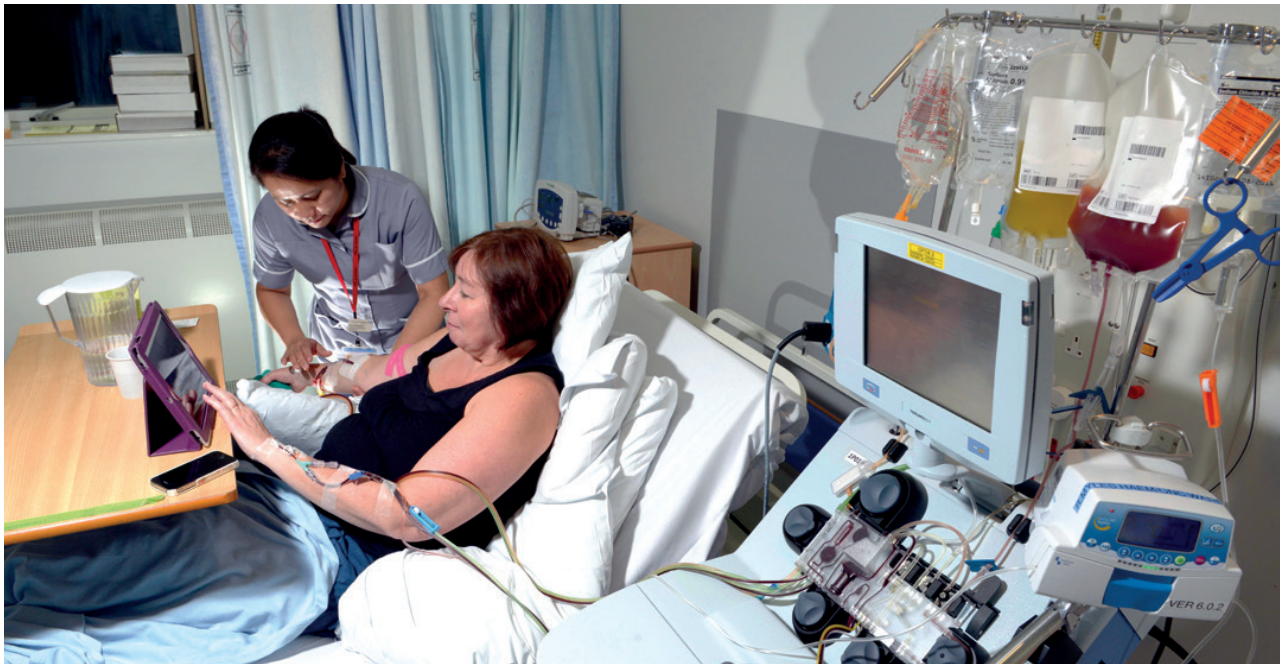

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A woman donates blood stem cells at St George's Hospital in London. Stem cells harvested here can help patients with sickle-cell disease and leukaemia.

STEM CELLS

Creating a cure-all

Stem-cell transplantation can cure sickle-cell disease, but so far this has been limited to a lucky few. That is changing fast.

BY ANDREW R. SCOTT

Like all patients with sickle-cell disease, Stephanie Alvarado-Ross had to cope with excruciating pain, anxiety and stress, and faced the likelihood of an early death. But after 20 years of suffering, that all changed in November 2013 when stem cells from her brother's bone marrow were infused into her bloodstream at the Dana-Farber/Boston Children's Cancer and Blood Disorders Center in Boston, Massachusetts. The donated cells repopulated Stephanie's bone marrow and produced healthy blood cells, delivering what will hopefully be a permanent cure. "I am only now beginning to realize what difference the transplant has made in my life," says Alvarado-Ross, who is now free of the disease. "I will no longer have to manage or worry about increasing complications or crises due to a chronic condition."

Alvarado-Ross received her life-changing transplant from a team led by Leslie Lehmann, clinical director of the centre's stem-cell transplantation programme. So far, Lehmann says, stem-cell transplant therapy, in which a patient's diseased bone marrow is killed off before being re-established with stem cells from a healthy donor's bone marrow, has cured about 600 people worldwide. But the procedure is not available to everyone[1]. It has generally been offered only to children and adolescents, because the disease causes organ damage that puts older patients at greater risk of life-threatening complications, including pulmonary hypertension, kidney failure and neurovascular problems.

Alvarado-Ross just squeaked in: at 20, she was nearly considered too old, but she was healthier than most patients of her age. She was also lucky to have a fully immunologically matched sibling donor — someone with identical human leukocyte antigens (HLAs) on the surface of their cells. Full HLA matching has been an absolute requirement for attempting stem-cell transplantation to ensure that the donor and host cells are compatible, but fewer than 10% of people with sickle-cell disease have such a donor available.

Transplantation has also involved the drastic approach of first completely destroying the patient's bone marrow — and hence their immune system — with chemotherapy and radiation, providing a clean home for the new cells. This step increases both the short- and long-term toxicity of the treatment, however, and if the donor cells fail to engraft, the patient's outlook is bleak. Other potential problems include graft-versus-host disease, in which the donated stem cells attack the recipient's cells, as well as infections and other complications. All this makes stem-cell transplantation risky, says Lehmann: there's a 90% cure rate but a 5% mortality rate.

But recent research is making the procedure more inclusive and safer. Small-scale trials of gentler techniques, which do not entirely destroy the recipient's bone narrow and use established drugs, are broadening the age range of potential recipients and increasing the pool of potential donors. It may not be long before transplants become a standard option for many more people, maybe even for fetuses in the womb.

GENTLE CONDITIONING

The first hint that a milder approach might work came from examining the blood of children whose bone marrow had been destroyed in the usual way before the transplant. In a small proportion of cases, donor cells were

found living alongside native cells that had managed to survive. Having as few as 11% of the donor cells in this 'mixed chimaera' state was sufficient to overcome the disease, and there was no evidence of graft-versus-host attack or immune rejection. The reasons for this mutual tolerance of host and donor cells remain unclear, says John Tisdale, a clinical researcher at the US National Heart, Lung, and Blood Institute in Bethesda, Maryland, and his team is investigating it. In the meantime, they have replicated the mixed-chimaera state in adults with a mild pretransplant regime that Tisdale likens to "killing weeds and replanting in their place, rather than killing off everything in a garden and starting all over again."

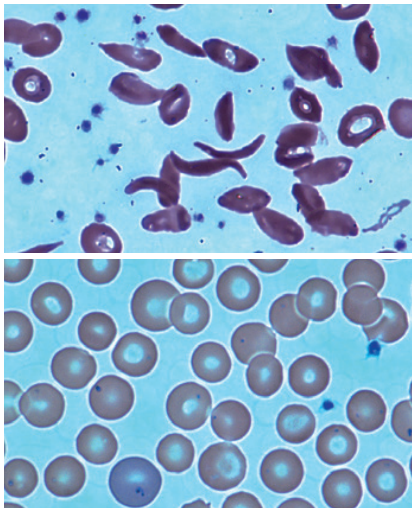
Tisdale's team replaced the combination of high-dose radiation, harsh chemotherapy and immunosuppression with a lower dose of radiation, immunosuppression with the drug rapamycin, and a novel monoclonal antibody. The antibody, called alemtuzumab, binds to and triggers the destruction of specific white blood cells in both recipient and donor that would otherwise cause either rejection or graft-versus-host disease.

In June 2014, Tisdale reported the results of a trial using this procedure[2] in sickle-cell patients between 16 and 65 years of age. Of the 30 subjects, 26 experienced successful transplants and, he says, "are effectively cured". Crucially, 15 of the patients with the highest level of engrafted donor cells were able to come off immunosuppressant drugs altogether, despite retaining a significant proportion of their original immune system, indicating that they had developed long-term immune tolerance of the graft. "This is one of our most exciting findings," Tisdale says.

DEEPENING THE DONOR POOL

Although Tisdale's work has widened the potential pool of patients by removing one of the biggest limiting factors — a patient's age — the stem-cell donations still had to come from the same small group of fully matched donors. Expanding this to half-matched donors would greatly increase the number of transplants that could be performed, as almost 90% of patients have such a donor in their immediate family.

For several years, haematologist Robert Brodsky and colleagues at Johns Hopkins University School of Medicine in Baltimore, Maryland, have been developing their own protocol that allows the use of half-matched transplants in adults, again without full destruction of the recipient's bone marrow[3]. Brodsky's procedure does not include antibody treatment but does use radiation and immunosuppressive drugs, including rapamycin. It also uses cyclophosphamide, a long-established chemotherapeutic agent. Brodsky explains that cyclophosphamide does not harm the engrafted stem cells, as these produce an enzyme called aldehyde dehydrogenase that



NIH MOLECULAR AND CLINICAL HEMATOLOGY BRANCH

Blood from a patient with sickle-cell disease before (top) and after (bottom) a stem-cell transplant.

renders them resistant to it. But the lymphocytes that cause graft-versus-host disease have very little of the enzyme and so are sensitive to the drug.

The main problem is getting the graft to take, says Brodsky, who had an initial success rate of only around 60% because of graft rejection problems. By modifying the procedure he has increased this to nearly 75%, still lower than the 87% achieved by Tisdale using fully matched donors. But the lower success rate is outweighed by a tenfold increase in the donor pool, he says, bringing a potential sickle-cell cure to many more patients.

Brodsky reports that other teams are following his lead, and says the more gentle procedure using post-transplant cyclophosphamide is spreading across the United States and overseas. But the numbers treated still remain low — in all, maybe 40 to 50 people worldwide — and an important part of getting more patients treated is educating the wider medical community. "Even in the United States, most patients are not seen in specialist centres," Brodsky says, and non-specialist doctors tend to be unaware that transplantation is becoming an option for adults.

Mickey Koh, director of stem-cell transplantation at St George's Hospital in London, is using a modified version of Tisdale's techniques. "Our first adult patient was treated in early 2011 and is now leading a normal life with no drugs required and no evidence of graft-versus-host disease," Koh reports. If such positive results continue to accumulate, he believes that stem-cell transplantation could soon become a standard treatment option for adults.

BACK TO THE WOMB

The most dramatic extension of the age range for stem-cell transplants, however, takes it all the way back to the womb, where the affected cells are replaced before birth. A team led by Alan Flake, director of the Children's Center for Fetal Research at the Children's Hospital of Philadelphia, Pennsylvania, has successfully treated sickle-cell disease *in utero* in mice and dogs[4]. He is now running a trial in monkeys, and is applying for regulatory approval for a small-scale clinical trial with human fetal patients within the next two years.

Flake and his colleagues collect stem cells from the mother and inject them into the bloodstream of the fetus using a fine needle guided by ultrasound scanning. No destruction of the fetal immune system is required because the fetal and maternal immune systems are naturally tolerant of one another. The most recent results in dogs achieved stable mixes of cells from mother and offspring, at levels that could make a therapeutic difference, in 40% of cases. More impressively, the trials also showed that the recipients were completely tolerant of the donor cells. If this can be replicated in humans, then even in cases when a graft does not take, a non-toxic follow-up transplant involving minimal bone-marrow damage could be given after birth, Flake says. The team hopes to increase the success rate for graft acceptance. But even if it remains at 40%, he says, "the only major risk of failure is that the child will still be born with sickle-cell disease, which would have happened anyway." His institution's discussions with the sickle-cell community have indicated that many women carrying an affected fetus would consider a fetal transplant if it were available.

These are all promising developments, but Brodsky and Koh raise issues that are often forgotten when sophisticated treatments are pioneered in developed nations. "Most sickle-cell patients are in Africa, where few countries have the resources to undertake these types of transplant," Brodsky says. He estimates that a transplant at his centre would cost around US$300,000 — a big upfront expense, even though it may well be recouped by avoiding the costly long-term treatment that would otherwise be required. Some people from Africa have travelled abroad for transplants, but it's an option limited to a financially privileged few. Such issues serve as a reminder that although stem-cell transplantation is broadening its reach, many sickle-cell patients cannot yet benefit. Bringing the emerging treatments to every part of the world that needs them will be an economic and political challenge as much as a scientific one. ■

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