THE CONSUMMATE NEURO-ONCOLOGIST

Michelle Monje's teenage project to aid the disabled led her to neurology and a research career that's bringing new hope for the treatment of childhood brain cancers and the mind-fog caused by chemotherapy.

Competitive figure skating was once a big part of Michelle Monje's life. By the time she was in middle school in the Bay Area of San Francisco, Monje was squeezing in as many as 35 hours of practice every week at the rink. Then her mother, who'd started at IBM in the late 60's as a computer programmer and worked her way up to the executive ranks, had a little chat with her. "She pointed out that dedicating that much time to a sport was great," Monje recalls, "but perhaps I should also think about how I'm going to be productive and contribute to the rest of the world." Just 13 at the time, Monje mulled the matter for a spell and came up with a precociously fitting answer: She created a figure skating program for children with Down syndrome.

The experience left Monje, who is today a researcher at the Ludwig Center at Stanford and a pediatric neuro-oncologist at Stanford University's School of Medicine, with an abiding interest in neurology. In keeping with her mother's advice, Monje has over the past quarter century made significant contributions to our understanding of the brain's postnatal plasticity and the neurological disorders caused by cancer therapies. She has also led the charge against a swiftly lethal childhood cancer of the brainstem known as diffuse intrinsic pontine glioma (DIPG), charting new approaches to the treatment of the long-neglected cancer and other high-grade gliomas that she is now—or soon will be—evaluating in clinical trials.

In 2018, Monje and her colleagues reported in *Cell* their dissection of the cellular interactions underlying an enduring fogging of the mind often caused by chemotherapy and identified a potential treatment for its mitigation. In another study, done in collaboration with Ludwig Stanford researcher Crystal Mackall and published in

MICHELLE MONJE

LUDWIG STANFORD

Photo by Flynn Larsen

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Nature Medicine, Monje and her colleagues applied an engineered immune cell therapy that, for the first time, almost eliminated DIPG in a mouse model of the incurable cancer.

"Ludwig funding has been really critical for my research program, as the flexible nature of the funding allows us to test new hypotheses and leads in real time, rather then needing to first write a specific grant proposal and then wait for the funding to do the work," says Monje. "This has allowed our research relevant to cancer stem cell biology to move forward more quickly than would otherwise have been possible."

Brainy pursuits

After completing high school in Danville, California, Monje went to Vassar College, where she initially planned to major in English. Her freshman advisor, neuroscientist Kathleen Susman, revived Monje's early interest in biology and neurology, beginning a long and cherished mentorship. Monje enrolled in many of Susman's courses and conducted research in her lab, even authoring a paper under her supervision. "Kate was enormously influential in my life," says Monje. "I really fell in love with the nervous system at Vassar."

In her first year of medical school at Stanford in 1998, Monje applied to the university's Neuroscience PhD program but deferred enrollment until she had completed her clinical rotations to ensure she still wanted to focus on neuroscience. The answer, she discovered, was yes. "Doing neurology and pediatric oncology in the clinic," Monje recalls, "I was really compelled by the patients I saw who were suffering from the long-term neurological side effects of cancer therapy."

Studies suggested that the cognitive decline associated with cranial radiotherapy, in particular, stemmed from neural dysfunction in the hippocampus, a region of the brain involved in emotion and memory. When she joined the Stanford PhD program, Monje asked Theo Palmer, who was studying neural stem cells in the hippocampus, if she could do her doctoral research in his lab exploring the phenomenon.

The project was a runaway success. Monje, Palmer and their colleagues reported in *Nature Medicine* in 2002 that the neural dysfunction was caused by changes in the hippocampal microenvironment induced by radiation. X-rays, they discovered, activated the brain's resident immune cells, or microglia, and the factors they secreted compromised the ability of stem cells to generate neurons. In 2003, Monje, Palmer and colleagues reported in *Science* that the anti-inflammatory drug indomethacin could restore hippocampal neurogenesis in mice after irradiation. Those findings laid the groundwork for clinical studies done by others that have since altered the delivery of cranial radiotherapy.

The initial discovery also challenged dogma.

It demonstrated that microglia aren't just defenders against microbial invasion but modulators of neural function as well—a novel idea at the time. "That was a really compelling concept for me," says Monje, "one that I have continued to study throughout my career: the way that different cells in the brain communicate and influence each other's ability to do their jobs."

Another experience in those years would shape Monje's career. While completing her continuity clinic requirement at Stanford under the supervision of pediatric neuro-oncologist Paul Fisher, she saw her first patient with DIPG, a nine-year-old girl. "It was astounding to me that we had no effective therapy for this cancer, that we knew so little about it even though it's one of the leading causes of childhood cancer-related death," she recalls. Monje decided that when she started her own lab someday she would work on the cancer.

Photo by Monty Rakusen

Glial groundwork

Getting her medical degree and PhD in 2004, and completing her internship the following year, Monje began her residency in neurology at a combined program of the Massachusetts General Hospital, the Brigham and Women's Hospital and Harvard Medical School. "I had wonderful mentors in neuro-oncology, and I considered staying there," says Monje. But Stanford's pull was stronger. Her husband, the prominent neuropsychiatrist Karl Deisseroth, whom she'd met in medical school, had accepted a job at their alma mater. Further, pediatric high-grade cancers that arise from glial cells—the collective term for cells that support, nourish and defend neurons—continued to fascinate Monje, and she was eager to learn more about their treatment under the guidance of her mentor Paul Fisher.

Monje returned to Stanford in 2008 to begin a fellowship in pediatric neuro-oncology and a postdoc with Phillip Beachy, who is also a member of the Ludwig Center. Beachy was investigating a cellular signaling pathway of importance to both neural development and the genesis of gliomas. Monje began addressing one of the biggest barriers to studying DIPG at the time: the lack of experimental model systems for the cancer. "There was very little tissue in the world to study," says Monje. "There were no cell cultures, no mouse models, and we knew nothing at the time about the genomic landscape of the cancer. It was really a black box."

Given its location, in a region of the brainstem known as the pons that controls several vital body functions, including breathing, the tumor was rarely biopsied. The tumor itself posed problems as well. "This isn't a golf ball in the middle of the brain," says Monje. "It is a diffuse, infiltrative disease that is intermingled with normal tissues." Monje's PhD advisor, Palmer, had

Photo by Flynn Larsen

pioneered techniques to culture normal stem cells taken from the brain a few hours after death, and Monje started by adopting those protocols for the generation of DIPG cultures.

She soon got a chance to put her procedures to the test. Among the first patients Monje encountered in her fellowship was a five-year-old boy with DIPG. As he neared death—most children diagnosed with DIPG die within the year—his family asked about donating his organs to medicine. Monje told them his corneas could be donated, and then asked if they might also consider donating his brain to science. They said yes. "As a parent, think about what that means," says Monje, who is herself a mother of four. "You're giving your child's brain to a researcher to study to help other children who get this disease in the future. It's amazing."

Monje established the first ever DIPG culture from that patient's cells in 2009 and immediately began sharing it with the small community of DIPG researchers. (Drug screens on DIPG cultures done by a consortium of researchers a few years later led to the identification in 2014 of a drug—panobinostat—that may slow DIPG growth. Monje, who led that study, is now overseeing a clinical trial testing the possibility.)

Examining pons tissue from a variety of noncancerous samples, Monje had noticed that a cell type known as the oligodendroglial precursor cell (OPC) was present in increased numbers exactly when these tumors tended

to emerge, most noticeably around age six. OPCs give rise to oligodendrocytes, which make the myelin that insulates neurons and gives the brain's white matter its color. That myelination continues well into the third decade of life, in distinctly timed and located waves. The cresting of those waves, Monje found, mapped neatly against the times and locations at which high grade gliomas arise in children. This suggested to her that DIPG arises from dysfunctional OPCs—a hunch now supported by multiple lines of evidence.

"That was an important thing to recognize at the start of my career," says Monje, "because I wanted to study not only the postnatal developmental processes that go wrong in glial malignancies, but also the normal developmental processes that might be central to the long-term effects of cancer therapy. Myelin biology appears to be central to both."

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Illuminating results

When Monje started her own lab in 2011, there was some debate about whether neurons regulate the myelination of their own axons—the thread-like projections that conduct signals between the cells. To find out, Monje applied a technique pioneered by her husband known as optogenetics, in which light is used to control the firing of specific neurons, causing minimal confounding damage to brain tissue. She and her colleagues reported in *Science* in 2014 that light-induced neural firing sent a brisk proliferative signal to OPCs, causing them to replicate and then differentiate into oligodendrocytes. These cells then remodeled the myelin structure in the active neural circuit—in this case the motor circuit of a mouse brain. Done repeatedly, this remodeling improved function of the limb controlled by that circuit, suggesting that the plasticity of myelination is essential to neurological function. That itself was a landmark discovery.

Next, Monje and her team repeated their experiment in mice bearing high-grade human gliomas in the same neural circuit. They reported in *Cell* in 2015 that neural firing drove the growth of a variety of glial malignancies, including DIPG. One of the key messengers of that proliferative signal, they found, is a version of a neural protein known as neuroligin-3.

In 2017, Monje and her colleagues reported in *Nature* that, upon neural firing, Neuroligin-3 is snipped by an enzyme in neurons named ADAM10, generating a secreted protein fragment that induces cancer cell proliferation. "We found that if we blocked that enzyme pharmacologically using drugs that are already in the clinical pipeline, we could robustly slow tumor growth in mice," says Monje. She is now working with two brain cancer consortia to test the potential

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therapy in clinical trials for all childhood high-grade gliomas and for glioblastoma in adults.

Demolishing DIPG

Monje has continued to refine her lab's protocols for obtaining DIPG cultures. As part of that effort, her graduate student examined whether some molecule on DIPG cells might be grasped with antibodies to improve their isolation from the sticky, myelin-rich tissue in which they grow. An antibody panel unearthed a complex sugar chain named GD2. The sugar, it turned out, is found at uncommonly high levels in the 80% of DIPG tumors driven by a mutation known as H3K27M.

As it happened, Crystal Mackall, a leader in the field of cell-based immunotherapies, had just arrived at Stanford (where she too is a member of the Ludwig Center). Mackall's lab had recently engineered immune cells—chimeric antigen-receptor T cells (CAR-T)—to target and destroy GD2-bearing cells, which are found on other tumors as well. Mackall immediately agreed to test her anti-GD2 CAR-T cells on Monje's DIPG models.

The results, reported in *Nature Medicine* in 2018, were unprecedented. Given systemically to the mice, the CAR-T cells traveled to the brain and tore into the DIPG tissue, leaving only a few dozen cancer cells in their wake. "I didn't believe it the first time because I'd never seen anything do that," says Monje. "I think it was the sixth time my poor graduate student and a postdoc in Mackall's lab did this experiment that I really believed the results. It was just night and day. It was incredible."

There are risks, of course. The ferocity of the CAR-T attack in the cramped precincts of the brain stem caused inflammation that killed some of the mice. Further, the survival of cells that do not express GD2 suggests even the CAR-T therapy may not promise a complete cure. But Monje and Mackall think the brain inflammation is clinically manageable and are now preparing a trial to evaluate the therapy for children with high-grade gliomas rich in GD2.

Clearing a fog

On Fridays Monje sees patients, primarily survivors of childhood cancer and kids with high-grade gliomas enrolling in one of her clinical trials. The former often have long term neurological issues caused by chemotherapy—anxiety, impaired attention and memory dysfunction. These sequalae remain a central focus of Monje's research.

After starting her lab in 2011, Monje had examined brain tissue from patients who had been treated with the chemotherapy methotrexate and noticed in them an abnormal depletion of OPCs. To study the phenomenon, she began constructing a

Photo by Flynn Larsen

mouse model to mimic the exposure and effects of the chemotherapy on the human brain.

Studies using that model revealed that methotrexate shifts microglia into an activated state for six months or more after its administration. This chronic activation, Monje and her colleagues reported in *Cell* in 2018, muddles the brain's microenvironment enough to compromise cells known as astrocytes, which help nourish and link neurons and generally keep things in balance. It also disrupts the ability of OPCs to replenish themselves and to differentiate into oligodendrocytes. "The cells were getting stuck between the precursor cell state and the mature, oligodendrocyte cell state," explains Monje. All this disrupted the myelination of neurons, compromising their function—which is why mice treated with methotrexate displayed many of the same behavioral symptoms seen in patients.

To see if the pathological cascade could be reversed, Monje and her colleagues gave the mice a CSF-1R inhibitor, a drug that selectively depletes microglia. The effects were striking: OPCs, astrocytes and the myelination of neurons all normalized, and the neurological symptoms dissipated in the mice. "This is very exciting because CSF-1R inhibitors are already in clinical trials, already being used in humans," says Monje. More preclinical work must be done before the treatment can be tested in patients undergoing chemotherapy, says Monje. On the other hand, she is excited to finally have a strategy to develop. In her hands, that strategy is more than likely to yield results.