

Neuroblastoma Genomic Signatures Predict Survival of Patients

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Layperson's Summary

Neuroblastoma, a tumor of the sympathetic nervous system, is the most common solid tumor in children outside the brain. Neuroblastoma spreads (metastasizes) to many parts of the body in 50% of patients before being diagnosed. Treatment has improved for patients with metastatic neuroblastoma, but still only 40% survive. With current clinical and laboratory testing, it is not possible to predict which patients with metastatic disease will survive and which will succumb. If this could be done at diagnosis, those predicted to do well would receive current or possibly even less toxic therapies, whereas those predicted to do poorly would receive new therapies that may improve their chance of survival. In our first study, we determined the activity (level of expression) of essentially all human genes in 102 untreated metastatic neuroblastomas with "gene chips". We discovered for the first time a 55 gene "signature" (genomic signature) that predicts the likelihood of survival for patients who are otherwise clinically indistinguishable. The gene signature classified patients into subgroups with poor (16%) or excellent (79%) chances of long-term survival.

We now propose to extend this work to a larger number of patients, including those whose tumors do or do not have many copies of a cancer gene called MYCN, to identify gene signatures that predict how each kind of metastatic tumor will respond to therapy and to develop clinically applicable gene signature tests. Information obtained about gene signatures also will lead to the discovery of genes and networks of interacting genes that are important in cancer cell behavior that could be targeted in new therapeutic strategies aimed at providing more efficacious treatment. Collectively, this research will make it possible to individualize care for each patient based on definition of a tumor's gene signature. This new opportunity for studying tumor genes should lead to improving the survival of children with metastatic neuroblastoma.

Scientific Summary

Neuroblastoma is a common childhood tumor, and approximately 40% of patients have aggressive metastatic disease (stage 4) when diagnosed. With improved therapy, survival has increased to 35-40%. **Problem:** It is not possible at diagnosis to predict which patients will be long-term progression-free survivors (PFS) and which will succumb to disease. **Hypothesis:** Molecular "signatures" that are derived from microarray analyses of tumor RNA and DNA will define subgroups that have either excellent or poor outcomes. We discovered a 55 gene signature from expression profiles of MYCN gene non-amplified tumors that identifies patients with 79% and 16% PFS. **Overall goal:** Continue developing therapeutically relevant genomic classifiers for these patients. **Specific aims:** 1) Determine if RNA expression profiles predict PFS. 2) Determine if DNA signatures based upon loss of heterozygosity and copy number amplification or deletion predict PFS and if combining DNA and RNA signatures improves accuracy of prediction. **Research Design:** MYCN amplified and non-amplified tumors will be analyzed as separate groups because they are clinically and biologically distinct. Tumors are available from the Children's Oncology Group and other collaborators with annotation and clinical follow-up. Probes from RNA and DNA arrays or sets of probes with biologic relevance (e.g., pathways) that are significant for PFS will be used to build molecular classifiers, which will be internally and externally validated. **RNA:** Initially, expression profiling with Human Exon (HuEx) and standard HG 133 microarrays will be compared to determine if HuEx signatures have similar or better accuracy in predicting PFS. The optimal platform will be used to discover signatures with approximately 339 tumors. TaqMan® Low Density Arrays (TLDA) will be designed for signature genes and tested on the same RNAs to validate microarray data. Finally, an independent external validation set of approximately 210 tumors will be tested with both microarray and TLDA assays to confirm clinical applicability of signatures. **DNA:** DNA from the same 339 specimens originally used for RNA studies will be tested with high density 250K SNP arrays to determine if DNA and DNA + RNA signatures predict PFS. If so, TaqMan® DNA assays will be designed to validate SNP array results. Last, DNA from the external validation set of tumors will be tested to confirm potential clinical value of signatures. **Summary:** Accurate prediction of long-term PFS using genomic classifiers will facilitate assignment of treatment and development of potentially more effective therapy.

Budget

| Personnel | | Salary | % Effort | Salary requested | Employee Benefits | Total |
|--|------------------------|----------|----------|------------------|-------------------|-----------------|
| Robert Seeger, MD | Principal Investigator | | 20% | \$0 | \$0 | \$0 |
| Shahab Asgharzadeh, MD | Co-Investigator | | 20% | \$6,953 | \$2,190 | \$9,143 |
| Richard Sposto, PhD | Co-Investigator | | 10% | \$0 | \$0 | \$0 |
| Roger Pique-Regi, MS | Bioinformatician | \$25,622 | 20% | \$5,124 | \$1,179 | \$6,303 |
| Cathy Liu, MS | Res Specialist | \$52,510 | 20% | \$10,502 | \$2,415 | \$12,918 |
| Personnel Subtotal | | | | \$22,579 | \$5,784 | \$28,363 |
| Supplies (see Budget Justification for details) | | | | | | \$21,110 |
| Total Direct Cost | | | | | | \$49,473 |
| Indirect Cost | | | | | | \$4,947 |
| Total Cost | | | | | | \$54,420 |

Budget Justification

Personnel

Salaries and wages have been calculated in accordance with the University of Southern California, Department of Pediatrics Academic Salary Scale and the Childrens Hospital Los Angeles (CHLA) Staff Personnel Pay Plan, and employee benefits have been calculated using the figures agreed upon by CHLA and the DHHS Audit Agency. Benefit rates used are 31.5% of salary for academic personnel and 23% of salary for staff personnel.

Robert C. Seeger, M.D.; Principal Investigator; 20% effort, no salary requested. Dr. Seeger has overall responsibility for this investigation. He sets priorities, designs experiments, and analyzes data with Co-Investigators Drs. Asgharzadeh and Sposto. He supervises laboratory personnel (Ms. Cathy Liu) performing expression profiling and TaqMan Low Density Array analysis and works with Ms. Liu to manage the tumor and nucleic acid bank. He is primarily responsible for collaborations with the Children's Oncology Group (Drs. John Maris and Wendy London; Mr. Robert Gerbing), with the German Neuroblastoma Group (Drs. Frank Berthold and Andre Oberthur), and with others to acquire well-annotated specimens for analysis. All professional fees generated by Dr. Seeger are turned over to the University Children's Medical Group.

Shahab Asgharzadeh, MD.; Co-investigator, Molecular Biologist and Bioinformatician, 20% effort, 5% salary requested. Dr. Asgharzadeh has recently joined the faculty as a tenure-track Assistant Professor of Pediatrics. He is trained clinically in Hematology-Oncology, and his research training is strong in molecular biology and bioinformatics. His faculty job profile is that of a physician-scientist (75% effort overall devoted to translational laboratory research), who will investigate genomics of neuroblastoma. He recently received a NIH K12 award for his research. He will continue to investigate gene expression to discover tumor signatures that predict outcome of patients with metastatic neuroblastoma. He also will analyze matched tumor and normal cell DNA to generate chromosomal LOH and gain/loss profiles of the same tumors for which we have expression profiles and will develop TaqMan SNP and qPCR assays for DNA signatures. All professional fees generated by Dr. Asgharzadeh are turned over to the University Childrens Medical Group.

Richard Sposto, Ph.D.; Co-investigator, Statistician; 10% effort, no salary requested. Dr. Sposto is a Professor of Research in the Departments of Preventive Medicine and Pediatrics and is Director of Biostatistics/Bioinformatics in the Division of Hematology - Oncology at CHLA. Dr. Sposto has been involved with pediatric cancer research through CCG (Children's Cancer Group) and COG (Children's Oncology Group) and at CHLA for 19 of the last 25 years. For most of this time he was also principal statistician for CCG/COG research effort in CNS tumors. He was the Group Statistician for COG from 2002 to 2004 before being recruited to the Division of Hematology – Oncology at CHLA. In this project, Dr. Sposto will collaborate in the development and validation of classifiers of outcome, a significant scientific interest of his, and with Dr. Asgharzadeh will supervise Mr. Pique-Regi, the M.S bioinformatician attached to this project.

Roger Pique-Regi, MS ; Bioinformatician,20% effort, 20% salary requested. Mr. Pique-Regi is a gifted PhD student mentored by Dr. Antonio Ortega at the USC Viterbi School of Engineering. He also works closely with Drs. Asgharzadeh and Sposto to analyze RNA and DNA data generated from various array technologies. He plans to pursue his PhD thesis with data generated from this project. He has implemented various algorithms within Matlab software and presented his results at two national bioinformatics conferences. Testimony to his skill and involvement is a second author position in the paper by Asgharzadeh, et al., which describes our findings with MYCN non-amplified neuroblastomas.

Cathy Liu, MS.; Research Specialist III, 20% effort, 20% salary requested. Ms. Liu is exceptionally able in molecular biology and is responsible for preparing RNA and DNA for microarray analyses. She also is experienced and precise at performing quantitative PCR and RT-PCR testing including TaqMan Low Density Array (TLDA) analyses. She prepares the frozen tumor sections for isolation of RNA. She maintains the frozen specimens (tumor and bone marrow and blood cells) used in this research and provides storage information on RNA, DNA, and OCT blocks to Mr. Wakamatsu (database manager – no funding requested) for entry into the database. She also receives and prepares specimens for collaborations (e.g., Dr. Berthold's group).

Supplies

Note: Supplies are requested for approximately 55% of tumors that will be tested for gene expression during the first year of research. Funds are not requested for SNP profiling of tumor DNA. Additional funding to completely fund the research will be obtained from other sources.

| | Tumor Type | | Total no. tumors | unit cost | total yr 1 |
|--|--------------------------|---------|---------------------|--------------|---------------|
| | MYCN-A | MYCN-NA | | | |
| RNA | | | | | |
| Human Exon 1.0 XT | 16 | 24 | 40 | \$220 | \$8,800 |
| Affymetrix reagents for Exon set | 16 | 24 | 40 | \$120 | \$4,800 |
| Microarray Core Lab | 16 | 24 | 40 | \$80 | \$3,200 |
| | | | | | \$16,800 |
| | Number of ABI TLDA cards | | | | |
| TLDA cards(4 tumors per card) | 4 | 6 | 10 | \$325 | \$3,250 |
| TLDA card reagents | | | | | \$500 |
| | | | | | \$3,750 |
| Histology for QC and molecular correlation, 40 @ \$14 each | | | | | \$560 |
| TOTAL | | | | | \$21,110 |

1. The hybridization and scanning is performed in the Microarray Core Laboratory (Dr. Triche, Director).
2. Histology for quality control is for 2 sections/tumor (before and after sections used for preparation of RNA).