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Clinical Significance of *NTRK* Family Gene Expression in Neuroblastomas

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Abstract

Background—Neuroblastomas (NBs) are characterized by clinical heterogeneity, from spontaneous regression to relentless progression. The pattern of *NTRK* family gene expression contributes to these disparate behaviors. TrkA/*NTRK1* is expressed in favorable NBs that regress or differentiate, whereas TrkB/*NTRK2* and its ligand BDNF are co-expressed in unfavorable NBs, representing an autocrine survival pathway. We determined the significance of *NTRK* family gene expression in a large, representative set of primary NBs.

Patients and Methods—We analyzed the expression of the following genes in 814 NBs using quantitative real-time RT-PCR: *NTRK1*, *NTRK2*, *NTRK3*, *P75/NGFR*, *NGF*, *BDNF*, *IGFR1* and *EGFR*. Expression (high vs. low) was dichotomized by median expression value and compared to clinical and biological variables as well as outcome.

Results—High *NTRK1* expression was strongly correlated with favorable age, stage, *MYCN* status, histology, ploidy, risk group and outcome ($p < 0.0001$ for all). However, it did not add significantly to the panel of prognostic variables currently used for cooperative group trials. *NTRK2* expression was associated with risk factors but not with outcome. High NGF expression was also associated with most risk factors and weakly with unfavorable outcome.

Conclusions—High expression of *NTRK1* is strongly associated with favorable risk factors and outcome in a large, representative population of NB patients. It did not add significantly to the current risk prediction algorithm, but it may contribute to future expression classifiers. Indeed, prospective assessment of *NTRK1* and *NTRK2* expression will identify tumors that would be candidates for *NTRK*-targeted therapy, either alone or in combination with conventional agents.

Keywords

neuroblastoma; TrkA/*NTRK1*; TrkB/*NTRK2*; TrkC/*NTRK3*; *P75/NGFR*; *NGF*; *BDNF*; expression; prognosis

Introduction

Neuroblastoma (NB) is the most common extracranial solid tumor of childhood. This tumor arises from the sympathetic nervous system and accounts disproportionately for childhood

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Conflict of Interest

None of the authors have any conflicting financial interests with any of the material, data, results or conclusions in this manuscript.

cancer deaths [1]. NBs demonstrate clinical heterogeneity, from spontaneous regression to relentless progression. Different patterns of genetic change have been identified that are associated with these disparate clinical behaviors [2]. In addition, we and others have determined that the pattern of NTRK family gene expression likely contributes to this clinical heterogeneity [3,4].

***NTRK1* and *NTRK3* are highly expressed in favorable NBs**

We showed that high *TrkA/NTRK1* expression in primary NBs was associated with favorable clinical features and inversely associated with *MYCN* amplification [5]. Furthermore, we [5,6] and others [7–13] have shown that *NTRK1* expression is predictive of a favorable outcome. Some NBs also express *P75/NGFR*, and this increases the sensitivity and specificity of NTRK receptors for their cognate ligands [14]. However the clinical importance of *NGFR* expression is less clear [7]. Human *NTRK1* maps to 1q32-41 [15], but specific mutations or activating rearrangements are quite rare [16,17]. Low-stage NBs usually express high *NTRK1* and differentiate in vitro in the presence of NGF, but undergo apoptosis in its absence [6,18]. This in vitro behavior suggests the NGF/*NTRK1* pathway may be responsible for in vivo differentiation or regression of favorable NBs. We demonstrated that full-length *NTRK3* is expressed in about 25% of primary NBs, and these represent a subset of *NTRK1*-expressing tumors [19]. These tumors are associated with lower age and stage, and they lack *MYCN* amplification [19,20]. None expressed *NT3* mRNA, and no *NTRK3* mutations have been reported to date in NBs.

***NTRK2* and BDNF are highly expressed in unfavorable NBs**

Full-length *NTRK2* and its ligand BDNF are expressed in more aggressive NBs and expression is highly correlated with *MYCN* amplification ($p < 0.001$) [21], whereas truncated *NTRK2* (lacking a tyrosine kinase domain) is expressed in more differentiated tumors, such as ganglioneuroblastoma. Addition of BDNF to *NTRK2*-expressing NB cell lines causes enhanced cell survival in serum-free media [21]. Others found similar results in NB cells induced to express *NTRK2* by retinoic acid treatment [22]. Thus, the expression of both BDNF and full-length *NTRK2* probably represents an autocrine or paracrine survival pathway that is important for aggressive NBs [21–23]. This *NTRK2*/BDNF pathway likely contributes to this aggressive behavior by promoting invasion, metastasis, angiogenesis and drug resistance [22,24–27]. We cloned human *NTRK2* and mapped it to 9q22 [28], but so far we have not found mutations or activating rearrangements in NBs.

In this study, we examined 814 representative primary NBs for *NTRK* family gene expression using quantitative real-time RT-PCR to determine the potential prognostic value of assessing gene expression. We also assessed the impact of *NTRK1* expression on predicting outcome after adjusting for clinical and biological variables that are currently used for risk stratification in the Children's Oncology Group (COG). Our data suggest that high *NTRK1* expression is strongly associated with favorable clinical and biological features, and it is highly predictive of outcome as an individual variable. However, it did not add to the existing risk prediction algorithm, perhaps because it is so strongly correlated with all of the clinical and biological factors currently used for risk stratification.

Patients and Methods

Patients and tumor samples

For these expression studies, we selected 814 NB samples from patients treated on recent NB interventional protocols of the COG. Patients were diagnosed between 1995 and 2008 and were selected to be representative based on age, disease stage by the International Neuroblastoma Staging System (INSS) [29], and the prevalence of *MYCN* amplification

[30–32]. All patients were consented and enrolled on COG Biology protocol ANBL00B1, and clinical information was maintained and analyzed in the COG Statistical Office (WBL, Gainesville, FL). Institutional Review Boards at the participating institutions gave approval for ANBL00B1. Tumor samples were snap frozen, and tumor RNA samples were obtained from the COG Nucleic Acid Bank. We analyzed expression using a quantitative, real-time RT-PCR (qRT-PCR) technique on customized low-density array (LDA) microfluidic cards. We used RQ values for analysis of gene expression, but DDC values gave similar results. The *NTRK* family genes analyzed included: *NTRK1*, *NTRK2*, *NTRK3*, *NGFR*, *NGF*, and *BDNF*, as well as the expression of *EGFR* and *IGF1R*, two other receptor tyrosine kinases (RTKs) implicated in NB pathogenesis. We normalized gene expression to *GAPDH*, *UBC*, *HPRT1* and *IPO8*. Expression (high vs. low) for each was compared to clinical and biological variables as well as outcome.

Prognostic variables

We correlated expression of these genes with clinical and biological risk variables: patient age at diagnosis (<18 mo vs. ≥18 mo); INSS stage (1, 2, 3, 4S vs. 4) [29]; *MYCN* amplification status (nonamplified vs. amplified) [2,30–32]; cell ploidy (DNA index: hyperdiploid vs. diploid) [31,33,34]; Shimada histopathology (favorable versus unfavorable) [35,36]; 1p status (normal vs. aberration) [37,38]; 11q status (normal vs. aberration) [37,39]; and risk group (low/intermediate vs. high) according to COG criteria [40]. We also tested the predictive ability of *NTRK* family gene expression for event-free survival (EFS) and overall survival (OS) in univariate and multivariable analyses.

Quantitative assessment of gene expression

We used qRT-PCR to measure *NTRK* family gene mRNA expression. We reverse transcribed 1–2 µg RNA using the Applied Biosystems (AB, Foster City, CA) High Capacity cDNA Archive Kit and a standard protocol. Reactions were performed in a 20 µl total volume. Prepared cDNA was stored for 24 hours or less at 4°C, or at –20°C for long-term storage. Quantitative gene expression analysis was performed via TaqMan qRT-PCR with AB TaqMan LDA microfluidic cards. Custom-designed arrays contained 8 sample-loading ports, each with 16 detectors in triplicate, for 48 reaction chambers per port, and a total of 384 reactions per card. Twelve primer/probe sets (detectors) for genes of interest and 4 endogenous controls were included on each card (results of four other genes are not reported here). qRT-PCR was performed using the ABI PRISM 7900HT Sequence Detection System (AB). Gene expression for each sample-detector pair was measured as a fold change in amplification relative to a calibrator sample. First-pass analysis was performed using the comparative Ct method with ABI PRISM SDS 2.2.2 and RQ Manager software. Amplification efficiencies of all genes were assumed to be approximately equal to the efficiencies of the endogenous controls (within 5%).

Internal gene expression controls

Prior to assay selection, 2 endogenous control LDA cards were run with 16 representative samples to determine which endogenous control detectors to include in the study. GeNorm [41] was used to select the 3 endogenous control primer/probes with the most stable expression among the 16 samples. ABI custom arrays must include either 18S RNA or *GAPDH*; of the two, *GAPDH* was more stably expressed and was included. *IPO8*, *UBC*, and *HPRT1* were the 3 additional endogenous controls selected from the 16 because they were the most stably expressed, and there was precedent for their use in the literature [41].

Statistical analyses

In addition to normalization to internal controls, we performed further analysis prior to statistical analysis, using Integromics' RealTime StatMiner software. With this software, the most stable endogenous controls for the entire sample set were selected via the software's internal GeNorm application. The geometric mean of the expression of these 2 endogenous controls was used to normalize samples, using human fetal brain total RNA converted to cDNA as a calibrator sample (ClonTech, Cat. No. 636526). *NTRK* family gene expression levels were dichotomized into low and high using the median expression value. Other cut points were tested, but none gave significantly better discrimination. Univariate analyses: We used Fisher's exact test to test for associations of the expression level versus risk factors in a pairwise fashion. Risk factors included: patient age at diagnosis, INSS stage, *MYCN* amplification status, tumor cell ploidy, Shimada histopathology, 1p deletion, and 11q deletion.

A Wilcoxon test was used to test for the association of expression of each gene with each of the dichotomized risk factors. Due to multiple comparisons of prognostic and gene expression variables, p-values <0.001 were considered statistically significant. EFS time was calculated from the time of study enrollment until the time of the first relapse, progressive disease, secondary malignancy, or death, or until last contact. OS time was calculated until the time of death, or until last contact. We used the Kaplan-Meier method to generate survival curves for each gene expression subgroup (<median, median), and curves were compared using a log rank test (p<0.005 for statistical significance). EFS and OS rates were expressed as the 5-year point estimate $\pm$ the standard error, with standard errors calculated by the method of Peto. Multivariable analyses: We used Cox proportional hazards regression to determine if *NTRK* expression is prognostic of EFS or OS in one model adjusting for age, stage, and *MYCN*, and in another model adjusting for stage, histology, and ploidy.

Results

Correlation of Gene Expression with Prognostic Variables

We analyzed the expression of *NTRK* family genes (*NTRK1*, *NTRK2*, *NTRK3*, *NGFR*, *NGF*, and *BDNF*), as well as the expression of *EGFR* and *IGF1R* in 814 representative NBs. Table I shows the distribution of patients by COG risk factors, which was representative of an unselected population. Table II shows the p-values from the Wilcoxon test and highlights those associations with a p-value of ≤ 0.001 . We found the following statistically significant associations: *NTRK1* expression with all of the risk factors; *NTRK2* expression with age, *MYCN* and ploidy; *NGFR* expression with age and *MYCN*; *NGF* expression with age, *MYCN*, ploidy and risk; *BDNF* expression with age and ploidy; and *IGF1R* expression with age and ploidy; neither *NTRK3* nor *EGFR* expression had significant association with any risk factors.

Correlation of Gene Expression with Outcome

The overall cohort had 5-year EFS and OS of $69\% \pm 2\%$ and $76\% \pm 2\%$, respectively, which is consistent with the combined outcome on recent low-, intermediate- and high-risk COG trials. Table III shows the log-rank p-values and the 5-year EFS and OS for all genes. For *NTRK1*, patients with high expression have statistically significantly better EFS and OS than those with low expression (Figures 1A and 1B). For *NGF*, patients with low expression have statistically significantly better OS than those with high expression (Figures 2A and 2B). None of the other *NTRK* family genes or other RTKs analyzed had a significant association with outcome.

Multivariable Analysis of Risk Factors with *NTRK1* Expression

We also performed multivariable analysis of EFS considering the clinical variables of patient age and INSS stage, as well as biological variables of *MYCN* copy number (amplified or not), tumor cell ploidy (diploid or hyperdiploid) and Shimada histopathology (favorable or unfavorable) currently in use in the COG. We wanted to determine if *NTRK1* expression would contribute to predicting outcome in addition to or instead of one of these variables. Although *NTRK1* expression was significantly predictive of event-free survival as an independent variable, it was not significant after adjusting for age, stage and *MYCN* amplification (Model B; Table IV), nor was it significant after adjusting for stage, histology, and ploidy (Model C; Table IV).

Discussion

NBs have heterogeneous clinical behavior that is only partially predicted by the clinical and biological variables currently used in cooperative group studies in the US, Europe and Japan. Therefore, it would be beneficial to have additional prognostic markers, particularly if they could also identify tumors that would be candidates for targeted therapy. We and others have determined that the pattern of expression of NTRK neurotrophin receptors is associated with clinical behavior. Furthermore, these receptors likely contribute to this behavior, because transfection of null NB cells with either *NTRK1* or *NTRK2* leads to in vitro and in vivo behaviors that mimic those seen in primary tumors that express either receptor endogenously [24,26].

There have been a number of studies focusing on the relationship between NTRK receptor expression (mainly NTRK1) and other prognostic variables as well as survival. Recently, several groups have found that high levels of NTRK1 confer a favorable prognosis, and conversely low levels of NTRK1 are associated with a more unfavorable prognosis. De Souza studied 64 NB patients and found that those with high mRNA NTRK1 expression had better overall survival regardless of their INSS risk group [42]. However, the presence of NTRK1 mRNA expression conferred a significant survival advantage only in the intermediate risk group. Hishiki studied 106 patients and found that high levels of NTRK1 correlated with favorable risk factors (young age, low stage, favorable histology and non-amplified *MYCN* status), whereas low NTRK1 expression indicated a prognosis [9]. Shimada studied 265 neuroblastic tumors (including NBs, ganglioneuroblastomas and ganglioneuromas) and showed that NTRK1 expression was not an independent predictor of poorer outcome when the model included other associated prognostic variables like stage, histology and *MYCN* status [11]. Schramm and colleagues studied 68 patients with expression profiling in an attempt to obtain a precise prediction of the clinical behavior and risk stratification [10]. In their studies, NTRK1 expression demonstrated a strong association with a specific gene expression pattern and was correlated with specific biologic subtypes. They reported that NTRK1 expression was most useful when used to stratify patients in an intermediate risk category. Two groups have looked at combining NTRK1 and/or NTRK2 with other genes thought to be important in NB to correlate gene expression and prognosis [12,13]. These two studies support the importance of the NTRK family of receptors in NB, but they studied a small number of patients (51 and 44 patients, respectively). Moreover, no prior studies have examined all three NTRK receptors (*NTRK1*, *NTRK2* and *NTRK3*) as well as their cognate ligands and the low-affinity co-receptor, NGFR.

We initially examined expression of the NTRK ligands NGF, BDNF, NT3 and NT4, in addition to three NTRK receptors (222 patients). However, the overall expression of NT3 and NT4 was so low that we dropped them from subsequent studies and analysis (data not shown). Nevertheless, we have made a comprehensive assessment of the expression of NTRK family receptors and ligands in a large and representative group of 814 COG patients

treated on contemporaneous protocols. For comparison, we also examined the expression of two additional RTKs (EGFR and IGF1R) that have been implicated in the biology and behavior of NBs [43–46]. However, neither showed a significant correlation with outcome in this cohort.

Our results demonstrate a highly significant association between *NTRK1* expression and all clinical/biological prognostic variables, and it was strongly and independently predictive of EFS and OS ($p < 0.0001$ for all). High *NTRK1* was associated with younger age, lower stage, normal *MYCN* copy number, hyperdiploid tumor DNA content and favorable histopathological features, as well as favorable outcome (EFS and OS). All these associations were identified as highly significant, even after adjusting for potential misidentifications due to multiple comparisons. Although we and others have shown such associations before (see above), this is the largest study to date (5–10 fold larger than almost all prior studies), and the correlation with outcome was particularly strong. *NTRK1* did not add significantly to outcome prediction after correction for age, stage and *MYCN* amplification in a multivariable model. This is likely due to the very strong correlation of high *NTRK1* expression with younger age, lower stage and the absence of *MYCN* amplification. Nevertheless, assessment of *NTRK1* and *NTRK2* expression will identify tumors that would be candidates for NTRK-targeted therapy.

We and others have shown a clear association between *NTRK2* expression (along with its cognate ligand, BDNF) and unfavorable biological characteristics, such as invasion, metastasis, angiogenesis, drug resistance and unfavorable outcome [22,24–27]. Surprisingly, although *NTRK2* expression was associated with several prognostic variables (age, *MYCN* status, ploidy), it was not associated with unfavorable outcome in this study. The primers used were designed to detect total *NTRK2* expression, so an association of full-length *NTRK2* expression with outcome may have been obscured by simultaneous detection of isoforms lacking the tyrosine kinase domain.

NGF expression was associated with several prognostic variables (age, normal *MYCN* copy number, ploidy). Unexpectedly, high NGF expression was associated with unfavorable OS (but not EFS), using our adjusted cutoff of $p < 0.005$. However, the overall level of NGF expression was very low, so some of these statistical associations may have occurred by chance. It is hard to explain how high NGF expression would be associated with an unfavorable outcome. NGF would promote survival of NTRK1-expressing cells. However, activation of NTRK1/TrkA causes neuronal differentiation and cessation of growth. Also, NGF does not bind to the constitutively active NTRK1-III isoform [47], so it should not activate this unfavorable isoform. NTRK3, NGFR, BDNF and IGFR were each associated with one or two prognostic variables (age, *MYCN* copy number and/or ploidy) but not with outcome. Although we and others have shown that the EGFR pathway may have biological relevance in selected NBs [43–46], there was no association of EGFR expression with any prognostic variable or with outcome in this study.

Assessment of *NTRK1* expression may provide additional prognostic information, especially in identifying low-risk patients. Measuring expression of *NTRK1* (or other genes) by quantitative RT-PCR as a prognostic variable may be challenging to apply prospectively to a large group of patients. However, *NTRK1* expression had strong prognostic significance using a variety of cut points (10%, 25%, 50%, 75% or 90%), which would make broad application easier. Also, the expression *NTRK1* could be combined with a small panel of other disease-related genes using the LDA array approach to produce an expression classifier, which would be more quantitative, reproducible and easy to standardize compared to an expression microarray approach. It will be important to distinguish different NTRK1 and NTRK2 isoforms, as alternate isoforms of the same receptor can have the opposite

effect on NB behavior. For example, the TrkAIII isoform has been associated with constitutive activation and more aggressive behavior [3,47]. In order to assess the expression of NTRK family genes comprehensively in a very large and representative group of patients (814), our study detected only total NTRK1 expression. However, there are now at least 5 NTRK1 isoforms, and we are in the process of assessing NTRK1 isoform expression in a subset of patients to determine the potential relevance of each isoform.

Prospective assessment of *NTRK1* and *NTRK2* expression will also identify tumors that are likely to respond to NTRK inhibitors either alone or in combination with conventional agents. We [48,49] and others [50] have shown that coadministration of NTRK inhibitors with conventional chemotherapeutic agents greatly enhances their efficacy, presumably by removing an important autocrine/paracrine survival pathway. Therefore, identification of *NTRK2* expressing tumors will permit the rational selection of high-risk patients for inclusion of NTRK inhibitors in their therapeutic regimen. Indeed, NTRK inhibitor therapy may also be useful as monotherapy or in combination to treat favorable NBs expressing *NTRK1* that have bulky disease or disease impinging on vital structures (spinal cord compression, liver enlargement with respiratory compromise). Therefore prospective assessment of NTRK family genes may not only provide valuable prognostic information, but also it would assist the rational selection of patients most likely to benefit from NTRK inhibitor therapy.

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Abbreviations

RT-PCR	reverse transcriptase polymerase chain reaction
NGF	nerve growth factor
BDNF	brain-derived neurotrophic factor
LDA	low-density array

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Figure 1.

Figure 2.

Table I

Distribution of Patients by Risk Factors (n=814)

Risk factors	Frequency
Age	
< 18 months	383 (47%)
18 months	431 (53%)
INSS Stage	
Stage 1,2,3,4s	495 (61%)
Stage 4	319 (39%)
<i>MYCN</i>	
Not Amplified	663 (82%)
Amplified	145 (18%)
Missing	6
Ploidy	
Hyperdiploid	525 (65%)
Diploid	284 (35%)
Missing	5
Shimada	
Favorable	423 (54%)
Unfavorable	356 (46%)
Missing	35
1p	
No LOH	357 (81%)
LOH	86 (19%)
Missing	371
11q	
No LOH	305 (73%)
LOH	112 (27%)
Missing	397
Risk	
Low/Intermediate	484 (59%)
High	330 (41%)

P-values from the Wilcoxon Tests of Association for Gene Expression (RQ value) with Risk Factors

Table II

Gene	LDA	Age (n=814)	Stage (n=814)	MYCN (n=808)	Ploidy (n=809)	Shimada (n=779)	1p (n=443)	11q (n=417)	Risk (n=814)
NTRK1		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0023	<0.0001
NTRK2		<0.0001	0.2180	<0.0001	0.0001	0.1674	0.0019	0.0024	0.0052
NTRK3		0.8991	0.0371	0.5574	0.0017	0.1191	0.0215	0.2818	0.0226
NGFR		<0.0001	0.1026	<0.0001	0.0042	0.0260	0.0023	0.4492	0.0439
NGF		<0.0001	0.1095	0.0002	<0.0001	0.0312	0.1575	0.0643	0.0001
BDNF		<0.0001	0.9232	0.7514	<0.0001	0.0317	0.6939	0.7320	0.0921
EGFR		0.0107	0.7749	0.4611	0.0071	0.0931	0.1542	0.2482	0.8724
IGF1R		<0.0001	0.6077	0.7672	<0.0001	0.0213	0.5095	0.7421	0.0591

* Values less than 0.001 considered significant

Table III

Log rank p-values and 5-year EFS and OS for Gene Expression (RQ value) *

Gene RQ Dichotomized by the Median	N	5-year EFS ± SE (%)	5-year OS ± SE (%)	EFS p-value	OS p-value
Overall cohort	814	69 ± 2	76 ± 2	N/A	N/A
<i>NTRK1</i>					
< median	407	60 ± 3	66 ± 3	<0.0001	<0.0001
median	407	78 ± 3	86 ± 2		
<i>NTRK2</i>					
< median	407	70 ± 3	77 ± 3	0.4570	0.1480
median	407	68 ± 3	74 ± 3		
<i>NTRK3</i>					
< median	407	69 ± 3	78 ± 3	0.5083	0.1773
median	407	68 ± 3	74 ± 3		
<i>NGFR</i>					
< median	407	66 ± 3	74 ± 3	0.1499	0.2239
median	407	71 ± 3	78 ± 3		
<i>NGF</i>					
< median	407	73 ± 3	81 ± 3	0.0076	0.0016
median	407	64 ± 3	71 ± 3		
<i>BDNF</i>					
< median	407	71 ± 3	78 ± 3	0.1822	0.1670
median	407	67 ± 3	74 ± 3		
<i>EGFR</i>					
< median	407	68 ± 3	75 ± 3	0.4600	0.4633
median	407	70 ± 3	76 ± 3		
<i>IGF1R</i>					
< median	407	68 ± 3	74 ± 3	0.5149	0.2406
median	407	70 ± 3	78 ± 3		

* Values less than 0.005 considered significant

Table IV

Multivariable analyses

Model	Significant adverse factor	n	p-value	Hazard ratio (95% confidence interval)
A – Unsupervised, backwards selection *	Stage 4	776	<0.0001	3.2 (2.3, 4.4)
	Unfavorable histology		<0.0001	3.1 (2.2, 4.5)
	Diploidy		0.0459	1.3 (1.01, 1.7)
B – Supervised comparison of <i>NTRK1</i> to age, stage, and <i>MYCN</i>	Stage 4	808	<0.0001	3.7 (2.7, 5.1)
	<i>MYCN</i> amplified		<0.0001	1.8 (1.4, 2.5)
	Age 18mo		0.0008	1.7 (1.3, 2.4)
	<i>NTRK1</i> < median		0.9017	1.0 (0.7, 1.3)
C – Supervised comparison of <i>NTRK1</i> to stage, histology, and ploidy	Stage 4	776	<0.0001	3.2 (2.3, 4.4)
	Unfavorable histology		<0.0001	3.0 (2.1, 4.3)
	Diploidy		0.0656	1.3 (1.0, 1.7)
	<i>NTRK1</i> < median		0.3412	0.9 (0.7, 1.2)

* Variables tested in Model A and found to be not statistically significant were: age, *MYCN*, 11q LOH, 1p LOH, *NTRK1*, NGF, and *MYCN*RQ. Forward selection produced the same result.