Contribution: T.B. performed the statistical analyses; and T.B. and M.C. cowrote the paper.

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Response

Estimating frequency of cancer stem cells in a mouse model of adult T-cell leukemia/lymphoma

Bonnefoix and Callanan, using a statistical analysis, have challenged our report on the frequency of cancer stem cells (CSCs) in a nonobese diabetic–severe combined immunodeficiency (NOD/SCID) mouse model of adult T-cell leukemia/lymphoma.[1] While we agree (as discussed below) that there are limitations in estimating frequency of CSCs in our model, we have clearly documented the existence of CSCs using xenografting of candidate CSCs into NOD/SCID mice. Specifically, we identified candidate CSCs in a side population (0.06%) that mostly overlapped with a minor population of $CD38^{-}CD71^{-}CD117^{+}$ cells (0.03%). Furthermore, we found that 10^2 candidate CSCs could regenerate the original lymphoma when transplanted into the NOD/SCID mice. From the limiting dilution transplantation assay (LDTA) data in Table 2 (in our article), we estimated the frequency of candidate CSC to be around 0.01% on the basis of the observation that 10^4 SLCs could regenerate the original tumor. However, we agree that we have used only limited transplantation data to determine this frequency and that more detailed studies would be required to accurately calculate this. In this regard it is important to note that the experimental conditions used would certainly influence frequency estimates. In our study, candidate CSCs could regenerate the original tumor only when analyzed at 60 days after transplantation, suggesting that the duration of analysis could also affect the LDTA data. In this respect it has already been shown that modification of xenotransplantation assays in NOD/SCID mice can also dramatically change the detection frequency of CSCs.[2]

We should also highlight that the cell population we identified by LDTA would likely include not only CSCs but also other tumorigenic cells, and the existence of such cells in addition to the candidate CSCs would also influence our estimates of CSC frequency.

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