One form of pediatric cancer has, over the years, resolutely resisted treatment. But one pediatric oncologist and a team of UVM researchers are focused on changing that fact.

the Neuroblastoma challenge

by JENNIFER NACHBUR

It is one of the shining successes of medical science in the last half-century: the immense growth of survival rates from childhood cancer. In the past 50 years, those rates have increased from 10 percent to nearly 80 percent, with cure rates varying according to cancer type. Nearly 100 percent of childhood leukemia cases, for example, are now curable. The chance of survival for brain tumor patients is now 50 percent. That trend, however, does not extend to neuroblastoma, an often-fatal cancer that affects very young children. The neuroblastoma challenge has become the focus of groundbreaking clinical and basic science research by physicians and scientists at the Vermont Cancer Center.

Every 16 hours, a child with neuroblastoma dies in the United States. A large majority of neuroblastoma cases — about 95 percent — occur before five years of age.

“In the U.S., there are about 700 new cases each year of neuroblastoma,” says Giselle Sholler, M.D., a pediatric oncologist and assistant professor of pediatrics who is leading a Phase 1 neuroblastoma treatment clinical trial at UVM. “Although it’s not a high number of patients, it’s a high number of deaths from cancer.”

Sholler explains that there are two “peaks” of neuroblastoma that can occur. The first, which occurs in infants less than one year old, has a 95 percent survival rate and sometimes regresses spontaneously, without treatment. The second is at the opposite end of the spectrum: Stage IV metastatic neuroblastoma.
The most common solid tumor in children outside of brain tumors, neuroblastoma develops in the cells of the sympathetic nervous system. Best known for its role in the body’s “fight-or-flight” response, the sympathetic nervous system includes a chain of nerves that runs from the cervical neck down to the pelvis. About two-thirds of neuroblastomas actually begin in the abdomen. Tumors typically originate in the adrenal glands or in the nerve cells in the sympathetic nerve ganglia — or cell clusters — in the abdomen. Other tumor origins can include the sympathetic ganglia of the chest or neck, or in the pelvis and, more rarely, in the spinal cord.

Children with metastatic neuroblastoma must undergo a rigorous course of treatment to address their advanced disease, which has usually metastasized to the bone marrow and sometimes the liver and bone as well. Following six rounds of very aggressive, intensive inpatient chemotherapy, a patient will have surgery to remove any remaining tumor — a challenge due to the invasive nature and location of this type of tumor. The next step in treatment is two autologous (self-donated) bone marrow transplants. Patients receive additional therapies, including retinoic acid and/or an antibody against neuroblastoma, after which therapy is stopped and the patient is considered in remission.

Unfortunately, 70 percent of patients are expected to experience a recurrence. After relapse, the disease is so aggressive that the chance of survival is less than five percent.

Despite daunting circumstances, the children and families confronted with neuroblastoma are remarkably resilient, hopeful and motivated. Two families in particular, the Londons of New York City, and the Hutchisons of San Diego, have waged a heroic and passionate effort to find new treatments for children with relapsed neuroblastoma. Both families found their way to Dr. Sholler, the Vermont Cancer Center (VCC) and UVM after hearing about her research at a medical conference. Via the neuroblastoma parents’ “grapevine,” they spread the word that the Londons of New York City, and the Hutchisons of San Diego, have waged a heroic and passionate effort to find new treatments for children with relapsed neuroblastoma.

Sholler, who is getting ready to submit these findings for publication. “We’re now looking at what is the best combination of chemotherapy to use with nifurtimox, and that will guide us in writing our Phase 2 trial, which we anticipate opening shortly after the Phase 1 study closes in the fall. We need to find effective treatments for children with relapsed neuroblastoma.”

Neuroblastoma researchers Rae Nishi, Ph.D. (left); Jennifer Straub, Ph.D. (above); Umadevi Wesley, Ph.D. (at right)

The goal, for this phase, is to identify the maximum safely-tolerated dose of nifurtimox.

Experimental treatment options for children with relapsed neuroblastoma are available through several research groups, including New Advances in Neuroblastoma Therapy (NANT) and the Children’s Oncology Group (COG). Most of the patients Dr. Sholler has been seeing on her Phase 1 trial have already received NANT-COG therapies.

Sholler maintains regular communication with the neuroblastoma parents’ consortium. She speaks with families via conference call every other Wednesday, often joined by several “special guests,” including leaders from companies investigating therapeutics and clinicians and scientists from other institutions who are interested in collaborating with her. Serving as moderator with parent Neil Hutchison, Sholler provides updates on the clinical and basic science research taking place at the VCC.

The researchers and parents work together to discuss new approaches that would benefit the children. Their goal, in addition to working to make the latest therapies available to kids, is to one day establish a neuroblastoma translational research program at the VCC.

In a step towards that goal, Sholler chaired the first “Developments in Neuroblastoma Research Symposium” at UVM in March, an event that attracted over 100 scientists, physicians, students and family members of neuroblastoma patients. While continuing to care for patients and run the Phase 1 trial, Sholler is also conducting basic science research, in collaboration with Marcus Rosenberg, M.D., Ph.D., assistant professor of pathology, Nicholas Heinz, Ph.D., professor of pathology, and Laurent Beard, M.D., Ph.D., assistant professor of gynecology/ oncology at Brown University, to gain a better understanding of nifurtimox’s potential as a treatment alone or in combination with chemotherapy, as well as designing and testing new therapies.

“ar in our laboratory mouse models, we have shown that nifurtimox treatment alone reduces tumor size,” says Sholler. "We’re now looking at what is the best combination of chemotherapy to use with nifurtimox, and that will guide us in writing our Phase 2 trial, which we anticipate opening shortly after the Phase 1 study closes in the fall. We need to find effective treatments for children with relapsed neuroblastoma.”

Rae Nishi, Ph.D., professor of anatomy and neurobiology, directs the Neuroscience Graduate Program at UVM and has served as a mentor to Sholler. An established expert in programmed cell death and cell-to-cell interactions during nervous system development, Nishi expanded her focus to include neuroblastoma and launched two pilot projects — one run by Sholler and the other by Jennifer Straub, Ph.D., a postdoctoral fellow whose graduate work at the University of Rochester centered on cell death in the nervous system during development. In 2006 Nishi convened a neuroblastoma research team, which collaborates on research and meets regularly to share data and related information. In addition to Nishi, the group includes Sholler, Straub and Umadevi Wesley, Ph.D., research assistant professor of microbiology and molecular genetics.
As a graduate student, Straub's research centered on nerve growth factor signaling through a receptor called TrkB, which belongs to a family of proteins called Trk receptors. One of those proteins — TrkB — is normally a brain-derived neurotrophic factor (BDNF), its bonding partner, make up two of the major biological markers found in aggressive neuroblastoma tumors in children. Although TrkB was present in these tumors, no one had ever seen it expressed during the development of the sympathetic nervous system.

Using a chick embryo model, Straub set out to determine whether BDNF/TrkB signaling plays a role in the development of the sympathetic nervous system. She observed molecular activity through the early stages of sympathetic nervous system formation and made a significant discovery: TrkB is present, but only for a very transient period during early development. Straub then removed the TrkB-positive cells and put them into culture with BDNF. The evidence showed that BDNF can stimulate rapid growth of the TrkB-positive cells in a manner similar to its role in neuroblastoma tumors. “That’s one of the reasons why they proliferate and become so aggressive,” adds Straub. Based on her findings, Straub hypothesized that elevated BDNF levels in the environment of developing sympathetic nerve cells and active TrkB receptors together can potentially lead to a neuroblastoma tumor. This past February, she, Sholler and Nishi published this in vitro work in the open source scientific journal BMC Developmental Biology.

Straub is currently testing the hypothesis that elevated levels of BDNF can stimulate proliferation in vivo by adding BDNF in chick embryos. To accomplish this, she windows the eggs and adds BDNF to the eggs at different stages of development. Another ongoing project entails creating a constitutively active — or constantly active — mutation in the TrkB protein in a chick embryo model. Nishi is leading newly-funded research to approach finding causes and cures for childhood cancers. Collaborators on the project include UVM scientists Felix Eckenstein, Ph.D., professor of anatomy and neurobiology, and Mercedes Rincon, Ph.D., associate professor of medical science and director of UVM’s Transplant/Knockout Mouse Facility, as well as researchers at New York University School of Medicine and the University of California at San Francisco.

“We’re proposing to make a new transgenic mouse — actually a “double” transgenic mouse — which will allow us to confirm whether or not turning on TrkB causes tumors to form,” says Nishi. “In these mice, my co-investigators and I will be able to turn the gene on at different times with doxycycline during the development of the sympathetic nervous system,” explains Nishi, “which will allow us to see when the cells are most susceptible to becoming cancerous.”

The fourth member of the neuroblastoma research team, Umadevi Wesley, Ph.D., studied the role of DPP4 (dipeptidyl-peptidase IV), a protease or protein found on the cell surface in normal and skin cancer cells, during her postdoctoral fellowship and as a research scientist at Memorial Sloan-Kettering Cancer Center. Wesley discovered that DPP4 was highly expressed in normal melanocytes — specialized cells in the skin — but in melanoma tumor cells, the protease was absent, suggesting that DPP4 is necessary to keep the cell in a normal phenotype. Without it, cancer cell growth is promoted and melanoma tumors occur.

The next phase of her research involved securing tumor samples from melanoma patients. First, Wesley isolated a DPP4 gene from a normal melanocyte, and then she put it into a vector — or gene transporter — to express the protein. “We wanted to find out what would happen if we restored the expression of DPP4 in melanoma cells,” explains Wesley. “When we did that, its effect was amazingly striking — the DPP4 turned the melanoma cells into normal melanocyte cells.”

The project traveled to Vermont when Wesley joined the UVM faculty in 2001. Aiming to determine whether DPP4 functions as a tumor suppressor, she then looked at neuroblastoma, which originates from the same neural precursor cells as melanoma cells. In neuroblastoma, neural crest stem cells (NCSC) fail to differentiate. When she reintroduced DPP4 into neuroblastoma cell lines, it induced the differentiation and cell death necessary to produce neuronal neuronal cells, further proving DPP4’s critical role and potential as a treatment target.

Neuroblastoma cells grow very fast, but unlike melanoma cells, they are also very angiogenic — they form large blood vessels which help the tumor invade other parts of the body. Funded through UVM’s Neuroscience Center of Biomedical Research Excellence grant, Wesley is currently using cell culture and mouse models to further understand the role of DPP4 in regulating growth factors, such as FGF (fibroblast growth factor) and SDF (stromal cell-derived factor), which is normally cleared by DPP4, but, when DPP4 is absent, plays a role in metastasis to other parts of the body, including bone marrow.

Wesley is also investigating the role of DPP4 as a tumor suppressor gene for neuroblastoma. In the near future, she will be using knockout mice without DPP4 and tissue samples from neuroblastoma patients to help confirm her hypothesis that DPP4 is the suppressor gene for neuroblastoma.

Nishi considers the transgenic mouse the key to connecting many of the projects in the neuroblastoma study group together and moving the research forward. The mouse will assist in understanding how cancer develops and will also be a new model in which current promising drugs from Sholler and Wesley’s labs can be tested to determine their efficacy in killing neuroblastoma cells. “We’re hoping we’ll progress as planned and be able to request additional funding from Alex’s Lemonade Stand in December,” says Nishi. “If we have the mouse and it behaves the way we expect it to, then we can go to NIH.”

So, day by day, the neuroblastoma challenge goes on: molecule by molecule in the lab, and patient by patient in the clinic. “It is these young patients, and their parents, whom we need to always keep in mind,” said Giselle Sholler at the opening of the “Developments in Neuroblastoma” conference this spring. “They live with this disease every minute of every day. It is for them that we’re here.” And it is for them, and with them, that their mission continues.