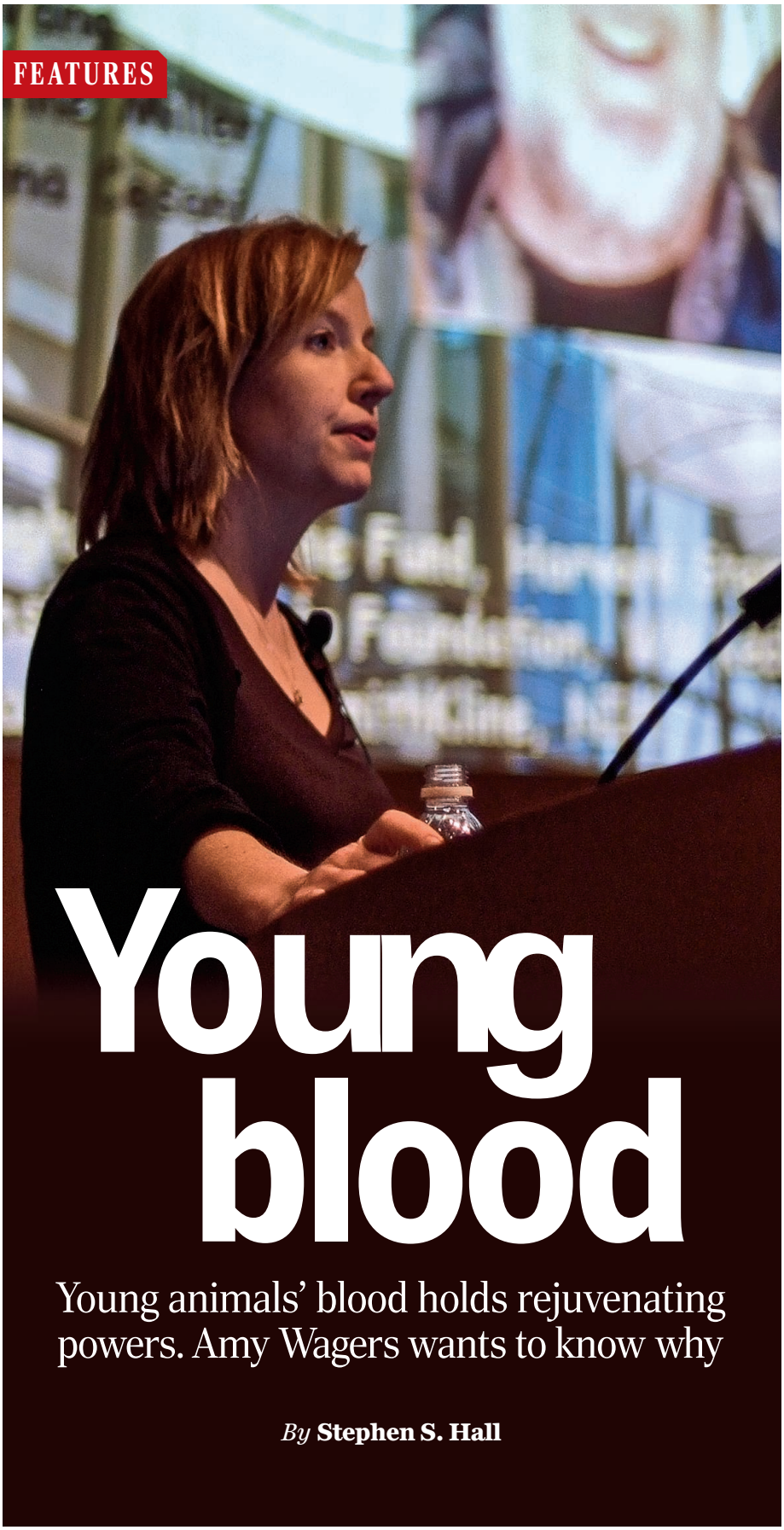
FEATURES



Young blood

Young animals' blood holds rejuvenating powers. Amy Wagers wants to know why

By **Stephen S. Hall**

Amy Wagers was about halfway through a 90-minute talk to a group of Boston-area science teachers last July when she showed her Arnold Schwarzenegger slides. One picture depicted the former governor of California as a buff young bodybuilder; the other showed a more recent, flabbier, and stooped version of the Terminator playing tennis. If a picture is worth a thousand words, the slide served as a two-volume treatise illustrating the point that Wagers, a Harvard University stem cell scientist, wanted to make: As humans age, our muscles fail to maintain and regenerate as they once did.

"I'm not saying aging is a disease," Wagers hastened to add, once her audience had stopped laughing. "But it is associated with increased incidence of particular types of diseases."

Wagers's quiet voice barely carried over an aspirating aquarium at the side of the science lab at Dover-Sherborn High School. But the 30 or so educators gathered at the Summer Science Institute avidly followed the narrative of her recent work—especially when Wagers moved on to her "fountain of youth" slides.

In a series of experiments that have captivated both the field of regenerative medicine and its many lay spectators, Wagers and a diverse army of collaborators have shown that when the blood of a young mouse circulates through the murine equivalent of an old geezer, startling physiological changes occur. Many of the trademark depredations of old age—withering muscles; stiff, oversized hearts; cognitive decline; and even the fraying of the myelin coating that insulates nerve fibers—are slowed, repaired, or even reversed.

"We became convinced that there was something in the blood" responsible for the dramatic effects, Wagers told the teachers. Indeed, after a difficult search, she and colleagues have recently isolated a molecule from "young blood," growth differentiation factor 11 (GDF11), that appears to rejuvenate the architecture of the heart, the vasculature of the brain, and the bulk of skeletal muscle—at least in animals. As Wagers told the group, "GDF11, which is this 'fountain of youth' kind of factor for the heart, is also a fountain-of-youth factor for the skeletal muscle and for the brain. It is generally a good protein for rejuvenation."

"Rejuvenation" has always been a loaded crossover term in biology. Quickly raising her hand, one young high school science teacher from Walpole could barely spit out the question on everyone's mind. "You have to wonder, given what you're seeing … I mean,

PHOTO: JUN SEITA/FLICKR

it seems like—well, when are you going to market this!"

The teachers also circled around an issue that has dogged stem cell researchers ever since the isolation of the first human embryonic stem (ES) cells in 1998 suddenly made the potential of regenerative medicine seem real. "Are we talking about extending life," asked another teacher, "or are we talking about improving the quality of life? The ethical issues are huge!"

"Yeah, yeah," Wagers nodded in agreement, before quickly walking the conversation back to biology. "I don't want to leave you with the impression that this molecule explains everything," she said. "It's absolutely certain that it does not." Indeed, other groups studying the rejuvenating properties of young blood are eyeing different proteins, factors, and pathways. Still, Wagers predicted GDF11 would have some sort of role in medicine within 4 years. "I guess the overarching image that I want to leave you with," she said, "is that perhaps aging is an imbalance of signals, and maybe we can restore the balance."

Balance may be the most important word in Wagers's vocabulary these days. She is tending a raft of collaborations, which produced 14 papers last year. She is still struggling to regain her own balance since an episode of misconduct in 2010 rocked her lab and resulted in the retraction of two high-profile papers. And, as an inveterate skeptic who made an early mark by challenging overstated claims by other researchers, she is aiming for balance between scientific enthusiasm about the "rejuvenation factor" and realism about what it might mean for medicine.

> ***"It's obviously a fascinating idea that something in the blood can potentially reverse aging. But you'd like to know how these things work."***
>
> **Toren Finkel,** National Heart, Lung, and Blood Institute

WAGERS, 41, IS OFTEN DESCRIBED as a "rising star" in the field, but it has been a challenging ascent. By her own admission, Wagers was "pathologically shy" as a child—to the point where she refused to speak to people in public, including restaurant waiters and store clerks, using her younger sister to communicate for her. The first time she gave a scientific talk, as a teenager, she "fainted dead away" and dropped to the floor. "If anyone had told me, 'By the way, Amy, you'll be speaking in front of huge audiences pretty much every week,' I would have run screaming from this career at that point," she admits.

She attributes the shyness, in part, to a peripatetic childhood in which her family relocated almost once a year because of her father's job as an electrical engineer in the telecommunications industry. Although nominally from Ohio, her family hopscotched around the country. "Sometime in the fourth grade, when I was at the playground, I wouldn't play with anybody," Wagers recalls. "The teachers asked me why, and I said, 'Oh, we're just going to move in a year. It doesn't really matter.' "

Wagers received her undergraduate degree in biology from Northwestern University in 1994 and continued her graduate work there in the laboratory of immunologist Geoffrey Kansas, where she earned her Ph.D. in 1999. She had contacted a number of labs about a postdoctoral fellowship, but didn't hear back from the one she most wanted to join: the Stanford University laboratory of noted stem cell scientist Irving Weissman in Palo Alto, California. "Geoff Kansas called me up," Weissman recalls, "and said, 'Irv, you're going to make the worst mistake of your life' if you don't talk to her."

He did, and she joined his lab. Although Weissman found Wagers "sort of quiet and unassuming" at first, he quickly realized that she was "terrific at managing collaborations" and taking on projects. "I could not saturate her with potential ideas or experiments or where we would go. She would just take on one after another, and get the results in a very clear way."

By that time, a national debate was raging over the ethics of studying ES cells. The bearded and burly Weissman became one of the most implacable and articulate scientific voices defending the use of ES cells in research. Working with Weissman, Wagers says, "landed me squarely in the middle of that debate."

A key ethical flashpoint was whether adult stem cells, such as the hematopoietic cells that form blood, could function just as well as embryo-derived cells in potential therapeutic applications. Several prominent groups had claimed that these bone-marrow cells could differentiate into other tissues, including muscle, heart, and brain cells. To test the claims, Wagers introduced a marker into mouse hematopoietic stem cells and transplanted just one of the cells into a recipient mouse. She could then see whether any new brain, heart, or muscle cells were descended from the transplanted cell.

Aside from what turned out to be rare "fusion events," where blood-forming stem cells and muscle-forming stem cells physically merged, Wagers and Weissman saw no sign that adult hematopoietic stem cells from bone marrow could form muscle. "Little Evidence for Developmental Plasticity of Adult Hematopoietic Stem Cells," declared their 2002 *Science* paper (27 September, p. 2256). ("Irv originally wanted it to be 'No Evidence,' " Wagers says, "but I think I talked him into 'Little Evidence.' ")

"My work was basically refuting those who were saying, 'Well, we don't need embryonic stem cells because we have adult stem cells,' " Wagers says.

"It was deeply unpopular," Weissman recalls. "What Amy showed was that not only was she smart and able to do experiments, but strong and courageous about standing up for what she did."

The "little evidence" paper was among the first of what Wagers now laughingly refers to as her "negative data" oeuvre—studies that challenged or disproved previously published claims. That body of work even inspired a bit of endogenous slang in the Weissman lab: To "wagerize" was to rigorously refute a research claim. The "negative data" papers also marked a turning point in Wagers's career, because some of the work involved pairs of mice with linked circulatory systems. That was her introduction to a curious 19th century laboratory creation that is now playing a starring role in aging research: the parabiotic mouse.

IN 1864, A FRENCH ZOOLOGIST named Paul Bert described an unusual surgical technique that merged the circulation systems of two animals into one. The technique involved making facing incisions in the flanks of two animals (mice, for example), and then sewing the skin of the two together, so that their flesh would be in contact. As the wound healed, capillaries from one animal would infiltrate the tissue of the other, Bert observed, "so as to create an exchange of nutrients by establishing a common circulatory system." Nearly a century would pass before scientists realized that parabiosis, as the technique became known, offered a powerful approach to studying aging and regeneration.

During the 1950s, Cornell University researcher Clive McCay, famous for having shown in the 1930s that caloric restriction extended lifespan in laboratory animals, and colleagues reported that stitching a young mouse to an old mouse (the technical term is heterochronic parabiosis) seemed to rejuvenate the older animals. But in an age before high-powered molecular analysis, the Cornell researchers had little chance of explaining

this surprising effect. Weissman himself had published parabiosis experiments as a high school student growing up in Great Falls, Montana, but for the most part the idea of using parabiosis to study the biology of aging fell out of fashion.

That began to change about 10 years ago, when Stanford biologist Thomas Rando and Irina Conboy, a postdoc in his lab, in collaboration with Wagers and Weissman, resurrected the technique with the thought of looking at how young blood affected the function of aging adult stem cells, such as the "satellite cells" that help maintain and repair muscle. These cells become less effective at regenerating tissue as an animal ages. The Stanford group sewed young mice together with old mice to see if there was anything in the blood that influenced the stem cells.

"We didn't invent parabiosis," Rando says, "but we revived the use of the technique." The results, published in 2005 in *Nature*, confirmed its power: The Stanford team found that sharing blood with young mice caused muscle stem cells in old mice to recapture their youthful ability to proliferate and regenerate muscle.

What remained unclear was the trigger—did something in young blood spark rejuvenation, or did something in old blood inhibit it? Rando, Tony Wyss-Coray, also at Stanford, and Conboy, who established her own lab at the University of California (UC), Berkeley, have pursued the answer ever since. So has Wagers, who moved to Harvard Medical School in 2004. As soon as her lab was up and running at Harvard's Joslin Diabetes Center, she launched a broad campaign to identify what she and others in the field coyly began to refer to as "systemic factors" in young blood.

Wagers set up multiple collaborations to explore the effects of those factors in different organs and tissues—with Richard Lee, a cardiologist at Brigham and Women's Hospital in Boston, to look at the heart; with Robin Franklin, whose lab at the University of Cambridge studies spinal cord injuries and ways to restore the myelin coating to nerve fibers; and with Lee Rubin, who studies neurodegenerative diseases at Harvard.

Just as the work was taking off, however, she had to take an involuntary and unofficial detour to deal with what she now describes as "the darkest time in my career, if not my life."

IN THE SUMMER OF 2010, Wagers became aware of a potential problem in a figure that had appeared as part of a *Nature* paper published earlier that year. First author Shane Mayack, a postdoc in her lab, had led the study, which claimed that the young blood phenomenon also affected hematopoietic stem cells in old mice, enhancing their blood-forming ability. One illustration in the supplemental material appeared to duplicate data from a 2008 *Blood* paper by Mayack and Wagers.

The possibility of impropriety hit Wagers like "a punch to the gut." She alerted Harvard Medical School officials, who launched an inquiry. Without waiting for the outcome, however, Wagers moved immediately to retract the *Nature* paper and contacted editors at *Blood*. "The first thing I thought was: I have to fix this."

A subsequent 2012 analysis by the federal Office of Research Integrity (ORI) concluded that Mayack had "engaged in research misconduct"; among the transgressions identified by ORI was the use of a figure from another, unrelated paper "to falsely represent [Mayack's] own experiment" and the use of an illustration from an online source as original data. The ORI report said Mayack also "falsely" relabeled identical flow cytometry plots to represent them as different experimental results.

The episode left Wagers psychologically bruised and scientifically sobered. "All of the waste in the research dollars; all of the waste in the time and effort of people in our lab and in other labs. And then the enormous amount of waste of the efforts of the people who track it down and document it. It's just horrible." Several online commentators at the website Retraction Watch faulted Wagers for lax oversight, but she wonders how lab chiefs can guard against the kind of false representations documented by ORI. "You can try very hard not to be deceived, but if you are, you are," she says. (According to the *Federal Register*, Mayack "neither admits nor denies" the ORI findings, and

Young blood versus old blood

Factors in "young blood" activate stem cells and rejuvenate organs and cells in old mice. Factors in "old blood" appear to inhibit regenerative capacity in young mice.

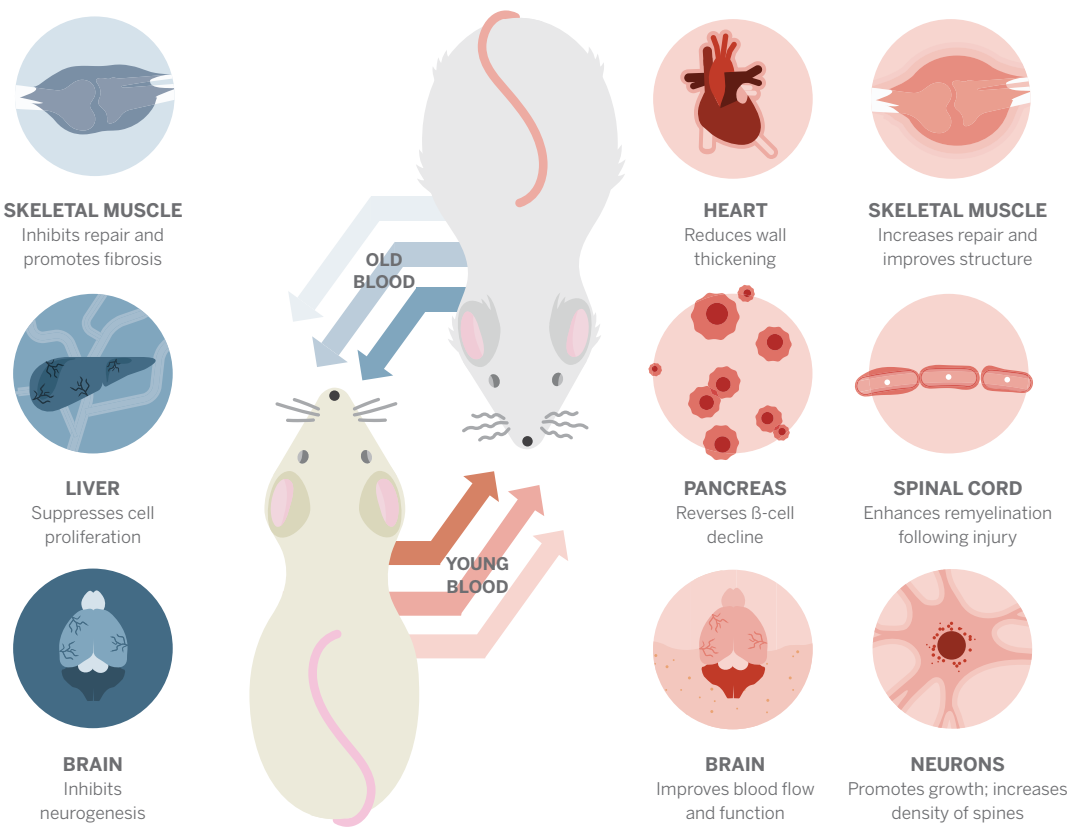


ILLUSTRATION: G. GRULLON/*SCIENCE*

Mayack later issued a statement admitting mistakes but denying misconduct.)

Despite coverage of the retractions in *The Boston Globe* and *The New York Times*, the episode of misconduct did not dim Wagers's star at Harvard; the university granted her tenure in 2012, and her former mentor Weissman applauded her quick reaction to the incident. "To her credit," he says, "she didn't try to wait the fuss out."

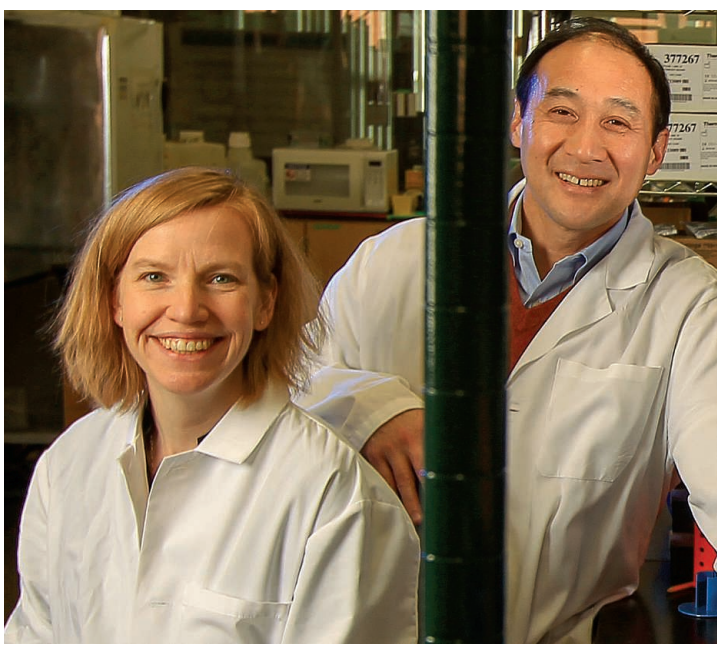
DURING THIS DIFFICULT TIME, the search for rejuvenating "systemic factors" in young blood went on, in Wagers's lab and elsewhere. But as she told the group of schoolteachers, "It's really hard to find something in the blood."

The Harvard researchers looked at numerous possibilities: proteins, fats, hormones. "It was all coming up negative," Lee recalls. "After about a year of work, we were starting to get concerned that we were never going to find this factor." Then the group enlisted the aid of a biotech firm in Boulder, Colorado, called SomaLogic Inc. The company used an advanced protein-capture technology to produce a list of 13 possible factors that seemed to be present at high levels in young blood and diminished levels in old blood. Out of this baker's dozen, the group ultimately zeroed in on GDF11.

"It's not a well-studied molecule," Wagers says. The protein is a member of the transforming growth factor β superfamily, a large group of related proteins that perform a wide variety of signaling functions regulating growth, differentiation, and immune function. Wagers's lab is still trying to figure out which cells make GDF11 and why levels decline with age. But using commercially available supplies, her team proceeded to test the rejuvenating potential of the molecule by injecting it into old mice.

The results were striking. In 2013, Wagers, Lee, and their colleagues reported in *Cell* that injection of GDF11 alone has the same effect on the hearts of old mice as an infusion of young blood: It significantly reversed cardiac enlargement (hypertrophy), which often causes the kind of heart failure commonly seen in older people. And this spring, in two papers in *Science*, Wagers, Lee, and their colleagues showed that raising GDF11 to youthful levels rejuvenated muscle stem cells, reversing age-related impairments and producing greater strength and endurance in older mice. Working with Rubin, they also reported that GDF11 bolstered circulation in older mouse brains, which promoted the formation of new neurons and ultimately improved brain function.

The reaction outside the lab has run the gamut from cautious excitement to over-the-top cultural enthusiasm (online commentators gushed about the potential for "vampire therapy"). "It's obviously a fascinating idea that something in the blood can potentially reverse aging," says Toren Finkel, who heads the Center for Molecular Medicine at the National Heart, Lung, and Blood Institute in Bethesda, Maryland. "That's sort of the holy grail for aging research. … But you'd like to know how these things work, what the mechanism is."



Amy Wagers and Richard Lee studied the rejuvenating effect of GDF11, a blood-borne factor.

PHOTO: THE BOSTON GLOBE/GETTY IMAGES

Other researchers caution that the work still needs to be reproduced and note that growth factors have the potential to initiate or accelerate cancers.

Wagers is the first to acknowledge that the biology is far from settled. "This is a complicated and robust system of regulation, so there's likely multiple signals." Indeed, Rando's lab at Stanford has been pursuing blood-borne factors in older mice that seem to suppress stem cell activity and blunt their regenerative capacity. Conboy's lab at UC Berkeley has recently reported that levels of the hormone oxytocin in the blood decline with age, and increased amounts of oxytocin seem to play a major role in activating adult muscle stem cells and improving muscle regeneration. And Wyss-Coray and colleagues reported this past May in *Nature Medicine* that infusions of "young blood plasma" reversed the neural and cognitive impairments of old mice—largely by rejuvenating the function of synapses. "Interestingly, none of us ended up with the same pathway in any of this," Wagers says.

Despite her team's impressive rodent results, Wagers suspects injecting GDF11 directly into patients would be "not ideal," because such an approach would bypass the tight biological regulation governing the molecule and perhaps increase the risk of side effects. "Minimally, we could use it as a biomarker for predicting outcome" and monitoring other treatments for age-related conditions, she says. Citing unpublished data on nearly 2000 elderly heart patients followed for roughly 9 years, for example, Peter Ganz of UC San Francisco and colleagues have reported at meetings that lower levels of GDF11 in the blood predicted higher rates of heart attack, stroke, congestive heart failure, and overall mortality.

Harvard has filed patents on the GDF11 work, and other groups are quickly moving their own young blood findings toward the clinic. Stanford doctors plan to begin transfusing plasma from young blood donors into patients with Alzheimer's disease, and Conboy says clinical testing of oxytocin, already a Food and Drug Administration–approved drug, and other blood-borne factors are under discussion by UC Berkeley scientists.

Wagers is acutely aware of the dangers of going overboard about GDF11's promise. But she has also been chastised, on the most personal level, for being too negative about potential therapies. Ten years ago, when one of her "negative" studies shot down the notion that bone marrow cells could treat heart attack victims, she got an earful from an outraged heart attack patient: her father. "He was angry at me for publishing that work. That kind of helped me understand the depth, the real power of hope in that way," Wagers recalls.

"You want to be enthusiastic about the potential, the real potential of the science," she continues. "On the other hand, you have to turn around and say, 'But not yet.' It's just a really hard message—to be clear that the hope is real, but that it will take time, and we can't tell you the path to get there." ■

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