



UNIVERSITY OF CHICAGO:

TED MULLIN FUND *for*
PEDIATRIC SARCOMA RESEARCH



PREPARED FOR MARY HENRY AND RICK MULLIN
AND THE TED MULLIN FUND
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A decade ago, external funding was not a possibility for sarcomas—neither industry nor government would support research into these diseases that, while life-threatening, impact a relatively small population. Thanks to the partnership with the Mullin family and the work done in memory of Ted Mullin, the University of Chicago has been able to enhance its team of experts and we now have the opportunity to make a dramatic difference in the research and treatment of this devastating disease.

As you well know, philanthropy, in the form of private donations for basic science research, is essential in ultimately finding a cure for sarcoma. Unlike research in other more common types of cancer, where large-scale clinical trials involving thousands of patients have enabled investigators to gain insights into risk factors and effective treatments, breakthroughs for sarcoma patients will come from basic and translational research, like the kind taking place at the University of Chicago.

IMPACT OF THE TED MULLIN FUND

The Ted Mullin Fund has allowed the University of Chicago to allocate essential seed funding to Dr. Stephen Skapek's study of tumor suppressor genes and how their abnormality contributes to sarcoma, specifically rhabdomyosarcoma, biology. Dr. Skapek is focusing on this type of sarcoma, which is particularly common in children and young adults because of its potential implications on the treatment of other sarcomas. Although currently, we can cure many patients with rhabdomyosarcoma—especially those with tumors that have not spread to other areas of the body—current treatments incorporating surgery, high doses of chemotherapy and radiation therapy can be associated with severe, and in some cases, life-threatening side effects. In situations where tumors cannot be removed surgically, only about 20% of the children are cured—and this number has not significantly improved despite nearly 30 years of research conducted by national collaborative clinical research groups. Innovative approaches are essential to cure more children with rhabdomyosarcoma and other types of sarcoma.

Historically, treatment for rhabdomyosarcoma has focused on trying to kill cancer cells by using radiation or chemotherapy, which causes damage to the genetic material (DNA) in the nucleus of the cells. When the cancer cell “recognizes” that its genetic material has been damaged, it has two choices—either to try to repair the damage using molecular machinery that is present in all cells or, if the damage is too severe, to activate a program in which the cell commits “suicide” in a program called apoptosis. Unfortunately, in children with advanced stages of rhabdomyosarcoma, chemotherapy and radiation usually fail to work because the cancer cells evolve and either more effectively repair the DNA damage or fail to activate the program to induce apoptosis. In either case, the cancer cells survive and continue to grow, and Dr. Skapek has developed the following four new strategies to surmount these fatal obstacles:

Can we induce skeletal muscle differentiation in rhabdomyosarcoma cells?

Dr. Skapek has been using a high throughput screen to identify new drug compounds or genetic manipulations that can promote muscle differentiation in myoblasts. Next, he planned to verify the “hits” using a secondary validation assay and finally, test the “hit” compounds on a panel of human rhabdomyosarcoma cell lines and tumors grown in experimental animals.

Dr. Skapek’s team successfully completed the planned high-throughput screen with the muscle differentiation assay they developed at the University of Chicago. They used a genetic approach with a “library” of roughly 700 RNA molecules, each of which that can “knock-down” the expression of one of roughly 700 kinases. Kinases are enzymes in the cell that carry out a very specific function – transferring a phosphate group onto a protein. Kinases are among the most important signaling proteins within a cell. For example, a number of kinases are known to block muscle differentiation and to help a cell proliferate. Further, in the past 10-15 years, a large number of new drugs have been developed to block the activity of individual kinases. By carrying out this screen, Dr. Skapek and team anticipated finding kinases that, when blocked, would promote muscle differentiation. These would then be potential targets that could be blocked with a drug in rhabdomyosarcoma to promote muscle differentiation and arrest cell proliferation.

Dr. Skapek and his team found just over 20 “hits” in their screen. That is, they identified just over 20 kinases that, when knocked down, promoted muscle differentiation and slowed the proliferation of the muscle cells. They have used computational tools to show that most of these kinases lie in biochemical “pathways” that are predicted to regulate proteins that are already known to be important in controlling the cell cycle and muscle differentiation. But, the individual kinases have not (yet) been implicated as regulators of either process. As such, by carrying out this screen, Dr. Skapek has done exactly what he hoped to do.

At the University of Texas Southwestern Medical Center, Dr. Skapek anticipates carrying this work further by first verifying that the “hits” in the screen will impede muscle differentiation in a complementary assay. Having done this, he will (a) investigate whether similar inhibition of these kinases slows growth or promotes differentiation in rhabdomyosarcoma cells, and (b) whether these kinases are mutated or expressed to high levels in rhabdomyosarcoma samples. In parallel, he plans to submit a grant proposal to the NIH to seek R01 funding (\$1.25M over 5 years) to expand this work.

Can we uncover mechanisms by which rhabdomyosarcoma cells become resistant to chemotherapy?

Dr. Skapek is using a mouse xenograft model in which human rhabdomyosarcoma cells grow as a tumor in experimental mice. He is treating the tumor-bearing mice with cyclophosphamide to induce remission and collecting the tumor tissue when the tumor recurs (which almost always happens). Through collaborations with other University of Chicago scientists to use cutting-edge DNA and RNA sequencing tools, he is sequencing all of the genetic material in the primary tumors and the tumors that relapsed after chemotherapy. By comparing these results with DNA and RNA from

tumor specimens collected and archived at the University of Chicago and from the Children's Oncology Group, he will develop ways to "target" the genes that are expressed in the recurrent tumors.

Dr. Skapek's initial analyses of these data did not reveal clear-cut genetic changes that correlate with resistance to vincristine which is commonly used in rhabdomyosarcoma therapy. This interesting finding suggests another, fundamentally different, mechanism of drug resistance: the existence of a subset of RMS cells that are capable of surviving vincristine chemotherapy without relying on new mutations. Due to the need to focus on other projects outlined in this report, little additional progress was made on this effort.

Can we identify a "signature" of proteins or genes that are expressed in rhabdomyosarcoma and that correlate with prognosis?

Dr. Skapek and his team have two, complementary approaches here: First, as a member of the Children's Oncology Group (COG) and vice-chair for biology studies in the Soft Tissue Sarcoma Committee of the COG, Dr. Skapek has obtained a "catalog" of all of the genes that are expressed in approximately 100 individual rhabdomyosarcoma tumor specimens. With a University of Chicago collaborator (Dr. Samuel Volchenbom), he is correlating the complex pattern of gene expression with different sarcoma subtypes and with clinical outcome. Importantly, they are applying sophisticated geometric and topological models to essentially describe the "shape" of tumor based on the expression of many thousands of genes. There is general agreement among oncologists and cancer biologists that by more precisely identifying those tumors that are not likely to be cured (by using these molecular genetic and computational tools), they will be better able to apply novel therapies.

In a parallel approach, Dr. Skapek has partnered with investigators in the Institute for Genomics and Systems Biology at the University of Chicago to determine the sequence of all of the genes that are expressed in a panel of 20 different rhabdomyosarcoma tumor specimens. This approach has the potential to reveal genes that have undergone a mutation as the sarcoma develops. In many cases of more common types of cancers, these mutated genes have been used to develop new drugs that specifically "target" the abnormal protein generated by the mutant genes.

In this past year, in collaboration with Dr. Volchenbom, Dr. Skapek has applied computational instruments to determine how expression of genes in human rhabdomyosarcoma reflects the gene expression changes that occur in normal muscle development. They have used gene expression data available from colleagues in the Children's Oncology Group and found that recognized subtypes of rhabdomyosarcoma seem to reflect different stages of normal muscle development. They are excited about this finding, in part, because the mathematical tools applied in this analysis have not been widely used for this type of work. Under Dr. Volchenbom's guidance, the findings are being confirmed. In Dr. Skapek's lab, they hope to use molecular biology tools to verify the key findings from the computational studies. Following this, they intend to submit the findings for publication, and potentially continue this line of work with other sarcoma subtypes.

Can we use “functional imaging” of a sarcoma to determine how well chemotherapy is working – after just the first dose of chemotherapy?

Emerging evidence from clinical trials of several types of sarcoma supports the concept that typical measurements of tumor size do not accurately predict who will ultimately be cured. This has been shown to be true for rhabdomyosarcoma and also for bone sarcoma. In the latter case, tumors rarely actually get smaller during chemotherapy; the first real evidence as to how well the chemotherapy is working comes from microscopic studies of the tumor obtained nearly three months after therapy was initiated. At that stage, if there is a “poor response,” it may be too late because changes in treatment at that point do not seem to alter the poor outcome.

In collaboration with other University of Chicago physicians, Dr. Skapek has written a new clinical trial in which they treat children (and young adults) with osteosarcoma using a chemotherapy regimen that was developed at Chicago. Three weeks following the initial dose of chemotherapy, they will obtain a PET scan, which measures how much glucose (sugar) is being used by the tumor cells. Decreased glucose use correlates with the viability (or health) of the tumor cells; but PET scans have not been used as an early marker of chemotherapy effects for sarcoma patients. Dr. Skapek predicts early PET scans will reveal which tumors are likely to be eradicated with chemotherapy. This will allow us to intervene with alternative treatment at an earlier point. This clinical trial is being supported using institutional funds. His immediate plan is to obtain “pilot data” on a small number of patients to support the overlying hypothesis; with these data, he will seek additional, federal funds to support a larger, multi-center study at the University of Chicago.

In this past year, 2 patients with osteosarcoma were enrolled. At this time, there is not yet information on the hypotheses being tested. Dr. Skapek anticipates attempting to open this trial at UT Southwestern Medical Center in Dallas.

THANK YOU

The Mullin Family should take enormous pride in all that we have already accomplished together. We cannot think of a better way to honor Ted’s memory than knowing that his untimely death has helped so many others and will continue to create advances in the field for many years to come. Thank you for both your personal support and your tireless enthusiasm for fundraising around our pediatric sarcoma research vision to improve outcomes for pediatric sarcoma patients.