Authors’ reply

Eran Kopel and colleagues wonder whether an association between the Y chromosome and coronary artery disease (CAD) identified by us in the West of Scotland Coronary Prevention Study (WOSCOPS) might have been confounded by the effect of haplogroup I on response to treatment with pravastatin. We think that this is unlikely. Neither the prevalence of those treated with pravastatin nor concentrations of LDL cholesterol (the measure of therapeutic response to statins) were significantly different between men with haplogroup I and those with all other lineages of the Y chromosome in WOSCOPS (table 2 in our paper).1 Furthermore, introduction of both LDL cholesterol and pravastatin-based treatment as independent variables into the multiple regression model had no effect on the association between haplogroup I and CAD in this cohort (supplementary table 4).1

Laure Case and colleagues provide an important evolutionary perspective to our findings. The series of studies in fruit flies and rodents clearly shows that the Y chromosome is an important regulator of immunity and a potential contributor to autoimmune diseases in experimental models.4,5 The question as to whether the effect of the Y chromosome on cardiovascular disease is mediated by its euchromatin (through protein-coding genes), its heterochromatin (through epigenetic mechanism), or a combination of both is intriguing and requires further studies.

We declare that we have no conflicts of interest.

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Cardiosphere-derived cells for heart regeneration

Raj Makkar and colleagues (March 10, p 895)1 report that cardiosphere-derived cells (CDCs) reduce scarring after myocardial infarction. Since there were no improvements in patients’ ejection fraction, it would be essential to address the following two points.

First, CDCs contain subpopulations, including mesenchymal cells. It would be interesting to know which fractions are responsible for the clinical effects, and whether the mechanisms of action are direct (differentiation into cardiomyocytes or vascular endothelial cells), indirect (cell–cell contact or paracrine effects), or both. Paracrine effects would include enhanced cardiomyogenesis or angiogenesis, suggesting that it would be crucial to define secreted cytokine profiles. On the other hand, although cardiac tissues have been known to contain cardiac fibroblasts,2 the use of cardiospheres is able to minimise fibroblast contamination, because only non-adherent cardiospheres are plated to create CDCs.3

Second, it would be interesting to examine whether CDCs from the hearts of patients with myocardial infarction are different from CDCs of healthy individuals. Not only cell-autonomous mechanisms, but also non-cell-autonomous (environmental) mechanisms might be at play in CDC infarction after myocardial infarction.

We declare that we have no conflicts of interest.


Correspondence

There are many other reported that this issue was not addressed. As skewness, one could be concerned being addressed. Since Makkar and colleagues without the presence of skewness problems with the use of these tests skewed), a test or ANOVA analysis being greater than the means. There would benefit from a better description, since there is no mention of any of the specific analyses used to obtain the p values.

The sample sizes are small for each of the groups, and many of the continuous variables have skewed distributions, as suggested by the SDs being greater than the means. There are special statistical concerns when analysing data with a small sample size and a skewed distribution. For example, the analysis shown in figure 4A has a p value of 0.02 for comparing the cardiosphere-derived cell group versus control group. If the data had a normal distribution (ie, not skewed), a t test or ANOVA analysis could be used. However, there are problems with the use of these tests without the presence of skewness being addressed. Since Makkar and colleagues did not mention the issue of skewness, one could be concerned that this issue was not addressed. There are many other reported p values that are also subject to this potential concern.

Additionally, Makkar and colleagues table includes variables that seem to differ between the groups. There was no mention of whether such differences were present or absent. Differences would need to be adjusted for in statistical analyses. If such analyses could not be done owing to the small sample size, the study limitations should mention this topic.

The addressing of these potential statistical concerns would assure the reader that the proper statistical analyses were done.

We declare that we have no conflicts of interest.

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The Article by Raj Makkar and colleagues1 has important potential. However, the statistical analyses would benefit from a better description, since there is no mention of any of the specific analyses used to obtain the p values.

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The addressing of these potential statistical concerns would assure the reader that the proper statistical analyses were done.

We declare that we have no conflicts of interest.

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Authors’ reply

Shigego Masuda points out the lack of a sizable effect on ejection fraction. The modest changes in ejection fraction in CADUCEUS (38.8% at baseline vs 41.2% at 6 months in the patients treated with cardiosphere-derived cells [CDCs]) are entirely consistent with the known relation between scar size and ejection fraction after myocardial infarction.1 Only a minor change in ejection fraction is predicted to accompany the reduction of scar size from 25.2% to 17.5% noted at 6 months.2 Despite the small effect on the ejection fraction, the striking increases in regional function seen in the zone of injury in CDC-treated patients support the notion that new working myocardium has been generated.

Heterogeneity is intrinsic to all clinically used cell types. Bone-marrow-derived mesenchymal stromal cells (MSCs) are highly heterogeneous in terms of cell surface markers or functional properties. By comparison, CDCs are relatively uniform: fewer than 25% of CDCs used in CADUCEUS express CD90, which has been argued to mark cardiac MSCs or cardiac fibroblasts, and fewer than 5% of CDCs are positive for discoidin-domain-containing receptor 2 (a marker of fibroblasts) or a smooth muscle actin (a marker of myofibroblasts). Nevertheless, we continue to investigate the roles of various subpopulations of CDCs.3 So far, we know that the c-kit+ subpopulation and the CD90+ subpopulation are both inferior in terms of functional efficacy relative to unselected CDCs, but much remains to be learned.

The available evidence overwhelmingly supports an indirect mechanism of action, with both soluble factors and contact-dependent cell-cell interactions having a role; moreover, both cardiomyogenesis and angiogenesis are affected.4 Regarding the cytokine profiles of CDCs, we know that CDCs are robust biological factories with a well balanced secretory profile compared with other cell types and subfractions.5

We have found that CDCs from diseased hearts do not have compromised regenerative capacity compared with CDCs from healthy hearts.4 Moreover, allogeneic CDCs have equivalent effectiveness to syngeneic CDCs, and, at least in preclinical models, the use of allogeneic CDCs is safe.6 Joshua Fogel and Vladimir Znamensky raise an important issue surrounding analyses of early-phase clinical trials, and one which we had highlighted and addressed in the supplementary materials published online with the CADUCEUS paper. To reiterate, small sample sizes inherent in phase 1 studies introduce the possibility of skewed data and thus violation of the assumption of normality in parametric tests. The Shapiro-Wilk test was used to test for normality of continuous data. If the normality assumption was not violated, we proceeded with parametric statistical tests (independent-samples t test,
ANOVA, paired-samples t test). When normality could not be established, non-parametric tests were used (Mann-Whitney U test, Kruskal-Wallis test, Wilcoxon test). Even when we used non-parametric tests exclusively, the reported differences in scar mass, viable mass, scar size, and regional function remained highly significant. Baseline characteristics were compared by use of Fisher’s exact test and did not differ between the CDC and control group (as expected given the rigorous randomisation). We did not prespecify any covariate adjustments based on potential differences between groups because of the limited sample size; subgroup analyses were not done.

CADUCEUS was a proof-of-concept study designed to determine whether CDCs derived from an endomyocardial biopsy were safe to administer after myocardial infarction, and to gauge efficacy in a preliminary manner. As with any phase 1 study, caution should be applied when interpreting the results. Nevertheless, our finding that administration of CDCs is safe, coupled with the evidence of efficacy in achieving therapeutic regeneration, favours progression to a phase 2 study.

The following individuals are associated with Capricer Inc: EM (as stockholder and adviser), KM (as consultant), and RRS (as employee). RM and AM declare that they have no conflicts of interest.

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1 Wu E, Ortiz JT, Tejedor P, et al. Infarct size by contrast enhanced cardiac magnetic resonance is a stronger predictor of outcomes than left ventricular ejection fraction or end-systolic volume index: prospective cohort study. Heart 2008; 94: 730–36.


The Lancet’s support for clinical trials in children and young people

In March, 2012, the European Medicines Agency (EMA) issued a call for more clinical trials to be done in children. Despite 5 years of European Union incentives to increase paediatric research, the EMA estimates that only 12% of clinical trials involve children. As a result, many medicines are still prescribed to children off licence.

Unlike many journals, The Lancet actively promotes an inclusive approach to research, stating that “We encourage researchers to enrol women and ethnic groups into clinical trials of all phases.” No encouragement is given to include children, although pharmacokinetic and pharmacodynamic variability is often greater between adults and children than between adults of different sex or ethnicity.

Decisions about clinical trial participants are clearly beyond The Lancet’s immediate control. However, extension of its policy to support clinical trials in children would be an important symbolic step towards safer and more evidence-based treatment for an often vulnerable group of patients.

I declare that I have no conflicts of interest.

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1 Sukkar E. European researchers are urged to do more trials in children, conference hears. BMJ 2012; 344: e2350.

2 The Lancet. Types of article and manuscript requirements. http://www.thelancet.com/lancet-information-for-authors/article-types-manuscript-requirements (accessed April 5, 2012).

In answer to questions about the Francis Crick Institute

The Francis Crick Institute will open in 3 years’ time in central London, UK. It will emphasise high-quality discovery research and a translational agenda that promotes advances in clinical care and creates new economic opportunities. The attracting and nurturing of early-career researchers, and support for the biomedical research endeavour throughout the UK, lie at the heart of our strategy.

Richard Horton (May 19, p 1862) suggests that the Crick cannot have a new science strategy because “there are already two separate and well advanced strategies (those of the National Institute for Medical Research [NIMR] and London Research Institute [LRI]) that have to be integrated together”. It is true that the Crick will assimilate very high quality researchers from NIMR and LRI, and will reflect the strategies of their respective funders, the Medical Research Council (MRC) and Cancer Research UK. The novelty of the Crick strategy is founded on the integration and synergy of the two institutes’ portfolios, levered by multidisciplinary and interdisciplinary research and comprehensive clinical links through the three university partners: King’s College London, Imperial College London, and UCL (University College London). The funders’ enthusiasm for the Crick project and commitment to its continued funding is a vote of confidence both in the quality of the scientists involved and in the new scientific opportunities offered. In addition to researchers from NIMR and LRI, the Crick will house around