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Can we please stop arguing about bone marrow-derived cells for cardiac regenerative therapy?

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Abstract

Almost seven years have passed since the initial publication reporting that bone marrow cells regenerate infarcted myocardium. The subsequent years produced hundreds of investigations that ran the gamut of findings from validation to disproof. Undeterred by the concurrent debate, clinical trials ensued to test the efficacy and safety of bone marrow derived cell population for autologous therapy in clinical treatment of myocardial disease. In the following conversational exchange, two scientists with distinct perspectives weigh the pros and cons of pursuing bone marrow stem cell therapy and look toward finding a consensus of where the future lies for regenerative medicine and the heart. The conclusion is that the two camps may not be as far apart as it may seem from the rancor in literature and at meetings, and the potential of one day achieving regenerative therapy is indeed a vision that both parties enthusiastically share.

Sussman: I think we can agree that therapeutic implementation of regenerative approaches for the myocardium will depend upon finding a cell population that can do the job. Bone marrow stem cells are attractive primarily from an availability standpoint as well as autologous therapy, but the literature on their efficacy as a population for mediating cardiac regeneration is mixed. This includes your Nature paper from a couple of years back (1) as well as others from various labs indicating that potential for transdifferentiation is miserably low (or non-existent altogether). Contrast those findings with observations primarily driven by the Anversa group (as well as many others) documenting the capacity of bone marrow derived stem cells to transdifferentiate into cardiogenic lineages that mediate significant reparative processes and we have a dilemma. Do we pursue the use of bone marrow-derived stem cell populations as a viable approach for mediating cardiac regeneration or look for a different cell type with allegedly more robust regenerative capacity? If we are going to move things in a therapeutically relevant direction, then the use of bone marrow derived cells has already begun in clinical trials and the stands the greatest chance of paying off in the short run. Do you think that planning such trials is a waste of time and energy with this cell population? I submit that your Nature

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paper infers that such studies offer a lot of false hope, are futile, and based upon a presumptuous idea that is not supported by the literature.

Murry: Before we dig too deeply into how to proceed, I always think it's useful to clearly define what we're discussing. Regeneration of the infarcted heart implies creation of new myocardium, with myocytes that are electromechanically coupled to the host tissue and an appropriate coronary vasculature and connective tissue to support function. Regeneration of the heart is a long way from a clinical reality, and I would submit, one that has rarely (if ever) been achieved in the experimental laboratory. For this reason, I don't think it's useful to talk about which cell type is going to regenerate hearts in clinical trials any time soon. On the other hand, repairing the heart means that one is restoring structure and function toward a more useful physiological state. Repair can proceed through a variety of mechanisms, such as remuscularization, revascularization, prevention of adverse remodeling, promoting survival of residual myocytes and vessels etc. Numerous physiological studies, including several from my own lab, indicate that cell transplantation can enhance function of the infarcted heart. This indicates that repair is something that we can strive for in the clinic within a reasonable time frame.

The question of whether marrow-derived cells in general, and hematopoietic stem cells in particular, can transdifferentiate into cardiomyocytes and promote widespread regeneration has been hotly debated for more than 5 years. In my opinion, this is one of the most rigorously disproven hypotheses in the history of cardiovascular research. Let us be clear, however, and stress that virtually all groups find the occasional cardiomyocyte (with a frequency of $\sim 10^{-4}$) that carries lineage markers suggesting marrow derivation, or possibly fusion of a leukocyte with a cardiomyocyte. Such rare cells cannot contribute meaningfully to contractile function. Since marrow derivatives are unable to generate physiologically significant numbers of cardiomyocytes, what do we make of the considerable body of evidence that suggests function is improved? That cardiac repair can occur through non-myogenic mechanisms is one of the biggest lessons learned from this field, and it would be foolish to discount the efficacy data, just because a mechanism was disproved. Rather, we need to focus on identifying the non-myogenic mechanisms through which these and other cells function and learn to exploit it better. We also need to focus on subsequent generations of approaches that offer hopes for true remuscularization, because if we can get this far with non-myogenic approaches, we should be able to do still better with myogenesis.

As for the clinical trials with marrow derivatives, so long as safety profiles continue to be favorable, these should proceed as planned. Medicine has a long history of developing drugs, surgical procedures etc. that have benefit without understanding their full mechanism of action. (We are still learning about aspirin, beta-blockers and angioplasty, for example.) It is important that we adjust our clinical expectations to fit scientific realities, however, because this is the only way to rationally improve cell-based therapies. One factor in addition to safety must be factored into the cost of these trials, however: The public does not have infinite resources, nor does it have infinite patience. If we raise false hopes by prematurely taking cell therapy to the clinic, we might reasonably expect the public, and our peers, to conclude that this field has failed to produce and no longer deserves support. By way of perspective, it took nearly 20 years for the first clinical stem cell therapy, bone marrow transplantation, to go from initially lethal results to a reliable success. It would be a pity to see the door close on the promising field of cardiac repair, just because in our haste and enthusiasm we pushed the wrong cells or promised unachievable results.

Sussman: I agree we need to frame the debate here carefully. Two issues are in play: 1) the transdifferentiation for bone-marrow derived cells into cardiogenic cell types, and 2) the potential for bone-marrow derived cells to serve a role in therapeutic myocardial regenerative

medicine. It is important that we not blur the two together, as the issues and points to be made are radically different. The former question is primarily rooted in work with a multitude of experimental models, whereas the latter issue depends in part upon critical assessment of numerous clinical studies. You appropriately laid out the terms of what would be the ideal standard for myocardial regeneration, but it is both unrealistic and short sighted to expect that initial forays into a new research endeavor will produce regeneration with the complexity and magnitude you describe. For a field that was literally non-existent a few years ago, I submit that remarkable progress has been made on both fronts you mention and that the level of stem cell-mediated repair will only get better with time. For the sake of clarity in keeping our discussion focused I will avoid dragging into the fray the question of the appropriate cell type, but I also have little doubt that such a specific cell, when it is unequivocally identified, will be a major advance toward the goal of rebuilding the heart. Yes, your lab and many others have tried shoveling a plethora of different cell types into the myocardium with rather disappointing outcomes. From lack of appropriate differentiation to generation of electrical abnormalities it is clear that many pluripotent or multipotent cell types used in the past are not well suited to the job of myocardial regeneration. Keeping in mind that many of the studies you refer to were done in a era when endogenous repair mechanisms were not even considered, we have no clue as to the contribution of the donated population versus recruited cells in the myocardium toward improvement of function. Given that these previous studies failed to show reproducible substantial benefit (and possibly lethal adverse complications), it is prudent to consider alternative strategies.

You stated that the hypothesis of bone marrow derived transdifferentiation "is one of the most rigorously disproven hypotheses in the history of cardiovascular research", but then go on to contradict yourself by stating that such events do occur with low frequency (let's leave out the issue of fusion; we can debate that in the next treatise). So which is it, Chuck? Do these cells transdifferentiate? If they do transdifferentiate, albeit at a relatively low frequency, then our goal should be to enhance the process and not continue pointless arguments about whether such events occur? Rare and unusual events are the cornerstone of great research advances, with some of the most significant advances from unusual and intriguing observations. There is already an avalanche of published studies from multiple labs showing the type of "rare" transdifferentiation events you describe, so it's not difficult to observe. All these labs and their publications aren't wrong. Transdifferentiation is currently just an inefficient process and our efforts should be focused upon enhancing the ability of the cells to do our bidding as has paid off handsomely for other clinical fields such as bone marrow reconstitutions or skin engraftment. Let's not forget that our studies are in their infancy and we are literally changing a paradigm that has stood for decades. Again, your point of the basis for improvement with non-myogenic mechanisms for improvement of function misses the forest through the trees. The big picture view is that the non-myogenic cells are likely acting to potentiate and promote the very endogenous repair mechanisms that under normal circumstances are relatively inefficient. Contributions of donated populations may be modest, but their influence upon the resident cells may be dramatic. The goal is to manipulate the donated population to enhance their activity as well. Many labs agree that the single biggest problem is short term survival and engraftment/persistence of the donated cell population. If only a few percent of the donated cells are around 1–2 days after transfer and we are seeing measurable benefits, then imagine what might occur if the donated cells stuck around, engrafted, and became part of the functional myocardium with enhanced efficiency? The levels of improvement would likely also be similarly profoundly increased. There's the clinically relevant outcome we're all looking for.

On the other question regarding clinical trials and therapeutic implementation, the ship has sailed on that and the results of initial studies are in. In a meta analysis of 18 such studies the conclusion was that bone marrow derived stem cell transplantation is associated with modest but significant improvements in physiologic and anatomic parameters in patients with heart

disease (2). The therapy is safe and efficacious: improvement in cardiac function with bone marrow in patients with acute MI and low ejection fraction is ~5–6% ejection fraction units, at least as good as that after reperfusion therapy with angioplasty or thrombolysis. And that is with only one shot of bone marrow and at an arbitrary dose. If we had listened to the advice of the proclaimed savants of the stem cell field, then bone marrow clinical studies should not even have been done! We are in a nascent stage of research and development of a revolution in the way medicine is practiced by moving from pharmacologic drug-based therapy to regenerative medicine. While we need to be prudent in our implementation of the bone marrow cells in the clinical arena, there is no reason to drown the enthusiasm generated by initially promising findings. We can focus public attention on the positive aspects of this innovative and creative field or engage in divisive, nonproductive debates that make our community appear confused and clueless to a public that wants to support and believe in our ability to deliver the promises foretold from multiple fronts. If you are truly concerned about public perception, then I suggest we stop debating the utility of bone marrow derived cells and get on with research to understand how to select, enhance, and potentiate cellular repair mechanisms in a logical, unbiased, and open minded fashion. And don't even get me started on paracrine factors... :)

Murry: There is actually quite a bit of consensus in our positions. We both agree that multiple cell types provide physiological benefit to the infarcted heart, and that enhancement of endogenous repair mechanisms are the likely basis for this. We both recognize the impediment posed by death of the transplanted cells and expect enhanced benefit from increasing cell survival. We agree that the clinical trials point to safety in intracoronary delivery of bone marrow cells, and we both think that carefully planned trials in humans should proceed. Regarding clinical efficacy, we have some residual disagreement. You appear to believe that efficacy is demonstrated. The jury is still out for me. There have only been 4 randomized controlled trials to date, and speaking of ships that have sailed, the days of uncontrolled, historically controlled or non-randomized controlled trials have passed for this field. Three of the four randomized trials showed either no benefit or transient benefit that did not persist for 6 months. Only the REPAIR AMI trial showed sustained benefit. In my opinion the evidence for efficacy is modest, at best. We clearly differ on the promise of using marrow cells to form cardiomyocytes. We cannot get this to happen in Seattle, despite many years' effort. On the other hand, just last week we induced human ESCs to form 180 million beating human cardiomyocytes for transplantation and molecular biology studies. These cells form significant amounts of human myocardium after transplantation and prevent the progression of heart failure when transplanted into the infarcted heart (3). The readership of the Journal will need to decide whether they think bone marrow transdifferentiation is a promising strategy when planning their next experiments or submitting their next grants.

Since our last salvo, however, there has been an important breakthrough in stem cell science. Three groups have shown that adult human fibroblasts can be reprogrammed with just 4 genes to pluripotent states resembling human embryonic stem cells in terms of colony morphology, gene expression profiles, global DNA methylation patterns and the ability to form teratomas [4–7]. Yamanaka and colleagues showed, using our group's protocol for directed differentiation, that these "induced pluripotent stem (iPS) cells" will readily form human cardiomyocytes that beat spontaneously in the dish. The process has already been refined to a 3-gene process that excludes the oncogene, c-Myc, and therefore should improve safety (7). The next step with development of these cells is to reprogram them without using integrating viruses; this work is underway in multiple laboratories. The ability to reprogram adult cells to pluripotency has several implications. First, it removes ethical concerns associated with hESCs because no embryo destruction is involved. Second, it makes possible the development of patient-specific stem cells. These could be used for isogenic cell replacement strategies that would avoid immune rejection, and they also could provide cells to study complex genetic

traits in the bona fide genetic background, e.g. cardiomyocytes from patients with cardiomyopathy. I think the availability of iPS cells will bring together the adult and embryonic stem cell camps and allow the community to focus on optimal strategies to enhance remuscularization, revascularization and restore homeostasis within the infarcted heart.

Sussman: It is indeed gratifying that we share many of the same perspectives in this emerging field. I'd like to point out that with regard to our divergent points of view on the topics of clinical efficacy and transdifferentiation a few facts should be kept in mind. First, the variable and inconsistent nature of clinical efficacy will more than likely boil down to differences in the protocols used and lack of understanding related to the optimization of preparations as well as best choices for suitable patient populations. The most straightforward approach to resolving these discrepancies is with additional carefully documented clinical trials, and no other approach will provide the insight we need to move the field forward if we abandon this important avenue of research. My second point has to do with broadening the scope of this debate beyond just bone marrow, which I feel justified in doing since you invoked recent studies on reprogramming of somatic cells. My lab as well as many other well respected research groups have used selected c-kit⁺ cell populations derived from bone marrow as well as from other tissues that generate cells populations expressing various cardiogenic markers including those of cardiomyocytes following adoptive transfer to cardiomyopathically injured recipients [8–12]. While the process may be relatively inefficient at present, there is every reason to expect that the remarkable salutary effects mediated by adoptive transfer of stem cells in experimental animal models will readily be applied to translational therapy once underlying mechanisms are more fully understood. In other words, things are promising at present and will likely get much better in the next few years with greater comprehension of the emerging stem cell regenerative medicine. It is easy to become enthused about relatively nascent observations of reverse-engineered somatic cells, but it is far too early to become evangelical about their potential relative to what we have already got working [13]. Like so many other candidate stem cell types gone before them, capabilities and limitations of iPS cells remain to be defined, and I don't need to lecture you on the problems of ES cell-based therapy for cardiac repair [14]. At the moment, the best characterized populations of regenerative cells we have in hand are selected and possibly genetically engineered c-kit⁺ cells, mesenchymal stem cells, or endothelial progenitor cell populations and those promising cell types are most likely to provide the results that you, I, the research community, clinicians, and patients are most eager to embrace as we move forward refining the initial observations of bone marrow cell therapy for myocardial regeneration.

Murry: There are of course no data demonstrating superiority of any of the populations of cells you describe, because no groups have performed head-to-head comparisons. Anyone proclaiming to know that one cell type is best stands on very thin ice. For this reason it is prudent for the basic and clinical research communities to look critically for themselves and draw their own conclusions. What does seem clear is that this field will be advanced by studies that address mechanisms underlying physiological improvements with cell transplantation. These include rigorous tests of the differentiated fates assumed by stem cells, the factors they secrete, and how they interact with host myocardial and inflammatory cells. If basic scientists can accomplish these tasks in the next several years they will provide critical guideposts to physicians conducting the next generation of clinical trials.

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Biographies

Dr. Mark Sussman is a tenured Professor of Biology at San Diego State University and key investigator at the San Diego State University Heart Research Institute. His current Akt/PKB-related research involves the surprising effects of nuclear Akt/PKB signaling for cardiomyocytes including anti-hypertrophic signaling, induced downstream mediators of cell survival including Pim-1 kinase, stem cell-related signaling in the pathologically challenged myocardium, and the potentiation of survival and regenerative capacity for cardiac stem cells. Dr. Sussman's research has been supported by grant awards from the National Institutes of Health and the American Heart Association since establishing his independent laboratory in 1995. He was recognized as an Established Investigator of the National American Heart Association in 2000. Currently, Dr. Sussman serves on multiple leadership and review

committees for the American Heart Association at both affiliate and national organization levels. He has authored over 85 peer-review articles and is a popular speaker at national and international venues with over 100 invited presentations in the last 15 years. Dr. Sussman also serves as Chair of the Cardiac Contractility, Hypertrophy, and Failure (CCHF) study section for the National Institutes of Health as well as a member of editorial boards for several journals including *Circulation Research*, the *Journal of Biological Chemistry*, the *American Journal of Physiology (Heart and Circulatory Physiology)*, and the *Journal of Molecular and Cellular Cardiology*. His laboratory at San Diego State University serves as a training ground for undergraduate, masters and doctoral students, and postdoctoral trainees who are now mentored under Dr. Sussman's guidance. Recently, he has championed efforts to bring together cardiovascular researchers in the San Diego academic community with a collaborative research program project grant that pools knowledge and resources in the San Diego research community to further studies on the molecular and cellular basis of heart failure.

Charles (Chuck) Murry is Professor of Pathology and Bioengineering at the University of Washington in Seattle. Murry is Director of the Center for Cardiovascular Biology and Co-Director of the newly formed Institute for Stem Cell and Regenerative Medicine at the South Lake Union research complex. He obtained his Ph.D. and M.D. from Duke University, and did a fellowship in vascular biology at the University of Washington under Stephen M. Schwartz, M.D., Ph.D. His awards include a Burroughs Wellcome Career Award in Biomedical Sciences in 1996, the Presidential Early Career Award in Science and Engineering in 2000 and 3 awards for outstanding teaching in basic sciences. The Murry Lab's research focuses on myocardial infarction and strategies to enhance the hearts response to injury. Active projects explore the

molecular mechanisms that underlie the heart's normal wound healing processes and in developing molecular and cell-based approaches to improve infarct repair, with a special emphasis on adult and pluripotent stem cells. They are a multidisciplinary group, doing basic work in molecular biology and regulation of gene expression, cell biology, tissue engineering, animal models of disease and analyses of human tissues.