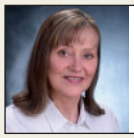
NIH stated the programme was paused was due to the 'scientific foundations of this trial'.

What happens next remains to be seen. The retraction of 31 scientific papers represents a large body of work and has implications not only for researchers going forward, but also for journals, institutions, peers and ultimately patients who all supported, funded and interacted with the trials. Researchers in the same field are reluctant to publicly discuss the case for fear of being tainted by association and the facts of the matter have not been laid bare. Full answers to the who, what, where, when, and why questions would clarify how this happened at one of the US's top institutions. Questions also arise about the peer-review and due diligence processes that allowed such a breach to occur and continue. Without clarity and transparency, those questions remain.



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References

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Clearing up the mist: cardiomyocyte renewal in human hearts

For almost 20 years, there has been much of a research-based focus towards cell replacement therapy for heart diseases. This therapeutic approach is based on an initial finding that showed that the myocardium could be regenerated by progenitor cells residing either in the heart or bone marrow. Today, we know that bone marrow cells cannot differentiate into cardiomyocytes under physiological conditions, and if they do at all, resident cardiac progenitor cells only contribute to new cardiomyocytes with an insignificant biological relevance. However, there is a body of evidence that suggests that cardiomyocytes in the mammalian heart, including the human heart, can be replaced during homeostasis and under disease conditions. Cardiomyocyte renewal has been difficult to quantify due to the biological features of cardiomyocytes as well as methodological difficulties.

First and foremost, cardiomyocyte cell cycle activity does not necessarily lead to cell duplication. Instead, premature cell cycle exit leads to multinucleation and polyploidization at different stages of development and heart growth. Therefore, markers of cell cycle activity and mitosis, such as Ki-67 and phospho-Histone H3, are not sufficient to determine whether or not a cardiomyocyte undergoes cytokinesis, particularly after cardiac infarction or in heart disease. Cardiomyocyte proliferation determined with these markers is also overestimated whenever polyploidy occurs. More recent studies have shown that the cellular localization of anillin and Aurora B kinase serves as good markers in distinguishing dividing cardiomyocytes from polyploidy, thereby allowing a more reliable quantification of myocyte proliferation.

Even having accurate molecular markers for proliferation, which provide a good view of the number of dividing cells at any given time, is insufficient to reliably estimate the number of cells that are generated or added to the tissue over years or decades. For instance, if a dividing cardiomyocyte belongs to a fast cycling sub-population, the estimated cell turnover will be overestimated when the same high renewal rate is assumed for the entire cardiomyocyte population. Similarly, the fact that apoptosis can be detected in the cardiomyocyte compartment at all ages and in heart disease has led to the conclusion that new cardiomyocytes are generated to maintain the pool of cardiomyocytes. Again, utmost care needs to be taken when estimating turnover rates based on rare events such as apoptotic myocytes. Because the duration of apoptosis in human cardiomyocytes is not exactly known, any extrapolation regarding the cell death rate per year or even decade is unreliable. Even small changes in the assumed apoptosis duration will immensely impact turnover estimates.

Moreover, cardiomyocytes are surrounded by other cell types with much higher proliferative indexes, and with cell nuclei close to the cardiomyocyte cell membrane. Thus, a prerequisite for any quantification must be to unequivocally identify and potentially isolate cardiomyocyte nuclei to assess cardiomyocyte renewal. Since genetic labelling of cells is not possible in humans, we used pericentriolar material 1 (PCM-1) as a reliable marker for adult cardiomyocyte nuclei. PCM-1 is a protein that is associated with the centrosome complex and microtubules during cell cycle, but forms perinuclear aggregates in mature cardiomyocytes. Therefore, PCM-1 can be used to easily co-localize with proliferation markers and isolate cardiomyocyte nuclei for downstream analyses.

To study cardiomyocyte turnover in humans, we developed a strategy to retrospectively determine the age of human cells. This strategy takes advantage of the dramatic increase in atmospheric radiocarbon (^{14}C) due to the above ground nuclear bomb tests conducted in the 1950s and 1960s. During DNA synthesis, dividing cells integrate ^{14}C into genomic DNA with a concentration that corresponds to the atmospheric ^{14}C at the time, thereby creating a birth mark in the DNA. By comparing the measured genomic ^{14}C concentration with documented atmospheric ^{14}C levels, the average birth date of a cell population can be inferred. Using PCM-1 to isolate cardiomyocyte nuclei and through ^{14}C dating, we showed that human cardiomyocytes renewed at a low, but detectable, rate of around 1% per year in young individuals, which decreased to values less than 0.5% per year in old individuals (*Figure 1*).

Although the annual renewal rate is low, about 40% of the cardiomyocyte population in a healthy heart is exchanged over an entire human life-span, suggesting that this renewal is necessary for the maintenance of normal heart function. To date, there are a number of studies that show comparable cardiomyocyte turnover rates in

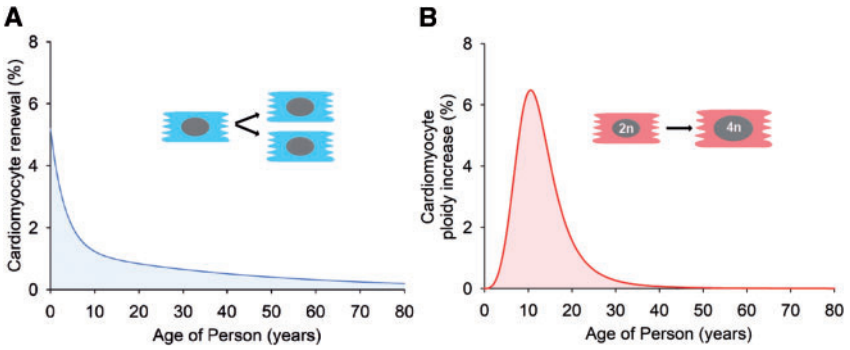

Figure 1 Cardiomyocyte renewal and ploidy in human hearts. (*A*) Cardiomyocyte renewal is highest in young hearts decreasing to values below 1% per year in adult hearts. (*B*) In neonatal hearts almost all cardiomyocyte nuclei are diploid (2n). Ploidy related DNA synthesis increases in childhood, and decreases to non-detectable levels in adulthood.

other mammals, suggesting that mammalian hearts retain the capacity to renew myocytes. Richard Lee's group took an elegant approach to labelling newly-formed adult cardiomyocytes. His team delivered a non-radioactive tracer, ^{15}N-thymidine, to mice of various ages over several weeks in order to mark cycling cardiomyocytes. This long-time infusion showed a baseline cardiomyocyte production rate of around 0.76% per year. Cardiomyocyte renewal was observed to be even higher after cardiac infarction when corrected for polyploidy and multinucleation.

The source of these new cardiomyocytes has also been debated for over a decade. Initial studies suggested stem and progenitor cells as the main source of adult-born cardiomyocytes, but recent genetic fate-mapping studies have painted a different picture. Molkentin wanted to know whether c-kit expressing cells that were attributed to become cardiac stem cells really had stem cell potential and could generate new cardiomyocytes. His team created a transgenic mouse line that allowed the selective labelling of c-kit-expressing putative stem cells and their progeny. They followed the progeny of c-kit cells during embryogenesis, perinatal period, adulthood, and after cardiac infarction by selectively inducing Cre-recombination in these cells. However, in contrast to previous studies, the rate of c-kit-derived cardiomyocyte production was found to be extremely low in all the tested conditions (<0.01% per year), indicating that c-kit cells did not contribute to new cardiomyocyte formation in a biologically meaningful manner. Moreover, most of the recombined cardiomyocytes were found to be a result of cell fusion and not of c-kit differentiating into cardiomyocytes. Although transgenic fate-mapping strategies have limitations such as recombination efficacy and heterozygosity in the modified allele, these results were replicated and confirmed by independent investigators using different transgenic lines.

Today, most studies in lower vertebrates and mammals point to pre-existing cardiomyocytes, and not cardiac stem cells, as the source of adult-born cardiomyocytes. These cardiomyocytes de-differentiate, re-enter into the cell cycle, and undergo cytokinesis to produce new myocytes. The underlying mechanism of this process has been under intensive investigation in order to promote cardiomyocyte proliferation and generate new functional myocardia. Recent studies have shown that cardiomyocyte proliferation can be regulated on several levels by directly manipulating the cell cycle, reprogramming myocytes, delivering small molecules and regulatory RNA, or using extracellular matrix proteins and secreted proteins as stimulators of proliferation.

Although the initial hype of viewing the heart as an organ harbouring a large number of stem and progenitor cells comparable to the bone marrow and gut has not held true, there is good evidence that the mammalian heart generates new cardiomyocytes throughout its life at a low annual rate. With this knowledge, it might be possible to augment this process in injured and diseased hearts to generate new myocardia, thereby improving cardiac function.

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