We appreciate the authors’ interest in our recent Commentary on Cutting Edge Science that discussed their recent article. We constructed what we believed to be a balanced review of their 2013 article published in Cell as yet another chapter in the c-kit+ cardiac stem cell regeneration story. We gave significantly more discussion to the positive findings of the article in question, although, like any good commentary, we discussed perceived shortcomings and topics that deserve more study. The authors’ rebuttal identified 3 main issues of concern with our Commentary, and we are delighted to address these issues again in greater detail.

First, the authors suggest that we disparaged their isoproterenol (ISO) injury model. This was not our intent, and we even stated that the ISO injury model “is suited to address the question of cardiac regeneration.” Also, both the Molkentin and Houser laboratories have used excess ISO to injure the heart by chronic administration. The authors suggest that their single injection of ISO mimics the complex and poorly understood clinical syndrome termed Takotsubo cardiomyopathy. However, patients with Takotsubo cardiomyopathy can fully recover with medical treatment in days to a few weeks, suggesting that it is nothing more than temporary myocardial stunning, which is also consistent with the authors’ own previous work in animals that also showed recovery of cardiac function 6 days after a single dosage of ISO. Six days is clearly too short for cardiomyocytes to be generated from cardiac stem cells, fully differentiate, and then restore function to the heart, suggesting that the loss of cardiac function with the ISO model is primarily due to stunning.

Second, the authors stated that we were skeptical of their results showing homing of injected cardiac stem cells into hearts 28 days after ISO injury plus 4 days of 5-FU treatment. In fact, we thought that these were exciting new findings and the results, as presented clearly, supported the conclusions. However, we were surprised that 4 days of 5-FU could abolish all inflammatory responses for the next 21 days in ISO-injured hearts. The idea that short-term 5-FU treatment fully inhibited inflammatory responses for such a prolonged time period was not well studied in the report and, therefore, clearly deserves additional experimentation.

Third, with respect to c-kit lineage labeling in which the Kit promoter was added to a lentiviral construct to drive cre recombinase expression, the undeniable fact is that the packaging limit for the lentivirus platform used by the authors was <5 kb, and such a short region of the Kit promoter is known not to be properly expressed in vivo. As shown in Figure 1 of the authors’ rebuttal, the Cairns et al5 article describes 3 kit promoter–driven transgenes of 6.7 kb (construct 1), 10.2 kb (construct 2), and 14.7 kb (construct 3). They could not have used construct 2 to create the lentivirus, as their rebuttal is attempting to mislead the readers into believing (they used the phrase “derived from”) to suggest that their ≤5-kb lentiviral Kit promoter–containing construct is the same as the 10.2-kb construct from Cairns et al). Even the 10.2- and 14.7-kb constructs produced ectopic expression 20% of the time in independent lines of transgenic mice, and construct 2 showed only 20% concordance with fetal liver and bone marrow (sites of known c-kit expression), suggesting that these promoter regions do not recapitulate true Kit allele expression in vivo. Another issue is that the authors misrepresented this entire line of experimentation in the original Cell article. Online Figure IV described Kit-GFP transgenic mice as a means of validating the lentiviral Kit promoter fragment, but these transgenic mice have a larger piece of the Kit promoter (construct 3 from Cairns et al). Clearly, this control experiment is irrelevant and does not validate the fidelity of the Kit-cre lentivirus. More than that, numerous studies have shown that the Kit promoter region is complex, and even constructs containing >100 kb of the regulatory region do not faithfully reproduce c-kit expression in vivo; hence, the fragmented 5 kb Kit promoter/enhancer region used in the lentivirus to drive cre recombinase is far from a definitive fate mapping approach.

In summary, we remain confident that we provided a fair, balanced, and data-driven commentary about the original report. The topic is clearly important, and in our view, there is still more work to be done before the role of c-kit+ cardiac stem cells in cardiac repair is clearly defined.

Disclosures
None.

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Response to Torella et al
Jeffery D. Molkentin and Steven R Houser

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