Science

Adult Stem Cells Get on Patients' Nerves

My Autumn 2016 column reviewed the recent clinical use of adult stem cells for patients who had experienced two different neurological impairments: stroke and multiple sclerosis. In a stroke, lack of oxygen damages nerves within the brain; in doing so, it produces a physiological trauma event. Multiple sclerosis, by contrast, is caused by autoimmune attack, that is, the body's immune cells go awry and attack its own tissues as if they were foreign invaders. This disease targets the ensheathing layer of cells surrounding major nerves and, similar to removing the insulation from an electric wire, results in short circuits that impede normal conductance. In those previous reports, adult stem cells showed the ability to heal damage caused by either trauma or autoimmune attack. This is especially significant because no known therapies do more than pause or slow these neurological conditions. The adult stem cell trials, however, induced repair of damage, even placing some multiple sclerosis patients into remission.

Two recent papers add to the burgeoning evidence for adult stem cells' reparative abilities in multiple sclerosis, even over the long term. Previous studies have found suppression, even remission, of neurological impairment but have not followed the patients for more than two or three years. Both of these new studies followed patients up to five years after their transplantation. In one multicenter trial, twenty-four patients with relapsing-remitting multiple sclerosis—where symptoms come and go at intermittent intervals—were treated with high-doses of chemotherapy drugs to induce immunosuppression, followed by transplantation with the patient's own (autologous) adult stem cells. After treatment, patients received no further therapies or medications. The treatment provided long-term remission in most patients: 91 percent showed no progression of disease, almost 87 percent showed no relapse, and 86 percent showed no further nerve damage on MRI examination.[1]

1. Richard A. Nash et al., "High-Dose Immunosuppressive Therapy and Autologous HCT for Relapsing-Remitting MS," *Neurology* 88.9 (February 28, 2017): 842–852, doi: 10.1212/WNL.0000000000003660.

A second paper by a larger multicenter group incorporated data from a larger pool of clinical centers and patients: twenty-five centers in thirteen countries reported results for 281 patients with multiple sclerosis, 78 percent of whom had aggressive, progressive forms of the disease.[2] Patients were followed for a median of over six years, with some patients followed up to sixteen years. Again, researchers used immunosuppression to knock out the rogue immune cells attacking the patient's body before transplanting autologous stem cells. This produced lasting remissions in most cases: overall survival at five years was 93 percent, and almost half of all patients were still in remission at the five-year mark. The results from both studies are very significant, not just because of their long-term survival rates, but because most patients are still in remission years after their transplantations and have not needed additional therapies. Again, no other multiple sclerosis therapy has put patients into remission.

Myasthenia gravis, another autoimmune disease that causes neurological impairment, destroys receptors at the junction between nerves and muscles, which reduces neuromuscular coordination by blocking the transmission of nerve impulses. Although symptoms are manageable and tend to be less severe than those of multiple sclerosis, they still can be disabling, and no known treatment leads to remission. A group from the University of Ottawa, in Canada, reports that adult stem cell transplants similar to those used to treat multiple sclerosis have shown promising results.[3] Stem cells were collected from seven patients with severe myasthenia gravis prior to intense conditioning that used chemotherapy to destroy the autoimmune cells causing the disease. Following this, the patients received autologous stem cell transplants. All of the patients showed no residual symptoms; their remissions were stable and long-lasting, ranging from 2.5 to over 12 years without symptoms; and they required no further treatments for myasthenia gravis. While this is only a small cohort of patients, the results are stunning and point to further opportunities for treating the disease.

Adult Stem Cells for Cartilage and Bone

Another example where adult stem cells are moving rapidly into the clinic is the use of adipose (fat)-derived adult stem cells for joint and cartilage replacement. An international research consortium has shown a decrease in pain and increase of function using autologous stem cells to treat osteoarthritis.[4] Osteoarthritis is the most common musculoskeletal problem seen in adults, and the inflammation and damage to cartilage and bone lead to significant pain and disability. In France and Germany, eighteen patients with severe osteoarthritis received injections of autologous

2. Paolo A. Muraro et al., "Long-Term Outcomes after Autologous Hematopoietic Stem Cell Transplantation for Multiple Sclerosis," *JAMA Neurol*ogy, February 20, 2017, e-pub, doi: 10.1001/jamaneurol.2016.5867.

3. Adam Bryant et al., "Myasthenia Gravis Treated with Autologous Hematopoietic Stem Cell Transplantation," *JAMA Neurol*ogy 73.6 (June 2016): 652–658, doi: 10.1001/jamaneurol.2016.0113.

4 Yves-Marie Pers et al., "Adipose Mesenchymal Stromal Cell-Based Therapy for Severe Osteoarthritis of the Knee: A Phase I Dose-Escalation Trial," *Stem Cells Translational Medicine* 5.7 (July 2016): 847–856, doi: 10.5966/sctm.2015-0245.

adipose-derived stem cells into the knee joint, with three different groups receiving increasing doses. Not only was the procedure shown to be safe, but the patients experienced what the researchers termed "significant improvements in pain levels and function" as compared to their original, pre-injection condition. While this was only a small trial, the results are very encouraging for using adult stem cells to treat osteoarthritis under controlled conditions.

A Swiss group has also documented the effectiveness of cell-based repairs for cartilage injuries. In a preliminary report from an ongoing study, ten patients with damaged or missing knee cartilage were treated with bioengineered cartilage grafts.[5] The study found an interesting source for the chondrocytes (cartilage-generating progenitor cells) used in the grafts: the nasal septum. The research group reasoned that the chondrocytes from this region could be grown more readily in culture and were easier to manipulate when creating the grafts. A small piece of nasal septum was removed from each patient, and the cells were dissociated to obtain chondrocytes. The cells were then grown in the laboratory and embedded in a matrix composed of extracellular components (glycosaminoglycan and type II collagen) that stimulated differentiation into cartilage. The bioengineered cartilage grafts were then placed into the articular joints to replace the patient's damaged cartilage. Patients were followed up to twenty-four months after the surgery and showed significant improvement in terms of pain, function of the joint, and quality of life. Imaging showed that the grafts were filling in the joint defects and developing normal cartilage. While these results need further validation, adult stem cells are already moving into the clinic in many locales as primary or adjunct treatments for cartilage and joint problems.

Another international collaboration set itself a more formidable goal: developing a construct that can be used for resurfacing an entire joint that has been damaged by osteoarthritis.[6] Adult stem cells were taken from adipose tissue, which could be a readily available source for any patient, and seeded into a large (22 mm diameter) matrix constructed of woven organic fibers, to which the cells readily attached. The matrix was also seeded with viral-based DNA vectors that would transduce the cells and add specific genes once they began to grow in the matrix. In this test, two genes were added: a marker gene to show whether cells had been genetically engineered, and the interleukin-1 receptor antagonist (IL-1Ra) gene, which acts as an anti-inflammatory agent to prevent degradation of the new cartilage. After growth in the laboratory for twenty-eight days, the cells had retained the shape of the matrix and were producing both new cartilage and the anti-inflammatory protein. These bioengineered cartilage patches show great promise for repairing and resurfacing an entire joint area.

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5. Marcus Mumme et al., "Nasal Chondrocyte-Based Engineered Autologous Cartilage Tissue for Repair of Articular Cartilage Defects: An Observational First-in-Human Trial," *Lancet* 388.10055 (October 22, 2016): 1985–1994, doi: 10.1016/S0140-6736(16)31658-0.

6. Franklin T. Moutos et al., "Anatomically Shaped Tissue-Engineered Cartilage with Tunable and Inducible Anticytokine Delivery for Biological Joint Resurfacing," *Proceedings of the National Academy of Sciences USA* 113.31 (August 2, 2016): E4513–E4522, doi: 10.1073/pnas.1601639113.

Gunning for New Skin Using Adult Stem Cells

More than 1.25 million burns are reported each year in the United States. For years, adult stem cells have been used as components of normal skin grafts for burns and wounds, but the results are often unsatisfactory. Now, purified adult stem cell applications are being developed to repair skin injuries. Previous reports have documented the use of a spray-on technique—termed the "skin gun"—for applying autologous stem cells to burns and surface wounds, quickly providing a sterile seal and inducing more rapid skin regrowth than is usually seen with standard grafts.[7] Recent work has verified the utility of this spray-on technique for treating burns of various origins. Jörg Gerlach's laboratory at the University of Pittsburgh has been one of the leaders in this area and recently published two papers on using sprayed-on adult stem cells to treat burns. One paper provides case studies of six patients, each of whom suffered second-degree burns from a different source: gas, chemicals, electricity, gasoline, hot water, and tar.[8] In each case, the patient's burn was treated by spraying on autologous stem cells obtained from skin and used immediately without further cell culture. For every case discussed, the sprayed-on cells resulted in healthy, cosmetically normal skin re-growth. On average, patients were discharged from the hospital in just over a week.

In its second paper, the group used skin donations from patients undergoing other procedures to calculate the amount of skin necessary to derive enough adult stem cells for spraying onto wounds.[9] Skin cells were isolated from twenty-eight patients and grown in the laboratory under standardized conditions, with cell counts taken at various intervals. The researchers also developed methods of calculating a burn's surface area. Isolated cells were sprayed onto test surfaces to determine the number of cells needed to achieve a specific amount of surface coverage for potential burns and wounds. The results were impressive. On average, they achieved a coverage ratio of 1:80, that is, one square inch of harvested skin for stem cells could cover approximately eighty times the surface area. This far exceeds the usual efficiency of traditional skin-grafting methods. Additional results and calculations suggest the ratio could potentially be expanded to 1:100, providing significant wound coverage from a minimal amount of healthy harvested skin and maximizing the benefits for burn patients in a minimum amount of treatment time. The calculations will be very useful in treating patients and decreasing their recovery time.

7. Jörg C. Gerlach et al., "Method for Autologous Single Skin Cell Isolation for Regenerative Cell Spray Transplantation with Non-Cultured Cells," *International Journal of Artificial Organs* 34.3 (March 2011): 271–279, doi: 10.5301/IJAO.2011.6508; and Jörg C. Gerlach et al., "Autologous Skin Cell Spray-Transplantation for a Deep Dermal Burn Patient in an Ambulant Treatment Room Setting," *Burns* 37.4 (June 2011): e19–e23, doi: 10.1016/j.burns.2011.01.022.

8. Roger Esteban-Vives et al., "Second-Degree Burns with Six Etiologies Treated with Autologous Noncultured Cell-Spray Grafting," *Burns* 42.7 (November 2016): e99–e106, doi: 10.1016/j.burns.2016.02.020.

9. Roger Esteban-Vives et al., "Calculations for Reproducible Autologous Skin Cell-Spray Grafting," *Burns* 42.8 (December 2016): 1756–1765, doi: 10.1016/j.burns.2016.06.013.

Dietary Preparation for Stem Cell Transplantation

Adult stem cell transplantations for blood and marrow diseases, such as anemias and leukemias, require conditioning the patient and the marrow, usually through chemotherapy or irradiation, essentially to open up a space in the marrow for transplanted cells to occupy. Such conditioning has been successful, and it is now a standard medical practice in adult stem cell transplantations for such conditions, with increasing success rates as the technique is refined. A review of data through the end of 2012 found that over one million such transplantations for blood and marrow diseases have been conducted.[10] A similar review shows that globally these applications in clinical therapy are growing rapidly.[11] Such transplantations still carry risks, especially from potentially life-threatening toxicities associated with the conditioning regimens. A collaboration between the United States and Japan has found a much gentler conditioning regimen that will work in mice. Some of the twenty normal amino acids used by the body cannot be synthesized in our own cells. These essential amino acids are supplied through our diet. It has been known for some time that depriving cells in culture of certain essential amino acids places them into a quiescent state rather than a growth state.[12] A team led by Yuki Taya found that mouse hematopoietic (blood-forming) stem cells in the bone marrow are sensitive to deprivation of the essential amino acid valine, which could be used as a non-toxic conditioning method for stem cell transplantation in these animals.[13] Feeding mice a valine-deficient diet for two weeks sufficiently conditioned them for a stem cell transplant. After the procedures, the donor cells produced 10 to 15 percent of the recipients' blood cells. Moreover, they observed none of the toxicities or complications normally seen with chemotherapy or irradiation conditioning. While this technique of essential amino acid deprivation looks very promising for bone-marrow stem-cell transplants, several facets of the method, such as increasing the transplant efficiency, still need to be worked out before it will be ready for human trials. Nonetheless, this new method of conditioning promises easier transplants ahead.

David A. Prentice

10. Alois Gratwohl et al., "One Million Haemopoietic Stem-Cell Transplants: A Retrospective Observational Study," *Lancet Haematology* 2.3 (March 2015): e91–e100, doi: 10.1016/S2352-3026(15)00028-9.

11. D. Niederwieser et al., "Hematopoietic Stem Cell Transplantation Activity Worldwide in 2012 and a SWOT Analysis of the Worldwide Network for Blood and Marrow Transplantation Group including the Global Survey," *Bone Marrow Transplantation* 51.6 (June 2016): 778–785, doi: 10.1038/bmt.2016.18.

12. See R.A. Tobey and H.A. Crissman, "Preparation of Large Quantities of Synchronized Mammalian Cells in Late G1 in the Pre-DNA Replicative Phase of the Cell Cycle," *Experimental Cell Research* 75.2 (January 1973): 460–464, doi: 10.1016/0014-4827(72)90453-3.

13. Yuki Taya et al., "Depleting Dietary Valine Permits Nonmyeloablative Mouse Hematopoietic Stem Cell Transplantation," *Science* 354.6316 (October 20, 2016): 1152–1155, doi: 10.1126/science.aag3145.

Science Abstracts

Bone Marrow Transplantation

D. Niederwieser et al., **Hematopoietic stem cell transplantation activity worldwide in 2012 and a SWOT analysis of the Worldwide Network for Blood and Marrow Transplantation Group including the global survey**, *Bone Marrow Transplant* 51.6 (June 2016): 778–785, doi: 10.1038/bmt.2016.18 • Data on 68 146 hematopoietic stem cell transplants (HSCTs) (53% autologous and 47% allogeneic) gathered by 1566 teams from 77 countries and reported through their regional transplant organizations were analyzed by main indication, donor type and stem cell source for the year 2012. With transplant rates ranging from 0.1 to 1001 per 10 million inhabitants, more HSCTs were registered from unrelated 16 433 donors than related 15 493 donors. Grafts were collected from peripheral blood (66%), bone marrow (24%; mainly non-malignant disorders) and cord blood (10%). Compared with 2006, an increase of 46% total (57% allogeneic and 38% autologous) was observed. Growth was due to an increase in reporting teams (18%) and median transplant activity/team (from 38 to 48 HSCTs/team). An increase of 167% was noted in mismatched/haploidentical family HSCT. A Strengths, Weaknesses, Opportunities, Threats (SWOT) analysis revealed the global perspective of WBMT to be its major strength and identified potential to be the key professional body for patients and authorities. The limited data collection remains its major weakness and threat. In conclusion, global HSCT grows over the years without plateauing (allogeneic > autologous) and at different rates in the four World Health Organization regions. Major increases were observed in allogeneic, haploidentical HSCT and, to a lesser extent, in cord blood transplantation.

Burns

Roger Esteban-Vives et al., **Calculations for reproducible autologous skin cell-spray grafting**, *Burns* 42.8 (December 2016): 1756–1765, doi: 10.1016/j.burns.2016.06.013 • Non-cultured, autologous cell-spray grafting is an alternative to mesh grafting for larger partial- and deep partial-thickness burn wounds. The treatment uses a suspension of isolated cells, from a patient's donor site skin tissue, and cell-spray deposition onto the wound that facilitates re-epithelialization. Existing protocols for therapeutic autologous skin cell isolation and cell-spray grafting have defined the donor site area to treatment area ratio of 1:80, substantially exceeding the coverage of conventional mesh grafting. However, ratios of 1:100 are possible by maximizing the wound treatment area with harvested cells from a given donor site skin tissue according to a given burn area. Although cell isolation methods are very well described in the literature, a rational approach addressing critical aspects of these techniques are of interest in planning clinical study protocols. We considered in an experimental study the cell yield as a function of the donor site skin tissue, the cell density for spray grafting, the liquid spray volume, the sprayed distribution area, and the percentage of surface coverage. The experimental data was then used for the development of constants and mathematical equations to give a rationale for the cell isolation and cell-spray grafting processes and in planning for clinical studies.

Roger Esteban-Vives et al., **Second-degree burns with six etiologies treated with autologous noncultured cell-spray grafting**, *Burns* 42.7 (November 2016): e99–e106, doi: 10.1016/j.burns.2016.02.020 • Partial and deep partial-thickness burn

wounds present a difficult diagnosis and prognosis that makes the planning for a conservative treatment versus mesh grafting problematic. A non-invasive treatment strategy avoiding mesh grafting is often chosen by practitioners based on their clinical and empirical evidence. However, a delayed re-epithelialization after conservative treatment may extend the patient's hospitalization period, increase the risk of infection, and lead to poor functional and aesthetic outcome. Early spray grafting, using non-cultured autologous cells, is under discussion for partial and deep partial-thickness wounds to accelerate the re-epithelialization process, reducing the healing time in the hospital and minimizing complications. To address planning for future clinical studies on this technology, suitable indications will be interesting. We present case information on severe second-degree injuries after gas, chemical, electrical, gasoline, hot water, and tar scalding burns showing one patient per indication. The treatment results with autologous non-cultured cells support rapid, uncomplicated re-epithelialization with aesthetically and functionally satisfying outcomes. Hospital stays averaged 7.6 ± 1.6 days. Early autologous cell-spray grafting does not preclude or prevent simultaneous or subsequent traditional mesh autografting when indicated on defined areas of full-thickness injury.

Jörg C. Gerlach et al., **Autologous skin cell spray-transplantation for a deep dermal burn patient in an ambulant treatment room setting**, *Burns* 37.4 (June 2011): e19–e23, doi: 10.1016/j.burns.2011.01.022 • While superficial partial-thickness burn wounds are treated conservatively and split-thickness skin grafting (STSG), with or without mesh expansion, is unequivocally indicated for full-thickness burns, the indication for STSG to a deep partial-thickness burn wound remains obscure, if not controversial. Delayed healing with its associated complications (i.e., hypertrophic scarring, contracture, infection, and unsatisfactory psycho-social adjustment) [1] must be balanced against overgrafting, particularly when the hands and face are involved [2]. Delays in deep-dermal wound healing can typically be appreciated by week 2 of conservative management. Unfortunately, this is a particularly problematic stage for traditional skin grafting. Autologous single-cell skin grafting as an alternative treatment in this situation has been described previously by Wood and co-workers [3–5]. Much like a traditional STSG, autologous skin-cell transplantation is based on removing healthy skin, at a superficial dermal depth, using a dermtome, from an on-prominent, unburned area of the body in an operative setting. Skin cell isolation is performed immediately after harvest and spray-grafting to the burn site is applied during the same operative session. In comparing skin cell spray-grafting with STSG in the deep dermal wound, Gravante et al. found skin cell grafting to be well tolerated and similar in results to STSG [6]. The advantage of a reduced donor skin area for spray-grafting, however, is associated with an on-desirable prolonged general anesthesia time required for the cell isolation. Cell spray-grafting that could be enabled in an outpatient treatment room setting without general anesthesia would present advantages.

Experimental Cell Research

R.A. Tobey and H.A. Crissman, **Preparation of large quantities of synchronized mammalian cells in late G1 in the pre-DNA replicative phase of the cell cycle**, *Exp. Cell Res*. 75.2 (January 1973): 460–464, doi: 10.1016/0014-4827(72)90453-3 • Chinese hamster cells, line CHO, can be synchronized in late G1 by growth in isoleucine-deficient medium for 30 h followed by resuspension in fresh, complete medium containing either hydroxyurea (to 10^{-3} M) or cytosine arabinoside (to 5 μg/ml) for 10 h. Cells which are then washed and resuspended in fresh, complete medium without drugs initiate DNA synthesis shortly thereafter, then commence dividing within 7 h. The technique yields large quantities of cells suitable for biochemical studies of events associated with initiation of genome replication and completion of interphase.

International Journal of Artificial Organs

Jörg C. Gerlach et al., **Method for autologous single skin cell isolation for regenerative cell spray transplantation with non-cultured cells**, *Int J Artif Organs* 34.3 (March 2011) 271–279, doi: 10.5301/IJAO.2011.6508 • *Background:* There is a therapeutic gap for patients with deep partial-thickness wounds (Grade IIb) of moderate size that were initially not treated with split- or mesh grafting to avoid over-grafting, but developed delayed wound healing around two weeks after injury—at which time grafting is typically not indicated anymore. Delayed wound healing is often associated with esthetically unsatisfactory results and sometimes functional problems. An innovative cell isolation method for cell spray transplantation at the point of care, which eliminates cell culture prior to treatment, was implemented for this population of burn patients in our center. *Methods*: Autologous skin cell spray transplantation was initiated by taking healthy skin. The dermal/epidermal layers were separated using enzymatic digestion with 40 min dispase application, followed by 15 min trypsin application for basal kerationcyte isolation, 7 min cell washing by centrifugation, followed by transferring the cells for spraying into Ringer lactate solution. The procedure was performed on site in a single session immediately following the biopsy. After sharp wound debridement, cells were immediately transplanted by deposition with a cell sprayer for even distribution of the cell suspension. *Results and conclusions*: Eight patients were treated (mean age 30.3 years, mean burn total body surface area 14%, mean Abbreviated Burn Severity Index (5 points). The mean time to complete re-epithelialization was 12.6 days. All patients exhibited wound healing with improved esthetic and functional quality. Our initial experience for the use of non-cultured cells using a two-enzyme approach with cell washing suggests shortened time for wound closure, suggesting that the method may potentially avoid longer-term complications.

JAMA Neurology

Adam Bryant et al., **Myasthenia gravis treated with autologous hematopoietic stem cell transplantation**, *JAMA Neurol* 73.6 (June 2016): 652–658, doi: 10.1001/jamaneurol.2016.0113 • *Importance:* Some patients with myasthenia gravis (MG) do not respond to conventional treatment and have severe or life-threatening symptoms. Alternate and emerging therapies have not yet proved consistently or durably effective. Autologous hematopoietic stem cell transplant (HSCT) has been effective in treating other severe autoimmune neurologic conditions and may have similar application in MG. *Objective:* To report 7 cases of severe MG treated with autologous HSCT in which consistent, durable, symptom-free, and treatment-free remission was achieved. *Design, Setting, and Participants:* This retrospective cohort study reports outcomes at The Ottawa Hospital, a large, Canadian, tertiary care referral center with expertise in neurology and HSCT, from January 1, 2001, through December 31, 2014, with a median follow-up of 40 months (range, 29-149 months). Data collection and analysis were performed from February 1 through August 31, 2015. All patients with MG treated with autologous HSCT at The Ottawa Hospital were included. All had persistent severe or life-threatening MG-related symptoms despite continued use of intensive immunosuppressive therapies. *Interventions:* Autologous hematopoietic stem cell grafts were mobilized with cyclophosphamide and granulocyte colony-stimulating factor, collected by peripheral blood leukapheresis, and purified away from contaminating lymphocytes using CD34 immunomagnetic selection. Patients were treated with intensive conditioning chemotherapy regimens to destroy the autoreactive immune system followed by graft reinfusion for blood and immune reconstitution. *Main Outcomes and Measures:* The primary outcome was MG disease activity after autologous HSCT measured by frequency of emergency department visits and hospitalizations and Myasthenia Gravis Foundation of America (MGFA)

clinical classification, MGFA therapy status, and MGFA postintervention status. Safety outcomes included all severe autologous HSCT–related complications. *Results:* Seven patients underwent autologous HSCT, 6 for MG and 1 for follicular lymphoma with coincident active MG. Mean (SD) ages at MG diagnosis and at autologous HSCT were 37 (11) and 44 (10) years, respectively. Five patients (71%) had concurrent autoimmune or lymphoproliferative illnesses related to immune dysregulation. All patients had distinct clinical and electromyographic evidence of MG (MGFA clinical classification IIIb-V). All patients achieved durable MGFA complete stable remission with no residual MG symptoms and freedom from any ongoing MG therapy (MGFA postintervention status of complete stable remission). Three patients (43%) experienced transient viral reactivations, and 1 (14%) developed a secondary autoimmune disease after autologous HSCT, all of which resolved or stabilized with treatment. There were no treatment- or MG-related deaths. *Conclusions and Relevance:* Autologous HSCT results in long-term symptom- and treatment-free remission in patients with severe MG. The application of autologous HSCT for this and other autoimmune neurologic conditions warrants prospective study.

Paolo A. Muraro et al., **Long-term outcomes after autologous hematopoietic stem cell transplantation for multiple sclerosis**, *JAMA Neurol*, e-pub February 20, 2017, doi: 10.1001/jamaneurol.2016.5867 • *Importance:* Autologous hematopoietic stem cell transplantation (AHSCT) may be effective in aggressive forms of multiple sclerosis (MS) that fail to respond to standard therapies. *Objective:* To evaluate the long-term outcomes in patients who underwent AHSCT for the treatment of MS in a large multicenter cohort. *Design, Setting, and Participants:* Data were obtained in a multicenter, observational, retrospective cohort study. Eligibility criteria were receipt of AHSCT for the treatment of MS between January 1995 and December 2006 and the availability of a prespecified minimum data set comprising the disease subtype at baseline; the Expanded Disability Status Scale (EDSS) score at baseline; information on the administered conditioning regimen and graft manipulation; and at least 1 follow-up visit or report after transplant. The last patient visit was on July 1, 2012. To avoid bias, all eligible patients were included in the analysis regardless of their duration of follow-up. Data analysis was conducted from September 1, 2014 to April 27, 2015. *Exposures:* Demographic, disease-related, and treatment-related exposures were considered variables of interest, including age, disease subtype, baseline EDSS score, number of previous disease-modifying treatments, and intensity of the conditioning regimen. *Main Outcomes and Measures:* The primary outcomes were MS progression-free survival and overall survival. The probabilities of progression-free survival and overall survival were calculated using Kaplan-Meier survival curves and multivariable Cox proportional hazards regression analysis models. *Results:* Valid data were obtained from 25 centers in 13 countries for 281 evaluable patients, with median follow-up of 6.6 years (range, 0.2-16 years). Seventy-eight percent (218 of 281) of patients had progressive forms of MS. The median EDSS score before mobilization of peripheral blood stem cells was 6.5 (range, 1.5-9). Eight deaths (2.8%; 95%CI, 1.0%-4.9%) were reported within 100 days of transplant and were considered transplant-related mortality. The 5-year probability of progression-free survival as assessed by the EDSS score was 46%(95%CI, 42%-54%), and overall survival was 93%(95%CI, 89%-96%) at 5 years. Factors associated with neurological progression after transplant were older age (hazard ratio [HR], 1.03; 95%CI, 1.00-1.05), progressive vs relapsing form of MS (HR, 2.33; 95%CI, 1.27-4.28), and more than 2 previous disease-modifying therapies (HR, 1.65; 95%CI, 1.10-2.47). Higher baseline EDSS score was associated with worse overall survival (HR, 2.03; 95%CI, 1.40-2.95). *Conclusions and Relevance:* In this observational study of patients with MS treated with AHSCT, almost half of them remained free from neurological progression

for 5 years after transplant. Younger age, relapsing form of MS, fewer prior immunotherapies, and lower baseline EDSS score were factors associated with better outcomes. The results support the rationale for further randomized clinical trials of AHSCT for the treatment of MS.

Lancet

Marcus Mumme et al., **Nasal chondrocyte-based engineered autologous cartilage tissue for repair of articular cartilage defects: an observational first-in-human trial**, *Lancet* 388.10055 (October 22, 2016): 1985–1994, doi: 10.1016/S0140-6736(16)31658-0 • *Background:* Articular cartilage injuries have poor repair capacity, leading to progressive joint damage, and cannot be restored predictably by either conventional treatments or advanced therapies based on implantation of articular chondrocytes. Compared with articular chondrocytes, chondrocytes derived from the nasal septum have superior and more reproducible capacity to generate hyaline-like cartilage tissues, with the plasticity to adapt to a joint environment. We aimed to assess whether engineered autologous nasal chondrocyte-based cartilage grafts allow safe and functional restoration of knee cartilage defects. *Methods:* In a first-in-human trial, ten patients with symptomatic, post-traumatic, full-thickness cartilage lesions (2–6 cm2) on the femoral condyle or trochlea were treated at University Hospital Basel in Switzerland. Chondrocytes isolated from a 6 mm nasal septum biopsy specimen were expanded and cultured onto collagen membranes to engineer cartilage grafts (30 × 40 × 2 mm). The engineered tissues were implanted into the femoral defects via mini-arthrotomy and assessed up to 24 months after surgery. Primary outcomes were feasibility and safety of the procedure. Secondary outcomes included self-assessed clinical scores and MRI-based estimation of morphological and compositional quality of the repair tissue. This study is registered with ClinicalTrials.gov, number NCT01605201. The study is ongoing, with an approved extension to 25 patients. *Findings:* For every patient, it was feasible to manufacture cartilaginous grafts with nasal chondrocytes embedded in an extracellular matrix rich in glycosaminoglycan and type II collagen. Engineered tissues were stable through handling with forceps and could be secured in the injured joints. No adverse reactions were recorded and self-assessed clinical scores for pain, knee function, and quality of life were improved significantly from before surgery to 24 months after surgery. Radiological assessments indicated variable degrees of defect filling and development of repair tissue approaching the composition of native cartilage. *Interpretation:* Hyaline-like cartilage tissues, engineered from autologous nasal chondrocytes, can be used clinically for repair of articular cartilage defects in the knee. Future studies are warranted to assess efficacy in large controlled trials and to investigate an extension of indications to early degenerative states or to other joints.

Lancet Haematology

Alois Gratwohl et al., **One million haemopoietic stem-cell transplants: a retrospective observational study**, *Lancet Haematol* 2.3 (March 2015): e91–e100, doi: 10.1016/S2352-3026(15)00028-9 • *Background:* The transplantation of cells, tissues, and organs has been recognised by WHO as an important medical task for its member states; however, information about how to best organise transplantation is scarce. We aimed to document the activity worldwide from the beginning of transplantation and search for region adapted indications and associations between transplant rates and macroeconomics. *Methods:* Between Jan 1, 2006, and Dec 31, 2014, the Worldwide Network for Blood and Marrow Transplantation collected data for the evolution of haemopoietic stem-cell transplantation (HSCT) activity and volunteer donors in the 194 WHO member states. *Findings:* 953,651 HSCTs (553 350 [58%] autologous and 400,301 [42%] allogeneic) were reported by 1516 transplant centres from 75 countries. No transplants were done in countries with fewer than 300,000 inhabitants, a surface area less than 700 km2, and a

gross national income per person of US$1260 or lower. Use of HSCT increased from the first transplant in 1957 to almost 10,000 by 1985. We recorded a cumulative total of about 100,000 transplants by 1995, and an estimated 1 million by December, 2012. Unrelated donor registries contributed 22.3 million typed volunteer donors and 645,646 cord blood products by 2012. Numbers of allogeneic HSCTs increased in the past 35 years with no signs of saturation (R2 = 0·989). Transplant rates were higher in countries with more resources, more transplant teams, and an unrelated donor infrastructure. *Interpretation:* Our findings show achievements and high unmet needs and give guidance for decisions; to grant access for patients, to provide a donor infrastructure, and to limit overuse by defining risk and region adapted indications for HSCT as an efficient and cost-effective approach for life-threatening, potentially curable diseases.

Neurology

Richard A. Nash et al., **High-dose immunosuppressive therapy and autologous HCT for relapsing-remitting MS**, *Neurology* 88.9 (February 28, 2017): 842–852, doi: 10.1212/WNL.0000000000003660 • *Objective:* To evaluate the safety, efficacy, and durability of multiple sclerosis (MS) disease stabilization after high-dose immunosuppressive therapy (HDIT) and autologous hematopoietic cell transplantation (HCT). *Methods:* High-dose immunosuppression and autologous transplantation for multiple sclerosis (HALT-MS) is a phase II clinical trial of HDIT/HCT for patients with relapsing-remitting (RR) MS who experienced relapses with disability progression (Expanded Disability Status Scale [EDSS] 3.0–5.5) while on MS disease-modifying therapy. The primary endpoint was event-free survival (EFS), defined as survival without death or disease activity from any one of: disability progression, relapse, or new lesions on MRI. Participants were evaluated through 5 years posttransplant. Toxicities were reported using the National Cancer Institute Common Terminology Criteria for Adverse Events (AE). *Results:* Twenty-five participants were evaluated for transplant and 24 participants underwent HDIT/HCT. Median follow-up was 62 months (range 12–72). EFS was 69.2% (90% confidence interval [CI] 50.2–82.1). Progression-free survival, clinical relapse-free survival, and MRI activity free survival were 91.3% (90% CI 74.7%–97.2%), 86.9% (90%CI 69.5%–94.7%), and 86.3% (90% CI 68.1%–94.5%), respectively. AE due to HDIT/HCT were consistent with expected toxicities and there were no significant late neurologic adverse effects noted. Improvements were noted in neurologic disability with a median change in EDSS of 20.5 (interquartile range 21.5 to 0.0; p 5 0.001) among participants who survived and completed the study. *Conclusion:* HDIT/HCT without maintenance therapy was effective for inducing long-term sustained remissions of active RRMS at 5 years.

Proceedings of the National Academy of Sciences USA

Franklin T. Moutos et al., **Anatomically shaped tissue-engineered cartilage with tunable and inducible anticytokine delivery for biological joint resurfacing**, *Proc Natl Acad Sci USA* 113.31 (August 2, 2016): E4513–E4522, doi: 10.1073/pnas.1601639113 • Biological resurfacing of entire articular surfaces represents an important but challenging strategy for treatment of cartilage degeneration that occurs in osteoarthritis. Not only does this approach require anatomically sized and functional engineered cartilage, but the inflammatory environment within an arthritic joint may also inhibit chondrogenesis and induce degradation of native and engineered cartilage. The goal of this study was to use adult stem cells to engineer anatomically shaped, functional cartilage constructs capable of tunable and inducible expression of antiinflammatory molecules, specifically IL-1 receptor antagonist (IL-1Ra). Large (22-mm-diameter) hemispherical scaffolds were fabricated from 3D woven poly(ε-caprolactone) (PCL) fibers into two different configurations and seeded with human adipose-derived stem cells (ASCs). Doxycycline (dox)-inducible lentiviral vectors containing eGFP or IL-1Ra transgenes were immobilized

to the PCL to transduce ASCs upon seeding, and constructs were cultured in chondrogenic conditions for 28 d. Constructs showed biomimetic cartilage properties and uniform tissue growth while maintaining their anatomic shape throughout culture. IL-1Ra–expressing constructs produced nearly 1 µg/mL of IL-1Ra upon controlled induction with dox. Treatment with IL-1 significantly increased matrix metalloprotease activity in the conditioned media of eGFP-expressing constructs but not in IL-1Ra–expressing constructs. Our findings show that advanced textile manufacturing combined with scaffold-mediated gene delivery can be used to tissue engineer large anatomically shaped cartilage constructs that possess controlled delivery of anticytokine therapy. Importantly, these cartilage constructs have the potential to provide mechanical functionality immediately upon implantation, as they will need to replace a majority, if not the entire joint surface to restore function.

Science

Yuki Taya et al., **Depleting dietary valine permits nonmyeloablative mouse hematopoietic stem cell transplantation**, *Science* 354.6316 (October 20, 2016): 1152–1155, doi: 10.1126/science.aag3145 • A specialized bone marrow microenvironment (niche) regulates hematopoietic stem cell (HSC) self-renewal and commitment. For successful donor-HSC engraftment, the niche must be emptied via myeloablative irradiation or chemotherapy. However, myeloablation can cause severe complications and even mortality. Here we report that the essential amino acid valine is indispensable for the proliferation and maintenance of HSCs. Both mouse and human HSCs failed to proliferate when cultured in valine-depleted conditions. In mice fed a valine-restricted diet, HSC frequency fell dramatically within 1 week. Furthermore, dietary valine restriction emptied the mouse bone marrow niche and afforded donor-HSC engraftment without chemoirradiative myeloablation. These findings indicate a critical role for valine in HSC maintenance and suggest that dietary valine restriction may reduce iatrogenic complications in HSC transplantation.

Stem Cells Translational Medicine

Yves-Marie Pers et al., **Adipose mesenchymal stromal cell-based therapy for severe osteoarthritis of the knee: a phase I dose-escalation trial**, *Stem Cells Transl Med* 5.7 (July 2016): 847–856, doi: 10.5966/sctm.2015-0245 • Osteoarthritis (OA) is the most widespread musculoskeletal disorder in adults. It leads to cartilage damage associated with subchondral bone changes and synovial inflammation, causing pain and disability. The present study aimed at evaluating the safety of a dose-escalation protocol of intra-articular injected adipose-derived stromal cells (ASCs) in patients with knee OA, as well as clinical efficacy as secondary endpoint. A bicentric, uncontrolled, open phase I clinical trial was conducted in France and Germany with regulatory agency approval for ASC expansion procedure in both countries. From April 2012 to December 2013, 18 consecutive patients with symptomatic and severe knee OA were treated with a single intra-articular injection of autologous ASCs. The study design consisted of three consecutive cohorts (six patients each) with dose escalation: low dose (2×10^6 cells), medium dose (10×10^6), and high dose (50×10^6). The primary outcome parameter was safety evaluated by recording adverse events throughout the trial, and secondary parameters were pain and function subscales of the Western Ontario and McMaster Universities Arthritis Index. After 6 months of follow-up, the procedure was found to be safe, and no serious adverse events were reported. Four patients experienced transient knee joint pain and swelling after local injection. Interestingly, patients treated with low-dose ASCs experienced significant improvements in pain levels and function compared with baseline. Our data suggest that the intra-articular injection of ASCs is a safe therapeutic alternative to treat severe knee OA patients. A placebo-controlled double-blind phase IIb study is being initiated to assess clinical and structural efficacy.