Protocol NMTRC009

Molecular-guided therapy for the treatment of patients with relapsed and refractory childhood cancers

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Contact Information:

STUDY CHAIR:

Giselle Sholler, MD
NMTRC Chair
100 Michigan Avenue NE MC 272
Grand Rapids, MI 49503
Tel: (616) 267-0332
Fax: 616-391-2785
giselle.sholler@helendevoschildrens.org

STUDY VICE CHAIR:

Jeff Trent
Translational Genomics Research Institute (TGen)
President and Research Director
445 N 5th Street
Phoenix, AZ 85004
602-343-8419
jtrent@tgen.org

Safety Officer:

Melinda Merchant, PhD, MD
AstraZeneca
Senior Medical Director, Oncology
Translational Medical Unit
Early Clinical Development
35 Gatehouse Dr, Waltham MA 02451
Tel: +1 781-491-5292
melinda.merchant@astrazeneca.com

STUDY COMMITTEE MEMBERS:

Genevieve Bergendahl, RN Clinical Program Director NMTRC 100 Michigan Avenue NE MC 272 Grand Rapids, MI 49503	Tel: (616) 267-0335 Fax: 616-391-2785 E-Mail: Genevieve.bergendahl@helendevoschildrens.org
Alyssa VanderWerff Clinical Program Coordinator NMTRC 100 Michigan Avenue NE MC 272 Grand Rapids, MI 49503	Tel: (616) 267-0327 Fax: 616-391-2785 E-Mail: Alyssa.VanderWerff@helendevoschildrens.org
TBDClinical Project Coordinator NMTRC 100 Michigan Avenue NE MC 272 Grand Rapids, MI 49503	Tel: (616) 391-0807 Fax: 616-391-2785 E-Mail:
Abhinav Beeravally Nagulapally Computational Biologist NMTRC 100 Michigan Avenue NE MC 272 Grand Rapids, MI 49503	Tel: (616) 267-0346 Fax: 616-391-2785 E-Mail: Abhinav.BeeravallyNagulapally@helendevoschildrens.org
William Ferguson, MD Pediatric Hematology-Oncology Cardinal Glennon Children's Medical Ctr Saint Louis University School of Medicine 1465 S. Grand Blvd. St. Louis, MO	Tel: (314) 577-5638 Fax: (314) 268-4081 fergusws@slu.edu
William Roberts, MD Pediatric Hematology/Oncology Rady Children's Hospital San Diego 3020 Children's Way MC5035 San Diego, CA 92123-4282	Tel: (858) 966-5811 Fax: (858) 966-8035 wroberts@chsd.org
Don Eslin, MD Arnold Palmer Hospital for Children Pediatric Hematology/Oncology 50 W. Sturtevant St. Orlando, FL 32806	Tel: 321-841-8588 Don.Eslin@orlandohealth.com

Jacqueline Kraveka, DO Medical University of South Carolina Division of Pediatric Hematology-Oncology 135 Rutledge Avenue, Room 489 MSC 558 Charleston, SC 29425	Tel: 843-792-2957 Fax: 843-792-8912 kravekjm@musc.edu
Amy Lee Bredlau, MD Director, Pediatric Brain Tumor Program Assistant Professor, Sullivan Scholar Medical University of South Carolina 135 Rutledge Avenue, MSC 558 Charleston, SC 29425	Phone: 843-876-2872 Fax: 843-792-8912 bredlau@musc.edu
Javier Oesterheld, MD Levine Children's Hospital 1000 Blythe Blvd Charlotte, NC 282033	Tel: 704-381-9900 Fax: 704-381-9901 Javier.oesterheld@carolinashealthcare.org
Deanna Mitchell, MD Michigan State University Helen DeVos Children's Hospital 100 Michigan NE Grand Rapids, MI 49503	Tel: 616-391-2086 Deanna.Mitchell@ helendevoschildrens.org
Micheal Isakoff, MD Assistant Professor of Pediatrics Connecticut Children's Medical Center Division of Hematology / Oncology 2J 282 Washington Street Hartford, CT 06106	Tel: 860-545-9630 Fax: 860-545-9622 Misakoff@connecticutchildrens.org
Kathleen Neville, MD, MS Arkansas Children's Hospital Director, Experimental Therapeutics in Pediatric Oncology Program Associate Professor of Pediatrics 1 Children's Way; Slot 512-23 Little Rock, AR 72202	Office: (501)364-4405kaneville@uams.edu
Randal K. Wada, MD Associate Professor of Pediatrics and Nursing University of Hawaii Kapiolani Medical Center for Women and Children 55 Merchant Street, 26th Floor Honolulu, HI 96813	Office/Lab: (808) 586-2963 randalw@hawaii.edu

Francis Eshun, MD Center for Cancer and Blood Disorders Phoenix Children's Hospital 1919 Thomas Road Phoenix, AZ 85016	feshun@phoenixchildrens.com 602-933-0920
Devang Pastakia, MD Vanderbilt-Ingram Cancer Center 397 Preston Research Building 2220 Pierce Ave Nashville, TN 37232	Phone: 615-875-6129 Email: devang.pastakia@Vanderbilt.Edu
Virginia Harrod MD, PhDChief-Neuro-OncologyDell Children's Blood and Cancer Center 1301 Barbara Jordan Blvd, Ste 401 Austin, Texas 78723	512-628-1900 office VLHarrod@seton.org
Mark Fluchel, MD Primary Children's Hospital Division of Pediatric Hematology/Oncology 100 North Mario Capecchi Drive, 4th Floor Salt Lake City, UT 84113	Phone: 801-662-4700 Fax: 801-662-4705 email: mark.fluchel@imail.org
Valerie I. Brown, MD, PhD Associate Professor of Pediatrics Division of Pediatric Oncology/Hematology Clinical Director, Experimental Therapeutics Attending Physician, Hematopoietic Stem Cell Transplant Program Penn State Milton S. Hershey Medical Center and Children's Hospital 500 University Dr. P.O. Box 850, MC H085 Hershey, PA 17033-0850	Phone: 717-531-1042 Email: vbrown1@hmc.psu.edu
Daniel Marc Rubin ND FABNO Medical Director Naturopathic Specialists, LLC 7331 E Osborn Dr Suite 330 Scottsdale, AZ 85251	Phone: 480-990-1111 email: rubinhere@me.com

Jeffrey Bond, PhD NMTRC 100 Michigan Avenue NE MC 272 Grand Rapids, MI 49503	Tel: (802) 656-4608 jeffrey.bond@ helendevoschildrens.org
Gina K. Hanna, PharmD Pediatric Oncology Clinical Trials Pharmacist Dana-Farber Cancer Institute Yawkey Center for Cancer Care Research Pharmacy Y-5 | 450 Brookline Ave | Boston, MA 02215	phone 617.582.8125 | fax 617.632.4616 ginak_hanna@dfci.harvard.edu
Meghan M. Devine, PharmD Clinical Specialist, Pediatric Hematology and Oncology Pharmacy Services-Levine Children's Hospital at Carolinas Medical Center 1000 Blythe Blvd Charlotte, NC 28203	Phone: 704-381-7049 Meghan.Devine@carolinashealthcare.org
Jeff Trent Translational Genomics Research Institute (TGen) President and Research Director 445 N 5th Street Phoenix, AZ 85004	602-343-8419 jtrent@tgen.org
Matt Huntelman Translational Genomics Research Institute (TGen) Associate Professor, Neurogenomics Division 445 N 5th Street Phoenix, AZ 85004	602-343-8730 mhuentelman@tgen.org
John Carpten Translational Genomics Research Institute (TGen) Deputy Director of Basic Science 445 N 5th Street Phoenix, AZ 85004	602-343-8819 jcarpten@tgen.org
Jeff Keifer Translational Genomics Research Institute (TGen) Research Associate Professor 445 N 5th Street Phoenix, AZ 85004	602-343-8667 jkiefer@tgen.org

David Craig Translational Genomics Research Institute (TGen) Deputy Director, Informatic Sciences and Professor and Director, Neurogenomics Division 445 N 5th Street Phoenix, AZ 85004	602-343-8747 dcraig@tgen.org
William Burleson Translational Genomics Research Institute (TGen) Product Delivery Manager 445 N 5th Street Phoenix, AZ 85004	602-343-8503 wburleson@tgen.org
Apurva Patel Translational Genomics Research Institute (TGen) Director, Enterprise Applications 445 N 5th Street Phoenix, AZ 85004	602-343-8404 apatel@tgen.org
Janine LoBello, DO Ashion Analytics, LLC Laboratory Director 445 N 5th Street Phoenix, AZ 85004	602-343-8606 jlobello@ashiondx.com

INVESTIGATOR SIGNATURE SHEET

I have read the attached protocol amendment and agree that it contains all the necessary details for performing the study.

I will review copies of the amended protocol and of the preclinical and clinical information on the test article, which was furnished to me by the Sponsor, to all members of the study team responsible to me who participate in the study. I will discuss this material with them to assure that they are fully informed regarding the test article and the conduct of the study.

Once the protocol has been approved by the Institutional Review Board (IRB)/Independent Ethics Committee (IEC), I will not modify this protocol without obtaining the prior approval of the Sponsor and of the IRB/IEC. I will submit the protocol modifications and/or any informed consent form (ICF) modifications to the Sponsor and the IRB/IEC, and approval will be obtained before any modifications are implemented.

I understand the protocol and will work according to it, the principles of Good Clinical Practice (GCP) [current International Conference of Harmonization (ICH) guidelines], and the Declaration of Helsinki (1964) including all amendments up to and including the Scotland revision (2000) and notes of clarification added in 2002 and 2004.

Investigator's Signature	Date

Investigator's Printed Name

Investigational Site Name

TABLE OF CONTENTS

PROTOCOL SYNOPSIS

PROTOCOL TITLE	Molecular-guided therapy for the treatment of patients with relapsed and refractory childhood cancers
PROTOCOL NUMBER	NMTRC009
PHASE OF DEVELOPMENT	Feasibility

OBJECTIVES	**Primary**
	Determine feasibility of using tumor samples to assess genomic sequencing using predictive modeling to make real-time treatment decisions for children with relapsed/refractory cancers
	
	Secondary
	• To determine the activity of treatments chosen based on:
	  • Overall response rate (ORR)
	  • Progression free survival (PFS)
	• To continue to evaluate the safety of allowing a molecular tumor board to determine individualized treatment plans
	• (Voluntary) To explore the relationship between tumor phenotype/genotype and response by permitting use of tumor tissue in a correlative biologic study
	• To compare PFS interval to PFS intervals of previous chemotherapy regimens since relapse for each subject.
	• Sub analysis examination of disease types.

STUDY DESIGN	A prospective open label, multicenter study to evaluate the feasibility of molecularly guided therapy in patients with relapsed or refractory childhood cancers A total of 200 evaluable subjects with childhood cancer that are refractory to or have relapsed on conventional therapy will be treated with molecular guided therapy. Additional patients may enroll through compassionate access if no other treatment options are available. Subjects will be evaluated in 3 strata: • Stratum 1: 50 subjects with refractory/relapsed neuroblastoma • Stratum 2: 50 subjects with relapsed or refractory brain tumors • Stratum 3: 100 subjects with refractory or relapsed rare tumors Guided therapy will allow the use of individualized therapeutic combinations (up to 4 agents). All subjects will be followed for survival, disease response, progression and safety. All subjects will be treated according to the discretion of the treating oncologist and study committee (minimum 3 oncologists and one pharmacist). Extent of disease will be measured and assessed for changes throughout the course of the study.

ELIGIBILITY	**Inclusion Criteria** *1.* Subjects must have proven pediatric cancer with confirmation at diagnosis or at the time of recurrence/progression and clinical determination of disease for which there is no known effective curative therapy or disease that is refractory to established proven therapies fitting into one of the following categories: a. **Neuroblastoma**- Patients that have relapsed following standard of care therapy (such as high risk patients, patient presenting after age 15 months or MYCN amplified, and only following (for eligible patients) high-dose chemotherapy followed by hematopoietic stem cell transplantation and maintenance therapy with retinoic acid and antibody therapy) or having progressed during standard of care therapy and non-responsive/progressive to accepted curative chemotherapy. b. **Brain Tumors** i. Medulloblastomas (At relapse after standard of care therapy [surgery, chemotherapy and/or radiation] and/or non-responsive/progressive on accepted curative therapy) ii. Gliomas (At relapse after standard of care therapy [surgery and/or radiation and/or chemotherapy] and/or non-responsive/progressive on accepted curative therapy) iii. Diffuse Intrinsic Pontine Glioma/DIPG/Diffuse Midline Tumor- (At diagnosis, as there is no known curative therapy) iv. Glioblastoma Multiforme- (At diagnosis, as there is no known curative therapy) v. Ependymomas (At relapse after standard of care therapy [surgery with or without radiation] and/or non-responsive/progressive on accepted curative therapy) vi. Choroid plexus tumors (At relapse after standard of care therapy [surgery] and/or non-responsive/progressive on accepted curative therapy) vii. Craniopharyngiomas (At relapse after standard of care therapy [surgery or suppressive therapy] and/or non-responsive/progressive on accepted curative therapy) viii. Dysembryoplastic neuroepithelial tumors (DNETs) (At relapse after standard of care therapy [surgery] and/or non-responsive/progressive on accepted curative therapy) ix. Meningiomas (At relapse after standard of care therapy [surgery] and/or non-responsive/progressive on accepted curative therapy) x. Primitive Neuroectodermal Tumors (PNETs)/CNS Embryonal Tumors (At relapse after standard of care therapy [surgery, chemotherapy, and/or radiation] and/or non-responsive/progressive on accepted

curative therapy)

xi. Germ cell tumors (At relapse after standard of care therapy [surgery, and/or radiation and/or chemotherapy] and/or non-responsive/progressive on accepted curative therapy)

xii. Atypical Teratoid Rhabdoid Tumor (ATRT) (At relapse after standard of care therapy [surgery, chemotherapy and/or radiation] and/or non-responsive/progressive on accepted curative therapy)

c. **Rare Tumors:**

i. Soft tissue sarcoma

Rhabdomyosarcoma (At relapse after standard of care therapy [surgery, and/or radiation, chemotherapy] and/or non-responsive/progressive to accepted curative chemotherapy)

Non-rhabdomyosarcoma (At relapse after standard of care therapy [surgery, and/or radiation, chemotherapy] and/or non-responsive/progressive to accepted curative chemotherapy)

ii. Bone

Ewings sarcoma (At relapse after standard of care therapy [surgery, and/or radiation, chemotherapy] and/or non- responsive/progressive to accepted curative chemotherapy)

Osteosarcoma (At relapse after standard of care therapy [surgery, chemotherapy] and/or non-responsive/progressive to accepted curative chemotherapy)

iii. Renal

Wilms tumor (At relapse after standard of care therapy [surgery, and/or radiation, chemotherapy] and/or non- responsive/progressive to accepted chemotherapy)

Renal cell carcinoma (At relapse after standard of care therapy [surgery, chemotherapy] and/or non-responsive/progressive to accepted curative chemotherapy)

Malignant rhabdoid tumor (At diagnosis, as there is no known curative therapy)

Clear Cell Sarcoma- (At relapse after standard of care therapy [radiation, chemotherapy] and/or non-responsive/progressive to accepted curative chemotherapy)

iv. Germ Cell tumors (At relapse after standard of care therapy [surgery, chemotherapy] and/or non-responsive/progressive to accepted curative chemotherapy)

v. Liver Tumors (At relapse after standard of care therapy [surgery, chemotherapy] and/or non-

	responsive/progressive to accepted curative chemotherapy) vi. Carcinomas- (At relapse after standard of care therapy and/or non- responsive/progressive to accepted curative chemotherapy) vii. Desmoid tumors- (At relapse after standard of care therapy and/or non- responsive/progressive to accepted curative chemotherapy) viii. Malignant Peripheral Nerve Sheath Tumors (MPNST)- (At diagnosis, as there is no known curative therapy). ix. Pheochromocytoma (At relapse after standard of care therapy and/or non- responsive/progressive to accepted curative chemotherapy). x. Ethesioneuroblastoma (At relapse after standard of care therapy and/or non- responsive/progressive to accepted curative chemotherapy). xi. Neuroendocrine tumors (At relapse after standard of care therapy and/or non- responsive/progressive to accepted curative chemotherapy). xii. Desmoplastic small round cell tumor (At relapse after standard of care therapy and/or non-responsive/progressive to accepted curative chemotherapy). xiii. Melanoma (At relapse after standard of care therapy and/or non- responsive/progressive to accepted curative chemotherapy). 2. Subjects must be age >12 months at enrollment 3. Subjects must be age $\leq$ 21 years at initial diagnosis 4. Subjects must have measurable disease as defined by RESIST v1.1 at the time of biopsy and tumor must be accessible for biopsy. Tumor samples submitted for analysis must contain >50% viable tumor tissue to qualify. In addition, subjects with disease confined to the bone marrow are eligible to enroll if the degree of marrow involvement is expected to be >50%. Note: Subjects who are expected to have no evidence of disease after surgical removal of their tumor are still eligible for this trial if their disease would normally require adjuvant chemotherapy treatment after surgery despite NED status. 5. Current disease state must be one for which there is currently no known effective therapy 6. Specimens will be obtained only in a non-significant risk manner and not solely for the purpose of investigational testing. 7. Lansky or Karnofsky Score must be $\geq$ 50 8. Subjects without bone marrow metastases must have an ANC >

	750/µl to begin treatment. 9. Subjects with CNS disease currently taking steroids must have been on a stable dose of steroids for 2 weeks prior to their biopsy and must not have progressive hydrocephalus at enrollment. 10. Adequate liver function must be demonstrated, defined as: a. Total bilirubin ≤ 1.5 x upper limit of normal (ULN) for age AND b. ALT (SGPT) < 10 x upper limit of normal (ULN) for age 11. A negative serum pregnancy test is required for female participants of child bearing potential (≥13 years of age or after onset of menses) 12. Both male and female post-pubertal study subjects need to agree to use one of the more effective birth control methods during treatment and for six months after treatment is stopped. These methods include total abstinence (no sex), oral contraceptives ("the pill"), an intrauterine device (IUD), levonorgestrol implants (Norplant), or medroxyprogesterone acetate injections (Depo-provera shots). If one of these cannot be used, contraceptive foam with a condom is recommended. 13. Informed Consent: All subjects and/or legal guardians must sign informed written consent. Assent, when appropriate, will be obtained according to institutional guidelines **Exclusion Criteria** 14. Subjects who have received any cytotoxic chemotherapy within the last 7 days prior to biopsy 15. Subjects who have received any radiotherapy to the primary sample site within the last 14 days (radiation may be included in treatment decision after biopsy). 16. Subjects receiving any investigational drug concurrently. 17. Subjects with uncontrolled serious infections or a life-threatening illness (unrelated to tumor) 18. Subjects with any other medical condition, including malabsorption syndromes, mental illness or substance abuse, deemed by the Investigator to be likely to interfere with the interpretation of the results or which would interfere with a subject's ability to sign or the legal guardian's ability to sign the informed consent, and subject's ability to cooperate and participate in the study **Additional criteria:** Subjects willing to participate in the correlative biologic studies will sign an additional consent form to provide additional tumor tissue.

ESTIMATED NUMBER OF PATIENTS/GEOGRAPHIC REGIONS	200 evaluable (220 total) relapsed/refractory pediatric cancer patients. Additional patients may enroll through compassionate access if no other treatment options are available. Patients will enroll at: Arnold Palmer Children's Hospital, FL St. Louis University/Cardinal Glennon Children's Medical Center, MO Helen DeVos Children's Hospital, MI Levine Children's Hospital, NC Connecticut Children's Medical Center, CT Children's Mercy Hospital, Kansas City MO Rady Children's Hospital of San Diego, CA Medical University of South Carolina, SC Monroe Carrell Jr. Children's Hospital at Vanderbilt, TN Kapiolani Medical Center for Women and Children, HI Dell Children's Hospital,TX Texas Children's Hospital, Baylor College of Medicine,TX Primary Children's Hospital, UT Children's Hospital and Clinics of Minnesota, MN Penn State Milton S. Hershey Medical Center and Children's Hospital, Hershey, PA Arkansas Children's Hospital – Little Rock, AR International Sites American University of Beirut Medical Center, Lebanon La Timone; Marseille, France
LENGTH OF STUDY	Accrual to this study is estimated to be approximately 3-4 years.
STUDY ASSESSMENTS	Refer to Table of Assessments for timing of study procedures.
CRITERIA FOR EVALUATION	Feasibility Measures: Primary objective: The definition of feasibility for this study will include: **"Enrollment onto study, genomic profile, analysis and report generation completed, tumor board held with treatment decision, treatment review completed, start of treatment, and completion of 1 cycle of therapy."** Efficacy Measures: Secondary objectives Response: To determine the overall response rate (ORR) by the presence of radiologically assessable disease by cross-sectional CT or MRI imaging and/or by MIBG or PET scans. ♦ Duration of response, defined as the period of time from when measurement criteria are met for complete response (CR) or partial response (PR), whichever is first recorded, until the first date that recurrent or progressive disease (PD) is objectively documented (taking as reference for PD the smallest measurements recorded since the treatment started) ♦ The assessment of response will include the initial measurable

	targets and will be performed after cycle 2, then after every other cycle. • A subject will be defined as a responder if CR/PR is observed at any time of the treatment • Clinical response will be seen if stable disease and decrease in tumor markers by ≥ 50% or CR of bone marrow. • Time to progression, defined as the period from the start of the treatment until the criteria for progression are met taking as reference the screening measurements • Comparisons of ORR and PFS for the first and second cohorts of 100 patients (i.e., those who enroll prior to versus after the implementation of Amendment 3.0, respectively) will be performed. <u>Safety Measures: Secondary objectives</u> • Safety analysis will be conducted on all subjects who have received at least one dose of therapy, and will include the frequency of all reported adverse events and laboratory abnormalities as well as frequency of dose interruptions, dose reductions and treatment discontinuation.
STATISTICS/SAMPLE SIZE ESTIMATE	200 evaluable subjects Patients who enroll following implementation of Amendment 3.0 (second cohort of 100 patients) will have their tumor biopsies analyzed using an updated Drug Prediction Report (Appendix III). Overall Response Rate (ORR) and Progression Free Survival (PFS) for this group will be compared to those of the first 100 patients who received treatment based upon the original Drug Prediciton Report and Pharmacopeia Library.

TABLE OF PROCEDURES AND ASSESSMENTS

Procedure	**Screening**	**Cycle 1 Day 1**	**Cycle 1 Day 8**	**Cycle 1 Day 15**	**Cycle 1 Day 22 (if applicable)**	**Subsequent Cycles Day 1**	**Off Therapy/ 30 Day Follow-up**
Informed consent	X						
Demographics	X						
Medical history	X	X	X	X	X	X	
Baseline Symptom Form	X	X					
Concurrent meds	X	X				X	X
Physical exam	X	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X	X
Height	X	X				X	
Weight	X	X				X	X
Karnofsky or Lansky play score	X	X		X		X	X
CBC w/diff,	X	X	X	X	X	X	X
Serum chemistry *	X	X		X		X	X
Tumor Markers **	X	X				X	X
Adverse events			X	X	X	X	X
Surgical resection &/or diagnostic biopsy	X						
MRI or CT***	X	Radiologic measurements will be performed every 8 weeks or every other cycle (whichever occurs first) through Cycle 6, after which they may be performed as per institutional standard.					
MIBG/PET ***	X						
Bone Marrow (any subject suspected to have BM disease) ^	X	Should be repeated every 8 weeks or every other cycle (whichever occurs first) only if positive at study entry; after Cycle 6, may be performed as per institutional standard.					
B-HCG	X						
EKG (as indicated)		X					

* Serum electrolytes, BUN, Creatinine, Bilirubin, ALT, AST
**LDH and/or Specific Tumor Markers for defined disease (if available)
***Type of scan as indicated for disease type
^Standard of Care Bone Marrow

1 Background

1.1 Study Disease

Relapsed or refractory neuroblastoma, brain tumors, and rare childhood tumors.

1.2 Rationale

Lethality of Relapsed/Refractory Pediatric Cancers is an Unmet Clinical Need.

Although improvements in the past 40 years have led to markedly improved survival rates of approximately 80% overall for pediatric cancers, patients with relapsed, rare and advanced stage tumors still have a very poor prognosis. Soft tissue sarcomas account for ~ 7% of pediatric cancers, with 850-900 new cases annually in the United States [1]. Rhabdomyosarcoma, the most common soft tissue sarcoma in children and adolescents, affects nearly 350 children in the United States each year [1]. Patients with metastatic rhabdomyosarcoma have a poor prognosis with an overall survival rate of ~30% [2]. The non-rhabdomyosarcoma soft tissue sarcomas affect ~500 children each year. Patients with metastatic non-rhabdomyosarcoma soft tissue sarcomas have long-term survival < 20% [3]. Malignant bone tumors (mainly Ewing's Sarcoma and Osteosarcoma) account for 6% of childhood cancers, with ~400 cases of osteosarcoma and 200 cases of Ewing's Sarcoma diagnosed annually [1]. Patients with localized osteosarcoma, who have a poor response to pre-operative chemotherapy have a survival rate of around 50%, while patients with metastatic osteosarcoma or Ewing's sarcoma disease at presentation, have a survival of < 20% [4, 5]. Renal cancers account for ~6% of pediatric cancer diagnoses, with 95% of cases being Wilms Tumor, which has a very favorable prognosis [1]. However, rare and high risk renal tumors such as metastatic anaplastic Wilms tumor, clear cell sarcoma of the kidney, renal cell carcinoma, and malignant rhabdoid tumor have an inferior outcome, with survival of < 20% [6, 7]. For all these patients survival remains poor, despite use of dose-intensified multi-agent chemotherapy and stem cell transplantation. Therefore, new approaches such as targeted therapies are warranted.

Neuroblastoma is the most common extracranial solid tumor in children, with approximately 700 new diagnoses per year. It accounts for 7% to 10% of childhood cancers [8, 9]. Whereas the prognosis for infants with neuroblastoma is generally good, currently only 30% of children diagnosed after 12-15 months of age survive despite aggressive multimodal therapies [10, 11]. Even with high-dose chemotherapy (HDC) followed by hematopoietic stem cell transplantation (HSCT) and maintenance therapy with retinoic acid the 5-year event-free survival remains below 50% [12, 13]. Long-term survival of patients who are treated with conventional therapies following relapse is <5%. As such, neuroblastoma accounts for 15% of all pediatric cancer deaths in the United States [14]. Consequently, the evaluation of new drugs is strongly needed in this disease.

Recent evidence has established the genetic heterogeneity of neuroblastoma and revealed the existence of several major molecular subsets that collectively may provide prognostic value for future disease management [15] [16]. While the poor prognosis for older neuroblastoma patients underscores the need for new treatment strategies, the elucidation of specific biologic subsets of neuroblastoma suggests a way to improve disease management. The current standard-of-care

treatments for relapsed neuroblastoma include a variety of Phase II or Phase I studies that generally have only modest response rates (10%-35%) [11]. Even in responding patients, tumors often go on to further rapid relapses and novel strategies to treat this patient population are urgently needed. There are currently few treatment options from which pediatric oncologists can select with any degree of confidence to improve the management of multiply recurrent neuroblastoma patients. The current strategy is to add salvage therapies based on the anecdotal experience of the treating physician, which often leads to drug-related toxicity but may or may not extend life. The identification of agents that target specific molecular pathways associated with the development and/or progression of neoplastic diseases holds promise. Improved approaches that identify in a more rational (data-driven) fashion combinations of existing agents that are likely to be effective should result in a survival benefit in the clinical setting, while avoiding the toxicity associated with agents that are unlikely to be beneficial. [17].

CNS tumors are the most common solid tumors in children, with 3000-4000 new diagnoses each year. CNS tumors account for approximately 25-30% of all childhood cancers. Though many of these CNS tumors are quite treatable, the 5-year survival for pediatric CNS tumors is only 71%[18]. Patients who have relapsed and refractory CNS tumors are those most likely to succumb to their disease, in spite of aggressive chemotherapy and radiation, often followed by HSCT[19, 20]. Therefore, new treatment strategies are needed for control of CNS tumors.

There is a growing focus on treating CNS tumors based on the biology of the disease[21, 22] rather than with “one size fits all” therapy. Risk stratification in CNS tumors based on biology is increasingly understood to be a priority. For example, medulloblastoma has been broken into 4 biologic categories with different genetic mutations and different clinical outcomes[23-25]. Similar to neuroblastoma, these differences in the biology and clinical outcome of patients with medulloblastoma suggest new strategies to improve disease management. Ongoing clinical trials of upfront therapy for medulloblastoma are attempting to incorporate molecularly guided therapies. The current standard-of-care treatments for relapsed CNS tumors include Phase I and Phase II therapies that often have few, if any, responding patients[26, 27]. Again reflecting the challenges in neuroblastoma, responding patients with high grade CNS tumors often experience multiple relapses and are in dire need of novel treatment strategies. Improved approaches that can inform treatment regimens based on the biology of the disease could result in a survival benefit in this challenging patient population.

Molecular Networks as the Drug Target.

It is now firmly established that cancer results from perturbations in the molecular networks within cellular systems that disturb the normal homeostatic state [28-30]. Fluctuations in these networks can result from genetic or epigenetic cellular events and/or changes in the molecular constitution of the tumor microenvironment, which collectively dictate the phenotype of the biological system. The molecular networks engaged during tumor development and/or progression are complex. Molecular networks have evolved to provide the ultimate level of cellular plasticity, allowing cells to adapt to or exploit extracellular cues within the local milieu [31]. The complexity of a tumor system is further exaggerated by the inherent genomic instability seen in many neoplasms, which leads to an accelerated micro-evolutionary process that results in further cellular and tumor sub-system heterogeneity [32, 33]. This variability combined with the adaptability of many molecular pathways provides a somewhat predictable and highly probable path to resistance for any given agent that targets a subset of cellular systems within a tumor’s molecular and genetic repertoire [34]. A fundamental challenge in the area of targeted cancer treatment is how to identify optimal therapeutic combinations that can treat heterogeneous tumors that are both highly adaptive and that exhibit significant inter- and intra-patient variation [28, 35-37]. Our proposal outlines an approach by which we can utilize our expanding knowledge of molecular networks and the mechanisms of action of a growing pharmacopeia [38, 39] in

conjunction with standardized biomarker assessments to deliver targeted combinations of effective therapies to pediatric cancer patients.

Practice and Promise of Biomarkers for Patient Treatment Planning.
A panel of individual biomarkers has recently been identified that can be used in the clinical setting to identify neuroblastoma patients most likely to respond to a specific therapy. For example, activation of the ALK gene through sequence variation mutations has been identified in a subset of neuroblastoma patients, and small-molecule inhibition of the ALK-encoded receptor tyrosine kinase caused cytotoxicity in affected neuroblastoma cell lines [40]. Such studies, in conjunction with a large body of in vitro and in vivo data, have further demonstrated that the efficacy of specific treatment modalities is dependent upon the molecular constitution of the tumor, and that the observed variations in tumor response to current therapies is attributable in large part to disease heterogeneity at the molecular level.

While individual biomarkers may be predictive of responses to specific therapies, especially in the context of front-line treatment, a different approach to the management of patients with refractory disease is needed in order to identify effective treatments from the catalog of available agents. Advances in informatics and molecular technologies, coupled with our expanding knowledge of molecular networks and mechanism of action of the existing pharmacopeia, provide a great opportunity in translational medicine to develop a model that more accurately predicts tumor response. The studies outlined in this proposal are a step in the development of personalized oncology, in which each patient is truly treated individually based upon the systematic molecular analysis of their disease. Our project is focused on testing the merits of a specific data-driven predictive method through which any number of drugs in our current knowledge base has a chance of being recommended, of these only the FDA approved drugs listed in Appendix III will be used. Although this novel approach to personalized therapeutics brings with it to the concern that toxicities that may arise from novel combinations of agents, it is strongly felt by the investigators that the logical interpretation of molecular data by a committee of highly qualified physicians and pharmacists in order to identify drug candidates for consideration in the treatment of a patient population with few alternative options is a worthwhile endeavor. The systematic biomarker-driven approach outlined in this proposal, by tailoring a combination of drugs targeting the specific molecular composition of a tumor—irrespective of tumor classification or anatomical origin— provides a feasible alternative to the conventional approach that targets specific organs or tissues without consideration of the underlying tumor biology.

A Pilot Trial Testing the Feasibility of using Molecular-Guided Therapy in Patients with Refractory or Recurrent Neuroblastoma.

The primary objective of this pilot study was to evaluate the feasibility of using predictive modeling based on genome-wide mRNA expression profiles of neuroblastoma tumor biopsies to make real-time treatment decisions. Feasibility was defined as the completion of the following in a two week time period: tumor biopsy, quality RNA extraction, mRNA U133 2+ Affymetrix gene chip hybridization, analysis utilizing a series of predictive methodologies, report generation, tumor board review with formulated treatment plan, and medical monitor review. Subjects were not treated on this study. There were 5 subjects enrolled between April-June 2010 with multiply relapsed or refractory neuroblastoma. Subjects had received between 2-13 previous relapsed therapies and were between 2-6.5 years post diagnosis. All subjects had soft tissue disease which was able to be biopsied. All biopsies were adequate by pathology evaluation (>75% viable tumor) and RNA quality (>6.5 RIN). Gene chips were completed in 3-7 days, report generation was 1-5 days, tumor board was 1-3 days, medical monitor sign off was 1 day. The total time was 10-12

days for all subjects. The tumor board which consisted of 4-10 pediatric oncologists from sites across the US and a pediatric oncology pharmacist was able to create individualized therapy regimens for all subjects. Correlative biology specimens were obtained and grown in culture. Mice xenografts of 3/5 subjects were established. Cultures and xenografts are able to be used for validation studies of predicted drug sensitivity. In conclusion it is feasible to obtain real-time genomic profiling for molecularly guided therapy for use in treatment decision making.

A Feasibility Trial using Molecular-Guided Therapy in Patients with Refractory or Recurrent Neuroblastoma.

The primary objective of this feasibility study was to evaluate the feasibility and safety of using predictive modeling based on genome-wide mRNA expression profiles of neuroblastoma tumor biopsies to make real-time treatment decisions. Feasibility was defined as "enrollment onto study, quality mRNA obtained, gene chip completed, tumor board held, medical monitor review and approval, start of treatment by 21 days post biopsy/surgical resection date, and completion of 1 cycle of therapy." Sixteen (16) subjects were enrolled to this study, all with multiply relapsed or refractory neuroblastoma. Subjects were between 1-11 years post diagnosis. All subjects had soft tissue disease which was able to be biopsied. All biopsies were adequate by pathology evaluation (>75% viable tumor) and RNA quality (>6.5 RIN). 2 subjects were deemed ineligible due to tumor type after biopsy. Gene chips were completed in 3-8 days (95% CI: 3.8 – 6.8), report generation took 0-3 days (95% CI: 0.0 – 1.5), tumor board took 1-6 days (95% CI: 1.6 – 4.2), medical monitor sign off took 1-2 days (95% CI: 0.8 – 1.4). The total time from date of biopsy to tumor board was 6-11 days (95% CI: 7.5 – 10.2) for all subjects and 7-20 days to treatment (95% CI: 8.9 – 16.1), with an average of 12.4 days. The tumor board which consisted of 4-15 pediatric oncologists from sites across the US and a pediatric oncology pharmacist was able to create individualized therapy regimens for all subjects. Correlative biology specimens were obtained and grown in culture. Mice xenograft establishment is ongoing. Cultures and xenografts are able to be used for validation studies of predicted drug sensitivity. Triplicate biopsies showed reproducible sets of drugs and the comparatively large differences in drug lists between patients show that the drug lists are tailored to patients. Comparison of RNA expression profiles with RNA sequencing from each patient showed strong correlation.

The study endpoint showed that 14/14 patients were feasible to enroll, biopsy, genomic analysis, tumor board review and patient treatment was met. All regimens were found to be safe by the medical monitor proving that the molecular tumor board was able to make safe decisions similar to hospital tumor boards making treatment decisions without molecular information on the patients. It was felt that the pharmacy review for evaluating drug-drug interactions and deciding on laboratory and safety testing was an important part of the tumor board (this is not usually present at hospital tumor boards) and will continue to be incorporated in this and future trials. All patients tolerated therapy well and were treated without any serious adverse events related to study drugs. Clinical benefit was seen 64% of patients. This study shows that it is feasible to create therapeutic treatment plans based on genomic profiling in real time and that patients are able to be treated safely on a tumor board derived molecular guided therapy using existing medications

In this study we proposed to study reproducibility by applying it to triplicate samples obtained by dissecting each of five solid tumors. The primary goal of the reproducibility study is to evaluate the variation among sets of drug therapies associated with the same tumor. Secondary goals are 1) to compare the variation among sets of drug therapies from the same tumor with variation

between tumors from distinct patients and 2) to evaluate variation associated with earlier steps in the process, in particular, expression profiles and comparative expression profiles.

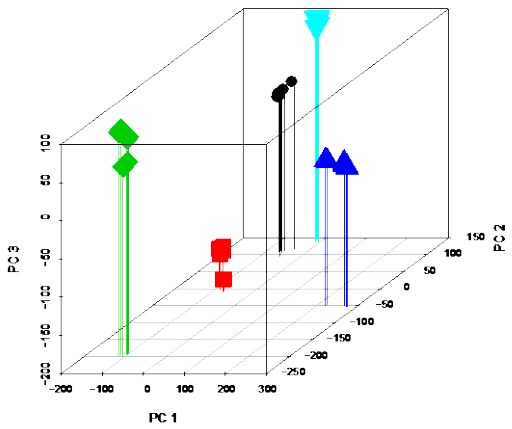


Figure 1 Principal Component Analysis. The first three principal component scores, PC1-PC3, illustrate the observation that the variation associated with the six samples for each biopsy (all of which have the same symbol and color) is small relative to the variation between biopsies.

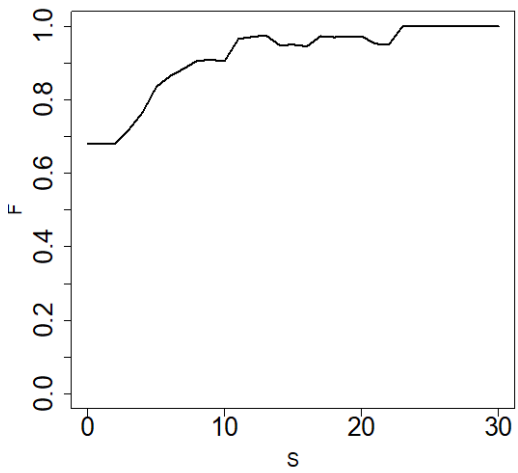


Figure 2. Reproducibility as a function of the threshold score. The reproducibility averaged over patients, replicates, and drugs is 0.68 (the intercept with the horizontal axis). As the threshold score increases the reproducibility increases to 1.

Distance-based nonparametric multivariate analysis of variance [41, 42] allowed us to reject the null hypothesis that variation between biopsies can be accounted for by the variation within biopsies (p=0.001). That the variation among expression profiles associated with the same biopsy is small compared with the variation between expression profiles associated with different biopsies is apparent from Principal Component Analysis (Figure 1).

Sets of drug therapies

Similarly, the variation among drug sets associated with the same biopsy is small compared with the variation among drug lists associated with different biopsies (p=0.001). The reproducibility averaged over patients, replicates, and drugs is 0.68. As the threshold score increases the reproducibility increases to 1 (Figure 2).

A Feasibility Trial using Molecular-Guided Therapy in Patients with Relapsed and Refractory Childhood Cancer.

The primary objective of this study was to determine feasibility of using tumor samples to assess genomic mRNA expression arrays and DNA Mutation Panels using predictive modeling to make real-time treatment decisions for children with relapsed/refractory cancers. Feasibility was defined as "enrollment onto study, RNA expression profile completed, DNA Mutation Panel completed, genomic analysis and report generation, tumor board held with treatment decision, treatment review completed and start of treatment by 21 days post biopsy/surgical resection date,

and then completion of 1 cycle of therapy." This study has completed accrual and final results will be reported at a future time point.

Conclusion
Collectively, the early preclinical and current clinical data suggests that this integrated approach is highly feasible and safe in pediatric cancers and that it is reproducible among repeat samples within patients. Based upon the significant need for a more rational-based approach to therapeutic selection in these difficult to treat pediatric tumors continued development of this approach is warranted. The area of genomic analysis is advancing rapidly and recently DNA exomes and RNA sequencing are now available by CLIA certified laboratories. We have adapted our analysis to move from RNA expression profiling to RNA sequencing and DNA mutation panel to DNA exomes. This will be evaluated in this study for use by the molecular tumor board in treatment decision making.

2 STUDY OBJECTIVES

2.1 Primary Objectives

Determine feasibility of using tumor samples to assess genomic sequencing using predictive modeling to make real-time treatment decisions for children with relapsed/refractory cancers.

2.2 Secondary Objectives

- To determine the activity of treatments chosen based on:
  - Overall response rate (ORR)
  - Progression free survival (PFS)
- To continue to evaluate the safety of allowing a molecular tumor board to determine individualized treatment plans
- (Voluntary) To explore the relationship between tumor phenotype/genotype and response by permitting use of tumor tissue in a correlative biologic study
- To compare PFS interval to PFS intervals of previous chemotherapy regimens since relapse for each subject.
- Sub analysis examination of disease types.

2.3 Subject Enrollment and Sample Procurement and Data Processing

Eligible subjects will be enrolled onto this study at each site following a registration process that includes receipt of a signed subject consent form and a copy of the required baseline laboratory tests. Subjects will undergo a scheduled surgical resection and/or diagnostic biopsy procedure. Common sample identifiers will be provided by the NMTRC Lead Study Coordinator to the participating site and will be used by all parties throughout the project to ensure sample, data and report alignment between participating organizations. At the time of tissue resection or biopsy, fresh tumor samples and a blood sample will be committed for this specific research study (referred to as "Primary Samples"), and prepared immediately per Appendix II and section 9.3. Primary Samples will be sent to Ashion Analytics, LLC, which is a CLIA-certified laboratory housed at the Translational Genomics Reserach Institute (TGen). Samples will be reviewed by local pathology to confirm that the sample sent to TGen contains >50% tumor cell viability which is the minimum required for successful analysis. The absence of an adequate tumor specimen from an enrolled subject will make them not eligible to continue on the study. Tumor

samples will also be sent for research purposes (referred to as "Secondary Samples.") A portion of the tumor tissue biopsy will be shipped overnight to the Sholler Pediatric Oncology Translational Research Laboratory on dry ice.

Upon arrival of the tumor specimens at Ashion and the Sholler Pediatric Oncology Translational Research Laboratory, the samples will be logged and immediately processed according to details in Section 9.

2.4 DNA and RNA Sequencing

Sequencing for this study will be performed at Ashion Analytics, LLC (Ashion). Ashion is a CLIA-certified laboratory developed by TGen in order to perform whole exome and RNA sequencing in a clinical laboratory to ensure the accuracy, reliability, and quality of the data. The sequencing will be performed on the Illumina HiSeq 2500 Sequencer. Quality thresholds will include metrics such as base calling quality, coverage, allelic read percentages, strand bias, and alignment quality. Once data has passed all QC thresholds, the de-identified data will be preprocessed and uploaded directly to the NMTRC TransMed software for creation of the NMTRC Drug Prediction Report.

Data Flow

At enrollment in the study, a patient record will be created and the tumor and blood samples for the patient will be given a sample identifier and stored in the NMTRC laboratory management database (LIMS). The samples, tagged with the LIMS sample identifier, will then be sent to the CLIA laboratory in Phoenix, AZ. Once there, whole exome sequencing and RNA sequencing will be performed on the tumor sample. Whole exome sequencing will also be performed on a blood sample from the patient. For exome sequencing, fastq files are aligned with BWA 0.6.2 to GRCh37.62 and the SAM output are converted to a sorted BAM file using samtools 0.1.18. BAM files are subjected to indel realignment, mark duplicates, and recalibration steps in this order with GATK 2.2 where dpsnp135 is used for known SNPs and 1000 Genomes' ALL.wgs.low_coverage_vqsr.20101123 is used for known indels. Lane level sample BAMs are then merged with Picard 1.79 if they are sequenced across multiple lanes. Variant calling is done with Unified Genotyper and the output VCF files are recalibrated with VariantRecalibrator from GATK 2.2. SnpEff 3.0, and SnpSift 1.7 is then used to annotate these VCF files with database version GRCh37.66.For RNA sequencing, lane level fastq files are appended together if they are across multiple lanes. These fastq files are then aligned with TopHat 2.0.6 to GRCh37.62 using ensembl.63.genes.gtf as GTF file and the resulting BAM files are assembled with Cufflinks 2.0.2 using the same GTF file and ensembl.63.genes.MASK.gtf as the mask file. HTSeq 0.5.3 is used to count the number of reads spanning each gene. Changes in transcript expression are calculated with Cuffdiff 2.0.2. For novel fusion discovery, reads were trimmed to 50 base pairs and then aligned with TopHat-Fusion 2.0.6. At the completion of RNA Sequencing, two files will be produced that will be consumed by the personalized medicine reporting engine. The first of these files is a VCF file that specifies the variants that were observed in the tumor sample. The second file is an FPKM file that specifies the expression levels of genes in the tumor sample. Both of these files will be in the VCF 4.1 format - an example of the VCF 4.1 format for expression is shown in the appendix. At the completion of the whole exome sequencing, a single file VCF file containing the variants for the tumor sample and identifying them as either somatic or germ line by comparison to the normal blood sequence. The tumor variants will be in VCF4.1 spec generated by Seurat 2.5. An example of a variant (for the V600E mutation of BRAF) is shown in the appendix below where AR is allele ratio, DP1 is depth of normal, DP2 is depth of tumor, LN1 is length. The effect of the variant is extracted with the following regular expressions, regex is EFF=/\((.*)\)/.

Patient data captured on the Ashion server is encrypted and pushed via a file transfer server (FTP) for secure delivery of data to authorized NMTRC study personnel. The LIMS system contains a file detection system that will identify when samples are made available on the FTP site. These files, tagged with the sample identifiers will be pulled from the FTP site and imported into the LIMS system. The LIMS system will then process the three files into a personalized medicine report as described in the algorithms section.

2.5 NMTRC Drug prediction report

The following describes the NMTRC report generation which utilizes data generated from Ashion (as described in section 2.4 above).

Predicted Therapeutic Compounds: At this time, there are currently greater than 200 drugs within the NMTRC drug library each with previously defined or inferred molecular targets. For the purposes of this protocol, the tumor board will only use the drugs listed in Appendix III.

Algorithms

There will be three drug matching algorithms utilized in this molecularly guided therapy protocol.

1. Direct variant/drug matching
2. Inferred variant/drug matching
3. Expression of biomarkers/drug targets (biomarker expression)

The algorithms are described in detail below. Although there will not be formal ranking of the drug calls, there are different levels of confidence in the three algorithms and as such, the drugs will be listed in the report in the order of the confidence in the algorithms that identified them as candidate therapies for the patient (Direct variant first, followed by inferred variants, followed by biomarker expression). This is because sequence variants (whether direct or inferred) provide stronger evidence of tumor dependence than simple gene expression calls, with direct drug/variant relationships being the highest confidence of all three. Within the biomarker expression, the drugs will be listed in descending order from most to least differentially expressed.

Variant drug matching

The NMTRC LIMS system has a database that contains a table linking drugs, variants, and genes with evidence from peer reviewed literature. The patient's tumor variants from the VCF files produced by Ashion are linked to the variants and genes in this table to identify potential therapies. This matching provides the output for direct variant/drug matching and inferred variant drug/matching. The inferred and direct refer to the strength of the evidence supporting the relationship between the drug and the variant. The first level of confidence, direct evidence indicates that the literature indicates that 1) the variant has been directly associated with a change in response to the therapy (either becoming more sensitive or more resistant) and the gene is the target of the drug or 2) the variant has been directly shown to activate the pathway for which the drug is a target or 3) the variant has been associated with changes in response to the drug due to indirect mechanism including but not limited to transportation or metabolism. Evidence of direct activation of the drug target by the variant is considered a sensitivity indicator, unless there is specific evidence that the variant induces resistance to the drug. The second level of confidence,

inferred evidence, indicates that the variant is in a gene that is a direct target of a drug and is computationally predicted to have biological impact in the gene, but the variant has not been directly associated with changes in response to drug or activation of target. For inferred variants the variant must be a somatic mutation (present in the tumor only) and also have a minor allele frequence from the 1000 genomes project which is less than 0.5%.Whether the evidence is direct or inferred will be indicated by a column in the report. The evidence that resulted in the drug selection will be provided as a hyperlink in the table.

Biomarker Expression

The third algorithm utilizes the gene expression as measured by RNASeq. A consistent step for the NMTRC methodologies outlined in this proposal is the conversion of normalized probe set intensities to a relative measure of transcript abundance. We utilize a standardized z-score method (described below in Signal Pre-Preprocessing and Z-Score Calculation) to determine statistically significant differences in each sample relative to a whole body reference and relative to cancer reference. This method assigns a z-score to each probe set based upon the number of standard deviations from the normal sample population mean. This provides a good assessment of transcript "differences" relative to a whole body reference or cancer reference sets which may not necessarily be associated with large fold changes in transcript expression. Having established a consistent data input of relative transcript abundance in each subject's tumor specimen, the following analytical methods will be applied to the standardized data to predict drug efficacy.

The method is fundamentally very straightforward. There are two classes of expression rules. The first of these rules are biomarker rules and are of the type "overexpression of gene g1 implies sensitivity (or insensitivity) to drug d1" or "underexpression of gene g2 implies sensitivity (or insensitivity) to drug d2" whereas the second is "g3 is the target of drug d3, thus overexpression of g3 implies sensitivity to d3". The NMTRC LIMS contains a database of drugs and the genes along with a column that implies whether over or under expression of that gene indicates sensitivity (or insensitivity) to the drug and the evidence for that rule. In order for a gene to be called over or underexpressed a method is required to identify calls. This is described in more detail in the next section.
Gene expression calls

Two distinct perspectives are consistent with using over- or underexpression to guide therapy but suggest different sets of reference samples and different statistical tests. If gene expression level is interpreted to reflect sensitivity of a cell to a drug then elevated (alternatively, depressed) expression should be *anomalous* by comparison with normal cells throughout the body (the whole body normal reference). The drug should then kill the tumor cells before it kills normal cells. We call elevated (depressed) expression relative to samples of the whole body Normal Reference using a standard score (Z-score), referred to as the NRZ.

In contrast, if gene expression level in a cancer cell is interpreted as reflecting comparative drug sensitivity among cancer cells then elevated expression should be called based on its *rank* among other cancer samples (the cancer reference). We call elevated (alternatively, depressed) expression relative to the Cancer Reference based on the Cumulative distribution, referred to as the CRC statistic.

We report indications based on both the NRZ and CRC, based on only the NRZ, and based on only the CRC. For each such indication we report both the NRZ and the CRC.

Normal Reference Z-score (NRZ)

$$NRZ = \frac{X_q - \bar{X}_R}{s_R}$$

where X is the normalized expression of Anders and coworkers [43]. q indicates the query (cancer) sample, $\bar{X}_R$ is the reference sample mean of X, and s_R is the reference sample standard deviation of X. The NRZ is consistent with the statistic we used previously for microarray-based expression measurements. We use a threshold of 2, that is, NRZ>2 indicates overexpression NRZ < -2 indicates underexpression. The normal reference set comprises 42 normal tissues, organized into 21 tissue types: Adrenal Gland, Brain, Bronchus, Corpus uteri, Corpus Uteri, Esophagus, Gland, Heart, Large Intestine, Liver, Lymph Node, Nipple, Prostate Gland, Salivary Gland, Skeletal Muscle, Skin, Small Intestine, Spleen, Testis, Tonsil, Uterus. Many (28) of the samples are also given a sub-type designation, for example, Brain: Hippocampus.

Cancer Reference Cumulative statistic (CRC)

$$CRC = \begin{cases} E_{i\in C}[Z_i > 2] & \text{Overexpression} \\ E_{i\in C}[Z_i < -2] & \text{Underexpression} \end{cases}$$

where $E_{i\in C}[\,]$ indicates an average over the set, C, of cancer *cases*, $Z_i = \frac{X_q - \bar{X}_i}{s_C}$ is the standard score for the query by comparison with cancer case i, $\bar{X}_i$ is the mean associated with samples from case i , and s_C is the sample standard deviation, obtained from C. CRC estimates the proportion of cancer samples that are less extreme than the query, that is, a higher CRC indicates more strongly for treatment based on the rule. For example, with an overexpression rule, CRC = 1 means that expression in the query sample was higher than in all cancer reference samples by at least 2 standard deviations. We have chosen to call samples that are in the top quartile (CRC>0.75) as elevated or depressed in expression. We also identify genes support by both NRZ and CRC when NRZ > 2 and CRC > 0.5.

In contrast to the use of the use of the NRZ, with which the objective is to call anomalous expression, comparison with the cancer reference requires estimating the quantile associated with X_q. The proportion of cancer samples that express a gene at a level below the query sample might be estimated using the empirical cumulative distribution function, for example, in the case of overexpression

$E_{i\in C}[X_q > X_i]$.

However, variation arises as a result of biological differences between tumors and as a result of differences in observations of the same tumor. If all tumors were biologically identical with respect to expression of a gene then quantiles would reflect exclusively the observation and variation would not be relevant. Since our cancer reference set includes samples representing pediatric oncology tumors we can estimate the variation *within* cancer case and therefore estimate the proportion of cancer cases for which expression of the gene falls significantly below (overexpression) or above (underexpression) the query.

Report:

The process of report generation begins when all of the sequencing data is available on the FTP server. If gene matches gene in the database then aberration is checked. For each gene/variant that

matches a rule in the rules database, a row will be generated in the report. All variants supported by direct evidence will be listed first in the report indicating their greater clinical significance and an indication that the relationship is supported by direct evidence. If an aberration meets the criteria for an inferred indicator of drug response (described in the algorithms section) it will generate a line in the report which indicates that the relationship between the drug and gene is inferred. For each differentially expressed biomarker indicating/not indicating, a line will be generated in the report. For all rows in the report there will be a link to the PubMed article that supports the identified aberration/drug relationship. The set of rules will be in a read only database that will be inaccessible for changes during the trial.

The software has been validated to show that:

1. 100% reproducible output given the same input.
2. Simple yet sophisticated rules management.
3. Cross reference data with several open source data sets.
4. Generation of a clinically understandable, actionable report.

Positive mutation findings from the whole exome and RNA sequencing will be verified using Sanger Sequencing. While all attempts will be made to have this data available to the tumor board, due to the potential length of time to process Sanger Sequencing samples, these results may not be available until after the tumor board has met and the patient has started protocol treatment. If Sanger Sequencing results differ from the original sequencing, the results and the subject has already started protocol treatment, this information will be communicated to the treating site Principal Investigator who will then communicate this information to the patient and a mutual decision about continued treatment will be made.

3 Treatment Protocol Decision

3.1 Decision Making Rules for Treatment Regimens

Treatments protocols will be generated from the molecular tumor board meeting utilizing the information contained in a report generated on the basis of genomic analysis of the gene expression profile of the subject's tumor. The molecular tumor board consists of pediatric oncologists, pharmacists, bioinformations, genomicists, and pathologists (as available). Specific treatment details will consist of a regimen chosen from a guided list of agents implicated in critical molecular signaling pathways summarized in the guided therapy reports and may include one standard chemotherapy agent not included on the report. All agents are listed in the current FDA approved pharmacopoeia with age appropriate dosing, but will differ amongst individual subjects.

3.1.1 Decision rules for the committee will include:

1. All drugs with predicted efficacy will be reported to the tumor board. At this time, FDA approved and over the counter medications predicted by one of the different predictive methodologies under evaluation will be included. For these panels the analytical methods are independently applied to the standardized and normalized (relative to whole body and cancer reference sets) gene sequencing data derived from the profiling of each subject's tumor. Each method is associated with an objective statistical threshold that must be

exceeded in order for a specific drug to be predicted and reported for consideration. Molecular-based predictions of drug efficacy will be supplemented with evidence gathered from searching of the literature, clinical trials and the internet.

2. Drugs must be FDA approved or available over the counter with established standard and safe age appropriate dosing schedules.
3. Potential drug choices will be analyzed with regards to safety, mechanism, availability and cost. Focus will be on low-toxicity, targeted therapies.
4. Drug combinations will be allowed, up to a maximum of 4 agents. Literature searches will be conducted to assemble data on previously established and tested regimens. These regimens will be given priority.
5. A pharmacist will analyze potential drug interactions between the guided agents and the subject's routine medications and supplements. For drug interactions and known toxicities the following databases will be used: MicroMedex LexiComp, Facts and Comparisons, Natural Medicines Database.

Subjects' history and previously received treatments will be reviewed. Drugs which a subject has failed will be given low priority and used only if there is a rationale for synergy in combination therapy.

3.1.2 Recommended priorities for determining drug dosing to be used in individualized treatment plans include the following:

1. For a given proposed individualized combination of drugs the first priority to establish doses will be to identify the same combination of drugs in a peer-reviewed journal article or presented as a reviewed abstract, or which is part of an ongoing peer-reviewed clinical trial registered with clinical trials.gov.
2. When a proposed individualized combination of drugs has not previously been reported, the process to establish doses will be to then identify individual members of the proposed combination that have been used in combination with other cytotoxic agents similar to those being considered for combination therapy. The source of information will be a peer-reviewed journal article or presented as a reviewed abstract, or which is part of an ongoing peer-reviewed clinical trial registered with clinical trials.gov.
3. When a proposed individualized combination of drugs have no available combination data, dosing guidelines will start with the MTD determined from a phase I/II pediatric study. Reference information will be based upon either a peer-reviewed journal article or presented as a reviewed abstract, or which is part of an ongoing peer-reviewed clinical trial registered with clinical trials.gov. Doses will be reduced to compensate for potential additive toxicities of combination agents.

Please note that many of the drugs that will be considered for proposed individualized combination therapy in this protocol are not considered cytotoxic agents and therefore will not have previously been specifically tested as part of conventional antineoplastic protocols. Examples of these medications may include anti-seizure or anti-cholesterol agents. These non-cytotoxic agents will be used at standard doses based on recommendations of an experienced pediatric pharmacist and described pharmacy programs that are used to evaluate potential interactions and combination dosing for all medications. The final treatment regimen will be subjected to a 12 hour in depth review and evaluation for safety and then be signed off by one of the protocol pharmacists. The treatment memo will then be discussed with the subject and their parents (if the subject is < 18 years of age).

3.2 Additional Biopsies:

Subjects that are having additional biopsies done as standard of care during the course of the study, when deemed appropriate by study PI, may complete the study process again and have new reports generated on the latest tumor. A new tumor board discussion will occur utilizing all rules of this protocol and the new reports. The subject may then change treatment regimens based on the decision of the tumor board. All protocol requirements and specifications will be followed as if it were a new biopsy.

3.3 Safety Evaluations

The principal investigator and the study chairs will evaluate reported serious adverse events and other toxicities in real time. Study chair and PI meetings will occur at least monthly. The principal investigator will meet with the clinical research team at frequent and regularly scheduled intervals to determine treatment modifications and treatment based toxicities.

Any safety concern or new information that might affect either the safety or the ethical conduct of this trial will be immediately forwarded to the study chairs in written form. The NMTRC will be responsible for informing the central IRB and DSMB. If trends in toxicities are noted or stopping rules are met, the study chair will temporarily suspend enrollment while reviewing the episodes with the DSMB. The DSMB will convene every 6 months for evaluation of all safety data.

Study will be on hold for safety monitoring by DSMB review when:

1. Any deaths deemed related to the study drug by the treating PI while on study or occurring less than 30 days after medications ended or 2 serious adverse events possibly related to protocol within 60 days.
2. Any other reason that the NMTRC feels it is in the best safety interest of the subjects.

Subjects will be required to either come off study or be dose reduced per treating physician standard of care if one of the following toxicities related to treatment regimen occurs. Toxicity will be graded according to the NCI-Common Terminology Criteria for Adverse Events (CTCAE v4.0).

Non-Hematologic toxicity:

· ≥Grade 3 non-hematological toxicity not resolved to <Grade 2 or baseline within 2 weeks excluding nausea, vomiting, anorexia, hypertension, electrolyte abnormalities corrected with oral supplementation.

Hematologic toxicity:

· Grade 4 neutropenia lasting ≥14 days;

· Grade 4 neutropenia with Grade 4 infection

3.4 Efficacy Evaluations

At the times indicated in Section 4 and the Table of Procedures and Assessments, scans will be obtained to evaluate response data for subjects enrolled in this study. Efficacy will be assessed according to criteria outlined in Section 6 (Efficacy Assessments) to evaluate the activity and potential benefit of the chosen treatments protocol in this subject population.

3.5 Biological Studies

Subjects will have an opportunity to participate in correlative biological studies on a voluntary basis. This study will be used to correlate in vivo and in vitro predictive drug responses. This study may be able to contribute to our knowledge of molecular determinants of response to therapy and/or development of biomarkers to help guide future therapy.

3.6 Treatment during waiting period

Subjects will be allowed to receive one cycle of standard of care or treating physician chosen therapy after their biopsy and before starting tumor board defined therapy in order to prevent progression during this waiting period. Subjects that opt to have this cycle of therapy will need to have recovered from hematological effects (to baseline) and have repeat scans of their primary tumor sites and any sites being followed for study efficacy before starting tumor board defined therapy.

4 SUBJECT SELECTION

4.1 Inclusion Criteria

1. Subjects must have proven pediatric cancer with confirmation at diagnosis or at the time of recurrence/progression and clinical determination of disease for which there is no known effective curative therapy or disease that is refractory to established proven therapies fitting into one of the following categories:
   a. **Neuroblastoma**- Patients that have relapsed following standard of care therapy (such as high risk patients, patient presenting after age 15 months or MYCN amplified, and only following (for eligible patients) high-dose chemotherapy followed by hematopoietic stem cell transplantation and maintenance therapy with retinoic acid and antibody therapy) or having progressed during standard of care therapy and non-responsive/progressive to accepted curative chemotherapy.
   b. **Brain Tumors**
      i. Medulloblastomas (At relapse after standard of care therapy [surgery, chemotherapy and/or radiation] and/or non-responsive/progressive on accepted curative therapy)
      ii. Gliomas (At relapse after standard of care therapy [surgery and/or radiation and/or chemotherapy] and/or non-responsive/progressive on accepted curative therapy)
      iii. Diffuse Intrinsic Pontine Glioma/DIPG/Diffuse Midline Tumor- (At diagnosis, as there is no known curative therapy)
      iv. Glioblastoma Multiforme- (At diagnosis, as there is no known curative therapy)
      v. Ependymomas (At relapse after standard of care therapy [surgery with or without radiation] and/or non-responsive/progressive on accepted curative therapy)

vi. Choroid plexus tumors (At relapse after standard of care therapy [surgery] and/or non-responsive/progressive on accepted curative therapy)

vii. Craniopharyngiomas (At relapse after standard of care therapy [surgery or suppressive therapy] and/or non-responsive/progressive on accepted curative therapy)

viii. Dysembryoplastic neuroepithelial tumors (DNETs) (At relapse after standard of care therapy [surgery] and/or non-responsive/progressive on accepted curative therapy)

ix. Meningiomas (At relapse after standard of care therapy [surgery] and/or non-responsive/progressive on accepted curative therapy)

x. Primitive Neuroectodermal Tumors (PNETs)/CNS Embryonal Tumors (At relapse after standard of care therapy [surgery, chemotherapy, and/or radiation] and/or non-responsive/progressive on accepted curative therapy)

xi. Germ cell tumors (At relapse after standard of care therapy [surgery, and/or radiation and/or chemotherapy] and/or non-responsive/progressive on accepted curative therapy)

xii. Atypical Teratoid Rhabdoid Tumor (ATRT) (At relapse after standard of care therapy [surgery, chemotherapy and/or radiation] and/or non-responsive/progressive on accepted curative therapy)

c. **Rare Tumors:**

i. Soft tissue sarcoma

Rhabdomyosarcoma (At relapse after standard of care therapy [surgery, and/or radiation, chemotherapy] and/or non-responsive/progressive to accepted curative chemotherapy)

Non-rhabdomyosarcoma (At relapse after standard of care therapy [surgery, and/or radiation, chemotherapy] and/or non-responsive/progressive to accepted curative chemotherapy)

ii. Bone

Ewings sarcoma (At relapse after standard of care therapy [surgery, and/or radiation, chemotherapy] and/or non- responsive/progressive to accepted curative chemotherapy)

Osteosarcoma (At relapse after standard of care therapy [surgery, chemotherapy] and/or non- responsive/progressive to accepted curative chemotherapy)

iii. Renal

Wilms tumor (At relapse after standard of care therapy [surgery, and/or radiation, chemotherapy] and/or non- responsive/progressive to accepted chemotherapy)

Renal cell carcinoma (At relapse after standard of care therapy [surgery, chemotherapy] and/or non- responsive/progressive to accepted curative chemotherapy)

Malignant rhabdoid tumor (At diagnosis, as there is no known curative therapy)

Clear Cell Sarcoma- (At relapse after standard of care therapy [radiation, chemotherapy] and/or non- responsive/progressive to accepted curative chemotherapy)

iv. Germ Cell tumors (At relapse after standard of care therapy [surgery, chemotherapy] and/or non-responsive/progressive to accepted curative chemotherapy)

v. Liver Tumors (At relapse after standard of care therapy [surgery, chemotherapy] and/or non- responsive/progressive to accepted curative chemotherapy)
vi. Carcinomas- (At relapse after standard of care therapy and/or non-responsive/progressive to accepted curative chemotherapy)
vii. Desmoid tumors- (At relapse after standard of care therapy and/or non-responsive/progressive to accepted curative chemotherapy)
viii. Malignant Peripheral Nerve Sheath Tumors (MPNST)- (At diagnosis, as there is no known curative therapy)
ix. Pheochromocytoma (At relapse after standard of care therapy and/or non-responsive/progressive to accepted curative chemotherapy).
x. Ethesioneuroblastoma (At relapse after standard of care therapy and/or non-responsive/progressive to accepted curative chemotherapy).
xi. Neuroendocrine tumors (At relapse after standard of care therapy and/or non-responsive/progressive to accepted curative chemotherapy).
xii. Desmoplastic small round cell tumor (At relapse after standard of care therapy and/or non- responsive/progressive to accepted curative chemotherapy).
xiii. Melanoma (At relapse after standard of care therapy and/or non-responsive/progressive to accepted curative chemotherapy).

2. Subjects must be age >12 months at enrollment
3. Subjects must be age ≤ 21 years at initial diagnosis
4. Subjects must have measurable disease as defined by RESIST v1.1 at the time of biopsy and tumor must be accessible for biopsy. Tumor samples submitted for analysis must contain >50% viable tumor tissue to qualify. In addition, subjects with disease confined to the bone marrow are eligible to enroll if the degree of marrow involvement is expected to be >50%.

   Note: Subjects who are expected to have no evidence of disease after surgical removal of their tumor are still eligible for this trial if their disease would normally require adjuvant chemotherapy treatment after surgery despite NED status.
5. Current disease state must be one for which there is currently no known effective therapy
6. Specimens will be obtained only in a non-significant risk manner and not solely for the purpose of investigational testing.
7. Lansky or Karnofsky Score must be ≥ 50
8. Subjects without bone marrow metastases must have an ANC > 750/µl to begin treatment.
9. Subjects with CNS disease currently taking steroids must have been on a stable dose of steroids for 2 weeks prior to their biopsy and must not have progressive hydrocephalus at enrollment.
10. Adequate liver function must be demonstrated, defined as:
    a. Total bilirubin ≤ 1.5 x upper limit of normal (ULN) for age AND
    b. ALT (SGPT) < 10 x upper limit of normal (ULN) for age
11. A negative serum pregnancy test is required for female participants of child bearing potential (≥13 years of age or after onset of menses)
12. Both male and female post-pubertal study subjects need to agree to use one of the more effective birth control methods during treatment and for six months after treatment is stopped. These methods include total abstinence (no sex), oral contraceptives ("the pill"), an intrauterine device (IUD), levonorgestrol implants (Norplant), or medroxyprogesterone acetate injections (Depo-provera shots). If one of these cannot be used, contraceptive foam with a condom is recommended.

13. Informed Consent: All subjects and/or legal guardians must sign informed written consent. Assent, when appropriate, will be obtained according to institutional guidelines

4.2 Exclusion Criteria

1. Subjects who have received any cytotoxic chemotherapy within the last 7 days prior to biopsy
2. Subjects who have received any radiotherapy to the primary sample site within the last 14 days (radiation may be included in treatment decision after biopsy).
3. Subjects receiving any investigational drug concurrently.
4. Subjects with uncontrolled serious infections or a life-threatening illness (unrelated to tumor)
5. Subjects with any other medical condition, including malabsorption syndromes, mental illness or substance abuse, deemed by the Investigator to be likely to interfere with the interpretation of the results or which would interfere with a subject's ability to sign or the legal guardian's ability to sign the informed consent, and subject's ability to cooperate and participate in the study

Additional criteria:

Subjects willing to participate in the correlative biologic studies will sign an additional consent form to provide additional tumor tissue.

5 STUDY PROCEDURES AND ASSESSMENTS

5.1 Enrollment of Subjects

All subjects (or subjects' legal representatives) must provide written informed consent before any study specific assessments may be performed. The NMTRC will keep a "possible enrollment list" for all sites combined. Subjects will be allowed to enroll in the order that they are added to that list. Prior to consent of the subject, the NMTRC research coordinator will be contacted (via e-mail) to place subject on this possible enrollment list. The NMTRC coordinator will then reply with study space availability. If a spot is not available at that time, the site will be contacted as soon as a spot does open up (based on the subject's order on the list). If a spot is available at the time, the potential subject will undergo consent and completion of all required screening procedures and certification of all inclusion and exclusion criteria by the Investigator. If the subject fits all enrollment criteria, the site will again contact the coordinator at the NMTRC for official enrollment confirmation and unique subject identifier assignment. In addition, a study enrollment form will be e-mailed or faxed to the coordinator at the NMTRC. A subject may NOT be enrolled on trial until official approval from the NMTRC is received. When a spot becomes available, the first subject from the list will be contacted and will have 5 working days with which to enroll in the study. If this subject does not enroll by that time then the subject forfeits his/her spot and the next subject will be offered that spot.

5.2 Screening

The Investigator is responsible for keeping a record of all subjects screened for entry into the study and subsequently excluded.

The following screening procedures must be performed within 21 days (14 days preferred) prior to date of biopsy*. Studies must be done *after* last previous treatment for malignancy:

1. Signed informed consent form. All subjects (or subjects' legal representatives) must provide written informed consent before any study specific assessments may be performed. Signed informed consent form for voluntary participation in correlative biologic analysis;
2. Complete medical and surgical history, including documentation of the evidence of malignancy and prior treatments for cancer. Include all other pertinent medical conditions and a careful history of all prior medical treatments;
3. Demographics;
4. Physical examination (including height and weight), noting all abnormalities and sites of palpable neoplastic disease;
5. Vital signs, including temperature, pulse rate, and blood pressure;
6. Karnofsky Performance status/Lansky Play status (Appendix I);
7. Complete Screening Baseline Symptom form;
8. CBC with differential;
9. Serum electrolytes (sodium, potassium, chloride, bicarbonate), blood urea nitrogen (BUN), creatinine, albumin, Bilirubin, ALT, and AST;
10. LDH and/or Specific Tumor Markers for defined disease (if available);
11. Serum pregnancy test for female subjects of child bearing potential (onset of menses or ≥13 years of age);
12. CT or MRI of measurable disease sites (as indicated for tumor type);
13. Solid tumor biopsy for "Primary Sample" with remaining tumor tissue used in correlative biologic studies handled per section 9.3 and submitted per Appendix II.

    For subjects that cannot provide a solid tumor biopsy; a bone marrow core biopsy sample will replace the solid tumor sample as the "Primary Sample." Bone Marrow must have adequate tumor (defined as >50% tumor) in order to qualify as "Primary Sample."

    ***NOTE: If standard-of-care sample collection occurred within 12 weeks prior to enrollment and after last previous treatment for malignancy, and frozen sample remains, a subject may enroll using previously collected samples. Samples must additionally have been snap frozen within 20 minutes of collection and kept in a -70/-80 freezer.**

14. Concomitant medications/therapies including documentation of steroid use and dose;
15. Confirmation of inclusion and exclusion requirements.

Following completion of all required screening procedures and certification of all inclusion and exclusion criteria by the Investigator, the coordinator at the NMTRC will be contacted (via e-mail or phone call), at which time the subject will be enrolled in the trial and a unique subject number assigned.

The following screening procedures must be done prior to the first dose of study drug, but are not required to be done before biopsy.

1. MIBG/PET scan (if indicated for tumor type). Other imaging appropriate for tumor type may also be considered.
2. Any subject that is suspected to have possible bone marrow disease: Bone marrow aspirate and biopsy.
3. Completion (by study staff) of pre-study case report forms.
4. Any additional labs or testing required by the tumor board.

For subjects that receive one cycle of treatment while waiting to start tumor board defined treatment (per section 2.11) or if patient did not receive treatment but more than 28 days since last assessment has occurred prior to starting study treatment: The following additional screening procedures must be performed AFTER the last dose of this additional cycle of therapy and PRIOR to the first dose of tumor board defined treatment:

1. Repeat CT or MRI of primary disease sites and any disease sites being followed for study endpoints.
2. CBC with differential- subject must be hematologically recovered to baseline before starting tumor board defined treatment.

5.3 Study Procedures/Study Interventions

5.3.1 Study Visits- Cycle 1 Day 1 (may have been done within 5 days of treatment start/day 1, unless otherwise indicated as "same day")

Subjects will return to the enrolling clinic at Day 1 of cycle 1 for evaluations. The following evaluations will be conducted prior to starting study drug:

1. Physical examination (including body weight) and medical history (**same day**);
2. Karnofsky Performance status/Lansky Play status-Appendix I (**same day**);
3. Vital signs, including height, weight, temperature, pulse rate, and sitting blood pressure (**same day**);
4. CBC with differential;
5. Serum electrolytes, BUN, creatinine, Bilirubin, ALT, AST;
6. LDH and/or Specific Tumor Markers for defined disease (if available);
7. Baseline EKG (as indicated);
8. Cycle 1 Day 1 Baseline Symptom Form (**same day**);
9. Review and recording of concomitant medications (**same day**);
10. Completion (by study staff) of Case Report Forms;
11. Any additional labs or testing required by the tumor board.

5.3.2 Study Visits- Cycle 1 Day 8 (± 3 days)

Subjects will return to the enrolling clinic at Day 8 of cycle 1 for evaluations. The following evaluations will be conducted at those time points:

1. Physical examination and medical history;
2. Vital signs, including height, weight, temperature, pulse rate, and sitting blood pressure;
3. CBC with differential;
4. Monitoring of AEs;
5. Review and recording of concomitant medications;
6. Completion (by study staff) of Case Report Forms;
7. Any additional labs or testing required by the tumor board.

5.3.3 Study Visits- Cycle 1 Day 15 (± 3 days)

Subjects will return to the enrolling clinic at Day 15 of cycle 1 for evaluations. The following evaluations will be conducted at those time points:

1. Physical exam and medical history;
2. Karnofsky Performance status/Lansky Play status-Appendix I;
3. Vital signs, including temperature, pulse rate, and blood pressure (sitting);
4. CBC with differential;
5. Serum electrolytes, BUN, creatinine, Bilirubin, ALT, AST;
6. Review and recording of concomitant medications;
7. Monitoring of AEs;
8. Completion (by study staff) of Case Report Forms;
9. Any additional labs or testing required by the tumor board.

5.3.4 Study Visits- Cycle 1 Day 22 (± 3 days)- for 28 day cycles only

Subjects will return to the enrolling clinic at Day 22 of cycle 1 for evaluations. The following evaluations will be conducted at those time points:

1. Physical examination and medical history;
2. Vital signs, including height, weight, temperature, pulse rate, and sitting blood pressure;
3. CBC with differential;
4. Monitoring of AEs;
5. Review and recording of concomitant medications;
6. Completion (by study staff) of Case Report Forms;
7. Any additional labs or testing required by the tumor board.

5.3.5 Subsequent Cycles (Maintenance Protocol Treatment Cycles) (up to 14 day window from end of previous cycle to start Day 1 of the next Cycle treatment per treating institutions standard of care)

Drug administration will be according to guidelines in previous cycles with dose modifications if needed per Section 5.1.1. The following evaluations will be performed on day 1 of each cycle as indicated. Evaluations may be performed within 5 days prior to dosing unless otherwise indicated. Subsequent cycles (cycles 2 and beyond) may be given at the subject's home institution. If subjects are to receive subsequent cycles at a home institution, this will occur only at COG (Children's Oncology Group) member hospitals or those specifically approved by theSite Principal Investigator.

1. Physical examination and medical history;
2. Karnofsky Performance status/Lansky Play status-Appendix I;
3. Vital signs, including height, weight, BSA, temperature, pulse rate, and sitting blood pressure (**same day**);
4. CBC with differential;
5. Serum electrolytes, BUN, creatinine, Bilirubin, ALT, AST;
6. LDH and/or Specific Tumor Markers for defined disease (if available);
7. Review and recording of concomitant medications;
8. Monitoring of AEs (must be done on Day 1);
9. Completion (by study staff) of Case Report Forms;
10. Any additional labs or testing required by the tumor board.

5.3.6 Reevaluation during Subsequent (Maintenance) Cycles

Subjects will be assessed for disease at the end of cycle 2 and every other cycle or every 8 weeks*, whichever occurs first, with the following (timing should be during the last week of the cycle):

1. CT or MRI of measurable disease sites (same scans as done at study entry);
2. MIBG/PET scan (if indicated for tumor type). Other imaging appropriate for tumor type may also be considered;
3. Any subject with suspected bone marrow disease and with positive bone marrow disease at study entry: Bone marrow aspirate and biopsy.

*After Cycle 6, reevaluations procedures in section 5.3.6 may be done per institutional standard.

5.3.7 Off Therapy/30 Day Follow-Up Visit

Subjects will be followed up at either the study clinic or by telephone within 30 (+7) days after the last dose of treatment. Last dose of treatment is defined as the last day that the subject received a tumor board recommended treatment. The following evaluations will be conducted:

1. Review and recording of concomitant medications;
2. Monitoring of AEs and review of concurrent illnesses;
3. Completion (by study staff) of Case Report Forms.

Any subject with suspected study drug <u>related</u> toxicities at the follow-up visit must be followed until all current drug <u>related</u> adverse events have resolved to baseline or ≤ Grade 2 or stabilization of the event. This may require additional clinical assessments and laboratory

tests. The follow-up results will be recorded on the appropriate page of the CRF, as well as in the subject's source documentation.

6 Protocol Drugs

Specific treatment details will consist of a regimen chosen from a guided list of agents implicated in critical molecular signaling pathways and/or from signature-based predictions of drug efficacy summarized from the guided therapy report. All agents are listed in the current pharmacopoeia for human use, but will differ amongst individual subjects. The treatment regimens will be discussed with families and will include review of known side effects per Pharmacopeia Library (Appendix III) (information obtained from package insert and from MicroMedex, LexiComp, Facts and Comparisons, and Natural Medicines Database), serious adverse effects of possible new drug combinations, and any additional clinical monitoring that may be recommended by the tumor board. The family will be given the option to proceed with therapy and if the family decides to proceed with the tumor board's treatment decision they will be asked to sign a treatment specific memo.

Drugs will be prescribed by the enrolling institution per treating hospital protocol and administered per FDA guidelines and tumor board recommendations.

6.1 Treatment modifications

Dosing delays and modifications will be at the discretion of the treating Principal Investigator and their institutional pharmacist and based upon the selected therapy, subject response and practiced dosing regimens. Decisions will be made on the basis of clinical expertise of the physicians and pharmacists and with the subject's welfare being the principal concern. Informational websites such as drug bank (Micromedex, Lexicomp, Facts and Comparisons, Natural Medicines Database) will be utilized to identify potential adverse events associated with individual treatments. The study pharmacist will be available to consult on decisions made with respect to changes and modification made due to potential drug related adverse events.

6.2 Concomitant Medications and Treatments

All intercurrent medical conditions will be treated at the discretion of the Investigator according to acceptable community standards of medical care. All concomitant medications and treatments will be documented on the appropriate case report form.

The following medications are not permitted during the trial:

- Any cytotoxic chemotherapy
- Any other investigational treatment
- Any other systemic anti-neoplastic therapy including, but not limited to, immunotherapy, hormonal therapy, targeted therapies, anti-angiogenic therapies, or monoclonal antibody therapy

The following medications/treatments may be administered as follows:

- Any radiotherapy administered with palliative intent/pain control or recommended by tumor board as part of therapeutic regimen for best patient care.
- Prophylactic filgrastim, pegfilgrastim or oprelvekin; these hematopoietic growth factors may be administered according to ASCO, ASH, or institutional guidelines to treat an established cytopenia
    - If subject experiences a prolonged neutropenia greater than 72 hours during Cycle 1; consider using filgrastim or pegfilgrastim during all subsequent cycles.

- Erythropoietin, blood products, anti-emetics, steroids, and transfusions may be administered at the discretion of the Investigator based on established criteria.

7 Efficacy Assessments

7.1 Tumor Assessments/Scans

Tumor assessments/imaging studies must be obtained at baseline, at the end of cycle 2 and again after every other cycle (or every 8 weeks, whichever occurs first). After Cycle 6, may be done per institutional standard. The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up.

All radiological images must be available for source verification. Images may be submitted for extramural review for final assessment of antitumor activity.

Subjects who come off study should have a final end of study disease-specific assessment done when possible.

7.2 Scan Submission:

All required study scans (CT's, MRI's, MIBG's and PET's) will be de-identified and sent to the NMTRC. All study required de-identified scans will be uploaded to HIPAA compliant database (if available) or sent on disc to:

Alyssa VanderWerff
NMTRC
Clinical Program Coordinator
100 Michigan Avenue NE MC 272
Grand Rapids, MI 49503
Tel: (616) 267-0327
E-Mail: Alyssa.VanderWerff@ helendevoschildrens.org

7.3 Response Criteria

Overall response rate (ORR) in subjects with radiologically assessable disease will be determined by CT or MRI by cross-sectional imaging, MIBG/PET scans, and/or bone marrow assessment.

Response Assessment: Each subject will be classified according to their "best response" for the purposes of analysis of treatment effect. Best response is determined from the sequence of the objective statuses described below.

Response Criteria for Subjects with Solid Tumors: This study will use the (RECIST) Response Evaluation Criteria in Solid Tumor (version 1.1) from the NCI[44]. Key points are that a maximum of 5 target lesions are identified and that changes in the ***largest*** diameter (uni-dimensional measurement) of the tumor lesions are used in the RECIST v1.1 criteria.

- Measurable disease: The presence of at least one lesion that can be accurately measured in at least one dimension with the longest diameter at least 10 mm (CT scan slice thickness no greater than 5 mm). The investigator will identify up to 5 measurable lesions to be followed for response. Previously irradiated lesions must demonstrate clear evidence of progression to be considered measurable.
- Serial measurements of lesions are to be done with CT or MRI, using the same method of assessment is to be used to characterize each identified and reported lesion at baseline and during follow-up.

- Quantification of Disease Burden The sum of the longest diameter (LD) for all target lesions will be calculated and reported as the disease measurement.

- **Complete Response (CR):** Disappearance of all target and non-target lesions.
- **Very Good Partial Response (VGPR):** Greater than 90% decrease of the disease measurement for CT/MRI lesions, taking as reference the disease measurement done to confirm measurable disease at study entry. Non-target CT/MRI lesions stable to smaller in size.
- **Partial Response (PR):** At least a 30% decrease in the disease measurement, taking as reference the disease measurement done to confirm measurable disease at study enrollment. No new lesions or progression of any non-target measurable lesion.
- **Stable Disease (SD):** Neither sufficient decrease to qualify for PR or sufficient increase to qualify for PD from study entry.
- **Progressive Disease (PD):** At least a 20% increase in the sum of the disease measurements for measurable lesions, taking as reference the smallest disease measurement recorded since the start of treatment (nadir), and minimum 5 mm increase over the nadir or the appearance of one or more new lesions.

Response Criteria for Subjects with Bone Marrow Disease:

- Those subjects with morphologic evidence of disease by routine H and E staining (NSE staining only is not evaluable) will be evaluable to assess bone marrow response.
- **Complete response:** No tumor cells detectable by routine morphology on two consecutive bilateral bone marrow aspirates and biopsies done at least three weeks apart after study entry.
- **Progressive disease**: Tumor seen on morphology on two consecutive bone marrows done at least three weeks apart in subjects who had NO tumor in bone marrow at study entry. (Note: Subject may be declared as progressive disease in bone marrow after only one diagnostic bone marrow at the discretion of the treating physician after discussion with the study chair.)
- **Stable disease**: Persistence of an amount of tumor in the bone marrow by morphology that does not meet criteria for either complete response or progressive disease.

Response Criteria for Subjects with MIBG or PET Positive Lesions

- Subjects who have a positive MIBG or PET scan at the start of therapy will be evaluable for MIBG or PET response. All MIBG's and PET scans will be performed at the research institution and then centrally reviewed.
- **Complete response** = complete resolution of all positive lesions
- **Partial response** = resolution of at least one positive lesion, with persistence of other MIBG positive lesions.
- **Stable disease** = no change in scan in number of positive lesions (includes subjects who have same number of positive lesions but decreased intensity)
- **Progressive disease** = Development of new positive lesions

The intensity of MIBG uptake is not to be considered in the above institutional evaluation.

Duration of response:

Duration of response is defined as the period of time from when measurement criteria are met for complete response (CR) or partial response (PR), whichever is first recorded, until the first date that recurrent or progressive disease (PD) is objectively documented (taking as reference for PD the smallest measurements recorded since the treatment started).

The assessment of response will include the initial measurable targets and will be performed after the second cycle, then after every other cycle (or 8 weeks, whichever occurs first). Serial results of bone marrow aspirates, biopsies and urinary catecholamines will be reviewed for responding subjects to confirm response or lack of progression.

Clinical Response

A subject will be defined as having a clinical response if they have stable disease or better and a decrease in their tumor markers by ≥ 50%. Clinical response will also be defined as a subject that has a complete clearing of a previously positive bone marrow while on study.

Progression Free Survival

Time to progression, defined as the period from the first day of administration of study drug until the criteria for progression are met taking as reference the screening measurements, will be assessed.

8 Sample Size Justification

Feasibility

Since this is primarily a feasibility trial, the definition of feasibility for this study will include:

"Enrollment onto study, genomic profile, analysis and report generation completed, tumor board held with treatment decision, treatment review completed, start of treatment, and completion of 1 cycle of therapy."

Our preliminary results for 5 subjects indicates that we have a sample collection success rate of 5/5 (100%) subjects, a genetic report profile success rate of 5/5 (100%) within the 7-10 day time window as well as a treatment agreement success rate of 5/5 (100%) within the 5 day time window. The average time (+/-SD) for completion of the genetic profile was 6.6 days (+/-1.7 days) with a median of 7 days. The average time (+/-SD) for time to Treatment was 3.2 days (+/-0.8 days) with a median of 3 days. Data preliminary to date for each of these three feasibility markers are very consistent with our anticipated performance standards.

Our initial feasibility trial continues to show that it is feasible and safe to use RNA expression analysis and a DNA mutation panel for tumor boards creating treatment decisions for patients without curative options. This study will ask the feasibility of utilizing DNA exomes and RNA sequencing in a tumor for molecular guided therapy.

Assumptions: The feasibility outcome is a binary measure for each of the three clinical strata with a lower success null value of 50% which would require reconsideration of the full process and an upper success value of 75% for the alternative which would be deemed worthy of further consideration for an efficacy trial at the next phase. A binomial distribution was used for the testing process with a combination of Type I error levels (10%) and Power (75%). The basic design overall is a MiniMax approach which minimizes the overall sample size. **The MiniMax design with an interim look will require an overall sample size of n = 16. Each of the three clinical strata will use a two-stage decision rule.**

The Two-Stage Stopping Rule for each of the three strata is as follows:

Stage 1: Stop and accept the null hypothesis if the observed feasibility rate is less than or equal to 5/9. Otherwise, continue to stage 2.

The probability of stopping for futility is 0.746 when Ho is true and 0.166 when Ha is true.

Stage 2: Stop and accept the null hypothesis if the observed feasibility rate is less than or equal to 10/16. Otherwise, stop and reject the null hypothesis.

As of Amendment 3.0, 100 subjects have been enrolled and all feasibility measures for all strata have been met. To gain additional feasibility with the new Drug Prediction Report and additional safety and efficacy data, additional subjects will be enrolled to meet a revised goal of 200 total evaluable subjects.

The expanded access for 100 patients will enroll following implementation of Amendment 3.0. Their tumor samples will be analyzed using an updated Drug Prediction Report (Appendix III). Overall Response Rate (ORR) and Progression Free Survival (PFS) for this group will be compared to those of the first 100 patients who received treatment based upon the original Drug Prediciton Report and Pharmacopeia Library.

9 Laboratory Evaluations

9.1 Specimens to be Collected, Schedule and Amount

Required samples will be collected at the start of study. Recommended/Secondary (volunteer/optional) samples will also be collected at the start of study. Additionally, optional bone marrow samples will be collected every 6-8 weeks per Study Procedure Table.

9.2 Tumor collection and correlative biology studies.

Fresh tumor will be flash frozen and placed in kit for Ashion along with 4-6ml blood sample in an EDTA tube. Viable, fresh tumor from tumor biopsy should be placed in cell growth media using sterile technique for, cell line and xenograft generation, one vial of tumor in RNA later, and two vials of fresh frozen tumor to be shipped to the Pediatric Oncology Translational Research Laboratory for RNA analysis. Samples should be labeled with subject's study number, date of extraction and sample contents. If excess tissue is available, then snap frozen tumor tissue approximately 5mm in size should be wrapped in foil, snap frozen in liquid nitrogen and stored at -80ºC. Tissue samples will be coded. Fixed tissue remaining from diagnostic evaluation may be used. Bone marrow samples will also be sent to the Pediatric Oncology Translational Research Laboratory for tumor cell isolation and culture.

At the Pediatric Oncology Translational Research Laboratory tumor cells will be grown to 70% confluency in cell growth media. Cell lines will be maintained in culture for biology studies. These will include determining the growth curves and responsiveness of cells derived from these tumors to a variety of agents identified on the report *in vitro* (both the chosen regimen as well as alternate options). Cells will be injected into the inguinal fat pad of NOD SCID mice for generation of subject xenografts. These mice will be used for correlative drug testing experiments based on predictive models. Evaluation of mechanism of action of new drug combinations will be evaluated in mice models and in cell culture through immunohistochemical staining, phosphoproteomics, PCR, and western blotting. These models will allow us the opportunity to evaluate alternate drug combinations that might have been superior to the chosen regimen.

9.3 Detailed Procedure For Sending Subject Samples:

At screening, subjects will have biopsy samples sent. The main sample for study report will be referred to as the "Primary Sample."

The "Primary Sample" will be a current solid tumor biopsy or core bone marrow biopsy (with >50% viable tumor) along with a 4-6 mL blood sample collected in an EDTA tube.

If a solid tumor sample cannot be obtained and the subject's bone marrow is <50% tumor then they will be ineligible for study.

In addition to the "Primary Sample", if subjects have signed the additional consent for optional sample collection, they will also have these secondary samples sent as follows.

9.3.1 Sample Procurement and Shipping at Screening:

De-identified subject tumor samples will be sent on all subjects as following:

Label all tubes with subject's unique identifier, date/time of sample collection, and contents (i.e. solid tumor, bone marrow, blood).

9.3.1.1 Send the following Required Primary Samples for All Tumor Types (along with appropriate form)

1. **Required- Sample 1 (tumor)**

   Solid Tumor or Bone Marrow Core Biopsy (if solid tumor is not available)- You will be provided a kit for tumor collection. This kit will include instructions, collection tubes and shipping instructions.

   For sample 1 follow collection instructions in Appendix II (flash freeze tumor sample **within 20 minutes** of harvest, or sooner when possible) and ship overnight on at least 1kg of dry ice to:
   *Note: Do NOT place sample in aluminum/tin foil at any time.

   Ashion Analytics, LLC
   445 N 5th Street, Suite 468
   Phoenix, AZ 85004
   Phone: 602-343-8796
   Lab Fax: 602-343-8545
   email: customerservice@ashiondx.com

   For Biopsies- refer to Instructions for Core/Needle Biopsy Specimens for sample size instructions.
   For Surgical resections- size of sample should be about the size of a pencil eraser. Minimum amount of tumor sample required is 50-200 mg of tissue.

2. Required- Sample 2 (blood):
Send 4-6 mL of blood in EDTA tube (ambient – prefer to have shipped on frozen gel pack in summer months May to October) to:

Ashion Analytics, LLC
445 N 5th Street, Suite 468
Phoenix, AZ 85004
Phone: 602-343-8796
Lab Fax: 602-343-8545
email: customerservice@ashiondx.com

9.3.1.2 **Send the following** Recommended secondary tumor **samples** along with appropriate forms listed **in order of priority** (please work your way down the list of tumor samples until you either run out of available sample or have completed all samples. Once this occurs move on to Bone Marrow samples:

You will be provided a kit for tumor collection (fresh snap frozen and in culture media). Follow collection instructions and ship as directed overnight.

3. Sample 3:

With sterile technique and within 20 minutes add tumor sample or needle core biopsy to T25 flask containing cell growth media (provided), seal with parafilm, and overnight at ambient temperature to:

Ping Zhao
Pediatric Oncology Translational Research Laboratory
Coopers Landing
1345 Monroe Ave, Suite 121
Grand Rapids, MI 49505
Ph: 616-486-8645
Ping.zhao@helendevoschildrens.org

4. Sample 4:

Add tumor or needle core biopsy to cryo-vial, snap freeze and ship on at least 1kg of dry ice to:

Ping Zhao
Pediatric Oncology Translational Research Laboratory
Coopers Landing
1345 Monroe Ave, Suite 121
Grand Rapids, MI 49505
Ph: 616-486-8645
Ping.zhao@helendevoschildrens.org

5. **Sample 5:**

Add tumor or needle core biopsy to cryo-vial, snap freeze and ship on at least 1kg of dry ice to:

Ping Zhao
Pediatric Oncology Translational Research Laboratory
Coopers Landing
1345 Monroe Ave, Suite 121
Grand Rapids, MI 49505
Ph: 616-486-8645
Ping.zhao@helendevoschildrens.org

6. **Sample 6:**

Add tumor or needle core biopsy to RNA Later and ship overnight at ambient temperature to:

Ping Zhao
Pediatric Oncology Translational Research Laboratory
Coopers Landing
1345 Monroe Ave, Suite 121
Grand Rapids, MI 49505
Ph: 616-486-8645
Ping.zhao@helendevoschildrens.org

9.3.1.3 For all Tumor Types: Send the following Recommended secondary blood **sample** (along with appropriate forms):

Blood- Send 4-6 mL of blood in tubes (tubes and instructions will be provided).

1. **Sample 7:**

Send PAX DNA Gene tubes (ambient) to:

Ping Zhao
Pediatric Oncology Translational Research Laboratory
Coopers Landing
1345 Monroe Ave, Suite 121
Grand Rapids, MI 49505
Ph: 616-486-8645
Ping.zhao@helendevoschildrens.org

9.3.1.4 **For Neuroblastoma Subjects or Any subject with suspected bone marrow disease Only- Send the following Recommended secondary bone marrow samples listed in order of priority** (along with appropriate forms):

1. **Sample 8:**

Bone Marrow Aspirate- Send 4 mL of bone marrow aspirate (preferably bilateral) in green top (sodium heparin) tube(s) priority overnight at room temperature to: (Please alert site 2-3 days prior to procedure. Contact site to confirm shipment as well.)

Ping Zhao
Pediatric Oncology Translational Research Laboratory
Coopers Landing
1345 Monroe Ave, Suite 121
Grand Rapids, MI 49505
Ph: 616-486-8645
Ping.zhao@helendevoschildrens.org

Notes: If all samples are collected, you will be collecting 8 samples and mailing **4** packages. The samples above are listed in decreasing priority.

* Sample 1 to Ashion (frozen on dry ice)
** Sample 2 to Ashion (ambient with frozen gel pack)
***Sample 3, 6, 7, & 8 to Pediatric Oncology Translational Research Laboratory (ambient).
****Samples 4 & 5 to Pediatric Oncology Translational Research Laboratory (frozen).

******* Keep collection flask (sample 3) at 4° C. until ready to use.

Please contact Ping Zhao if you have any trouble with the preparation or packaging of the samples. Ping.zhao@helendevoschildrens.org

9.3.2 For Neuroblastoma Subjects or Any subject with suspected bone marrow disease Only- Sample Procurement and Shipping at the end of Cycle 2 and every two cycles after that:

Send the following Recommended samples (along with appropriate forms) to the Pediatric Oncology Translational Research Laboratory

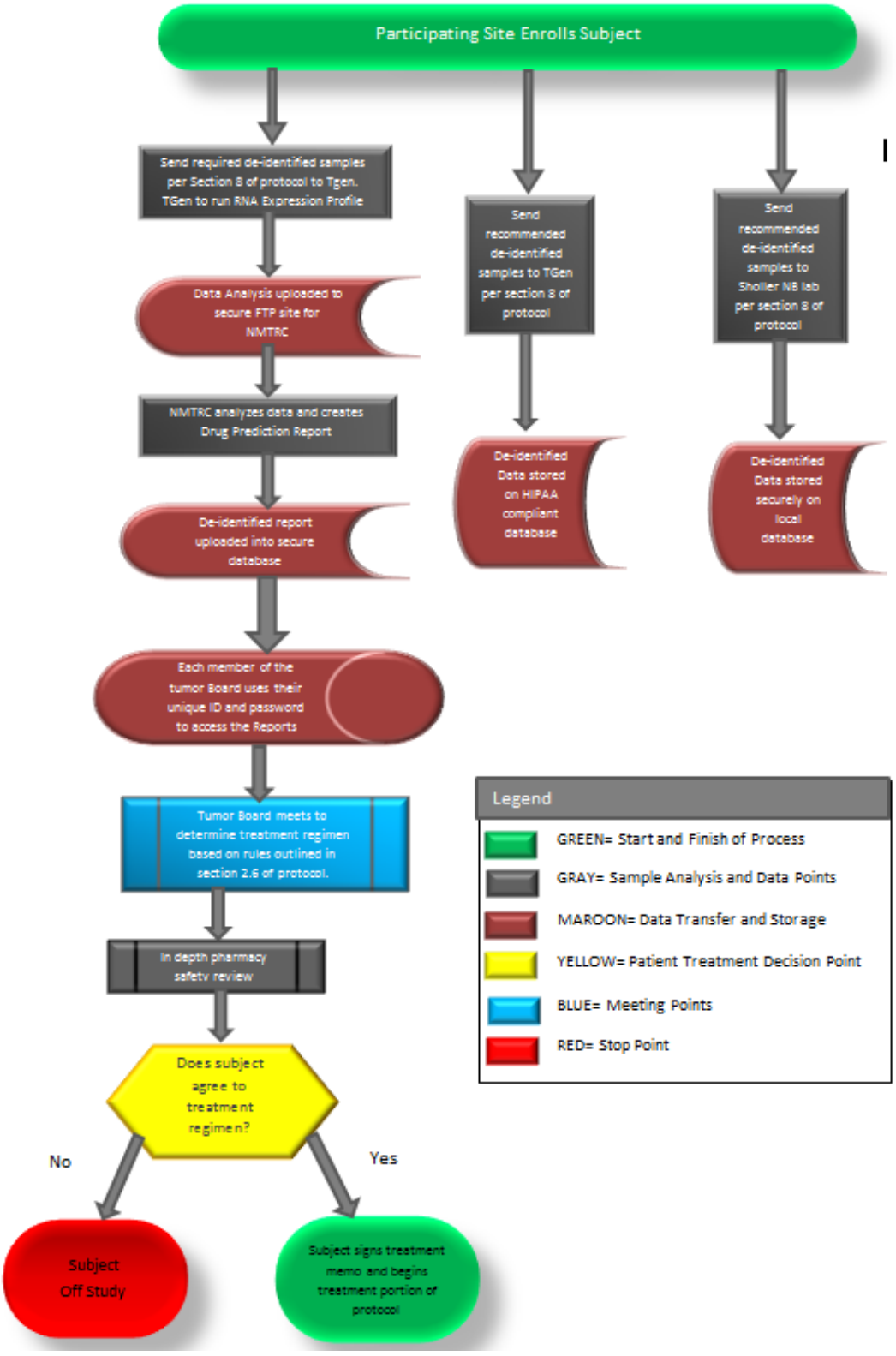
1. **Bone Marrow Aspirate-** Send 4 mL of bone marrow aspirate (preferably bilateral) in green top (sodium heparin) tube(s) priority overnight at room temperature to:

Ping Zhao
Pediatric Oncology Translational Research Laboratory
Coopers Landing
1345 Monroe Ave, Suite 121
Grand Rapids, MI 49505
Ph: 616-486-8645
Ping.zhao@helendevoschildrens.org

9.4 Storage

Blood and tumor samples collected for any studies performed in this protocol, and any other components from the processed cells, may be stored indefinitely to research scientific questions related to cancer and/or study drugs. The subject retains the right to have the sample material destroyed at any time by contacting the principal investigator.

9.5 Plan for Communication of Samples and Data



9.6 Plan for Communication of Collaborating Sites

Communication between centers will be critical in this trial. Prior to consent of the subject, the NMTRC research coordinator will be contacted (via e-mail). If a spot is available at the time, the potential subject will undergo consent and completion of all required screening procedures and certification of all inclusion and exclusion criteria by the Investigator. If the subject fits all enrollment criteria, the site will again contact the coordinator at the NMTRC who will notify TGen, Ashion, Spectrum Health, and the Pediatric Oncology Translational Research Laboratory in preparation for collection and processing of tissue sample. The research coordinator at NMTRC will organize the convening of members of the tumor board for discussion of the current case once the full report is available. In addition, a study enrollment form will be faxed to the coordinator at NMTRC, a unique subject identifier will be assigned and the enrollment form will be sent back to the participating site. The NMTRC will contact all sites via e-mail if the study enrollment is on hold or closed at any time.

Subject samples will be collected at each institution and follow the flow chart above. Once the reports are generated they will be sent to NMTRC/Dr. Sholler. The Principal Investigator enrolling the patient will prepare a tumor board presentation. The presentation and reports will be sent to all tumor board members by the NMTRC coordinator. There will be a meeting of the tumor board within 4 days of receiving the report to decide on the treatment plan recommendation. The recommendation will be reviewed in depth for safety by the Pharmacist. The treatment plan and memo will be discussed with the subject and their parents and upon their signed agreement the treatment will begin within 1 week of the discussion with parents.

Each site will also participate in a 1 hour monthly video or teleconference meeting to review study progress and subjects currently enrolled. There will also be a yearly face-face study committee meeting hosted by a member institution.

10 ADVERSE EVENT REPORTING

10.1 Definitions

10.1.1 Adverse Event

An ***adverse event*** is any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

An untoward medical event which occurs outside the period of follow-up as defined in the protocol will not be considered an adverse event unless related to study drug. Worsening of a medical condition for which the efficacy of the study drug is being evaluated will not be considered an adverse event.

10.1.2 Unexpected Adverse Event

An ***unexpected adverse event*** is one for which the nature or severity of the event is not consistent with the applicable product information as outlined in package insert filed with the FDA.

10.1.3 Serious Adverse Event

A ***serious adverse event*** is any untoward medical occurrence that:

- Results in death
- Is life-threatening (an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Results in a congenital anomaly or birth defect
- Other important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed above. Examples of such events are intensive treatment in an emergency room for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

The term "severe" is often used to describe the intensity (severity) of an event; the event itself may be of relatively minor medical significance (such as a severe headache). This is not the same as "serious", which is based on subject/event outcome or action criteria usually associated with events that pose a threat to a subject's life or functioning.

10.1.4 Documenting Adverse Events

The Investigator should elicit information regarding the occurrence of adverse events through open-ended questioning of the subject, physical examination and review of laboratory results.

All adverse events, whether serious or not, will be described in the source documents and Grade 3 or higher (per CTCAE 4.0) related and unexpected adverse events that occur while on study should be captured on the adverse event case report form. All Baseline AE's will be captured on a separate baseline Adverse event case report form. All Grade 3 or higher related and unexpected new events, as well as those that worsen in intensity or frequency relative to baseline, which occur after administration of study drug through the period of protocol-specified follow-up, must be captured.

Information to be reported in the description of each adverse event includes:

- A medical diagnosis of the event (if a medical diagnosis cannot be determined, a description of each sign or symptom characterizing the event should be recorded)
- The date of onset of the event and relatedness to treatment regimen.
- The date of resolution of the event and whether the event is serious or not
- Action taken; drug treatment required; non-drug treatment required; hospitalization or prolongation of hospitalization required; diagnostic procedure performed; subject discontinued from the study
- Outcome: complete recovery or return to baseline; unknown/lost to follow-up; adverse event persisting; subject died (notify the NMTRC immediately)

Adverse events will be collected for 30 days following the last treatment or until a new treatment is started. Suspected study drug-related toxicity at the 30 day follow-up visit must continue to be followed until resolution to baseline or ≤ Grade 2 or stabilization of the event.

10.1.5 Expedited Reporting of Serious Adverse Events

All fatal or life–threatening adverse events must be reported to the NMTRC immediately by telephone, fax, or e-mail within 24 hours of knowledge of the event. If full information is not known, additional follow-up by the Investigator will be required.

All other serious adverse events that are related and unexpected (not listed in the relevant appendices) which occur any time after the subject has been consented up to 30 days after the last dose of treatment, and are possibly, probably, or definitely related to the research must be reported to the study chair and appropriate regulatory authorities (local IRB and FDA if required) within 7 days of notification of the event.

The Investigator must report all serious adverse events reported to regulatory authorities in an expedited manner to the local IRB or IEC. All serious adverse events must be followed until resolution or stabilization. Unanticipated problems involving risk to subjects or others, serious adverse events related to participation in the study, and all volunteer deaths related to participation in the study should be promptly reported to the NMTRC.

NMTRC Reporting Contact

Please report all expedited reports to:

Alyssa VanderWerff and Genevieve Bergendahl, RN
NMTRC
Tel: (616) 267-0327
E-Mail: Alyssa.VanderWerff@helendevoschildrens.org
E-Mail: Genevieve.Bergendahl@helendevoschildrens.org

10.1.6 Grading and Relatedness of Adverse Events

10.1.6.1 Grading of Severity of an Adverse Event

Each adverse event (Grade 2 or higher) will be graded for severity per the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE V 4.0), and these criteria must be used in grading the severity of adverse events. The criteria can be found at: http://ctep.cancer.gov/reporting/ctc.html.

Grading of Severity of an Adverse Event Not Listed in Published Criteria:
For those adverse events which are not listed as part of the NCI CTCAE V 4.0, the same grading system should be used, where:

- **Mild** corresponds to an event not resulting in disability or incapacity and which resolves without intervention
- **Moderate** corresponds to an event not resulting in disability or incapacity but which requires intervention
- **Severe** corresponds to an event resulting in temporary disability or incapacity and which requires intervention
- **Life-threatening** corresponds to an event in which the subject was at risk of death at the time of the event
- **Fatal** corresponds to an event that results in the death of the subject

10.1.6.2 Relatedness to Study Drug

The Investigator must attempt to determine if an adverse event is in some way related to the use of the study drug and define an attribution category. This relationship should be described as follows:

RELATIONSHIP	ATTRIBUTION	DESCRIPTION
Unrelated to investigational agent/intervention	Unrelated	The AE *is clearly **NOT** related* to the intervention. The event is clearly due to causes distinct from the use of the study drug, such as a documented pre-existing condition, the effect of a concomitant medication, or a new condition which, based on the pathophysiology of the condition, and the pharmacology of the study drug, would be unrelated to the use of the study drug.
	Unlikely	The AE *is **doubtfully related*** to the intervention. Adverse event does not have temporal relationship to intervention, could readily have been produced by the subject's clinical state, could have been due to environmental or other interventions, does not follow known pattern of response to intervention, does not reappear or worsen with reintroduction of intervention.
Related to investigational agent/intervention	Possible	The AE ***may be related*** to the intervention. The event follows a reasonable temporal sequence from administration of the study drug and the event follows a known response pattern to the study drug BUT the event could have been produced by an intercurrent medical condition which, based on the pathophysiology of the condition, and the pharmacology of the study drug, would be unlikely related to the use of the study drug OR the event could be the effect of a concomitant medication.
	Probable	The AE ***is likely related*** to the intervention. The event follows a reasonable temporal sequence from administration of the study drug, the event follows a known response pattern to the study drug AND the event cannot have been reasonably explained by an intercurrent medical condition OR the event cannot be the effect of a concomitant medication.
	Definite	The AE ***is clearly related*** to the intervention. The event follows a reasonable temporal sequence from administration of the study drug, the event follows a known response pattern to the study drug and based on the known pharmacology of the study drug, the event is clearly related to the effect of the study drug. The adverse event improves upon discontinuation of the study drug and reappears upon repeat exposure.

11 SUBJECT WITHDRAWAL AND TRIAL DISCONTINUATION

11.1 Criteria for Subject Off-Therapy

Subjects will be removed from the study therapy for the following reasons:

- Subject completes protocol defined therapy (a minimum of one cycle)
- Progressive neoplastic disease
- Subject or guardian withdraws consent to continue in the trial
- Subject develops an intercurrent illness that precludes further participation, or requires a prohibited concomitant treatment
- The Investigator withdraws the subject in the subject's best interests
- Subject is lost to follow-up (defined as the inability to contact the subject on 3 separate occasions over a period of 2 weeks)
- Administrative reasons (e.g., the subject is transferred to hospice care)
- An adverse event, which in the opinion of the Investigator, precludes further trial participation or fulfills the protocol requirements for withdrawal
- Death

11.2 Criteria for Subject Off-Study

Subjects may be withdrawn from the study completely which includes withdrawal from survival follow-up for the following reasons:

- Completion of all study requirements
- Subject or guardian withdraws consent to continue in the trial (if this occurs, no further study visits or data may be collected)
- Subject is lost to follow-up (defined as the inability to contact the subject on 3 separate occasions over a period of 2 weeks)
- Death

11.3 Trial Discontinuation

The Neuroblastoma and Medulloblastoma Translational Research Consortium (NMTRC) may discontinue the trial as a whole or at an individual investigational site at any time. Reasons for early trial discontinuation may include, but are not limited to, unacceptable toxicity of treatment regimens, a request to discontinue the trial from a regulatory authority, protocol violations at an investigational site, violations of good clinical practice at an investigational site, or poor enrollment. The NMTRC will promptly inform all Investigators in the event of premature study discontinuation and provide all Investigators with instructions regarding the disposition of subjects still on study. Should the study be terminated prematurely, all case report forms and any other study material will be returned to the NMTRC.

12 DATA ANALYSIS

12.1 Data Quality Assurance

Electronic and paper case report forms will be checked for correctness against source document data by the independent study monitor. If any entries into the CRF are incorrect, incomplete or

illegible, the study monitor will ask the Investigator or the study site staff to make appropriate corrections.

12.2 Data Safety Monitoring Board (DSMB)

An independent Data Safety and Monitoring Board (DSMB) will oversee the conduct of the study. The members of this Board will receive database summaries, including adverse event reports, and will convene either in person or via teleconference according to section 3.5. The Board will be responsible for decisions regarding possible termination and/or early reporting of the study.

12.3 Process and Feasibility Analysis

Each of the process measures including the total time to treatment initiation and time to completion of the first cycle of treatment will be described using standard descriptive methods such as means, medians, standard deviations, and 95% confidence intervals. The total time duration to treatment initiation and time to completion of the first cycle of treatment will also be examined using Kaplan-Meier plots since some patients who are considered process failures or who do not complete the full duration to the completion of the first cycle of treatment may contribute censored data. The formal hypothesis testing of the feasibility or success is based upon the 2-stage Mini-Max testing process outlined in Section 8: *Sample Size Justification.* Further quantification of the feasibility success rate will be based upon the percentage of the n = 16 patients in each strata who achieve the specified overall time frame limits. This success rate will be supplemented with an exact 95% confidence interval using a Binomial distribution model due to the small sample size involved.

12.4 Secondary Data Analysis

Overall response rates (ORR) will be examined using rates for each of the clinical response categories supplemented using exact 95% confidence intervals. Progression free survival (PFS) data for each of the three clinical strata will be examined using Kaplan-Meier time to event plots. Differences in the PFS for the current treatment protocol with historical PFS values will be explored using Cox proportional hazard rate methods when possible. Patients that enroll through compassionate access will be included in secondary data analysis.

13 ADMINISTRATIVE PROCEDURES

13.1 Subject Informed Consent

No study related procedures will be performed until a subject or a subject's legal representative has given written informed consent. The NMTRC will provide the site Investigators with a sample informed consent document that conforms to all the requirements for informed consent according to ICH GCP and US FDA guidelines (21 CFR 50). However, it is up to each site Investigator to provide a final informed consent that may include additional elements required by the Investigator's institution or local regulatory authorities. The IRB/EC for each investigational site must approve the consent form document prior to study activation; changes to the consent form during the course of the study may also require IRB/EC approval. The informed consent document must clearly describe the potential risks and benefits of the trial, and each prospective participant must be given adequate time to discuss the trial with the Investigator or site staff and to decide whether or not to participate. Each subject who agrees to participate in the trial and who signs the informed consent will be given a copy of the signed dated and witnessed document. The original copy of the signed dated and witnessed informed consent document will be retained by

the Investigator in the study files. After the tumor board has made a therapy decision and that decision has been reviewed by the pharmacist, a therapy specific treatment memo will be signed.

The Investigator must also obtain authorization from the subject to use and/or disclose protected health information in compliance with the Health Insurance Portability and Accountability Act (HIPAA). Written HIPAA authorization may be obtained as part of the informed consent process.

13.2 Ethical Conduct of the Study and IRB/IEC Approval

The study will be conducted according to the principles of the 2004 version of the Declaration of Helsinki, the International Conference on Harmonization Guidance on Good Clinical Practice and the requirements of all local regulatory authorities regarding the conduct of clinical trials and the protection of human subjects.

The Investigator will submit the protocol, the informed consent and any other material used to inform subjects about the trial to the local IRB/IEC for approval prior to enrolling any subject into the trial. The IRB/IEC should be duly constituted according to applicable regulatory requirements. Approval must be in the form of a letter signed by the Chairperson of the IRB/IEC or the Chairperson's designee, must be on IRB/IEC stationary and must include the protocol by name and/or designated number. If an Investigator is a member of the IRB/IEC, the approval letter must stipulate that the Investigator did not participate in the final vote, although the Investigator may participate in the discussion of the trial. The Investigator will also inform the IRB/IEC of any serious adverse events that are reported to regulatory authorities and will provide to the IRB/IEC a final summary of the results of the trial at the conclusion of the trial.

Any amendments to the protocol will be done through the NMTRC, and will be submitted to the coordinating IRB/IEC for review and written approval before implementation.

13.3 Monitoring

An independent study monitor will make regularly scheduled trips to the investigational site to review the progress of the trial. The actual frequency of monitoring trips will depend on the enrollment rate and performance at each site. At each visit, the monitor will review various aspects of the trial including, but not limited to, screening and enrollment logs; compliance with the protocol and with the principles of Good Clinical Practice; completion of case report forms; source data verification; facilities and staff.

During scheduled monitoring visits, the Investigator and the investigational site staff must be available to meet with the study monitor in order to discuss the progress of the trial, make necessary corrections to case report form entries, respond to data clarification requests and respond to any other trial-related inquiries of the monitor.

In addition to the above, representatives of the NMTRC or government inspectors may review the conduct/results of the trial at the investigational site. The Investigator at each site must promptly notify the NMTRC of any audit requests by regulatory authorities.

A separate monitoring plan will be provided for additional monitoring guidelines.

13.4 Pre-Study Documentation

Prior to initiating the trial, the Investigators at each site will provide to the NMTRC the following documents:

• A signed NMTRC009 Investigator Agreement Form

• A current curriculum vitae for the Principal Investigator and each sub-investigator listed on the Investigator Agreement Form

• A copy of the Investigator's medical license from the state in which the study is being conducted

• A letter from the IRB or EC stipulating approval of the protocol, the informed consent document and any other material provided to potential trial participants with information about the trial (e.g., advertisements)

• A copy of the IRB- or EC-approved informed consent document

• Current IRB membership list for IRB's without a multiple project assurance number or an IRB organization number under the Federal Wide Assurance program (www.ohrp.osophs.dhhs.gov).

• A signed Investigator Signature Sheet for each amendment put through local IRB- Found on page 9 of this protocol (original)

• A completed conflict of interest and financial disclosure form (copy of original) from each person listed in the Investigator Agreement Form.

• Current laboratory certification for the reference laboratory

• A list of current laboratory normal values for the reference laboratory

13.5 Confidentiality

It is the responsibility of the investigator to insure that the confidentiality of all subjects participating in the trial and all of their medical information is maintained. Case report forms and other documents submitted must never contain the name of a trial participant. Each subject in the trial will be identified by a unique identifier that will be used on all CRF's and any other material submitted to the NMTRC. Case Report Forms for this study will be both paper and electronic. Electronic data will be stored in a HIPAA compliant data center. All case report forms and any identifying information must be kept in a secure location with access limited to the study staff directly participating in the trial.

Personal medical information may be reviewed by representatives of the NMTRC, of the IRB or of regulatory authorities in the course of monitoring the progress of the trial. Every reasonable effort will be made to maintain such information as confidential.

The results of the study may be presented in reports, published in scientific journals or presented at medical meetings; however, subject names will never be used in any reports about the study.

13.6 Source Documents

The Investigator will maintain records separate from the case report forms in the form of clinical charts, medical records, original laboratory, radiology and pathology reports, pharmacy records, etc. The Investigator will document in the clinic chart or medical record the name and number of the trial and the date on which the subject signed informed consent prior to the subject's participation in the trial. Source documents must completely reflect the nature and extent of the subject's medical care, and must be available for source document verification against entries in

the case report forms when the monitor visits the investigational site. All information obtained from source documents will be kept in strict confidentiality.

13.7 Record Retention

The Investigator will retain the records of the study for 15 years. The NMTRC will notify Investigators when retention of study records is no longer required. All study records must be maintained in a safe and secure location that allows for timely retrieval, if needed.

Study records that must be retained include copies of case report forms, signed informed consents, correspondence with the IRB or IEC, source documents, clinic charts, medical records, laboratory results, radiographic reports and screening/enrollment logs.

Should the Investigator relocate or retire, or should there be any changes in the archival arrangements for the study records, the NMTRC must be notified. The responsibility for maintaining the study records may be transferred to another suitable individual, but the NMTRC must be notified of the identity of the individual assuming responsibility for maintaining the study records and the location of their storage.

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Appendix I: Performance Status/Scores

Performance Status Criteria
Karnofsky and Lansky performance scores are intended to be multiples of 10

ECOG (Zubrod)		Karnofsky		Lansky*	
Score	Description	Score	Description	Score	Description
0	Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease	100	Fully active, normal.
		90	Able to carry on normal activity, minor signs or symptoms of disease.	90	Minor restrictions in physically strenuous activity.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work.	80	Normal activity with effort; some signs or symptoms of disease.	80	Active, but tires more quickly
		70	Cares for self, unable to carry on normal activity or do active work.	70	Both greater restriction of and less time spent in play activity.
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours	60	Required occasional assistance, but is able to care for most of his/her needs.	60	Up and around, but minimal active play; keeps busy with quieter activities.
		50	Requires considerable assistance and frequent medical care.	50	Gets dressed, but lies around much of the day; no active play, able to participate in all quiet play and activities.
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.	40	Mostly in bed; participates in quiet activities.
		30	Severely disabled, hospitalization indicated. Death not imminent.	30	In bed; needs assistance even for quiet play.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.	20	Often sleeping; play entirely limited to very passive activities.
		10	Moribund, fatal processes progressing rapidly.	10	No play; does not get out of bed.

*The conversion of the Lansky to ECOG scales is intended for NCI reporting purposes only.

Appendix II: Biology Studies Collection and Shipping forms

Instructions for Core/Needle Biopsy Specimens

Interventional Radiologist:

Standard Pathology Sample

1. Obtain first core biopsy (if possible; otherwise 2nd core)
    a. At least 18 gauge guiding needle or larger in caliber
    b. Remove excess blood from the sample with sterile saline rinse (provided in the kit) without damaging the sample

2. Place core (for standard pathology) in neutral buffered formalin container per standard practices

3. Send tissue to pathology

Primary Sample – sent to Ashion

4. Obtain second core biopsy from same track according to institutional protocol

5. ***DO NOT PLACE SAMPLE INTO FORMALIN*****.** Flash Freeze sample within 20 minutes of extraction and place in Tube 1 provided by the NTMRC. Note time of extraction and time of flash freezing.

6. Upon generation of a pathology report, please e-mail the NMTRC with the percent tumor in Sample 1.

Pathologist:

Place Tube 1 with the patient sample and Ashion Shipping Form in the shipping kit. Affix the label to FedEx shipping bag and place the kit in the bag for shipment. Please ship via FedEx (if you do not have a scheduled FedEx pickup, call 800-GO-FEDEX - 800-463-3339).

Note- Ashion is unable to generate a patient molecular report without knowing the percent viable tumor in the sample (Must be >50% to move forward) - Thank you

For questions please call the NMTRC at (616) 267-0327 or e-mail nmtrc@helendevoschildrens.org

Instructions for Incision/Excision Surgery Specimen

Surgeon:

1. Obtain surgical excision/biopsy (operating room or office)
    a. **DO NOT PLACE SAMPLE INTO FORMALIN**
    b. Please place sample directly (not wrapped) into the provided sterile saline (0.9% NaCl without dextrose), place in a labeled container and note time of extraction on container label.

2. Send to Pathology/Histology ASAP; please hand deliver to pathology within 20 minutes. NEVER AT ANY TIME PUT SAMPLE IN TIN/ALUMINUM FOIL.

Pathologist/Pathology Assistant:

1. Do not ink specimen (prior to completing steps 2-4 below)

2. Section specimen with a sterile blade on a clean surface. Select tumor tissue that appears non-necrotic; avoid normal tissue. Obtain a specimen for histopathological analysis at your institution, avoiding normal or necrotic tissue.

3. Obtain a sample with a maximum dimension of 0.5 cm^3 (approximately the size of a pencil eraser). Minimum amount of tumor sample required is 50-200mg of tissue.

4. Flash Freeze sample within 20 minutes of extraction and place in Tube 1 provided by the NTMRC. Note time of time of flash freezing.

5. Place Tube 1 with the patient sample and Ashion shipping form in the shipping box with dry ice. Affix a shipping label to the FedEx shipping bag and place the kit in the bag for shipment. Please ship via FedEx (if you do not have a scheduled FedEx pickup, call 800-GO-FEDEX - 800-463-3339)

6. Handle any remaining tissue as per your institutional protocols and in accordance with physician order

7. Upon generation of a pathology report, please e-mail the NMTRC with the percent tumor in Sample 1.

Note- Ashion is unable to generate a patient molecular report without knowing the percent viable tumor in the sample (Must be >50% to move forward) - Thank you

For questions please call the NMTRC at (616) 267-0327 or e-mail nmtrc@helendevoschildrens.org

Appendix III: Pharmacopeia Library

Drug Table:

Acetazolamide	Dinutuximab	Leflunomide	Raloxifene hydrochloride
Adalimumab	Docetaxel	Lenalidomide	Ramipril
Albendazole	Donepezil	Lomustine	Rifampin
Aldesleukin	DOXOrubicin	Lovastatin	Rituxumab
Alemtuzumab	Doxycycline	Luteolin	Romidepsin
Alfuzosin	Enalapril	Mechlorethamine	Rosuvastatin calcium
Anakinra	Epirubicin	Mercaptopurine	Ruxolitinib
Anastrozole	Ergocalciferol	Mesalamine	Simvastatin
Atorvastatin	Eribulin	Methotrexate	Sirolimus
Atovaquone	Erlotinib	Methyl CCNU	Sorafenib
Azacitidine	Ethambutol	MitoMYCIN	Streptozocin
Balsalazide	Ethosuximide	Mitotane	Sulfasalazine
Bevacizumab	Etodolac	Mitoxantrone	Sulindac
Bexarotene	Etoposide	MK4	Sunitinib
Bortezomib	Everolimus	Nabumetone	Tacrolimus
Brentuximab	Famotidine	Naproxen	Tamoxifen
Bumetanide	Felodipine	Nelarabine	Temozolomide
Bupropion	Fenoprofen	Nilotinib	Temsirolimus
Capecitabine	Fexofenadine	Nivolumab	Teniposide
Captopril	Flucytosine	Octreotide	Thalidomide
Carboplatin	Fludarabine	Ofatumumab	Thioguanine
Carmustine	Fludrocortisone	Olaparib	Thiotepa
Celecoxib	Fluorouracil	Omeprazole	Trabectidin
Ceritinib	Fuoxetine	Orlistat	Trametinib
Cetuximab	Fluvastatin	Oxaliplatin	Tofacitinib
Cisplatin	Fluvoxamine	Oxaprozin	Tolcapone
Cladribine	Gabapentin	Paclitaxel	Tolmetin
Clofarabine	Gefitinib	Palbociclib	Topiramate
Clonidine	Gemcitabine	Pamidronate	Topotecan
Clopidogrel	Griseofulvin	Panitumumab	Torsemide
Clotrimazole	Guanfacine	Panobinostat	Trastuzumab
Colchicine	Hydroxyurea	Pazopanib	Trazodone
Cortisone	Ibuprofen	Peg-asparaginase	Tretinoin
Crizotinib	IDArubicin	Pembrolizumab	Ursolic acid
Cyclophosphamide	Ifosfamide	Pemetrexed	Valproic acid
Cyclosporine	Imatinib	Pentostatin	Valrubicin
Cytarabine	Indomethacin	Phenelzine	Vandetanib
Dacarbazine	Irinotecan	Piperazine	Vemurafenib
Dantrolene	Ipilimumab	Piroxicam	Verapamil
Dasatinib	Isotretinoin	Plerixafor	VinBLAStine
DAUNOrubicin	Ixabepilone	Pravastatin sodium	VinCRIStine
Decitabine	Ketoprofen	Prazosin hydrochloride	Vinorelbine
Denileukin diftitox	Ketorolac	PrednisoLONE	Vismodegib
Dexamethasone	Lansoprazole	Prochlorperazine	Vorinostat
Diazoxide	Lapatinib	Propylthiouracil	Zolendronic acid
Diclofenac		Rabeprazole sodium	

Naturopathic Drug Table:

ALA	Calcitriol	Garlic	Modified citrus pectin
Ascorbic acid	Cordyceps	Ginger (6-gingerol)	Myrrh (Guggulsterone)
Astragalus membranaceu s	Coriolus (versicolor)	Green Tea	Pycnogenol
Berberine	Curcumin	Indole-3-Carbinol	Quercetin
Black Cumin (thymoquinone)	Fermented wheat germ extract	Lycopene	Rosemary
Bromelain	Frankincense	Melatonin	Soy Isoflavones
			Sulforaphane

Appendix IV- Example expression file format for the VCF 4.1:

##fileformat=VCFv4.1

##DESeq

##ALT=<ID=EXP,Description="Differential Expression">

##INFO=<ID=IMPRECISE,Number=0,Type=Flag,Description="Imprecise ">

##INFO=<ID=END,Number=1,Type=Integer,Description="End position of gene ">

##INFO=<ID=GENEID,Number=1,Type=String,Description="Ensembl gene ID ">

##INFO=<ID=GENE,Number=1,Type=String,Description="Common gene symbol ">

##INFO=<ID=BASEMEAN,Number=1,Type=Float,Description="Average of baseMeanA and baseMeanB ">

##INFO=<ID=BASEMEANA,Number=1,Type=Float,Description="Average normalized counts for control sample(s) ">

##INFO=<ID=BASEMEANB,Number=1,Type=Float,Description="Average normalized counts for affected sample(s) ">

##INFO=<ID=FOLDCHANGE,Number=1,Type=Float,Description="Fold Change (Affected/Control) ">

##INFO=<ID=LOG2FC,Number=1,Type=Float,Description="Log2 Fold Change (Affected/Control) ">

##INFO=<ID=PVALUE,Number=1,Type=Float,Description="p-value ">

##INFO=<ID=PADJ,Number=1,Type=Float,Description="Multiple hypothesis testing corrected p-value ">

#CHROM POS ID REF ALT QUAL FILTER INFO

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Example variant from a VCF file for BRAF V600E mutation:

```
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aA
aaAAtAaAAAAaaaaAAAAAaAaaaaAaaAAAAAAaAaAAAAAAAaaaAAAAAAaaaAAAAAAaaaaAAaaAaA
aa
aaaAaAAAAAAAAAAAAaaaAAaAAaaAaaAa;PILEUP2=TTTtaATaaATtTtTttTttttttttaaAaat
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Ta
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Aa
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tA
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VC
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diated_decay|CODING|ENST00000479537|2|1),NON_SYNONYMOUS_CODING(MODERATE|MI
SS
ENSE|gTg/gAg|V600E|766|BRAF|protein_coding|CODING|ENST00000288602|15|1),
ENSE|UTR_
3_PRIME(MODIFIER|||||BRAF|nonsense_mediated_decay|CODING|ENST00000497784|1
6|
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