Meta-analysis torpedoes blood substitutes

Trials of hemoglobin-based blood substitutes have been dogged by clinical and regulatory setbacks and even attracted ethical spats over patient consent. But now a controversial meta-analysis published in the May 21 issue of the *Journal of the American Medical Association* (*JAMA* **299**, 2304–2312, 2008) threatens to cast a cloud over the entire field. The paper's authors, Charles Natanson and his colleagues at the US National Institutes of Health, conclude that current-generation blood substitutes pose a 30% elevated risk of death and a nearly threefold greater risk of heart attack than do standard products (e.g., saline) to correct volume. The meta-analysis includes data from 16 randomized, controlled clinical trials of products tested by five companies: Baxter International, Hemosol Biopharma, Biopure, Northfield Laboratories and Sangart.

The *JAMA* article elicited impassioned responses from blood-substitute companies that now find themselves in dire straits—falling stock prices, clinical trials in limbo and a barrage of negative press. A. Gerson, Greenburg, Cambridge, Massachusetts–based Biopure's vice president for medical affairs, fired off a letter to *JAMA*'s editors insisting the meta-analysis unfairly lumped the company's data with those from other firms, generating results he says aren't relevant to Biopure's own products. In response, Natanson claims the evidence for heightened risk of death and heart attack from the collective data is "overwhelming," and insists clinical testing should have been halted long ago.

The *JAMA* publication coincided with a two-day meeting cosponsored by the US Food and Drug Administration (FDA) and attended by nearly 350 people, including company representatives. At the meeting, agency officials concurred that current blood substitutes produce excess mortality and heart attacks. "For this reason, a careful weighing of potential risks and benefits will be needed to permit any future trials of the current products," writes Jay Epstein, director of the FDA's Office of Blood Research and Review, in an e-mail to *Nature Biotechnology*.

Alan Schechter, chief of molecular medicine at the National Institute of Diabetes and Digestive and Kidney Diseases in Bethesda, Maryland says, "Assuming the meta-analysis was appropriate and valid, [it] poses a big barrier to further clinical trials with current generation agents…and that's not good for the companies trying to develop these products."

Blood substitutes come in two forms: the widely available volume expanders—saline, Ringer's Lactate and D5W (a water-based 5% dextrose solution)—or oxygen therapeutics that mimic the blood's ability to transport oxygen. All current oxygen therapeutics, including those assessed in the *JAMA* study, are hemoglobin-based oxygen carriers (HBOCs). These products consist of free hemoglobin, the protein in red blood cells that binds oxygen in the lungs and releases it elsewhere in the body.

All HBOC companies aim to create an effective oxygen carrier that remains stable in storage at room temperature for long periods. So far, scientists have been unable to replicate blood's capacity to transport oxygen safely. The big hurdles come with managing free hemoglobin. In the body, hemoglobin is packaged into red blood cells, protecting the molecule from degradation and limiting its ability to interact dangerously with other molecules. But free hemoglobin readily breaks down into smaller molecular 'dimers' that rapidly wind up in urine. To avoid that problem, first-generation HBOCs cross-linked hemoglobins into larger molecules that would, in

A safe, economic substitute could resolve blood shortages in the battlefield, emergency rooms and developing countries.

IN brief

UK passes hybrids

The UK Parliament has voted to allow the generation of human-animal hybrid embryos, creating the most liberal legal framework anywhere in the world for embryonic stem cell research. The move confirms that the Human Fertilisation and Embryology Authority acted within its jurisdiction when it gave permission in January to scientists at King's College London and Newcastle University to work on generating embryos by fusing enucleated animal oocytes with the nuclei of adult human cells (*Nat. Biotechnol.* **26**, 252, 2008). Embryonic stem cell lines produced as a result cannot be used in therapies but are expected to be useful as disease models. One immediate beneficiary was ReNeuron, of Surrey, UK, which saw its share price double, although its products are based on fetal stem cell lines. CEO Michael Hunt said, "Our hope is that the UK's reputation for supporting such pioneering early-stage stem cell research will be mirrored by further support for later-stage translational research activities." In Germany, researchers no longer have to fear a possible prison sentence for working on human embryonic stem cell lines created after January 2002. The German Federal Parliament voted in April to allow scientists to use up to 500 stem cell lines from abroad, as opposed to the 20 previously allowed, extending the qualifying date for importing lines to May 1, 2007. Brazil's Supreme Court ruled in May that scientists can lawfully conduct embryonic stem cell research, subject to certain caveats, such as not allowing the embryo to be destroyed. *—Nuala Moran*

Tighter gene tests

A report issued by the Secretary's Advisory Committee on Genetics, Health and Society (SACGHS) urges better oversight for genetic tests. The panel, commissioned by the US Department of Health and Human Services, identifies various gaps in the regulation of genetic testing and calls for better coordination between federal, state and other agencies to improve the oversight model. SACGHS members also recommend that public and private sectors adopt measures to assure public health and safety when conducting and interpreting results from clinical genetic testing. Although mandated to review the validity and utility of genetic testing, the panel recognizes that their recommendations "could well be applied more broadly to improve the quality of all laboratory tests." Indeed, they call on the US Food and Drug Administration (FDA) and the Centers for Medicare & Medicaid Services (CMS)—the two federal agencies with principal regulatory authority over genetic testing—to overhaul clinical testing with "establishment of a mandatory test registry." The panel also urges the FDA "to strengthen monitoring and enforcement efforts against laboratories and companies that make false and misleading claims about laboratory tests, including direct-to-consumer tests." *—Jeff Fox*

IN brief

Biofuels, take two

The next-generation biofuels industry got a boost in May when Congress passed new laws under the 2008 Farm Bill that support the use of lignocellulosic feedstocks. The bill creates a tax credit of $1.01 per gallon of cellulosic ethanol, decreases the tax credit for corn-based ethanol by six cents to $0.45 and provides $320 million in loan guarantees for the construction of next-generation biofuels plants. It also increases to $120 million funding for R&D in feedstock development and biofuel production efficiency and provides payments to farmers near biorefineries to help them transition to energy crops. Key are the bill's incentives for farmers to commit to growing energy crops before biorefineries are built. "It's difficult to put up a biorefinery until you have an assured supply of biomass. But it's difficult for growers to want to plant large acreage of dedicated energy crops until they're assured a market in the form of a biorefinery," says Anna Rath, vice president of commercial development at Ceres, in Thousand Oaks, California. "So from our perspective, the [farm bill] takes care of that chicken-and-egg problem." President George Bush vetoed the bill May 21, saying it would subsidize wealthy farmers, including married couples making up to $1.5 million per year, and allow crops to be subsidized at any price. Congress the next day overrode the veto, enacting 14 of the bill's 15 titles. The bill, as of press time is expected to be passed into law. *—Emily Waltz*

50 cancers to be sequenced

The recent launch of the International Cancer Genome Consortium (ICGC) looks set to flood DNA databases with unprecedented genomic detail on up to 50 types of cancer. The initiative—a collaboration of more than a dozen major research organizations around the globe—will generate a "comprehensive description of genomic, transcriptomic and epigenomic changes in 50 different tumor types and/or subtypes." Each member is expected to take on one or more cancers from a list agreed by the ICGC who will coordinate the program. Each project will involve sourcing and sequencing both tumor and non-tumor tissue from some 500 patients at an estimated cost of around $20 million. Participants are expected to find their own funding and, to maximize the public benefit, will not file any patent applications. Instead, the data will be made available to selected investigators. Some question whether this money—the total cost is $1 billion—is well spent. Only in part, says Stephen Elledge of the Center for Genetics and Genomics at Harvard Medical School in Boston. "If the goal is to cure cancer and not just to describe it, there needs to be more money for functional genomics rather than just sequencing," he says. The consortium, which includes institutes from Canada, China, France, India, Japan, Singapore, the UK and the US, is about to begin the process of selecting which cancers will make it onto the sequencing shortlist. *—Henry Nicholls*

Table 1 How the substitutes line up[a]

Company	Development phase (country)	Product
Biopure	Launched (South Africa) Phase 3 (Canada)	Hemopure
Northfield Laboratories (Evanston, Illinois)	Phase 3 (US)	PolyHeme (glutaraldehyde-polymerized human hemoglobin modified by pyridoxylation)
Sangart (San Diego, California)	Phase 3 (multicenter, Europe)	Hemospan PS (polyethylene glycol cross-linked human hemoglobin)
HemoBioTech (West Dallas, Texas)	Phase 1 (Zaire)	HemoTech

[a]Clinical development of Hemolink (*O*-raffinose cross-linked human hemoglobin) by Hemosol Biopharma (whose assets were acquired from Hemosol Corp, Mississauga, Canada, in July 2007) was discontinued in 2005; Baxter International's HemAssist program was discontinued in 1998.

theory, remain in the circulation longer. This approach was taken by Deerfield, Illinois–based Baxter's pioneering product HemAssist (diaspirin cross-linked hemoglobin), which was purified from donated human blood. HemAssist ran into trouble, however, when trauma patients receiving it in clinical trials died at higher-than-expected rates. Baxter terminated the product voluntarily in 1998.

Although it has never been confirmed, scientists suspect HemAssist's clinical setbacks—and those of subsequent HBOCs—resulted from vascular hypertension caused by constriction of patients' blood vessels. Schechter says the hypertension probably occurs because free hemoglobin also binds to, and inactivates, nitric oxide, which helps regulate vasodilatation. Nitric oxide binding—a major concern at the FDA meeting—continues to plague blood-substitute companies today. Barry Scott, the vice president for business development at Biopure, argues these concerns are likely to be overblown. However, a US Navy protocol for testing Biopure's product, Hemopure (glutaraldehyde-polymerized bovine hemoglobin modified by pyridoxylation), in clinical trials was put on hold in 2002. Although the Navy still argues in favor of testing Hemopure, neither Biopure nor the FDA would comment on the rationale for halting the protocol.

William Hoffman, formerly Biopure's Chief Medical Officer and now medical director of intensive care at Massachusetts General Hospital, blames the harmful reactions developed in a clinical trial involving anemic patients who were given Hemopure during orthopedic surgery. Biopure continues to offer Hemopure to US hospitals for trauma treatment on a compassionate-use basis. "We don't deny that Hemopure binds nitric oxide; all [HBOCs] do," Biopure's Scott says. "We know what the potential adverse events are, but you'll get adverse events with any clinical product. Our aim isn't to reproduce blood; we're merely aiming to bridge clinical care until real blood is available."

Other companies claim they're now 'decorating' hemoglobins with molecules to limit nitric oxide binding. San Diego–based Sangart, for instance, expands the radius of its HBOC, Hemospan, with polyethylene glycol, explains H. Franklin Bunn, a Harvard Medical School professor who sits on the company's advisory board. Bulking Hemospan with polyethylene glycol distances

SELECTED research collaborations

Partner 1	Partner 2	$ (millions)
Alnylam (Cambridge, Massachusetts)	Takeda (Osaka, Japan)	1,000
Symphogen (Lyngby, Denmark)	Genentech (S. San Francisco, California)	*
DuPont (Wilmington, Delaware)	Genencor (Rochester, New York)	140

* Financial details not disclosed.

the hemoglobin molecule from endothelial tissue surfaces to prevent nitric oxide binding. Likewise, West Dallas–based HemoBioTech's product, HemoTech, made with bovine blood, links hemoglobin molecules with adenosine 5′-triphosphate, *O*-adenosine and reduced glutathione. According to the company, this encourages vasodilation and stimulates erythrocyte production.

HemoBioTech managed to stay out of the *JAMA* study, possibly because the only human clinical trial data for its product HemoTech come from a small 1990 study in Zaire, on a population of nine children with anemia. The company's CEO, Arthur Bollon, isn't planning on disputing the conclusions from the meta-analysis: "We argue that ours is the only true second-generation product, because we use pharmacology [i.e., adenosine] to overcome toxicity, and the other products don't," he says. Bollon would not discuss the Zaire trial results in detail, beyond pointing out that toxicity had not been a concern and the data had been shared with the FDA.

For his part, Natanson claims the companies were all uncooperative and released data grudgingly, in some cases only if prodded by the threat of lawsuits. Natanson acknowledges that meta-analyses do not discriminate among different products. But he also concludes that products from four of the five companies evaluated had almost identical profiles, with elevated rates of mortality and heart attacks. This suggests that the HBOCs operate as a single class of compounds. Sangart was the exception, possibly because the number of patients tested with its product was too small to make such estimates meaningful.

Those companies present at the FDA meeting acknowledged toxicity concerns but attributed the mortality to the patient population tested. For instance, Sangart's Hemospan was tested during hip-replacement surgery on older patients who were more susceptible to adverse events, according to Peter Keipert, Sangart's vice president for clinical and regulatory affairs. Natanson responds that "each of the companies ascribed poor outcomes to patients; each had a different reason, which varied among the specific populations tested."

Natanson is also sharply critical of the FDA, suggesting agency policies contributed to lengthy delays between the time when clinical trial data were collected and the time they were made public. Natanson points out that "data on a large proportion of patients (approximately 75%) in Hemopure trials, all of which were completed by 2000, have still not been published. These data only became publicly known after Public Citizen in Washington, DC, sued the FDA, which [finally revealed them] at a December, 2006 FDA advisory meeting."

Companies in this sector are continuing to explore overseas options. Each has ongoing or planned trials in various countries in Europe or the developing world. And some even feel it is not all gloom and doom. John Olson, a blood researcher at Rice University in Houston, Texas, points out that patients treated with blood substitutes have experienced positive outcomes. "I understand safety concerns and efforts to counterbalance overenthusiasm from the companies. But damnation of the whole field with an almost religious fury is reminiscent of the condemnation of evolution by right-wing creationists. There is a need for hemoglobin-based oxygen carriers and the question is how best to make them safe and effective," he argues.

Charlie Schmidt *Portland, Maine*

IN their words

"That's akin to shooting an arrow and having it land on a wall and then drawing a target around it. It's an attempt to resurrect a trial that has failed."

FDA's Richard Pazdur on companies' attempts to salvage drugs that failed their primary endpoints by looking at a subgroup of patients. (*Business Week*, May 21, 2008)

"It's really been an honor system thing. If somebody tells us that a pharmaceutical company pays them $80,000 a year, I don't even know how to check on that."

Robert Alpern, dean of Yale School of Medicine commenting on the case of a Harvard child psychiatrist Joseph Biederman's failure to disclose earnings of at least $1.6 million in consulting fees from drugmakers. (*New York Times*, June 8, 2008)

"This project is really a celebration of the mutt. Most people who are interested in our service own mutts. That's a breed of one, and you'll never get that again."

Lou Hawthorne, chief executive of biotech company BioArts, commenting on their service for cloning dogs, set up in partnership with Hwang's Sooam Biotech Research Foundation in South Korea. (*ABC News*, May 23, 2008)

"You can bet these bully tactics will have an effect. Look for greater demands by the FDA for cancer programs to not use the accelerated approval pathway."

GenVec's senior vice president of clinical development Mark Thornton on US Senator Charles Grassley's (R, Iowa) request that the Government Accountability Office launch an inquiry into whether the FDA behaved appropriately in granting the "accelerated approval" of Avastin. (*Wall Street Journal*, May 29, 2008)

Details
Takeda will pay $ 100 million upfront to use Alnylam's RNAi technology for five years to develop treatments for cancer and metabolic disease. Under the terms of the agreement, Takeda will have the right of first negotiation to develop and commercialize Alnylam programs for the Asian market. Alnylam will have the option to co-develop and co-commercialize Takeda's linked programs in the US on a 50/50 basis. Takeda will also pay $50 million for short-term technology transfers.
Genentech will make an upfront payment and an equity investment in Symphogen to apply the Danish company's Symplex antibody discovery technology platform to identify novel drug candidates against three undisclosed infectious agents. Symphogen is eligible to receive milestone payments and royalties on any products developed and commercialized by Genentech that may result from this collaboration. The total value of the agreement could exceed $330 million. Genentech will obtain exclusive worldwide license for candidates developed through this agreement.
The companies entered an agreement to form DuPont Danisco Cellulosic Ethanol LLC, a 50/50 global joint venture to develop and commercialize a next generation biofuel produced from nonfood sources. The partners plan an initial three-year investment of $140 million, which will target corn stover and sugar cane bagasse. Future targets include multiple lignocellulosic feedstocks including wheat straw, a variety of energy crops and other biomass sources. The joint venture's technology which will combine Genencor's enzyme technologies and Dupont's proprietary ethanologen technology for high yield, developed with the US Dept of Energy National Renewable Energy Laboratory, can be used as a 'bolt-on' to existing ethanol plants, allowing them to accept cellulosic feedstocks, or as a stand-alone cellulosic ethanol facility. The joint venture expects to produce commercial volumes of cellulosic ethanol by 2012.