The Neuroblastoma Virtual Tumor Bank (VTB)
2008 Summary of Accomplishments
September 29, 2008

The funding provided by The Neuroblastoma Children's Cancer Society (NCCS) has made it possible to perform the following national and international work which falls outside the purview of the Children's Oncology Group:

1. Development of a computer SAS macro for faster specimen selection for national and international laboratory research projects;
2. Generation of 16 statistical technical reports, including one that resulted in a paper accepted to the prominent, high-impact journal *Nature*;
3. Addition of data to differentiate patients who died of their neuroblastoma from those who have died from some other cause (toxicity or accidental cause);
4. Addition of data to track which patients' data are used on which project in order to avoid using a given patient's data for both hypothesis generation and validation;
5. Updating the database with more recent information about the duration of time that the patients have survived;
6. Incorporation of new data from the Biopathology Center's FreezerWorks database and the Children's Hospital of Philadelphia; and,
7. Generation of detailed documentation of the system of programs required to support and maintain the VTB data, as well as to document the database structure.

1. Macro for specimen selection

Until recently, much of the process to select specimens from the Virtual Tumor Bank (VTB) database was manual. The funding from NCCS allowed us to develop a more automated computer SAS macro for consistent and efficient selection of specimens. Eligibility criteria are used to define a subset of patients' specimens from the VTB database, and then specimens are randomly selected from that subset. We now have a macro template that can be easily modified for a given specimen selection request. The new macro permits the selection process to be done in a very short period of time; therefore, the research process for a cure has been accelerated.

Specimen selection was made for 20 projects, each performed with a two-day turnaround time from the time of request (Table 2.a).

As of December 3, 2007, the total number of tumor specimens with complete outcome data that were available for selection for laboratory research projects is 4,021 (Table 1.a). Availability of other types of specimens and data are shown in Tables 1.b-1.e.

2. Generation of statistical technical reports

The statistical analysis of investigator-initiated correlative biology studies is work that is not funded by the COG. With the funding from the NCCS, we have performed specimen selection on 20 projects (Table 2.a), and been able to provide 16 technical reports on over the past year. The data from the VTB (survival dataset and the biology dataset) are linked to the laboratory results and analyzed. The results are communicated back to the investigators in the form of statistical technical reports. Subsequent to the technical reports, we have collaborated on 10

manuscripts for journal publication in the last year, including a manuscript currently in press with the prominent, high-impact journal *Nature* (Table 2.b).

3. Identification of patients who died from neuroblastoma

For clinical trials, a bad outcome of any kind is typically used as a measure of the lack of benefit of a given treatment. However, in biological studies of the natural history and genetic causes of neuroblastoma, it is important to analyze only the disease-related bad outcome, and exclude deaths that were due to toxicity or accidents. In other words, we need to be able to tell if the patient died of the disease rather than just knowing they died. Based on this information, another dataset is in the process of being developed that will allow calculation of progression-free survival (PFS) rates in addition to the event-free survival (EFS) more typically employed in clinical trials. This will be very important data for the analysis of national and international microarray data projects.

4. Track use of specimens by project

A program and database have been added that allows us to keep track of which patients were selected for a given project. This information is critical in order to prevent using a given patient's data for both hypothesis generation and validation of a neuroblastoma gene or risk factor. For example, the patients from Dr. John Maris's TARGET project (a full genome study that will be used to identify genetic mutations as potential therapeutic targets), will be excluded from possible selection on any other project with similar goals.

5. Update survival duration data

The overall survival dataset used for analysis was updated to include the most recently activated COG protocols, and this allows accurate follow-up for patients who enroll on multiple studies either sequentially or simultaneously. This required incorporating several datasets for each new study, including information from several sources, each with a different way of formatting variables.

6. Adaptation to changing data inputs to the VTB

The Biopathology Center (BPC) has changed the way they collect and disseminate their data, separating the data into older data representing all the neuroblastoma legacy data, and newer data in a system called FreezerWorks. We have made changes to our programs and data structure to adapt to these improved inputs. In addition, the format and content of the data transmissions from the Children's Hospital of Philadelphia has changed, and required a significant programming effort.

7. Documentation of the VTB database structure and computer programs

A continuous work in progress is maintaining an up-to-date detailed description of each part of the VTB database structure and system of computer programs. This documentation shows the location of the various files and data along, which programs are run to create the various datasets, and the names of the datasets. In addition, key datasets are copied to a shared drive in order to permit sharing of the data and programs by other statisticians in COG.

Table 1.a
Specimens in the Neuroblastoma Virtual Tumor Bank (VTB) as of December 3, 2007*
The first number is the total number of specimens, and the number in parenthesis is the count of those with complete biology and survival data available for analysis.

Specimen	Specimen Type	BPC Lab	Maris Lab	Reynolds Lab	Seeger Lab	Total
1	SP (Snap Frozen Primary Tumor)	3494 (3312)	746 (709)	0	0	4240 (4021)
2	POCT (Primary Tumor in OCT)	580 (536)	0	0	0	580 (536)
3	FP (Fixed Primary Tumor [Formalin/Paraffin Block])	325 (293)	0	0	0	325 (293)
4	SM (Snap Frozen Metastatic Tumor)	10 (8)	0	0	0	10 (8)
5	MOCT (Metastatic Tumor in OCT	3 (2)	0	0	0	3 (2)
6	FM (Fixed Metastatic Tumor [Formalin/Paraffin Block])	4 (4)	0	0	0	4 (4)
7	SN (Snap Frozen Normal Tissue)	51 (48)	0	0	0	51 (48)
8	NOCT (Normal Tissue In OCT)	3 (2)	0	0	0	3 (2)
9	FN (Fixed Normal Tissue [Formalin/Paraffin Block])	58 (55)	0	0	0	58 (55)
10	BM ASP-Frozen Bone Marrow Aspirate	2 (1)	0	0	0	2 (1)
12	FBM (Frozen Ficolled Bone Marrow Aspirate)	927 (873)	0	0	0	927 (873)
13	SERA (Frozen Serum)	876 (802)	0	0	0	876 (802)
14	BLOOD (Frozen Blood/Peripheral Blood)	10 (7)	0	0	0	10 (7)
15	FBLD (Frozen Ficolled Blood/Peripheral Blood)	1666 (1585)	706 (671)	0	0	2372 (2256)
16	TPS (Touchprep Slides/Touchimprint Slides	558 (509)	0	0	0	558 (509)
17	H_E (Hematoxilyn and Eosin Stained Slides)	308 (269)	0	0	0	308 (269)
18	UNST (Unstained Slides [from paraffin block])	121 (107)	0	0	0	121 (107)
19	Tumor RNA	0	1072 (990)	0	0	1072 (990)
20	Tumor DNA	0	1579 (1478)	0	0	1579 (1478)
21	Blood DNA	0	1951 (1856)	0	0	1951 (1856)
22	Cell Lines	0	0	50 (40)	0	50 (40)
23	Tumor	0	0	0	783 (729)	783 (729)
26	Plasma	922 (868)	0	0	0	922 (868)

* The annual update of the VTB has been delayed due to change in personnel at the BPC.

Table 1.b
Banked Slides at BPC

Specimen Type	BPC Lab
Block	256 (196)
Coverslips Unstained	1 (1)
EM Blocks	27 (24)
EM Prints	1 (1)
Scroll/Ploidy	202 (179)
Slide/Photo	1 (1)
Stained BM Aspirate Slides	19 (16)
Stained BM Biopsy Slides	43 (33)
Stained Bone Marrow clot Slides	1 (0)
Stained Peripheral Blood Slides	2 (2)
Stained Slides	2973 (2523)
Unstained BM Aspirate Slides	35 (30)
Unstained BM Biopsy Slides	35 (26)
Unstained BM Clot Slides	2 (0)
Unstained Peripheral Blood	19 (19)
Unstained Slides	3479 (2961)

Table 1.c
UAB Serum Bank

Patients with available serum
676 (662)

Table 1.d
CHOP genetic data

Type	Counts	
11q LOH	374 LOH (356)	812 No loss (763)
1p LOH	278 LOH (264)	956 No loss (900)
Unbalanced 11q	183 Unbalanced (175)	186 Balanced (176)
17q gain	200 No gain (180)	106 Gain (98)

Table 1.e
Number of Patients with genetic results using the Allumina 550-K Platform

Type	Patients with Genetic Analysis Completed	Pts w/ Specimen In the VTB
Blood DNA	281 (281)	258
Tumor DNA	488 (488)	478

Table 2.a
List of projects making use of specimens and/or data from the VTB

Protocol Number	PI	Title
2007-06	Zage	The role of the EGFR degradation pathway in neuroblastoma
2007-07	Attiyeh	Integrated analysis of neuroblastoma genomics
2007-08	Kraveka	Role of bioactive sphingolipids in neuroblastoma progression
2007-09	Stallings	Genomic Profiling of miRNA loci in neuroblastoma
2007-10	Hogarty	Neuroblastoma TARGET Initiative
2003-03Am1	Look	SHP2 mutations in the Leopard Syndrome region in human neuroblastomas
2007-11	Asgharzadeh	
2007-12	Wagner	Assessment of MGMT phenotype in neuroblastoma patients
2006-10Am1	Look	Resequencing activation domains of SHP2 in Neuroblastoma
2006-10Am2	Look	Resequencing for activating mutations in HRAS, KRAS and NRAS in neuroblastoma
2007-13	DuBois	Evaluation of NET gene polymorphisms in relation to neuroblastoma MIBG tumor uptake
2008-01	Vermuelen	Neuroblastoma Prognostic Gene Signature Validation study
2008-02	Hogarty	Resequencing the Neuroblastoma cancer genome
2008-03	Maris	ALK translocations in Neuroblastoma
2008-04	Cohn	Genome-wide analysis of Favorable Stage Neuroblastomas with MYCN amplification
2008-05	Klein	Raman Spectroscopy Detects and Distinguishes Neuroblastoma and Related Tissues in Fresh and (Banked) Frozen Specimens
2007-08Am1	Kraveka	Role of Bioactive Sphingolipids in Neuroblastoma Progression
2008-06	Lieuw	GATA3 mutations in neuroblastoma
2008-07	Khan	Diagnosis of Small Round Blue Cell Tumors (SRBCTs) using multiplex RT-PCR
2008-09	Nishi	

Table 2.b
List of manuscripts generated from data and/or specimens from the VTB

1. Rani E. George, Takaomi Sanda, Megan Hanna, Stefan Frohling, William Luther II, Jianming Zhang, Sergey Zozulya, Wendy B. London, Vlad Gregor, Patrick McGrady, Nathanael S.Gray, Thomas R. Webb, Liquan Xue, D. Gary Gilliland, Heidi Greulich, Stephan W. Morris, Matthew Meyerson, A. Thomas Look. Inhibitor-sensitive mutations in the ALK receptor tyrosine kinase provide a therapeutic target in neuroblastoma. *Nature* (in press)

2. JM Maris, YP Mosse, JP Bradfield, C Hou, S Monni, RH Scott, S Asgharzadeh, EF Attiyeh, SJ Diskin, M Laudenslager, C Winter, K Cole, JT Glessner, C Kim, EC Frackelton, T Casalunovo, AW Eckert, M Capasso, EF Rappaport, C McConville, WB London, RC Seeger, N Rahman, M Devoto, SFA Grant, H Li, H Hakonarson. Chromosome 6p22 Locus Associated with Clinically Aggressive Neuroblastoma. *New England Journal of Medicine* 2008 Jun 12;358(24):2585-93.

3. Tomoyuki Fujita, Jun Igarashi, Erin R. Okawa, Takahiro Gotoh, Jayanthi Manne, Venkatadri Kolla, Jessica Kim, Huaqing Zhao, Bruce R. Pawel, Wendy B. London, John M. Maris, Peter S. White,and Garrett M. Brodeur. CHD5, a Tumor Suppressor Gene Deleted from 1p36.31 in Neuroblastomas. *Journal of the National Cancer Institute* 2008 Jul 2;100(13):940-9.

4. Andrew D J Pearson*, Susan L Cohn*, Wendy B London, Tom Monclair, Peter F Ambros, Andreas, Faldum, Barbara Hero, Tomoko Iehara, David Machin, Veronique Mosseri, Katherine K Matthay, for the INRG Task Force. The International Neuroblastoma Risk Group (INRG) Classification System. *Journal of Clinical Oncology* 2008 (in press) [* = share first authorship]

5. Tom Monclair, Garrett M Brodeur, Peter F Ambros, Hervé Brisse, Giovanni Cecchetto, Keith Holmes, Michio Kaneko, Wendy B London, Katherine K Matthay, Jed G Nuchtern, Dietrich von Schweinitz, Susan L Cohn, and Andrew D J Pearson *for the INRG Working Group*. The International Neuroblastoma Risk Group (INRG) Staging System. *Journal of Clinical Oncology* 2008 (in press)

6. Rochelle Bagatell, Maja Beck-Popovic, Wendy B. London, Yang Zhang, Andrew D. J. Pearson, Katherine K. Matthay, Tom Monclair, Peter F. Ambros, Susan L. Cohn. Significance of *MYCN* Amplification in INSS Stage 1 and 2 Neuroblastoma: A Report from the International Neuroblastoma Risk Group (INRG) Database. *Journal of Clinical Oncology* 2008 (in press)

7. Steven G. DuBois, Wendy B. London, Yang Zhang, Katherine K. Matthay, Tom Monclair, Peter F. Ambros, Susan L. Cohn, Andrew Pearson, Lisa Diller. Lung Metastases in Neuroblastoma at Initial Diagnosis: A Report from the International Neuroblastoma Risk Group (INRG) Project. *Pediatric Blood and Cancer* 2008 (in press)

8. Elizabeth A. Beierle, Nicole A. Massoll, Melissa K. Li, William H. Donnelly, Joseph

Hartwich, Tracy Clarke, Martha Campbell-Thompson, Elena V. Kurenova, Vita M. Golubovskaya, William G. Cance, Patrick McGrady, and Wendy B. London. Focal Adhesion Kinase Expression in Human Neuroblastoma: Immunohistochemical and Real-Time PCR and Analyses. *Clinical Cancer Research* 2008 Jun 1;14(11):3299-305.

9. Jennifer Schneiderman, Wendy B. London, Garrett M. Brodeur, Robert P. Castleberry, A. Thomas Look, and Susan L. Cohn. Clinical Significance of *MYCN* Amplification and Ploidy in "Favorable" Stage Neuroblastoma *Journal of Clinical Oncology* 2008 Feb 20;26(6):913-8.

10. Chizuko Okamatsu, Wendy B. London, Arlene Naranjo, Michael D. Hogarty, Julie M. Gastier-Foster, A. Thomas Look, Michael LaQuaglia, John M. Maris, Susan L. Cohn, Katherine K. Matthay, Robert C. Seeger, Tsutom Saji, Hiroyuki Shimada. Clinicopathological Characteristics of Ganglioneuroma and Ganglioneuroblastoma: A Report from the CCG and COG Studies. 2008 (submitted)