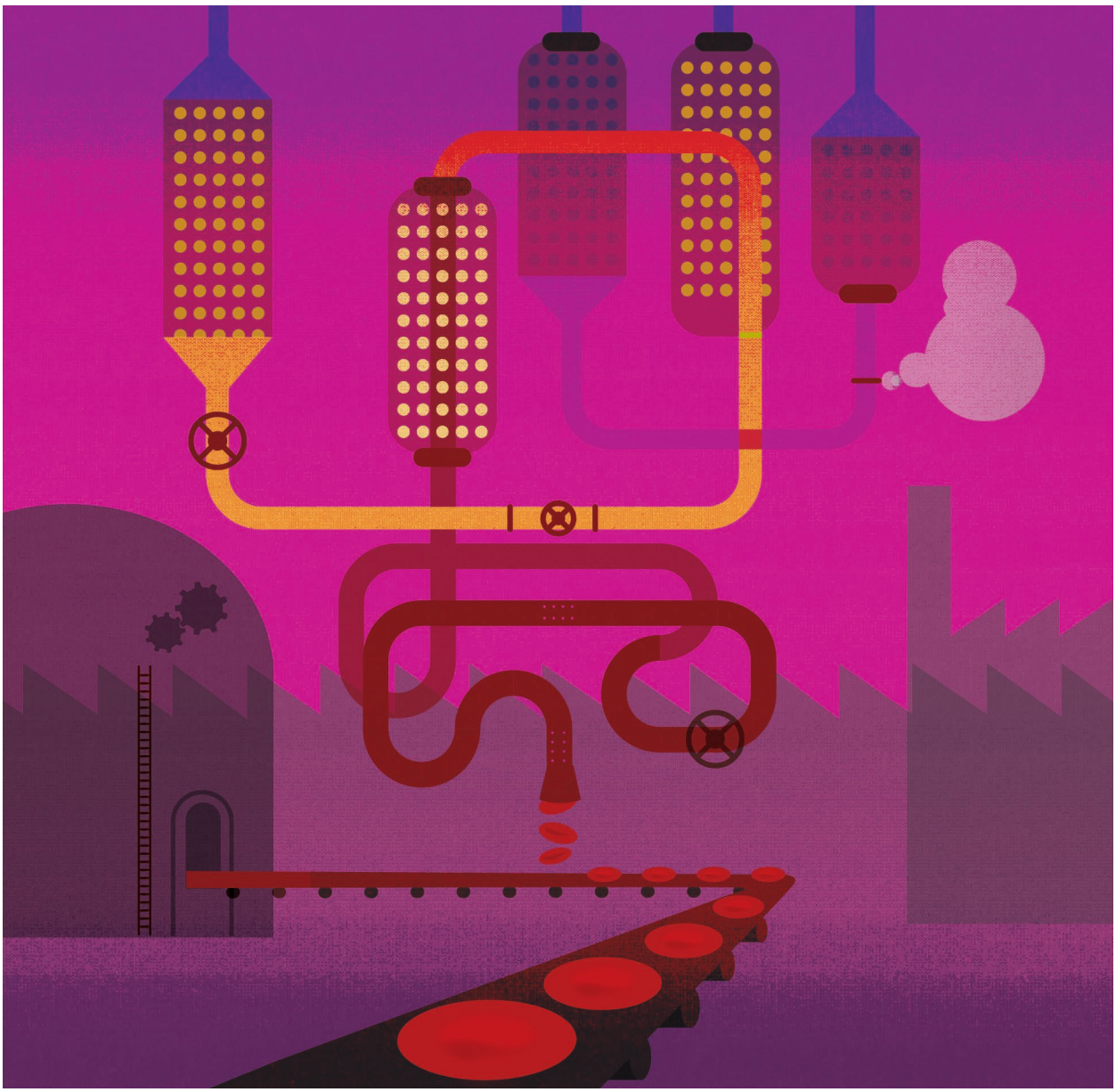
DOING WITHOUT DONORS

Stem cells and artificial substitutes could ease the dependence on blood donations.



ANDREW BAKER

BY ELIE DOLGIN

Each year, at about 13,000 collection centres worldwide, phlebotomists stick needles in the veins of healthy volunteers and amass in excess of 110 million donations of blood. The volume collected is enough to fill 20 Olympic-sized swimming pools — but it's nowhere near to meeting the medical demand for whole blood or its components. To fill the gap, an enterprising group of stem-cell biologists and bioengineers hopes to produce a safe, reliable and bottomless supply of on-demand blood substitutes in the laboratory.

According to Robert Lanza, a pioneer of stem cell therapies and head of global regenerative medicine at Astellas Pharma in Marlborough, Massachusetts, current technologies are not yet ready to compete with the real stuff. "We're not going to put blood banks out of business any time soon," he says. But in the near future, artificial blood products could be approved for use when transfusions are not otherwise an option, such as during combat or in people with a religious objection to receiving blood transfusions. And therapies that rely on reprogrammed stem cells to produce components of blood might also help transfusion centres to relieve shortages or to avoid donor-derived contamination. They might even obviate the need for patients requiring bone-marrow transplants to find immune-matched donors.

"There's going to be a huge blood crisis as the supply goes down and the demand goes up in our ageing population," says Jonathan Jahr, an anaesthesiologist with the David Geffen School of Medicine at the University of California, Los Angeles. "Many of these products could fill critical niches in special circumstances when blood is not available or not an option."

Platelets will probably be the first blood product to be made from a type of stem cell created by the genetic rewiring of adult cells — induced pluripotent stem (iPS) cells — to enter widespread clinical testing. In part, that's because of the huge medical need that could be met by these life-saving Band-Aids of the bloodstream, which are frequently given to people undergoing organ transplants, treatment for cancer or surgery to stop or prevent bleeding.

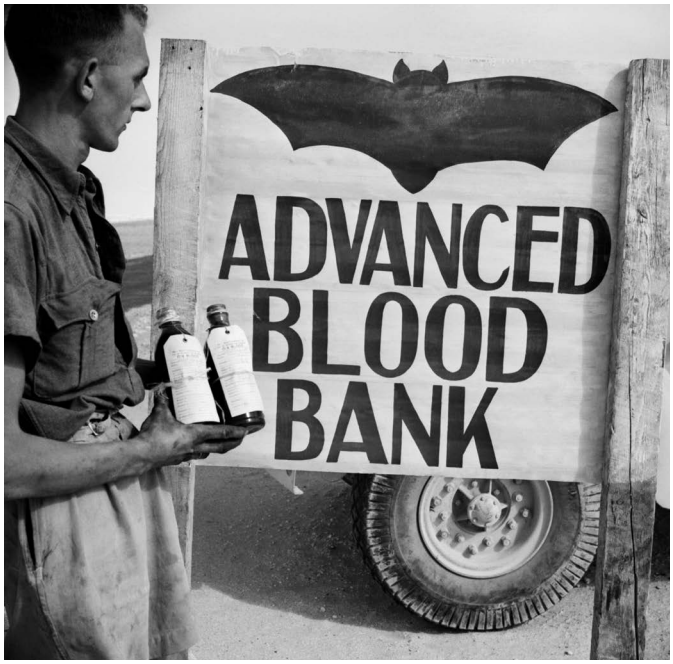
The demand for platelets is fuelled by limits on their storage. Currently, platelets must be kept at room temperature to maintain their shape and function, which means they have a shelf life of only five days. Blood banks therefore face the unfortunate situation of having to discard platelets for which the expiry date has passed; and hospitals can run out of platelets when there are unexpected spikes in demand, as might happen after a mass-casualty disaster or public-health emergency, or simply when donation rates are down.

The use of iPS cells in other settings has been stymied by safety concerns. In particular, regulators have been wary of approving clinical trials of iPS-cell derivatives for fear that the reprogramming process renders the cells carcinogenic. But those concerns can probably be put to rest with platelets derived from iPS cells, says Mortimer Poncz, a haematologist at the Children's Hospital of Philadelphia in Pennsylvania. As he points out, because platelets lack a cell nucleus, they do not contain the DNA needed for mutations and tumours to form. And if any nucleus-containing cells did happen to contaminate the platelet production line, there is a solution that does not affect platelet function. "You simply irradiate the product," Poncz says, which kills off those cells but leaves the desired platelets intact.

The absence of carcinogenicity has drawn several companies — including Megakaryon Corporation in Kyoto, Japan, and PlatOD in Paris, France — into the business of using iPS-cell technology to make replacement platelets. "It's really ideal for a first-in-man pluripotent therapy," says Lanza.

But growing platelets by the plateful is no easy feat. Scientists need to turn iPS cells into platelet 'mother' cells known as megakaryocytes, which normally live in bone marrow. Those must then be coaxed to shoot out tentacle-like projections called proplatelets, each beaded with teardrop-shaped swellings that eventually form the blood-clotting cells of interest.

Researchers in Japan, led by Hiromitsu Nakauchi and Koji Eto, the stem-cell scientists behind Megakaryon, first succeeded in deriving



SGT. CHETWYN/ IWM VIA GETTY IMAGES

A British Army blood bank in Egypt, during the Second World War.

functional platelets from human pluripotent stem cells almost a decade ago[1]. Generating megakaryocytes turned out to be straightforward, but the cells typically produced only two or three platelets each — nowhere near the thousands made by the body's own megakaryocytes.

Protocols have since improved, but not enough to make large-scale production possible. "The big bottleneck in our field is that we've never been able to get megakaryocytes to produce enough platelets each to make this commercially feasible," says Jonathan Thon, co-founder of Platelet Biogenesis in Boston, Massachusetts, a company that aims to test platelets derived from iPS cells in clinical trials.

FAKE IT 'TIL YOU PLATELET

An easier alternative might be to make completely synthetic platelets. Ashley Brown, a bioengineer at North Carolina State University in Raleigh, created a fake platelet out of a squishy hydrogel that sticks to strands of fibrin, one of the main proteins involved in blood clotting. In a rat model of traumatic injury, these platelet-like particles staunched bleeding much more quickly than did 100 times as many fresh platelets[2]. Brown cautions, however, that she has yet to test the blood-clotting power of her platelet stand-ins in any animal larger than a rat. "Once you scale up to pigs," she says, "the dynamics can be quite different and you can see some very unexpected results."

Erin Lavik, a polymer engineer at the University of Maryland, Baltimore County, found this out the hard way when she tested clot-building nanoparticles in both rats and pigs. The experimental therapy passed testing in rats with flying colours, but in as-yet unpublished experiments in pigs, the artificial platelets triggered a rapid immune reaction that caused blood vessels to widen, leading to increased bleeding rather than wound closure.

Joseph Italiano, a platelet biologist at the Brigham and Women's Hospital in Boston, applauds these efforts to create platelet mimics, but even if they're proved to be safe, he doubts they'll achieve the full functionality of the real thing. In the past decade, he says, scientists have begun to realize that platelets do much more than thwart blood loss. They are also involved in tissue regeneration, blood-vessel remodelling and the immune response. "It's almost easier to describe things they're not involved in," says Italiano, who co-founded Platelet Biogenesis with Thon. And because the many functions of platelets aren't entirely understood, "it's hard to emulate them *de novo*."

Thon joined Italiano's lab in 2008, after completing a PhD at the

University of British Columbia in Vancouver, Canada, where he worked on methods for extending the shelf life of platelets. But he had grown disillusioned with the existing donor-blood-based system, and instead decided to dedicate himself to the challenge of growing platelets in the lab. He focused on developing a microfluidic device that could produce more platelets per megakaryocyte than had been achieved in culture plates.

As Thon reported in 2014, his bioreactor-on-a-chip — composed of tiny channels moulded in silicone that replicate some of the shear stresses and other physiological characteristics of bone marrow — produced around 30 platelets per iPS-cell-derived megakaryotye[3], a yield 3–8 times greater than those achieved by the researchers at Megakaryon or PlatOD. Thon continued to work at the Brigham until August 2017, when he decided to dedicate himself to full-time research at Platelet BioGenesis.

He and a team of staff scientists are now focused on improving the platelet system in two ways — starting with the bioreactor itself. According to Lea Beaulieu, a platelet biologist at the company, the academic prototype went through four major upgrades. "Now, we're trying to create one module that we can multiplex," Beaulieu says, as she holds up the latest iteration to reveal the serpentine channels that run though the device's thick plastic.

There is also the question of which cells to place in the bioreactor — and that involves choosing a particularly industrious starter cell line. Brad Dykstra, a stem cell biologist at Platelet Biogenesis, has spent most of the past nine months evaluating three clinical-grade iPS cell lines for their potential to make platelets in the lab. "Each of our cell lines has its own personality," Dykstra explains, as he moves a tissue-culture plate under a microscope to show a clump of cells in their growth phase, matted together in a shape that roughly resembles a map of Australia. That cell line is the most efficient at making megakaryocytes, whereas the megakaryocytes derived from another line produce more proplatelets. The third cell line is something of a low-quality rebel: "We call it our angry teenager," Dykstra says.

Thon now has to choose between megakaryocyte quality and quantity when deciding which iPS cell line and bioreactor design to take forward. He hopes to begin testing in animals by early 2018, with clinical trials to follow a couple years after. "As of today," Thon says, "we are right on schedule."

SEEING RED

After lab-grown platelets, red blood cells could be next. These oxygen-carrying cells (also known as erythrocytes) could be produced from a universal donor, thereby allowing their transfusion regardless of blood type. And a manufactured product could eliminate the risk of blood-borne infections, a considerable problem when pathogens emerge for which reliable tests don't yet exist (see page S19).

But even if researchers do develop a successful differentiation and maturation protocol — much of the recipe remains to be worked out — the economics of producing commercially viable red blood cells will impede their widespread adoption. As Marc Turner, medical director of the Scottish National Blood Transfusion Service in Edinburgh, UK, points out, "There is no clear path to manufacture this at a scale and cost that is tractable." Turner leads a consortium called Novosang that aims to mass-produce red blood cells from iPS cells. "We certainly can't do that at the moment," he says. "We're nowhere near it."

Red blood cells considerably outnumber platelets in a bag of donated blood, meaning that any manufactured alternative would need to be made cheaply and with massive efficiency. And donor-derived red blood cells can be stored for up to six weeks in a refrigerator (and even longer in a freezer), which gives blood banks and hospitals the flexibility to manage their inventory in a way that's not possible with platelets.

Instead of trying to compete with the relatively inexpensive and plentiful pool of donated red blood cells, Linzhao Cheng, a stem-cell biologist at the Johns Hopkins University School of Medicine in Baltimore, Maryland, says he is "looking for niche applications" for his iPS-cell-derived erythrocytes. In one project, Cheng is working with John Roback, medical director of the Emory University Hospital Blood Bank in Atlanta, Georgia, to make personalized red blood cells grown from the stem cells of people with sickle-cell disease.

The researchers are focusing on this group of blood disorders because of the high rate of immune responses to donor blood products in affected people. Among all recipients of transfusions, an estimated 2–5% develop antibodies to mismatched marker proteins found on the surface of red blood cells. However, in people with sickle-cell disease, which mainly affects those of African descent but is treated with blood products from an overwhelmingly white donor pool, the unwanted immune reaction is much more common, occurring in almost 1 in 3 transfusion recipients.

Many of these patients require regular transfusions to improve the oxygen-carrying capacity of their blood but can only find a few compatible donors. For such patients, a personalized, compatible source of red blood cells, even though expensive, might prove to be cost-effective, says Roback. "iPS-cell-derived red blood cells could dramatically improve our ability to treat their anaemia," he says.

> "MANY OF THESE PRODUCTS COULD FILL CRITICAL NICHES."

A cheaper alternative could be an oxygen-carrying blood substitute. And although a cell-based product would be preferable for people with sickle-cell disease, there are some situations in which an artificial blood that lacks the delicacy and nuance of living cells could be just what the doctor ordered. On the battlefield, for example, where more than 90% of potentially survivable deaths are due to uncontrolled blood loss[4], a blood replacement that provides temporary transport of oxygen to the body's tissues and helps to revive casualties in shock could save lives. Importantly, such a product would need to be stable for long periods without refrigeration.

"What you really want is something that can be freeze-dried and stored at ambient temperatures," says Allan Doctor, a paediatric critical-care physician who is developing a biosynthetic, artificial red blood cell at Washington University School of Medicine in St Louis, Missouri. The same would be true for a life-threatening situation in a remote environment, for a person with a religious objection to blood transfusion, or for an ambulance in a city — at least as a stop-gap until the patient makes it to hospital. "A medic would simply have to mix it with water, shake it and inject it," Doctor explains. "Then it can be used in some back alley in Fallujah, on a cruise ship, on Mars, on a submarine — wherever."

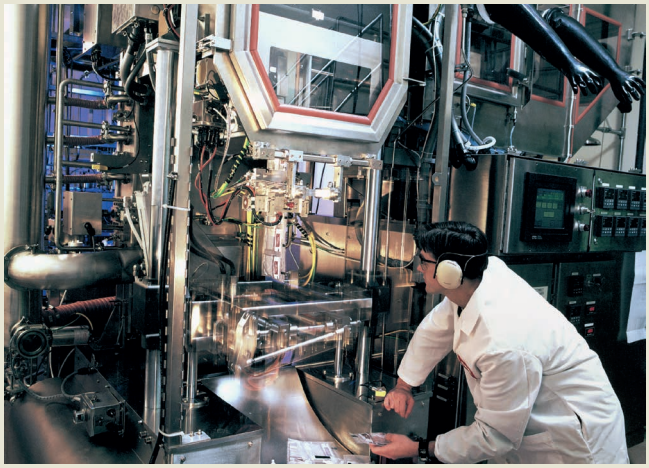
IN A NUT SHELL

Doctor is developing his reconstitutable blood substitute through a spin-off company called KaloCyte that he co-founded in St Louis. The product, known as ErythroMer, comprises a polymer shell that surrounds a huge payload of haemoglobin, the oxygen-binding protein found in blood (isolated from human red blood cells stripped of their cell membrane), and a small molecule that facilitates oxygen release. Importantly, the shell allows oxygen through while slowing the diffusion of nitric oxide, a signalling molecule that gets scooped up by haemoglobin, causing blood vessels to tighten and spasm — a problem seen with some unencapsulated haemoglobin-based oxygen carriers, many of which faltered in clinical testing (see 'A meta-problem for blood substitutes').

At the 2017 annual meeting of the International Society on Oxygen Transport to Tissue in August, Doctor and Dipanjan Pan, co-inventor of ErythroMer and a synthetic chemist at the University of Illinois at Urbana-Champaign, reported that their product rapidly stabilized blood oxygen levels in a rat model of haemorrhagic shock. The blood substitute also mopped up nitric oxide from the blood at a rate lower than did normal red blood cells. "We're guardedly optimistic that this represents a breakthrough," Doctor says.

CASE STUDY

A meta-problem for blood substitutes



The production of Hemopure, a blood substitute from HbO_2 Therapeutics.

The clinical development of oxygen-carrying blood substitutes has a chequered history. The first such oxygen transporter to enter late-stage clinical testing, HemAssist, was found to increase death rates in patients with serious or life-threatening injury. And an ethical firestorm erupted when another experimental therapy, PolyHeme, was administered to unconscious patients who were unable to give their consent.

But the real blow to these haemoglobin-based oxygen carriers (HBOCs) came when a 2008 meta-analysis purported to show that the blood substitutes significantly increased the risks of death and heart attack[7]. That finding put a black mark on the entire class of drugs. "It delayed the whole industry for 10 years," says Jonathan Jahr, an anaesthesiologist at the University of California, Los Angeles.

There was only one problem. "The meta-analysis was seriously flawed," says Colin Mackenzie, an anaesthesiologist and physiologist at the University of Maryland School of Medicine in Baltimore.

Letters to the editor poured in, criticizing the authors for lumping together randomized trials from five different HBOCs, each of which had different properties and had been tested in different study populations with a variety of control conditions. According to news reports, an internal report by the US Navy blasted the US Food and Drug Administration (FDA) for rejecting applications to further test one of the leading HBOCs. Remarkably, Mackenzie and Jahr found that by simply removing HemAssist from the mix, the conclusions of the meta-analysis could be flipped entirely[8]. It wasn't all HBOCs that were dangerous — just that one long-abandoned product.

But the damage had already been done. In 2009, Northfield Laboratories of Evanston, Illinois, which developed PolyHeme, filed for bankruptcy. So did the other major player at the time, Biopure of Cambridge, Massachusetts. A Russian industrialist snapped up Biopure's assets for US$4 million, creating OPK Biotech. Within five years, however, that company too was in financial straits, and it was sold to become what is now HbO_2 Therapeutics, headquartered in Souderton, Pennsylvania.

HbO_2 is distributing its HBOC to clinics worldwide on a compassionate-use basis, says chief executive and co-founder Jerry Kowalski. The product, a formulation of bovine haemoglobin called Hemopure, has been approved for use in South Africa, where more than 2,000 people have received it. In the United States and Europe, a veterinary formulation is available for dogs. In 2018, Kowalski hopes to launch a phase III clinical trial in the United States to test Hemopure as a treatment for anaemia in people for whom receiving donor blood is not an option, such as Jehovah's Witnesses or those with sickle-cell disease who have developed an immune reaction to red-blood-cell transfusions. HBOCs derived from human, cow or even lugworm (*Arenicola marina*) haemoglobin might not be far behind.

"A better understanding of the complex chemistry of HBOCs is emerging, and there is hope that application of this understanding will lead to a reduction in their toxicity," says Abdu Alayash, an expert in blood substitutes at the FDA in Bethesda, Maryland.

Although commercial interest in the technology has waned, a few companies have stuck with the idea of developing a red-blood-cell substitute. "This is a huge opportunity," says Abraham Abuchowski, chief executive officer and chief scientific officer of Prolong Pharmaceuticals, South Plainfield, New Jersey, which has an oxygen transfer agent called Sanguinate in phase II testing. "There are many clinical conditions that need something like this." E.D.

Theoretically, the biggest breakthrough for the blood replacement field — and one that could spell the end of blood donation — would be the ability to make bespoke blood stem cells from a patient's own tissue. Such a personalized yet primordial blood cell could produce all components of blood, including red blood cells and platelets, as well as the white blood cells of the immune system required by people with leukaemia and other blood disorders in need of bone-marrow transplants. If blood stem cells could be made from a patient's skin or other healthy tissues, transplant recipients would no longer be reliant on finding compatible donors.

This year, two research groups in the United States came enticingly close to cooking up blood stem cells in the lab. A team led by George Daley, a stem-cell biologist at Boston Children's Hospital, developed a way to turn human iPS cells into cells with all the features of blood stem cells. However, their gene-expression profile doesn't exactly match that of true blood stem cells, and the lab-grown cells do not engraft as robustly as those found naturally in the bone marrow or umbilical-cord blood[5]. They're "tired" blood stem cells, Daley says.

Working in mice, stem-cell scientist Shahin Rafii and his colleagues at Weill Cornell Medicine in New York City had greater success in making bona fide blood stem cells, starting with cells isolated from the lungs[6]. In collaboration with Hans-Peter Kiem, a cell-therapy researcher at the Fred Hutchinson Cancer Research Center in Seattle, Washington, Rafii and his team are now testing whether the same approach can work in fat cells from a newborn macaque. According to Raphael Lis, a researcher in Rafii's lab, the team has succeeded at making small numbers of macaque blood stem cells in a dish. "Right now, we are just working on expanding the culture to a clinical dose," he says.

Later this year, they plan to reintroduce about 500,000 lab-grown blood stem cells into the same monkey after wiping out its bone marrow. If the cells take hold, and then self-renew and produce healthy immune cells and all the other components of blood — without unwanted side effects — trials in humans could be next. ■

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1. Takayama, N. *et al. Blood* **111,** 5298–5306 (2008).
2. Brown, A. C. *et al. Nature Mater.* **13,** 1108–1114 (2014).
3. Thon, J. N. *et al. Blood* **124,** 1857–1867 (2014).
4. Eastbridge, B. J. *et al. J. Trauma Acute Care Surg.* **73,** S431–S437 (2012).
5. Sugimura, R. *et al. Nature* **545,** 432–438 (2017).
6. Lis, R. *et al. Nature* **545,** 439–445 (2017).
7. Natanson, C., Kern, S. J., Lurie, P., Banks, S. M. & Wolfe, S. M. *JAMA* **299,** 2304–2312 (2008).
8. Mackenzie, C. F. *et al. Am. J. Ther.* **22,** e115–e121 (2015).