL-34 microglia favoured HIV-1 infection, induced inflammation and a neurotoxic response, and formed an important reservoir for virus particles (Mathews, et al., 2019).

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).

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 enhanced the synthesis of type I collagen by decreasing expression of the collagenase MMP-1 (Preisser, et al., 2014). As with HCV, hepatitis B virus (HBV) infections could lead to hepatic fibrosis and chronic inflammation in which IL-34 was involved. In a rat pre-clinical model, 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, the level of IL-34 detected in blood plasma differed according to the phases of chronic HBV infection and correlated with progression of liver fibrosis and poor prognosis (Wang, et al., 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 in serum. As observed in HCV infections (Preisser, et al., 2014), IL-34 levels correlated with the chronicity of the HBV infection. However further studies are needed to clarify the 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) 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 receptors in an ERK- and STAT3-dependent manner (Kong, et al., 2019).

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).

IL-34 plays an important role during viral infections in other species. In fish, Xue *et al.* 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 inhibiting anti-inflammatory factors such as IL-10 and the transforming growth factor β1 (TGF-β1) (Xue, et al., 2019). Amphibian populations are dramatically affected by the frog virus 3 (FV3) ranavirus (Grayfer & Robert, 2016). In *Xenopus laevis* M-CSF and IL-34 polarised macrophage differentiation with distinct functionalities (Grayfer & Robert, 2016). Both subtypes of macrophage expressed specific pattern recognition receptors (PRRs) that were essential for pathogen recognition. IL-34-activated macrophages highly expressed antiviral interferon genes, showing better anti-FV3 properties than M-CSF-activated

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

2.3 Effects of IL-34 in tumour-associated macrophage polarisation

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 are considered to be heterogenous populations that originate in adult circulating myeloid precursors and "tissue-resident" macrophage precursors (Locati, et al., 2020). TAMs are responsible for driving pro- or anti-inflammatory responses by controlling immunocompetent, stroma and vascular cells according to the tumour microenvironment (TEM), (Mantovani, et 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 inflammation leading to the anti-tumour response (Williams, et al., 2016). However, depending on the surrounding TEM, TAMs can acquire M1, M2 or dual polarisation (Wang, et al., 2019). Recent advances in TAM functions and their involvement in cancer and inflammatory diseases have been reviewed in (Wang, et al., 2019; Kumar, et al., 2020; Zhou, et al., 2020).

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 the pro-tumorigenic effect of IL-34-derivated TAMs. Segaliny *et al.* demonstrated that IL-34 macrophage extravasation and polarisation to the M2 phenotype promoted osteosarcoma proliferation and metastasis expansion (Ségaliny, et al., 2015). In lung cancer, tumour cells 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 activation (Baghdadi, et al., 2016). Moreover, the co-expression of both cytokines, M-CSF and IL-34, was expressed by lung cancer cells, and correlated with an increase in the TAM 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 that may bind to CD115 (Ségaliny, et al., 2015). This new cytokine may result in specific functions of myeloid and cancer cells during lung cancer development (Baghdadi, et al., 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 IL-34-derivated TAM population (Arora, et al., 2018). Taken together, these observations suggest that IL-34 may play an essential role in both the pro-tumorigenic polarisation of TAMs and tumour progression, as revealed in various other cancers. In colorectal cancer, high levels of IL-34 correlated with TAM infiltration and a poor prognosis for the affected patients (Kobayashi, et al., 2019). In ovarian cancer, cytotoxic chemotherapy enhanced the expression 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; Noda, et al., 2019). However, the results were more contrasted in breast cancer in which IL-34 displayed different prognosis properties depending on the cancer subtype. High levels of 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 al., 2018).

3. Tissue-resident macrophages and IL-34

In healthy tissues, the local population of macrophages is sustained by autonomous proliferation (Hashimoto, et al., 2013). During pathologic episodes, the death of local macrophages induces the infiltration of circulating monocytes to reinforce the local population until proliferation of the remaining resident macrophages. These circulating monocytes end up acquiring tissue-resident macrophage features, indicating the presence of local signals that confer tissue-specific identities (van de Laar, et al., 2016). Several authors have suggested that the autonomous proliferation of local macrophages is regulated by the existence of a tissue "niche". Each tissue niche consists in cell-cell circuits based on the 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 of IL-34 in the differentiation of tissue-resident macrophages.

3.1 IL-34 in osteoclast differentiation and associated bone diseases

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

or osteoporosis. As part of the tissue-specific mononuclear phagocyte lineage, osteoclasts play an essential role in maintaining bone homeostasis. A key factor in the differentiation of 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 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 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, 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-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 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, et al., 2011). In the bone marrow, TNF-α stimulates the expression of IL-34 and M-CSF in osteoblast via the NFκB pathway (Yu, et al., 2014). In pathologic situations such as osteopetrosis, the spleen acts as a reservoir for osteoclast progenitors. In a vitamin D-dependent manner, vascular endothelial cells from the spleen expressed the IL-34 that was needed to maintain and conscribe osteoclast progenitors from the spleen to the bone tissue (Nakamichi, et al., 2012). Recently, a study using bone marrow macrophages from mice 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 presence of the protease inhibitor AG490, which also favoured the expression of SMAD7, an antagonist TGF-β protein for the STAT pathway (Cheng, et al., 2017). Segaliny *et al.* demonstrated that osteosarcoma cells expressed IL-34 and that this expression was regulated 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 glycosaminoglycan during tumour development. In relation with this pro-angiogenic effect, IL-34 promoted macrophage extravasation and polarisation to an M2 phenotype (Ségaliny, et al., 2015).

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 al., "in press"). Chemel *et al.* was the first to describe the correlation between IL-34 and inflammation levels in RA and found that IL-34 expression was upmodulated by IL-1β and TNF-α stimulation in synoviocytes (Chemel, et al., 2012). In contrast, two members of the transforming growth factor β family, BMP-2 and TGF-β1, were described as inhibiting expression of IL-34, acting as regulators of inflammation during RA (Chemel, et al., 2017). While macrophages are potential targets of IL-34, fibroblast-like synoviocytes were identified as new targets expressing CD115. The binding of IL-34 to its receptor activated the expression and secretion of IL-6 (via the JNK/P38/NF-κB signalling pathway), which in turn induced polarisation of naive T lymphocytes into a Th17 population (Wang, et al., 2018; Wang, et al., 2017). Moreover, IL-34 activated/inhibited the expression of RANKL/OPG by fibroblast-like synoviocytes and circulating monocytes promoting cartilage and bone destruction in an IL-17-dependent manner, which was consistent with the correlation 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 (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 correlated with poor prognosis. Moreover, the increase in IL-34 also correlated with the development of interstitial lung disease (ILD) (Kuzumi, et al., 2018). As SSc patients also 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 (Kuzumi, et al., 2018). Systemic lupus erythematosus (SLE) is characterised by an acute nephritis process. Wada *et al.* demonstrated that IL-34 and its two receptors, CD115 and PTP-ζ, were highly expressed in patients with lupus nephritis. IL-34 induced circulating monocytes and intrarenal macrophage proliferation and accumulation, together with B and T lymphocyte enrichment in kidney areas, promoting the inflammation process and tubular epithelial cell apoptosis (Wada, et al., 2019). In Sjogren's syndrome, Ciccia *et al.* showed that IL-34 expression increased in the ductal epithelial cells of inflamed salivary glands. The increase in IL-34 correlated with the expansion of pro-inflammatory monocytes and expression of IL-23 and IL-17, suggesting that IL-34 plays an important role during the inflammatory process in Sjogren's syndrome (Ciccia, et al., 2013).

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).

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

3.2 IL-34 in microglia differentiaton and neural disorders

Microglia are the resident macrophage population of the central neural system (CNS) and present neurotoxic and neuroprotective activities (Nakajima & Kohsaka, 2001). Microglia development depends on CD115 activity and, by consequence, its ligands M-CSF and IL-34 (Dai, et al., 2002). IL-34 is basically expressed by neurons whereas M-CSF is expressed by astrocytes, microglia and oligodendrocytes (Cahoy, et al., 2008; Mizuno, et al., 2011). M-CSF or IL-34 knockout mice highlighted the harmful impact of one of these two cytokines on the survival/differentiation of microglia populations in different regions of the CNS (Wang, et al., 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 adulthood (Greter, et al., 2012). In agreement with this, Easley-Neal *et al*. demonstrated the essential role played by IL-34 in microglia support during postnatal life using specific 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 was necessary for proper development of the grey matter while, in contrast, M-CSF was 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 matters, such as the cerebellum and the dentate gyrus, showed differential responses to anti-M-CSF antibodies with no effect in the denatate gyrus and partial depletion of the microglia in the cerebelum, whereas no effect was observed for anti-IL-34 antibodies. However, 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). 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 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 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 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 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 (O'Koren, et al., 2019).

As observed in other tissues, IL-34 plays an important role in pathogenic situations (Wright-Jin & Gutmann, 2019). Depending on the disease, IL-34 is considered a neuroprotective or a neurotoxic agent. In neurodegenerative diseases, IL-34 primarily produced by neurons promoted microglia differentiation and proliferation via the CD115 receptor in an Alzheimer disease (AD) mouse model (Mizuno, et al., 2011). AD was characterised by the production and accumulation of neurotoxicoligomeric β-amyloid peptides. IL-34-differentiated microglia were able to abolish the neurotoxic effects of β-amyloid peptides and derivates by the production of the insulin degrading enzyme (IDE), haeme oxygenase-1 (HO-1) and TGF-β (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-CSF-differentiated cells (Mizuno, et al., 2011; Ma, et al., 2012). A recent report revealed that IL-34 and M-CSF cytokines induced the differentiation of microglia into a $CD11c^{+}$ type (Wlodarczyk, et al., 2019). The $CD11c^{+}$ microglia is characterised by the expression of 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-

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

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

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 severe neurotoxic effects (Valcour, et al., 2012). In humanised mouse models, IL-34-differentiated microglia acted as a reservoir for virus particles, promoting the persistence of 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 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).

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).

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 macrophages. LCs are the first immune barrier of the skin and contribute to its homeostasis. As with microglia, LCs autonomously maintain their own population with minimal 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, 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 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 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 rudiment during embriogenesis in mice, and for cell maintenance in adult mice (Greter, et al., 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., 2016). M-CSF and IL-34 exhibited differential activities in LC renewal depending on the pathophysiological context. Neutrophil-derived M-CSFs drove the renewal of LCs in the course of skin injury, while IL-34-derived keratinocytes were the main drivers of LC renewal in intact skin (Greter, et al., 2012; Wang, et al., 2016). Reinforcing this observation, it has been shown that M-CSF - but not IL-34 - was responsible for LC differentiation and pro-inflammatory activity in Langerhans cell histiocytosis (Lonardi, et al., "in press"). Overall, 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").

IL-34 is highly expressed by tumour tissue in patients with melanoma, and its levels appear to depend on clinical status. An increase in IL-34 expression was observed in refractory melanomas that correlated with the expansion of $CD163^{+}$ differentiated M2 macrophages (Han, et al., 2018). Similarly, the RUNX1/CD115/IL-34 axis was responsible for the tumour rebound in BRAF inhibitor resistant melanoma. The emergence of a population resistant to 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 and proliferation (Giricz, et al., 2018).

3.4 IL-34 in gut macrophage differentiation and intestinal disorders

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 existence of a heterogeneous population of macrophages in the intestine, divided into two major populations: resident macrophages and inflammatory macrophages (Platt, et al., 2010; Geissmann, et al., 2010; Guilliams, et al., 2018). By using multi-parameter flow cytometry and lineage tracking techniques, Bain *et al.* investigated the heterogeneity of macrophage 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 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 expression, accompanied by high expression of CX3CR1, F4/80, CD64, CD11c, CD163 and 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 inflammatory bowel diseases (IBD), macrophage populations quickly turned to pro-inflammatory subsets characterised by high expression of Ly6C and CD14 (Bain, et al., 2013).

Intestine macrophage differentiation depends on activation of CD115 by its ligands, M-CSF and IL-34, which are differently distributed and expressed along the intestine. M-CSF was mainly expressed in the colon, whereas IL-34 was principally expressed in the ileum (Zwicker, et al., 2015). Both cytokines were produced by the intestinal epithelium in response to TNF-α via the NFκB pathway. Inflammation of the gut in IBD patients was characterised by an increase in IL-34 levels produced by mononuclear cells in the lamina propria. This increase in IL-34 induced macrophage differentiation and the expression of pro-inflammatory factors TNF-α 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 mucosal inflammation in CD patients (Franzè, et al., "in press").

Colorectal carcinoma cells express CD115 and can be considered as potential targets of CD115 ligands (Franzè, et al., 2016). IL-34 induced the expression of CCL20 in a DLD1 colorectal adenoma cell line via activation of the ERK1/2 pathway and stimulation of the inflammatory response. This suggests that IL-34 participates in the crosstalk between the 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 and induced the proliferation and invasion of colorectal cancer cells. Furthermore, HT29 cell proliferation implied activation of the ERK1/2 signalling pathway by IL-34 through CD115, 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-tumorigenic factor in IBD disease and colorectal carcinomas (Franzè, et al., 2018).

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 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 maintained by self-renewal and by bone marrow circulating monocytes (Hashimoto, et al., 2013). Depending on the microenvironment, KCs can be polarised into an M1 pro-inflammatory subtype leading to a Th1 response, or into an M2 anti-inflammatory subtype leading to a Th2 response. The latter is associated with inducing and maintaining immune tolerance during liver transplantation (Atif, et al., 2020). Rat and human regulatory T (Treg) cells $FOXP3^+$ $CD4^+$ or $CD8^+$ cells expressed IL-34 (Bézie, et al., 2015). Treg $FOXP3^+$ cells, the main players in immune tolerance, seemed sensitive to IL-34 (Atif, et al., 2020). IL-34 treatment effectively seemed to induce Treg $FOXP3^+$ cell proliferation by polarising $CD14^+$ macrophages that over-expressed arginase-1 and inducible NO synthase, both implicated in the inhibition of T lymphocyte proliferation (Bézie, et al., 2015). These data suggest the presence of a positive feedback loop between IL-34 expression and $FOXP3^+$ Tregs. Interestingly, the fact that IL-34 targeted $FOXP3^+$ Tregs, which are essential for inhibiting the anti-donor immune response during allografts, suggests that IL-34 plays a potential role in transplantation therapy (Bézie, et al., 2015). In agreement with these data, during pregnancy; IL-34 induced macrophage polarisation into an M2 state, suggesting that IL-34 may play a 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 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).

In non-alcoholic fatty liver disease (NAFLD), a positive correlation between IL-34 levels in 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 $MIP3\alpha^{+}/CCL20^{+}$ macrophage differentiation, which promoted the production of collagen by hepatic stellate cells and subsequently development of fibrosis (Preisser, et al., 2014; Shoji, et al., 2016). The role of IL-34 in fibrosis was recently confirmed. Soluble egg antigen (SEA) produced by schistosome egg impairs NF-κB activation in hepatic stellate cells. This inhibits TNF-α, blocking the IL-34-associated liver fibrosis (Chen, et al., 2019). Consequently, IL-34 may be used as an independent marker for liver fibrosis in NAFLD (Shoji, et al., 2016).

Conclusion

Final macrophage differentiation relies on the action of specific growth factors and their specific receptors. The studies discussed in this review have shown that of these factors, the "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., 2008). Both cytokines share a common receptor, CD115, and their binding to the receptor activates the same signalling pathways. However, the similarities between M-CSF and IL-34 ended there. Each cytokine presented specific domains of interaction with CD115 and the nature of these interactions generated differential activation of the receptor (Liu, et al., 2012). IL-34 induced a strong but transitory phosphorylation of CD115 tyrosines, followed by rapid downregulation of the receptor (Chihara, et al., 2010). These differences in CD115 activation 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 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; 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.

Under normal physiology conditions, and depending on their microenviroment, circulating monocytes could be differentiated by M-CSF and IL-34 into specific non-resident macrophages with a "pro-inflammatory" M1 phenotype or in an "anti-inflammatory" M2 phenotype. Treating monocytes with IL-34 primarily induced macrophage differentiation into an M2 phenotype (Foucher, et al., 2013). The ability of each cytokine to induce different 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 frogs (Popovic, et al., 2019), contributing to and offering new potential animal models for advancement in knowledge of both cytokines and macrophage differentiation.

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 and proliferation of osteoclast precursos, and may replace M-CSF during osteoclastogenesis (Yu, et al., 2014). In the CNS, IL-34 is necessary for maintaining microglia during adulthood (Greter, et al., 2012), as well as for the proper development of the grey matter (Easley-Neal, et al., 2019) and the retinal microglia population (O'Koren, et al., 2019). In the skin, IL-34 has shown that it is essential for the development of LCs in the skin rudiment, the differentiation/proliferation of LCs in the dermis, and its absence compromised the presence 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 important for macrophage differentiation in other organs such as the gut (Zwicker, et al., 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 unclear and recent advances in single cell omics and organoid techniques may be helpful tools for better understanding the molecular and physiological cytokine-specificity of each subset of IL-34-derived macrophages, from non-resident to tissue-resident populations.

Depending on the tissue and microenviroment, the cytokine IL-34 has a positive or negative role and can be considered the Dr. Jekyll and Mr. Hyde cytokine. On the one hand, IL-34 acts a beneficial factor with high potential as a therapeutic molecule. In the CNS, IL-34-derived microglia have a neuroprotetive function by favoring neural myelination and survival in AD (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-34 by neurons in prion infections helps microglia proliferation and neuroprotection (Wang, et al., 2012). Moreover, IL-34 induces microglia resistance to West Nile virus infection (Vasek, 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 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 IL-34 can be used as a powerful tool for improving graft generation and allograft tolerance.

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, et al., 2014; Wang, et al., 2018), to autoimmune diseases such as RA (Galligan & Fish, 2017; Wang, et al., 2018; Wang, et al., 2017), systemic sclerosis (Kuzumi, et al., 2018), systemic 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 osteosarcoma (Ségaliny, et al., 2015), lung cancers (Baghdadi, et al., 2016), melanoma (Han, et al., 2018), colorectal cancer (Franzè, et al., 2018; Kobayashi, et al., 2019), ovarian tumours (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 high levels of IL-34 in serum or tissue fluids correlated with poor prognosis and survival, and 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 variety of tumours. IL-34 favoured tumour cell proliferation and metastasis by promoting IL-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. However, there were some exceptions, such as in the breast luminal B and HER2 tumour subtypes, where high levels of IL-34 were associated with better survival and a good prognosis. Therapies against TAMs have been developed in recent years. Reducing and

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

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

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Figure legends

Figure 1. Macrophage ontogeny and implications of IL-34 during macrophage differentiation. Depending on their origin, macrophages are divided into two different populations: tissue-resident macrophages and non-resident macrophages. Tissue-resident macrophages originate in the embryonic yolk sac, foetal liver and the bone marrow. Tissue-resident macrophages are capable of self-renewal of their own population (round arrows). However, in pathogenic situations, non-resident macrophages can migrate into the affected tissues and replenish the local populations by acquiring tissue specificities. Depending on the tissue, IL-34 drives macrophage differentiation, proliferation, maintenance, migration, and adhesion. Non-resident macrophages originate in the bone marrow and spleen. Circulating monocytes can extravasate and migrate to different tissues where, through the actions of different growth factors, they induce their polarisation into M1 or M2 subtypes. M1 macrophages detect pathogenic particles or inflammatory molecules such as LPS or INT-γ and display pro-inflammatory functions by secretion of pro-inflammatory factors such as TNF-α, IL-6 and Il-12. M2 macrophages are sensitive to molecules such as IL-4 or IL-13 and 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 bacterial, viral infection or inflammation, IL-34 can act as a pro- or anti-viral/inflammatory 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	K_{on}	K_{off}
M-CSF / CD115	**hydrophilic**	**1 pM**	**6.29×10^{7} s^{-1} M^{-1}**	**6.55×10^{-5} s^{-1}**
IL-34 / CD115	**hydrophobic**	**34 pM**	**1.7 x 107 s-1 M^{-1}**	**6.03×10^{-4} s^{-1}**

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

Table 2: IL-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 Ileum. 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 NFκB 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-1α 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)

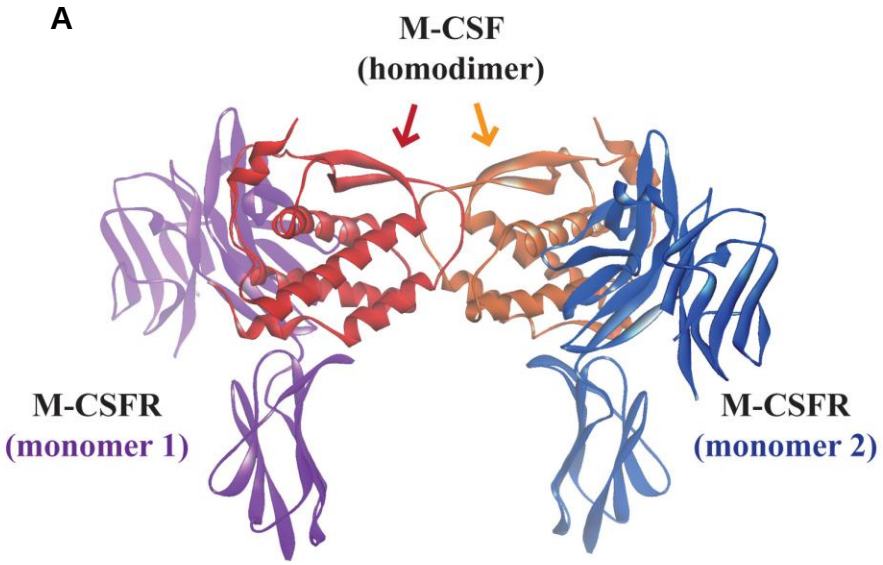
macrophage differentiation into tumour-associated macrophages (TAMs), which are characterised by an M2-like phenotype that promotes tumour proliferation, angiogenesis and metastasis. The capacity of IL-34 to act in a positive or negative direction is tissue- and microenvironment-dependent.

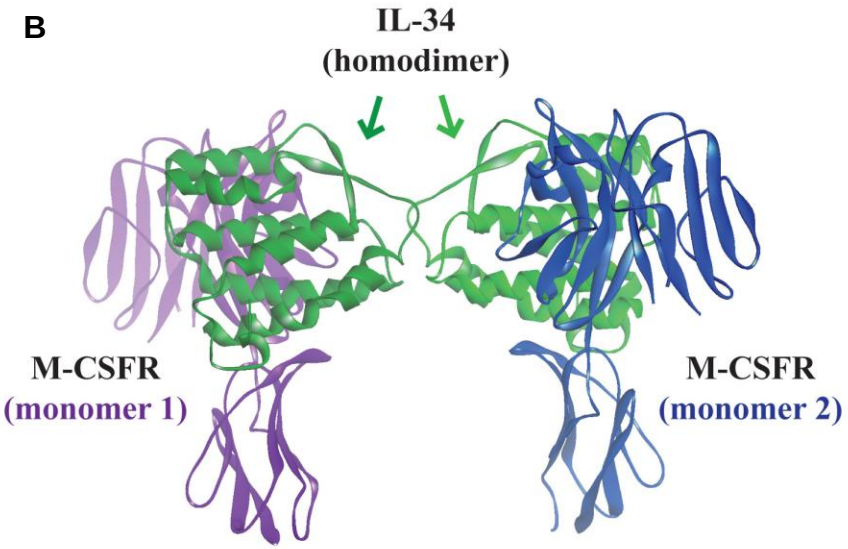
Figure 2. Molecular modelling of CD115 binding to its ligands. A) Representation of the three-dimensional crystal structure of the M-CSF/CD115 complex. In red and orange: monomers of M-CSF; and in blue and purple: monomers of CD115. **B**) Representation of the three-dimensional crystal structure of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

Figure 3. IL-34 signalling pathways involved in macrophage differentiation and non-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 CD115 or to syndecan-1 receptors expressed at the cell surface of monocytes/macrophages. The binding of IL-34 to CD115 induces activation of CD115 through auto-phosphorylation of the different tyrosines present in the cytosolic domain of CD115. Compared to M-CSF, IL-34 induces strong and transient activation of CD115, as well as rapid downregulation of CD115. These differences between the two cytokines imply differential activation of downstream signalling pathways that result in a diversity of macrophage biological processes such as differentiation, proliferation, survival or migration. The binding of IL-34 to the chondroitin chains of syndecan-1 results in *in vitro* phosphorylations of the tyrosines Y708 and Y723 of CD115, suggesting that the complex IL-34/syndecan-1 can act as a regulator of CD115 activity. Moreover, IL-34/syndecan-1 interaction regulates macrophage migration. **B**) IL-34 expression in the microenvironment of epithelial cells, fibroblasts and tumour cells can 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 PTP-ζ and controls inhibition of migration and proliferation of tumour cells lines such as glioblastoma U251.

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 IL-10. Macrophages 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 analysed by means of flow cytometry using specific antibodies for both M1-like macrophages (CD14, CD86 and CD64) and M2-like macrophages (CD163, CD200R and CD206). **B**) Comparison of macrophage differentiation after treatment with the cytokines GM-CSF (20 ng/ml), M-CSF (50 ng/ml) and IL-34 (50 ng/ml) alone or in combination with IFN-γ, IL-4 or IL-10 as performed in A. The three cytokines in combination with IFN-γ were able to induce 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 IL-10. No effect of IL-34 was observed in CD14 expression. Overall, IL-34 was able to induce M1 and M2 macrophage differentiation with a specific increase in CD163, an M2 marker.


A
M-CSF
(homodimer)
M-CSFR
(monomer 1)
M-CSFR
(monomer 2)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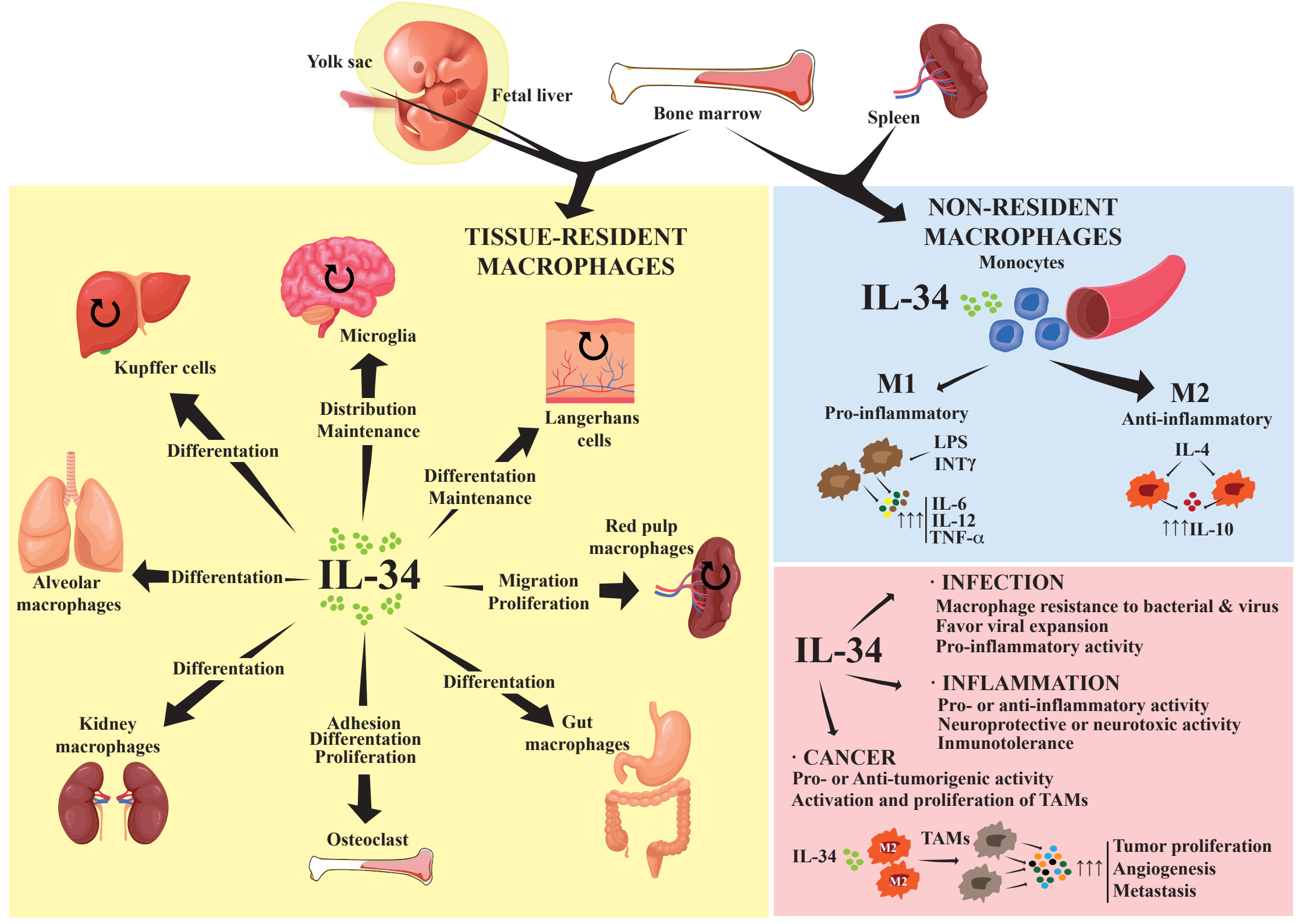
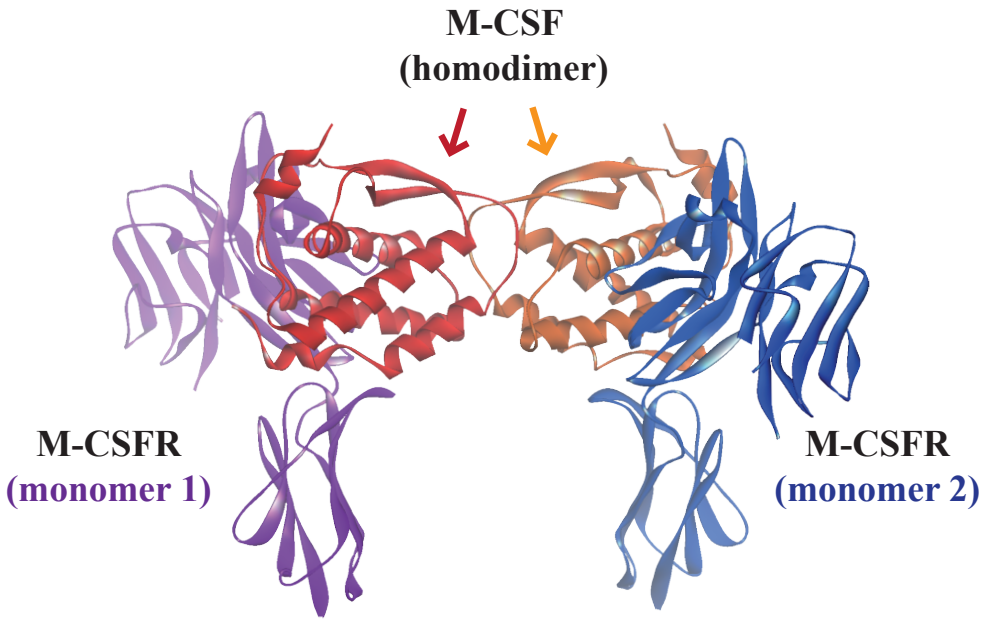
IL-34
(homodimer)
M-CSFR
(monomer 1)
M-CSFR
(monomer 2)


Yolk sac
Fetal liver
Bone marrow
Spleen
TISSUE-RESIDENT MACROPHAGES
NON-RESIDENT MACROPHAGES
Monocytes
IL-34
M1
Pro-inflammatory
LPS
INTγ
IL-6
IL-12
TNF-α
M2
Anti-inflammatory
IL-4
IL-10
Kupffer cells
Microglia
Distribution
Maintenance
Langerhans cells
Differentation
Differentation
Maintenance
Alveolar macrophages
Differentation
IL-34
Migration
Proliferation
Red pulp macrophages
Differentation
Differentation
Kidney macrophages
Adhesion
Differentation
Proliferation
Osteoclast
Gut macrophages
IL-34
· INFECTION
Macrophage resistance to bacterial & virus
Favor viral expansion
Pro-inflammatory activity
· INFLAMMATION
Pro- or anti-inflammatory activity
Neuroprotective or neurotoxic activity
Inmunotolerance
· CANCER
Pro- or Anti-tumorigenic activity
Activation and proliferation of TAMs
IL-34
M2
M2
TAMs
Tumor proliferation
Angiogenesis
Metastasis

A



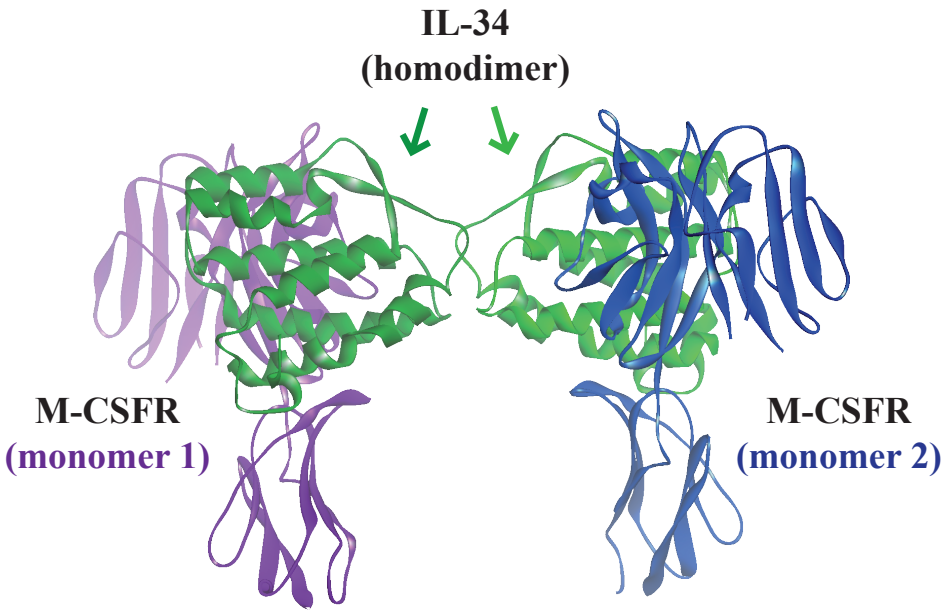
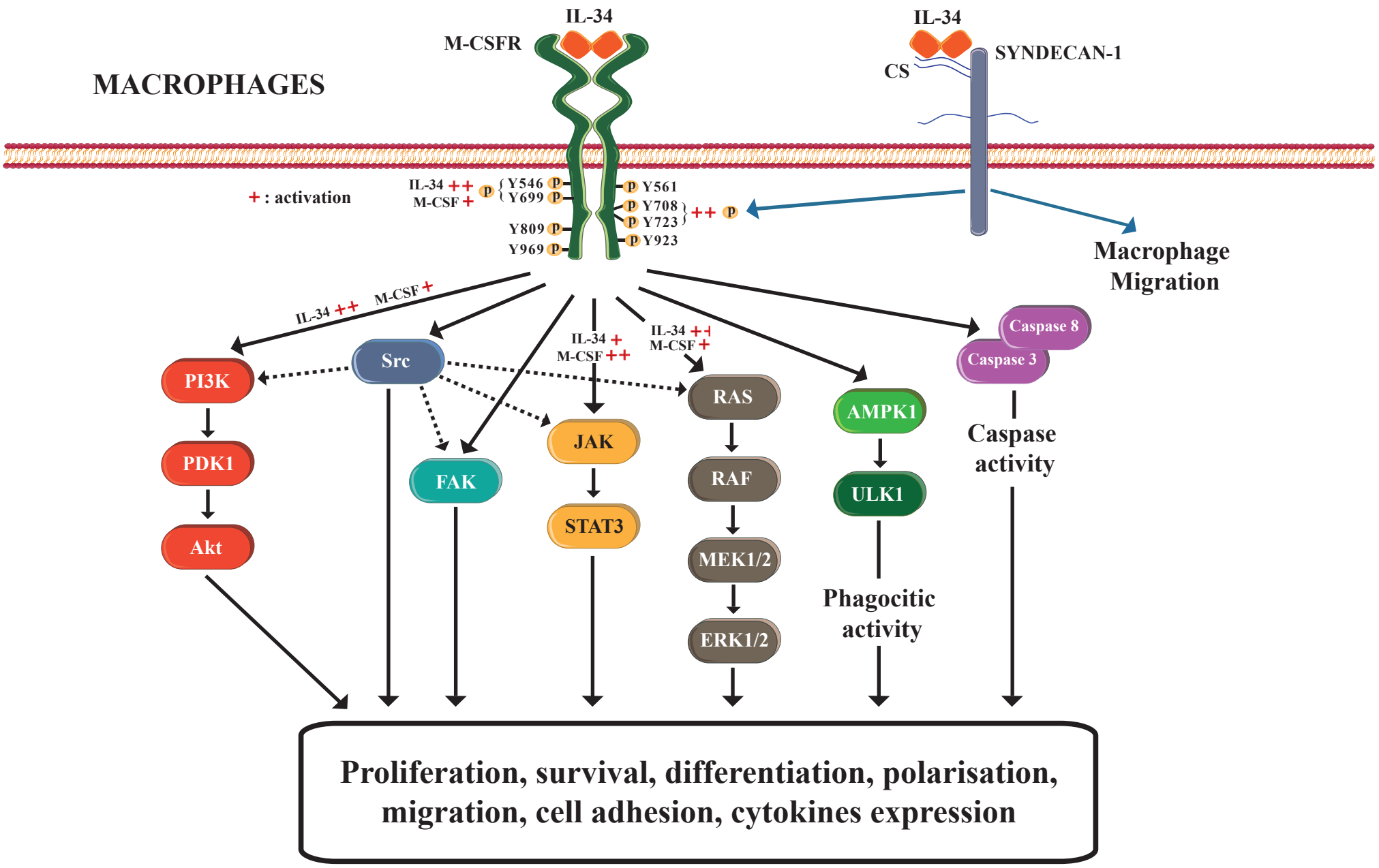


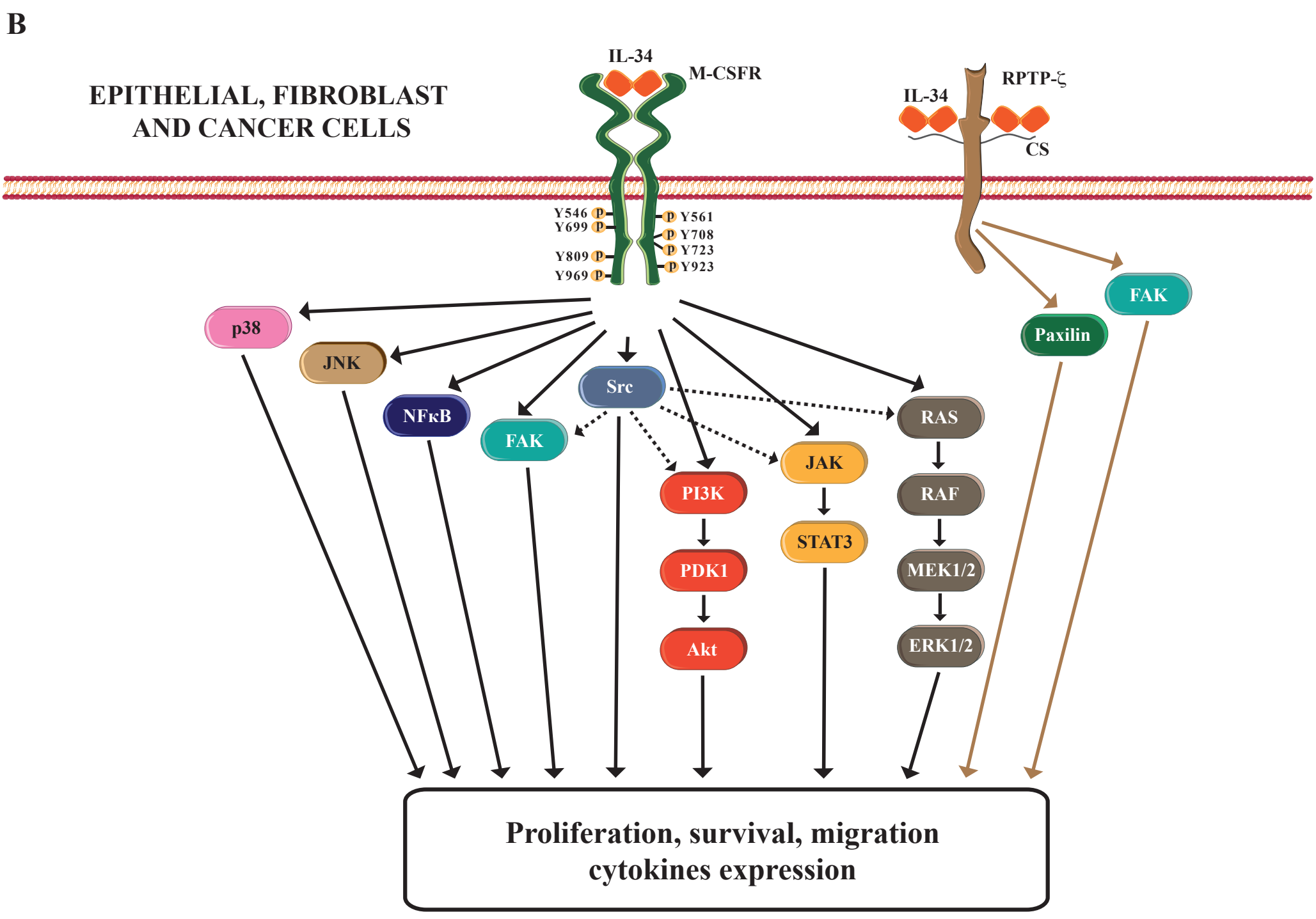
Table 1

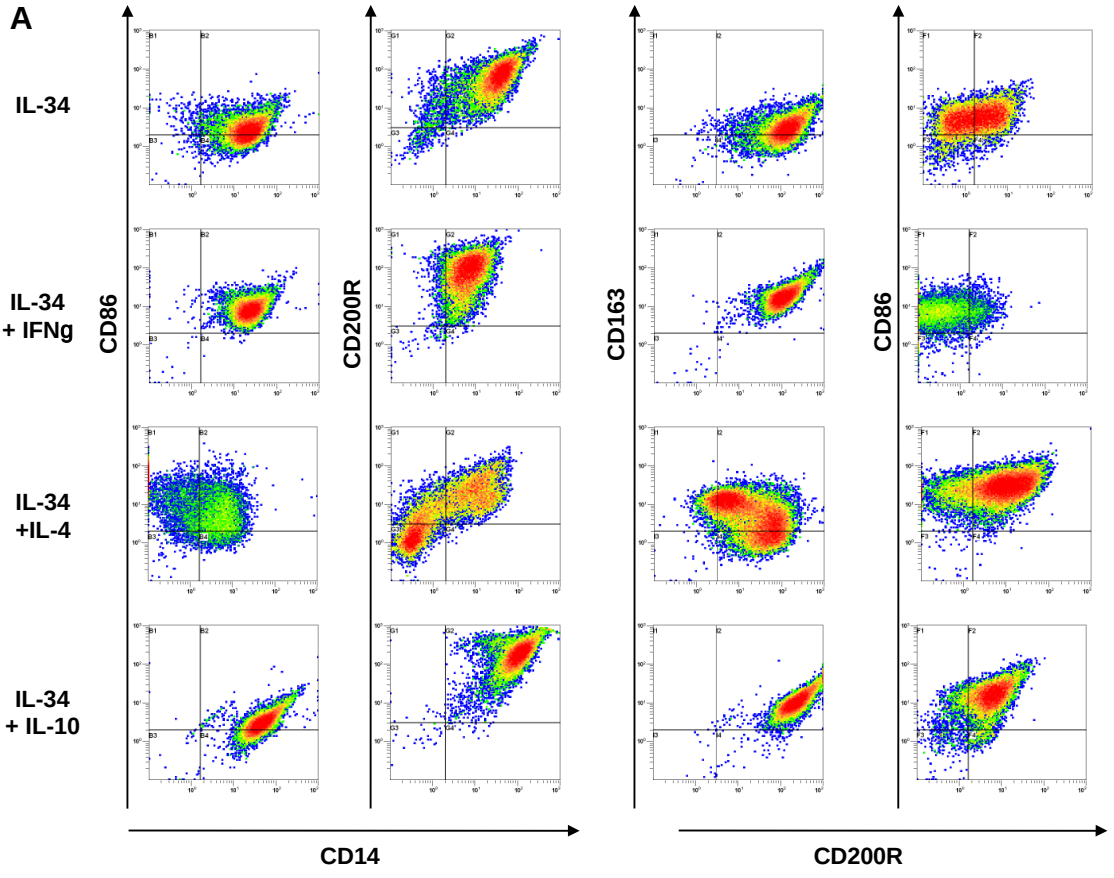
	Bound nature	K_D	K_{on}	K_{off}
M-CSF : M-CSFR	hydrophilic	1 pM	$6.29 \times 10^{7}\ s^{-1}\ M^{-1}$	$6.55 \times 10^{-5}\ s^{-1}$
IL-34 : M-CSFR	hydrofobic	34 pM	$1.7 \times 10^{7}\ s^{-1}\ M^{-1}$	$6.03 \times 10^{-4}\ s^{-1}$

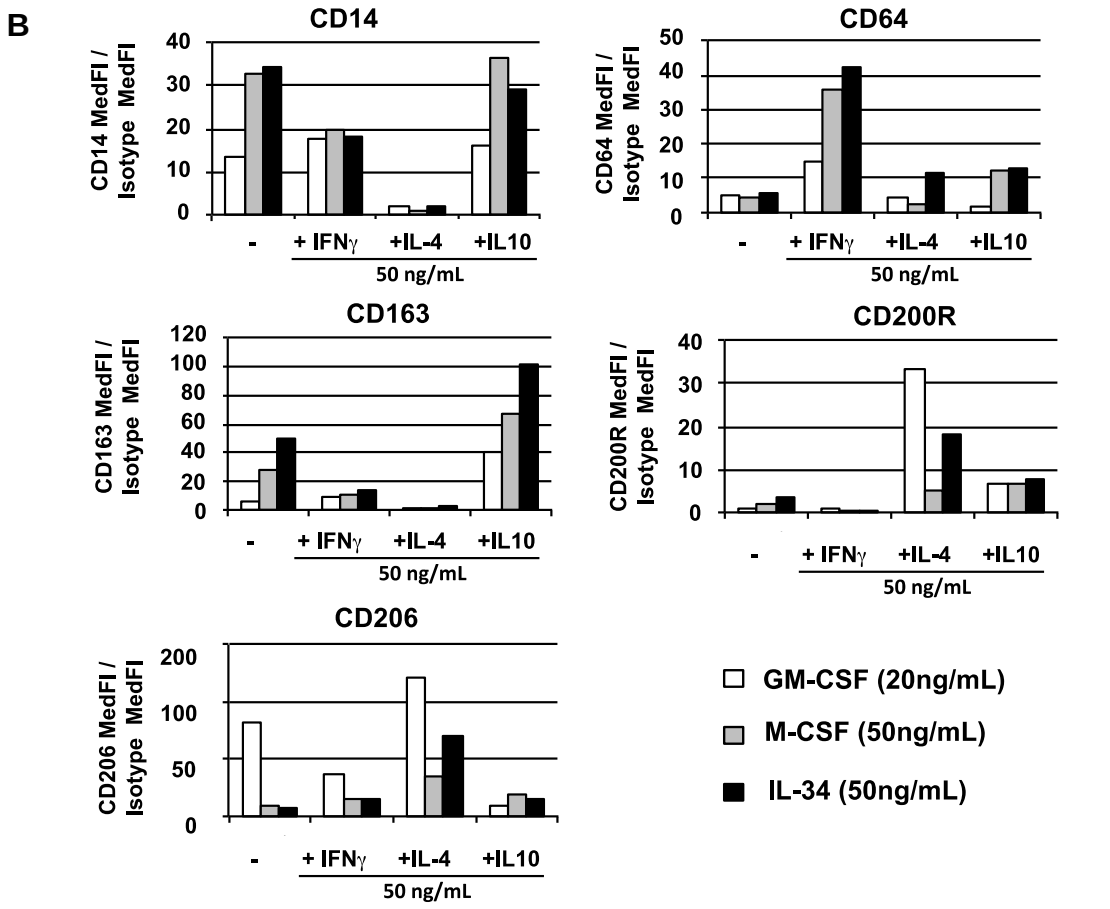
A

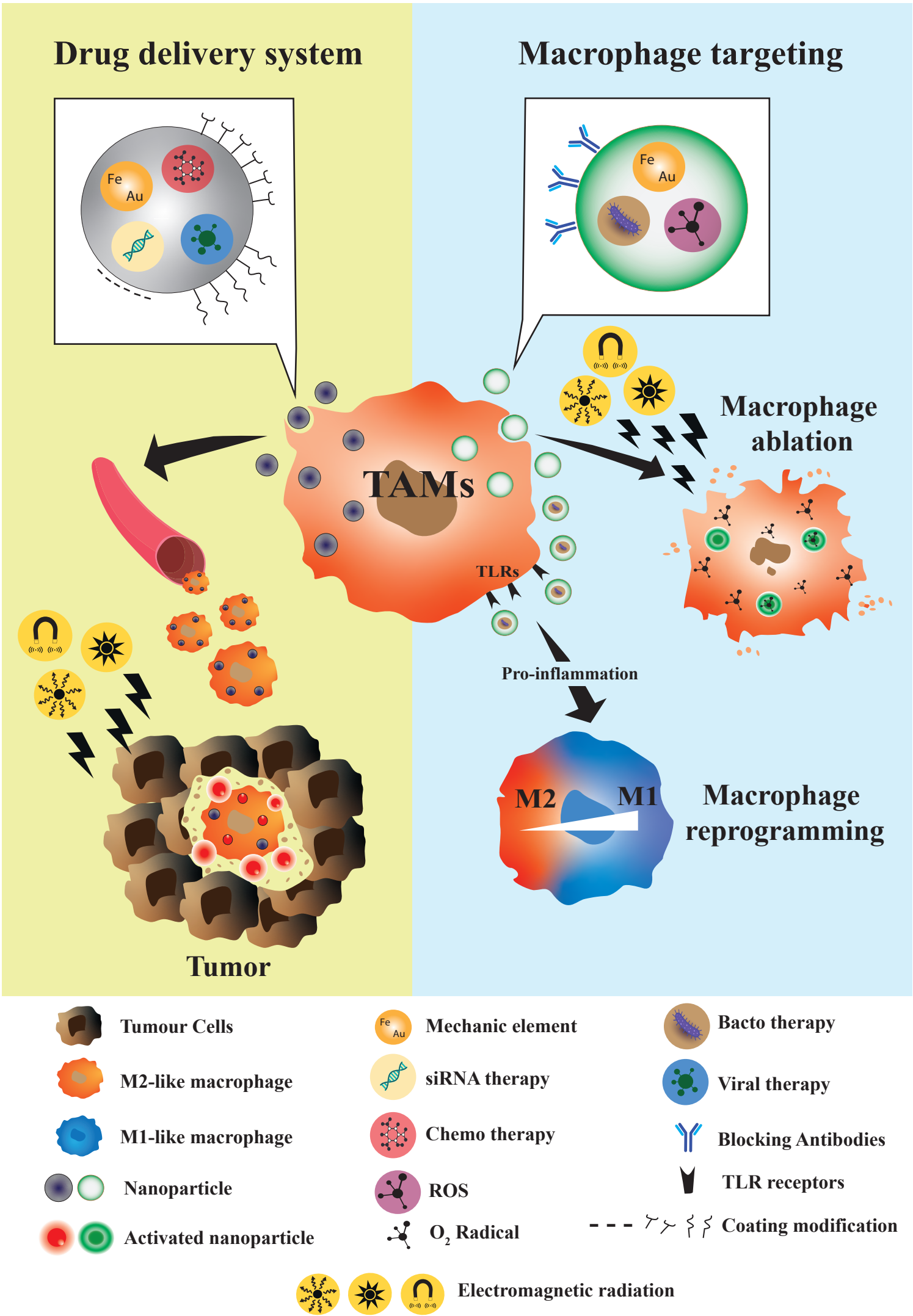

MACROPHAGES
IL-34
M-CSFR
IL-34
CS
SYNDECAN-1
+ : activation
IL-34 ++
M-CSF +
Y546
Y699
Y561
Y708
Y723
++
Y809
Y969
Y923
Macrophage Migration
IL-34 ++ M-CSF +
IL-34 + M-CSF ++
IL-34 ++ M-CSF +
PI3K
PDK1
Akt
Src
FAK
JAK
STAT3
RAS
RAF
MEK1/2
ERK1/2
AMPK1
ULK1
Phagocitic activity
Caspase 8
Caspase 3
Caspase activity
Proliferation, survival, differentiation, polarisation, migration, cell adhesion, cytokines expression

B


EPITHELIAL, FIBROBLAST AND CANCER CELLS
IL-34
M-CSFR
IL-34
RPTP-ζ
CS
Y546
Y699
Y561
Y708
Y723
Y809
Y969
Y923
p38
JNK
NFκB
FAK
Src
PI3K
PDK1
Akt
JAK
STAT3
RAS
RAF
MEK1/2
ERK1/2
Paxilin
FAK
Proliferation, survival, migration cytokines expression


A
IL-34
IL-34 + IFNg
IL-34 +IL-4
IL-34 + IL-10
CD86
CD200R
CD163
CD86
CD14
CD200R


B
CD14
CD14 MedFI / Isotype MedFI
CD64
CD64 MedFI / Isotype MedFI
CD163
CD163 MedFI / Isotype MedFI
CD200R
CD200R MedFI / Isotype MedFI
CD206
CD206 MedFI / Isotype MedFI
- + IFNγ +IL-4 +IL10
50 ng/mL
GM-CSF (20ng/mL)
M-CSF (50ng/mL)
IL-34 (50ng/mL)


Drug delivery system
Macrophage targeting
Fe
Au
TAMs
TLRs
Macrophage ablation
Pro-inflammation
M2
M1
Macrophage reprogramming
Tumor
Tumour Cells
M2-like macrophage
M1-like macrophage
Nanoparticle
Activated nanoparticle
Mechanic element
siRNA therapy
Chemo therapy
ROS
O2 Radical
Bacto therapy
Viral therapy
Blocking Antibodies
TLR receptors
Coating modification
Electromagnetic radiation