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Citation for published version:
Hume, DA, Caruso, M, Ferrari-Cestari, M, Summers, KM, Pridans, C & Irvine, KM 2019, 'Phenotypic impacts of CSF1R deficiencies in humans and model organisms', Journal of Leukocyte Biology. https://doi.org/10.1002/JLB.MR0519-143R

Digital Object Identifier (DOI):
10.1002/JLB.MR0519-143R

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published In:
Journal of Leukocyte Biology

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REVIEW

Phenotypic impacts of CSF1R deficiencies in humans and model organisms

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Abstract
Mφ proliferation, differentiation, and survival are controlled by signals from the Mφ CSF receptor (CSF1R). Mono-allelic gain-of-function mutations in CSF1R in humans are associated with an autosomal-dominant leukodystrophy and bi-allelic loss-of-function mutations with recessive skeletal dysplasia, brain disorders, and developmental anomalies. Most of the phenotypes observed in these human disease states are also observed in mice and rats with loss-of-function mutations in Csf1r or in Csf1 encoding one of its two ligands. Studies in rodent models also highlight the importance of genetic background and likely epistatic interactions between Csf1r and other loci. The impacts of Csf1r mutations on the brain are usually attributed solely to direct impacts on microglial number and function. However, analysis of hypomorphic Csf1r mutants in mice and several other lines of evidence suggest that primary hydrocephalus and loss of the physiological functions of Mφs in the periphery contribute to the development of brain pathology. In this review, we outline the evidence that CSF1R is expressed exclusively in mononuclear phagocytes and explore the mechanisms linking CSF1R mutations to pleiotropic impacts on postnatal growth and development.

KEYWORDS
CSF1R, Mφs, neurodegeneration, osteoclasts, transgenic

1 INTRODUCTION

Mφs are an abundant cell population in all major organs and adapt at each location to perform specific functions in physiology and homeostasis.1–4 Mφ proliferation and differentiation is controlled by signals from the Mφ-CSF receptor (CSF1R), in response to 2 alternative ligands, Mφ CSF1 and IL-34. Mφs generated in vitro from monocytes or bone marrow progenitors, by cultivation in CSF1, have been widely used as models for the study of Mφ biology in multiple species.5–8 In the mouse, Mφs also depend upon exogenous CSF1 for survival.9 In other species, including humans, rats, pigs, sheep, goats, cattle, horses, water buffalo, and even chickens, CSF1 is induced during Mφ differentiation. Mature Mφs themselves express high levels of CSF1 mRNA and are effectively autocrine for the pro-survival signal (data in ref. 10–13). Notwithstanding the apparent differences in expression among species, the 2 functional ligands, CSF1 and IL-34 are conserved across species from chicken and fish through to humans14–17 and an intronic enhancer that regulates CSF1R expression is conserved from reptiles to humans.18 The CSF1R gene was originally recognized by its relationship to the transforming oncogene of a feline leukemia virus19 and was known as the Fms protooncogene. Aside from the functions in Mφs, in both mouse and human (and in other mammals; www.biogps.org) CSF1R is also expressed in placental trophoblasts driven by a separate promoter and transcription start site to those used in Mφ lineage cells.20

In 2011, the human CSF1R gene was recognized as the site of point mutations underlying autosomal dominant adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP).21 (MIM: 221820, also known as HDLS). Subsequent studies have revealed many additional CSF1R mutations associated with ALSP...
(reviewed in ref. 22 and 23) and unified the diagnosis with a num-
ber of other disease entities with similar presentation (e.g., pigmented
orthochromatic leukodystrophy or POLD). The brain pathology and
symptoms of ALSP vary significantly between affected individuals
and the disease has been misdiagnosed antemortem as various other
dementias and neurodegenerative diseases. For example, Sassi et al.24
identified 3 likely pathogenic CSF1R mutations in a cohort of 465 late-
onset Alzheimer’s patients.

A distinct recessive disease has more recently been associated
with loss-of-function alleles at the CSF1R locus.25,26 Patients lacking
CSF1R in the recessive disease had almost complete loss of microglia,
the MΦs of the brain, as well as defects in skeletal development and
osteosclerosis. As far as we are aware, no patients have been described
in whom there is homozygous loss-of-function mutation in either CSF1
or IL34. The contact amino acids involved in interactions in the CSF1-
CSF1R and IL-34-CSF1R complexes have been dissected in crystal
structures.27,28 In the large human exome sequence collection ExAC
(exac.broadinstitute.org), there are no non-synonymous mutations
in CSF1 that potentially affect binding of CSF1 to the receptor, and
only 2 rare variants in IL-34 (E111K and W116G) that could alter IL34
binding. However, there is likely to be expression variation between
individuals. SNPs within the CSF1 locus have been associated with
Paget’s disease, an abnormality of bone resorption,29 most likely
associated with overexpression of CSF1 and excessive osteoclast acti-
vation. The level of circulating CSF1 in a very large cohort of coronary
artery disease patients was correlated with distinct and relatively com-
oncis-acting variants at the CSF1 locus and in turn with susceptibility
to disease.30 In this article, we will critically review the interpretation
of studies of Csf1, IL34, and Csf1r mutations in experimental animals
and their relevance to the human genetic diseases.

1.1 | The cell-type and tissue specificity of Csf1r expression

A key piece of knowledge required to interpret the impact of Csf1r
mutations is the site of transcript and protein expression. The main site
of expression of Csf1r (aside from expression in placental trophoblasts)
is undoubtably in cells of the monocyte/MΦ lineage. Reporter trans-
genesis driven by the Csf1r promoter have been used to locate MΦs
throughout embryonic development and in tissues of adult mice.31-33
rats,34 sheep,35 and even chickens.14,15 The same mouse Csf1r pro-
moter driving Cre recombinase has also been used in lineage trace
experiments in the embryo36 without any evidence of expression out-
side myeloid lineages.

During mouse embryonic development, Csf1r mRNA is first
detected in the ectoplacental cone early after implantation and in
isolated MΦ-lik cells in the yolk sac.37 Localization of Csf1r mRNA
by whole mount in situ hybridization is consistent with restriction
to myeloid cells in the embryo.37 Although they are abundant and
actively involved in clearance of apoptotic cells,37 MΦs in the embryo
are not required for organogenesis. Ablation of Csf1r-dependent MΦs
by anti-CSF1R treatment of the mother has no effect on embryonic
development38 and Csf1r-deficient mice and rats are indistinguishable
from their littermates at birth.39,40

The restriction of Csf1r expression to cells of the MΦ lineage is also
supported by network analysis in both mouse and human systems. In
mouse development, the appearance of Csf1r mRNA is strongly cor-
related with expression of other known MΦ markers in a time course
of embryo gene expression.41 In the large promoter-based transcript-
omic atlas produced by the FANTOM Consortium, there is a single
cluster of MΦ-specific transcription start sites in both mouse and
human.13 There is no detectable Csf1r expression in non-myeloid
primary cells or cell lines of multiple lineages. Interestingly, Csf1r
mRNA is also tightly correlated with a MΦ signature in gene expres-
sion profiles from a wide range of human solid tumors42 suggesting
that it is exclusively expressed by tumor-associated MΦs and ectopic
expression in tumor cells is not common. The molecular basis for MΦ-
specific transcription of Csf1r and reporter gene expression has been
reviewed elsewhere.1-20 An inducible Fas-based suicide gene driven by
the Csf1r promoter (the so-called MAFIA mouse) has been applied to
functional studies of MΦs in vivo without evidence of ectopic expres-
sion or adverse impacts on other cell types.43

In spite of this compelling evidence that Csf1r expression is
restricted to cells of the monocyte/MΦ lineage, the recent studies of
the Csf1r homozygous mutation in human patients25,26 and reviews of
Csf1r roles in embryonic and postnatal development (e.g., ref. 44)
cite a small number of studies that claim to demonstrate expression
of Csf1r in non-hematopoietic cells including neuronal progenitors,
intestinal and renal epithelial cells, and cells of the female reproduc-
itive system. If these reports are correct, then some of the pleiotropic
impacts of Csf1r mutations in patients and experimental animals
might be attributed to defects in non-myeloid cells. It is therefore
timely to re-evaluate the evidence from these reports for non-myeloid
expression of Csf1r.

There are several caveats to each of the studies claiming func-
tional expression of Csf1r outside the mononuclear phagocyte lin-
eage, notably in relation to the specificity of anti-CSF1R Abs. In the
mouse brain, Sierra et al.45 provided detailed evidence that the Csf1r-
EGFP reporter gene produced by our laboratory32 is restricted in its
expression to microglia and perivascular MΦs at all stages of post-
natal development and in injury and ageing models. Using additional
Csf1r reporter genes in mice and distinct anti-CSF1R Abs, we31-32 and
others46,47 have shown that expression of the transgene and Csf1r
protein is restricted to microglia and MΦs at all stages of brain de-
velopment. By contrast, Nandi et al.48 reported that Csf1r mRNA was
expressed in neuronal progenitors and used Ab staining to demon-
strate high levels of expression of Csf1r protein in these cells in the
early postnatal period. The specificity of their Ab binding was based
upon lack of binding to Csf1r-/- mouse brains, but curiously, the Ab
did not appear to detect microglia in wild-type mice. Their study would
suggest quite high levels of Csf1r mRNA in the developing mouse brain.
This is clearly not evident from analysis of deep RNAseq data, includ-
ing the time course of embryonic development41 in which expres-
sion is low and tightly correlated with increased expression of other
known MΦ-specific transcripts. In detailed network analysis of the

transcriptomes of isolated cells from mouse and human brain, Csf1r is clearly part of a microglia-Mφ signature.49,50

Based upon conditional deletion of Csf1r with a Nestin-cre transgene and direct impacts of added CSF1 or IL-34 to "microglia-free" forebrain cultures (selected based upon Nestin-EGFP expression), Nandi et al.48 proposed that CSF1 acts directly on neuronal stem cells. The interpretation of these studies depends on the view that the Nestin promoter is not active in microglia. However, Nestin mRNA is detectable in isolated microglia at levels higher than total brain mRNA and CSF1R protein in the brain in microglia and Mφ populations. Furthermore, conditional deletion of Csf1r-Cre-dependent EGFP reporter, to demonstrate apparent induction of Csf1r in injured neurons. They detected EGFP by Ab staining rather than direct imaging of EGFP fluorescence, so even if the signal reflects some level of inducible promoter activity, the signal is not quantitative. Finally, Luo et al.53 generated a conditional knockout by crossing a floxed allele of Csf1r to CaMKIIα-Cre to delete the Csf1r gene in neurons. These mice were reported to be more susceptible to kainic acid-induced injury. But the controls in this case were mice that lacked the CaMKIIα-Cre transgene. There is a need for caution in interpreting both this result and those obtained with Nestin-cre. High-level expression of cre recombinase can clearly have impacts on cellular function.54 It is entirely possible that expression of cre in neurons directly impacts their functions including the production of CSF1 and IL-34 and the sensitivity to toxic challenge. In overview, we consider there is compelling evidence for the exclusive expression of Csf1r mRNA and CSF1R protein in the brain in microglia and Mφs.

In the intestine, in Csf1r- conditional deletion of Csf1r, and Csf1r−/− mice, Paneth cells were lost and there was disordered differentiation of epithelia including an excess of goblet cells.55-57 In the mouse, Csf1r was apparently expressed functionally by Paneth cells and by other epithelial cells in both small and large intestine based upon staining with a commercial rabbit polyclonal Ab against CSF1R.55-57 Conditional deletion of Csf1r with a tamoxifen-inducible Villin-cre reproduced intestinal epithelial disruptions associated with Csf1r mutation leading to the conclusion that Csf1r function is intrinsic to epithelial cells.55 Consistent with the cautionary note above about cre recombinase, Bohin et al.58 subsequently showed that tamoxifen-inducible Villin-cre activation per se led to DNA damage and cleavage of cryptic LoxP sites in intestinal stem cells. A secondary concern with the inducible cre recombinase system is that tamoxifen is not a neutral agonist, especially when applied to Mφ biology (reviewed in ref. 1). The role of Csf1r in intestinal homeostasis was reinvestigated in our group by exploring the impact of treatment of mice with a purified blocking rat mAb against mouse CSF1R. Prolonged treatment completely depleted lamina propria Mφs and also disrupted epithelial differentiation leading to increased goblet cells and the loss of microfold (M) cells.59 However, we did not reproduce the expression of Csf1r in epithelial cells. Instead, we demonstrated that the expression of both Csf1r mRNA and Csf1r-EGFP expression was completely restricted to lamina propria Mφs. Figure 1B shows the intimate association between crypt-associated Mφs and intestinal stem cells. This conclusion was supported by mRNA analysis of isolated intestinal cell populations. Furthermore, conditional deletion of Csf1r using a constitutive Villin-cre had no effect on epithelial differentiation. Importantly, Paneth cells were not actually depleted by anti-CSF1R; their expression of markers such as lysozyme and defensins was lost indicating that Mφs control their differentiation rather than their survival.59 In the rat, we also saw no effect of the Csf1r null mutation on the presence of Paneth cells or on overall villus architecture.40 We have not yet investigated whether epithelial function is regulated by interactions with Mφs in this species.

Resident Mφs are an abundant interstitial population in the kidney and Menke et al.60 claimed that CSF1 signals directly to renal tubular epithelial cells to promote repair of hypoxic injury. Inducible expression of Csf1 in damaged tubular epithelial cells has been reproduced by multiple authors. Exogenous CSF1 administration promoted epithelial repair and anti-CSF1R treatment, CSF1R kinase inhibition or genetic deletion of Csf1 increased the pathology or delayed resolution in several different injury models60-63 Menke et al.60 claimed that CSF1R protein and Csf1r mRNA were detectable in isolated mouse renal epithelial cells and Zhang et al.63 appeared to detect phosphorylated CSF1R in damaged renal epithelium using anti-CSF1R Ab. In the former study, protein detection was based upon the same rabbit anti-mouse CSF1R preparation used by Nandi et al.60 The authors noted that Csf1r mRNA expression was considerably lower than in Mφs and contamination by Mφs was not excluded. Based upon conditional deletion using an Ilgam-dipheria toxin receptor transgene, Menke et al.60 argued that Mφs make a minor contribution to CSF1-dependent repair. Our own study in a similar renal ischemia model, which reproduced the beneficial effect of CSF1 treatment, strongly favors the Mφ as the mediator of tissue repair and the exclusive responder to CSF1 administration.61 This conclusion is supported by subsequent
Expression of Csf1r reporter genes is restricted to macrophages. Panel (A) shows expression of cyan fluorescent protein in microglia in the hippocampus of the brain of MacBlue (Csf1r-GAL4VP16/UAS-ECP) mice. The neuronal cells of the pyramidal layer are stained for the neuronal marker NeuN. Reproduced from ref. 33 with permission. In the same study, both ECFP and Csf1r-EGFP were detected in disaggregated total brain by FACS exclusively in cells that co-expressed CD11b. Panel (B) shows an optical section of a 3D whole mount of the crypt of a small intestinal villus of Csf1r-EGFP mice, stained for the Paneth cell marker, lysozyme. Note the intimate association between EGFP+ cells and the basal membranes of epithelial cells. Phalloidin staining of F-actin is used as a counterstain and highlights the basolateral and apical regions of epithelial cells and the non-Mϕ cells (including endothelial cells and fibroblasts) in the lamina propria. Reproduced and modified from ref. 59 with permission and thanks to Dr. A Sehgal studies. Menke et al.60 described an apparent increase in Csf1r-EGFP expression in epithelial cells in response to renal injury but in our view, the apparent increase in EGFP fluorescence was attributable to Mϕ infiltration of the damaged epithelial layers and autofluorescence of tubular casts.61 In neither study was there any evidence of expression of the Csf1r-EGFP reporter genes in undamaged renal epithelium consistent with the original description of the transgene.31,32 As in the brain, the increasing abundance of Csf1r mRNA in the kidney during embryonic and postnatal development correlated closely with other Mϕ markers.41 In summary, the claim that CSF1 signals directly to renal tubular epithelial cells in any circumstance is not strongly supported.

There are high-affinity mAbs against murine CSF1R that detect expression in isolated monocytes and progenitors (e.g., ref. 60 and 64) but they have not been used successfully to detect the protein in tissues. In fact, it is intrinsically unlikely that the CSF1R protein is detectable at high levels in tissues because it turns over constantly upon ligand binding (see below). As an alternative, we developed an AF647-conjugated version of CSF1. This protein bound specifically to monocytes isolated from the blood of mice and rats and when injected into mice localized specifically to tissue Mϕs.31,40,65

The restriction of CSF1R expression to Mϕs and microglia is also supported by studies using Csf1r kinase inhibitors. Elmore et al.51 reported the almost complete elimination of microglia from the mouse brain using selective CSF1R inhibitors. There was no evidence of a phenotypic impact and gene expression profiling revealed only the loss of known microglia-associated transcripts. Since this original report, the inhibitor PLX3397 has been used extensively in studies of the functions of microglia in brain and retinal development and homeostasis (e.g., ref. 66–69, and references therein) without any evidence of effects on non-myeloid cells. The lack of effect is actually surprising. Contrary to the way it is portrayed explicitly in many publications, PLX3397 is not a specific CSF1R kinase inhibitor; it is also an effective inhibitor of related kinases KIT and FLT370–72 and likely mediates its effects on microglia in part by interacting with other kinase targets. Another orally available CSF1R kinase inhibitor, GW2580, also penetrates the brain but unlike PLX3397, it prevents microglial proliferation/self-renewal without impacting on survival.46

Taking all of these data together, we believe there is no reason to consider Csf1r expression outside of myeloid lineages in the interpretation of mutant phenotypes in experimental animals or humans.

1.2 | CSF1R signal transduction

The binding of CSF1 or IL34 to CSF1R and the downstream signaling events have been reviewed in detail by Stanley and Chitu.73 CSF1 signal transduction has mostly been studied in mature Mϕs, osteoclasts, or cell lines, and mainly in mouse systems where CSF1 is not
produced by Mφs themselves and endogenous/autocrine CSF1 signaling is therefore not an issue. For obvious reasons, the CSF1 response is also commonly studied in cells that have been deprived of growth factor to allow up-regulation of surface receptor and the analysis of a synchronous response to receptor ligation.

In broad outline, studies of CSF1 signaling have shown that ligand binding induces dimerization of the receptor and release of the kinase domain from an auto-inhibited conformation leading to initial tyrosine phosphorylation and ubiquitination of a membrane proximal domain. Trans-phosphorylation of individual tyrosine residues in the intracellular domain then provides a scaffold for recruitment of several different effector pathways linked separately to survival, increased cell motility, proliferation, and specific gene regulation. One of these pathways is the classical SOS-GRB2-RAS-RAF-MAPK pathway that was first dissected in detail in the analysis of "sevenless/pointed" pathway in Drosophila. The same pathway from CSF1R through SOS/GRB2, RAS, RAF, and the MAP kinases ERK1/ERK2 leads in Mφs to phosphorylation of the transcription factor ETS2 on the pointed domain. ETS2 then interacts with AP1 transcription factors on a conserved Ras response element to activate transcription of urokinase plasminogen activator (Plau) in the Drosophila eye.

Following initial signal generation, the SOS-GRB2 complex dissociates from the receptor, there is further tyrosine and serine phosphorylation and a cascade of ubiquitination culminating in degradation of both ligand and receptor in lysosomes. Internalization and degradation of the receptor is not blocked by inhibitors of receptor kinase activity. Further signaling, therefore, requires continuous synthesis of new receptors on the cell surface. The nature of the response to CSF1 depends upon the duration and magnitude of that continued stimulation. Removal of CSF1 from bone marrow-derived Mφs (BMDM) at any time leads to rapid loss of expression of Plau mRNA and CSF1 must be present continuously in order for cells to enter S phase and subsequently undergo cell division. Because CSF1 is internalized and degraded, binding at 37°C is irreversible and ligand is depleted from the medium. The degradation of CSF1 by proliferating mouse bone marrow-derived Mφs is saturated at concentrations that are required to drive entry into the S phase if the cell cycle. As a consequence of the rapid degradation of the ligand, the dose-response curve for CSF1 action on any measured outcome in cell culture is very steep. It is actually not a concentration dependence, but a titration of the amount of CSF1 available per cell per hour. If the cells exhaust the supply of growth factor at any time, the signaling cascade terminates. For example, the CSF1-dependent phosphorylation of the MAP kinases ERK1/ERK2 and of their target ETS2 in Mφs is sustained for as long as CSF1 is present. The outcome of signaling also depends upon the cell population. Mature peritoneal Mφs are more effective at internalization and degradation of CSF1 than BMDM but do not undergo proliferation; they can compete in vitro for the available CSF1. The Mφs of the liver clear CSF1 from the blood thereby maintaining a low circulating concentration (~20 ng/ml) that is less than saturating for Mφ-mediated clearance by the receptor (~70–100 ng/ml).

The circulating CSF1 concentration in vivo is also sub-stimulatory for monocyte production by the bone marrow and for proliferation and regulated gene expression in resident tissue Mφs. As a consequence, the entire mononuclear phagocyte system can respond to increased CSF1 availability. Administration of CSF1 or a CSF1-Fc fusion protein (which has a longer circulating half-life) leads to both expansion of the blood monocyte pool and proliferation of resident Mφs in all organs. It also induces expression by Mφs of Plau and other target genes with similar regulatory elements (e.g., Mmp9). A striking and unexpected consequence of CSF1-Fc treatment is extensive hepatocytopenia arising to rapid expansion of the size of the liver in mice, rats, and pigs. This finding indicates that Mφs contribute to the homeostatic regulation of liver size relative to body size. Resident Mφs have relatively low rates of proliferation/self-renewal as noted in the original descriptions of the mononuclear phagocyte system (reviewed in refs. 1 and 2). Nevertheless, liver Mφs, and most other tissue Mφ populations do require CSF1R signals for continued survival and in mice, they are rapidly and sustainably depleted in response to anti-CSF1R treatment.

Like all signaling pathways, CSF1R signaling at multiple levels is subject to feedback controls, many of which are themselves CSF1-inducible (reviewed in ref. 73). They include protein tyrosine phosphatases, serine phosphatases, dual-specificity phosphatases (DUSP), inositol phosphatases, and suppressors of cytokine signaling. Deficiencies in any of these feedback mechanisms can lead to uncontrolled CSF1R signaling and each of them is therefore a potential epistatic modifier of the effect of CSF1R mutations. For example, mutation of the SHP1 hematopoietic tyrosine phosphatase (encoded by Ptpn6) in the motheaten mouse leads to constitutive activation of ERK1/2-dependent ETS2 phosphorylation and expression of CSF1R target genes such as Plau in Mφs and factor-independent survival. As a consequence of the separation of internalization and degradation from kinase activity and the presence of so many feedback pathways the efficiency of signaling can be controlled at multiple levels. For example, an old study showed that glucocorticoids actually shift the CSF1 dose-response curve for induction of Plau and proliferation in mouse BMDM so that cells consume and degrade more CSF1 to produce the same outcome. One of the conserved actions of glucocorticoids in mouse and human Mφs is to induce the expression of the MAPK kinase inhibitor DUSP1, which would act to reduce the effective activation of ERK1/2 and efficiency of downstream transmission of the CSF1R signal. The CSF1R/glucocorticoid axis may also be relevant to understanding variable penetrance of CSF1R mutant phenotypes.

1.3 The phenotypes associated with CSF1, IL34, and CSF1R mutations in mice

Most of our current knowledge of mononuclear phagocyte adaptation is derived from studies of inbred mice. In terms of Mφ-specific and inducible gene expression, mouse and human Mφs are substantially different from each other. Recent comparative analysis of mouse and human microglial expression signatures also revealed significant differences in gene expression driven by species-specific variation
TABLE 1  Summary of mutations of Csf1r, Csf1, and Il34 in rodents

| Gene/species | Genetic background | Survival | References |
|-------------|-------------------|----------|------------|
| Csf1<sup>op/op</sup> mouse | Mixed B6/C3H | Adult, bone phenotype resolves with age | 39,92–95 |
| Csf1<sup>op/op</sup> mouse | Inbred C57BL/6 | Lethal | 106 |
| Csf1<sup>op/op</sup> x Cre | Inbred C57BL/6 | Osteopetrosis | 157 |
| Csf1<sup>op/op</sup> rat | Inbred Fischer | Adult, no resolution with age | 119 |
| Csf1<sup>+/−</sup> mouse | Mixed 129/Sv and B6/C3H | >80 days, bone phenotype resolves with age | 39 |
| Csf1<sup>+/−</sup> mouse | Inbred C57BL/6 | Few survive to wean | 106 |
| Csf1<sup>−/−</sup> mouse | Mixed FVB/NJ | <4 weeks | 86 |
| Csf1<sup>−/−</sup> mouse | Inbred DA/D2 | Few survive to wean | 47,102 |
| Csf1<sup>−/−</sup> rat | Mixed DA/SD | >12 weeks | 40 |
| Csf1<sup>−/−</sup> rat | Inbred DA | ~12 weeks | Unpublished |
| Csf1<sup>−/−</sup> rat | Outbred SD | >12 weeks | Unpublished |
| Il34<sup>−/−</sup> Lux2/Lux2 mouse | Inbred C57BL/6 | Adult, fertile | 110 |

in cis-regulatory elements and transcriptional regulators. Nevertheless, the biology of CSF1R is conserved in mammals and the mutations in rodents have provided clear insights into Mφ functions in development. Table 1 summarizes the mutations that have been studied in laboratory animals and the impacts of genetic background.

The osteopetrotic mouse is a spontaneous mutation first described in detail by Marks and Lane. The original characterization included a failure of tooth eruption, excessive accumulations of bone and compromised marrow cavities, increases in bone matrix formation, and hypophosphatemia associated with primary deficiency of bone-resorbing osteoclasts. Subsequently, 2 groups identified an inactivating mutation of Csf1 (a single base insertion before codon 88, which results in a premature stop codon after 21 bases) in these mice, now referred to as Csf1<sup>op/op</sup>. The original description of the Csf1<sup>op/op</sup> mouse noted that the osteoclast deficiency and reduction in bone marrow cellularity resolves with age and extramedullary hematopoiesis in the spleen is corrected. In the spleen of mutant mice, there is a substantial expansion of committed Mφ progenitors, but CSF2 (GM-CSF), which can also act upon these shared progenitors, was not required for age-dependent correction. Age-dependent resolution of the Csf1<sup>op/op</sup> bone phenotype has been attributed to signals from other tyrosine kinase receptors/ligands notably VEGFA and FLT3. Most subsequent studies of Csf1/−/− mice also lead to reduced Mφ numbers in most organs and there are numerous pleiotropic impacts including compromised postnatal growth, extensive skeletal abnormalities, defects in sensory neuronal systems, abnormal intestinal organization, Paneth cell deficiency, relative male and female infertility, delayed beta cell development in the pancreatic islets, reduced mammary gland development, defective angiogenesis and lymphangiogenesis, partial B cell deficiency, and altered neurogenesis and brain development (reviewed in refs. 44 and 100). Among many applications, Csf1<sup>op/op</sup> mice have been used to infer the role of CSF1-dependent Mφs in the control of cholesterol metabolism and the development of atherosclerosis (reviewed in ref. 101).

Targeted mutation of the Csf1r locus in mice by insertion of a reporter gene within exon 3 revealed a very similar phenotype to the Csf1<sup>op/op</sup> including an expansion of committed Mφ progenitors in the spleen. As in the case of the ligand mutation, the bone marrow cellularity and osteoclast deficiency resolved with age. In this original study, the Csf1r mutation was generated on the inbred 129SvJ genetic background and crossed to the Csf1<sup>op/op</sup> line on a mixed C57BL6/C3HHe/FJ genetic background on which the mutation is maintained by the Jackson Laboratories to enable comparison of single and double mutations. With this complex cross, the majority of single or double homozygous Csf1r<sup>−/−</sup> and Csf1<sup>op/op</sup> mice survived to adulthood (at least 80 days). The phenotypic impacts of the 2 mutations were not additive because the double homozygote phenotype was largely indistinguishable from either single homozygote. Both receptor and ligand mutant animals were reportedly able to generate progeny, albeit their fertility was greatly compromised. Small differences in bone phenotype and marginally greater pre-weaning mortality in Csf1r<sup>−/−</sup> mice provided some indication of the existence of a second Csf1r ligand. Unfortunately, the original study of the mutation on the mixed genetic background did not examine the brain. Based upon subsequent studies, the Csf1r<sup>−/−</sup> mice that survived beyond weaning were presumably microglia-deficient but not severely affected.

Most subsequent studies of Csf1r mutant mice, including a second conditional mutation (deletion of exon 5) made by another laboratory, have been carried out on 1 of 2 inbred genetic backgrounds (FVB, C57BL/6) where the perinatal lethality is much more penetrant and very few mice survive to weaning. In a subsequent study, Dai et al. examined the bone phenotype of juvenile Csf1r<sup>−/−</sup> mice on an inbred background (FVB/NJ) in greater detail. These mice died within the first 4 weeks of life. In juvenile mutants, they observed chondrodysplasia and defects in collagen matrix organization and mineralization and defects in osteoblasts. Using a novel approach, they transplanted fetal femoral anlagen from Csf1r<sup>−/−</sup> mice into wild-type mice and showed that infiltrating recipient osteoclasts permitted the generation of normal cortical bone in the transplanted tissue. Although these authors suggested that Mφs were not involved in resolution of the bone osteoblast function in this model, subsequent studies of the control of osteoblast calcification indicate that the loss of Mφs associated with the bone surface probably also contributes to.
The rat as an alternative rodent model of Csf1 relative to the equivalent to CSF1 in that transgenic expression of Il34 in both mice and humans.

Another group studied a more limited backcross of the Csf1op/op to this genetic background and found that the homozygotes could be maintained to adulthood with careful husbandry and feeding. The idiosyncrasies of C57Bl/6 mice as a model for Mϕ biology have been reviewed elsewhere. One relevant feature is that they have an intrinsically low bone density. Female C57BL/6 develop spontaneous osteoporosis at a relatively young age, and this can be blocked by anti-CSF1R treatment. If anything, the ligand mutation in C57BL/6 mice is more severe than the receptor mutation. Curiously, heterozygous mutation of Csf1r abolished the pre-weaning mortality of the Csf1op/op mutation. There is a similar paradox in the case of Flt3, which encodes a receptor tyrosine kinase (FLT3) related to CSF1R. FLT3 and CSF1R are both present on the cell surface of a shared bone marrow progenitor of the monocyte-dendritic cell lineage in mice. Mutation of the ligand gene (Flt3l) has an even greater impact than mutation of the receptor gene (Flt3) on dendritic cell maturation. Dural et al. proposed that FLT3 is retained on the cell surface in the absence of Flt3l and effectively cross-competes for signaling molecules on progenitor cells with CSF1R. On the other hand, the absence of FLT3 permits increased CSF1R signaling and partial rescue of the dendritic cell deficiency. The potential cross-talk between tyrosine kinase receptors may be relevant to mutant CSF1R phenotypes and variable penetrance in humans discussed below.

Leaving aside whether the differences in apparent severity of ligand and receptor mutations are dependent on genetic background, another explanation for any increased severity of the Csf1r mutation relative to the Csf1op/op was provided by the identification of the second ligand, IL-34. The knockout of the mouse Il34 gene (removal of Exons 3–5; inbred C57BL/6 background) revealed a substantial but incomplete depletion of microglia in the brain and loss of Langerhans cells in the skin. IL-34 activation of CSF1R appears functionally equivalent to CSF1 in that transgenic expression of Il34 under the control of the Csfr1 promoter rescued the phenotypes of the Csf1op/op mouse (at least on an inbred FVB/NJ background). The impact of the Il34 mutation is consistent with the major sites of expression of Il34 in both mice and humans.

Within the mouse brain, Il34 and Csfr1 have distinct and largely non-overlapping distributions across regions, and the knockouts of the 2 ligands accordingly show distinct impacts on regional microglial densities. Studies in vitro do not support the idea that CSF1 and IL-34 have any differential signaling effects on microglia. A second receptor for IL-34, PTPRZ, has been identified but thus far no phenotype has been described in the mouse Il34 knockout that is incompatible with effects solely mediated by CSF1R. Recent studies have extended the analysis of differential Il34 dependency to distinct niches in the retina and to Mϕs of the kidney. By contrast to mice, extensive profiling of human brain regions in the FANTOM5 project did not indicate a significant excess of IL34 over CSF1 mRNA, nor any region specificity. However, the transcriptomic data do identify separate promoters/transcription start sites associated with expression of IL34 in skin and brain in both mouse and human.

As discussed above, some impacts of Csf1r mutations on the brain have also been attributed to direct actions on neuronal cells. Erblit et al., on the other hand, demonstrated clearly that Csf1r mRNA and protein and Csf1r reporter genes were expressed exclusively in microglia. They described in detail the progressive postnatal enlargement of the lateral ventricles in Csf1r−/− mice on the C57BL/6N background and argued that many of the impacts of the mutation may be secondary to severe hydrocephalus. Consistent with this view, and further highlighting the impact of genetic background, mutations in the genes encoding the junctional adhesion molecule JAM3 and the FYN kinase also produce severe hydrocephalus when crossed to the C57BL/6 background with phenotypic consequences in the brain parenchyma resembling the Csf1r knockout.

1.4 The rat as an alternative rodent model of Csf1r deficiency

There have been considerably fewer studies of CSF1R signaling biology in the rat. The toothless rat (Csf1tl/tl) has a frame-shift mutation in the Csf1 gene that ablates function. Most studies of the Csf1tl/tl rat have focused on the bone phenotype and the control of tooth eruption. By contrast to the Csf1op/op mouse, which retains some osteoclasts and recovers with age, the Csf1tl/tl rat has an almost complete loss of osteoclasts, chondrodysplasia and unremitting osteopetrosis that was only partly overcome by postnatal CSF1 administration. Like Csf1op/op and Csf1r−/− mice, Csf1tl/tl rats also exhibited severe postnatal growth retardation, which was associated with deficiencies in the growth-hormone/IGF1 axis. We recently generated a Csf1r knockout rat by homologous recombination in embryonic stem cells. Like the Csf1op/op mice, these rats had a complete deficiency of osteoclasts and also lacked bone-associated Mϕs (osteomacs) that are required for osteoblast maintenance and both endochondral and intramembranous ossification. Despite the lack of osteoclasts, the bone phenotype of mutant rats was distinct from the mouse with much greater preservation of a marrow cavity and no evidence of extramedullary hematopoiesis in the spleen. Unlike Csf1r−/− inbred mice, the large majority of mutant rats survived beyond weaning. Figure 2 shows a comparison of the skeletal phenotypes of adult wild-type and mutant rats.

Analysis of female Csf1r−/− rats at 11–12 weeks revealed complete loss of specific Mϕ populations, including microglia, Langerhans cells, splenic marginal zone Mϕs, and peritoneal Mϕs whereas other Mϕ populations were partly CSF1R-independent. Both sexes are likely to be infertile. The gonads and secondary sexual organs were poorly-developed in both males and females and the differential growth of males versus females was abolished. One phenotype of the Csf1r−/− rats, which was not observed in mutant mice, was the complete loss of visceral adipose tissue. Despite the complete absence of microglia in Csf1r−/− rats, there was limited evidence of brain pathology aside from mildly enlarged lateral ventricles, thinning of the corpus callosum, and altered differentiation of dopaminergic neurons. Expression profiling
1.5 | Growth deficiency in CSF1R-deficient animals

Mutations of Csfr in both mice and rats lead to reduced body weight. The growth retardation in Csfr−/− rats was reportedly associated with an almost complete loss of circulating IGF1. The Csfr−/− rats were indistinguishable from litter mates at birth but in common with Csfr−/−/− rats, their growth rate declined rapidly. The impacts of Csfr mutations have some obvious similarities to growth hormone (GH)/IGF1 mutations (reviewed in ref. 126). Like Csfr−/−/− animals, GH-deficient (Ghr−/−) or GH receptor-deficient (Ghr−/−) mice are born normal size and the growth defect manifests from around 2 weeks of age. Igf1 deficiency has a greater impact on embryonic growth than Ghr or Ghr mutation but as is the case with Csfr mutation, the perinatal lethality depends on genetic background. Although the liver is the main source of IGF1 in the circulation, conditional deletion of Igf1 in hepatocytes did not cause a substantial reduction in postnatal growth. Mouse Mφs grown in CSF1 also express very high levels of Igf1 mRNA initiated from a separate promoter from that used in the liver. Chitu and Stanley have argued that the growth deficiency in Csfr or Csfr mutant animals is secondary to the bone/skeletal abnormality; essentially a consequence of osteoclast deficiency. They noted that transgenic expression of human CSF1 in mouse bone from an osteoblast-specific promoter was able to overcome the growth and skeletal defects in Csfrop/op mice. This finding does not argue against a central role for CSF1 in growth control since CSF1 was also elevated in plasma in these transgenic mice and the impact on circulating IGF1 was not measured. Chang et al. confirmed the expression of Igf1 mRNA in inflammatory Mφs in adipose tissue, but conditional deletion of Igf1 in myeloid cells using LysM-cre did not produce a change in circulating IGF1, growth, or body composition. However, LysM-cre does not produce efficient recombination in most resident tissue Mφ populations (see ref. 130). Expression profiling of the liver of Csfr−/− rats revealed a relatively small reduction in Ghr and Igf1, probably insufficient to explain the growth retardation and suggesting there is not a primary GH deficiency. There are many other possible axes of regulation that might explain the loss of control of circulating IGF1 by CSF1R-dependent Mφs. IGF1 has a very short plasma half-life unless bound by members of the IGFBP family. Altered expression of these proteins in liver or in other tissues, or changes in their posttranslational modification, could lead to indirect impacts on circulating IGF1. Additionally, recent studies have shown that IGF1 is generated in muscle as a pro-IGF1 form that requires processing. Conditional deletion of muscle-specific IGF1 expression can also reduce circulating IGF1 and impair somatic growth whereas Mφ-expressed Igf1 appears essential for muscle regeneration following injury. As well as contributing directly as a source of IGF1 production, Mφs are obvious candidates for a role in proteolytic processing of both pro-IGF1 and IGFBPs. Whereas the precise mechanism is unclear and probably complex, CSF1R mutation clearly impacts the GH-IGF1 axis and many of the pleiotropic consequences in rodents are probably linked to that impact.

1.6 | A hypomorphic Csfr mutation in mice

The transcriptional regulation of the Csfr gene has been analyzed in detail in both mice and humans (reviewed in ref. 20). The major Mφ-specific transcription start site region contains multiple binding sites.
What do the rodent models tell us about the conserved intronic enhancer element (FIRE)? To test the function of reporter genes in *M Csf1r* was no apparent brain phenotype in mice expressing FIRE in its normal genomic context, we deleted the 300 bp sequence from the mouse germ line to produce *Csf1r*ΔFIRE/ΔFIRE mice. Given the level of conservation, we anticipated a phenocopy of the *Csf1r* knockout. Instead, we found a selective loss of certain tissue Mφ populations, notably complete absence of microglia, Langerhans cells of the epidermis, and heart, kidney, and peritoneal Mφs. However, most major Mφ populations expressed *Csf1r* normally and/or were unaffected in *Csf1r*ΔFIRE/ΔFIRE mice; the animals have normal bones, grow normally, and are fertile. Furthermore, the mutation distinguishes the brain-associated and perivascular Mφ populations, which were retained despite their complete loss of microglia, and of *Csf1r* mRNA and other microglial markers in the brain, there was no apparent brain phenotype in *Csf1r*ΔFIRE/ΔFIRE mice and no detectable change in expression of any genes that are not clearly myeloid-associated. These mice, along with the *Csf1r* null mutation on outbred genetic backgrounds in mice and rats, tell us that the functions of microglia in development are largely redundant. The elimination of microglia in adults has no obvious pathological impact and seems to protect against several forms of brain injury. Similarly, the microglial deficiency in *Il34* mutant mice (~80% in most brain regions) has no apparent phenotypic consequences in the steady state but does compromise the ability of mice to deal with pathology associated with viral infection of the brain. These findings support the view that the effects of *Csf1r* deletion on the brain may be attributable in part to the primary hydrocephalus, which does not occur in *Csf1r*ΔFIRE/ΔFIRE or the *Il34*−/− mice and is greatly delayed in *Csf1r*−/− rats. The effects of *Csf1r* inhibitors such as PLX3397 discussed above and *Csf1r* knockouts may also depend in part upon systemic effects including the loss of circulating IGF1 described above. For example, we suspect that impacts of the microbiome on microglial numbers and maturation depend indirectly upon peripheral CSF1 deficiency and consequent changes in peripheral Mφ populations.

### 1.7 What do the rodent models tell us about the impact of human CSF1R mutations?

ALSP is an adult-onset degenerative disease of the brain leading to multiple cognitive, behavioral, and motor dysfunctions. The historical hallmark of the disease is white matter destruction leading to the loss of axons and myelin, thinning of the corpus callosum, calcification, axonal swellings (spheroids), and the accumulation of pigmented Mφs. The point mutations in CSF1R associated with ALSP (reviewed in refs. 22 and 23, and annotated in Ensembl) are concentrated in the intracellular tyrosine kinase domain. The entire intracellular domain of CSF1R is highly conserved across vertebrate species and even the chicken CSF1R can signal to support proliferation and differentiation when expressed in mammalian cells. Without exception ALSP-associated mutations alter amino acids that are conserved in the chicken receptor. We expressed CSF1R containing a range of human mutations in the BaF3 factor-dependent cell line where wild-type receptor can sustain CSF1-independent proliferation. Whereas each of the mutant proteins was defective in signaling, they were expressed on the cell surface at comparable levels to the wild-type receptor, bound CSF1, and were internalized in response to binding. In an ALSP patient heterozygous for a CSF1R point mutation that abolishes kinase activity, we predict that 75% of receptor dimers will be signaling defective; either homodimers of the mutant protein (25%) or heterodimers with one mutant and one wild-type subunit (50%). These receptor dimers will nevertheless bind and internalize CSF1 thereby competing with the remaining 25% of functional receptor dimers. As discussed above, mutant receptors may also compete for signaling pathways with other receptors such as FLT3 that would otherwise mitigate the impact of CSF1R deficiency. Hence, our data support a dominant negative model for ALSP.

The alternative to a dominant-negative model of disease is to propose that the mutant proteins have no function and reduction of normal peptides to 50% (haploinsufficiency) is enough to cause disease. Neither the animal models nor the human disease patients support this proposal. In rats and mice heterozygous null mutation of *Csf1r* is not dosage compensated. Monocytes and Mφs in blood and bone marrow of heterozygous mutants express half the level of *Csf1r* mRNA and protein. This is also the case in *Csf1r*ΔFIRE/+ mice. In the expression profiles of the brains of heterozygous *Csf1r*+/− rats and *Csf1r*ΔFIRE/+ mice, there is a 50% reduction in *Csf1r* mRNA. Nevertheless, there is no significant change in any other transcript in response to the 50% loss of *Csf1r*. The lack of dosage compensation is rather surprising since CSF1 can induce down-regulation *Csf1r* mRNA in Mφs. One might have anticipated that reduced CSF1R signals would permit up-regulation of expression from the wild-type allele. A further puzzling finding is that despite the 50% reduction in *Csf1r* mRNA and CSF1R protein in heterozygous mutant mice no increase in circulating CSF1 was detected. It seems that haploinsufficiency for *Csf1r* has little impact in mice or rats and is unlikely to explain the human dominant disease.

By contrast to the ALSP-associated mutations, the CSF1R mutations described in the recessive syndrome with skeletal symptoms all appear to result in complete or partial loss of function or expression of the protein. Some lead to the generation of premature stop codons and nonsense-mediated mRNA decay, others involve cryptic splice acceptors leading to greatly reduced expression of the wild-type mRNA. Heterozygous carriers are therefore "models" for genuine haploinsufficiency. The recessive disease varies greatly between individuals from infant mortality to adult-onset. Monies et al. described a truncating null mutation (Y540*) in CSF1R in a consanguineous family. The mutation would abolish the intracellular domain including the kinase region and apparently led to early postnatal lethality and severe brain and skeletal phenotypes. These individuals may be the only report of definitive human CSF1R null individuals; their heterozygous parents and siblings were asymptomatic. Guo et al. reported another null mutation (Q481*) as one of the compound heterozygous alleles in an individual with recessive skeletal dysplasia and brain disorders. Similar to p.Y540*, p.Q481* would abolish...
the intracellular part of the receptor. The RT-PCR analysis for cells derived from this patient demonstrated that mRNA from the mutant allele is subjected to complete NMD. All the heterozygous carriers of Q481* and Y540* in the 2 families were asymptomatic. Guo et al.25 attribute some of the variation in phenotype in the recessive disease to the residual production of wild-type CSF1R mRNA (in the case of splice variation) or some retained signaling function of the mutant CSF1R encoded by one of the alleles. One disease-associated variant, P132L, present as a compound heterozygote with the definitive loss-of-function allele (Q481*), affects an amino acid in the domain 2 ligand binding domain. While this amino acid is not involved directly in binding either CSF1 or IL3427,28 the proline is immediately adjacent to a conserved cysteine involved in the immunoglobulin fold, and is conserved in chickens.142 Both this mutation, and a mutation in the tyrosine kinase domain (K627del) that was also present as a compound heterozygote in affected individuals, retained some biological activity when expressed in a reporter system.25 Oosterhof et al.26 described a family where affected individuals were homozygous for H643Q mutation in the kinase domain (also a conserved amino acid in chicken) and heterozygous family members were unaffected. They speculate that by contrast to ALSP mutations, the H643Q mutant protein retains some residual kinase activity. This would clearly be testable in the BaF3 cell line system.65

A small number of studies have identified loss-of-function mutations in CSF1R as the primary causal mutation in ALSP patients and have advocated haploinsufficiency as a mechanism underlying the disease.145–147 The proposal is supported by ALSP-like symptoms developing in a subset of aged male heterozygous Csf1r mutant mice on the C57BL/6 background,148 a background on which the homozygous mutant is lethal prior to weaning. But in this model, there was a substantial increase in microglial numbers throughout the brain compared to wild-type mice. A detailed analysis of the brains of multiple ALSP patients revealed the direct reverse of the mouse phenotype.147 By contrast to other leukodystrophies, in which microglia appear increased and activated, there were greatly reduced microglial numbers, uneven distribution, and focal changes in morphology referred to as dysplasia. The loss of microglia was associated with a marked loss of detectable CSF1R protein and the microglial marker CD11b on Western blots in clear contrast to increased detection in other disease states exhibiting microgliosis. In ALSP patients with heterozygous loss-of-function mutations and a diagnosis of ALSP the expression of the wild-type allele at the protein level in microglial progenitors is not known. The lack of any evidence of disease in family members with heterozygous complete loss of function alleles of CSF1R associated with the recessive disease25,26 strongly supports the view that haploinsufficiency per se is not likely to explain the pathology of ALSP.

Although coding variations in the intracellular domain of CSF1R are comparatively rare, examination of the ExAC database (exac.broadinstitute.org) reveals several (T672H, A629S, T621M, T600M, T587I, R549H, R549C) detected in multiple individuals that affect amino acids in the intracellular kinase domain that are conserved in birds. By inference these mutations are likely to compromise receptor activity. If that is the case, ALSP may be the tip of a less penetrant iceberg of CSF1R-dependent microglial dysfunction (likely diagnosed as other disease entities in the absence of CSF1R genotyping or autopsy22,150) or alternatively, these variants might indeed produce complete loss of function but cause disease only in homozygotes. Coding variants are considerably more common in the extracellular domain but none affects contact amino acids involved directly in ligand binding. However, Yeh et al.151 recently described a CSF1R variant (H362R) with a high-allele frequency in the East Asian population. They presented evidence that this change within the receptor dimerization domain partly compromises signaling, but others found that the variant did not prevent autophosphorylation.147

One of the two recent reports of the recessive disease26 promoted the zebrafish as an alternative model of Csf1r deficiency, in part because the mutation in inbred mice is apparently much more severe than the human disease. It is certainly the case that the function of Csf1r in the generation of Mφs and microglia is conserved in fish16,17,152 as it is in birds that provide an alternative tractable model in which development can be monitored in ovo.15 We have produced a Csf1r deletion in chicks and observed the same severe growth retardation (post-hatch) seen in mice and rats (DAH, A. Balic; unpublished). One complexity of working with zebrafish, other than the quite distinct skeletal and hematopoietic biology, is that much of the genome is duplicated and there are two Csf1r loci with partially redundant functions. The generation of an allelic series with graded loss of 1 to 4 copies of Csf1r indicated that microglial numbers are sensitive to Csf1r dosage and further, that as in mice and rats (see above) some peripheral Mφ populations were less dependent upon Csf1r.152 However, as noted above, 50% loss of Csf1r in the brain of rats and mice does not compromise microglial numbers. The phenotypes of mouse and rat Csf1r mutations on inbred and outbred backgrounds show the same range of phenotypes as observed in human patients and provide an informative and relevant model to test therapies such as bone marrow transplantation. The clear impact of genetic background implies the existence of epistatic modifiers of disease phenotype, likely providing an additional explanation for the diversity of age-of-onset. Indeed, one might consider the possibility that consanguineous couples could also generate homozygosity for epistatic modifiers of the impact of CSF1R mutations in their affected progeny.

Adult-onset patients with homozygous CSF1R mutations have not been reported to exhibit the severe postnatal growth retardation seen in mice and rats.25 We suggest that the impact of mutation on the GH/IGF1 axis requires the complex loss of CSF1-CSF1R activity and peripheral Mφ populations and it may have contributed to the more severe cases with infant mortality. In any case, the absence of this phenotypic impact supports the argument above that osteopetrosis per se is insufficient explanation for growth retardation. Otherwise the differences from mutant animal phenotypes are not that great. It is clear from the mouse and rat mutations of Csf1r that microglia and osteoclasts are CSF1R-dependent. Other populations that share CSF1R dependence with microglia, such as Langerhans cells, peritoneal Mφs, and heart and kidney Mφs, have not been studied in either ALSP or homozygous/compound heterozygous mutant patients. ALSP patients,
who certainly do have residual CSF1R activity, do not exhibit the skeletal phenotypes described in the recessive disease but they do show ectopic calcification in the brain.146 As discussed above, the osteoclast deficiency in outbred mouse Csf1r<sup>−/−</sup> mice resolves with age, indicating that there are other factors that can compensate for the loss of CSF1R. Similarly, our Csf1r<sub>ΔFIRE/ΔFIRE</sub> mice lack microglia but they have normal M<sub>φ</sub>s and osteoclasts in the bone despite the lack of Csf1r expression in those cells. We can conclude therefore that microglia are likely more sensitive than osteoclasts to disruption of CSF1R signals. In simple terms, we suggest that the autosomal dominant and recessive diseases associated with CSF1R mutations are part of the same spectrum of CSF1R insufficiency, with ALSP patients retaining enough CSF1R activity to maintain osteoclasts and most tissue M<sub>φ</sub> populations. That said, the impact of the ALSP-associated mutations on peripheral M<sub>φ</sub> biology has not been adequately considered in dissecting disease mechanisms. Several recent studies have emphasized the relationship between resident tissue M<sub>φ</sub>s and the peripheral nervous system (e.g., ref. 153) and we have identified very large numbers of M<sub>φ</sub>s in smooth and skeletal muscle; loss of function of these cells could potentially contribute to motor defects in ALSP patients.1 Kapfer cells were apparently unaffected in the livers of ALSP patients<sup>149</sup> but they are also relatively CSF1R-independent in animals.40 There is clearly some impact of ALSP mutations in the periphery, since there is a defect in the CSF1-dependent generation of so-called non-classical monocytes in patients.<sup>154</sup> So, further studies of peripheral M<sub>φ</sub> populations in ALSP patients are needed in order to fully understand the disease process.

2 | CONCLUSIONS

In overview, we suggest that mouse and rat mutations provide informative and predictive models of the pathology of human CSF1R deficiency provided account is taken of genetic background and the variable sensitivity of different M<sub>φ</sub> populations to CSF1R loss of function. The evidence for functional expression of CSF1R in non-hematopoietic cells is not compelling and accordingly all of the phenotypes associated with mutation of CSF1R or its ligands can be attributed to their impacts on mononuclear phagocyte biology. In keeping with that conclusion, all of the pleiotropic impacts of a Csf1r mutation in mice can be overcome by neonatal bone marrow transplantation.155,156 The rat Csf1r<sub>−/−</sub> model with improved postnatal viability offers the opportunity to test therapies that might reverse the adverse phenotypes of human CSF1R mutations later in postnatal development or even in adults. Transplantation and other interventions proven efficacious in mouse and rat models are likely to provide insight into the human condition and may offer promise to patients with these rare diseases.

ACKNOWLEDGMENT

Research in our laboratory is made possible by core support from the Mater Foundation. The Translational Research Institute receives funding from the Australian Government.

AUTHOR CONTRIBUTIONS

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