Letter by Murry et al Regarding Article, “Embryonic Stem Cell–Derived Cardiac Myocytes Are Not Ready for Human Trials”

Our group recently reported a proof of concept study showing that human embryonic stem cell–derived cardiomyocytes (hESC-CMs) can regenerate significant myocardium in the infarcted hearts of nonhuman primates.1 A Commentary by Sussman and colleagues casts an unfavorable light on this study and concludes that hESC-CMs are not ready for use in patients.2 We had some difficulty recognizing our study in the authors’ caricature and encourage readers to read the original report before drawing conclusions.

They made 3 meritorious critiques: group sizes were too small for statistical comparison, insufficient cardiac mechanics analyses were performed, and the arrhythmias could be serious in patients with heart disease. We agree with these limitations and clearly pointed them out. The small group sizes were necessitated by the nature of primate research: it is expensive, labor-intensive, and ethical considerations require the minimum numbers possible. The limited mechanical analysis stemmed from small group size. Cardiac MRI was intentionally deferred to ongoing definitive studies (with larger numbers) analyzing cardiac function. Finally, we fully recognize the seriousness of ventricular arrhythmias. This prompted us to instrument the monkeys for ECG telemetry and monitor their rhythm more closely than any cell therapy group has done previously. If we had taken the more common approach to cardiac monitoring (≤24 hours), we might have erroneously concluded that our cell therapy caused no arrhythmias. We reported the arrhythmias as a main finding in the abstract, as a major in-

print figure (Figure 4), and a main point of the discussion.

Other proffered criticisms have less merit. They suggested that the quality of our science does not justify using primates and imply a reckless rush to the clinic. This belies the painstaking approach we have taken to cardiac repair over nearly 2 decades. These studies began in cell culture, learning first to control the differentiation of pluripotent cells.3 We then developed mouse and rat models that allowed careful analysis of cardiac structure and function. We have studied >1000 rodents with hESC-CM grafts, providing unequivocal proof that these cells preserve cardiac geometry and enhance systolic function postinfarction.4 Importantly, we perform necropsies on every animal, and although we have reported rare epithelial cysts,5 we have never seen a teratoma, suggesting that these can be avoided with good control of differentiation. From this knowledge base, we extended our work into the guinea pig, whose heart rate is substantially slower than the mouse or rat, and we provided the first evidence that, not only do hESC-CMs enhance cardiac structure and function, they electrically couple with the host myocardium and beat in synchrony.6 This is a sine qua non for true heart regeneration that has not been achieved with any other cell population. Only after this step did we choose to study primates. Importantly, our Nature paper never stated that hESC-CMs are ready for the clinic—we were surprised by this straw man argument.

Because of editorial word limits, other criticisms are rebutted in point-by-point format.

Critique: Primates are poor models for human myocardial infarction.

Response: Primates are closer genetically than other species and are >10× larger than guinea pigs (our previous model). Their resting heart rate of 120 is close enough to test human cells in a meaningful environment. Moreover, the Food and Drug Administration recognizes primates as highly relevant preclinical models.

Critique: Gene targeting requires whole genome sequencing before such cells can be used clinically.

Response: We created GCaMP3+ hESC-CMs to determine whether grafts coupled electrically with monkey myocardium. These were never intended for use in humans; thus these safety concerns are irrelevant.

Comment: Authors fail to provide evidence for regenerated myocytes.

Response: We grew >0.5 g/heart of new myocardium, >10-fold more than reported in other studies.

Comment: Therapy will require lifelong immunosuppression.

Response: Lifelong immunosuppression is not optimal, but the entire field of transplantation medicine is built on it. Also, creation of universal donor cells may obviate immunosuppression.

Comment: No basis to suggest the arrhythmias resolve over time.

Response: Ventricular arrhythmias in all cell-treated animals peaked early then decreased in frequency. In the animal studied for 3 months postengraftment, arrhythmias peaked early and were undetectable from 3 weeks to 3 months. Because of the importance of this issue, ongoing studies are addressing it in detail.

Comment: No basis to suggest arrhythmias caused no distress.

Response: The animals were inspected regularly and followed by continuous (24/7) video surveillance. They remained conscious, ate and drank normally, and groomed themselves during arrhythmic episodes.

Critique: Analysis of cell purity before and after transplantation is flawed.

Response: cTnT (cardiac troponin T) flow cytometry has been extensively validated by our group and others against multiple cell type markers.8-11 We demonstrate many images of the grafted cells and show that >98% of human cells are cardiomyocytes (extended data Figure 8A).

Critique: Graft muscle fiber orientation looks like endogenous muscle.

Response: This is a compliment. Yes, our human myocardium is bundled into oriented muscle fascicles. We validated its human origin by green fluorescent protein immunostaining and in situ hybridization for human pan-centromeric sequences.

Critique: Sarcomeres and intercalated disks are not clear, and grafts resemble scar tissue.

Response: It is difficult to understand how our grafts can simultaneously resemble endogenous muscle and scar tissue.
Readers should judge the quality of the human sarcomeres and intercalated disks for themselves in Figures 1B and 2B2Q.

**Critique:** Authors should have used their prosurvival cocktail to enhance engraftment.

**Response:** We did (Methods, paragraph 1).

**Critique:** Time points were inconsistent, and animal age was not uniform.

**Response:** This was a time course study, so inconsistency was by design. The different ages reflect differences in patient populations and revealed that engraftment is not age dependent.

In summary, we concur that our study was not perfect and pointed out the most serious limitations ourselves. However, it is worth emphasizing several positives: we generated 10x more purified human cardiomyocytes and grew more human myocardium than in any previous studies; we showed that grafts were electromechanically coupled with the host; and we identified ventricular arrhythmias as a challenge to overcome en route to clinical translation. We are working on the questions raised by this study in a careful and systematic manner, and we think that pluripotent human stem cells have much potential in regenerative medicine.

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**Disclosures**

Drs Murry and Laflamme are scientific founders and equity holders of BEAT Biotherapeutics.

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**References**

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