LETTER

On the utility of CSF1R inhibitors

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We refer to an article pointedly titled "CSF1R inhibition by a small-molecule inhibitor is not microglia specific; affecting hematopoiesis and the function of macrophages" in PNAS (1). This Brief Report addresses a straw man. Thirty years of studies on CSF1 signaling in peripheral populations, assessed via small-molecule inhibitors, antibodies, and mutations, document effects outside of the brain (reviewed in ref. 2). It is nevertheless the case that microglia are unusually dependent on CSF1R signals compared to peripheral macrophage populations. Biallelic loss of function, dominant negative gain of function, and hypomorphic mutations of the *CSF1R* gene in mice, rats, and humans lead to partial or complete loss of microglia and progressive neuropathology while partly sparing many peripheral macrophage populations (1, 2). Accordingly, CSF1R inhibitors with the ability to cross the blood–brain barrier are a useful tool for manipulating microglia in the healthy and diseased central nervous system (reviewed in ref. 3).

The authors of this report focus on the effects of a specific inhibitor, PLX5622, on hematopoiesis and multiple hematopoietic lineages (1). As presented, the results are uninterpretable. The dose of PLX5622 and precise source of chow were not provided, the duration of treatment was arbitrary (3 wk), there is apparently a single experiment with $n = 5$, the sex of the animals was not recorded (female C57Bl6 mice are osteoporotic), and they were aged anywhere from 6 mo to 12 mo. There is not even direct evidence of microglial depletion. Critical data are missing for all flow cytometry datasets—there is no information on use of Fluorescence Minus Ones/unstained controls to assess spectral overlap and no representative flow plots to assess gating strategy.

The first published study utilizing PLX5622 explored various peripheral myeloid subsets, including neutrophils, lymphocytes, monocytes, eosinophils, and basophils via flow cytometry (4), and other extensive analyses have followed, including peripheral cell assessment after 6 mo of treatment (5). The authors (1) provide no explanation for their divergent findings. The extensive impacts the authors report on bone marrow progenitors and lymphoid cells cannot be due solely to CSF1R inhibition. One of us has recently confirmed that CSF1R protein is expressed exclusively by committed cells of the mononuclear phagocyte lineage and absent from hematopoietic stem cells (6). Blockade of CSF1R with anti-CSF1R antibody depletes many tissue macrophage populations without impacting myelopoiesis (7), and a hypomorphic *Csf1r* mutation in mice that ablates CSF1R in the marrow has no effect on myelopoiesis but does impact microglia (8). PLX5622 is 20-fold more active against CSF1R than the related kinases KIT and FLT3 in cell-free assays (5). Assuming their findings are reproducible, it seems likely that the authors have administered sufficient dose to impact these related molecules which are essential for normal hematopoiesis.

In summary, we feel that the title of this work (1) greatly overstates its significance. CSF1R kinase inhibitors used at appropriate doses and with appropriate controls remain a valid experimental tool and have potential clinical utility.

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