

Purified Blood Stem Cells Show Promise for Stage 4 Breast Cancer



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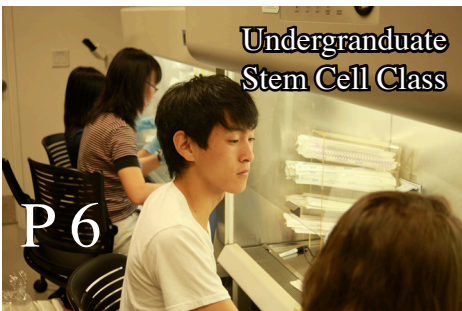
Survival of stage-4 breast cancer patients improves with stem cell treatment, study finds

A new long-term study of women with stage-4 breast cancer at the Stanford University School of Medicine is likely to revive a decade-old debate about high-dose chemotherapy as a treatment option. Specifically, researchers found that a greater proportion of patients who received the aggressive treatment 12 to 14 years ago, followed by a rescue with their own, specially purified blood stem cells that had been purged of cancer, survived compared with those who were rescued with unmanipulated blood grafts.

The study, although small, is the first to analyze the long-term outcomes of women who received their own (autologous) stem cells that had undergone this purification process. While high-dose chemotherapy followed by autologous blood stem cell transplantation was largely discarded at the end of the 1990s — interim analyses of several then-ongoing phase-3 clinical trials suggested it produced no better outcomes

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High-dose chemo and purified stem cells

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than other forms of treatment — women in this report received blood stem cells that had been prepared very differently.

“Most people in the oncology community feel that this issue is a done deal, that high-dose chemotherapy does not work for patients with breast cancer,” said associate professor of medicine, Judith Shizuru, MD, PhD. “But our study suggests that the high-dose therapy strategy can be modified to include the use of cancer-free purified blood stem cells to yield better overall outcomes in women with advanced breast cancer.”

Shizuru is the senior author of the research, which was published online last week in *Biology of Blood and Marrow Transplantation*. She and the study’s first author, Antonia Mueller, MD, along with hematologist Robert Negrin, MD, chief of Stanford’s Blood and Bone Marrow Transplant Program, followed the outcome of a number of women with metastatic breast cancer who enrolled in the mid- to late 1990s in a small phase 1-2 study to assess the effectiveness and feasibility of using highly purified stem cells from circulating blood, instead of an unmanipulated blood graft, for transplantation. The women in the study were treated at either Stanford Hospital & Clinics or the Barbara Ann Karmanos Cancer Institute in Detroit. At present, women with metastatic breast cancer have few options. “For over 10 years, women with metastatic breast cancer have not been offered high-dose chemotherapy treatments,” said Shizuru, who is also a member of the Stanford Cancer Institute. High-dose chemotherapy is considered to be an aggressive treatment because, in addition to killing cancer cells, it also destroys a patient’s blood forming system. Therefore, such patients need to be rescued with stem cells that can restore blood production, which includes red blood cells, platelets and infection-fighting white blood cells. To increase the proportion of blood-forming stem cells in the bloodstream patients routinely receive drugs that “mobilize” the stem cells out of the bone marrow into the blood.

Unfortunately, studies by many groups have shown that cancer cells often stowaway in the blood as well and may cause an eventual relapse.

As a result, in the mid-1990s Stanford researchers headed by professors of medicine Karl Blume, MD, Robert Negrin, MD, and professor of pathology Irving Weissman, MD, wondered if there was a way to overcome this problem. They opted to use antibodies that recognized newly identified markers on the surface of the blood stem cells to purify the stem cells away from regular blood and any roving cancer cells. They then used this purified population of stem cells in 22 women with metastatic breast cancer who enrolled in the trial from December 1996 to February 1998. Then they waited as the years passed.

Last year, Mueller and the research team began to compare the progression-free and overall survival of their experimental group to those of a group of 74 women who received identical chemotherapy treatments between February 1995 and June 1999 but who received unmanipulated, mobilized peripheral blood.

Although the overall numbers are small, the difference in survival 12 to 14 years after therapy is stark: Five of the 22 women (23 percent) who received the purified stem cells are still alive, four of whom have no sign of disease. Their median overall survival was 60 months. In contrast, just seven of the 74 women (9 percent) who received the untreated cells are living, five of whom have no sign of disease. Their median overall survival was 28 months.

“Even with this small sample size, this paper demonstrates much-better overall and progression-free survival in those patients who received cancer-free stem cells,” said Weissman, the Virginia & D.K. Ludwig Professor for Clinical Investigation in Cancer Research at the medical school and co-author of the paper. “It is important to use these findings as a basis for future trials not only for breast cancer, but also other cancers in which autologous transplants are used to enable high-dose chemotherapy.”

One of the institute's own succumbs to cancer

Angela Lee Riepel, PhD

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The institute is mourning the loss of our friend and colleague, Angela Lee Riepel, PhD. Angela died August 29th, 10 years after being diagnosed with a gastrointestinal stromal tumor or GIST. For over the past year, Angela was the grant writer for Dr. Irv Weissman's lab where she wrote grants, evaluated applicants and helped edit manuscripts and fellowship applications for students and postdocs, but she has been an integral member of Stanford's scientific community since 1991 when she entered the PhD program in Cancer Biology. Angela did her thesis work in the laboratory of Dr. Jane Parnes where she studied the effects of CD45, BSAP and CD8 α on thymocyte development. After earning her PhD, she went on to a postdoctoral position in the lab of neurobiologist Dr. Robert Sapolsky in the department of Biology where she investigated stroke and seizure therapeutics. After completing her postdoc, she continued on in the Department of Biology working with Dr. Robert Simoni as a lecturer and coordinator of the Undergraduate Honors Program.

It was during her postdoc that she was first diagnosed with GIST. She was treated surgically and with molecularly targeted therapies that had a remarkable response that nearly eradicated her large tumor burden. Her tumor ultimately became resistant to the drug and Angela went on to participate in numerous clinical trials in an effort to identify a new treatment that would eliminate her tumor once and for all. Most of these trials were conducted in Boston and required Angela and her husband Rob to make multiple trips and often stay for weeks or months at a time away from family, friends and work.

Throughout the time since her diagnosis, Angela remained committed not only to returning to health, but to science, work, family and friends. She volunteered her time and energy in many



Angel Lee Riepel, PhD

ways, including being a member of Stanford's Institutional Review Board, as well as serving as scientific advisor and writing articles for the GIST Support International and Life Raft GIST support groups and teaching scientific experimentation to schoolchildren. As recently as July, Angela was still riding her bike, going to the gym and cooking gourmet dinners with friends. She always kept in touch with old friends and brought people together. She was a force to be reckoned with. During one holiday season as a graduate student, she got everyone in the lab that played an instrument to bring it in and organized caroling in the atrium of MSLS. Angela always had a positivity about her; she just refused to be negative, refused to give in. If you said something negative about yourself in her presence, she would admonish you not to. Whenever something bad happened to a friend or colleague, Angela was quick to act, reach out to that person, ask them to lunch. She never shied away from people in pain. She was always there to ease it with her warmth and candid kindness.

Angela enjoyed being back at work at the Institute in the Weissman lab where she could continue her scientific career. She loved talking and writing

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Angela Lee (continued from previous page)

science, fostering collaborations and mentoring trainees. Irv Weissman credits Angela with being the inspiration to begin studies using anti-CD47 for GIST. "That research will go on in her memory," he says. "She was selfless. No one ever knew she was in pain. She was brave in the face of it. She often did her work from clinics or hospital beds. She didn't slow down in her work even the week that she was dying. She was a strong person whose life was cut way, way too short."



A service for Angela was held in Stanford Memorial Church

Below are additional comments from Angela's other mentors here at Stanford illustrating the broad impact she had and the depth of the love and admiration she engendered.

Jane Parnes, Angela's PhD advisor:

"I always admired Angela, and in particular, her fierce independence, determination, and optimism. As a graduate student, she came into the lab with a desire to work on a project that she had chosen independently and was not bothered by the fact it was a new direction for the lab. Angela always knew what she wanted to do and had the initiative and self-confidence to strive for her goals even when others would have given up."

Bob Simoni, Chair of the Department of Biology:

"Angela was liked, respected and admired by everyone with whom she interacted; students, staff and faculty. In her Lecturer position, she

helped students navigate the requirements for Honors research from finding a faculty mentor to completing their Honor thesis. During her tenure in Biology, she was often exhausted and frail from treatments for her illness but never complained or failed to meet her responsibilities. Angela will be greatly missed not just as a terrific staff member but as an inspiration to all who encounter life's challenges."

Robert Sapolsky, Angela's postdoctoral advisor:

"Angela Lee joined my lab in 1999 and spent three years in it as a post-doc, her first foray into being a neurobiologist. While in the lab, she did some superb work on neuronal gene therapy. Specifically, she designed viral vectors to overexpress activity-dependent potassium channels; these hyperpolarized neurons, blocking their excitability and buffering them from the damaging effects of seizures. In the years following her diagnosis, amid working for the Bio Department and then in the Weissman lab, Angela continued an association with my lab. In the periods when she felt strong enough, she carried on her research, producing a paper in 2010. As her energy flagged, she came in to help, providing extra hands for someone in a marathon of assays, teaching techniques to new undergrads, editing people's papers.

"Those activities reflected so much of who Angela was – the tenacity with which she fought for her health, her urgent need to help and contribute and avoid becoming merely her disease, her mind-boggling resilience. Angela and the battle she put up were both extraordinary.

"Angela taught us a huge lesson – to recognize just how much grace humans can achieve in the face of a never-ending nightmare. And she gave us a huge gift – the constant reminder of why we do what we do, the reminder that our job is to find ways to keep people from being taken from us too soon. Angela will be deeply missed."

A memorial service was held Thursday, September 15, 2011 in Stanford Memorial Church.

Discovery may eliminate potentially lethal side effect of stem cell therapy

With the right combination of growth factors, skill and patience, the laboratory tissue culture dish promises to yield stem cells for any type of tissue. But within these batches of newly generated cells lurks a big potential problem: Any remaining embryonic stem cells — those that haven't differentiated into the desired tissue — can go on to become dangerous tumors called teratomas when transplanted into patients.

ISCBRM researchers have developed a way to remove pluripotent human embryonic stem cells from their progeny before the differentiated cells are used in humans.

"The ability to do regenerative medicine requires the complete removal of tumor-forming cells from any culture that began with pluripotent cells," said Irving Weissman, MD, director of the Stanford Institute for Stem Cell Biology and Regenerative Medicine.

"We've used a combination of antibodies to weed out the few undifferentiated cells that could be left in the 10 or 100 million differentiated cells that make up a therapeutic dose."

Weissman pointed out that the production of therapeutic cells from pluripotent stem cells for regenerative medicine was a major goal of Proposition 71, the ballot measure that established the California Institute for Regenerative Medicine to allocate \$3 billion to advance stem cell science. CIRM funded this research.

The scientists believe the technique could also be used to remove residual tumor-initiating cells from populations of cells derived from induced pluripotent stem, or iPS, cells. These cells may also be useful for therapy but, unlike embryonic stem cells, iPS cells are created in the laboratory from adult tissue.

"Commonly used differentiation protocols for embryonic stem and iPS cells often give rise to mixed cultures of cells," said research associate Micha Drukker, PhD. "Because even a single undifferentiated

cell harbors the ability to become a teratoma, we sought to develop a way to remove these cells before transplantation."

Drukker is the senior author of the research, published online Aug. 14 in *Nature Biotechnology*. Stanford medical student Chad Tang is the first author.

Weissman, who is also the Virginia and D.K. Ludwig Professor for Clinical Investigation and Cancer Research and a member of the Stanford Cancer Institute, is a co-author. The research was conducted in his lab.

Teratomas are the Frankensteins of the tumor world — a hodgepodge of tissues like teeth, hair and bone. They owe their remarkable composition to the fact that the cells from which they arise early in development are pluripotent. In fact, the ability to form teratomas in animals is a defining feature of true pluripotent cells. But the very feature that confirms a cell's pluripotency also makes it potentially dangerous to use therapeutically. That's why Tang, Drukker and Weissman decided to try to develop an antibody that would recognize and bind to only pluripotent cells and enable their removal from a mixture of cells. Although a few such antibodies already existed, they were not specific enough on their own to completely weed out the tumor-causing cells.

The researchers found one newly generated antibody that was highly specific for a previously unknown marker on undifferentiated cells that they termed stage-specific embryonic antigen-5, or SSEA-5. Combining anti-SSEA-5 with two other antibodies known to bind to pluripotent cells completely separated the pluripotent from the differentiated cells, although the researchers did see some smaller, less-diverse growths in some cases.

A longer version of this article can be found at <http://stemcell.stanford.edu>

Stem cell class gives undergrads hands-on experience

The first year of college is always a whirlwind of experiences. But after spring quarter, eleven Stanford freshmen went home for summer with something more to share than dorm room shenanigans and late-night cram sessions. They did something relatively few scientists have done: they created beating heart cells out of human embryonic stem cells.

“It was amazing,” said freshman bioengineering major Javier Guinard. “Getting to work with human embryonic stem cells in the laboratory was a great experience. You can learn from lectures, but sitting at a lab bench with the cells in your hands gives you a really good glimpse into what a scientist’s life is like.” The hands-on laboratory experience Guinard and his classmates gained was part of a new curriculum developed by experts at the School of Medicine that is designed to transform students with little or no scientific background into well-trained potential workers fluent in the science, ethics and legal aspects of stem cell biology.

“We wanted to know whether we could fully immerse the students in stem cell research in a way that integrated biology with practical instruction like lab work, while also talking about the ethical and legal dimensions of what they were doing,” said bioethicist and course designer Christopher Scott. “I think we demonstrated that you can design a course that contains both hard science and the humanities that will give students practical skills they can use in a future career.”

The class, Medicine 83Q, was called “Ethical, Legal and Social Dimensions of Stem Cell Research” and was offered by the Office of the Vice Provost of Undergraduate Education as one of Stanford’s Introductory Studies for freshman and sophomores. Scott, who directs the medical school’s Program on Stem Cells in Society, received \$500,000 from the Course, Curriculum and Laboratory Improvement program of the National Science Foundation in 2009 to design the curriculum as part of his grant, “Workforce training for stem cell research.”

The curriculum will eventually be targeted to a slightly older group of students: those who have finished their bachelor degrees in a biology or non-biology-related science field and who want to gain an understanding of the basic biology of stem cells and how to work with them in a laboratory.

But Scott and other stem cell researchers involved in the class first wanted to try out the program close to home, and Stanford undergraduates were the ideal test subjects.



In addition to Stanford, the curriculum was also piloted at City College of San Francisco, San Francisco State University and Middlesex Community College in Massachusetts.

The Stanford course was unusual because it incorporated a laboratory component in which the students each used the human embryonic stem cell line H9 to differentiate them into beating heart cells. When they got back to the classroom, they discussed not only the scientific reasons why the stem cells had differentiated into particular cell types, but also when and how the H9 cell line was derived, how its federal funding status has varied during the past several years and ethical considerations surrounding how consent was obtained from the individuals who donated the embryos from which stem cell lines are created.

“Most of us came in with an interest in stem cells, but didn’t know much about them,” said freshman bioengineering major Shaheen Jeeawoody. “By the end of the class, I had learned exactly what they are. And the ethics and policy debates encouraged us to see the issues surrounding human stem cell research from a variety of perspectives.”

A longer version of this article can be found at <http://stemcell.stanford.edu>