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## Comment on "Little Evidence for Developmental Plasticity of Adult Hematopoietic Stem Cells"

Wagers *et al.* (1) investigated the possibility of marrow cell plasticity at the single cell level, as we did (2). Although they found "little evidence for developmental plasticity"—i.e., marrow cells giving rise to mesoderm (blood), endoderm (liver), and ectoderm (brain)—their experiment substantially differed from ours and is therefore not a clear refutation of our previous work.

Wagers *et al.* used 6- to 12-week-old donor and 10- to 14-week-old recipient mice. Our donor and recipient mice were age-matched at 4 to 6 weeks old. Although age may not affect the rate of hematopoietic engraftment, it may affect plasticity potential. Hematopoietic stem cells (HSCs) have different phenotypes in younger (3 to 5 weeks) than in older (5 weeks) mice. For example, murine HSCs express the CD34 antigen in mice younger than 5 weeks, but not older (3).

We analyzed engraftment 11 months following stem cell transplantation, while Wagers *et al.* analyzed mice from 4 to 9 months after transplantation. It is unclear from (1) how many mice were analyzed at different time points, how much donor-derived marrow engraftment each mouse had, and whether there was an association between time of sacrifice and presence of donor-derived hepatocytes or Perkinje cells. For example, Wagers *et al.* reported that their mice had 0.03 to 71.6% peripheral blood engraftment, but engraftment levels in the four mice analyzed were not specified, nor was the level of blood engraftment stated for the time that the solid tissues were examined.

More important, the differently purified stem cells likely represent different rare subpopulations with functionally different levels of plasticity. We isolated and purified our stem cells through functional isolation involving lineage depletion, elutriation,

bone marrow homing, and separation of small, G<sub>0</sub>/G<sub>1</sub> cells. We have shown (4) that elutriated, phenotypically negatively selected cells are enriched for stem cell engraftment following a homing step to the marrow *in vivo*. In contrast, Wagers *et al.* used purely phenotypic fluorescence-activated cell sorting (FACS) to select for c-kit<sup>+</sup>Thy1.1<sup>lo</sup>Lin<sup>-</sup>Sca-1<sup>+</sup> (KTLS) cells.

The most important difference in methods may be our inclusion of AA4.1 antibody in our Lin cocktail, a step that removed hematopoietic progenitors and possibly allowed selection for an "earlier" stem cell with more plasticity potential. The parabiotic experiments performed by Wagers *et al.* would of course include the cell population we tested, but they did not assess engraftment following injury (including whole body or targeted irradiation) in that model. Thus, the absence of evidence of plasticity in the parabiotic model may simply indicate the need for injury to elicit a plasticity response.

The methods used for engraftment detection may also differ in sensitivity. We used the Y chromosome as a marker of marrow cell origin. Any cell derived from the engrafted population, by any mechanism (fusion or differentiation), would contain a Y chromosome. Wagers *et al.* used the green fluorescent protein (GFP) transgene expressed from the chicken  $\beta$ -actin promoter with a cytomegalovirus (CMV) enhancer. Although this promoter was expressed in all cell types tested, there is no evidence that all marrow cells would express GFP when and if they differentiated into nonhematopoietic cells. In addition, it is uncertain whether the level of any transgene in a newly "transdifferentiated" cell would always be detectable above background autofluorescence in different tis-

sues. For example, following transplantation of male Rosa marrow cells into lethally irradiated female wild type mice, the spleen showed >90% engraftment by Y chromosome analysis, but cells expressing  $\beta$ -galactosidase expression represented <50% of the splenic cells (5). This is important, given that the Rosa mouse is commonly said to show "ubiquitous" transgene expression, as Wagers *et al.* claimed for their relatively uncharacterized GFP<sup>+</sup> transgenic mouse. It is possible that transplantation and expansion of the donor-derived cell population could affect transgene expression. Therefore, transgene expression, even if driven by a presumed "ubiquitously" active promoter, is a less rigorous and nonoptimal means for identifying donor-derived cells.

In summary, the Wagers *et al.* study differs significantly from our prior work and clearly shows that further experimentation is necessary to determine the factors that inhibit or promote adult cell plasticity responses.

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