

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

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1 Introduction

Neuroblastoma is the most common extracranial solid tumor in children. While the prognosis for infants with neuroblastoma is good, only 30% of children diagnosed after 12-15 months of age survive despite aggressive multimodal therapies. 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. Through genomic profiling, identification of agents targeting specific molecular pathways associated with the development and/or progression of neoplastic diseases holds promise.

PRIMARY OBJECTIVE

A pilot study was completed to evaluate the feasibility of using predictive modeling based on genome-wide mRNA expression profiles from neuroblastoma tumor biopsies to make real-time treatment decisions. Feasibility was defined as the completion of the following sequential evaluations in a two week time period: tumor biopsy, quality RNA extraction, mRNA Affymetrix U133 2.0 Plus GeneChip® hybridization, analysis utilizing a series of predictive methodologies, report generation, tumor board review with formulated treatment plan, and medical monitor review.

STUDY DESIGN

This was an open label, prospective feasibility study in patients with refractory or recurrent neuroblastoma. Enrollment and consent → biopsy → mRNA analysis → report generation → tumor board → review by Medical Monitor → Treatment regimen final (FDA-approved drugs with pediatric dosing)

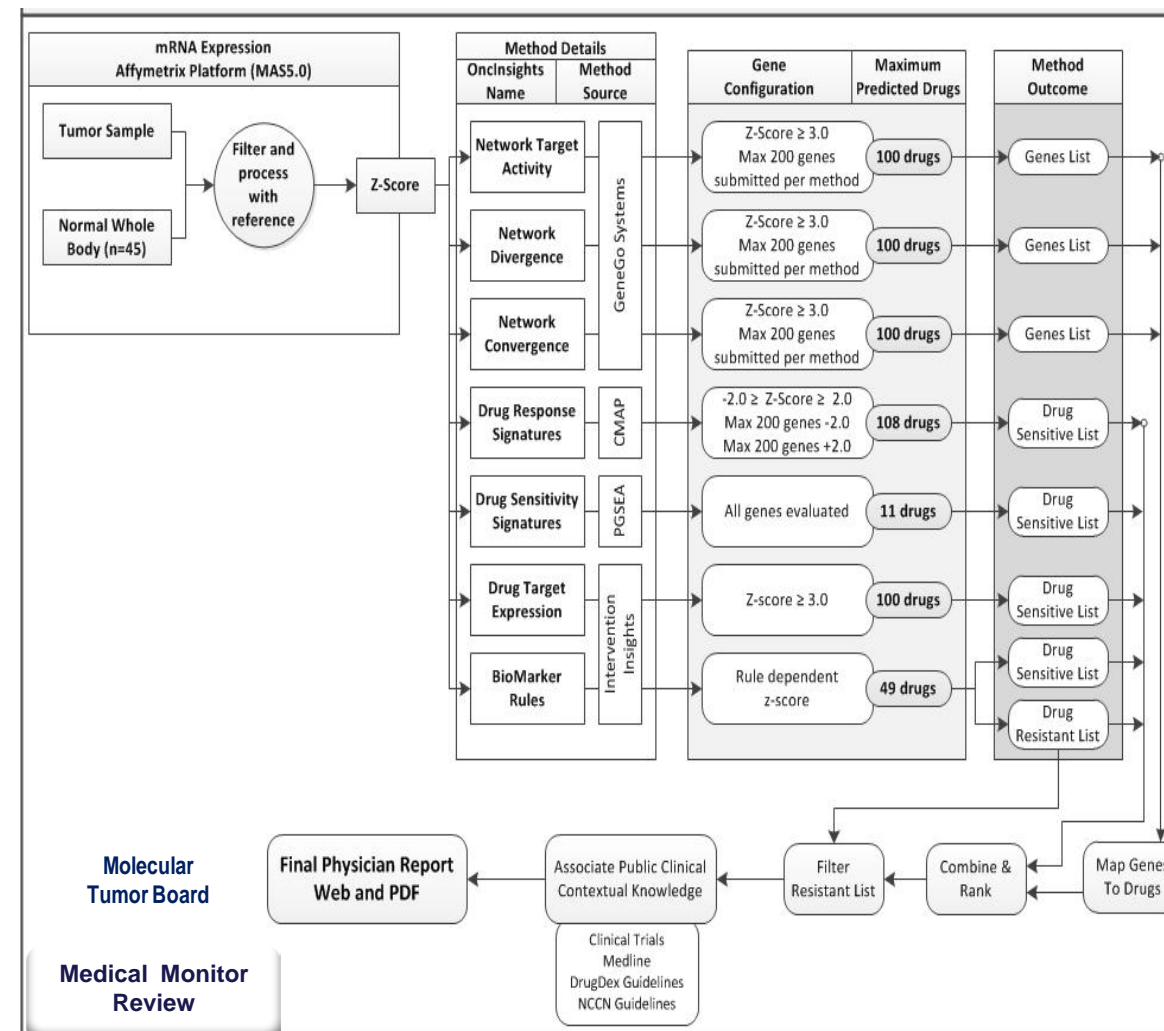
Sample Procurement and gene expression profiling: To pass quality control tumor samples were read by clinical pathology for a ≥ 75% viable tumor by nuclei, and < 20% necrosis. If the sample passed this criteria, it was processed by CRL (Clinical Reference Laboratory). The RNA extraction, amplification, Affymetrix U133 2.0 Plus GeneChip® hybridization, and scanning procedures utilized CLIA certified CRL standard protocols. Passing criteria include; (i) RIN > 6.5 using the Agilent 2100 BioAnalyzer; (ii) RNA 260/280 and 260/230 absorbance ratios > 1.8 by NanoDrop; (iii) total cDNA yield ≥ 5 µg/30 µl; (iv) cDNA 260/280 and 260/230 absorbance ratios ≥ 1.8 by NanoDrop. Data files were processed using the Affymetrix Expression Console™ and the MAS5.0 statistical algorithm.

2 Genomic Analysis and Generation of Onclnsights™ Report

Intervention Insights utilizes a series of predictive methodologies and reporting tools developed at the Van Andel Research Institute and offered through the Onclnsights™ service (see www.interventioninsights.com).

Gene expression data from tumor is compared to a reference sample set to obtain a relative gene expression profile and gene probe set that is represented by a z-score. The z-score depicts the expression of the tumor in terms of the number of standard-deviations from the mean expression used as a reference. Tumor genes with a positive z-score are over-expressed, whereas those with a negative z-score are under-expressed. After this pre-processing step, data are then submitted to the following collection of methodologies to identify potential agents for consideration:

Biomarker Rules: Predefined/published rules from a drug biomarker knowledgebase where efficacy of a drug is associated with the expression of a specific molecular marker.
Drug Target Expression: Identifies over-expressed (z-score≥3) drug targets
Network Target Activity: Predicts activity of drug targets based on topological analysis developed in partnership with GeneGo.
Drug Response Signatures: Connectivity Map concept developed by Broad Institute. Genomic consequence of drug exposure connects drug effect to disease signatures.
Drug Sensitivity Signatures: Implementation of Parametric Gene Set Enrichment Analysis (PGSEA) method using the NCI-60 cell line sensitivity signatures.



Onclnsights™ Report Generation: Upon execution of the five analytical methodologies, a compiled Onclnsights™ report was generated. The report is highly interactive, allowing the physician and reviewing tumor board to quickly navigate to the underlying knowledge and evidence at multiple levels. While the total drug pool available to this study is currently 182 FDA approved drugs, only those with established pediatric dosing (n=108) were used.

3 Medical History

Subject #	Relapsed/ Refractory	Age/ stage at diagnosis	Time to first relapse	#of prior relapsed therapies	Disease at study entry	Time since diagnosis
1	Relapsed	8ys (Stage 2B)	1 ½ yrs	10	Progressive	5 ½ yrs
2	Relapsed	3yrs (Stage 4)	4 months	2	Stable Disease	2 yrs
3	Refractory	4yrs (Stage 4)	---	7	Stable Disease	5 ½ yrs
4	Relapsed	3yrs (Stage 4)	2 ½ yrs	7	Progressive	5 yrs
5	Refractory	4 yrs(Stage 4)	---	13	Progressive	6 ½ yrs

4 Results of Feasibility

Subject #	Biopsy Date	Days to CRL RNA Chip	Days to Report	Days to Tumor Board	Days to Medical Monitor Sign-off	Total Days
1	4/16/10	3	5	1	1	10
2	5/10/10	7	1	2	1	11
3	5/12/10	5	1	3	1	10
4	5/18/10	7	<1	1	2	10
5	6/7/10	4	4	3	1	12

Subject #	RNA RIN	ALK Mutation	DNA Profile NexGen Sequencing
1	9.5 Pass	p.Phe1174Val PHOX2B pAla227Leu	Completed
2	6.9 Pass	No	Completed
3	8.1 Pass	No	Amplification
4	9.3 Pass	No	Amplification
5	9.8 Pass	p.Phe1174Leu	Completed

5 Conclusions

It is feasible to complete genomic profiling and create individualized treatment plans in real-time for patients

- All subjects had acceptable mRNA quality with completion of chip analysis
- All reports generated, tumor board held, individualized treatment plan agreed and approved by medical monitor completed in ≤12 days
- All tumor cells grown in culture with 3/5 mice models actively followed to date
- NextGen sequencing possible for all patients

Future Direction: Opening of a therapeutic study with the objective of predictive analysis, creation of individualized treatment plan, and completion of one cycle of therapy

ACKNOWLEDGEMENTS