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Cardiac stem cells in the post-Anversa era

At the turn of the century, prevailing dogma stated that the adult mammalian heart was incapable of self-repair. Postnatal growth reflected increases in cardiomyocyte size alone rather than through increases in cell number. This dogma was shaken by the demonstration that bone marrow cells could be used to regenerate heart muscle. The subsequent discovery that adult hearts contained cells that expressed the haematological stem cell marker c-Kit led to a large body of literature, mostly from Piero Aversa's laboratory, which advanced the premise that cardiac c-Kit+ cells were clonogenic, multipotent, and capable of self-renewal (i.e. genuine heart stem cells). While this hypothesis was popularized and espoused by many, the validity of Anversa's findings were questioned early on by several investigators who failed to reproduce key findings.[1,2]

On 14 October 2018, the Harvard Medical School and Brigham and Women's Hospital brought an end to this chapter as 31 papers from the lab pioneering heart c-Kit+ cells were recommended for retraction because the validity of the scientific data was uncertain. While the full identity of the papers affected is still unknown, the *New England Journal of Medicine* promptly issued an expression of concern that the data presented in two (heretofore) landmark papers in cardiac regeneration may not be reliable[3] and outright retracted a 2011 paper demonstrating evidence for human lung c-Kit+ stem cells.[4]

On the heels of multiple corrections,[5–11] institutional settlements,[12] lawsuits,[13] and prior retractions,[14] it appears much of the literature supporting resident (*in situ*) c-Kit+ cells having any role in cardiac repair is open to question. The impact of this verdict is only now starting to be understood and has led many to question the concept of heart stem cells in the post-Anversa era.

Is the human heart capable of self-repair?

Yes. Archaeological carbon-14 dating conclusively established that half of all cardiomyocytes are renewed over an individual lifespan.[15] This 'repopulation' decreases with advanced years. For example, at 25 years old almost 1% of cardiomyocytes turn-over every year compared with only ~0.5% turnover after 75 years. Such numbers—low but definitely not zero—have been confirmed by others using complementary methods in experimental animals.[16,17]

Do resident c-Kit+ cells contribute cardiomyocytes to the heart?

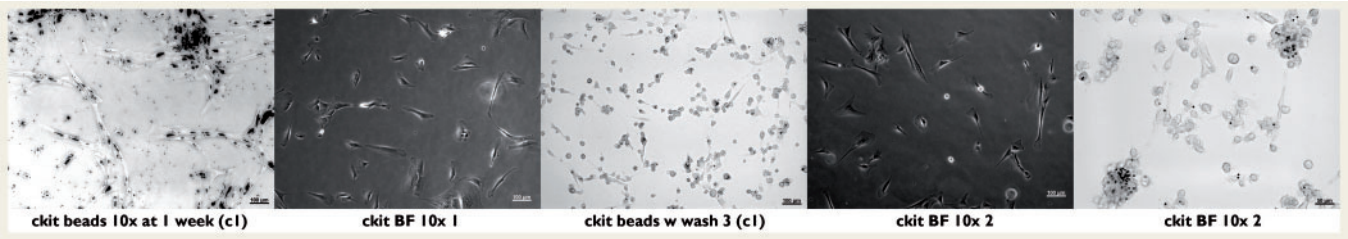
No. Reports began to emerge 10 years ago questioning the cardiomyogenic potential of c-Kit+ cells.[18–20] Recent lineage tracking from multiple labs using complimentary techniques has established that endogenous cardiac c-Kit+ cells do not generate cardiomyocytes.[21–23]

Do resident heart stem cells exist?

Probably not. Early reports panned through tissue lysate and heart sections for cells expressing embryonic or haematological stem markers in hopes of identifying cells that could be enticed to express cardiac markers in culture. In the absence of lineage tracking, the origin of the cells discovered is uncertain and very well may represent extra-cardiac contamination. It follows that cardio myogenesis seen before or after injury likely arises from myocardial de-differentiation only.[24] Although cardiosphere-derived cells (CDCs) are clonogenic and multipotent *in vitro*,[25] they have long been recognized not to function as cardiac progenitors after transplantation *in vivo*.[26]

What are heart-derived cell therapeutics?

In 2004, Messina *et al.* demonstrated a mixed population of CD105+ CD45-cells, explant-derived cells that spontaneously emigrate from heart tissue plated in culture.[27] Forensic analysis showed these cells are intrinsically cardiac with no detectable seeding from extra-cardiac organs.[28] To enable cell expansion to clinical 'doses', explant-derived cells have been antigenically selected or sphere cultured to generate c-Kit+ cells or CDCs, respectively (see *Figure 1*). Independent labs have shown that both c-Kit+ cells (6 labs) or CDCs (45+ labs) improve heart function when delivered after injury. Unfortunately, studies providing direct comparisons between either cell type are often difficult to interpret as divergent cell culture methods or patient comorbidities influence cell potency; however, within CDCs, the small c-Kit+ cell fraction does not contribute to and is not necessary for, the observed gains in function.[29]



What do we know about heart c-Kit+ cells?

Not as much as we thought! *Ex vivo* expanded c-Kit+ cells were inspired by the Anversa literature and it was thought, until recently, that robust cell numbers persisted for many years after intramyocardial injection.[30] The *in situ* c-Kit+ cell findings, which largely emanated from the well-funded Anversa lab, were directly extended to *ex vivo*

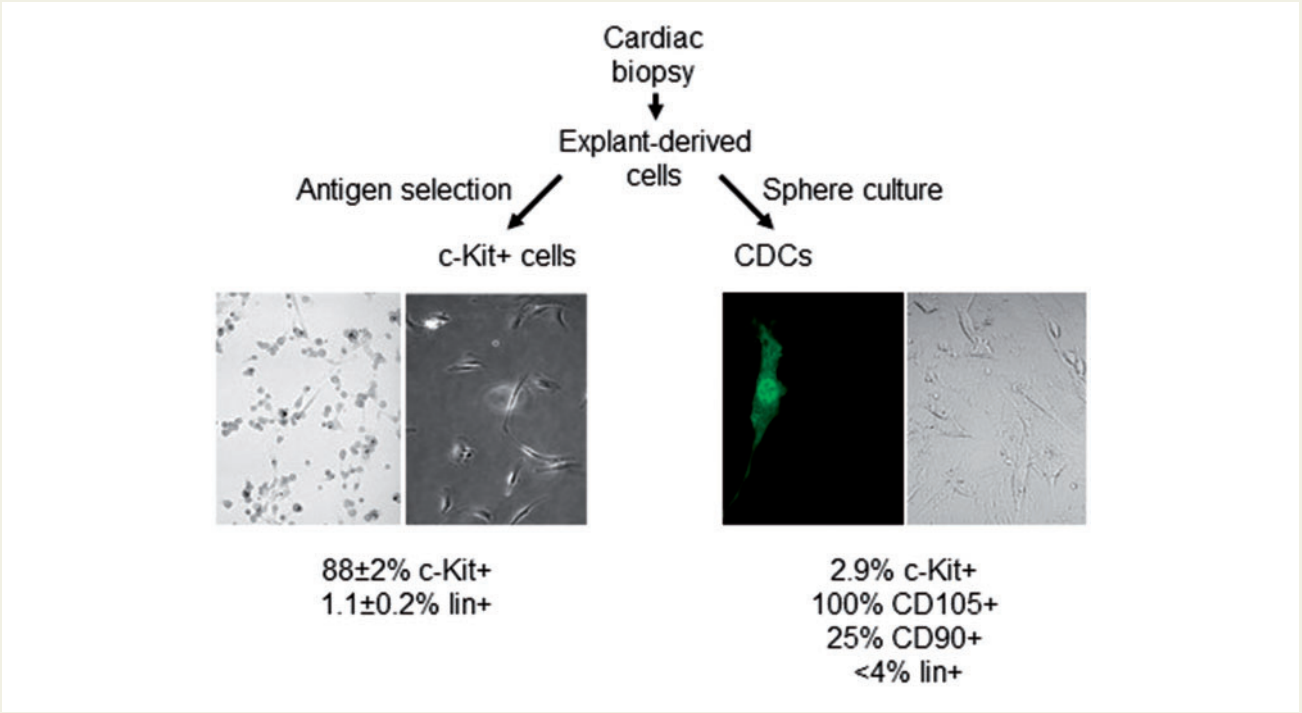


Figure 1 Schematic outline of heart-derived cell therapeutic manufacturing and identity. Explant-derived cells are cultured from myocardial tissue for antigenic selection (c-Kit+ cells, left panels) or sphere culture (CDCs, right panels) prior to expansion. Representative c-Kit+ cell images demonstrate freshly isolated human c-Kit+ cells (left panel, black dots, beads from magnetic-activated cell sorting) and during cell expansion (right panel, low confluence to highlight cell morphology). Representative images of CDCs cultured from transgenic mouse tissue expressing the c-Kit reporter (green fluorescent protein)[18] highlighting the proportion of c-Kit+ cells within. Also shown is flow cytometry characterization from the SCIPIO (c-Kit+ cell trial, left panel)[35] and CADUCEUS (CDC trial, right panel)[41] trials contrasting the antigenic identity of each heart-derived cell therapeutic used in clinical trials.

expanded c-Kit+ cells. Since then, it has been concretely established that few transplanted cells engraft beyond a few days.[31] This surprising observation revealed that c-Kit+ cells were evanescent, and thus not functioning as stem cells.

This realization came very late for c-Kit+ cells, unlike CDCs, which have been known for >10 years to be effective despite little persistence of injected cells beyond 4 weeks (i.e. 2–3% of the initial injectate).[32,33] Fortunately, the CDC literature provides a clear template for these investigations with several articles listing comprehensive proteomic analysis, cytokine over-expression/subtraction data supporting causation, exosome profiling data and microRNA addition/subtraction data supporting a causative role in post infarct repair.[34]

Although very late in the game, a great deal of the basic phenotyping work is not yet known about c-Kit+ cells; including the fundamental differences between heart-derived and extra-cardiac c-Kit+ cells. It may be that c-Kit+ cells stimulate many of the immunomodulatory (macrophage polarization) and trophic (angiogenic, anti-apoptotic, mitotic and anti-scarring) endogenous repair mechanisms already identified in the CDC literature but much waits to be uncovered.

Are c-Kit+ cells dead?

Reports of their death have been greatly exaggerated. The 2011 Phase 1 SCIPIO Trial demonstrated intra-coronary injection of c-Kit+ cells was safe and provided encouraging hints of efficacy as shown by increases in cardiac ejection fraction, New York Heart Association (NYHA) class and viable myocardium.[35] But the subsequent 2014 expression of concern by *The Lancet*[36] reflected cell product characterization, identity and manufacturing which were both done in Boston by Dr Anversa's team.[37] The impact of recent events on interpretation of the SCIPIO Trial is still not known but may emerge as the journals affected by the list of articles recommended for retraction receive more information.

The CONCERT HF Trial (ClinicalTrials.gov Identifier: NCT02501811) began in 2015 to explore the effects of combining heart-derived c-Kit+ cells with blood mesenchymal stem cells on post infarct repair.[38] This trial was based upon two preclinical studies suggesting combined therapy increases transplanted cell engraftment to enhance cell treatment outcomes.[39,40] With the Harvard c-Kit+ cell retractions, the NIHBLI paused the trial on 29 October 2018 to provide the Data and Safety Monitoring Board (DSMB) an opportunity to review the literature supporting the scientific foundations of the trial. Given the invasive nature of the trial (and the observation that a patient died during endomyocardial biopsy), this caution is appreciated to ensure that sufficient pre-clinical insight and clinical equipoise still exist in the new post-Anversa era.

At best, the future of heart c-Kit+ cells is uncertain. With the astounding number of key publications likely to be retracted, it may

very well be that adult c-Kit+ cells are not fundamentally different enough from other heart-derived cells to warrant efforts exploring clinical efficacy beyond the multiple clinical trials completed or underway using CDCs or the CDC secretome.

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A Time for Reflection and Reassurance

American cardiologist Jonathan A Epstein MD considers the causes and consequences of the Harvard University investigation into scientific misconduct

In late November 2018, Jonathan Epstein, Professor of Medicine at the Perelman School of Medicine, University of Pennsylvania, wrote a definitive article in *JAMA* calling for a complete rethink on cardiac stem cell biology following the retraction of 31 papers linked to the Harvard Medical School (HMS) laboratory headed by Piero Anversa. Although the dust has not yet settled and further fall-out from the Anversa case is likely, Epstein suggests it is incumbent on the scientific community to stand up and take account of events going forward.

As a founding co-director of the Penn Institute for Regenerative Medicine and a senior researcher with interests in the molecular mechanisms of cardiovascular development, Epstein is well-placed to understand the background to the unravelling of Anversa's professional reputation. He describes the chain of events which led to Harvard's public retraction of the papers in late 2018 as a 'catastrophe' that went on for too long and consumed a large amount of public money in funding. 'If Anversa's basic premise on cardiac stem cells turns out to be predominantly fraudulent, it resets the entire field which had focused, perhaps inappropriately, to a very large extent on the existence of these cells for therapeutic purposes'.

The processes that led to the fabricated data gaining ground remain unaccounted for. Epstein says: 'We still don't have all the necessary information to fully evaluate how best to avoid a catastrophe like this again. We don't currently know all the papers which have been identified as containing potentially fraudulent information, we don't know the details of the settlement with the National Institutes of Health (NIH), and therefore, what grant application materials might be untrustworthy. Information regarding the many steps involved would be useful for a full review'. Against this environment of uncertainty, the institutions and organizations involved or involved by default, in the Anversa case need to establish whether warning signs were ignored or whether existing internal checks and balances were insufficient. 'This not only relates to the Brigham and HMS, but also to the NIH and the other foundations and funding agencies that supported the work, and to the journals where the work was published'.

Epstein believes that the process of peer review in major scientific journals remains broadly effective and operates in a fair and balanced way. Although peer review plays a useful role in the vetting of good quality science, he suggests it is very difficult to catch out a 'determined cheater', and it is not appropriate to rely on peer review to oust such an individual. 'I think it's worth reflecting on whether the community of reviewers and editors who participated in these particular papers was broad enough to avoid potential conflicts and whether all voices were listened to equally. That much of the work was published in the flagship cardiovascular journals of the American Heart Association is a cause for concern, as is the fact that in many cases the co-authors of the discredited papers were also in editorial control of the publications'.

Although safeguards exist at different levels across the scientific, academic and publishing spheres, these should be evaluated for robustness. In relation to the HMS laboratory implicated in the research, it should be noted that concerns were raised about the way it was run. He says: 'This is thought to have precluded certain members of staff from participating in the interpreting of data or in potentially being aware of potential lack of transparency in how the results were handled throughout the process, and that's an area where institutions can look to make sure that trainees, postdocs and staff in the labs have the opportunity to speak up about practices that may not be perfectly appropriate'. Those working in related fields would now benefit from knowing that appropriate safeguards have been adopted and that academia is broadly committed to strengthening them.

The media frenzy that erupted when the retractions were first made public sparked off headlines around the world. The widespread public media coverage, however, should be viewed as proportionate to the enormity and rarity of this event which is perhaps a once-in-a-generation deviation from the norm of good practice. It is not, Epstein suggests, a cause for concern about the state of biomedicine or the possibility of similar events occurring in future. 'My belief is that the vast majority of publicly funded research that is undertaken in this country is performed with decency and honesty and every good intention, and that the checks and balances in place do suffice to maintain integrity and excellence and we can be reassured by the high quality of research that we do'.