Paul Nabil Bustany Fund
for synovial sarcoma research
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NEWSLETTER

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The 2010 Istanbul Marathon: Sunrise Across the Bosphorus

Participants in the Istanbul Marathon (from back to front): Birna, Dannette, Bryndis, Thorarinn, Samir, Brynjar, Zeina, George, Kamran, Cyrus, Malene, Maria, Bryndis, Joy, Laufey, Anne, Asta, Halldor, Sigga, Steve, Dale, Renee, Christine, Cynthia, Eric, Claire, Rami, Sabrina, Apur. Not pictured: Asu, Emel, Gudrun, Jeff, Jon Pall, Katrin, Lisa, Maria, Mark, Rafik, Soha, Villi, Ylmaz.

The major fundraising event of 2010 was the Istanbul Marathon on October 17th with 42 PNB Fund participants who all paid their own way and were sponsored by over 250 donors.

The Istanbul Marathon is the only course in the world that includes two continents, Asia and Europe, in one race. As we gathered on a crisp October morning, we certainly felt a part of something special. There was the dream-like state of being awake and ready to run at 6 am. Even more, there was the joy of knowing that we were fulfilling Nabil's dream of bringing together his family and friends from around the world to Turkey.

We began the run on the Asian side of Istanbul and were soon crossing the Bosphorus Bridge, alongside 200,000 other participants, into the European side. We were met with a spectacular view, the white clouds filtering the morning sunlight across the sound, but it was only the beginning. We continued past many historic sites and across more bridges to the Golden Horn before the race ended in old Istanbul, where we crossed the finish line in the magnificent shadow of the Hagia Sophia.

Three of us ran the full marathon and the rest ran/walked 15k. For all of us, it was a triumphant day. On the fundraising side, we've raised $60,000 to-date, including donations from many people who are brand new to the Fund. It was also an inspiring experience. As one of our first-time marathon runners observed, "What I learned that day is that no matter how tired, worn out and exhausted you feel, you can always find a way to keep yourself going." These words remind us that, whatever the distance, participating in an event like the marathon is a test of one's mettle, faith, and perseverance.

The same can be said of the challenge to find a cure for synovial sarcoma. Research on this deadly cancer continues to be one of the least funded in the field. Nevertheless, a select group of doctors and scientists are making progress in genetic research and the PNB Fund continues to support their groundbreaking work. In February 2010, the Fund donated $45,000 to Dr. Marc Ladanyi's team at MSKCC and in June the Fund donated $30,000 to Dr. Mario Capecchi's team at the University of Utah. In the following sections you'll read their latest research reports—what's notable about their reports is not only the advancements they've made in synovial sarcoma research, but their optimism and enduring commitment to finding a cure.

The Istanbul Marathon was a remarkable event. Thanks are due to our organizers, Asu Okyay and Christine Bustany, to our runners, walkers and cheerleaders, and to all of those who continue to support our fundraising effort.

The Board Members of the Paul Nabil Bustany Memorial Fund for Synovial Sarcoma Research are: Rami Badawy, Christine Bustany, Laufey Bustany, Samir Ted Bustany, Alex Kaplan, Asu Okyay, Alex Rafal, Anthony Rizzo, and Sabrina Tom.

Oncogene Addiction in Synovial Sarcoma
by Malay Haldar, MD and Kevin B. Jones, MD

The readership of this newsletter need not be reminded of the critical need for novel therapeutic strategies to improve survival and reduce treatment morbidity for patients with synovial sarcoma. The Paul Nabil Bustany Fund for Synovial Sarcoma Research has graciously supported the Mario Capecchi Genetics Laboratory at the University of Utah Huntsman Cancer Institute for the last 3 years in efforts to develop genetically engineered mouse models for this lethal disease for the purpose of understanding the underlying molecular mechanisms and to develop and test novel therapeutic strategies.

Translocations are genetic abnormalities caused by rearrangements of parts between different chromosomes. These often lead to breaking and joining of unrelated genes residing within affected chromosomes resulting in formation of chimerical genes. Synovial sarcomas are associated with a specific translocation between chromosome X and 18 that leads to formation of a signature chimerical gene comprised partly by SYT gene from chromosome 18 and partly by SSX gene from chromosome X. The expression of SYT-SSX chimerical protein is specific to synovial sarcoma tumor cells and is not seen in any other tumor or normal cells of the body. This suggested that SYT-SSX fusion protein may be responsible for the induction of this tumor. Based upon this assumption, we previously developed a genetically engineered mouse model for this rare and lethal human disease by expressing the human SYT-SSX fusion protein in mouse muscle progenitor cells. These mice developed synovial sarcoma like tumors at an early age. The histology and molecular signature of the mouse tumors closely resembled the human counterpart. Additionally, we discovered that expression of the SYT-SSX fusion protein leads to tumors only when it is expressed within muscle progenitor cells of the engineered mice. Expression in other tissue type did not lead to tumor formation. This suggested that SYT-SSX fusion protein is an oncogene that induces synovial sarcoma when expressed in "permissive" cell types.

While the aforementioned studies demonstrated that SYT-SSX fusion protein could induce synovial sarcoma, it is not clear whether continued expression of this fusion gene is required for disease progression (as opposed to disease induction). Certain oncogenes are known to be required throughout the lifetime of a tumor cell, a concept known as "oncogene addiction". If the tumor cells

are dependent or "addicted" to a certain protein for their survival, it makes that protein a valuable therapeutic target. We are currently investigating whether synovial sarcoma cells demonstrate "addiction" to SYT-SSX fusion protein. In our original mouse model, we had the ability to express SYT-SSX in any chosen tissue type. However, once turned on, there was no way to modulate subsequent expression of SYT-SSX. To test for oncogene addiction, we needed a new mouse model where we can not only turn on expression of SYT-SSX to induce tumors, but also turn it off to see the effects of the absence of SYT-SSX on tumor progression. To develop such a model, we decided to combine two well-known tools in genetic engineering: Cre-LoxP based conditional systems and tetracycline-inducible system. The Cre-LoxP system ensures that SYT-SSX is only expressed in specific tissue type and not globally since global expression of SYT-SSX causes embryonic lethality. The tetracycline inducible system allows SYT-SSX expression only in the presence of the antibiotic doxycycline administered via food or water. By combining these two strategies, we developed a mouse model where SYT-SSX expression cannot only be targeted to specific tissue, but can also be modulated at any time via exposure to doxycycline.

Once we had these mice, our first goal was to induce synovial sarcoma by exposing them to doxycycline. This entails prolonged exposure to various concentration of doxycycline to figure out the optimal doxycycline regimen that will most consistently induce synovial sarcoma. Our results show that tumor induction by doxycycline is highly dependent upon the age of the mouse when such exposure is begun as well as the dosage of doxycycline. We have now successfully optimized the timings and dosing of doxycycline regimen that leads to tumor formation in nearly 100% of the mice. Analysis of the tumors generated demonstrated high degree of similarity to human synovial sarcomas. We have now started the final and the most crucial phase of the study—what happens when we turn off SYT-SSX expression in synovial sarcoma? Briefly, our strategy is to divide engineered mice into control groups without doxycycline and an experimental group exposed to doxycycline regimen. Once the experimental group develops tumor, half of them will be taken off doxycycline while the remaining half will continue on doxycycline. The response of the tumor to the presence and absence of doxycycline will be monitored closely by physical examination and imaging at regular intervals. If evidence of tumor stabilization or tumor regression is seen in the absence of SYT-SSX, these tumors will be subjected to rigorous pathological analysis such as histology, immunohistochemistry, expression profiling, etc.

If we observe tumor regression in the absence of SYT-SSX, the importance of the observation goes beyond confirming oncogene addiction. Understanding the molecular mechanisms responsible for tumor regression in the absence of SYT-SSX will provide us with new insights that could be exploited to design more effective targeted therapies. Even if we do not observe tumor regression, this model will allow us to explore combinatorial therapeutic strategies where SYT-SSX expression can be knocked down with concurrent drug therapies to investigate whether they are more effective in combination. There are many more potential uses for such a versatile model and while we wait for our preliminary results, we are excited about the prospects.

Dr. Malay Haldar and Dr. Kevin B. Jones are Co-investigators at the Department of Human Genetics and Howard Hughes Medical Institute, University of Utah.

Major Events in Synovial Sarcoma Research
by Marc Ladanyi, MD

In this update, I would like to highlight three major events in our work on synovial sarcoma. First, our laboratory, as part of the MSKCC Sarcoma SPORE (Specialized Programs of Research Excellence) application, successfully competed for the NIH/NCI grant, which officially started in July of this year. Within this four project grant, we have Project #4, "Elucidating SYT-SSX-dependent histone code alterations to guide targeted epigenetic therapy for synovial sarcoma", a joint effort with the laboratory of Dr C. David Allis at Rockefeller University. The goal is to address the central role of SYT-SSX-dependent epigenetic alterations in the biology of synovial sarcoma from mechanistic, global genomic, and preclinical perspectives. The SYT-SSX fusion oncoprotein functions as an aberrant transcriptional protein that causes abnormal gene expression by interacting with enzymes that, modify the proteins (histones) that hold DNA (genes) in an active or inactive conformation. This project brings together expertise in fundamental histone biology with experience in synovial sarcoma cell line- and human tissue-based translational research to develop a deeper understanding of SYT-SSX-dependent histone alterations that could lead to more rational, more precisely targeted, and, hopefully, more effective epigenetic therapy for synovial sarcoma. Dr. Tatsuo Ito, a postdoctoral fellow

in my laboratory who is supported in part by the Paul Nabil Bustany Fund, generated key preliminary data for this application and is now continuing this project.

Secondly, 2010 saw the publication of the report of the Sarcoma Genome Project, entitled "Subtype-specific genomic alterations define new targets for soft-tissue sarcoma therapy", in the journal *Nature Genetics.* This is the result of a collaboration of the MSKCC Soft Tissue Sarcoma group with a group from the Broad Institute and Dana-Farber Cancer Institute, both in Boston. This consortium performed an integrative analysis of DNA sequence changes, DNA copy number changes and gene expression patterns in 207 sarcoma samples encompassing seven major subtypes, including 23 synovial sarcoma samples. This provided the largest amount of genomic data so far assembled on synovial sarcoma, highlighting frequent losses at chromosome region 3p and gains of chromosome region 12q.

Finally, a second postdoctoral fellow who focused on synovial sarcoma recently joined my laboratory, Dr. Yoshiyuki Suehara. Dr. Suehara brings a special expertise in proteomics and is currently working on follow-up studies on secernin. In a previous unpublished study, he found that secernin expression appears to be a significant predictor for metastasis and survival of patients with synovial sarcoma. He is now pursuing a number of projects to confirm and further understand this finding in our laboratory.

We are very grateful for the support provided by the Paul Nabil Bustany Fund for Synovial Sarcoma Research, which has provided seed money and support for our ongoing work on this deadly cancer.

Dr. Marc Ladanyi is the William Ruane Chair in Molecular Oncology at Memorial Sloan-Kettering Cancer Center in New York.

Upcoming Event: January 22 at Pasha Restaurant

We will be holding a benefit lunch at Pasha Restaurant on Saturday, January 22, at 12 pm. Guest speaker Dr. Marc Ladanyi will talk about the status of synovial sarcoma research and there will also be a slideshow of our recent event in Istanbul. For those who couldn't make it to Turkey, as well as for those who did, this will be a casual and friendly-family opportunity to participate in our ongoing fundraising efforts, so please consider joining us.

For more details, call or email Laufey at **(973) 539-8347** or **nach7377@yahoo.com**.

How to Donate

Option 1:
Write a check to The Paul Nabil Bustany Memorial Fund for Synovial Sarcoma Research. Send your check to: 15 Footes Lane, Morristown, NJ, 07960.

Option 2:
Donate online at **www.pnbustanyfund.org**.

The PNB Fund is a 501(c)(3) nonprofit organization. All donations are tax deductible.

The purpose of the PNB Fund is to raise money for basic scientific research on synovial sarcoma. The PNB Fund is entirely run by volunteers and all donations go directly to synovial sarcoma research. Thank you for your support.