# Science

*Adult Stem Cells Heal Hearts*

For over a decade, researchers have used adult stem cells from various tissue sources, including bone marrow, in attempts to repair damage caused by acute and chronic heart disease. However, some trials have reported equivocal results because of their small samples and diverse methodologies, which has led to divergent opinions on the efficacy and mechanism of action of stem cells. Meta-analyses, including a recent review by Anweshan Samanta and colleagues, indicate that the evidence does support the efficacy of stem cell treatments to improve the parameters of heart health and function as well as overall patient outcomes.[1] The predominant view is that stem cells show significant potential for healing hearts, and future trials will define the best routes of administration, doses, and cell sources to optimize the effectiveness of specific therapies.

Jorge Bartolucci and colleagues have contributed additional evidence of the efficacy of adult stem cells from an infrequently used source: umbilical cord blood.[2] The stem cells in umbilical cord blood, like those in umbilical cord tissue, are similar

1. Anweshan Samanta et al., "Bone Marrow Cells for Heart Repair: Clinical Evidence and Perspectives," *Minerva Cardioangiologica* 65.3 (June 2017): 299–313, doi: 10.23736/S0026-4725.16.04265-1. See also Muhammad R. Afzal et al., "Adult Bone Marrow Cell Therapy for Ischemic Heart Disease: Evidence and Insights from Randomized Controlled Trials," *Circulation Research* 117.6 (August 28, 2015): 558–575, doi: 10.1161/CIRCRESAHA.114.304792; and Vinodh Jeevanantham et al., "Adult Bone Marrow Cell Therapy Improves Survival and Induces Long-Term Improvement in Cardiac Parameters: A Systematic Review and Meta-Analysis," *Circulation* 126.5 (July 31, 2012): 551–568, doi: 10.1161/CIRCULATIONAHA.111.086074.

2. Jorge G. Bartolucci et al., "Safety and Efficacy of the Intravenous Infusion of Umbilical Cord Mesenchymal Stem Cells in Patients with Heart Failure: A Phase 1/2 Randomized Controlled Trial (RIMECARD Trial)," *Circulation Research*, e-pub September 26, 2017, doi: 10.1161/CIRCRESAHA.117.310712.

to those found in bone marrow but are present in higher concentrations. Moreover, cord-blood stem cells are more accessible than those derived from bone marrow, raise none of the ethical concerns of embryonic stem cells, and are unlikely to cause an autoimmune response. In the first clinical trial that used umbilical-cord-derived mesenchymal stem cells (MSCs) to treat patients with heart failure, fifteen patients received a single infusion of the MSCs, and fifteen patients received a placebo and standard heart care. Patients' heart function and health were assessed three months, six months, and twelve months after treatment, and all patients in the intervention group showed significant improvements in heart function (measured as the left ventricular ejection fraction), overall health, and quality of life. The study indicates once again that adult stem cells can positively affect heart repair and function, and it validates another source of stem cells.

*Cord-Blood Stem Cells Are Safe for Autism*

The precise cause of some neurological conditions, including autism spectrum disorder, remains unclear, but some evidence suggests that inflammation plays a role in these disorders. Given that stem cells can suppress inflammation, Geraldine Dawson and colleagues hypothesized that cord-blood stem cells can ameliorate autism symptoms. In a phase 1 FDA-approved clinical trial, researchers reinfused autologous cord-blood stem cells into twenty-five children (approximately two to six years old) who had been diagnosed with autism.[3] The children were followed for twelve months post-infusion to measure health and behavioral indicators. The authors determined that the infusion of autologous cord-blood stem cells is safe for children and significantly improves behavior across a wide range of outcome measures. The authors caution that these results are preliminary and come from a small, open-label study, but the positive indications from this initial investigation could provide guidance for larger, controlled clinical trials.

*Resetting the Immune System in Type 1 Diabetes*

Type 1, or juvenile, diabetes is an autoimmune disease that occurs when T lymphocyte white blood cells (T cells) destroy insulin-producing beta cells. The resultant insulin deficiency causes the hyperglycemic characteristic of the disease. Autologous adult stem cell transplantation is a potential treatment for autoimmune disease that could enable the pancreas to restore its beta cells, thereby ameliorating the hyperglycemic symptoms.[4] Initial reports from an international team led by Julio Voltarelli and Richard Burt show that high-dose immunosuppression followed by transplantation of autologous bone marrow stem cells sustained existing pancreatic

3. Geraldine Dawson et al., "Autologous Cord Blood Infusions Are Safe and Feasible in Young Children with Autism Spectrum Disorder: Results of a Single-Center Phase I Open-Label Trial," *Stem Cells Translational Medicine* 6.5 (May 2017): 1332–1339, doi: 10.1002/sctm.16-0474.

4. Ismé de Kleer et al., "Autologous Stem Cell Transplantation for Autoimmunity Induces Immunologic Self-Tolerance by Reprogramming Autoreactive T Cells and Restoring the $CD4^{+}CD25^{+}$ Immune Regulatory Network," *Blood* 107.4 (February 15, 2006): 1696–1702, doi: 10.1182/blood-2005-07-2800.

beta cells and restored normal glucose responsiveness in patients recently diagnosed with type 1 diabetes.[5] Kelen Malmegrim and colleagues have provided additional long-term evidence of the effectiveness of this technique in treating type 1 diabetes. This research has also contributed to the development of patient parameters for use in future clinical trials.[6] Twenty-one patients with type 1 diabetes achieved insulin independence and normoglycemic response after immunosuppression and transplantation. Patients were assessed every six months for up to eight years and ten months, with the longest remission lasting eight years and four months. Retrospectively, the researchers found that patient response could be divided into a short or prolonged remission response. In the prolonged-remission group, the levels of T regulatory cells increased significantly and remained elevated, while the levels of autoreactive T cells remained low. These data suggest that a key component of extended remission is the elicitation of an increased response in T regulatory cells, which can maintain the balance of the immune system and prevent autoimmunity reactions. Future trials will need to consider how to achieve this balance in more precise ways.

*Using iPSCs to Find Treatments for Mitochondrial Disease*

Mutations in mitochondrial DNA can lead to severely disabling, even lethal, disorders, many of which cause neurological degeneration.[7] My summer 2017 column discussed the genetic manipulation and creation of human embryos as well as the birth of the first three-parent baby in attempts to bypass mitochondrial mutations. It is telling that most of the existing techniques concentrate on creating human beings who do not carry the genetic mutations rather than on treating people who are living with mitochondrial disease. In one notable exception, Carmen Lorenz and colleagues have shown that human induced pluripotent stem cells can faithfully reproduce mitochondrial DNA syndromes in culture and thus can serve as model systems for discovering drug treatments.[8] Using iPSCs derived from patients who carry deleterious mitochondrial mutations, researchers generated neural progenitor cells which showed neural activity and faithfully modeled the physiological impairments caused by the mutation. Screening of FDA-approved drugs revealed a compound with the potential to partially restore the impaired functions of neural progenitors and

5. Julio C. Voltarelli et al., "Autologous Nonmyeloablative Hematopoietic Stem Cell Transplantation in Newly Diagnosed Type 1 Diabetes Mellitus," *JAMA* 297.14 (April 11, 2007): 1568–1576, doi: 10.1001/jama.297.14.1568; and Carlos E. B. Couri et al., "C-Peptide Levels and Insulin Independence Following Autologous Nonmyeloablative Hematopoietic Stem Cell Transplantation in Newly Diagnosed Type 1 Diabetes Mellitus," *JAMA* 301.15 (April 15, 2009): 1573–1579, doi: 10.1001/ jama.2009.470.

6. Kelen C. R. Malmegrim et al., "Immunological Balance Is Associated with Clinical Outcome after Autologous Hematopoietic Stem Cell Transplantation in Type 1 Diabetes," *Frontiers in Immunology* 8 (February 22, 2017), 167, doi: 10.3389/fimmu.2017.00167.

7. Valerio Carelli and David C. Chan, "Mitochondrial DNA: Impacting Central and Peripheral Nervous Systems," *Neuron* 84.6 (December 17, 2014): 1126–1142, doi: 10.1016/j .neuron.2014.11.022.

8. Carmen Lorenz et al., "Human iPSC-Derived Neural Progenitors Are an Effective Drug Discovery Model for Neurological mtDNA Disorders," *Cell Stem Cell* 20.5 (May 4, 2017): 659–674.e9, doi: 10.1016/j.stem.2016.12.013.

differentiated neurons. Additional work is needed to validate both the screening model and the targeted drugs, but the ability to use iPSCs as model systems provides a path forward toward developing treatments for individuals with mitochondrial diseases.

*Embryonic Stem Cells Accumulate Mutations*

One of the supposed advantages of embryonic stem cells is that they continue to proliferate almost indefinitely in culture. This continuous growth makes it easier for scientists to work with them in the laboratory, replicate experiments, and create large numbers of cells. But this rapid, continued growth resembles the growth of cancerous cells in culture, suggesting that the so-called advantage might actually hinder possible clinical applications and studies involving the normal proliferation and function of cells. Florian Merkle and colleagues sequenced the protein-coding genes of one hundred forty human embryonic stem cell lines and identified a number of lines that carried mutations in the important tumor suppressor P53.[9] These dominant negative mutations, most commonly seen in human cancers, confer a growth advantage to cells in culture. Such mutations are not easily screened for in embryonic stem cell lines, and point-mutation screens are not often applied, even on supposedly clinical-grade human embryonic stem cells that will be injected into patients. Previous studies have noted significant chromosomal aberrations after continued growth in culture, including trisomies and other aneuploidies, changes in copy number and mitochondrial DNA that are common in human cancers, and amplifications at specific sites on chromosome 20.[10] The current findings further highlight the practical problems, separate from the ethical breach, that plague human embryonic stem cells.

*Scaring Patients Away from Stem Cell Trials*

James Sherley asks an important question: why are some organizations and scientists trying to scare patients away from private stem cell clinics and trials?[11] He notes what seems to be a concerted effort by organizations, such as the National Institutes of Health and the International Society for Stem Cell Research, bloggers,

9. Florian T. Merkle et al., "Human Pluripotent Stem Cells Recurrently Acquire and Expand Dominant Negative P53 Mutations," *Nature* 545.7653 (May 11, 2017): 229–233, doi: 10.1038/nature22312.

10. Chad A. Cowan et al., "Derivation of Embryonic Stem-Cell Lines from Human Blastocysts," *New England Journal of Medicine* 350.13 (March 25, 2004): 1353-1356, doi: 10.1056/NEJMsr040330; Jonathan S. Draper et al., "Recurrent Gain of Chromosomes 17q and 12 in Cultured Human Embryonic Stem Cells," *Nature Biotechnology* 22.1 (January 2004): 53–54, doi: 10.1038/nbt922; Anirban Maitra et al., "Genomic Alterations in Cultured Human Embryonic Stem Cells," *Nature Genetics* 37.10 (October 2005): 1099–1103, doi:10.1038/ng1631; Claudia Spits et al., "Recurrent Chromosomal Abnormalities in Human Embryonic Stem Cells," *Nature Biotechnology* 26.12 (December 2008): 1361–1363, doi: 10.1038/nbt.1510; and Louise C. Laurent et al., "Dynamic Changes in the Copy Number of Pluripotency and Cell Proliferation Genes in Human ESCs and iPSCs during Reprogramming and Time in Culture," *Cell Stem Cell* 8.1 (January 7, 2011): 106–118, doi: 10.1016/j.stem.2010.12.003.

11. James L. Sherley, "Why Are Patients Scared Away from Private Stem Cell Treatment Clinics?," *Journal of Stem Cell Research and Medicine* 2.2 (May 8, 2017): 1–2, doi: 10.15761/JSCRM.1000120.

publications, and public service announcements to steer patients away from private clinics that are performing stem cell therapies. Critics often characterize these therapies as "unproven" and hold up FDA-approved clinical trials as a gold standard. Yet as Sherley notes, FDA-approved trials are also unproven—that is the essence of a trial, to test a therapy's safety and effectiveness. In particular, both private clinics and approved trials face difficulties when determining correct doses. Often clinicians assume that viable cells at a certain dose are being injected into patients, but the assumptions are rarely validated or even checked. Likewise, patients should be informed of the source of stem cells used in any trial, as this is an important aspect of informed consent, particularly when patients are considering whether they can accept a treatment in good conscience. Sherley encourages patients to demand quality assurance and standardization and admonishes the critics of clinics or trials to focus on advising patients on the questions they should ask no matter the setting.

*Egg Donors Face Health Risks without Complete Information for Consent*

Increasingly, fertility clinics are recruiting paid egg donors, despite a profound lack of information about the consequences for these young women. Beyond the significant ethical questions regarding current practices in the fertility industry, the recruitment of healthy young donors adds an additional layer regarding unknown health risks. While data exist on the short-term risks caused by ovarian hyperstimulation, the long-term risks of high-dose hormonal stimulation have not been assessed. Jennifer Schneider and colleagues describe case studies of five women who developed breast cancer after donating their eggs, even though they tested negative for genetic links and other risk factors.[12] The absence of long-term data coupled with the real danger highlights the fact that the fertility industry exposes young women to unknown risks and therefore cannot receive free and informed consent from them.

*Sperm–Egg Binding: Molecules at the Beginning of Life*

Fertilization marks the beginning of life for sexually reproducing organisms, and new research shows the important role of species-specific molecules in sperm–egg binding. Initial contact takes place between a sperm protein and the proteins of an outer, proteinaceous envelope just outside the egg cell membrane. Isha Raj and colleagues examined the molecular structures of the binding proteins in sperm and egg.[13] Using marine mollusks as a model system, they showed that the VERL protein on the egg has three binding-domain repeats in its structure, and the middle domain (repeat 2) binds to the sperm acrosomal protein lysin in a species-specific manner. They also showed that repeat 2 is homologous to the specific sperm-binding domain found on the mammalian ZP2 protein. This indicates that the species-specific binding

12. Jennifer Schneider, Jennifer Lahl, and Wendy Kramer, "Long-Term Breast Cancer Risk Following Ovarian Stimulation in Young Egg Donors: A Call for Follow-Up, Research and Informed Consent," *Reproductive BioMedicine Online* 34.5 (May 2017): 480–485, doi: 10.1016/j.rbmo.2017.02.003.

13. Isha Raj et al., "Structural Basis of Egg Coat–Sperm Recognition at Fertilization," *Cell* 169.7 (June 15, 2017): 1315–1326.e17, doi: 10.1016/j.cell.2017.05.033.

domains are highly conserved between invertebrates and vertebrates and that these functionally equivalent proteins definitely provide the essential species-specific binding of gametes. The study also showed a potential mechanism by which sperm penetrates the egg coat. After the sperm lysin binds to the egg VERL protein, the bound lysin proteins, which are highly positively charged, repel each other. The repulsion opens an area in the fibers of the egg coat, allowing the sperm to fuse with the egg membrane.

David A. Prentice

# Science Abstracts

### *Blood*

*Ismé de Kleer et al.*, **Autologous stem cell transplantation for autoimmunity induces immunologic self-tolerance by reprogramming autoreactive T cells and restoring the $CD4^+CD25^+$ immune regulatory network**, *Blood* 107.4 (February 15, 2006): 1696–1702, doi: 10.1182/blood-2005-07-2800 • Despite a rapidly accumulating clinical experience with autologous stem cell transplantation (ASCT) as a treatment for severe refractory autoimmune disease, data on the mechanisms by which ASCT induces immune tolerance are still very scarce. In this study, it is shown that ASCT restores immunologic self-tolerance in juvenile idiopathic arthritis (JIA) via 2 mechanisms. First, ASCT induces a restoration of the frequency of FoxP3 expressing $CD4^+CD25^{bright}$ regulatory T cells (Tregs) from severely reduced numbers before ASCT to normal levels after ASCT. This recovery is due to a preferential homeostatic expansion of $CD4^+CD25^+$ Tregs during the lymphopenic phase of immune-reconstitution, as measured by Ki67 and CD44 expression, and to a renewed thymopoiesis of naive mRNA FoxP3 expressing $CD4^+CD25^+$ Tregs after ASCT. Second, using artificial antigen-presenting cells to specifically isolate self-reactive T cells, we demonstrate that ASCT induces autoimmune cells to deviate from a proinflammatory phenotype (mRNA interferon-γ [IFN-γ] and T-bet high) to a tolerant phenotype (mRNA interleukin-10 [IL-10] and GATA-3 high). These data are the first to demonstrate the qualitative immunologic changes that are responsible for the induction of immune tolerance by ASCT for JIA: the restoration of the $CD4^+CD25^+$ immune regulatory network and reprogramming of autoreactive T cells.

### *Cell*

*Isha Raj et al.*, **Structural basis of egg coat–sperm recognition at fertilization**, *Cell* 169.7 (June 15, 2017): 1315–1326.e17, doi: 10.1016/j.cell.2017.05.033 • Recognition between sperm and the egg surface marks the beginning of life in all sexually reproducing organisms. This fundamental biological event depends on the species-specific interaction between rapidly evolving counterpart molecules on the gametes. We report biochemical, crystallographic, and mutational studies of domain repeats 1–3 of invertebrate egg coat protein VERL and their interaction with cognate sperm protein lysin. VERL repeats fold like the functionally essential N-terminal repeat of mammalian sperm receptor ZP2, whose structure is also described here. Whereas sequence-divergent repeat 1 does not bind lysin, repeat 3 binds it non-species specifically via a high-affinity, largely hydrophobic interface. Due to its intermediate binding affinity, repeat 2 selectively interacts with lysin from the same species. Exposure of a highly positively charged surface of VERL-bound lysin suggests that complex formation both disrupts the organization of egg coat filaments and triggers their electrostatic repulsion, thereby opening a hole for sperm penetration and fusion.

### *Cell Stem Cell*

*Louise C. Laurent et al.*, **Dynamic changes in the copy number of pluripotency and cell proliferation genes in human ESCs and iPSCs during reprogramming and time in culture**, *Cell Stem Cell* 8.1 (January 7, 2011): 106–118, doi: 10.1016/j.stem.2010.12.003 • Genomic stability is critical for the clinical use of human embryonic and induced pluripotent stem cells. We performed high-resolution SNP (single-nucleotide polymorphism) analysis on 186 pluripotent and 119 nonpluripotent samples. We report a higher frequency of subchromosomal copy number variations in pluripotent samples compared with nonpluripotent

samples, with variations enriched in specific genomic regions. The distribution of these variations differed between hESCs and hiPSCs, characterized by large numbers of duplications found in a few hESC samples and moderate numbers of deletions distributed across many hiPSC samples. For hiPSCs, the reprogramming process was associated with deletions of tumor-suppressor genes, whereas time in culture was associated with duplications of oncogenic genes. We also observed duplications that arose during a differentiation protocol. Our results illustrate the dynamic nature of genomic abnormalities in pluripotent stem cells and the need for frequent genomic monitoring to assure phenotypic stability and clinical safety.

*Carmen Lorenz et al.*, **Human iPSC-derived neural progenitors are an effective drug discovery model for neurological mtDNA disorders**, *Cell Stem Cell* 20.5 (May 4, 2017): 659–674.e9, doi: 10.1016/j.stem.2016.12.013 • Mitochondrial DNA (mtDNA) mutations frequently cause neurological diseases. Modeling of these defects has been difficult because of the challenges associated with engineering mtDNA. We show here that neural progenitor cells (NPCs) derived from human induced pluripotent stem cells (iPSCs) retain the parental mtDNA profile and exhibit a metabolic switch toward oxidative phosphorylation. NPCs derived in this way from patients carrying a deleterious homoplasmic mutation in the mitochondrial gene MT-ATP6 (m.9185T>C) showed defective ATP production and abnormally high mitochondrial membrane potential (MMP), plus altered calcium homeostasis, which represents a potential cause of neural impairment. High-content screening of FDA-approved drugs using the MMP phenotype highlighted avanafil, which we found was able to partially rescue the calcium defect in patient NPCs and differentiated neurons. Overall, our results show that iPSC-derived NPCs provide an effective model for drug screening to target mtDNA disorders that affect the nervous system.

### *Circulation*

*Vinodh Jeevanantham et al.*, **Adult bone marrow cell therapy improves survival and induces long-term improvement in cardiac parameters: A systematic review and meta-analysis**, *Circulation* 126.5 (July 31, 2012): 551–568, doi: 10.1161/CIRCULATIONAHA.111.086074 • *Background:* Despite rapid clinical translation and widespread enthusiasm, the therapeutic benefits of adult bone marrow cell (BMC) transplantation in patients with ischemic heart disease continue to remain controversial. A synthesis of the available data is critical to appreciate and underscore the true impact of this promising approach. *Methods and results:* A total of 50 studies (enrolling 2625 patients) identified by database searches through January 2012 were included. Weighted mean differences for changes in left ventricular (LV) ejection fraction, infarct size, LV end-systolic volume, and LV end-diastolic volume were estimated with random-effects meta-analysis. Compared with control subjects, BMC-treated patients exhibited greater LV ejection fraction (3.96%; 95% confidence interval, 2.90 to 5.02; $P<0.00001$) and smaller infarct size (-4.03%, 95% confidence interval, -5.47 to -2.59; $P<0.00001$), LV end-systolic volume (-8.91 mL; 95% confidence interval, -11.57 to -6.25; $P<0.00001$), and LV end-diastolic volume (-5.23 mL; 95% confidence interval, -7.60 to -2.86; $P<0.0001$). These benefits were noted regardless of the study design (randomized controlled study versus cohort study) and the type of ischemic heart disease (acute myocardial infarction versus chronic ischemic heart disease) and persisted during long-term follow-up. Importantly, all-cause mortality, cardiac mortality, and the incidence of recurrent myocardial infarction and stent thrombosis were significantly lower in BMC-treated patients compared with control subjects. *Conclusions:* Transplantation of adult BMCs improves LV function, infarct size, and remodeling in patients with ischemic heart disease compared with standard therapy, and these benefits persist during long-term follow-up. BMC transplantation also reduces the incidence of death, recurrent myocardial infarction, and stent thrombosis in patients with ischemic heart disease.

### *Circulation Research*

*Muhammad R. Afzal et al.*, **Adult bone marrow cell therapy for ischemic heart disease:**

**evidence and insights from randomized controlled trials**, *Circ Res* 117.6 (August 28, 2015): 558–575, doi: 10.1161/CIRCRESAHA.114.304792 • *Rationale:* Notwithstanding the uncertainties about the outcomes of bone marrow cell (BMC) therapy for heart repair, further insights are critically needed to improve this promising approach. *Objective:* To delineate the true effect of BMC therapy for cardiac repair and gain insights for future trials through systematic review and meta-analysis of data from eligible randomized controlled trials (RCTs). *Methods and results:* Database searches through August 2014 identified 48 eligible randomized controlled trials (enrolling 2602 patients). Weighted mean differences for changes in left ventricular (LV) ejection fraction, infarct size, LV end-systolic volume, and LV end-diastolic volume were analyzed with random-effects meta-analysis. Compared with standard therapy, BMC transplantation improved LV ejection fraction (2.92%; 95% confidence interval, 1.91 to 3.92; P<0.00001), reduced infarct size (-2.25%; 95% confidence interval, -3.55 to -0.95; P=0.0007) and LV end-systolic volume (-6.37 mL; 95% confidence interval, -8.95 to -3.80; P<0.00001), and tended to reduce LV end-diastolic volume (-2.26 mL; 95% confidence interval, -4.59 to 0.07; P=0.06). Similar effects were noted when data were analyzed after excluding studies with discrepancies in reporting of outcomes. The benefits also persisted when cardiac catheterization was performed in control patients as well. Although imaging modalities partly influenced the outcomes, LV ejection fraction improved in BMC-treated patients when assessed by magnetic resonance imaging. Early (<48 hours) BMC injection after myocardial infarction was more effective in reducing infarct size, whereas BMC injection between 3 and 10 days proved superior toward improving systolic function. A minimum of 50 million BMCs seemed to be necessary, with limited additional benefits seen with increasing cell numbers. BMC therapy was safe and improved clinical outcomes, including all-cause mortality, recurrent myocardial infarction, ventricular arrhythmia, and cerebrovascular accident during follow-up, albeit with differences between acute myocardial infarction and chronic IHD subgroups. *Conclusions:* Transplantation of adult BMCs improves LV ejection fraction, reduces infarct size, and ameliorates remodeling in patients with ischemic heart disease. These effects are upheld in the analyses of studies using magnetic resonance imaging and also after excluding studies with discrepant reporting of outcomes. BMC transplantation may also reduce the incidence of death, recurrent myocardial infarction, ventricular arrhythmia, and cerebrovascular accident during follow-up.

*Jorge G. Bartolucci et al.*, **Safety and efficacy of the intravenous infusion of umbilical cord mesenchymal stem cells in patients with heart failure: a phase 1/2 randomized controlled trial (RIMECARD trial)**, *Circ Res* 121.10 (October 27, 2017): 1192–1204, doi: 10.1161/CIRCRESAHA.117.310712 • *Rationale:* Umbilical-cord-derived mesenchymal stem cells (UC-MSCs) are easily accessible and expanded in vitro, possess distinct properties, and improve myocardial remodeling and function in experimental models of cardiovascular disease. While bone-marrow-derived mesenchymal stem cells (BM-MSCs) have been previously assessed for their therapeutic potential in individuals with heart failure and reduced ejection fraction (HFrEF), no clinical trial has evaluated UC-MSCs in these patients. *Objective:* Evaluate the safety and efficacy of the infusion of UC-MSCs in patients with chronic stable HFrEF. *Methods and results:* HFrEF patients under optimal medical treatment were randomized to intravenous infusion of allogenic UC-MSCs (Cellistem, Cells for Cells S.A., Santiago, Chile) (1x106 cells/Kg) or placebo (n=15 per group). UC-MSCs in vitro compared with BM-MSCs displayed a 55-fold increase in the expression of hepatocyte growth factor (HGF), known to be involved in myogenesis, cell migration and immunoregulation. UC-MSC-treated patients presented no adverse events related to the cell infusion, and none of the patients tested at 0, 15 and 90 days presented alloantibodies to the UC-MSCs (n=7). Only the UC-MSC-treated group exhibited significant improvements in left ventricular ejection fraction at 3, 6

and 12 months of follow-up assessed both through transthoracic echocardiography (p=0.0167 versus baseline) and cardiac magnetic resonance imaging (p=0.025 versus baseline). Echocardiographic LVEF change from baseline to month 12 differed significantly between groups (+7.07±6.22% vs +1.85±5.60, p=0.028). In addition, at all follow-up time points, UC-MSC-treated patients displayed improvements of NYHA functional class (p=0.0167 versus baseline) and MLHFQ (p<0.05 versus baseline). At study completion, groups did not differ in mortality, heart failure admissions, arrhythmias or incident malignancy. *Conclusions:* Intravenous infusion of UC-MSC was safe in this group of patients with stable HFrEF under optimal medical treatment. Improvements in left ventricular function, functional status and quality of life were observed in patients treated with UC-MSCs.

### *Frontiers of Immunology*

*Kelen C. R. Malmegrim et al.*, **Immunological balance is associated with clinical outcome after autologous hematopoietic stem cell transplantation in type 1 diabetes**, *Front Immunol* 8 (February 22, 2017): 167, doi: 10.3389/fimmu.2017.00167 • Autologous hematopoietic stem cell transplantation (AHSCT) increases C-peptide levels and induces insulin independence in patients with type 1 diabetes. This study aimed to investigate how clinical outcomes may associate with the immunological status, especially concerning the balance between immunoregulation and autoreactivity. Twenty-one type 1 diabetes patients were monitored after AHSCT and assessed every 6 months for duration of insulin independence, C-peptide levels, frequencies of islet-specific autoreactive $CD8^+$ T cells (CTL), regulatory lymphocyte subsets, thymic function, and T-cell repertoire diversity. In median follow-up of 78 (range 15–106) months, all patients became insulin-independent, resuming insulin after median of 43 (range 6–100) months. Patients were retrospectively divided into short- or prolonged-remission groups, according to duration of insulin independence. For the entire follow-up, $CD3^+CD4^+$ T-cell numbers remained lower than baseline in both groups, whereas $CD3^+CD8^+$ T-cell levels did not change, resulting in a CD4/CD8 ratio inversion. Memory CTL comprehended most of T cells detected on long-term follow-up of patients after AHSCT. B cells reconstituted to baseline levels at 2–3 months post-AHSCT in both patient groups. In the prolonged-remission group, baseline islet-specific T-cell autoreactivity persisted after transplantation, but regulatory T cell counts increased. Patients with lower frequencies of autoreactive islet-specific T cells remained insulin-free longer and presented greater C-peptide levels than those with lower frequencies of these cells. Therefore, immune monitoring identified a subgroup of patients with superior clinical outcome of AHSCT. Our study shows that improved immunoregulation may balance autoreactivity, endorsing better metabolic outcomes in patients with lower frequencies of islet-specific T cells. Development of new strategies of AHSCT is necessary to increase frequency and function of T and B regulatory cells and decrease efficiently autoreactive islet-specific T and B memory cells in type 1 diabetes patients undergoing transplantation.

### *JAMA*

*Carlos E. B. Couri et al.*, **C-peptide levels and insulin independence following autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus**, *JAMA* 301.15 (April 15, 2009): 1573–1579, doi: 10.1001/jama.2009.470 • *Context:* In 2007, the effects of the autologous nonmyeloablative hematopoietic stem cell transplantation (HSCT) in 15 patients with type 1 diabetes mellitus (DM) were reported. Most patients became insulin free with normal levels of glycated hemoglobin $A_{1c}$ ($HbA_{1c}$) during a mean 18.8-month follow-up. To investigate if this effect was due to preservation of beta-cell mass, continued monitoring was performed of C-peptide levels after stem cell transplantation in the 15 original and 8 additional patients. *Objective:* To determine C-peptide levels after autologous nonmyeloablative HSCT in patients with newly diagnosed

type 1 DM during a longer follow-up. *Design, setting, and participants:* A prospective phase 1/2 study of 23 patients with type 1 DM (aged 13–31 years) diagnosed in the previous 6 weeks by clinical findings with hyperglycemia and confirmed by measurement of serum levels of anti-glutamic-acid decarboxylase antibodies. Enrollment was November 2003–April 2008, with follow-up until December 2008 at the Bone Marrow Transplantation Unit of the School of Medicine of Ribeirão Preto, Ribeirão Preto, Brazil. Hematopoietic stem cells were mobilized via the 2007 protocol. *Main outcome measures:* C-peptide levels were measured during the mixed-meal tolerance test, before, and at different times following HSCT. Secondary end points included morbidity and mortality from transplantation, temporal changes in exogenous insulin requirements, and serum levels of $HbA_{1c}$. *Results:* During a 7- to 58-month follow-up (mean, 29.8 months; median, 30 months), 20 patients without previous ketoacidosis and not receiving corticosteroids during the preparative regimen became insulin free. Twelve patients maintained this status for a mean 31 months (range, 14–52 months), and 8 patients relapsed and resumed insulin use at low dose (0.1–0.3 IU/kg). In the continuous insulin–independent group, $HbA_{1c}$ levels were less than 7.0% and mean (SE) area under the curve (AUC) of C-peptide levels increased significantly from 225.0 (75.2) ng/mL per 2 hours pretransplantation to 785.4 (90.3) ng/mL per 2 hours at 24 months posttransplantation ($P < .001$) and to 728.1 (144.4) ng/mL per 2 hours at 36 months ($P = .001$). In the transient insulin–independent group, mean (SE) AUC of C-peptide levels also increased from 148.9 (75.2) ng/mL per 2 hours pretransplantation to 546.8 (96.9) ng/mL per 2 hours at 36 months ($P = .001$), which was sustained at 48 months. In this group, 2 patients regained insulin independence after treatment with sitagliptin, which was associated with increase in C-peptide levels. Two patients developed bilateral nosocomial pneumonia, 3 patients developed late endocrine dysfunction, and 9 patients developed oligospermia. There was no mortality. *Conclusion:* After a mean follow-up of 29.8 months following autologous nonmyeloablative HSCT in patients with newly diagnosed type 1 DM, C-peptide levels increased significantly and the majority of patients achieved insulin independence with good glycemic control.

*Julio C. Voltarelli et al.*, **Autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus**, *JAMA* 297.14 (April 11, 2007): 1568–1576, doi: 10.1001/jama.297.14.1568 • *Context:* Type 1 diabetes mellitus (DM) results from a cell-mediated autoimmune attack against pancreatic beta cells. Previous animal and clinical studies suggest that moderate immunosuppression in newly diagnosed type 1 DM can prevent further loss of insulin production and can reduce insulin needs. *Objective:* To determine the safety and metabolic effects of high-dose immunosuppression followed by autologous nonmyeloablative hematopoietic stem cell transplantation (AHST) in newly diagnosed type 1 DM. *Design, setting, and participants:* A prospective phase 1/2 study of 15 patients with type 1 DM (aged 14–31 years) diagnosed within the previous 6 weeks by clinical findings and hyperglycemia and confirmed with positive antibodies against glutamic acid decarboxylase. Enrollment was November 2003–July 2006 with observation until February 2007 at the Bone Marrow Transplantation Unit of the School of Medicine of Ribeirão Preto, Ribeirão Preto, Brazil. Patients with previous diabetic ketoacidosis were excluded after the first patient with diabetic ketoacidosis failed to benefit from AHST. Hematopoietic stem cells were mobilized with cyclophosphamide (2.0 g/$m^2$) and granulocyte-colony-stimulating factor (10 μg/kg per day) and then collected from peripheral blood by leukapheresis and cryopreserved. The cells were injected intravenously after conditioning with cyclophosphamide (200 mg/kg) and rabbit antithymocyte globulin (4.5 mg/kg). *Main outcome measures:* Morbidity and mortality from transplantation and temporal changes in exogenous insulin requirements (daily dose and duration of usage). *Secondary end points:*

Serum levels of hemoglobin $A_{1c}$, C-peptide levels during the mixed-meal tolerance test, and anti-glutamic-acid decarboxylase antibody titers measured before and at different times following AHST. *Results:* During a 7- to 36-month follow-up (mean 18.8), 14 patients became insulin-free (1 for 35 months, 4 for at least 21 months, 7 for at least 6 months; and 2 with late response were insulin-free for 1 and 5 months, respectively). Among those, 1 patient resumed insulin use 1 year after AHST. At 6 months after AHST, mean total area under the C-peptide response curve was significantly greater than the pretreatment values, and at 12 and 24 months it did not change. Anti-glutamic-acid decarboxylase antibody levels decreased after 6 months and stabilized at 12 and 24 months. Serum levels of hemoglobin $A_{1c}$ were maintained at less than 7% in 13 of 14 patients. The only acute severe adverse effect was culture-negative bilateral pneumonia in 1 patient and late endocrine dysfunction (hypothyroidism or hypogonadism) in 2 others. There was no mortality. *Conclusions:* High-dose immuno-suppression and AHST were performed with acceptable toxicity in a small number of patients with newly diagnosed type 1 DM. With AHST, beta cell function was increased in all but 1 patient and induced prolonged insulin independence in the majority of the patients.

### *Minerva Cardioangiologica*

*Anweshan Samanta et al.*, **Bone marrow cells for heart repair: clinical evidence and perspectives**, *Minerva Cardioangiol* 65.3 (June 2017): 299–313, doi: 10.23736/S0026-4725.16.04265-1 • More than 15 years ago, bone marrow cell (BMC) therapy for cardiac repair was hailed as a highly promising and revolutionary treatment approach that was poised to benefit countless patients with ischemic heart disease (IHD) and heart failure. The ensuing years have unfortunately witnessed endless controversy not only about the mechanisms of action of cardiac repair with cell therapy, but also regarding the efficacy of such approaches. Somewhat discordant results from smaller clinical trials with diverse study designs, BMC types, routes of injection, timing after myocardial infarction (MI), and other key study variables have been less than conclusive. Because of this uncertainty regarding outcomes of BMC therapy, a large number of meta-analyses have been performed, also with dissimilar findings. Although the field continues to evolve with the emergence of data from newer and larger clinical trials with more stringent design, the overall evidence does support efficacy of BMC injection in patients with IHD with regard to improvement in cardiac parameters as well as patient outcomes. Given the limitless potential of adult stem cell therapy in general, at this juncture, a careful appraisal of the cumulative evidence is critically necessary to appreciate the true impact of BMC therapy on injured hearts. This review will discuss the clinical evidence and perspectives from trials and meta-analyses of BMC therapy in patients with IHD that have accumulated in published literature.

### *Nature*

*Florian T. Merkle et al.*, **Human pluripotent stem cells recurrently acquire and expand dominant negative P53 mutations**, *Nature* 545.7653 (May 11, 2017): 229–233, doi: 10.1038/nature22312 • Human pluripotent stem cells (hPS cells) can self-renew indefinitely, making them an attractive source for regenerative therapies. This expansion potential has been linked with the acquisition of large copy number variants that provide mutated cells with a growth advantage in culture. The nature, extent and functional effects of other acquired genome sequence mutations in cultured hPS cells are not known. Here we sequence the protein-coding genes (exomes) of 140 independent human embryonic stem cell (hES cell) lines, including 26 lines prepared for potential clinical use. We then apply computational strategies for identifying mutations present in a subset of cells in each hES cell line. Although such mosaic mutations were generally rare, we identified five unrelated hES cell lines that carried six mutations in the *TP53* gene that encodes the tumour suppressor P53. The *TP53* mutations we observed are dominant negative and are the mutations most commonly seen in human

cancers. We found that the *TP53* mutant allelic fraction increased with passage number under standard culture conditions, suggesting that the P53 mutations confer selective advantage. We then mined published RNA sequencing data from 117 hPS cell lines, and observed another nine *TP53* mutations, all resulting in coding changes in the DNA-binding domain of P53. In three lines, the allelic fraction exceeded 50%, suggesting additional selective advantage resulting from the loss of heterozygosity at the *TP53* locus. As the acquisition and expansion of cancer-associated mutations in hPS cells may go unnoticed during most applications, we suggest that careful genetic characterization of hPS cells and their differentiated derivatives be carried out before clinical use.

### *Nature Biotechnology*

*Jonathan S. Draper et al.*, **Recurrent gain of chromosomes 17q and 12 in cultured human embryonic stem cells**, *Nat Biotechnol* 22.1 (January 2004): 53–54, doi: 10.1038/nbt 922 • We have observed karyotypic changes involving the gain of chromosome 17q in three independent human embryonic stem (hES) cell lines on five independent occasions. A gain of chromosome 12 was seen occasionally. This implies that increased dosage of chromosome 17q and 12 gene(s) provides a selective advantage for the propagation of undifferentiated hES cells. These observations are instructive for the future application of hES cells in transplantation therapies in which the use of aneuploid cells could be detrimental.

*Claudia Spits et al.*, **Recurrent chromosomal abnormalities in human embryonic stem cells**, *Nat Biotechnol* 26.12 (December 2008): 1361–1363, doi: 10.1038/nbt.1510 • Cultured human embryonic stem (hES) cells have a known predisposition to aneuploidy of chromosomes 12, 17 and X. We studied 17 hES cell lines by array-based comparative genomic hybridization (aCGH) and found that the cells accumulate other recurrent chromosomal abnormalities, including amplification at 20q11.21 and a derivative chromosome 18. These genomic changes have a variable impact at the transcriptional level.

### *Nature Genetics*

*Anirban Maitra et al.*, **Genomic alterations in cultured human embryonic stem cells**, *Nat Genet* 37.10 (October 2005): 1099–1103, doi: 10.1038/ng1631 • Cultured human embryonic stem cell (hESC) lines are an invaluable resource because they provide a uniform and stable genetic system for functional analyses and therapeutic applications. Nevertheless, these dividing cells, like other cells, probably undergo spontaneous mutation at a rate of $10^{-9}$ per nucleotide. Because each mutant has only a few progeny, the overall biological properties of the cell culture are not altered unless a mutation provides a survival or growth advantage. Clonal evolution that leads to emergence of a dominant mutant genotype may potentially affect cellular phenotype as well. We assessed the genomic fidelity of paired early- and late-passage hESC lines in the course of tissue culture. Relative to early passage lines, eight of nine late-passage hESC lines had one or more genomic alterations commonly observed in human cancers, including aberrations in copy number (45%), mitochondrial DNA sequence (22%) and gene promoter methylation (90%), although the latter was essentially restricted to 2 of 14 promoters examined. The observation that hESC lines maintained in vitro develop genetic and epigenetic alterations implies that periodic monitoring of these lines will be required before they are used in in vivo applications and that some late-passage hESC lines may be unusable for therapeutic purposes.

### *Neuron*

*Valerio Carelli and David C. Chan*, **Mitochondrial DNA: impacting central and peripheral nervous systems**, *Neuron* 84.6 (December 17, 2014): 1126–1142, doi: 10.1016 /j.neuron.2014.11.022 • Because of their high-energy metabolism, neurons are strictly dependent on mitochondria, which generate cellular ATP through oxidative phosphorylation. The mitochondrial genome encodes for critical components of the oxidative phosphorylation pathway machinery, and

therefore, mutations in mitochondrial DNA (mtDNA) cause energy production defects that frequently have severe neurological manifestations. Here we review the principles of mitochondrial genetics and focus on prototypical mitochondrial diseases to illustrate how primary defects in mtDNA or secondary defects in mtDNA due to nuclear genome mutations can cause prominent neurological and multisystem features. In addition, we discuss the pathophysiological mechanisms underlying mitochondrial diseases, the cellular mechanisms that protect mitochondrial integrity, and the prospects for therapy.

### *Reproductive Biomedicine Online*

*Jennifer Schneider, Jennifer Lahl, and Wendy Kramer*, **Long-term breast cancer risk following ovarian stimulation in young egg donors: a call for follow-up, research and informed consent**, *Reprod Biomed Online* 34.5 (May 2017): 480–485, doi: 10.1016/j.rbmo.2017.02.003 • In the USA and other countries, oocyte donation is gaining increasing importance. Although sufficient data exist on procedure-associated short-term risks for oocyte donors, such as ovarian hyperstimulation syndrome, long-term follow-up studies of egg donors are lacking, and their health risks are unknown. The lack of information may be misleadingly interpreted as lack of risk. Long-term hormone replacement therapy is recognized as a risk factor for breast cancer; the breast cancer risk of ovarian stimulation for egg donors is unknown but is a possibility. This commentary describes five individual cases of egg donors who developed breast cancer (four out of five women in their 30s) despite negative genetic testing results. Additionally, we summarize available studies of breast cancer in infertile women who experienced IVF. We emphasize the need to create egg donor registries that will facilitate long-term studies on egg donors. Until this information is available, we call for more realistic explanations to egg donors about the lack of knowledge of long-term risks as well as more transparent informed consent documents.

### *Stem Cells Translational Medicine*

*Geraldine Dawson et al.*, **Autologous cord blood infusions are safe and feasible in young children with autism spectrum disorder: results of a single-center phase I open-label trial**, *Stem Cells Transl Med* 6.5 (May 2017): 1332–1339, doi: 10.1002/sctm.16-0474 • Despite advances in early diagnosis and behavioral therapies, more effective treatments for children with autism spectrum disorder (ASD) are needed. We hypothesized that umbilical cord blood–derived cell therapies may have potential in alleviating ASD symptoms by modulating inflammatory processes in the brain. Accordingly, we conducted a phase I, open-label trial to assess the safety and feasibility of a single intravenous infusion of autologous umbilical cord blood, as well as sensitivity to change in several ASD assessment tools, to determine suitable endpoints for future trials. Twenty-five children, median age 4.6 years (range 2.26–5.97), with a confirmed diagnosis of ASD and a qualified banked autologous umbilical cord blood unit, were enrolled. Children were evaluated with a battery of behavioral and functional tests immediately prior to cord blood infusion (baseline) and 6 and 12 months later. Assessment of adverse events across the 12-month period indicated that the treatment was safe and well tolerated. Significant improvements in children's behavior were observed on parent-report measures of social communication skills and autism symptoms, clinician ratings of overall autism symptom severity and degree of improvement, standardized measures of expressive vocabulary, and objective eye-tracking measures of children's attention to social stimuli, indicating that these measures may be useful endpoints in future studies. Behavioral improvements were observed during the first 6 months after infusion and were greater in children with higher baseline nonverbal intelligence quotients. These data will serve as the basis for future studies to determine the efficacy of umbilical cord blood infusions in children with ASD.