LETTER

REPLY TO GREEN AND HUME:

Nonmicroglia peripheral immune effects of short-term CSF1R inhibition with PLX5622

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Our study (1) was designed to provide definitive direct data to the microglia community that PLX5622 does not act solely on microglia but also affects other peripheral macrophage populations. Many microglia papers have assumed or claimed that CSFR1 inhibition has no to minimal effects on peripheral immune cells.* The nonmicroglia effects of CSFR1 inhibition have direct ramifications for the interpretation of relevant experimental data; peripheral monocytes/macrophages engraft permanently into the retina after PLX5622 treatment (2, 3), causing remodeling and functional changes that affect studied outcome.

We reject the comment by Green and Hume (4) that PLX5622 dose and duration was not provided or that it was selected arbitrarily. A careful reading of our paper (1) and the literature reveals this information, which is in concordance with at least 14 papers using exactly the same dose (PLX5622 chow, 1,200 ppm) and duration (3 wk) (1–3, 5), some of which have been cited by Green et al. (6) in a prior review.† For the benefit of the community, we provide additional data in this Reply showing that even short-term (7 d or 10 d) treatment with PLX5622 results in similar nonmicroglia changes (Figs. 1 and 2) in younger animals (3 mo old; mixed gender). It should be noted that 7 d of either PLX3397, as shown by Green and coworkers (7), or PLX5622 (8) is inferior to 3 wk in depleting microglia. The number of animals was dictated by statistics, with a power of >90%. An equal or fewer number of animals have been previously used in other similar studies (9, 10).

During the review process of our original article, we provided supporting data on gating to the reviewers that was not published. We did not detect differences between males and females. The age of the animals (same in control and treated) was partially dictated by the time needed to generate bone marrow chimeras, treat animals with PLX, and allow them to recover before analysis. Moreover, most common human central nervous system pathologies often develop later in life. In this context, experiments closer to the age of 6 mo to 9 mo old in mice are more realistic and were chosen for this study. However, data in younger mice (with gating) are provided here with similar results (Figs. 1 and 2).

The studies cited by Green and Hume (4) suggesting microglia selectivity of CSF1R inhibition are with either biologics (antibodies), or genetic manipulations which have been challenged by recent publications (11, 12). Furthermore, they are not equivalent to small-molecule inhibitors which are known to have multiple off-target effects. Hence, the title of our manuscript is accurate and conveys the intended information regarding the nonmicroglia effects of CSF1R small-molecule inhibitors as commonly used by many in the scientific community.

CSF1R inhibition remains an important tool for immunologists. However, PLX treatment (even short term) can cause changes in nonmicroglia cells. Thus, future studies need to take this into account and incorporate appropriately designed experiments that allow for more accurate interpretation of experimental outcomes.

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Author contributions: F.L., D.G.V., and E.I.P. designed research; F.L. performed research; F.L., D.G.V., and E.I.P. analyzed data; and N.C., C.Z., J.C., D.G.V., and E.I.P. wrote the paper.

The authors declare no competing interest.

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Published January 14, 2021.

*For a partial listing, see Pubmed ID (PMID): 24742461, 24247719, 25497089, 28539419, 29915052, 31023885, and 30705270.

†For a partial listing, see PMID: 26232154, 29507754, 29472620, 31772011, 31295468, 31042769, 32029629, 28539419, and 30705270.

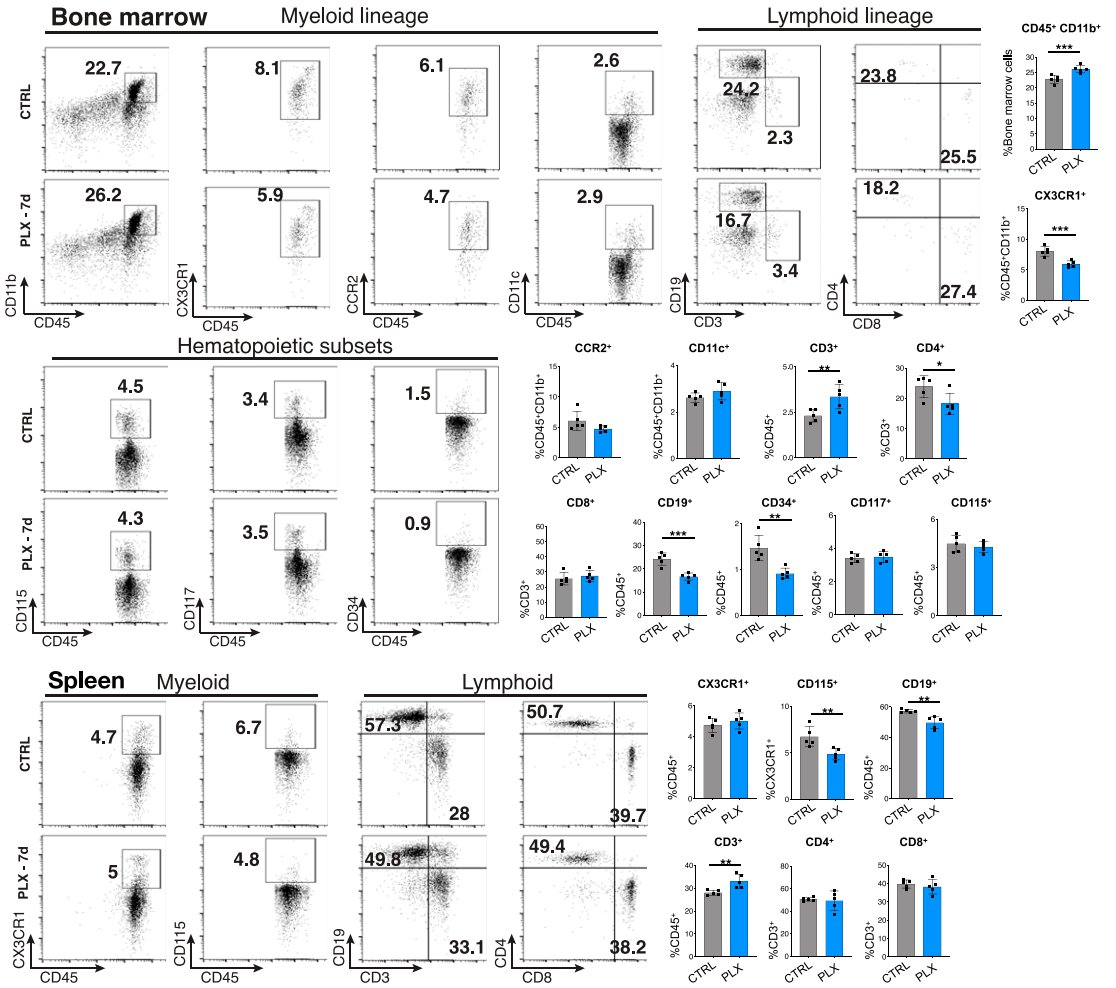

Fig. 1. Nonmicroglia peripheral immune effects after short-term CSF1R inhibition with PLX5622. Short-term exposure (7 d) to small-molecule CSF1R inhibition affects the myeloid and lymphoid compartments of the bone marrow and spleen. Flow cytometric analysis of bone marrow cells isolated from 3-mo-old CX3CR1$^{+/EGFP}$ mice treated with PLX5622 for 7 d shows suppression of CD11b^{+}, CX3CR1^{+}, CD3^{+}, CD19^{+}, and CD34^{+} bone marrow cells and CD115^{+}, CD19^{+}, and CD3^{+} splenic cells. $n = 5$ per group (3 mo old; mixed gender), mean ± SD, independent t test, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

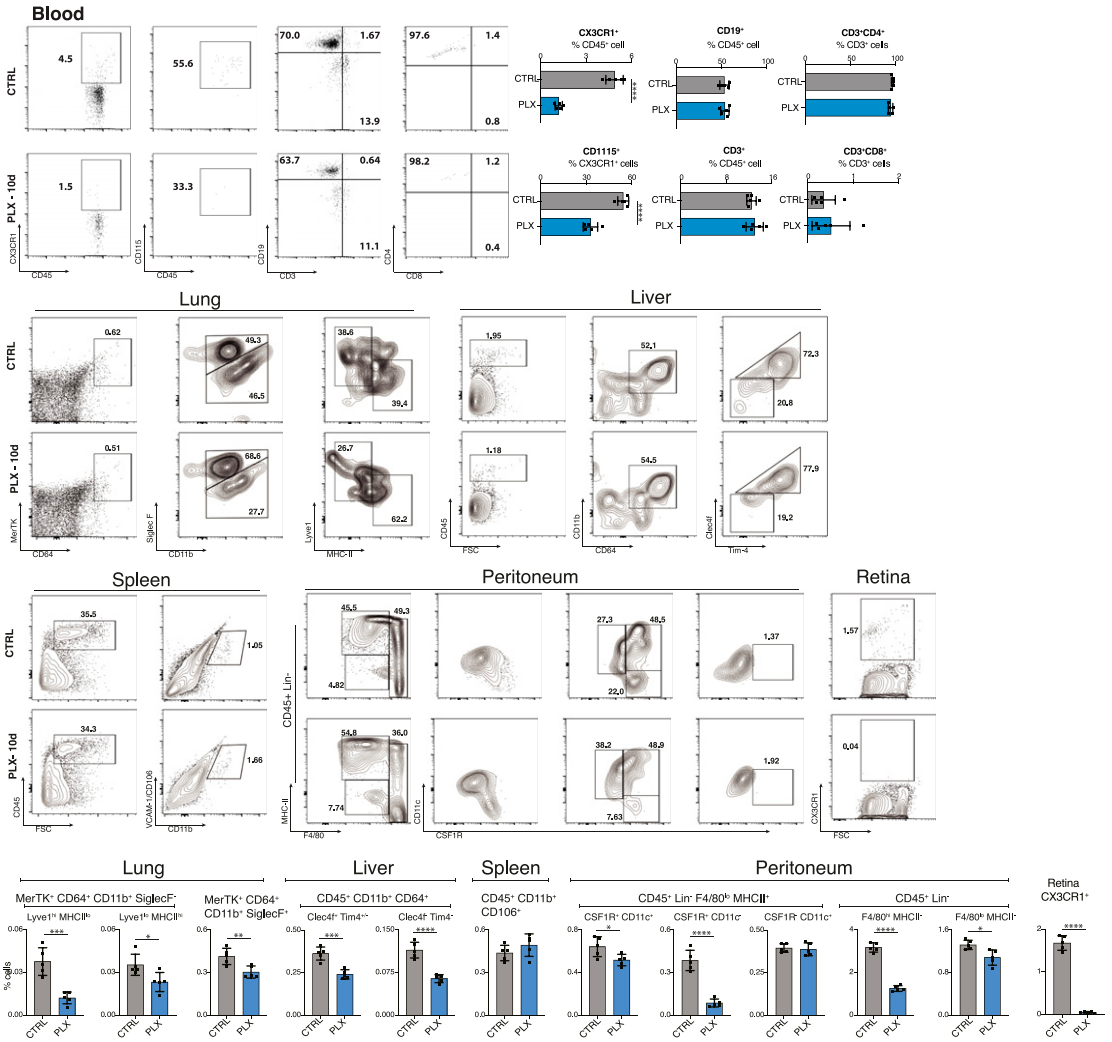

Fig. 2. Nonmicroglia peripheral immune effects after short-term CSF1R inhibition with PLX5622. Small-molecule CSF1R inhibitor, administered for 10 d, suppresses CX3CR1^{+} and CD115^{+} blood cells and reduces the number of resident and interstitial macrophages of the lung, liver, peritoneum, and retina, but not the CD45^{+} CD11b^{+} CD106^{+} cells of the spleen nor the F4/80^{lo} MHCII^{+} CSF1R- CD11c^{+} peritoneal macrophages; $n = 5$ per group (3 mo old; mixed gender), mean ± SD, independent t test, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

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