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REVIEW

Cytokines Focus

Emerging roles of IL-34 in health and disease

Iva Lelios, Dilay Cansever, Sebastian G. Utz, Wiebke Mildenberger, Sebastian A. Stifter, and Melanie Greter

Macrophages are part of the innate immune system and are present in every organ of the body. They fulfill critical roles in tissue homeostasis and development and are involved in various pathologies. An essential factor for the development, homeostasis, and function of mononuclear phagocytes is the colony stimulating factor-1 receptor (CSF-1R), which has two known ligands: CSF-1 and interleukin-34 (IL-34). While CSF-1 has been extensively studied, the biology and functions of IL-34 are only now beginning to be uncovered. In this review, we discuss recent advances of IL-34 biology in health and disease with a specific focus on mononuclear phagocytes.

Introduction

Colony-stimulating factor 1 (CSF-1; M-CSF) was originally defined by its ability to promote macrophage development from bone marrow precursors in vitro >40 yr ago (Stanley et al., 1978). Mice with an inactivating mutation in the *Csf1* gene ("osteopetrotic" mice [$Csf1^{op/op}$]) display severely reduced tissue macrophages, including osteoclasts, leading to osteopetrosis and other developmental defects (Wiktor-Jedrzejczak et al., 1990). Equally, most macrophages are absent in mice lacking the cognate receptor for CSF-1 ($Csf1r^{-/-}$ mice), highlighting the importance of CSF-1–mediated CSF-1R signaling for macrophage development (Dai et al., 2002; Erblich et al., 2011). Yet, $Csf1r^{-/-}$ mice have an overall more severe phenotype than mice lacking *Csf1*, leading to the proposed existence of an alternative ligand for CSF-1R (Dai et al., 2002). In 2008, IL-34 was identified as the additional ligand (Lin et al., 2008). In the past decade, many studies provided insight into IL-34 biology, but many questions remain unanswered, specifically in terms of its function. Here we review recent findings regarding IL-34, outline the impact of IL-34 on the development of the mononuclear phagocyte system, and discuss the current understanding of the role of IL-34 in pathology.

IL-34 and CSF-1 signaling through CSF-1R

IL-34 is a secreted homodimeric glycoprotein highly conserved among vertebrates and more conserved than CSF-1 among mammalian and avian species (Garceau et al., 2010; Lin et al., 2008). CSF-1, however, exists in three isoforms, which can be either secreted or membrane bound (Dai et al., 2004; Nandi et al., 2006). The dimers of IL-34 are noncovalently linked, in contrast to the disulfide-linked CSF-1 homodimers (Liu et al., 2012; Ma et al., 2012b; Pandit et al., 1992). Despite binding to the same receptor, IL-34 and CSF-1 do not share sequence homology, but their active regions are similar (Liu et al., 2012; Ma et al., 2012b). In vitro studies suggested that the two ligands could even form heterodimers (Ségaliny et al., 2015a), but whether this occurs in vivo and translates into biological activity remains unknown.

Both ligands bind to the same regions of the tyrosine kinase receptor CSF-1R, leading to phosphorylation and homodimerization of the receptor (Fig. 1). CSF-1 and IL-34 similarly support cell growth and survival of human monocytes and their differentiation into macrophages (Barve et al., 2013; Boulakirba et al., 2018; Chihara et al., 2010; Lin et al., 2008; Wei et al., 2010). Yet, some differences in signal transduction and polarization of macrophages were found. IL-34–stimulated macrophages induced eotaxin-1, while CSF-1–mediated signaling led to higher levels of MCP-1 (Chihara et al., 2010). IL-34 also promoted increased secretion of IL-10 and CCL17 in macrophages in comparison with CSF-1–exposed macrophages (Boulakirba et al., 2018). Interestingly, the two ligands seem to induce different transcriptional profiles in vivo in microglia, the resident macrophages in the brain parenchyma (Kana et al., 2019).

On the other hand, an overlapping function of the two ligands was demonstrated in transgenic mice lacking *Csf1* ($Csf1^{op/op}$) but expressing *Il34* under the control of the CSF-1 promoter. This rescued the developmental defects of $Csf1^{op/op}$ mice, suggesting that IL-34 can take over the function of CSF-1 if it is expressed in a similar spatiotemporal manner (Wei et al., 2010). In addition, osteoclast deficiency in $Csf1^{op/op}$ mice was compensated over time by IL-34 in the spleen (Nakamichi et al., 2012), indicating a

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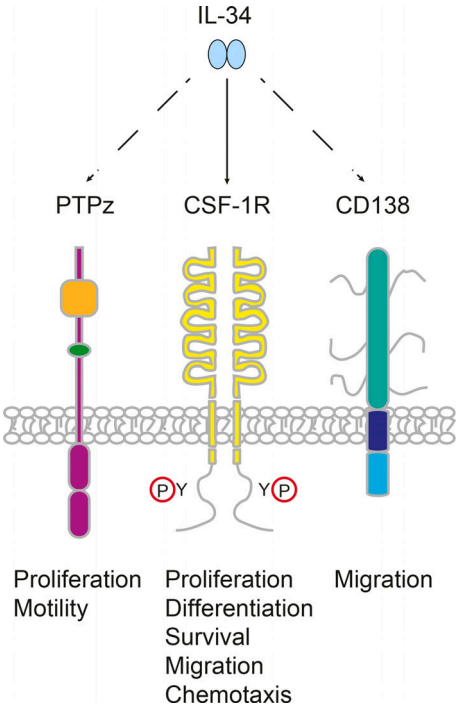

Figure 1. **IL-34 can bind three different receptors.** In addition to CSF-1R (CD115), which is the main receptor for IL-34, PTP-ζ and syndecan-1 (CD138) have recently been identified as alternative receptors (Nandi et al., 2013; Ségaliny et al., 2015b). IL-34–mediated receptor activation can induce several signaling pathways, including proliferation, survival, migration, polarization, and differentiation (Baghdadi et al., 2017; Guillonneau et al., 2017; Lafont et al., 2009; von Holst et al., 2006). Y, tyrosine; P, phosphorylation.

functional redundancy of IL-34 and CSF-1. Yet, in the absence of either IL-34 or CSF-1, no increase of the respective other ligand was observed, arguing against a compensatory impact (Wei et al., 2010). Thus, future studies may reveal potential differences in downstream CSF-1R signaling pathways triggered by either IL-34 or CSF-1 or whether similar CSF-1R signal transduction is determined by the regional ligand availability and/or abundance. This might also provide insight into the reason for the evolution of two ligands signaling through the same receptor.

IL-34 receptors

CSF-1R is the main receptor for IL-34. While it is virtually expressed by all macrophages, monocytes, myeloid precursors, and a subset of dendritic cells, some other nonhematopoietic cells were also suggested to express CSF-1R (Stanley and Chitu, 2014). Among those were placental trophoblasts, renal proximal tubular epithelial cells (TECs), and colonic epithelial cells (Huynh et al., 2013; Jokhi et al., 1993; Stanley and Chitu, 2014) and some neurons (Clare et al., 2018; Luo et al., 2013; Nandi et al., 2012). However, other studies (Erblich et al., 2011; Kana et al., 2019; Sehgal et al., 2018) could not provide evidence that CSF-1R is indeed expressed outside of the mononuclear phagocyte lineage (reviewed in Hume et al., 2019).

Recently, alternative receptors engaging with IL-34 have been proposed: the receptor-type protein-tyrosine phosphatase-ζ (PTP-ζ) and syndecan 1 (CD138; Fig. 1; Nandi et al., 2013; Ségaliny et al., 2015b). Within the central nervous system (CNS), the chondroitin sulfate proteoglycan PTP-ζ is expressed on neural progenitors, neurons, and glial cells and was shown to be involved in various neural functions (e.g., proliferation, migration, differentiation, synaptogenesis, and myelination; Lafont et al., 2009). Whether IL-34 indeed contributes to PTP-ζ signaling in vivo and whether IL-34 has overlapping functions with other PTP-ζ ligands remains to be shown. Furthermore, albeit with low affinity, IL-34 can bind syndecan-1, a type I transmembrane heparin sulfate proteoglycan (Ségaliny et al., 2015b). Syndecan-1 expression correlated with IL-34–induced CSF-1R activation in macrophages in vitro (Ségaliny et al., 2015b). Whether syndecan-1 modulates CSF-1R activities in-vivo remains unclear.

Tissue expression of IL-34 and CSF-1

Both IL-34 and CSF-1 seem to be broadly expressed in a variety of tissues in the steady state. *CSF1* mRNA can be identified in humans and mice in the brain, spleen, mammary gland, testis, kidney, and small intestine and also in human lymph nodes, bone marrow, uterus, and ovary (Chitu and Stanley, 2006; Sherr et al., 1985; Stanley et al., 1983; Yoshida et al., 1990). In addition, *Csf1* seems to be expressed in the heart, fat, pancreas, liver, and lung (Tabula Muris Consortium, 2018). At the protein level, CSF-1 was detected in the human heart and the murine lung (Bettina et al., 2016; Hohensinner et al., 2007).

The highest levels of IL-34 are found in the brain and the skin, where it is expressed by neurons and keratinocytes, respectively (Greter et al., 2012; Nandi et al., 2012; Wang et al., 2012). *Il34* is further expressed in various tissues, including human and mouse heart, lung, liver, kidney, and testes, and in human intestine, thymus, spleen, colon, prostate, and lymph nodes (Lin et al., 2008; Nakamichi et al., 2012; Tabula Muris Consortium, 2018; Wang et al., 2012, 2016a; Fig. 2 A). While most of these studies reported gene expression, IL-34 protein was described in the healthy murine lung and human skin, kidney, intestine, spleen, and brain (Baek et al., 2015; Bettina et al., 2016; Franzè et al., 2015; Greter et al., 2012; Lin et al., 2008). We also found that IL-34 protein was detectable across various mouse tissues, with highest expression in brain, skin, and lymph nodes (Fig. 2 B), consistent with published mRNA data. In many organs, the cell types producing IL-34, its distribution pattern compared with CSF-1, and its role in the function of tissue-resident macrophages remain to be investigated.

The role of IL-34 for microglia and Langerhans cells (LCs)

Many tissue macrophages are derived from embryonic precursors and self-maintain throughout adulthood (Ginhoux and Jung, 2014). LCs, the resident mononuclear phagocytes of the epidermis, are mostly derived from fetal liver monocytes (Hoeffel et al., 2012). In contrast, primitive macrophages generated in the yolk sac during early embryonic development give rise to microglia (Ginhoux et al., 2010). Similarly to most other tissue macrophages, the genesis of these two populations also depends on CSF-1R signaling (Ginhoux et al., 2006, 2010; Greter et al., 2012). However, while most tissue macrophages rely solely on CSF-1 for their development, CSF-1 appears to only partially contribute to the development of LCs

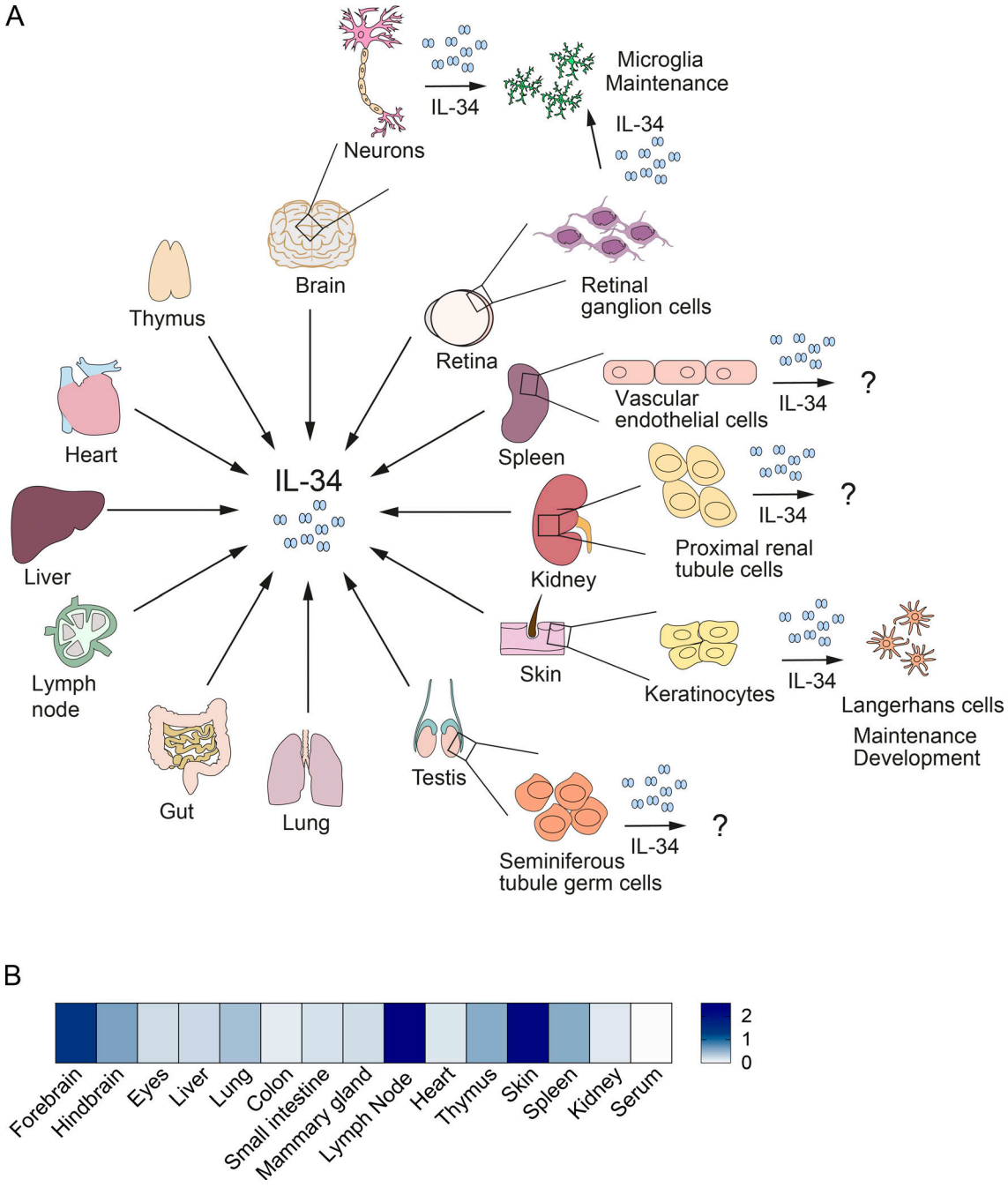

Figure 2. **Tissue expression of IL-34 in the steady state. (A)** Shown are tissues and cell types expressing IL-34 under physiological conditions (Lin et al., 2008; Tabula Muris Consortium, 2018). In the brain, IL-34 is produced by neurons, which control microglial homeostasis in adulthood (Greter et al., 2012; Nandi et al., 2012; Wang et al., 2012). The maintenance of retinal microglia in the inner plexiform layer depends on IL-34 produced by retinal ganglion cells (O'Koren et al., 2019). Epidermal keratinocyte–derived IL-34 is critical for the development and maintenance of LC (Greter et al., 2012; Wang et al., 2012). In the testis, IL-34 is secreted by seminiferous tubule germ cells and in the kidney by proximal renal tubule cells (Wang et al., 2012). Vascular endothelial cells in the spleen were also shown to produce IL-34 (Nakamichi et al., 2012). **(B)** Heatmap shows IL-34 concentration of various tissues from C57BL/6 mice quantified by ELISA (R&D Systems; $n = 3$). Background was assessed using tissue lysates from *Il34*-deficient mice. Color code is pg/µg total protein.

(Cecchini et al., 1994; Dai et al., 2002). Indeed, LC differentiation and maintenance are mostly dependent on IL-34, which is produced in the developing epidermis from embryonic day 17.5 onward. Consistent with this expression pattern, *Il34*-deficient mice display a reduction of LC precursors and absence of adult LCs (Greter et al., 2012; Wang et al., 2012, 2016a).

Within the brain, expression of CSF-1 and IL-34 is nonoverlapping and spatially distinct (Kana et al., 2019; Nandi et al., 2012). During embryogenesis, IL-34 is restricted to the marginal zone and cortical plate, whereas CSF-1 is expressed within the subventricular zone and the ventricular area. In the neonatal cortex, CSF-1 is detected in layer VI, and IL-34 is detected in

layers II–V (Nandi et al., 2012). Despite the presence of IL-34 in the developing brain, microglial development in mice is not dependent on IL-34 but requires CSF-1 (Greter et al., 2012; Kana et al., 2019). Yet, in zebrafish, a further role of IL-34 in the colonization of embryonic macrophages to the anterior head was demonstrated (Wu et al., 2018).

In contrast to development, adult microglia are reduced in *Il34*-deficient mice in regions correlating with high IL-34 expression, indicating that their maintenance depends on IL-34 (Greter et al., 2012; Wang et al., 2012). IL-34 is mainly produced by neurons, at the highest levels in the cortex, the hippocampus, and the striatum (Greter et al., 2012; Kana et al., 2019; Wang et al., 2012; Wei et al., 2010). In comparison, CSF-1 is predominantly expressed by astrocytes in the cerebellum, and in mice lacking CSF-1, cerebellar microglia were decreased, while in other regions, only moderate or no reduction of microglia was detected (Calvo et al., 1998; Ginhoux et al., 2010; Kana et al., 2019; Wegiel et al., 1998).

In addition to neurons, IL-34 is expressed by ependymal cells lining the ventricular system and in the choroid plexus (Nandi et al., 2012; Wang et al., 2012). Whether this influences the development or function of choroid plexus macrophages requires more studies. In the retina, IL-34 expression was detected in retinal ganglion cells, where it also controls the homeostasis of one subset of retinal microglia residing in the inner plexiform layer (O'Koren et al., 2019). Altogether, while CSF-1 is critical for microglial development, a region-specific expression of IL-34 and CSF-1 regulates microglial homeostasis, phenotype, and transcriptional profile.

IL-34 expression and function in pathological conditions

The importance of IL-34 for LCs and microglia has been described, but relatively little is known about its role in pathology. Here, we summarize studies showing correlations of IL-34 expression in a variety of diseases and discuss its potential function.

IL-34 in CNS pathology

In neuron–microglia cocultures, treatment of microglia with IL-34 triggered the upregulation of TGF-β, insulin-degrading enzyme, and the antioxidant enzyme heme oxygenase 1 (HO-1), which limited the neurotoxic effects of oligomeric amyloid-β 1–42 (Ma et al., 2012a; Mizuno et al., 2011). IL-34 administration to the CNS in vivo promoted microglial accumulation and improved cognitive function in the APP/PS1 mouse model of Alzheimer's disease (Mizuno et al., 2011), indicating a neuroprotective role of IL-34 via regulation of microglial function. Similarly, administration of IL-34 or CSF-1 reduced microgliosis and neuronal loss after kainic acid–induced excitotoxicity, a model for neurodegeneration (Luo et al., 2013; Wang et al., 2005). Finally, beneficial effects of IL-34 on neuropathology could also be shown in an animal model of prion disease where *Il34* deficiency led to accelerated PrP^{Sc} deposition and shorter survival (Zhu et al., 2016). Yet, terminally ill animals showed similar microglia numbers, proposing that increased susceptibility of *Il34*-deficient animals might be due to the decreased microglia numbers in steady state or altered functions in the absence of IL-34.

CSF-1R mutations are associated with neurodegenerative diseases, including adult-onset leukoencephalopathy with axonal spheroids and pigmented glia, a hereditary diffuse white matter disorder (Pridans et al., 2013; Rademakers et al., 2011). The underlying cause is the partial loss of CSF-1R signaling on microglia leading to microglial dysfunction. Whether decreased IL-34 or CSF-1–mediated CSF-1R transduction leads to adult-onset leukoencephalopathy with axonal spheroids and pigmented glia pathogenesis and whether mutations affecting either ligand would lead to a similar disorder remain to be shown.

IL-34 in rheumatoid arthritis (RA)

RA is a complex autoimmune disease characterized by synovial inflammation and hyperplasia, bone and cartilage destruction, and systemic disorders affecting different organs (McInnes and Schett, 2011). Many studies revealed an association between RA severity and IL-34 expression. Higher serum levels of IL-34 in RA patients correlated with a variety of RA features and were described as an independent risk factor for radiographic progression in RA (Moon et al., 2013; Tian et al., 2013; Chang et al., 2015; Zhang et al., 2015; Yang et al., 2016; Wang et al., 2018a). Similarly, higher levels of IL-34 in the synovial fluid in RA patients were indicative of increased inflammation in the synovium (Chemel et al., 2012; Hwang et al., 2012; Moon et al., 2013; Tian et al., 2013; Yang et al., 2016) and corresponded to higher histological severity of synovitis, increased hyperplasia, higher leukocyte counts, and increased levels of IL-6 and receptor activator of NF-κB ligand (Chemel et al., 2012; Moon et al., 2013; Wang et al., 2017). Synovial biopsies from RA patients showed that IL-34 is mostly expressed by fibroblast-like synovial cells (FLS) and endothelial cells (Chang et al., 2015; Chemel et al., 2012; Hwang et al., 2012). Moreover, IL-34 production in FLS from RA patients was increased by the proinflammatory cytokines TNF-α and IL-1β and was inhibited by TGF-β1 and BMP-2 (Chemel et al., 2012; Hwang et al., 2012; Wang et al., 2017). In comparison, while CSF-1 is also highly expressed by RA FLS, it is not enhanced upon TNF-α stimulation, indicating that the expression of the two cytokines is regulated by different mechanisms (Hwang et al., 2012). Consequently, serum levels of IL-34 could be used to predict the response to TNF-α antagonist therapy (Ding et al., 2015; Tian et al., 2013; Zhang et al., 2015). In line with these observations, injection of recombinant IL-34 in mice aggravated the disease severity of collagen-induced arthritis and promoted an increase in IL-6 and TNF-α (Zhang et al., 2018). Similarly, administration of CSF-1 exacerbated disease in models of arthritis (Bischof et al., 2000; Campbell et al., 2000). On the other hand, blocking CSF-1R in mice had a protective role in collagen-induced arthritis and was more effective than a TNF-α antagonist (Garcia et al., 2016). Nevertheless, it is not clear whether this effect was mediated only by neutralization of CSF-1R signaling or by the general depletion of macrophages upon CSF-1R blockade (Sauter et al., 2014). Altogether, IL-34 is induced by proinflammatory cytokines to sustain an inflammatory cascade promoting joint pathology. Future studies should further elucidate the precise mechanisms of IL-34 action in vivo and establish its possible role as a diagnostic or therapeutic factor.

IL-34 in systemic lupus erythematosus (SLE) and kidney disease

SLE is a chronic autoimmune disease affecting different organs (Tsokos, 2011). Increased serum levels of IL-34 in SLE patients correlated positively with SLE disease activity index, titers of anti–double-stranded DNA antibodies, and C-reactive protein and negatively with levels of C3 in serum (Wang et al., 2016b; Xie et al., 2018). IL-34 was also found to be an independent risk factor for the development of lupus nephritis, an SLE-caused kidney inflammation with high morbidity and mortality (Almaani et al., 2017; Cheng et al., 2019). Both IL-34 and CSF-1 are expressed in TECs of the kidney in both SLE patients and the MRL-Fas^{lpr} mouse model of lupus nephritis. *Il34*-deficient MRL-Fas^{lpr} mice showed decreased kidney pathology, reduced signs of multiorgan failure, decreased infiltration of monocytes and lymphocytes in the kidney, and lower intrarenal production of chemokines and proinflammatory cytokines. Thus, IL-34 appears to promote lupus nephritis pathogenesis by supporting the accumulation of macrophages in the kidney (Wada et al., 2019).

IL-34 and CSF-1 expression in TECs was also increased in another model of kidney pathology: ischemia–reperfusion acute kidney injury that can progress to chronic kidney disease (Baek et al., 2015). Similarly to lupus nephritis, IL-34 promoted kidney pathology and TEC destruction, as well as proliferation of kidney macrophages and myeloid cell infiltration. IL-34 deficiency resulted in significant reduction in kidney atrophy and myeloid cell accumulation (Baek et al., 2015). Overall, inflammation-induced IL-34 in the kidney promotes expansion and recruitment of myeloid cells to drive kidney destruction. Interestingly, CSF-1 was suggested to promote repair in models of acute kidney injury (Alikhan et al., 2011; Menke et al., 2009; Zhang et al., 2012), indicating opposing roles for CSF-1 and IL-34. How these two CSF-1R ligands can mediate opposing functions, however, remains to be defined.

IL-34 in other inflammatory diseases

Il34 mRNA was up-regulated in the salivary glands of patients with Sjögren's syndrome, a chronic autoimmune disorder of the exocrine glands (Ciccia et al., 2013; Fox, 2005). IL-34 correlated with the expression of proinflammatory cytokines TNF-α, IL-1β, IL-17, and IL-23p19 and with infiltration of inflammatory monocytes (Ciccia et al., 2013). In patients with Crohn's disease or ulcerative colitis, the two major forms of inflammatory bowel disease, IL-34 (and also CSF-1) was up-regulated in the inflamed colon epithelium through TNF-α–induced NF-κB signaling (Franzè et al., 2015; Zwicker et al., 2015).

In nonalcoholic fatty liver disease, levels of CSF-1 and IL-34 were highly increased in the liver and the serum of patients with nonalcoholic fatty liver disease. IL-34 concentration increased with fibrosis progression and could be used as an independent diagnostic marker for fibrosis (Shoji et al., 2016). Likewise, IL-34 was expressed by hepatocytes in liver fibrosis induced by hepatitis B or C infection and also correlated with increasing liver fibrosis (Preisser et al., 2014; Wang et al., 2018b). Consistent with these findings in the clinic, in vitro IL-34–differentiated monocytes up-regulated profibrotic factors and promoted collagen I production in hepatic stellate cells, supporting a pathogenic function for IL-34 in promoting fibrotic liver diseases (Preisser et al., 2014).

Altogether, increased levels of IL-34 correlate with many inflammatory, autoimmune, metabolic, and cardiovascular disorders (Chang et al., 2014; Fan et al., 2016; Li et al., 2012; Tao et al., 2017; Xi et al., 2018; Zorena et al., 2016). Yet, clear evidence for a function of IL-34 in many of these disorders is lacking.

IL-34 in skin inflammation

In contrast to the inflammatory diseases described above, acute skin inflammation (induced by UV light or in a mouse model of atopic dermatitis) results in down-regulation of IL-34 and increased expression of CSF-1 in the epidermis. This leads to a partial repopulation of LCs in *Il34*-deficient mice, suggesting that in inflammation, CSF-1 can replace IL-34 in supporting LC differentiation (Ginhoux et al., 2006; Greter et al., 2012; Wang et al., 2012, 2016a). Consistent with the findings in animal models, in patients with atopic dermatitis, IL-34 expression was reduced compared with skin from healthy volunteers (Esaki et al., 2015). Thus, in the skin, IL-34 appears to be a homeostatic cytokine, which is down-regulated in acute inflammation. Experimental systems designed to elevate local levels of IL-34 in skin could limit skin inflammation and thus result in clinical benefit.

IL-34 in cancer

Tumor-associated macrophages (TAMs) are implicated in tumor growth, metastasis formation, angiogenesis, and immune suppression/activation. Expression of CSF-1R as well as CSF-1 often correlates with poor prognosis in various types of cancer (Behnes et al., 2014; Espinosa et al., 2014; Kawamura et al., 2009; Liu et al., 2016; Richardsen et al., 2015; Scholl et al., 1994). Thus, the CSF-1R axis, which is involved in regulating the function of TAMs, represents an attractive target for immunotherapeutic strategies, and several approaches inhibiting CSF-1R are currently under clinical development (Cannarile et al., 2017; Kumari et al., 2018; Peyraud et al., 2017).

Regarding IL-34, emerging data also demonstrate its correlation with poor prognosis in several cancers, such as osteosarcoma and lung and breast cancer (Fig. 3; Baghdadi et al., 2018; Wang et al., 2015). Expressed by various types of cancer cells and further induced upon chemotherapy treatment (Baghdadi et al., 2016; Baud'Huin et al., 2010; Cioce et al., 2014; Franzè et al., 2017; Wang et al., 2015), IL-34 binds to CSF-1R on mononuclear phagocytes, which play distinct roles during different stages of tumor progression (Baud'Huin et al., 2010; Noy and Pollard, 2014; Ségaliny et al., 2015c; Zins et al., 2018). IL-34 contributes to new vessel formation and promotes tumor progression and metastasis via accumulation of TAMs (Ségaliny et al., 2015c), as was shown for CSF-1 (Curry et al., 2008; Lin et al., 2001). Embryonic stem cell–derived IL-34 was shown to polarize bone marrow–derived macrophages into macrophages that phenotypically and functionally resemble TAMs, showing increased levels of Arg-1, Tie-2, and TNF-α, and which contributed to angiogenesis and teratoma progression (Chen et al., 2014).

Another study showed that IL-34 produced by hepatocellular carcinoma cells increased TGF-α production by TAMs, which in turn inhibited the negative regulator of IL-34, miR-28-5p. This

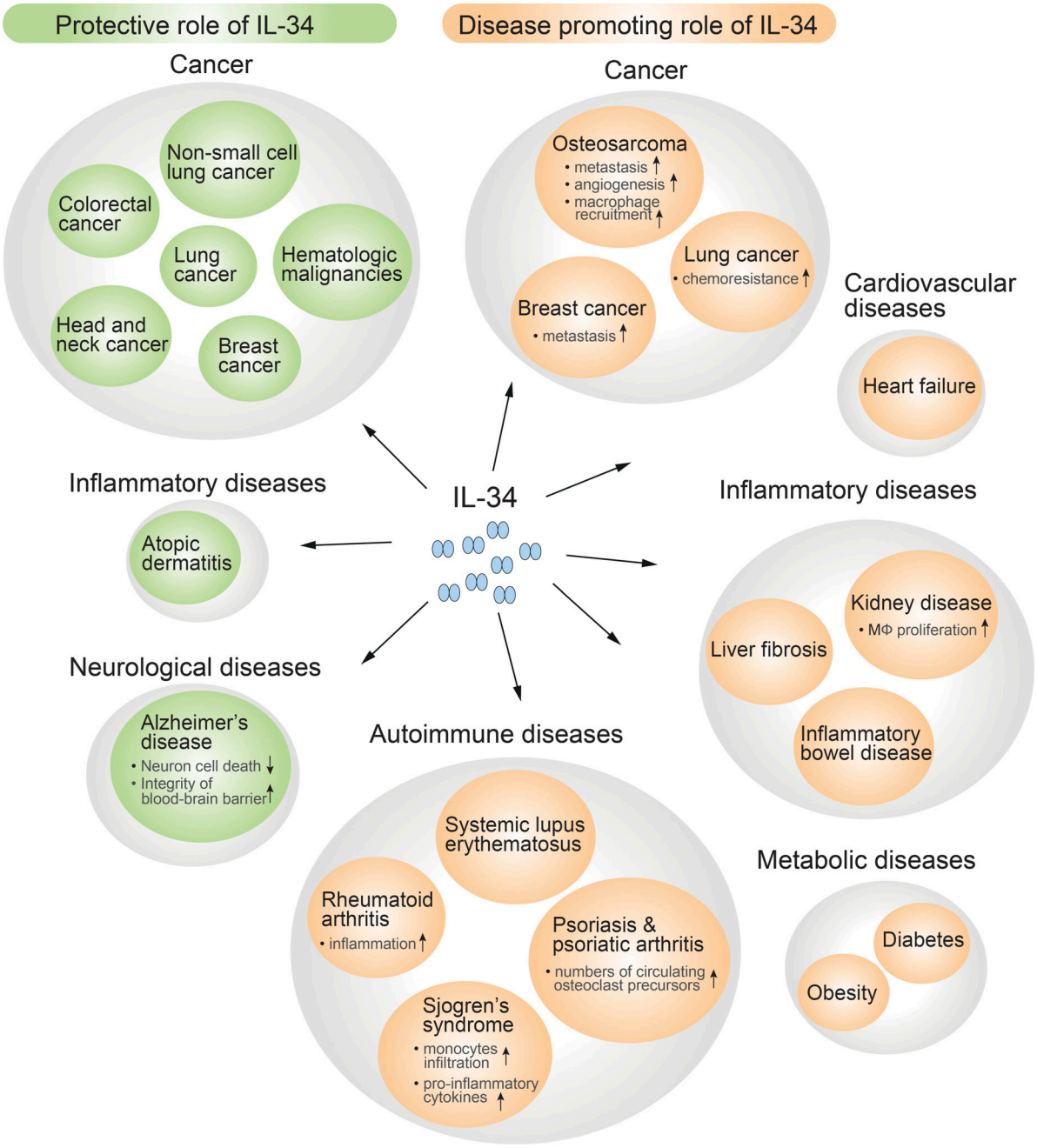

Figure 3. **Protective and disease-promoting roles of IL-34 in disease.** High expression of IL-34 correlates with disease severity in autoimmune diseases (Sjögren's syndrome [Ciccia et al., 2013; Fox, 2005], SLE [Wang et al., 2016b; Xie et al., 2018], psoriasis [Mizuno et al., 2011], and RA [Chang et al., 2015; Moon et al., 2013; Tian et al., 2013; Wang et al., 2017; Yang et al., 2016; Zhang et al., 2015]), inflammatory diseases (liver fibrosis [Shoji et al., 2016; Preisser et al., 2014; Wang et al., 2018b], kidney disease [Baek et al., 2015], and inflammatory bowel disease [Franzè et al., 2015; Zwicker et al., 2015]), cardiovascular disease (heart failure; Fan et al., 2016; Li et al., 2012; Tao et al., 2017; Xi et al., 2018), metabolic diseases (diabetes [Chang et al., 2014; Zorena et al., 2016] and obesity [Chang et al., 2014; Zorena et al., 2016]), and cancer (osteosarcoma [Ségaliny et al., 2015c], lung cancer [Baghdadi et al., 2018], and breast cancer [Wang et al., 2015]). In contrast, IL-34 plays a protective role in some diseases, such as atopic dermatitis (Esaki et al., 2015), Alzheimer's disease (Mizuno et al., 2011), and cancer (non–small cell lung cancer [Lee et al., 2008], colorectal cancer [Wang et al., 2015], breast cancer [Mizuno et al., 2009; Uhlen et al., 2017], lung cancer [Wang et al., 2015], hematologic malignancies [Wang et al., 2015], and head and neck cancer [Mizuno et al., 2009; Uhlen et al., 2017]).

feedback loop was suggested to modulate hepatocellular carcinoma growth and metastasis and to promote tumor progression. Moreover, patients with low miR-28-5p expression, high IL-34 levels, and high numbers of TAMs had a poor prognosis with shorter overall survival (Zhou et al., 2016).

A potential involvement of IL-34 in immunotherapy resistance was demonstrated in a patient with metastatic melanoma. Resistance to anti-programmed cell death protein 1 immunotherapy was acquired concomitant with enhanced expression of IL-34 and increased frequencies of CD163$^+$ macrophages (Han

et al., 2018). A similar observation was made for CSF-1 (Neubert et al., 2018). Altogether, while a direct role of IL-34 on TAMs and in tumor progression and immunotherapy resistance still has to be demonstrated, it is a promising potential candidate for novel therapeutic strategies.

Yet, both IL-34 and CSF-1 were also shown to negatively correlate with tumor progression. CSF-1R blockade or anti–CSF-1 treatment promoted metastasis formation in several animal models (Beffinger et al., 2018; Swierczak et al., 2014). Likewise, lower expression of IL-34 was found in a cohort of patients with non–small cell lung cancer and was related to poor survival (Wang et al., 2015). In addition, high expression of IL-34 also seemed to be favorable in some cases of hematologic malignancies, head and neck cancer, and breast and colorectal cancers (Mizuno et al., 2009; Uhlen et al., 2017; Wang et al., 2015). This suggests that the function of IL-34 in cancer may be multidimensional and can vary across different types of tumors and tissues.

Concluding remarks

IL-34 is a fairly new member of the family of "myelopoietic" cytokines. Originally described as a nonredundant growth factor specifically regulating LC development and microglial homeostasis, recent advances suggest that IL-34 is also expressed in a host of tissues other than skin and brain, in both the steady state and inflammation. However, whether IL-34 supports the generation, maintenance, or function of the resident macrophage compartment in these various organs requires further studies. In addition, the distribution pattern of IL-34 compared with CSF-1 in a given tissue might dictate the regulation of different macrophage subsets and their functions.

In pathological conditions, IL-34 seems to be involved in an array of diseases ranging from inflammation and autoimmunity to cancer. Nonetheless, while many human studies predominantly show a correlation between disease progression and IL-34 expression, very little is known about the actual function of IL-34. Additionally, whether IL-34 is beneficial or detrimental in a given pathology appears to be disease dependent, adding a layer of complexity to uncovering the biological roles of this cytokine. Thus, further research will reveal whether IL-34 indeed sustains an inflammatory disease, has proinflammatory or immunoregulatory properties, or is simply a hallmark of inflammation. This information will provide the necessary insight into whether IL-34 can serve as a predictive biomarker or even be exploited as a therapeutic target. Due to experimental design limitations, in many studies, including the ones discussed here, CSF-1 and IL-34 were not analyzed simultaneously. Thus, a further challenge is to carefully dissect similarities and differences between CSF-1 and IL-34 signaling and to identify the regulation and activities of both cytokines and their spatiotemporal expression pattern across tissues in pathological conditions. In cancer, for example, specific targeting of either CSF-1 or IL-34 instead of CSF-1R blockage, which eliminates most tissue macrophages, could be advantageous and could offer new perspectives for therapeutic interventions. Recent technologies (such as mass cytometry, multiparameter flow cytometric analysis, and single-cell RNA sequencing) combined with animal models and conditional mutagenesis should further help to advance our understanding of the interplay between IL-34, macrophages, and the immune system and could point toward novel disease mechanisms.

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References

Alikhan, M.A., C.V. Jones, T.M. Williams, A.G. Beckhouse, A.L. Fletcher, M.M. Kett, S. Sakkal, C.S. Samuel, R.G. Ramsay, J.A. Deane, et al. 2011. Colony-stimulating factor-1 promotes kidney growth and repair via alteration of macrophage responses. *Am. J. Pathol.* 179:1243–1256. https://doi.org/10.1016/j.ajpath.2011.05.037

Almaani, S., A. Meara, and B.H. Rovin. 2017. Update on lupus nephritis. *Clin. J. Am. Soc. Nephrol.* 12:825–835. https://doi.org/10.2215/CJN.05780616

Baek, J.-H., R. Zeng, J. Weinmann-Menke, M.T. Valerius, Y. Wada, A.K. Ajay, M. Colonna, and V.R. Kelley. 2015. IL-34 mediates acute kidney injury and worsens subsequent chronic kidney disease. *J. Clin. Invest.* 125: 3198–3214. https://doi.org/10.1172/JCI81166

Baghdadi, M., H. Wada, S. Nakanishi, H. Abe, N. Han, W.E. Putra, D. Endo, H. Watari, N. Sakuragi, Y. Hida, et al. 2016. Chemotherapy-induced IL34 enhances immunosuppression by tumor-associated macrophages and mediates survival of chemoresistant lung cancer cells. *Cancer Res.* 76: 6030–6042. https://doi.org/10.1158/0008-5472.CAN-16-1170

Baghdadi, M., H. Endo, Y. Tanaka, H. Wada, and K.I. Seino. 2017. Interleukin 34, from pathogenesis to clinical applications. *Cytokine.* 99:139–147. https://doi.org/10.1016/j.cyto.2017.08.020

Baghdadi, M., H. Endo, A. Takano, K. Ishikawa, Y. Kameda, H. Wada, Y. Miyagi, T. Yokose, H. Ito, H. Nakayama, et al. 2018. High co-expression of IL-34 and M-CSF correlates with tumor progression and poor survival in lung cancers. *Sci. Rep.* 8:418. https://doi.org/10.1038/s41598-017-18796-8

Barve, R.A., M.D. Zack, D. Weiss, R.H. Song, D. Beidler, and R.D. Head. 2013. Transcriptional profiling and pathway analysis of CSF-1 and IL-34 effects on human monocyte differentiation. *Cytokine.* 63:10–17. https://doi.org/10.1016/j.cyto.2013.04.019

Baud'huin, M., R. Renault, C. Charrier, A. Riet, A. Moreau, R. Brion, F. Gouin, L. Duplomb, and D. Heymann. 2010. Interleukin-34 is expressed by giant cell tumours of bone and plays a key role in RANKL-induced osteoclastogenesis. *J. Pathol.* 221:77–86. https://doi.org/10.1002/path.2684

Beffinger, M., P. Tallón de Lara, S. Tugues, M. Vermeer, Y. Montagnolo, I. Ohs, V. Cecconi, G. Lucchiari, A. Gagliardi, N. Misljencevic, et al. 2018. CSF1R-dependent myeloid cells are required for NK-mediated control of metastasis. *JCI Insight.* 3:e97792. https://doi.org/10.1172/jci.insight.97792

Behnes, C.L., F. Bremmer, B. Hemmerlein, A. Strauss, P. Ströbel, and H.J. Radzun. 2014. Tumor-associated macrophages are involved in tumor progression in papillary renal cell carcinoma. *Virchows Arch.* 464: 191–196. https://doi.org/10.1007/s00428-013-1523-0

Bettina, A., Z. Zhang, K. Michels, R.E. Cagnina, I.S. Vincent, M.D. Burdick, A. Kadl, and B. Mehrad. 2016. M-CSF mediates host defense during bacterial pneumonia by promoting the survival of lung and liver mononuclear phagocytes. *J. Immunol.* 196:5047–5055. https://doi.org/10.4049/jimmunol.1600306

Bischof, R.J., D. Zafiropoulos, J.A. Hamilton, and I.K. Campbell. 2000. Exacerbation of acute inflammatory arthritis by the colony-stimulating factors CSF-1 and granulocyte macrophage (GM)-CSF: evidence of macrophage infiltration and local proliferation. *Clin. Exp. Immunol.* 119: 361–367. https://doi.org/10.1046/j.1365-2249.2000.01125.x

Boulakirba, S., A. Pfeifer, R. Mhaidly, S. Obba, M. Goulard, T. Schmitt, P. Chaintreuil, A. Calleja, N. Furstoss, F. Orange, et al. 2018. IL-34 and CSF-1 display an equivalent macrophage differentiation ability but a different polarization potential. *Sci. Rep.* 8:256. https://doi.org/10.1038/s41598-017-18433-4

Calvo, C.F., A. Dobbertin, M. Gelman, J. Glowinski, and M. Mallat. 1998. Identification of CSF-1 as a brain macrophage migratory activity produced by astrocytes. *Glia.* 24:180–186. https://doi.org/10.1002/(SICI)1098-1136(199810)24:2<180::AID-GLIA3>3.0.CO;2-8

Campbell, I.K., M.J. Rich, R.J. Bischof, and J.A. Hamilton. 2000. The colony-stimulating factors and collagen-induced arthritis: exacerbation of disease by M-CSF and G-CSF and requirement for endogenous M-CSF. *J. Leukoc. Biol.* 68:144–150.

Cannarile, M.A., M. Weisser, W. Jacob, A.M. Jegg, C.H. Ries, and D. Rüttinger. 2017. Colony-stimulating factor 1 receptor (CSF1R) inhibitors in cancer therapy. *J. Immunother. Cancer.* 5:53. https://doi.org/10.1186/s40425-017-0257-y

Cecchini, M.G., M.G. Dominguez, S. Mocci, A. Wetterwald, R. Felix, H. Fleisch, O. Chisholm, W. Hofstetter, J.W. Pollard, and E.R. Stanley. 1994. Role of colony stimulating factor-1 in the establishment and regulation of tissue macrophages during postnatal development of the mouse. *Development.* 120:1357–1372.

Chang, E.-J., S.K. Lee, Y.S. Song, Y.J. Jang, H.S. Park, J.P. Hong, A.R. Ko, D.Y. Kim, J.-H. Kim, Y.J. Lee, and Y.-S. Heo. 2014. IL-34 is associated with obesity, chronic inflammation, and insulin resistance. *J. Clin. Endocrinol. Metab.* 99:E1263–E1271. https://doi.org/10.1210/jc.2013-4409

Chang, S.H., B.Y. Choi, J. Choi, J.J. Yoo, Y.-J. Ha, H.J. Cho, E.H. Kang, Y.W. Song, and Y.J. Lee. 2015. Baseline serum interleukin-34 levels independently predict radiographic progression in patients with rheumatoid arthritis. *Rheumatol. Int.* 35:71–79. https://doi.org/10.1007/s00296-014-3056-5

Chemel, M., B. Le Goff, R. Brion, C. Cozic, M. Berreur, J. Amiaud, G. Bougras, S. Touchais, F. Blanchard, M.F. Heymann, et al. 2012. Interleukin 34 expression is associated with synovitis severity in rheumatoid arthritis patients. *Ann. Rheum. Dis.* 71:150–154. https://doi.org/10.1136/annrheumdis-2011-200096

Chen, T., X. Wang, L. Guo, M. Wu, Z. Duan, J. Lv, W. Tai, H. Renganathan, R. Didier, J. Li, et al. 2014. Embryonic stem cells promoting macrophage survival and function are crucial for teratoma development. *Front. Immunol.* 5:275. https://doi.org/10.3389/fimmu.2014.00275

Cheng, Y., X. Yang, X. Zhang, and Z. An. 2019. Analysis of expression levels of IL-17 and IL-34 and influencing factors for prognosis in patients with lupus nephritis. *Exp. Ther. Med.* 17:2279–2283.

Chihara, T., S. Suzu, R. Hassan, N. Chutiwitoonchai, M. Hiyoshi, K. Motoyoshi, F. Kimura, and S. Okada. 2010. IL-34 and M-CSF share the receptor Fms but are not identical in biological activity and signal activation. *Cell Death Differ.* 17:1917–1927. https://doi.org/10.1038/cdd.2010.60

Chitu, V., and E.R. Stanley. 2006. Colony-stimulating factor-1 in immunity and inflammation. *Curr. Opin. Immunol.* 18:39–48. https://doi.org/10.1016/j.coi.2005.11.006

Ciccia, F., R. Alessandro, V. Rodolico, G. Guggino, S. Raimondo, C. Guarnotta, A. Giardina, G. Sireci, G. Campisi, G. De Leo, and G. Triolo. 2013. IL-34 is overexpressed in the inflamed salivary glands of patients with Sjogren's syndrome and is associated with the local expansion of pro-inflammatory CD14(^{bright})CD16^{+} monocytes. *Rheumatology (Oxford).* 52:1009–1017. https://doi.org/10.1093/rheumatology/kes435

Cioce, M., C. Canino, C. Goparaju, H. Yang, M. Carbone, and H.I. Pass. 2014. Autocrine CSF-1R signaling drives mesothelioma chemoresistance via AKT activation. *Cell Death Dis.* 5:e1167. https://doi.org/10.1038/cddis.2014.136

Clare, A.J., R.C. Day, R.M. Empson, and S.M. Hughes. 2018. Transcriptome profiling of layer 5 intratelencephalic projection neurons from the mature mouse motor cortex. *Front. Mol. Neurosci.* 11:410. https://doi.org/10.3389/fnmol.2018.00410

Curry, J.M., T.D. Eubank, R.D. Roberts, Y. Wang, N. Pore, A. Maity, and C.B. Marsh. 2008. M-CSF signals through the MAPK/ERK pathway via Sp1 to induce VEGF production and induces angiogenesis in vivo. *PLoS One.* 3:e3405. https://doi.org/10.1371/journal.pone.0003405

Dai, X.M., G.R. Ryan, A.J. Hapel, M.G. Dominguez, R.G. Russell, S. Kapp, V. Sylvestre, and E.R. Stanley. 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.* 99:111–120. https://doi.org/10.1182/blood.V99.1.111

Dai, X.M., X.H. Zong, V. Sylvestre, and E.R. Stanley. 2004. Incomplete restoration of colony-stimulating factor 1 (CSF-1) function in CSF-1-deficient *Csf1^{op}/Csf1^{op}* mice by transgenic expression of cell surface CSF-1. *Blood.* 103:1114–1123. https://doi.org/10.1182/blood-2003-08-2739

Ding, R., P. Li, D. Song, X. Zhang, and L. Bi. 2015. Predictors of response to TNF-α antagonist therapy in Chinese rheumatoid arthritis. *Clin. Rheumatol.* 34:1203–1210. https://doi.org/10.1007/s10067-015-2973-3

Erblich, B., L. Zhu, A.M. Etgen, K. Dobrenis, and J.W. Pollard. 2011. Absence of colony stimulation factor-1 receptor results in loss of microglia, disrupted brain development and olfactory deficits. *PLoS One.* 6:e26317. https://doi.org/10.1371/journal.pone.0026317

Esaki, H., D.A. Ewald, B. Ungar, M. Rozenblit, X. Zheng, H. Xu, Y.D. Estrada, X. Peng, H. Mitsui, T. Litman, et al. 2015. Identification of novel immune and barrier genes in atopic dermatitis by means of laser capture microdissection. *J. Allergy Clin. Immunol.* 135:153–163. https://doi.org/10.1016/j.jaci.2014.10.037

Espinosa, I., L. Catasus, E. D' Angelo, A. Mozos, N. Pedrola, C. Bértolo, I. Ferrer, G.F. Zannoni, R.B. West, M. van de Rijn, et al. 2014. Stromal signatures in endometrioid endometrial carcinomas. *Mod. Pathol.* 27:631–639. https://doi.org/10.1038/modpathol.2013.131

Fan, Q., X. Yan, H. Zhang, L. Lu, Q. Zhang, F. Wang, R. Xi, J. Hu, Q. Chen, W. Niu, et al. 2016. IL-34 is associated with the presence and severity of renal dysfunction and coronary artery disease in patients with heart failure. *Sci. Rep.* 6:39324. https://doi.org/10.1038/srep39324

Fox, R.I. 2005. Sjögren's syndrome. *Lancet.* 366:321–331. https://doi.org/10.1016/S0140-6736(05)66990-5

Franzè, E., I. Monteleone, M.L. Cupi, P. Mancia, F. Caprioli, I. Marafini, A. Colantoni, A. Ortenzi, F. Laudisi, G. Sica, et al. 2015. Interleukin-34 sustains inflammatory pathways in the gut. *Clin. Sci. (Lond.).* 129:271–280. https://doi.org/10.1042/CS20150132

Franzè, E., V. Dinallo, A. Rizzo, M. Di Giovangiulio, G. Bevivino, C. Stolfi, F. Caprioli, A. Colantoni, A. Ortenzi, A.D. Grazia, et al. 2017. Interleukin-34 sustains pro-tumorigenic signals in colon cancer tissue. *Oncotarget.* 9:3432–3445.

Garceau, V., J. Smith, I.R. Paton, M. Davey, M.A. Fares, D.P. Sester, D.W. Burt, and D.A. Hume. 2010. Pivotal Advance: Avian colony-stimulating factor 1 (CSF-1), interleukin-34 (IL-34), and CSF-1 receptor genes and gene products. *J. Leukoc. Biol.* 87:753–764. https://doi.org/10.1189/jlb.0909624

Garcia, S., L.M. Hartkamp, B. Malvar-Fernandez, I.E. van Es, H. Lin, J. Wong, L. Long, J.A. Zanghi, A.L. Rankin, E.L. Masteller, et al. 2016. Colony-stimulating factor (CSF) 1 receptor blockade reduces inflammation in human and murine models of rheumatoid arthritis. *Arthritis Res. Ther.* 18:75. https://doi.org/10.1186/s13075-016-0973-6

Ginhoux, F., and S. Jung. 2014. Monocytes and macrophages: developmental pathways and tissue homeostasis. *Nat. Rev. Immunol.* 14:392–404. https://doi.org/10.1038/nri3671

Ginhoux, F., F. Tacke, V. Angeli, M. Bogunovic, M. Loubeau, X.M. Dai, E.R. Stanley, G.J. Randolph, and M. Merad. 2006. Langerhans cells arise from monocytes in vivo. *Nat. Immunol.* 7:265–273. https://doi.org/10.1038/ni1307

Ginhoux, F., M. Greter, M. Leboeuf, S. Nandi, P. See, S. Gokhan, M.F. Mehler, S.J. Conway, L.G. Ng, E.R. Stanley, et al. 2010. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science.* 330:841–845. https://doi.org/10.1126/science.1194637

Greter, M., I. Lelios, P. Pelczar, G. Hoeffel, J. Price, M. Leboeuf, T.M. Kündig, K. Frei, F. Ginhoux, M. Merad, and B. Becher. 2012. Stroma-derived interleukin-34 controls the development and maintenance of langerhans cells and the maintenance of microglia. *Immunity.* 37:1050–1060. https://doi.org/10.1016/j.immuni.2012.11.001

Guillonneau, C., S. Bézie, and I. Anegon. 2017. Immunoregulatory properties of the cytokine IL-34. *Cell. Mol. Life Sci.* 74:2569–2586. https://doi.org/10.1007/s00018-017-2482-4

Han, N., M. Baghdadi, K. Ishikawa, H. Endo, T. Kobayashi, H. Wada, K. Imafuku, H. Hata, and K.I. Seino. 2018. Enhanced IL-34 expression in Nivolumab-resistant metastatic melanoma. *Inflamm. Regen.* 38:3. https://doi.org/10.1186/s41232-018-0060-2

Hoeffel, G., Y. Wang, M. Greter, P. See, P. Teo, B. Malleret, M. Leboeuf, D. Low, G. Oller, F. Almeida, et al. 2012. Adult Langerhans cells derive predominantly from embryonic fetal liver monocytes with a minor contribution of yolk sac-derived macrophages. *J. Exp. Med.* 209:1167–1181. https://doi.org/10.1084/jem.20120340

Hohensinner, P.J., C. Kaun, K. Rychli, A. Niessner, S. Pfaffenberger, G. Rega, R. de Martin, G. Maurer, R. Ullrich, K. Huber, and J. Wojta. 2007. Macrophage colony stimulating factor expression in human cardiac cells is upregulated by tumor necrosis factor-α via an NF-kappaB

dependent mechanism. *J. Thromb. Haemost.* 5:2520–2528. https://doi.org/10.1111/j.1538-7836.2007.02784.x

Hume, D.A., M. Caruso, M. Ferrari-Cestari, K.M. Summers, C. Pridans, and K.M. Irvine. 2019. Phenotypic impacts of CSF1R deficiencies in humans and model organisms. *J. Leukoc. Biol.* https://doi.org/10.1002/JLB.MR0519-143R

Huynh, D., D. Akçora, J. Malaterre, C.K. Chan, X.M. Dai, I. Bertoncello, E.R. Stanley, and R.G. Ramsay. 2013. CSF-1 receptor-dependent colon development, homeostasis and inflammatory stress response. *PLoS One.* 8: e56951. https://doi.org/10.1371/journal.pone.0056951

Hwang, S.-J., B. Choi, S.-S. Kang, J.-H. Chang, Y.-G. Kim, Y.-H. Chung, D.H. Sohn, M.W. So, C.-K. Lee, W.H. Robinson, and E.-J. Chang. 2012. Interleukin-34 produced by human fibroblast-like synovial cells in rheumatoid arthritis supports osteoclastogenesis. *Arthritis Res. Ther.* 14: R14. https://doi.org/10.1186/ar3693

Jokhi, P.P., G. Chumbley, A. King, L. Gardner, and Y.W. Loke. 1993. Expression of the colony stimulating factor-1 receptor (c-fms product) by cells at the human uteroplacental interface. *Lab. Invest.* 68:308–320.

Kana, V., F.A. Desland, M. Casanova-Acebes, P. Ayata, A. Badimon, E. Nabel, K. Yamamuro, M. Sneeboer, I.L. Tan, M.E. Flanigan, et al. 2019. CSF-1 controls cerebellar microglia and is required for motor function and social interaction. *J. Exp. Med.* 216:2265–2281. https://doi.org/10.1084/jem.20182037

Kawamura, K., Y. Komohara, K. Takaishi, H. Katabuchi, and M. Takeya. 2009. Detection of M2 macrophages and colony-stimulating factor 1 expression in serous and mucinous ovarian epithelial tumors. *Pathol. Int.* 59: 300–305. https://doi.org/10.1111/j.1440-1827.2009.02369.x

Kumari, A., O. Silakari, and R.K. Singh. 2018. Recent advances in colony stimulating factor-1 receptor/c-FMS as an emerging target for various therapeutic implications. *Biomed. Pharmacother.* 103:662–679. https://doi.org/10.1016/j.biopha.2018.04.046

Lafont, D., T. Adage, B. Gréco, and P. Zaratin. 2009. A novel role for receptor like protein tyrosine phosphatase ζ in modulation of sensorimotor responses to noxious stimuli: evidences from knockout mice studies. *Behav. Brain Res.* 201:29–40. https://doi.org/10.1016/j.bbr.2009.01.025

Lee, E.S., D.S. Son, S.H. Kim, J. Lee, J. Jo, J. Han, H. Kim, H.J. Lee, H.Y. Choi, Y. Jung, et al. 2008. Prediction of recurrence-free survival in postoperative non-small cell lung cancer patients by using an integrated model of clinical information and gene expression. *Clin. Cancer Res.* 14:7397–7404. https://doi.org/10.1158/1078-0432.CCR-07-4937

Li, Z., D. Jin, Y. Wu, K. Zhang, P. Hu, X. Cao, and Z. Chen. 2012. Increased serum interleukin-34 in patients with coronary artery disease. *J. Int. Med. Res.* 40:1866–1870. https://doi.org/10.1177/030006051204000525

Lin, E.Y., A.V. Nguyen, R.G. Russell, and J.W. Pollard. 2001. Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. *J. Exp. Med.* 193:727–740. https://doi.org/10.1084/jem.193.6.727

Lin, H., E. Lee, K. Hestir, C. Leo, M. Huang, E. Bosch, R. Halenbeck, G. Wu, A. Zhou, D. Behrens, et al. 2008. Discovery of a cytokine and its receptor by functional screening of the extracellular proteome. *Science.* 320: 807–811. https://doi.org/10.1126/science.1154370

Liu, H., C. Leo, X. Chen, B.R. Wong, L.T. Williams, H. Lin, and X. He. 2012. The mechanism of shared but distinct CSF-1R signaling by the non-homologous cytokines IL-34 and CSF-1. *Biochim. Biophys. Acta.* 1824: 938–945. https://doi.org/10.1016/j.bbapap.2012.04.012

Liu, H., H. Zhang, Z. Shen, C. Lin, X. Wang, J. Qin, X. Qin, J. Xu, and Y. Sun. 2016. Increased expression of CSF-1 associates with poor prognosis of patients with gastric cancer undergoing gastrectomy. *Medicine (Baltimore).* 95:e2675. https://doi.org/10.1097/MD.0000000000002675

Luo, J., F. Elwood, M. Britschgi, S. Villeda, H. Zhang, Z. Ding, L. Zhu, H. Alabsi, R. Getachew, R. Narasimhan, et al. 2013. Colony-stimulating factor 1 receptor (CSF1R) signaling in injured neurons facilitates protection and survival. *J. Exp. Med.* 210:157–172. https://doi.org/10.1084/jem.20120412

Ma, D., Y. Doi, S. Jin, E. Li, Y. Sonobe, H. Takeuchi, T. Mizuno, and A. Suzumura. 2012a. TGF-β induced by interleukin-34-stimulated microglia regulates microglial proliferation and attenuates oligomeric amyloid β neurotoxicity. *Neurosci. Lett.* 529:86–91. https://doi.org/10.1016/j.neulet.2012.08.071

Ma, X., W.Y. Lin, Y. Chen, S. Stawicki, K. Mukhyala, Y. Wu, F. Martin, J.F. Bazan, and M.A. Starovasnik. 2012b. Structural basis for the dual recognition of helical cytokines IL-34 and CSF-1 by CSF-1R. *Structure.* 20: 676–687. https://doi.org/10.1016/j.str.2012.02.010

McInnes, I.B., and G. Schett. 2011. The pathogenesis of rheumatoid arthritis. *N. Engl. J. Med.* 365:2205–2219. https://doi.org/10.1056/NEJMra1004965

Menke, J., Y. Iwata, W.A. Rabacal, R. Basu, Y.G. Yeung, B.D. Humphreys, T. Wada, A. Schwarting, E.R. Stanley, and V.R. Kelley. 2009. CSF-1 signals directly to renal tubular epithelial cells to mediate repair in mice. *J. Clin. Invest.* 119:2330–2342. https://doi.org/10.1172/JCI39087

Mizuno, H., K. Kitada, K. Nakai, and A. Sarai. 2009. PrognoScan: a new database for meta-analysis of the prognostic value of genes. *BMC Med. Genomics.* 2:18. https://doi.org/10.1186/1755-8794-2-18

Mizuno, T., Y. Doi, H. Mizoguchi, S. Jin, M. Noda, Y. Sonobe, H. Takeuchi, and A. Suzumura. 2011. Interleukin-34 selectively enhances the neuroprotective effects of microglia to attenuate oligomeric amyloid-β neurotoxicity. *Am. J. Pathol.* 179:2016–2027. https://doi.org/10.1016/j.ajpath.2011.06.011

Moon, S.-J., Y.-S. Hong, J.H. Ju, S.-K. Kwok, S.-H. Park, and J.-K. Min. 2013. Increased levels of interleukin 34 in serum and synovial fluid are associated with rheumatoid factor and anticyclic citrullinated peptide antibody titers in patients with rheumatoid arthritis. *J. Rheumatol.* 40: 1842–1849. https://doi.org/10.3899/jrheum.130356

Nakamichi, Y., T. Mizoguchi, A. Arai, Y. Kobayashi, M. Sato, J.M. Penninger, H. Yasuda, S. Kato, H.F. DeLuca, T. Suda, et al. 2012. Spleen serves as a reservoir of osteoclast precursors through vitamin D-induced IL-34 expression in osteopetrotic *op/op* mice. *Proc. Natl. Acad. Sci. USA.* 109: 10006–10011. https://doi.org/10.1073/pnas.1207361109

Nandi, S., M.P. Akhter, M.F. Seifert, X.M. Dai, and E.R. Stanley. 2006. Developmental and functional significance of the CSF-1 proteoglycan chondroitin sulfate chain. *Blood.* 107:786–795. https://doi.org/10.1182/blood-2005-05-1822

Nandi, S., S. Gokhan, X.M. Dai, S. Wei, G. Enikolopov, H. Lin, M.F. Mehler, and E.R. Stanley. 2012. The CSF-1 receptor ligands IL-34 and CSF-1 exhibit distinct developmental brain expression patterns and regulate neural progenitor cell maintenance and maturation. *Dev. Biol.* 367: 100–113. https://doi.org/10.1016/j.ydbio.2012.03.026

Nandi, S., M. Cioce, Y.G. Yeung, E. Nieves, L. Tesfa, H. Lin, A.W. Hsu, R. Halenbeck, H.Y. Cheng, S. Gokhan, et al. 2013. Receptor-type protein-tyrosine phosphatase ζ is a functional receptor for interleukin-34. *J. Biol. Chem.* 288:21972–21986. https://doi.org/10.1074/jbc.M112.442731

Neubert, N.J., M. Schmittnaegel, N. Bordry, S. Nassiri, N. Wald, C. Martignier, L. Tillé, K. Homicsko, W. Damsky, H. Maby-El Hajjami, et al. 2018. T cell-induced CSF1 promotes melanoma resistance to PD1 blockade. *Sci. Transl. Med.* 10:eaan3311. https://doi.org/10.1126/scitranslmed.aan3311

Noy, R., and J.W. Pollard. 2014. Tumor-associated macrophages: from mechanisms to therapy. *Immunity.* 41:49–61. https://doi.org/10.1016/j.immuni.2014.06.010

O'Koren, E.G., C. Yu, M. Klingeborn, A.Y.W. Wong, C.L. Prigge, R. Mathew, J. Kalnitsky, R.A. Msallam, A. Silvin, J.N. Kay, et al. 2019. Microglial function is distinct in different anatomical locations during retinal homeostasis and degeneration. *Immunity.* 50:723–737.e7. https://doi.org/10.1016/j.immuni.2019.02.007

Pandit, J., A. Bohm, J. Jancarik, R. Halenbeck, K. Koths, and S.H. Kim. 1992. Three-dimensional structure of dimeric human recombinant macrophage colony-stimulating factor. *Science.* 258:1358–1362. https://doi.org/10.1126/science.1455231

Peyraud, F., S. Cousin, and A. Italiano. 2017. CSF-1R inhibitor development: current clinical status. *Curr. Oncol. Rep.* 19:70. https://doi.org/10.1007/s11912-017-0634-1

Preisser, L., C. Miot, H. Le Guillou-Guillemette, E. Beaumont, E.D. Foucher, E. Garo, S. Blanchard, I. Frémaux, A. Croué, I. Fouchard, 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.* 60:1879–1890. https://doi.org/10.1002/hep.27328

Pridans, C., K.A. Sauter, K. Baer, H. Kissel, and D.A. Hume. 2013. CSF1R mutations in hereditary diffuse leukoencephalopathy with spheroids are loss of function. *Sci. Rep.* 3:3013. https://doi.org/10.1038/srep03013

Rademakers, R., M. Baker, A.M. Nicholson, N.J. Rutherford, N. Finch, A. Soto-Ortolaza, J. Lash, C. Wider, A. Wojtas, M. DeJesus-Hernandez, et al. 2011. Mutations in the colony stimulating factor 1 receptor (CSF1R) gene cause hereditary diffuse leukoencephalopathy with spheroids. *Nat. Genet.* 44:200–205. https://doi.org/10.1038/ng.1027

Richardsen, E., R.D. Uglehus, S.H. Johnsen, and L.T. Busund. 2015. Macrophage-colony stimulating factor (CSF1) predicts breast cancer progression and mortality. *Anticancer Res.* 35:865–874.

Sauter, K.A., C. Pridans, A. Sehgal, Y.T. Tsai, B.M. Bradford, S. Raza, L. Moffat, D.J. Gow, P.M. Beard, N.A. Mabbott, et al. 2014. Pleiotropic effects of extended blockade of CSF1R signaling in adult mice. *J. Leukoc. Biol.* 96:265–274. https://doi.org/10.1189/jlb.2A0114-006R

Scholl, S.M., C. Pallud, F. Beuvon, K. Hacene, E.R. Stanley, L. Rohrschneider, R. Tang, P. Pouillart, and R. Lidereau. 1994. Anti-colony-stimulating factor-1 antibody staining in primary breast adenocarcinomas correlates with marked inflammatory cell infiltrates and prognosis. *J. Natl. Cancer Inst.* 86:120–126. https://doi.org/10.1093/jnci/86.2.120

Ségaliny, A.I., R. Brion, B. Brulin, M. Maillasson, C. Charrier, S. Téletchéa, and D. Heymann. 2015a. IL-34 and M-CSF form a novel heteromeric cytokine and regulate the M-CSF receptor activation and localization. *Cytokine.* 76:170–181. https://doi.org/10.1016/j.cyto.2015.05.029

Ségaliny, A.I., R. Brion, E. Mortier, M. Maillasson, M. Cherel, Y. Jacques, B. Le Goff, and D. Heymann. 2015b. Syndecan-1 regulates the biological activities of interleukin-34. *Biochim. Biophys. Acta.* 1853:1010–1021. https://doi.org/10.1016/j.bbamcr.2015.01.023

Ségaliny, A.I., A. Mohamadi, B. Dizier, A. Lokajczyk, R. Brion, R. Lanel, J. Amiaud, C. Charrier, C. Boisson-Vidal, and D. Heymann. 2015c. Interleukin-34 promotes tumor progression and metastatic process in osteosarcoma through induction of angiogenesis and macrophage recruitment. *Int. J. Cancer.* 137:73–85. https://doi.org/10.1002/ijc.29376

Sehgal, A., D.S. Donaldson, C. Pridans, K.A. Sauter, D.A. Hume, and N.A. Mabbott. 2018. The role of CSF1R-dependent macrophages in control of the intestinal stem-cell niche. *Nat. Commun.* 9:1272. https://doi.org/10.1038/s41467-018-03638-6

Sherr, C.J., C.W. Rettenmier, R. Sacca, M.F. Roussel, A.T. Look, and E.R. Stanley. 1985. The c-*fms* proto-oncogene product is related to the receptor for the mononuclear phagocyte growth factor, CSF-1. *Cell.* 41: 665–676. https://doi.org/10.1016/S0092-8674(85)80047-7

Shoji, H., S. Yoshio, Y. Mano, E. Kumagai, M. Sugiyama, M. Korenaga, T. Arai, N. Itokawa, M. Atsukawa, H. Aikata, et al. 2016. Interleukin-34 as a fibroblast-derived marker of liver fibrosis in patients with non-alcoholic fatty liver disease. *Sci. Rep.* 6:28814. https://doi.org/10.1038/srep28814

Stanley, E.R., and V. Chitu. 2014. CSF-1 receptor signaling in myeloid cells. *Cold Spring Harb. Perspect. Biol.* 6:a021857. https://doi.org/10.1101/cshperspect.a021857

Stanley, E.R., D.M. Chen, and H.S. Lin. 1978. Induction of macrophage production and proliferation by a purified colony stimulating factor. *Nature.* 274:168–170. https://doi.org/10.1038/274168a0

Stanley, E.R., L.J. Guilbert, R.J. Tushinski, and S.H. Bartelmez. 1983. CSF-1--a mononuclear phagocyte lineage-specific hemopoietic growth factor. *J. Cell. Biochem.* 21:151–159. https://doi.org/10.1002/jcb.240210206

Swierczak, A., A.D. Cook, J.C. Lenzo, C.M. Restall, J.P. Doherty, R.L. Anderson, and J.A. Hamilton. 2014. The promotion of breast cancer metastasis caused by inhibition of CSF-1R/CSF-1 signaling is blocked by targeting the G-CSF receptor. *Cancer Immunol. Res.* 2:765–776. https://doi.org/10.1158/2326-6066.CIR-13-0190

Tabula Muris Consortium. 2018. Single-cell transcriptomics of 20 mouse organs creates a *Tabula Muris*. *Nature.* 562:367–372. https://doi.org/10.1038/s41586-018-0590-4

Tao, R., Q. Fan, H. Zhang, H. Xie, L. Lu, G. Gu, F. Wang, R. Xi, J. Hu, Q. Chen, et al. 2017. Prognostic significance of interleukin-34 (IL-34) in patients with chronic heart failure with or without renal insufficiency. *J. Am. Heart Assoc.* 6:e004911. https://doi.org/10.1161/JAHA.116.004911

Tian, Y., H. Shen, L. Xia, and J. Lu. 2013. Elevated serum and synovial fluid levels of interleukin-34 in rheumatoid arthritis: possible association with disease progression via interleukin-17 production. *J. Interferon Cytokine Res.* 33:398–401. https://doi.org/10.1089/jir.2012.0122

Tsokos, G.C. 2011. Systemic lupus erythematosus. *N. Engl. J. Med.* 365: 2110–2121. https://doi.org/10.1056/NEJMra1100359

Uhlen, M., C. Zhang, S. Lee, E. Sjöstedt, L. Fagerberg, G. Bidkhori, R. Benfeitas, M. Arif, Z. Liu, F. Edfors, et al. 2017. A pathology atlas of the human cancer transcriptome. *Science.* 357:eaan2507. https://doi.org/10.1126/science.aan2507

von Holst, A., S. Sirko, and A. Faissner. 2006. The unique 473HD-Chondroitinsulfate epitope is expressed by radial glia and involved in neural precursor cell proliferation. *J. Neurosci.* 26:4082–4094. https://doi.org/10.1523/JNEUROSCI.0422-06.2006

Wada, Y., H.M. Gonzalez-Sanchez, J. Weinmann-Menke, Y. Iwata, A.K. Ajay, M. Meineck, and V.R. Kelley. 2019. IL-34-dependent intrarenal and systemic mechanisms promote lupus nephritis in MRL-*Fas*^{lpr} mice. *J. Am. Soc. Nephrol.* 30:244–259. https://doi.org/10.1681/ASN.2018090901

Wang, Q., S. Yu, A. Simonyi, G.Y. Sun, and A.Y. Sun. 2005. Kainic acid-mediated excitotoxicity as a model for neurodegeneration. *Mol. Neurobiol.* 31:3–16. https://doi.org/10.1385/MN:31:1-3:003

Wang, Y., K.J. Szretter, W. Vermi, S. Gilfillan, C. Rossini, M. Cella, A.D. Barrow, M.S. Diamond, and M. Colonna. 2012. IL-34 is a tissue-restricted ligand of CSF1R required for the development of Langerhans cells and microglia. *Nat. Immunol.* 13:753–760. https://doi.org/10.1038/ni.2360

Wang, B., W. Xu, M. Tan, Y. Xiao, H. Yang, and T.S. Xia. 2015. Integrative genomic analyses of a novel cytokine, interleukin-34 and its potential role in cancer prediction. *Int. J. Mol. Med.* 35:92–102. https://doi.org/10.3892/ijmm.2014.2001

Wang, Y., M. Bugatti, T.K. Ulland, W. Vermi, S. Gilfillan, and M. Colonna. 2016a. Nonredundant roles of keratinocyte-derived IL-34 and neutrophil-derived CSF1 in Langerhans cell renewal in the steady state and during inflammation. *Eur. J. Immunol.* 46:552–559. https://doi.org/10.1002/eji.201545917

Wang, H., J. Cao, and X. Lai. 2016b. Serum interleukin-34 levels are elevated in patients with systemic lupus erythematosus. *Molecules.* 22:35. https://doi.org/10.3390/molecules22010035

Wang, B., Z. Ma, M. Wang, X. Sun, Y. Tang, M. Li, Y. Zhang, F. Li, and X. Li. 2017. IL-34 upregulated Th17 production through increased IL-6 expression by rheumatoid fibroblast-like synoviocytes. *Mediators Inflamm.* 2017:1567120. https://doi.org/10.1155/2017/1567120

Wang, S.-L., R. Zhang, K.-Z. Hu, M.-Q. Li, and Z.-C. Li. 2018a. Interleukin-34 synovial fluid was associated with knee osteoarthritis severity: a cross-sectional study in knee osteoarthritis patients in different radiographic stages. *Dis. Markers.* 2018:2095480. https://doi.org/10.1155/2018/2095480

Wang, Y.Q., W.J. Cao, Y.F. Gao, J. Ye, and G.Z. Zou. 2018b. Serum interleukin-34 level can be an indicator of liver fibrosis in patients with chronic hepatitis B virus infection. *World J. Gastroenterol.* 24:1312–1320. https://doi.org/10.3748/wjg.v24.i12.1312

Węgiel, J., H.M. Wiśniewski, J. Dziewiatkowski, M. Tarnawski, R. Kozielski, E. Trenkner, and W. Wiktor-Jedrzejczak. 1998. Reduced number and altered morphology of microglial cells in colony stimulating factor-1-deficient osteopetrotic *op/op* mice. *Brain Res.* 804:135–139. https://doi.org/10.1016/S0006-8993(98)00618-0

Wei, S., S. Nandi, V. Chitu, Y.G. Yeung, W. Yu, M. Huang, L.T. Williams, H. Lin, and E.R. Stanley. 2010. Functional overlap but differential expression of CSF-1 and IL-34 in their CSF-1 receptor-mediated regulation of myeloid cells. *J. Leukoc. Biol.* 88:495–505. https://doi.org/10.1189/jlb.1209822

Wiktor-Jedrzejczak, W., A. Bartocci, A.W. Ferrante Jr., A. Ahmed-Ansari, K.W. Sell, J.W. Pollard, and E.R. Stanley. 1990. Total absence of colony-stimulating factor 1 in the macrophage-deficient osteopetrotic (op/op) mouse. *Proc. Natl. Acad. Sci. USA.* 87:4828–4832. https://doi.org/10.1073/pnas.87.12.4828

Wu, S., R. Xue, S. Hassan, T.M.L. Nguyen, T. Wang, H. Pan, J. Xu, Q. Liu, W. Zhang, and Z. Wen. 2018. Il34-Csf1r pathway regulates the migration and colonization of microglial precursors. *Dev. Cell.* 46:552–563.e4. https://doi.org/10.1016/j.devcel.2018.08.005

Xi, R., Q. Fan, X. Yan, H. Zhang, H. Xie, G. Gu, Y. Xu, F. Wang, and R. Tao. 2018. Increased serum interleukin-34 levels are related to the presence and severity of cardiac dysfunction in patients with ischemic cardiomyopathy. *Front. Physiol.* 9:904. https://doi.org/10.3389/fphys.2018.00904

Xie, H.H., H. Shen, L. Zhang, M.Y. Cui, L.P. Xia, and J. Lu. 2018. Elevated serum interleukin-34 level in patients with systemic lupus erythematosus is associated with disease activity. *Sci. Rep.* 8:3462. https://doi.org/10.1038/s41598-018-21859-z

Yang, S., S. Jiang, Y. Wang, S. Tu, Z. Wang, and Z. Chen. 2016. Interleukin 34 upregulation contributes to the increment of microRNA 21 expression through STAT3 activation associated with disease activity in rheumatoid arthritis. *J. Rheumatol.* 43:1312–1319. https://doi.org/10.3899/jrheum.151253

Yoshida, H., S. Hayashi, T. Kunisada, M. Ogawa, S. Nishikawa, H. Okamura, T. Sudo, L.D. Shultz, and S. Nishikawa. 1990. The murine mutation osteopetrosis is in the coding region of the macrophage colony stimulating factor gene. *Nature.* 345:442–444. https://doi.org/10.1038/345442a0

Zhang, M.Z., B. Yao, S. Yang, L. Jiang, S. Wang, X. Fan, H. Yin, K. Wong, T. Miyazawa, J. Chen, et al. 2012. CSF-1 signaling mediates recovery from acute kidney injury. *J. Clin. Invest.* 122:4519–4532. https://doi.org/10.1172/JCI60363

Zhang, F., R. Ding, P. Li, C. Ma, D. Song, X. Wang, T. Ma, and L. Bi. 2015. Interleukin-34 in rheumatoid arthritis: potential role in clinical therapy. *Int. J. Clin. Exp. Med.* 8:7809–7815.

Zhang, L., M. Cui, L. Ding, L. Xia, J. Lu, and H. Shen. 2018. Interleukin-34 aggravates the severity of arthritis in collagen-induced arthritis mice by inducing interleukin-17 production. *J. Interferon Cytokine Res.* 38: 221–225. https://doi.org/10.1089/jir.2017.0095

Zhou, S.L., Z.Q. Hu, Z.J. Zhou, Z. Dai, Z. Wang, Y. Cao, J. Fan, X.W. Huang, and J. Zhou. 2016. miR-28-5p-IL-34-macrophage feedback loop modulates hepatocellular carcinoma metastasis. *Hepatology.* 63:1560–1575. https://doi.org/10.1002/hep.28445

Zhu, C., U.S. Herrmann, J. Falsig, I. Abakumova, M. Nuvolone, P. Schwarz, K. Frauenknecht, E.J. Rushing, and A. Aguzzi. 2016. A neuroprotective role for microglia in prion diseases. *J. Exp. Med.* 213:1047–1059. https://doi.org/10.1084/jem.20151000

Zins, K., G. Heller, M. Mayerhofer, M. Schreiber, and D. Abraham. 2018. Differential prognostic impact of interleukin-34 mRNA expression and infiltrating immune cell composition in intrinsic breast cancer subtypes. *Oncotarget.* 9:23126–23148. https://doi.org/10.18632/oncotarget.25226

Zorena, K., O. Jachimowicz-Duda, and P. Wąż. 2016. The cut-off value for interleukin 34 as an additional potential inflammatory biomarker for the prediction of the risk of diabetic complications. *Biomarkers.* 21: 276–282. https://doi.org/10.3109/1354750X.2016.1138321

Zwicker, S., G.L. Martinez, M. Bosma, M. Gerling, R. Clark, M. Majster, J. Söderman, S. Almer, and E.A. Boström. 2015. Interleukin 34: a new modulator of human and experimental inflammatory bowel disease. *Clin. Sci. (Lond.).* 129:281–290. https://doi.org/10.1042/CS20150176