# Brain surgeons turn to basic science to fight childhood brain cancer

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Teresa and Jamie Purzner came to Stanford to study medulloblastoma and search for a way to better treat the brain cancer. They faced many challenges, including the myriad difficulties of escorting a basic science finding through preclinical studies to testing in humans. Credit: Ethan Hill

In 2012, a pair of neurosurgery residents traded their scrubs for lab coats

in an effort to understand, at the most basic level, what causes medulloblastoma, the most common pediatric brain cancer.

Teresa Purzner, MD, Ph.D., is a hands-on, all-in physician.

As a neurosurgical resident at the University of Toronto in 2009, she rotated through a variety of specialties, including one in pediatric neurosurgery. During the three months of her pediatric rotation, she dealt with desperately ill children and their parents on a daily basis. Often, she participated in conversations that involved delivering bad news—telling parents, for example, that their child, suffering from a deadly brain tumor called a medulloblastoma, might have a chance of being cured, but the chemo and radiation treatment was likely to cause permanent cognitive and neurological damage.

"It is a devastating conversation," she recalled. "You're basically delivering, and living in, every parent's worst nightmare. One mother who lost her son shortly after diagnosis told me that she was thankful not to have had to put him through the treatment we were recommending."

When it all got to be too much, she and her husband, Jamie Purzner, MD, also a neurosurgical trainee, just walked away from their residencies. Frustrated with the challenges of treating children with the tumors, they put their clinical careers on hold to tackle the root of the problem: the cells in the brain that run wild during the tumor's development.

"A few brief conversations with the experts around us really sealed the deal," Teresa Purzner said. "It was clear that medulloblastoma was a tangible and interesting problem, and that amazing strides had already been made in understanding the link between developmental biology and medulloblastoma development."

The time was ripe, they felt, to bridge the gap between this new, conceptual understanding of the disease and the desperate need they'd witnessed in the clinic. But they needed to find the right place to do the necessary research.

The pair considered hundreds of laboratories in the United States and Canada. But rather than seeking out labs and investigators experienced in translating existing research results into clinical applications—a bench-to-bedside approach—they focused on laboratories drilling into the nuts and bolts of biological processes.

"Basic science is where fundamental discoveries occur," Teresa Purzner said, "and basic science can tell you whether a specific potential treatment is likely to be successful. But basic scientists often underestimate their value. They are at least as well-positioned as clinicians to figure out what the best target is from a biological perspective."

## Trading scrubs for lab coats

The couple shed their scrubs for lab coats in 2012. During the next six years, they worked as graduate students in the Stanford School of Medicine's Department of Developmental Biology to understand, at the most basic level, what causes the brain tumors.

Their unconventional career rewind has been uncommonly successful. The pair identified a potential new drug treatment for the disease, and with the support of Stanford SPARK—a program launched in 2006 to advance promising discoveries from the lab to the clinic—Teresa Purzner went on to test it in mice and to coordinate the launch of a phase-1 clinical trial that recently began enrolling patients. The two published their findings in *Science Signaling* in September 2018.

"This was completely unbiased discovery science," said developmental biologist Margaret Fuller, Ph.D., who advised the Purzners during the latter part of their graduate work. "Teresa and Jamie used an unbiased screen to identify a new component of a well-known developmental pathway, identified where in the pathway it functions and then showed that blocking this step can kill medulloblastoma cells implanted into mice. It's a remarkable achievement."

Along the way, the pair faced many challenges, including the myriad difficulties of escorting a basic science finding through preclinical studies in animals to testing in humans. Teresa Purzner took charge of marshaling support from funding agencies, national research consortiums and drug companies often wary of the fraught arena of clinical trials that enroll terminally ill children. The couple also started a family; their three children were born during their graduate school careers.

Not bad for some seemingly misplaced neurosurgeons.

"There are 101 valid reasons to not do what we did, and 100 more reasons why we should have failed once we decided to do it," Teresa Purzner said. "But we benefited from an amazing cast of collaborators at Stanford and elsewhere who spent hundreds and hundreds of hours helping to overcome many hurdles in the path to this trial. We were all very dedicated to doing everything possible to help these kids."

## In the lab (and home) of Matthew Scott

Matthew Scott, Ph.D., now professor emeritus at Stanford, was a developmental biologist in January 2012 when he received an email from Jamie Purzner inquiring about research positions. Scott was taken aback. "It's not often that neurosurgeons want to come train in my lab as graduate students," he said. "I thought, 'These people are doctors; they

don't really want to do research full time.' It was totally unbelievable, and unprecedented, for people with their training and skill."

Scott, who is married to Fuller, is known for his 1984 discovery in fruit flies of a short DNA sequence called a homeobox. Homeobox genes coordinate the activities of sets of other genes, acting within cells or groups of cells to control development. Proteins made from homeobox genes bind to specific DNA sequences throughout the genome to control genes used during early embryonic development to determine body patterning—ensuring that the wings, legs and abdominal sections fall neatly into place to generate the tiny flies drawn to the overripe fruit on your kitchen counter.

At first blush, none of this seems like something that would have especially interested pediatric neurosurgeons intent on discovering a new cancer treatment. But Scott also was known for identifying and studying signaling systems that allow groups of cells to communicate with one another during development —an area with more obvious relevance to the couple's interest. Mutations in some of these pathways, Scott found, are linked to the development of some types of cancers, including medulloblastoma.

In the early 1990s, the Scott lab began working on an important system called hedgehog signaling—first in fruit flies and later in mammals. It's named hedgehog because a mutation in the gene for a key protein in the signaling system results in fruit fly embryos that are spiny, like hedgehogs.

The hedgehog protein is produced and secreted by particular cells in the fruit fly embryo. When it binds to a receptor protein called patched on the surface of a cell, a cascade of activity is triggered that begins with proteins on the cell surface and ends with other proteins entering the nucleus. There, they stimulate the process by which genes lead to the

production of proteins that govern how cells multiply and develop. In the absence of hedgehog binding, patched keeps the pathway turned off.

In 1996, Scott's research into the pathway revealed that mutations in patched are often found in people with an inherited condition associated with frequent skin cancers and skeletal abnormalities called basal-cell nevus syndrome. When patched is missing or mutated, the hedgehog pathway is constantly active, and the cells receive ongoing signals to grow and divide. Carriers of patched mutations not only develop frequent basal-cell skin cancers, but they also often develop medulloblastomas.

The Scott lab then showed that during normal development, hedgehog signaling triggers growth of the cerebellum, the portion of the brain at the back of the head near the spinal cord. Loss of patched lets the normal growth signal happen when it should not. This explanation for the development of medulloblastomas piqued the Purzners' interest.

But choosing to come to America to work as graduate students put the Purzners in a funding gray area. They could no longer apply for grants meant to support Canadian clinicians doing research in Canada. They also couldn't qualify for funding meant for Americans. Although they eventually secured enough money to support themselves and their growing family, including funding from Stanford Bio-X, their financial stability was far from certain when they arrived at Stanford in June of 2012 in a truck packed with boxes they loaded the day after their last neurosurgical calls ended.

"We had no American bank account or credit cards and no plan for where we were going to live," Teresa Purzner recalled. "Matt and Margaret realized this and insisted we live with them until we found a place to rent."

Teresa Purzner with developmental biologist Matthew Scott, whose lab she and Jamie Purzner worked in. Timothy Archibald

## Most common childhood brain cancer

About 350 people a year in the United States are diagnosed with medulloblastoma, which develops in the cerebellum. It is the most common brain cancer in children. Even with the best treatments, only about 70 percent will live five years or more after their initial diagnosis and the prognosis for those who experience a recurrence is dire.

Frequently, the tumors spread to other parts of the brain and central nervous system. Treatment options are bleak and include whole brain and spinal radiation in combination with chemotherapy for as long as a year. Children are particularly susceptible to damage from these therapies because their brains are developing.

About 25% of all medulloblastomas are caused by mutations in genes for proteins involved in the hedgehog pathway, including patched. Although drugs that inhibit the pathway can often temporarily shrink tumors in patients, the cancer cells rapidly become resistant to the treatment when the cells develop mutations that reactivate the pathway. Targeting the very last step—the moment when the proteins reach the nucleus and bind to the DNA to turn genes on—should leave the cancer cells fewer options to wiggle out of the treatment, researchers believe. But how to do that?

The Purzners focused on the granule neuron precursor cells in the brain that give rise to hedgehog-associated human medulloblastomas. In mice, GNPs rapidly multiply between day one and day seven after birth in response to hedgehog pathway signaling. Between day seven and day 14, the proliferation rate slows and the cells begin to become granule neurons. After day 14, any remaining GNPs mature into granule neurons, which are the most common type of neuron in the brain.

Occasionally, however, GNPs ignore the normal developmental signals and keep multiplying after day 14. This increases the chance that the cells will accumulate additional mutations and become cancerous. Learning why this happens might be the key to stopping the rapid increase in medulloblastoma cells, the Purzners reasoned.

A chance encounter with Joshua Elias, Ph.D., assistant professor of chemical and systems biology, whose laboratory was one floor above Scott's, gave the Purzners an idea of how to start. The Elias lab focuses

on proteomics—the study of all aspects of proteins in a cell or tissue to learn how cells and tissues develop and function.For example, a cell often adds or removes small chemical tags, called phosphate groups, from proteins to control their function. For example, a phosphate tag in one location on a protein may cue it to bind to a second protein, move to another part of the cell or latch onto DNA to activate certain genes, whereas a tag in a different location on the same protein could trigger another set of biological outcomes. Conversely, removal of these phosphate groups can quickly inhibit the protein's activity.

The cell's ability to toggle a protein's activity in this way allows the cell to react quickly and appropriately to changing conditions or developmental stages. For researchers, the ability to chart changes in the patterns and locations of phosphate tags across a panel of proteins over time can provide an intimate look at the workings of a cell during development or disease progression.

## Combining expertise

Teresa Purzner decided to compare the pattern of phosphate tags, or protein phosphorylation, on GNP proteins isolated from the brains of newborn mice at day seven with those of GNPs isolated at day 14 and day one. Jamie Purzner, in contrast, focused on sussing out changes in which proteins are produced at different cell stages. Although Teresa Purzner's approach yielded more immediately promising results, they remained closely involved in each other's projects.

"It was pretty darn fun combining our expertise and thinking over the problems together from two perspectives," she said.

The Purzners found that the protein phosphorylation pattern of the rapidly dividing day seven GNP cells more closely resembles that of medulloblastoma cells than that of GNP cells on day one or day 14.

Further detective work homed in on a phosphate-adding protein called CK2 that is likely responsible for many of the phosphate-tagging events observed in day seven cells—including some that are critical to the last steps in the hedgehog pathway.

Blocking CK2 activity in mice during days three to seven left the animals with significantly fewer granule neurons than control animals had, the Purzners found. Furthermore, a CK2 inhibitor slowed or stopped the growth of mouse medulloblastoma cells implanted in mice—even cancer cells resistant to other hedgehog pathway inhibitors.

"We'd put these angry medulloblastoma cells into the flanks of mice and see complete tumor regression when CK2 was inhibited," Teresa Purzner said. "When we transplanted the medulloblastoma cells into the cerebella of mice, we found that, although the control animals had to be euthanized within 17 days due to cancer progression, 43 percent of mice treated with a CK2 inhibitor for 30 days lived past 100 days—basically until the experiment was terminated."

"This was astonishingly effective," Scott said. "The kinase acts very late in the hedgehog pathway, so it's difficult for the cancer cells to mutate around it. It's really a triumph of the application of basic science. The Purzners didn't start off looking for a protein involved in the hedgehog pathway. But once they did, Teresa Purzner embarked on shepherding this finding all the way from a basic science investigation to preclinical tests that have now launched a clinical trial."

Getting to the clinical trial wasn't easy, however.

## What to do next

"I had this beautiful, targeted small molecule inhibitor of CK2 that works in animals," Purzner said. "But I had absolutely no idea how to go

from there to get it to patients. This was far outside my realm of experience."

Enter Stanford SPARK. The program matches academic researchers with volunteers from the pharmaceutical, biotech and financial industries to streamline drug development and make it faster and cheaper. SPARK was founded in 2006 by Daria Mochly-Rosen, Ph.D., professor of chemical and systems biology at Stanford, who co-directs the program with Kevin Grimes, Ph.D., professor of chemical and systems biology.

"They started setting me up with world leaders—experts in every part of the drug development process to help me understand step-by-step what would be required to go from my discovery in the lab to a patient in the clinic," Purzner said. "It went from a seemingly impossible task to something difficult but achievable. And then we started just tackling each milestone one after the other."

Important steps included convincing a Taiwanese company called Senhwa Biosciences Inc., which was producing the only human-tested CK2 inhibitor, CX-4945, for use in a trial of basal-cell carcinoma, to agree to provide their drug for a pediatric clinical trial. Purzner was also able to secure the involvement of the Pediatric Brain Tumor Consortium, formed by the National Cancer Institute to improve the care of children with brain tumors across the country.

The FDA approved the phase 1-2 clinical trial of CX-4945 in children with hedgehog-pathway dependent medulloblastoma on Jan. 4, and the consortium's Central Institutional Review Board signed off on Feb. 28. The study opened on March 1.

## 'An absolute triumph'

"It's so exciting," Purzner said. "This took hundreds of hours and dozens

of people to accomplish because in many ways it was not a typical trial to put together. There were at least two or three times I thought, 'This could be the end. All of our work could be for nothing, and these kids are never going to get to see this drug.'"

Scott said, "This was an absolute triumph of the translation of a series of basic scientific discoveries into a clinical trial. In 1980, we identified the first mutations in hedgehog and patched in fruit flies.

"Sixteen years later we reported a connection with cancer; 16 years later we had our first FDA-approved drug targeting the hedgehog pathway in basal-cell carcinoma. So it took 32 years from pure, curiosity-driven 'Huh, that's interesting'—when we found some genes that control patterning in fly larva—to a point where patients were being treated. Now, 32 years is either way too long, or not too bad in the big picture of drug development. But Teresa did it in five," he said.

The Purzners have returned to Canada to complete their neurosurgical residencies.

It remains to be seen whether the CX-4945 will be safe and effective in children with hedgehog-dependent medulloblastoma. A success in mice doesn't always translate to humans. But Teresa Purzner'sintensive approach to solving the problem has led to a promising new target in the field.

"Having my own children gave me a very sobering perspective about what these families are going through," she said. "I didn't fully grasp just how heart-wrenching it would be to have a child with a serious medical issue until I had my own children. Getting to this clinical trial has been very emotional. And I'm not an emotional person. It is just such a huge relief to get to this point and know that I did what I came to do."