Efficacy and biomarker study of bevacizumab for hearing loss due to neurofibromatosis type 2 associated vestibular schwannomas

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Running head: Bevacizumab for NF2-related vestibular schwannoma

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ABSTRACT

PURPOSE: Neurofibromatosis type 2 (NF2) is a tumor predisposition syndrome characterized by bilateral vestibular schwannomas (VS) resulting in deafness and brainstem compression. This study evaluated efficacy and biomarkers of bevacizumab activity for NF2-associated progressive and symptomatic VS.

METHODS: Bevacizumab (7.5mg/kg) was given every 3 weeks for 46 weeks, followed by 24-weeks of surveillance off drug. The primary endpoint was hearing response defined by word recognition score (WRS). Secondary endpoints included toxicity, tolerability, imaging response using volumetric MRI analysis, durability of response, and imaging and blood biomarkers.

RESULTS: Fourteen patients (estimated to yield >90% power to detect an alternative response rate of 50% at alpha level of 0.05) with NF2, median age 30 (range, 14-79 years) with progressive hearing loss in the target ear (median baseline WRS 60%, range 13-82%) were enrolled. The primary endpoint, confirmed hearing response (improvement maintained $\geq$3 months), occurred in 5/14 patients (36%; 95%CI, 13-65%, $p<0.0001$). 8/14 patients (57%) had transient hearing improvement above the 95%CI for WRS. No patients experienced hearing decline. Radiographic response was seen in 6/14 (43%) target VS. Three grade 3 adverse events, hypertension (N=2) and immune-mediated thrombocytopenic purpura (N=1), were possibly related to bevacizumab. Bevacizumab treatment was associated with decreased free VEGF (not bound to bevacizumab) and increased PlGF in plasma. Hearing responses were inversely associated with baseline plasma HGF ($p=0.019$). Imaging responses were associated

with high baseline tumor vessel permeability and elevated blood levels of VEGF-D and SDF1α (p=0.037 and p=0.025, respectively).
CONCLUSION: Bevacizumab treatment resulted in durable hearing response in 36% of patients with NF2 and confirmed progressive VS-associated hearing loss. Imaging and plasma biomarkers showed promising associations with response that should be validated in larger studies.

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Introduction

Vestibular schwannomas (VS) are histologically benign tumors of the eighth nerve resulting in hearing loss, imbalance and brainstem compression. VS are common with roughly 3,000 new cases per year in the United States.[1] Surgery and radiation therapy (RT) achieve sustained control in >95% of sporadic, unilateral VS.[2-4] Germline inactivation of the gene *NF2* results in the rare tumor syndrome Neurofibromatosis type 2 (NF2) characterized by bilateral VS and multiple additional schwannomas, meningiomas and ependymomas.[5-7] NF2 associated VS cause higher morbidity as they are bilateral,[8-10] multi-lobular[11, 12] and have poor outcomes with standard therapies.[13-16] As a result, most people with NF2 develop significant hearing loss in young adulthood.[5,8]

Nearly 100% of VS express vascular endothelial growth factor (VEGF-A or VEGF).[17-19] Pharmacologic inhibition of VEGF in VS murine xenograft models decreases permeability and increases pericyte coverage consistent with vascular normalization.[20-22] Bevacizumab is a humanized IgG1 monoclonal blocking antibody specific for VEGF. Anecdotal experience with 31 individuals with NF2-associated VS treated with bevacizumab showed hearing improvement in 57% of people, making bevacizumab the first therapy to demonstrate functional and imaging responses in people with NF2.[17, 23] However, it also requires long-term administration and is associated with chronic toxicity.[24]

This study was conducted to: (1) prospectively confirm the hearing response (HR) rate in a well-defined patient population with NF2-associated VS hearing loss, (2)

define the duration of benefit on and off drug, and (3) identify biomarkers that may predict which individuals are most likely to benefit from bevacizumab.

Patients and Methods

This multi-institution, open label phase II trial enrolled subjects with NF2 and documented VS-associated hearing loss. The primary endpoint was the proportion of subjects with confirmed HR in the target ear. Secondary endpoints included the durability of HR, HR in non-target evaluable ears, change in VS volumetric MRI measures compared to baseline, safety, and the relationship between imaging and blood biomarkers and HR or radiographic response (RR). The trial was approved by site Institutional Review Boards and the NCI Cancer Therapy Evaluation Program (CTEP). Bevacizumab was supplied by Genentech through a Clinical Research and Development Agreement (CRADA) with CTEP. All subjects or their legal guardians provided informed consent.

Subjects ≥12 years old meeting National Institute of Health (NIH) or Manchester clinical criteria for NF2[25-27], with documented VS-associated hearing loss on serial audiograms over 24 months pre-enrollment, and a target ear baseline word recognition score (WRS) of <90% were eligible. Exclusion criteria included: prior anti-angiogenesis therapy, medical conditions incompatible with bevacizumab, and tumors not amenable to volumetric MRI analysis (Supplementary Data, Protocol).

Bevacizumab was given intravenously at 7.5 mg/kg every three weeks for 16 doses. Subjects were then assessed for 24 weeks off drug (Figure 1).

The target tumor was the VS with documented active, progressive hearing loss. Audiology examinations were performed at baseline, weeks 13, 25, 49, 60, and off study. WRS was assessed with a 100-word list of monosyllable words delivered via standardized methodology at a sound level determined to yield the optimal score for each participant.[28, 29] The 95% (p=0.05) critical difference table defined statistically significant increased WRS (HR) or decreased WRS (hearing decline) (Supplementary Table 1).[14,28,30] Confirmed HR was defined as an increase in WRS exceeding the 95% critical difference referenced to baseline and maintained across 2 evaluations over 3 months.

MRI brain was performed at baseline, weeks 13, 25, 49, and off study. Anatomical and functional imaging protocols were standardized across all sites on a Siemens 3T Verio with published protocols.[30, 31] Volumetric analysis was performed centrally using the anatomical sequences by independent radiologists blinded to treatment.[31] Enhancing tumor volume was outlined on post-contrast images. Median values of each parameter within enhancing tumor were computed. Double baseline MRI was performed to establish the test-retest variability in volumetric analysis of VS. Changes in VS volumes compared with baseline were determined for target and, when feasible, contralateral VS. RR definitions are: partial response (PR) ≥ 20% decrease in tumor volume, minor response (MR) 5% to 19% decrease in volume, progressive disease (PD) ≥ 20% increase in tumor volume and stable disease (SD) for all others. RR was confirmed at 3 months. Functional MRI sequences, dynamic contrast enhanced (DCE)-MRI to calculate K^{trans} (a measure of vascular permeability) and apparent

diffusion coefficient (ADC), were processed using custom-made software in Matlab (The MathWorks, Natick, Massachusetts), using published approaches.[32, 33]

Adverse events (AEs) were graded and attributed to bevacizumab according the Common Terminology Criteria for Adverse Events (CTCAE) v4.0 prior to infusion (every 3 weeks); physical examination every 6 weeks. Blood pressure was assessed weekly for the first six weeks and pre-infusion thereafter. For subjects <18 years old, bone toxicity was monitored with laboratory and imaging studies. (Supplemental Data, Protocol).

Circulating biomarkers were evaluated in peripheral blood pre-treatment, on treatment (weeks 25 and 49) and off treatment (week 72). Plasma samples were obtained from fresh blood, aliquoted, frozen, and analyzed for circulating VEGF, placental growth factor (PlGF), VEGF-C, VEGF-D, soluble VEGF receptor 1 (sVEGFR1 or sFLT1), basic fibroblast growth factor (bFGF), sTie-2, interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor-α (TNF-α), using multiplex enzyme-linked immunosorbent assay (ELISA) plates from Meso-Scale Discovery (Gaithersburg, MD). Hepatocyte growth factor (HGF), s-cMET, sVEGFR2, stromal cell-derived factor 1α (SDF1α), angiopoietins 1 and 2 (Ang1, Ang2) and carbonic anhydrase IX (CAIX) were measured using single analyte ELISA kits from R&D Systems (Minneapolis, MN). All samples were run in duplicate.

Statistical analysis

The primary endpoint was HR defined as increased WRS above the 95% critical threshold and maintained across at least two time points compared to baseline WRS.

Using a one-stage design based on a null hypothesis of response rate at 5%, a total of 14 subjects with confirmed progressive hearing loss were estimated to yield above 90% power to detect an alternative response rate of 50% at alpha level of 0.05. The trial requires 4 or more responders of 14 to reject the null hypothesis. Baseline patient and disease characteristics are presented with standard descriptive summaries. Proportion of HR was estimated using binomial distribution along with 95% confidence interval. The binomial exact test was used for testing proportions. Pearson correlation coefficient was used to estimate a correlation between continuous variables. All p-values are reported as 2-sided. All analyses were conducted using SAS software (version 9.2, SAS Institute).

Percent changes in the blood and imaging biomarkers from pre-, during and post-treatment were summarized using descriptive statistics. Blood biomarker analysis is reported per-patient and imaging biomarker analysis per-tumor. The differences before and during treatment in blood and imaging biomarkers were assessed with paired statistics. Signed Rank test was used to assess the significance of the change over time and Wilcoxon Sign-Rank test was used to test the difference between HR group and RR groups. Tumor reduction was calculated based on the percent change in volume from baseline to week 25 for all tumors. Correlation between RR for target VS and median ADC and K^{trans} at baseline was determined using the Spearman correlation test.

Results

Fourteen subjects (10 female), median age of 30 years (range, 14-79) were enrolled between November 2010 and August 2011(Table 1). Eight participants had prior surgery, 6 on the non-target ear, two bilaterally. Three participants had prior RT, one to target and two to non-target VS, 15-120 months prior to BEV (Supplementary Table 2). All subjects were evaluable for response and toxicity. Median baseline target ear WRS was 60% (range, 13-82%). Only 4/14 (28%) target ears had "serviceable" hearing (class A or B) per the American Academy of Otolaryngology-Head and Neck Society Hearing Committee guidelines (Supplementary Figure 1).[34] Nine of fourteen subjects (64%) were anacusic in the non-target ear.

Five of 14 subjects (36%; 95% CI: 13, 65%, $p<0.0001$) achieved the primary endpoint of confirmed HR in the target ear. This was achieved by week 13 in 4/5 subjects and maintained continuously throughout treatment. No subject had hearing decline while on bevacizumab, despite progressive hearing loss being required for enrolment. Of the five subjects evaluable for HR in the non-target ear, 4 had confirmed HR (80%, 95%CI: 28, 99%; Table 2). In total, 9/19 evaluable ears (47%, 95%CI: 24, 71%) achieved confirmed HR (Table 2). Pre- and post-treatment hearing scattergrams are presented in Supplementary Figure 1.

Bevacizumab was stopped after 12 months to assess durability of response. Three of five subjects (60%) with confirmed HR in the target ear maintained this 6 months off drug (Figure 2a). Similarly, 2/4 subjects with confirmed HR in the non-target ear maintained HR 6 months off drug. In total, 5/9 (target and non-target) ears with confirmed HR maintained this for 6 months off drug.

The median baseline target VS volume was 3.0cc (range 0.7-23cc). The mean difference in volume across the two baseline assessments was 0.02 cc (p=0.83), confirming the reproducibility of volumetric measurements. A total of 28 VS (14 target and 14 contralateral) were evaluable for RR. PR at any time point was achieved in 6/14 (43%) of target and 6/14 (43%) non-target ears (Table 2). Confirmed PR (imaging response maintained across two evaluation time points) was seen in 2/14 target VS (14%, 95%CI: 2, 43%). Maximal reduction was 39.7% at week 49. Confirmed MR occurred in 7/14 target VS (50%, 95%CI: 23, 77%). One person with confirmed MR had RT to the target ear 10 years earlier and theoretically, late recovery from RT could influence RR (Supplementary Table 2). No VS achieving MR or PR at any time point developed PD on treatment, but two VS with best response of SD developed PD at week 49 (Figure 2b). In the non-target VS, 3/14 tumors (21%, 95%CI: 5-51%) had confirmed PR and 6/14 (43%, 95%CI: 18, 71%) had confirmed MR. Of note, 2 non-target VS achieving PR had prior RT 15 and 48 months prior to enrolment that could potentially influence RR. In total, 5/28 (18%, 95%CI: 6, 37%) VS achieved confirmed PR and 13/28 VS (46%, 95%CI: 28, 66%) had confirmed MR. Of the 18/28 VS with confirmed PR or MR, 9 (50%) maintained durability of RR 6 months off of drug (Figure 2b).

There was no significant correlation between HR and RR when analyzed by subject or by target VS (r=0.34, 95%CI: -0.14, 0.82, p=0.23). There was also no significant correlation between WRS and tumor volume over time (R=0.287, 95%CI: -0.29, 0.71; p=0.32). Finally, there was no significant relationship between HR and baseline factors including age, gender or baseline WRS (Table 1).

There were 124 AEs possibly related to bevacizumab. Of these, 121 were classified as grade 1-2 (Table 3). The three grade 3 AEs were 2 episodes of hypertension that responded to monotherapy and one episode of idiopathic thrombocytopenia purpura (ITP) that required treatment termination at week 16, but resolved 6 months off drug. A second subject discontinued treatment after 13/16 planned doses due to required surgery for another tumor. No bone toxicity occurred in the 2 subjects <18 years old. Three of seven females with normal menstruation at baseline developed grade 1-2 irregular menstruation that resolved off treatment. There were 11 additional episodes of grade 1-2 bleeding (Table 3).

We explored potential associations between baseline functional imaging markers, ADC and K^{trans}, as well as changes in ADC and K^{trans} during treatment, with HR and RR. ADC and K^{trans} values were evaluable for 12 target VS and 9 contralateral VS. Baseline ADC values were not associated with HR or RR in target ears. However, dynamic changes in ADC from baseline to week 25 were associated with HR in target ears ($p=0.019$) with a median decrease in ADC of 9% in subjects with HR. K^{trans} was not significantly associated with HR, but baseline K^{trans} values were associated with RR at week 25 across all evaluable tumors ($p=0.037$, $n = 21$) and target VS achieving RR had higher baseline K^{trans} that non-responders (0.30 vs. 0.07, respectively, $p=0.051$).

Bevacizumab was associated with decreased plasma levels of free VEGF across all time points, in all subjects. At weeks 25 and 49, this was accompanied by increased levels of total VEGF, which significantly dropped post treatment (Figure 3 and Supplementary Table 3). Bevacizumab was also associated with increased plasma levels of PlGF (at all-time points), and VEGF-D and SDF1α at week 49 (Figure 3 and

Supplementary Table 3). Finally, bevacizumab was associated with a transient decrease in Ang2 levels at week 25 (Figure 3 and Supplementary Table 3).
HR was associated with lower baseline HGF (p=0.019), decreased plasma CAIX at weeks 25 and 49 (p=0.010 and p=0.035, respectively), and an increase in plasma sVEGFR2 at week 25 (p=0.004, Supplementary Figure 2, Supplementary Table 4). RR was associated with higher baseline levels of VEGF-D and SDF1α (p=0.037 and p=0.025, respectively); decreased s-cKIT at week 25 (p=0.023); and a decrease in sTie2 at week 49 (p=0.034) (Supplementary Table 5).

Discussion

The most common and universally life-altering consequence of NF2 is hearing loss, with the majority of affected individuals progressing to deafness in their third decade.[5, 6, 8, 10] Bevacizumab given on a compassionate-use basis to people with NF2 resulted in HR in 57% of evaluable patients.[17, 23] This outcome was unprecedented, heralding the possibility of effective therapy for these tumors. However, it left much uncertainty about the optimal patient population, dosing strategy and long-term durability. The results of this prospective efficacy study confirm the proportion of NF2 patients with symptomatic VS who achieve durable HR with bevacizumab (36%, 95% CI 13-65%, p<0.0001), the durability of response on and off drug and present several candidate biomarkers that may ultimately allow rational selection of patients for therapy.

HR was selected as the primary endpoint as it is clinically meaningful and provides evidence of drug activity given that durable hearing improvement with NF2-associated VS is improbable either spontaneously or with RT or resection.[8, 15, 16] HR

assessed by WRS is quantifiable, reliable and feasible for measuring hearing function over time.[28, 29] We required that statistically significant HR be maintained for at least 3 months to both overcome concerns about spurious HR and with the awareness that NF2-associated VS are chronic tumors for which short-term efficacy would have little value. Natural history data shows that only 16% of people with NF2 have spontaneous HR if baseline WRS is <90%.[8] The finding of a 36% (95% CI 13-65%) confirmed HR in people with NF2, documented progressive hearing loss and a median baseline WRS of 60% represents noteworthy therapeutic benefit. Moreover, the unconfirmed HR rate of 57% in this prospective study is identical to large retrospective series[17, 23] and far superior to spontaneous HR in natural history studies.[8]

Although bevacizumab was well tolerated in this study, there were 3 serious AEs and 3/7 females who had normal menstruation at baseline developed menstrual irregularities. However, all women recovered baseline menstrual function off bevacizumab. This experience echoes recent reports of ovarian failure in women with breast cancer treated with bevacizumab. Given the age of people with NF2 considered for treatment, this AE should be expressly discussed with women considering bevacizumab therapy and monitored during treatment.

An important finding is that 55% of subjects who achieved HR in any ear maintained this response for up to 6 months *off* of drug. Similar durability was seen with RR. These results suggest that after HR is achieved, multi-week dosing intervals or drug holidays capitalizing on the long half-life of bevacizumab may be feasible. Interestingly, analysis of antiangiogenic therapies across a variety of cancers also suggest alternative dosing strategies may be more efficacious based on markers of

vessel normalization and oxygenation.[35] Analysis of blood markers in this study is also consistent with this hypothesis. Specifically, we saw unexpectedly high baseline VEGF levels, comparable to those in brain cancer.[32, 33] Secondly, there was a sustained decrease in the circulating levels of free VEGF (with a corresponding increase in total VEGF) and a transient decrease in Ang2 during bevacizumab therapy; a pattern reminiscent of biologic response to anti-VEGF therapy in cancer,[35] but not previously recognized in non-malignant tumor syndromes like NF2. Thirdly, bevacizumab treatment was associated with increased plasma levels of PlGF, VEGF-D and SDF1α over time. These have been proposed as markers of resistance to anti-VEGF therapy in brain cancer,[20, 35] and may hold similar value as potential biomarkers for antiangiogenic therapy for benign nerve sheath tumors. Together these data indicate that circulating markers of vessel normalization and oxygenation may support alternative dosing strategies in both cancers and benign tumor syndromes.

Finally, the frequently observed absence of a significant correlation between hearing and tumor size in NF2-associated VS was borne out in this study. However, there were interesting associations between HR and dynamic changes in ADC from baseline to week 25 as well as lower absolute levels of HGF at baseline in patients achieving HR. These findings suggest that HR may be related to reduced tumor-associated edema and improved oxygenation rather than direct impact on tumor volume.[22] RR was associated with baseline K^{trans} and the degree of reduction in plasma free VEGF, VEGF-D and sTie-2 suggesting a pharmacodynamic relationship between targeting circulating VEGF and reducing hyperpermeable blood vessels.[20, 36] Lastly, the preliminary findings of Ang2 and sTie-2 changing in response to bevacizumab in people

with NF2 is notable since (a) similar patterns are observed with antiangiogenesis therapy in brain cancer, (b) Ang-2/Tie-2 is an important factor in proangiogenic pathways in general and (c) both proteins have been implicated in schwannomas.[20, 32, 37]

In conclusion, this prospective study confirms the efficacy and safety of bevacizumab in the subset of people with NF2 and progressive, symptomatic VS. The data, although from a small, single arm study, expand the understanding of required dosing intervals to maintain HR, potentially allowing lower doses over time, and identified several potential blood and imaging biomarkers that, if validated, will allow targeting therapy to the people with the highest likelihood of benefit. The ongoing subsequent study of bevacizumab for children and young adults with hearing loss due to NF2-associated VS (NCT01767792) will further investigate the findings from this study.

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Figure Legends:

Figure 1. Trial Schema.

Figure 2. (A) Change in word recognition score during treatment (through week 49) and off treatment (weeks 60 and 72) for target ears. Color-coded by best confirmed response (HR—green, SD—black). (B) Change in tumor volume during treatment (through week 49) and off treatment (weeks 60 and 72) for target ears, Color-coded by best confirmed response (PR—blue, MR—green, SD—black, PD—red)

Figure 3. Line graphs showing changes over time in plasma total VEGF (free + bound), free VEGF, PlGF and SDF1α for all participants (n=14). Anti-VEGF therapy with bevacizumab decreased the plasma levels of free VEGF, and increased the levels of antibody-bound VEGF, PlGF and SDF1α in people with NF2.

Supplementary Figures:

Supplementary Figure 1. (A) Scattergram of baseline hearing function for all target ears, as recommended by the Hearing Committee of the American Academy of Otolaryngology-Head and Neck Society (AAO-HNS). Color code: green—class A, yellow—class B, red—class C, blue—class D. (B) Scattergram of best change in hearing for all target ears after treatment with bevacizumab. Class A and B hearing are considered serviceable and class C and D are considered unserviceable.[34]

Supplementary Figure 2. Line graphs showing changes over time in relative free VEGF, total VEGF (free + bound), sVEGFR2, and CAIX in subjects with confirmed HR and confirmed RR versus non-responders. Panels A-D depict subjects with confirmed HR (n=5) vs. hearing non-responders (n=9). Panels E-H depict confirmed radiographic responders (n=9) vs. radiographic non-responders (n=5). Data are presented as median values with interquartile ranges; * indicates a significant difference between relative biomarker concentrations in responders and non-responders ($p< 0.05$).

Table 1: Baseline patient demographics and clinical characteristics.

	Total N=14	**Confirmed Hearing Response N=5**	**No Confirmed Hearing Response N=9**	**P-value**
Age – year				0.2
Median	30.5	26.0	32.0	
Range	14-79	14-33	14-79	
Sex – no .(%)				0.6
Male	4 (29)	1 (20)	3 (33)	
Female	10 (71)	4 (80)	6 (67)	
Race - no .(%)				
White	12 (87)	3 (60)	9 (100)	0.1
Karnofsky Performance Status no. (%)				0.7
90	7 (50)	3 (60)	4 (44)	
70- 80	7 (50)	2 (40)	5 (56)	
% Word Recognition Score Target Ear				0.7
Median	60.5	72.0	56.0	
Range	13-82	20-78	13--82	
Tumor Volume (cc) Target Ear				0.9
Median	3.0	2.3	3.4	
Range	0.7-23	1.2-22	0.7-23	

Table 2. Hearing and imaging response data during 12 months of bevacizumab treatment

	Overall response		Confirmed response*	
	No.	%	No.	%
Hearing Response				
Target ear	8/14	57%	5/14	36%
Contralateral ear	4/5	80%	4/5	80%
All ears	12/19	63%	9/19	47%
Imaging response				
Target vestibular schwannoma				
Partial response (≥20% decrease)	6/14	43%	2/14	14%
Minor response (5-19% decrease)	4/14	29%	7/14	50%
Stable disease	4/14	29%	5/14	36%
Progressive disease (≥20% increase)	2/14	14%	0/14	0%
Contralateral vestibular schwannoma				
Partial response (≥20% decrease)	6/14	43%	3/14	21%
Minor response (5-19% decrease)	6/14	43%	3/14	21%
Stable disease	2/14	14%	6/14	43%
Progressive disease (≥20% increase)	0/14	0%	2/14	14%

*Confirmed response was defined as maintained across 2 evaluations at least 3 months apart.

Table 3. Total number of adverse events possibly, probably, or definitively related to bevacizumab in 14 individuals with NF2 and progressive vestibular schwannomas.

Adverse Event: No. (%)	Grade 1	Grade 2	Grade 3
Abdominal pain	2 (14)		
Alanine aminotransferase increased	5 (36)	3 (21)	
Allergic rhinitis	1 (7)		
Anemia	1 (7)		
Anorexia	1 (7)	1 (7)	
Aspartate aminotransferase increased	8 (57)		
Bruising	2 (14)		
CPK increased	1 (7)		
Diarrhea	2 (14)	1 (7)	
Dizziness		1 (7)	
Dry skin	2 (14)		
Dyspepsia	2 (14)		
Dyspnea		2 (14)	
Electrocardiogram QT corrected interval prolonged	1 (7)		
Epistaxis	8 (57)	2 (14)	
Fatigue	9 (64)	4 (29)	
Headache	2 (14)		
Hemoglobinuria	1 (7)		
Hemolysis	1 (7)		
Hemorrhoidal hemorrhage	2 (14)		
Hoarseness	1 (7)		
Hyperglycemia	4 (29)		
Hypermagnesemia	2 (14)		
Hypertension			2 (14)
Hypomagnesemia	1 (7)		
Increased Blood Bicarbonate	1 (7)		
Irregular menstruation*	5 (71)	1 (14)	
Menorrhagia*	2 (29)	1 (14)	
Mucositis oral	1 (7)		

Nausea	5 (36)	2 (14)	
Oral hemorrhage	2 (14)		
Oral pain	1 (7)		
Palpitations	2 (14)		
Peripheral sensory neuropathy	1 (7)		
Platelet count decreased	2 (14)		
Proteinuria	7 (50)	3 (21)	
Rectal hemorrhage		1 (7)	
Respiratory, thoracic and mediastinal disorders	1 (7)		
Sore throat	4 (29)		
Thrombocytopenia Purpura			1 (7)
Vertigo	1 (7)		
Voice alteration	1 (7)		
Vomiting	1 (7)		
Weight gain	1 (7)		
Weight loss	1 (7)		
Wound complication	1 (7)		

The Adverse Events with asterisks are those events that could only have occurred among 7 female subjects. The percent represents number of events out of 7 female subjects with baseline normal menstruation.

Acknowledgements

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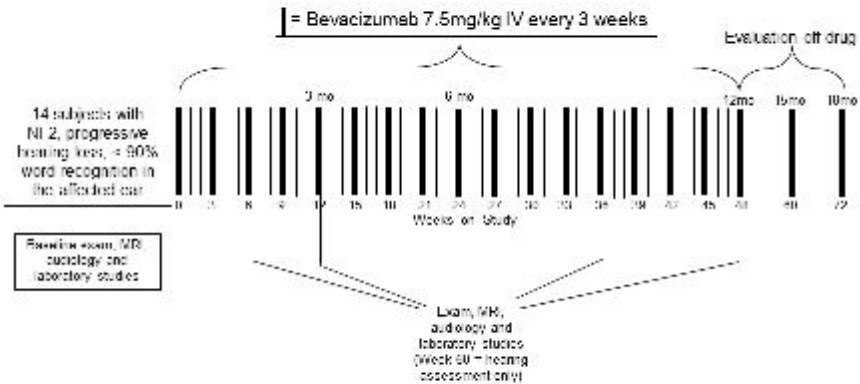
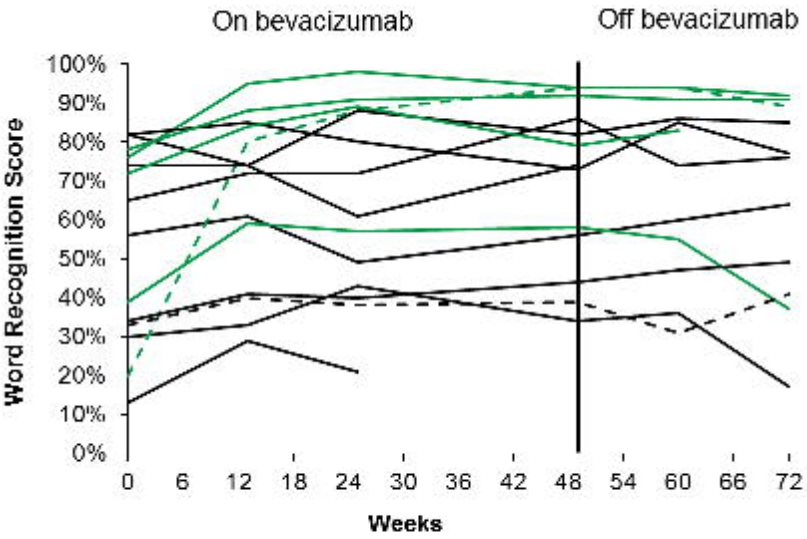
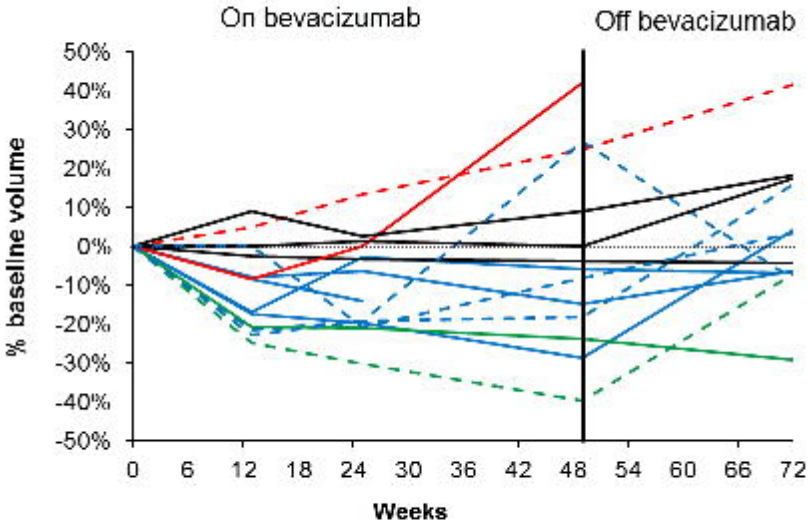
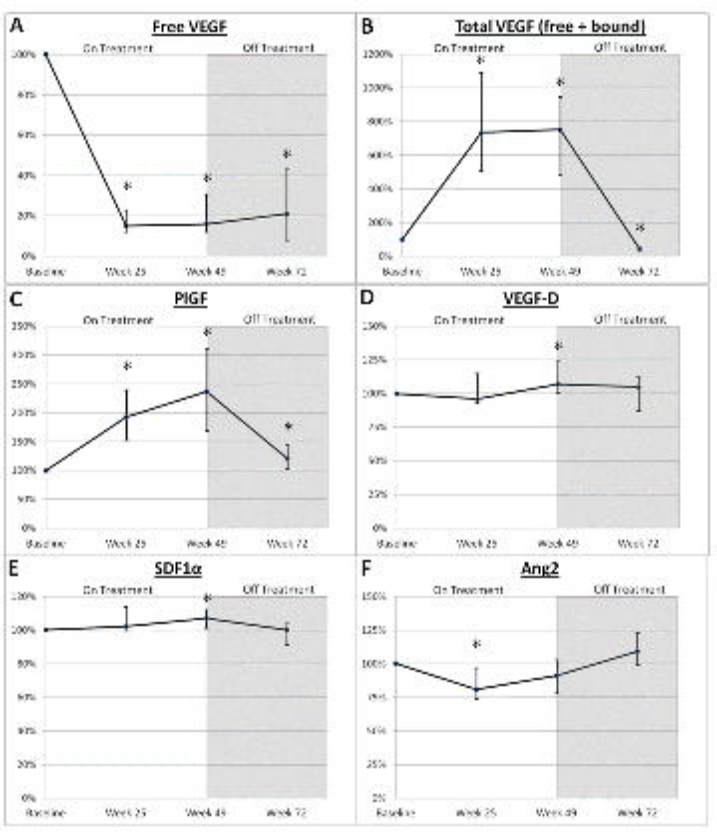

= Bevacizumab 7.5mg/kg IV every 3 weeks
Evaluation off drug
3 mo
6 mo
12mo
15mo
18mo
14 subjects with NI 2, progressive hearing loss, < 90% word recognition in the affected ear
Weeks on Study
Baseline exam, MR, audiology and laboratory studies
Exam, MR, audiology and laboratory studies (Week 60 = hearing assessment only)


On bevacizumab
Off bevacizumab
Word Recognition Score
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
6
12
18
24
30
36
42
48
54
60
66
72
Weeks


On bevacizumab
Off bevacizumab
50%
40%
30%
20%
10%
0%
-10%
-20%
-30%
-40%
-50%
% baseline volume
0
6
12
18
24
30
36
42
48
54
60
66
72
Weeks


A
Free VEGF
On Treatment
Off Treatment
Week 25
Week 49
Week 72
B
Total VEGF (free + bound)
On Treatment
Off Treatment
Week 25
Week 49
Week 72
C
PlGF
On Treatment
Off Treatment
Week 25
Week 49
Week 72
D
VEGF-D
On Treatment
Off Treatment
Week 25
Week 49
Week 72
E
SDF1α
On Treatment
Off Treatment
Week 25
Week 49
Week 72
F
Ang2
On Treatment
Off Treatment
Week 25
Week 49
Week 72

NCI Protocol #: 8248

Local Protocol #: NA_00034732

TITLE: Phase 2 Study of bevacizumab in children and adults with Neurofibromatosis type 2 and symptomatic vestibular schwannoma

Coordinating Center:	Johns Hopkins University
Trial Sponsor	JHU, Cancer Therapy Evaluation Program
NCI-supplied Agent	Bevacizumab
NSC#	704865
IND#	7921

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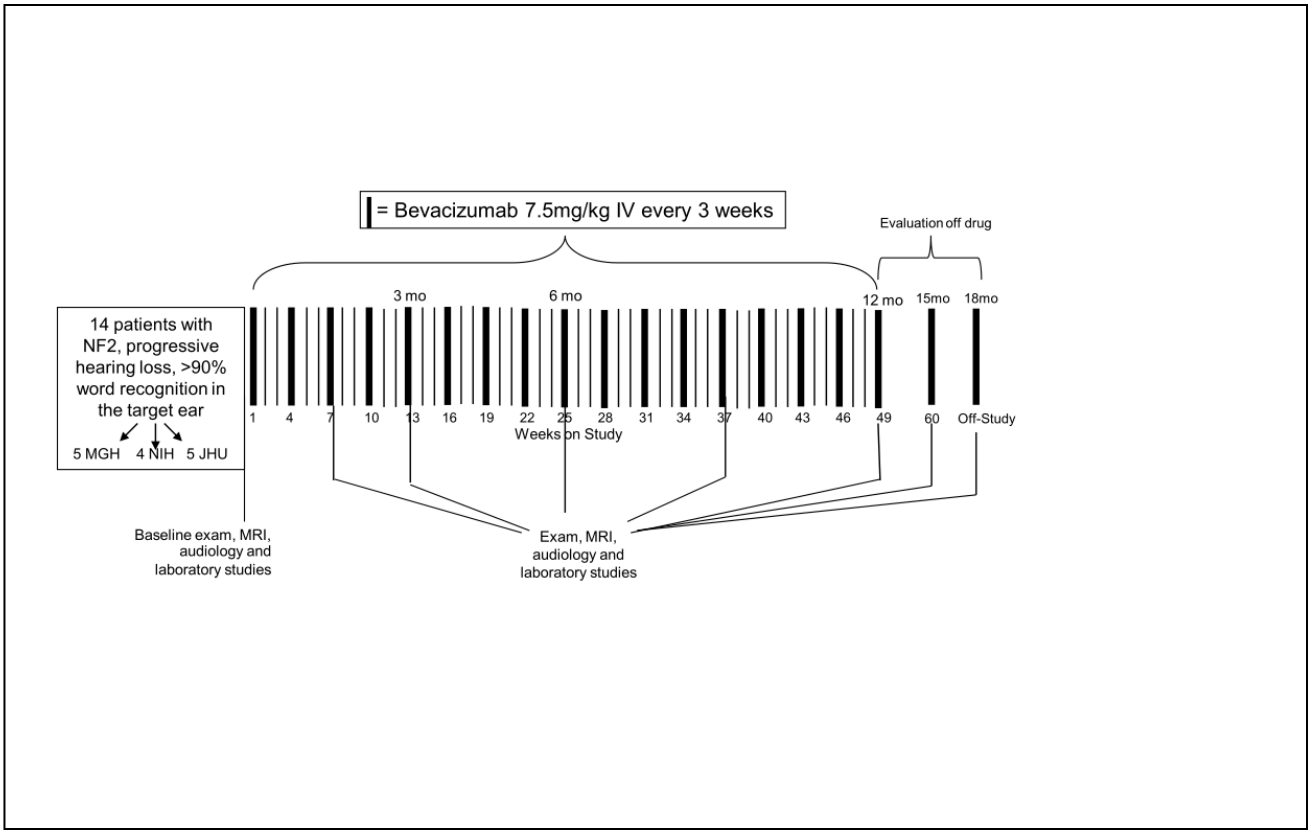
Responsible Data Manager:
Name: TBD

NCI Supplied Agent: Bevacizumab (rhuMAb VEGF), #704865

Protocol Type / Version # / Version Date: Revised, version #11, 2/20/12

SCHEMA

Figure 1. Study schema indicating treatment days, imaging, hearing and laboratory studies.



Précis

Background

- Patients with neurofibromatosis type 2 (NF2) develop tumors of the central and peripheral nervous system, including vestibular schwannomas, meningiomas, and ependymomas. Bilateral vestibular schwannomas (VSs) are the hallmark of NF2. As these tumors enlarge, they cause sensorineural hearing loss and, ultimately, complete deafness. The standard treatment options for VS include surgery, and in select cases radiation, which often result in hearing loss, cranial nerve dysfunction and other neurologic disability.
- Vestibular schwannomas demonstrate an angiogenic pattern of vasculature with increased microvascular density and size. Immunohistochemical studies show that 100% of tumors express vascular endothelial growth factor (VEGF). Our initial experience treating ten NF2 patients at risk for complete hearing loss with bevacizumab outside of a clinical trial showed promising results with 4/7 evaluable patients having significantly improved word recognition scores and 6/10 patients experiencing $\geq$ 20% reduction in tumor volume and 4/7 evaluable patients having significantly improved word recognition scores.
- Bevacizumab is a humanized IgG1 monoclonal antibody that binds all biologically active isoforms of human vascular endothelial growth factor (VEGF) with high affinity.

Objectives

- To determine the proportion of patients with hearing improvement with bevacizumab in patients with progressive hearing loss due to VS as assessed by word recognition scores.
- To determine the proportion of patients with radiographic improvement (decrease in VS volume by $\geq$20%) in VS with bevacizumab.
- To assess the safety and tolerability of bevacizumab 7.5mg/kg every 3 weeks for 12 months in patients with NF2 and progressive hearing loss.
- To explore the durability of response in both hearing and decreased tumor volume.
- To dissect the specific effect of bevacizumab treatment on the auditory system.
- To explore the biological effects of bevacizumab by measuring:
    - perfusion, permeability and vessel diameter using MRI tools including dynamic contrast enhanced (DCE) and apparent diffusion coefficient (ADC) measurements.
    - levels of circulating endothelial cells (CECs), circulating progenitor cells (CPCs), and plasma proteins (VEGF-A, VEGF-C, sVEGFR1, sVEGFR2, sVEGFR3, Col IV, SDF1a, IL-1beta, IL-6, IL-8, TNFalpha, G-CSF, Ang1, Ang2, sTie2, s-cKIT, MMP-1, MMP-2, MMP-3, MMP-9, MMP-10, PlGF, and bFGF).
- To explore the impact of bevacizumab therapy on health and hearing related quality of life (QOL) measures.
- To explore patient motivations and expectations for participation in a therapeutic trial for NF2

Eligibility

Adult and pediatric patients (12 years and older) with NF2 and evidence of active disease, defined as progressive hearing loss (with decrease in word recognition score) related to VS (i.e., not due to prior interventions such as surgery or radiation) in the preceding 24 months with a

word recognition score of <90% in the affected ear that is confirmed with study-specific audiometry testing. Patients with a progressive VS affecting their only hearing ear are considered particularly appropriate study candidates.

Design

- Bevacizumab will be administered intravenously at a dose of 7.5 mg/kg every three weeks (6 weeks = 1 treatment cycle).
- Response will be evaluated using the primary endpoint of hearing response (defined as exceeding the 95% critical difference for word recognition score) at 3-month intervals.
- The secondary endpoints will include:
    - Tolerability and safety
    - Radiographic response (defined as ≥ 20% decrease in tumor volume by MRI scan)
    - Vascular permeability (K^{trans}), relative cerebral blood volume/flow, mean transit time, and mean vessel diameter will be determined before and during therapy using perfusion-weighted MRI.
    - Changes in levels of circulating CECs, CPCs, VEGFR2+ monocytes and plasma proteins will be determined before and during therapy.
    - Patient reported QOL measures related to hearing including the Speech, Spatial and Qualities of Hearing Scale (SSQ), Tinnitus scale and the Short Form Health Survey-36.

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TABLE OF CONTENTS

1. OBJECTIVES

1.1. Primary Objective

The primary objective of this study is to determine the activity of bevacizumab for treatment of symptomatic vestibular schwannomas (VS) defined as progressive hearing loss in patients with neurofibromatosis type 2 (NF2) based on objective hearing response.

1.2. Secondary Objectives

The secondary objectives are:

- determine the safety and tolerability of bevacizumab in this patient population on an every three week dosing schedule of 7.5mg/kg for 12 months of therapy;
- assess the rate of radiographic response ($\geq$20% reduction in volume);
- determine the growth rate of VS using volumetric MRI analysis in comparison to 1-dimensional and 2-dimensional measurements;
- assess changes in function of the auditory system during bevacizumab treatment;
- assess the vascular permeability (K^{trans}), relative cerebral blood volume/flow, mean transit time, and mean vessel diameter from perfusion-weighted MRI;
- assess the change in circulating endothelial cells, circulating progenitor cells, and plasma angiogenic proteins in subjects receiving bevacizumab treatment;
- observe the impact of bevacizumab on non-VS tumors in patients with NF2 via whole body MRI;
- explore hearing related QOL measures throughout treatment;
- explore the effect of treatment with bevacizumab on vestibular function (to be evaluated at NCI only)

2. BACKGROUND

2.1 Neurofibromatosis type 2 (NF2)

An estimated 43,800 primary brain tumors were diagnosed in 2005 and 16,600 (38%) of these tumors were meningiomas or nerve sheath tumors. Surprisingly, meningiomas and vestibular schwannomas (VSs) are as common as all types of gliomas combined (1).

Neurofibromatosis 2 (NF2) is a tumor suppressor syndrome characterized by multiple schwannomas, meningiomas, and ependymomas. The birth prevalence is estimated to be 1 in 25,000 births and NF2 affects more than 10,000 individuals in the United States. The average age at onset of symptoms is 17 to 21 years.

Despite the benign histology of schwannomas, meningiomas, and ependymomas, NF2 patients experience significant morbidity and mortality related to their disease. Actuarial survival after diagnosis of NF2 is 85% at 5 years, 67% at 10 years, and 38% at 20 years (2). Bilateral VSs are the hallmark of NF2. As these tumors enlarge, they cause sensorineural hearing loss and, ultimately, complete hearing loss.

In addition, half of all NF2 patients have intracranial meningiomas and 75% have spinal tumors (i.e., schwannomas, meningiomas, ependymomas). Most patients require multiple surgeries during their lifetime. Morbidity related to NF2 is severe and includes early deafness, facial weakness (often resulting in poor chewing and swallowing and therefore, requiring PEG tube placement), blindness, seizures, hemiparesis and ultimately death related to the progression of tumors or complications of treatment.

There are two forms of hearing loss in patients with NF2. Gradual hearing loss is the rule, and it most commonly occurs with progression of tumor size over time. Although there is a rough correlation with tumor size, gradual hearing loss can occur with tumors of any size. Although surgical implantation of cochlear and auditory brainstem implants provides benefit for a small minority of patients, there is no widely effective treatment for this type of hearing loss. In addition, some patients can experience episodes of sudden hearing loss superimposed on baseline hearing dysfunction. Treatment with a short course of corticosteroids can often correct this acute hearing loss. The mechanism of acute hearing loss is not clearly understood but probably involves compression of the auditory nerve from tumor mass and associated edema. Previous studies of pressure within the internal auditory canal (IAC) of patients with VSs suggest that intracannalicular pressure is directly correlated with the amount of tumor in the IAC and may be inversely associated with preoperative hearing (3) . Thus, therapies that reduce edema, a common cause of increased pressure in brain tumors, are a rational approach to treating hearing loss in NF2 patients.

Current standard therapy for patients with NF2 is observation for stable tumors without neurologic symptoms. Surgery is the standard of care for progressive, symptomatic VS. Due to the large number of tumors encountered in the brain and spinal cord, surgical removal of all tumors is not possible or advisable. Iatrogenic morbidity after surgery for NF2-related lesions is, unfortunately, relatively common due to the intimate association between tumors and vital neurologic structures. For example, complete surgical resection of VSs often results in ipsilateral hearing loss except in a minority of tumors that are smaller than 1.5 cm and smooth in outline. In addition,, facial palsy, spinal fluid leaks and infection are all common complications of VS resection. As almost all patients with NF2 have bilateral vestibular schwannoma, a common scenario is early surgery for VS resection on the most active side with observation of the second VS. This often results in hearing loss on the side of surgery. When there is later progression of the contralateral VS, there is imminent risk of complete deafness. The current options for treatment at that time are surgery or radiation therapy. However, surgery carries a high risk of deafness itself as discussed above. In fact, specialists in NF2 associated tumors recommend nonoperative management of lesions in the only hearing ear whenever possible and reserve surgery for select situations, such as extensive brainstem compression (4).

Radiation has been used in a subset of tumors that progress despite surgical treatment or in individuals who are considered high risk for surgical complications. However, this modality should be used with caution since secondary malignancies after treatment have been reported (5). Specifically, the prevalence of nervous

system malignancy is very rare in population studies of NF2. In one study, 9 of 1242 cases reviewed across centers in North America and Europe were found to have a nervous system malignancy and the risk was limited to MPNST (compared to the general population) (6). In contrast, after radiation therapy for benign tumors such as vestibular schwannoma, roughly 5 of 106 patients developed a secondary malignancy. Hence, the prevalence of nervous system malignancies spontaneously in NF2 was 725 per 10^5 (95% confidence interval (CI) 253–1197 per 10^5) and after exposure to radiation therapy it was estimated at 4717 per 10^5 (95% CI 681–8753 per 10^5). This increase in incidence of nervous system malignancies is hypothesized to be related to the loss of the tumor suppressor gene in NF2 allowing greater susceptibility to the ionizing effects of radiation therapy (6). To date, more than 20 cases of malignancies (i.e. glioblastoma multiforme, rhabdomyosarcomas, malignant meningiomas) have been reported in patients with NF2 undergoing radiation therapy (7-12).

Regarding efficacy, retrospective single-center reviews suggest that stereotactic radiation can result in moderate rates of tumor control and poor long term hearing preservation. The best long term data is estimated from a center in Sheffield, England. They reported on 122 vestibular schwannomas in 92 patients with NF2 treated with RT and estimated tumor control at 8 years to be 50%. At 3 years they estimated 40% of patients to have preserved hearing, 40% of patients to have progressive hearing loss and 20% to have progressed to deafness. The long term risk of facial palsy was 5% (Rowe et al, 2008). In another study of 62 patients with NF2-related VS treated with stereotactic radiation therapy, hearing preservation was reported to be 73% at 1 year, 59% at 2 years, and 48% at 5 years after radiosurgery. Facial neuropathy occurred in 8% of patients (13). Overall, the data suggests that there can be short term tumor control and short term hearing preservation but that the long term efficacy (>5 years) of radiation therapy for NF2-related VS is moderate (<50% of patients with hearing) and there are unique risks with radiation therapy in NF2 patients including increased risk of secondary nervous system malignancy. There is currently no agreement in the field about the optimal timing and use of radiation therapy for NF2 patients, however most multidisciplinary specialty centers do not recommend this modality unless there are no other treatment options.

There are no known effective medical treatments for NF2-related tumors and no clinical trials with agents specifically targeting NF2-related vestibular schwannomas and hearing function have been performed in the past. Given the significant limitations of surgery and radiation therapy, and the devastating impact of these tumors, medical treatments are desperately needed. We have recently identified VEGF inhibition as a possible therapy. The initial experience treating ten NF2 patients at risk for complete hearing loss with bevacizumab outside of a clinical trial showed promising results with 6/10 patients experiencing $\geq$ 20% reduction in tumor volume and 4/7 evaluable patients having significantly improved word recognition scores (14). This study will provide preliminary, prospective data about the effect of bevacizumab in this group of patients (progressive hearing loss due to VS in patients with NF2) confirming the retrospective data reported, estimating the effects of bevacizumab on hearing, tumor size and quality of life and exploring possible biomarkers of this disease and its response to therapy.

2.2 Children and NF2

NF2 is traditionally thought to present in young adults. However, registry data has shown that 18% of patients with newly diagnosed NF2 are under the age of 15 years (15). Although manifestations of NF2 in children generally involve skin and ophthalmologic findings, there is an association between early development of symptoms and prognosis (16-18). There are rare cases with fulminant disease progression very early in life for which the currently available treatments of surgery and radiation therapy are insufficient resulting in significant neurologic morbidity or death due to relentless progression of tumor (19, 20). In one series of 12 patients diagnosed with NF2 before 18 years old, there was a high tumor burden with >75% of patients having vestibular schwannomas, other cranial nerve schwannomas or spinal cord tumors (21). At least 75% of the children had hearing loss and in the 58% of patients who underwent surgery for VS, none had preserved hearing post-operatively. Hence, the limited literature regarding NF2 in children suggests that there is a subpopulation that presents with early and severe disease.

2.3 Hearing loss and NF2

Hearing loss is a critical problem for patients with NF2. Unlike patients with sporadic VSs who can function with unilateral hearing loss, patients with NF2 are at risk for complete deafness. This hearing loss typically occurs during late adolescence or early adulthood and leads to social isolation and underemployment. Although hearing loss is related to VSs, the degree of hearing sensitivity (threshold) is only loosely correlated with tumor size. This dissociation may be due to direct compression of the auditory nerve, presence of intratumoral edema, disruption of blood flow to the cochlea, degeneration of certain cochlear structures, and distortion of auditory centers in the brainstem. There is evidence that the cochlea, as well as the 8th cranial nerve, is a site of both cell degeneration and the accumulation of precipitate in cases of VS (22) . This means that sites anywhere along the auditory pathways—from the periphery to the brainstem—may contribute to the observed loss of hearing function. Thus, we will use the full range of audiologic diagnostic tools available to assess function of the pathway.

Hearing is monitored in clinical practice by measuring pure tone thresholds, word recognition scores, brainstem auditory evoked responses (BAERs), and otoacoustic emissions (OAEs).

Pure tone thresholds measure the minimum sound level that an ear can perceive. Thresholds are typically measured at octaves and half-octaves from 250 Hz to 8000 Hz. An average of thresholds at 500, 1000, 2000 and 4000 Hz (PTA) can be used to characterize pure tone thresholds.

Word recognition scores measure the ability to recognize (as opposed to detect) auditory information. Patients are presented a list of 100 words at a level determined to yield the maximum score and the percentage identified correctly is the score. NF2 patients may exhibit “rollover” where the score decreases at a fully audible level so we will do a full 50 word list at the fully audible (high) level, and one additional 50 word list at a level 10-15dB below that level (the low level). An additional 50 words

will be added at the level of the highest score; the total of this list and the list on which the patient initially scored highest will generate a 100 word list which will be used and compared across visits. This study will use monosyllable lists and standardized recordings.

Otoacoustic emissions (OAEs) are sounds that are generated from within the cochlea as it acts to amplify incoming sounds. OAEs are a sensitive measure of cochlear health and often disappear after the cochlea has been damaged. The presence (significantly above noise-floor) of measurable emissions, called distortion product otoacoustic emissions (DPOAEs) across frequencies from 1000 to 8000 Hz will be used as an indicator of functioning cochlear regions.

Because tumors associated with NF2 have benign histology, overall survival is not an appropriate endpoint for clinical trials in this condition. Instead, the goal of treatment is to minimize neurologic morbidity (including hearing loss) and to defer surgical treatments that may cause iatrogenic dysfunction. For this reason, hearing function is the most important way to monitor the activity of new agents designed to treat VSs.

Word recognition is the measure most closely associated with daily hearing function since it measures the ability to comprehend speech (rather than "detect" it). If word recognition quality improves, the patient can converse successfully, even if a hearing aid is needed to make sounds sufficiently loud. Statistical methods have been developed to determine significant changes in this measure. Word recognition scores represent summary scores from a collection of binary endpoints (correct/incorrect responses) and thus follow a binomial distribution (e.g., non-Gaussian distribution). Although it is tempting to use a set change in word recognition score (e.g., fifteen percentage points) as a clinical response, this approach is inappropriate given the binomial model of variance. A more rigorous approach involves the use of the 95% (p=0.05) critical difference table (23) (Appendix C). The 95% critical differences have been used in previous studies (24) and in clinical trials evaluating the effect of drug treatment on hearing.

2.4 Angiogenesis and Imaging

Although many clinicians believe that hearing loss related to VSs is irreversible, there are cases with transient hearing restoration after a course of corticosteroids or decompression of the auditory canal without removal of the tumor. Our preliminary observations indicate that neutralizing VEGF can reverse profound hearing loss and subsequently maintain hearing. Based on these experiences, we hypothesize that the restoration of hearing function with bevacizumab is in part due to reduction in intraneural edema that compresses the auditory nerve. In this proposal we will investigate the mechanism(s) by which bevacizumab inhibits tumor growth and/or induces hearing recovery using advanced MRI techniques specifically designed to assess the vascular profile of tumors and the tumor environment.

Internal auditory canal (IAC) imaging. The vestibular and auditory nerves together comprise the 8th cranial nerve. The nerve exits the posterior brainstem, travels through the subarachnoid space in the IAC to terminate in the cochlea (auditory

nerve) and semicircular canals (vestibular nerve). Detailed imaging of the IAC is essential to identify and follow VSs in patients with NF2. MRIs must therefore include thin cuts through the IAC (3mm, no skip) in addition to the traditional pre- and post-contrast images (5 mm, 1 mm skip) that are performed for malignant brain tumors.

Volumetric analysis. Traditionally, VSs have been measured using either 1-dimensional measurements (in the long axis) or 2-dimensional measurements (calculated by taking the square root of the product of the short axis * long axis measured to the plane perpendicular to the face of the temporal bone) (25). However, the irregular shape of VSs, as determined by the unique anatomy of the IAC and cerebellopontine angle, makes it difficult for linear measurements to accurately and fully represent growth for the entire tumor. *As a result, VS growth may be underestimated by linear measurement criteria.* Semi-automated volumetric analysis overcomes these limitations since it uses data from 3 dimensions. The coefficient of variation (COV) ranges from 0.6% to 6.8% and is generally below 5% for lesions greater than 1 cc (26) . Volumetric analysis not only better reflects tumor size, but also allows accurate detection of smaller changes in tumor size compared to standard solid tumor response criteria (e.g., RECIST criteria). This ability to detect small changes in size is critical for tumors with slow growth rates such as VS, and, when used in clinical trials, helps limit exposure to potentially toxic and/or inactive agents. Trials for NF-related tumors, such as plexiform neurofibromas, are now routinely using volumetric changes as the primary endpoint, typically choosing a 20% increase for progression and 20% decrease for radiographic response (27-29) .

Vascular imaging. The development of anti-angiogenic therapies for tumors has led to a demand for imaging-based surrogate markers. It has been shown that T2*-weighted MRI scans are highly reproducible, and can be applied repeated to assess novel therapies in clinical trials (30). In addition to measuring the size of tumors with post-contrast images, dynamic contrast enhanced (DCE)-MRI can be used to measure the permeability of tumor vessels. This technique has been used to measure changes in the area under the contrast concentration vs. time curve (AUC) in patients with a variety of brain tumors including VSs, meningiomas, and gliomas (31) . Thus, it is technically feasible to use DCE-MRI to image tumors in the posterior fossa. Furthermore, the technique has been used in cancer patients receiving anti-angiogenic drugs such as SU5416 (32) and EMD 121974 (33), and within our own group with AZD2171 (34). Thus, these imaging modalities have become integral for many clinical trials assessing anti-angiogenic therapies for brain tumors.

2.5 Plasma Markers

The discovery of mechanism-based biomarkers can facilitate the efficient development of new anti-cancer medicines and potentially serve as true intermediate or surrogate end points for future clinical trials. Our study and others suggest that there are dose- and time-dependent increases in soluble VEGF and PlGF and decreases in soluble VEGFR following treatment with anti-angiogenic agents (34-38). Others have shown that circulating biomarkers can help predict tumor progression. For example, treatment with a VEGF aptamer in a preclinical model of angiogenesis revealed an upregulation of bFGF (39). This suggests that even in non-malignant

disease, compensatory upregulation of proangiogenic factors can develop during anti-angiogenic treatment.

Biomarkers of anti-angiogenic therapy in non-malignant disorders. To date, the only non-malignant disorder that is routinely treated with bevacizumab or other anti-VEGF agents is age-related wet macular degeneration. Although levels of angiogenesis-related protein have been measured in the vitreous of some patients, systemic levels of cytokines have not been followed since those patients receive local injections (40) . In this study we propose to follow patients with multiple benign tumors treated systemically. These tumors are slow growing and do not present the same genetic instability as seen in malignant tumors, we therefore do not expect the same level of "adaptation" to VEGF deprivation.

While there are no published reports on circulating cells in patients with NF2, several studies have shown higher intratumoral levels of progenitor cells. Our study will provide the first data in this patient population. Since patients with NF2 have multiple tumors and multiple tumor types, this information will be used to provide baseline levels and preliminary data for three factors: (i) as an indication of systemic response to treatment, where levels of VEGF and Plgf are expected to increase; (ii) as a marker of toxicity, since NF2 could potentially be treated for extended periods of time; and (iii) as a correlate to the functional MRI in order to further our understanding of the mechanisms of action of bevacizumab on tumor-associated vasculature. Although an increase of alternate angiogenic growth factor might not be directly attributable to the target vestibular schwannoma, this information will provide functional information when paired with changes in K^{trans} on perfusion MRI.

2.6 Whole Body Magnetic Resonance Imaging

Many patients with NF2 have a high burden of tumor. Although the hallmark of the disease is VS and progression of VS is the leading cause of morbidity and mortality in NF2, several other tumor types contribute to functional decline in NF2, including ependymomas, spinal schwannomas and peripheral schwannomas. In a recent study assessing the feasibility of whole body MRI to measure tumor burden in the neurofibromatoses, 6 of 14 patients (43%) with NF2 were found to have at least one peripheral tumor (41). Across all patients, the median number of peripheral tumors was 1 plexiform and 3 discrete tumors. However, in select patients the tumor burden was much higher (up to 63 tumors). Many patients with advanced VS at a young age will also have peripheral tumors (42). The advent of whole body MRI allows screening of NF2 patients for evidence of peripheral tumors and comparison between baseline and post-treatment tumor volumes (41).

There has been an increasing amount of evidence about the expression of VEGF in VS and the relationship between VEGF expression and tumor growth (14, 43-45). However, the expression of VEGF and influence of VEGF expression on tumor behavior is less well understood for peripheral schwannomas. Relatively low levels of VEGF mRNA expression or VEGF positive staining via immunohistochemistry were found in neurofibromas (n=14) or schwannomas (n=19) compared to MPNST (n=22) (46) . Another study found that 3/6 schwannoma samples stained positive for VEGF and 4/6 were positive for VEGFR-1 or -2, however, there was no correlation with

clinical course (47). In the only study to directly assess the VEGF expression in NF2 associated versus sporadic peripheral schwannomas, 8/10 schwannomas from patients with confirmed or presumed NF2 stained positive for VEGF. All 10 sporadic schwannomas investigated stained positive for VEGF and there was no significant difference between sporadic and NF2 associated peripheral schwannomas in mitotic index or microvascular density (48).

Our anecdotal clinical experience has been that some patients appear to have benefit in symptomatic peripheral schwannomas when being treated with bevacizumab for VS, but others do not. The development of whole body MRI technology allows detection of peripheral tumors with decreased risk to patients (peripheral tumors are visualized on STIR images that do not require exogenous contrast) and enhanced convenience (roughly 45 minute scan time). A screening MRI at baseline will allow identification of NF2 patients with peripheral tumor involvement. In patients with identified tumors (sensitivity cut-off is >2cm in size), follow-up whole-body MRIs will be done at 24 and 48 weeks. This will allow observation of whether peripheral tumors respond to treatment with bevacizumab. Such data will serve as preliminary information that may help the design of future trials for peripheral nerve sheath tumors.

2.7 Patient Hearing, Vestibular Function and Health Related Quality of Life

One of the major threats to quality of life in patients with NF2 is loss of hearing. Although early adulthood onset deafness is recognized by experts in the field as a major factor impacting quality of life (QOL) in NF2, this has not yet been directly assessed in this population (49, 50). There are studies assessing quality of life in patients with late onset hearing loss without NF2. These studies suggest that in adults with hearing loss, there is an association between degree of hearing impairment and social isolation, perceived disability and depression (51, 52). Moreover, there is some indication that although patients with late-onset hearing loss have decreased reported QOL at baseline, there is the possibility for improvement with effective therapies (51, 52). Studies in patients receiving cochlear implants for adult onset hearing loss suggest that specific improvement in functions such as communication abilities directly influenced perceived quality of life (53). Hence, it appears that for the most informative view of the impact of hearing dysfunction (and possible improvement) on patient perceived QOL, both general measures of QOL as well as specific function-based questionnaires are the most beneficial (54).

As noted above in section 2.3, word recognition score has been incorporated into the primary outcome because this variable is a direct measure of quality of life (e.g., ability to comprehend speech). In addition, we will explore patient reported QOL with the Short Form Health Survey-36 as a global measure of health related QOL (SF-36, Appendix H), the Speech, Spatial and Qualities of Hearing Scale (SSQ, Appendix I), and the Tinnitus Reaction Questionnaire (TRQ, Appendix J) as specific patient reported measures of impact of therapy on function. Finally, we will include three questions about the patients' experience of being involved in this clinical trial to explore how involvement in an experimental therapeutic trial may be influencing perceived QOL and vice versa (Appendix L). In additon, vestibular function will be evaluated as detailed in Appendix K (for subjects enrolled at the NCI only).

2.8 Bevacizumab

Bevacizumab is a humanized IgG1 monoclonal antibody (MAb) that binds all biologically active isoforms of human vascular endothelial growth factor (VEGF, or VEGF-A) with high affinity (k_d = 1.1 nM) (55). The antibody consists of a human IgG1 framework and the antigen-binding complementarity-determining regions from the murine anti-VEGF MAb A.4.6.1 (55-57).

Mechanism of Action

Of known proangiogenic factors, VEGF is one of the most potent and specific, and has been identified as a crucial regulator of both normal and pathological angiogenesis. VEGF is a secreted, heparin-binding protein that exists in multiple isoforms. Action of VEGF is primarily mediated through binding to the receptor tyrosine kinases, VEGFR-1 (Flt-1) and VEGFR-2 (KDR/Flk-1). The biological effects of VEGF include endothelial cell mitogenesis and migration, increased vascular permeability, induction of proteinases leading to remodeling of the extracellular matrix, and suppression of dendritic cell maturation. Neutralization of VEGF by A.4.6.1 or bevacizumab has been shown to inhibit the VEGF-induced proliferation of human endothelial cells *in vitro*, and decrease microvessel density, microvessel diameter and interstitial pressure in tumor xenografts *in vivo* (58). In patients, preliminary results from a neoadjuvant trial in rectal cancer demonstrated a decrease in blood perfusion/permeability and interstitial fluid pressure in tumors after one dose of bevacizumab (35).

Nonclinical Studies

The murine parent MAb of bevacizumab, A4.6.1, has demonstrated potent growth inhibition *in vivo* in a variety of human cancer xenograft and metastasis models, including those for SK-LMS-1 leiomyosarcoma, G55 glioblastoma multiforme, A673 rhabdomyosarcoma, Calu-6, and MCF-7 cell lines (55, 56, 59, 60). The antitumor activity was enhanced with the combination of A4.6.1 and chemotherapeutic agents compared to either agent alone. Combined blockage of the VEGF and other growth factor pathways (*e.g.*, EGFR or PDGFR) has also demonstrated additive effects *in vivo* (61, 62) . Associated with the anti-tumor activity of anti-VEGF MAbs were findings of reduced intratumoral endothelial cells and microcapillary counts as well as reduced vascular permeability and interstitial pressure

Nonclinical toxicology studies have examined the effects of bevacizumab on female reproductive function, fetal development, and wound healing. Fertility may be impaired in cynomolgus monkeys administered bevacizumab, which led to reduced endometrial proliferation and uterine weight as well as a decrease in ovarian weight and number of corpora lutea. Bevacizumab is teratogenic in rabbits, with increased frequency of fetal resorption, as well as specific gross and skeletal alterations. In juvenile cynomolgus monkeys with open growth plates, bevacizumab induced physeal dysplasia, which was partially reversible upon cessation of therapy. Bevacizumab also delays the rate of wound healing in rabbits. This effect appeared to be dose-dependent and characterized by a reduction of wound tensile strength.

Clinical Studies in Adults

To date, over 7000 patients have been treated in clinical trials with bevacizumab as monotherapy or in combination regimens (57) .

Pharmacokinetics

The pharmacokinetics (PK) of bevacizumab have been characterized in several phase 1 and phase 2 clinical trials, with doses ranging from 1 to 20 mg/kg administered weekly, every 2 weeks, or every 3 weeks. The estimated half-life of bevacizumab is approximately 21 days (range 11-50 days). The predicted time to reach steady state was 100 days. The volume of distribution is consistent with limited extravascular distribution.

Maximum Tolerated Dose

The maximum tolerated dose (MTD) of bevacizumab has not been determined; however, the dose level of 20 mg/kg was associated severe headaches (63). The dose schedule of either 10 mg/kg q2w or 15 mg/kg q3w is used in most phase 2 or 3 trials with only a few exceptions (e.g., the pivotal phase 3 trial in colorectal cancer, in which bevacizumab was given at 5 mg/kg q2w).

Clinical efficacy of bevacizumab

Clinical proof of principle for anti-VEGF therapy with bevacizumab has been observed in several solid tumors. In 1st- and 2nd-line metastatic colorectal cancer, combination of bevacizumab with 5-FU-based chemotherapy improved the overall survival (OS), progression-free survival (PFS) and response rate (RR) as compared to chemotherapy alone (64, 65). There was also improved overall survival in first-line non-small cell lung cancer (NSCLC) patients (E4599) treated with carboplatin/paclitaxel + bevacizumab compared with chemotherapy alone. Bevacizumab in combination with chemotherapy has been approved by the FDA for treatment in advanced/metastatic colorectal cancer (first and second lines) and in NSCLC.

In untreated advanced and metastatic breast cancer, addition of bevacizumab to paclitaxel also significantly improved the RR and PFS (E2100) (66). However, in the phase 3 trial in doxorubicin and paclitaxel-refractory metastatic breast cancer, the addition of bevacizumab to capecitabine did not show an improvement in PFS despite an increase in the RR (66) . In locally advanced and metastatic pancreatic cancer, a Phase III also failed to demonstrate OS or PFS advantage by adding bevacizumab to gemcitabine (CALGB 80303) (67).

Bevacizumab has been studied as monotherapy in renal cell cancer (mRCC). In a 3-arm, double-blind, placebo-controlled phase 2 trial (68), patients with previously treated stage IV RCC were randomized to high-dose (HD) bevacizumab (10 mg/kg q2w), low-dose (LD) bevacizumab (3 mg/kg q2w), or placebo. The study demonstrated a highly significant prolongation of time to progression (TTP) in the HD arm (4.8 months) as compared with the placebo (2.6 months) (hazard ratio = 2.55, $p = 0.0002$); the LD arm was associated with a smaller difference in TTP (3.0 months) of borderline significance; the tumor response rate was 10% in the HD arm but 0% in the LD and placebo groups. A Phase III study (BO17705) with bevacizumab (10 mg/kg/q2w) + interferon-alpha 2a versus interferon-alpha 2a +

placebo as first-line therapy in patients with advanced and/or mRCC demonstrated statistically significant and clinically relevant improvements in progression-free survival (10.2 vs. 5.4 months), and objective response rate (31.4 vs. 12.8%).

The Phase III study BO17706 indicated no statistically significant improvement in overall survival when bevacizumab (5 mg/kg/q2w) is added to the gemcitabine/erlotinib combination in the first-line treatment of advanced pancreatic cancer. The Phase III NCI-sponsored CALGB80303 study investigating the use of bevacizumab (10 mg/kg/q2w) combined with gemcitabine was prematurely terminated after the CALGB DSMB concluded that the futility boundary defined for the primary efficacy parameter (overall survival) had been crossed in a protocol-specified interim analysis (dated June 16th, 2006).

Additional clinical trials are ongoing in a variety of solid tumors and hematological malignancies using bevacizumab as monotherapy or in combination with chemotherapy, radiation, or other targeted/biological agents. To date, there have been no published studies on the efficacy and toxicity of bevacizumab for VS, meningiomas, or ependymomas, or for patients with NF2.

Clinical Studies in Children

Bevacizumab has been studied in the pediatric population in 3 clinical trials and two retrospective reports to date. Across all of these studies, with evaluation periods ranging from 1 month to 2 years, there have not been any DLTs or major toxicities seen in children treated with bevacizumab.

In a phase I trial in children with progressive solid tumors, 18 patients were assessable (received at least 1 course, 28 days, of treatment, range was 1-16 courses with a median of 3 courses). They evaluated doses of 5mg, 10mg or 15mg IV every 2 weeks. There were no DLTs observed at any dose and non-dose limiting toxicities were limited to mild increase in blood pressure (not meeting CTCAEv3 criteria), infusional reaction, mucositis, rash, proteinuria and lymphopenia. Bone toxicity was evaluated and was not seen albeit over short periods of observation (1-3 months) (69).

A retrospective study was then reported in children with refractory solid tumors in which bevacizumab was used between 2004 and 2006 (70). They found that across 15 patients (median age 14 years) treated for 1-23 months and dosed at 5-10mg/kg every 2-3 weeks for largely nervous system based tumors, there were no reports of DLTs and that the toxicities seen were mild and included hypertonia, proteinuria/hematuria, epistaxis, infusion related erythema and poor wound healing in a total of 8 patients.

Bevacizumab has been specifically evaluated in pediatric patients with brain disease. Packer et al studied bevacizumab and irinotecan in children with progressive low grade gliomas (71). The average age at treatment was 5 years old. Bevacizumab was doses at 10 mg/kg every 2 weeks and irinotecan at 125 mg/m2 every 2 weeks. Six of 10 patients remained on treatment for up to 22 months. There were two DLTs: transient leukoencephalopathy and grade 3 proteinuria. All other toxicities were non-dose-limiting toxicities (grade 1 and 2) included nausea and abdominal pain (2

patients) and increased obsessive/compulsive behaviors (1 patient), increased blood pressure (1 patient). There were no hemorrhages or bone toxicity seen with therapy up to 22 months.

In another trial, children with resistant, high-risk neuroblastoma metastatic to the skeleton were treated with bevacizumab 15mg/kg every 2 weeks plus 131I-3F8. This trial is ongoing, but thus far the preliminary results for 7 children have been reported and they have not seen skeletal toxicity or other DLT related to bevacizumab (72).

Finally, there is a brief report of 4 children with brainstem gliomas with presumed radiation induced necrosis treated with bevacizumab for a range of 1.5-7 months at a dose of 10mg/kg IV every 2 weeks (73). In this case series, there were 3 responses and no toxicities were reported.

In summary, there is published data about 54 pediatric patients with various advanced solid tumors (many of them specifically brain tumors) at bevacizumab doses from 5-15mg/kg every 2-3 weeks for a duration of therapy ranging from 1-23 months and there have been no reports of severe toxicity. Based on this review of the available data, it appears that the risk to children from bevacizumab is relatively small. Given that the enrollment criteria for this protocol includes progressive hearing loss with growing vestibular schwannomas for which no other therapy is available and the observation of 60% radiographic response and 57% hearing response in the retrospective evaluation of bevacizumab for patients with progressive VS and NF2, we are of the opinion that the demonstrated small risk of bevacizumab is balanced by potential benefit. We do not feel the available data suggests that we are subjecting adult or pediatric patients to increased risk that would require an IND. We have added the summary of the pediatric investigation of bevacizumab to the protocol and IND exemption statement.

Safety Profile

Based on clinical trials with bevacizumab as monotherapy or in combination with chemotherapy, the most common adverse events of any severity include asthenia, pain, headache, hypertension, diarrhea, stomatitis, constipation, epistaxis, dyspnea, dermatitis and proteinuria. The most common grade 3-4 adverse events were asthenia, pain, hypertension, diarrhea and leukopenia.

The major bevacizumab-associated adverse events identified in phase I to phase III trials include hypertension, proteinuria, arterial thromboembolic events, hemorrhage, congestive heart failure (CHF), gastrointestinal perforations, and wound healing complications. Other SAEs observed with bevacizumab therapy include reversible posterior leukoencephalopathy syndrome and fistula formation.

The following is a description of major adverse events associated with bevacizumab therapy. A list of Comprehensive Adverse Events and Potential Risks (CAEPR) in NCI-CTCAE v3.0 terms for bevacizumab is included in Section 7 of the protocol. Reference may also be made to the Investigators' Brochure and the FDA package insert (www.fda.gov/cder/foi/label/2004/125085lbl.pdf).

Infusion-Related Reactions. Infusion reactions with bevacizumab were uncommon (<3%) and rarely severe (0.2%). Infusion reactions may include rash, urticaria, fever, rigors, hypertension, hypotension, wheezing, or hypoxia. Currently, there is no adequate information on the safety of retreatment with bevacizumab in patients who have experienced severe infusion-related reactions.

Hypertension. Hypertension is common in patients treated with bevacizumab. The incidence of hypertension (all grade) is 20-30% across trials, with a mean increase of +5.5mmHg to +8.4mmHg for systolic pressure, or +4.1mmHg to +5.4mmHg for diastolic pressure. Incidence of grade 3 (hypertension requiring initiation of, or increase in, hypertensive medications) ranges from 7.8 to 17.9%. Grade 4 hypertension (hypertensive crisis) occurred in up to 0.5% of bevacizumab-treated patients.

Hypertension associated with bevacizumab can generally be controlled with routine oral drugs while bevacizumab is continued. However, incidents of hypertensive crisis with encephalopathy, including reversible posterior leukoencephalopathy syndrome (RPLS, see below), or cardiovascular sequelae have been rarely reported. Blood pressure (BP) should be closely monitored during bevacizumab therapy and the goal of BP control should be consistent with standard medical practice (74). Bevacizumab therapy should be suspended in the event of uncontrolled hypertension.

Proteinuria. Proteinuria has been seen in all bevacizumab studies to date, ranging in severity from mild asymptomatic increase in urine protein (incidence of about 38%) to rare instances of either grade 3 proteinuria (> 3.5gm/24 hour urine) (3%) or nephrotic syndrome (1.4%). Pathologic findings on renal biopsies in two patients showed proliferative glomerulonephritis. The risk of proteinuria may be higher in patients with advanced RCC or history of hypertension. There is also evidence from dose-finding trials that the rate of proteinuria may be dose related. Proteinuria will be monitored by urine protein level using urine analysis dipstick.

Hemorrhage. Overall, grade 3 and 4 bleeding events were observed in 4.0% of 1132 patients treated with bevacizumab in a pooled database from eight phase I, II, and III clinical trials in multiple tumor types. The hemorrhagic events that have been observed in bevacizumab clinical studies were predominantly tumor-associated hemorrhage and minor mucocutaneous hemorrhage.

Tumor-associated hemorrhage. Major or massive pulmonary hemorrhage/hemoptysis has been observed primarily in patients with NSCLC. In a phase 2 study in NSCLC, 6 cases of life-threatening (4 fatal) hemoptysis were reported among 66 patients treated with bevacizumab and chemotherapy (75); squamous cell histology was identified as the risk factor. In the phase III trial in non-squamous NSCLC (E4599), the rate of Grade ≥ 3 pulmonary hemorrhage was <1% in the control arm (carboplatin/paclitaxel) versus 2.3% in the chemotherapy plus bevacizumab arm (10/427 patients, including 7 deaths).

Gastrointestinal hemorrhages, including rectal bleeding and melaena have been reported in patients with colorectal cancer, and have been assessed as tumor-

associated hemorrhages. In the pivotal phase 3 trial in advanced colorectal cancer, the rate of GI hemorrhage (all grades) was 24% in the IFL/bevacizumab arm compared to 6% in the IFL arm; grade 3-4 hemorrhage was 3.1% for IFL/bevacizumab and 2.5% for IFL.

Serious tumor associated bleedings have also been observed in patients with pancreatic cancer, gastric cancer, CNS metastases, hepatoma, or varices treated with bevacizumab.

Mucocutaneous hemorrhage. Across all bevacizumab clinical trials, mucocutaneous hemorrhage has been seen in 20%-40% of patients treated with bevacizumab. These were most commonly NCI-CTC Grade 1 epistaxis that lasted less than 5 minutes, resolved without medical intervention, and did not require any changes in bevacizumab treatment regimen. There have also been less common events of minor mucocutaneous hemorrhage in other locations, such as gingival bleeding and vaginal bleeding.

Arterial Thromboembolic Events (ATE). The risk of arterial thromboembolic events is increased with bevacizumab therapy; such events included cerebral infarction, transient ischemic attack (TIA), myocardial infarction (MI) and other peripheral or visceral arterial thrombosis. A pooled analysis of five randomized studies showed a two-fold increase in these events (3.8% vs. 1.7%). ATE led to a fatal outcome in 0.8% patients with bevacizumab (vs. 0.5% without bevacizumab). The rate of cerebrovascular accidents (including TIA) was 2.3% vs. 0.5%, and the rates of MI 1.7% vs. 0.7%. Certain baseline characteristics, such as age and prior arterial ischemic events, appear to confer additional risk (76) . In patients $\geq$ 65 years treated with bevacizumab and chemotherapy, the rate of ATE was approximately 8.5%.

Aspirin is a standard therapy for primary and secondary prophylaxis of ATE in patients at high risk of such events, and the use of aspirin $\leq$ 325 mg daily was allowed in the five randomized studies discussed above, though safety analyses specifically regarding aspirin use were not preplanned. Due to the relatively small numbers of aspirin users and ATE events, retrospective analyses of the ability of aspirin to affect the risk of ATE were inconclusive. Further analyses of the effects of concomitant use of bevacizumab and aspirin are ongoing.

Venous thromboembolism (VTE), including deep venous thrombosis, pulmonary embolism and thrombophlebitis. In the Phase III pivotal trial in metastatic CRC, there was a slightly higher rate of VTE in patients treated with chemotherapy + bevacizumab compared with chemotherapy alone (19% vs. 16%). The incidence of NCI-CTC Grade $\geq$3 VTEs in one NSCLC trial (E4599) was higher in the bevacizumab-containing arm compared to the chemotherapy control arm (5.6% vs. 3.2%).

In clinical trials across all indications, the overall incidence of VTEs ranged from 2.8% to 17.3% in the bevacizumab-containing arms compared to 3.2% to 15.6% in the chemotherapy control arms. The use of bevacizumab with chemotherapy does not substantially increase the risk of VTE compared with chemotherapy alone. However,

patients with mCRC who receive bevacizumab and experienced VTE may be at higher risk for recurrence of VTE.

Gastrointestinal Perforation: GI perforations and/or fistula were rare but occurred at an increased rate in bevacizumab-containing therapies. The majority of such events required surgical intervention and some were associated with a fatal outcome. In the pivotal phase III trial in CRC (AVF2107), the incidence of bowel perforation was 2% in patients receiving IFL/bevacizumab and 4% in patients receiving 5-FU/bevacizumab compared to 0.3% in patients receiving IFL alone. GI perforation has also been reported in non-CRC tumors (e.g. gastric/esophageal, pancreatic and ovarian cancers) or nonmalignant conditions such as diverticulitis and gastric ulcer. GI perforation should be included in the differential diagnosis of patients on bevacizumab therapy presenting with abdominal pain or rectal/abdominal abscess.

Fistula: Fistula formations, including events resulting in death, have been observed in patients receiving bevacizumab in clinical studies and post-marketing reports. Fistulae in the GI tract are common (1-10% incidence) in patients with certain metastatic tumors such as colorectal cancer or cervical, but uncommon (0.1-1%) or rare (0.01-0.1%) in other indications. In addition, fistulae that involve areas other than the GI tract have also been observed (e.g. tracheoesophageal, bronchopleural, urogenital, biliary). Events were reported at various time points during treatment, ranging from 1 week to > 1 year following initiation of bevacizumab, with most events occurring within the first 6 months of therapy.

Wound Healing Complications. Bevacizumab delays wound healing in rabbits, and it may also compromise or delay wound healing in patients. Bowel anastomotic dehiscence and skin wound dehiscence have been reported in clinical trials with bevacizumab.

The appropriate interval between surgery and initiation of bevacizumab required to avoid the risk of impaired wound healing has not been determined. Across metastatic CRC trials, at least 28 days must have elapsed following major surgery before bevacizumab could be initiated; data suggested initiation of bevacizumab 29-60 days following surgery did not appear to increase the risk of wound healing complications compared to those treated with chemotherapy alone.

The optimal interval between termination of bevacizumab and subsequent elective surgery has not been determined. In the pivotal study in CRC, among patients who underwent major surgery while on study therapy, there was an increased rate of significant post-operative bleeding or wound healing complications in the IFL + bevacizumab arms vs. IFL alone [10% (4/40) vs. 0% (0/25)] (77). Decisions on the timing of elective surgery should take into consideration the half-life of bevacizumab (average 21 days, range 11-50 days).

If patients receiving treatment with bevacizumab require elective major surgery, it is recommended that bevacizumab be held for 4–8 weeks prior to the surgical procedure. Patients undergoing a major surgical procedure should not begin/restart bevacizumab until 4 weeks after that procedure (in the case of high-risk procedures

such as liver resection, thoracotomy, or neurosurgery, it is recommended that chemotherapy be restarted no earlier than 6 weeks and bevacizumab no earlier than 8 weeks after surgery).

Congestive Heart Failure (CHF). The risk of left ventricular dysfunction may be increased in patients with prior or concurrent anthracycline treatment. In phase III trials in metastatic breast cancer (AVF 2119g) in which all patients had received prior anthracyclines, CHF or cardiomyopathy were reported in 3% in the bevacizumab + capecitabine arm compared to 1% in the capecitabine-only arm (66). In a phase III trial of patients with previously untreated metastatic breast cancer (E2100), the incidence of LVEF decrease (defined as NCICTC Grade 3 or 4) in the paclitaxel + bevacizumab arm was 0.3% versus 0% for the paclitaxel alone arm.

In phase II study of 48 patients with refractory acute myelogenous leukemia treated with cytarabine, mitoxantrone, and bevacizumab, 5 cases of cardiac dysfunction (CHF or decreases to <40% in left ventricular ejection fraction, including AML trial) were reported. All but one of these subjects had significant prior exposure to anthracyclines as well.

Two additional studies investigated concurrent administration of anthracyclines and bevacizumab. In 21 patients with inflammatory breast cancer treated with neoadjuvant docetaxel, doxorubicin (cumulative doses at 240 mg/m2), and bevacizumab, no patients developed clinically apparent CHF; however, patients had asymptomatic decreases in LVEF to < 40% (78) . In a small phase II study in patients with soft tissue sarcoma, 2/17 patients treated with bevacizumab and high-dose doxorubicin (75 mg/m^2) developed CHF (one Grade 3 event after a cumulative doxorubicin dose of 591 mg/m^2, one Grade 4 event after a cumulative doxorubicin dose of 420 mg/m^2); an additional 4 patients had asymptomatic decreases in LVEF (79) .

Patients receiving anthracyclines or with prior exposure to anthracyclines should have a baseline MUGA or ECHO with a normal ejection fraction.

Reversible Posterior Leukoencephalopathy Syndrome (RPLS), Posterior Reversible Encephalopathy Syndrome (PRES), or similar leukoencephalopathy syndrome. RPLS/PRES are clinical syndromes related to vasogenic edema of the white matter and have rarely reported in association with bevacizumab therapy (<1%). Clinical presentations may include altered mental status, seizure, visual disturbance or cortical blindness, with or without associated hypertension. MRI scans are required for diagnosis. Typical findings are vasogenic edema (enhanced intensity in T2 and FLAIR sequences on non-contrast MRI) predominantly in the white matter of the posterior parietal and occipital lobes, and less frequently, in the anterior distributions and the gray matter.

RPLS/PRES is potentially reversible, but timely correction of the underlying causes, including control of BP and interruption of the offending drug, is important in order to prevent irreversible tissue damage. The safety of reinitiating bevacizumab therapy in patients previously experiencing RPLS is not known (80, 81) .

Neutropenia. In the phase III trial with IFL +/- bevacizumab in colorectal cancer, grade 3-4 neutropenia was 21% with bevacizumab + IFL vs. 14% with IFL (grade 4 neutropenia was 3% vs. 2%). Increased rates of severe neutropenia, febrile neutropenia, or infection with severe neutropenia (including some fatalities) have been observed in patients treated with some myelotoxic chemotherapy regimens plus bevacizumab. In a phase III in NSCLC, carboplatin and paclitaxel + bevacizumab arm was associated with increased rate of grade 4 neutropenia (27% vs. 17%), febrile neutropenia (5.4% vs. 1.8%), and infection with neutropenia (4.4% vs. 2.0%) with three fatal cases (82).

Additional Adverse Events. See the bevacizumab Investigator's Brochure for additional details regarding the safety experience with bevacizumab.

Fertility and Pregnancy. Clinical data are lacking regarding the immediate or long-term effect of bevacizumab on fertility and pregnancy. However, bevacizumab is known to be teratogenic and detrimental to fetal development in animal models. In addition, bevacizumab may alter corpus luteum development and endometrial proliferation, thereby having a negative effect on fertility. As an IgG1, it may also be secreted in human milk. Therefore, fertile men and women on bevacizumab studies must use adequate contraceptive measures and women should avoid breast feeding. The duration of such precautions after discontinuation of bevacizumab should take into consideration the half-life of the agent (average 21 days, ranging from 11 to 50 days).

Immunogenicity. As a therapeutic protein, there is a potential for immunogenicity with bevacizumab. With the currently available assay with limited sensitivity, high titer human anti-bevacizumab antibodies have not been detected in approximately 500 patients treated with bevacizumab.

2.9 Rationale

Although the NF2-related vestibular schwannomas are histologically benign, they demonstrate inexorable growth ultimately leading to bilateral hearing loss in adulthood. In addition, these tumors are in close proximity to the brain stem and are associated with multiple cranial neuropathies resulting in loss of facial muscle function, difficulty chewing or swallowing, visual impairment, and in some cases death due to brainstem compression. There is no effective medical treatment for NF2-related tumors. Given the limitations of surgery or radiation and the devastating impact of these tumors, innovative treatments are desperately needed.

Tumor angiogenesis is an important therapeutic target in patients with other brain tumors like glioblastoma, as well as other tumor types including lung, breast, and colon cancer. Vascular endothelial growth factor (VEGF) is a pleiotropic growth factor that mediates multiple functions through binding to VEGF receptors 1 and 2. An extensive literature supports a central role for VEGF in endothelial cell proliferation, survival, and migration (83). VEGF expression is up-regulated in many tumor types by environmental factors (e.g., hypoxia and low pH), genetic mutations (e.g., p53, EGFR) and indirectly by other growth factors (e.g., IL-1, and PDGF).

We have recently completed a study of 43 archival specimens of VSs. We found that VEGF was expressed in 100% of the cases examined and bound to the endothelium in 71% of NF2 VSs (84, 85). VEGFR2, the receptor commonly associated with active tumor angiogenesis, was found in only 31% of vessels which is significantly lower than in normal nerve (> 80% of the vessels) or in malignant brain tumors such as glioblastomas where all vessels express VEGFR2.

To gain further insight into the vascular abnormalities of schwannomas, we measured the density of vessels (MVD), their average size and perivascular cell coverage. Both MVD and size were increased in schwannomas while SMA-positive perivascular cells were reduced below 30%. These data conclusively demonstrate that schwannomas display an overall picture of active angiogenesis with more and larger vessels with abnormal cellular and molecular phenotype. Furthermore, they suggest that, for NF2-related tumors, neutralizing VEGF (using anti-VEGF antibodies) might be more effective than neutralizing VEGF receptors (using small molecule inhibitors of receptor tyrosine kinase).

It is widely accepted that the main effect of VEGF, secreted by tumor cells, is to stimulate angiogenesis through paracrine mechanisms. Agents that neutralize the VEGF pathway not only prevent growth of new vessels but also temporary "normalize" the abnormal, disorganized tumor vasculature into functional blood vessels thereby reducing peritumoral edema (86) . In an imaging study of 14 patients with recurrent glioblastoma (87), 7 patients experienced at least a 50% reduction in the size of the contrast-enhancing lesion with or without reduction in edema and an additional 3 patients with stable disease experienced a decrease in edema. A similar study was performed by our group in patients with recurrent glioblastoma who were treated with AZD2171, an oral pan-VEGF receptor inhibitor (34). In this study, 12 of 16 (75%) patients experienced > 25% in the size of the contrast-enhancing lesion. Furthermore, there was a significant reduction in the amount of cerebral edema associated with the tumors as determined by decrease in the ADC and FLAIR signal on MRI scans. Both the anti-tumor and anti-edema features of VEGF inhibition are likely to be of extreme importance for patients with vestibular schwannomas. Furthermore, in a series of 10 patients with bilateral VS at risk of complete hearing loss treated with bevacizumab at 5mg/kg every 2 weeks we found that the mean apparent diffusion coefficient (ADC), a measure of water motility within tissue and a radiographic marker of edema (13), decreased during treatment. A strong correlation was observed between ADC level at base line and percent change in tumor volume indicating that ADC level at base line might be a potential marker for response to anti-VEGF therapy (88).

Thus, there is a rationale for the study of anti-VEGF therapies in patients with primary brain tumors (including vestibular schwannomas and meningiomas). Given the importance of the VEGF pathway in vestibular schwannoma and meningioma and the putative role of edema in hearing loss, this is an especially attractive therapeutic target in NF2 patients.

We are thus proposing a phase II clinical trial for children $\geq$ 12 years of age and adults with NF2 to define the activity of bevacizumab in this population. Patients will

be carefully monitored for toxicity of bevacizumab and for response. Detailed monitoring for bony toxicity in children ages 12 through 17 years will be performed (Sections 2.10 and 10).

2.10 Correlative Studies Background

Preclinical studies have shown that blocking VEGF signaling pathways can inhibit the growth of tumors. For example, using DC101, a monoclonal antibody against VEGFR2, there is inhibition of glioma angiogenesis and growth (89). Additionally, targeting the VEGF pathway not only holds the promise to destroy tumor vessels, but can also potentially improve the function of tumor vessels. We, and others, have shown that anti-VEGF therapy using a tyrosine kinase inhibitor of the receptors VEGFR1, -R2, -R3 and PDGFRα and -β in recurrent gliomas induced sustained decrease in k^{trans} and ADC measured by functional MRI. All patients had significantly less edema and were able to decrease or completely stop their use of corticosteroids.

This study is designed to not only assess the benefit of anti-angiogenic therapy in NF2-related VSs, but also to understand the mechanism by which these changes occur. This goal requires novel clinical trial designs, monitoring volumetric radiographic response (29), functional parameters of vessels function (34), functional hearing (24) as well as known biomarkers of response and progression to anti-VEGF therapy (34, 35). These measures will help define the optimal duration of therapy for efficacy and possibly allow prediction of which patients are most likely to have a clinically beneficial response to VEGF blockade. Furthermore, since these patients harbor benign rather than malignant tumors, it is important to monitor closely systemic exposure to potentially toxic agents over a long period of time. This study is designed to establish the biological effect of bevacizumab in NF2-related VSs.

Hearing loss and NF2

Patients with NF2 are at high risk for complete hearing loss. Although hearing loss is related to the presence of VSs, the degree of hearing sensitivity (threshold) is only loosely correlated with tumor size. This dissociation may be due to direct compression of the auditory nerve, presence of intratumoral edema, disruption of blood flow to the cochlea, degeneration of certain cochlear structures, and distortion of auditory centers in the brainstem. There is evidence that the cochlea and the 8^{th} cranial nerve are sites of both cell degeneration and the accumulation of precipitate in cases of VS (22) . This means that sites anywhere along the auditory pathways—from the periphery to the brainstem—may contribute to the observed loss of hearing function. Thus, we will use the full range of audiologic diagnostic tools available to assess function of the pathway. Hearing will be monitored by measuring pure tone thresholds, word recognition scores, and otoacoustic emissions (OAEs) (please see section 2.3). This will allow an estimate of where bevacizumab appears to be most active in restoring hearing. In addition (at the NCI only), the effect of bevacizumab on vestibular function will be evaluated (Appendix K).

Functional MRI Imaging

The development of anti-angiogenic therapies for tumors has led to a demand for imaging-based assessment of vascular related changes in vivo. Several techniques have been developed for this purpose, and the hardware and software required for

these analyses is now widely available at academic centers. T2*-weighted MRI studies are highly reproducible across sites when similar acquisition protocols are used and have been used as a surrogate measures of biologic effect in vivo in clinical trials (30). In addition to measuring the size of tumors with post-contrast images, dynamic contrast enhanced (DCE)-MRI can be used to measure the permeability of tumor vessels. This technique has been used to measure changes in the AUC in patients with a variety of brain tumors including VSs, meningiomas, and gliomas (31). We have the required experience with these techniques to produce reproducible images that will allow the assessment of the effect of bevacizumab on the tumoral and peritumoral vasculature of VSs.

Plasma Biomarkers

Our study and others suggest that there are dose- and time-dependent increases in soluble VEGF and PlGF and decreases in soluble VEGFR following treatment with anti-angiogenic agents (34, 35, 37, 38) . Our studies have also shown that circulating biomarkers can help predict tumor progression. For example, progression of glioblastoma during treatment with AZD2171 is associated with significant increases in basic fibroblast growth factor (b-FGF) and stromal cell-derived factor 1 alpha (SDF-1α), whereas progression in hepatocellular carcinoma after sunitinib is correlated with increases in IL-6 and SDF-1α. Finally, we found that baseline sVEGFR1 concentration in plasma may predict response to bevacizumab therapy in rectal cancer patients {Duda et al., The Oncologist 2010}.

Tumor-derived angiogenic factors mobilize progenitor cells from the bone marrow and increase the survival/proliferation of populations of cells already present in the peripheral blood (e.g. circulating endothelial cells (CECs), circulating progenitor cells (CPCs) or VEGFR2+ monocytes (90). These effects are mediated through specific receptors present on these cells, such as VEGFR2 or –R1 (91), and the enumeration of CECs and CPCs in the peripheral blood has been proposed as an angiogenic marker (92). The number of CECs and CPCs, measured by flow cytometry, decreases significantly after bevacizumab therapy in rectal cancer patients, and high CEC levels post-treatment correlate with residual disease (93). In hepatocellular carcinoma patients, anti-VEGF treatment with sunitinib decreases the CPCs, and an increase in CPCs at any time point correlates with poor survival (37). Finally, in recurrent glioblastoma patients, an increase in CECs during AZD2171 treatment was seen in patients with rapid disease progression (38).

There is no data on cellular and molecular biomarkers for anti-VEGF agents in patients with NF2 and this study proposes to analyze baseline biomarkers with respect to tumor burden (volume) and outcomes (response) as well as the possible effect of bevacizumab on biomarker levels over time.

Whole Body MRI Assessment

There have been recent technological developments that allow rapid imaging of the whole body. 3D segmentation and computerized volumetry have been used to calculate the whole body tumor burden in a single imaging session in patients with NF1, NF2 and schwannomatosis (41). This study showed that the combination of

whole body MRI with tumor volumetry is feasible and reliably assesses total body tumor burden. Our anecdotal experience is that NF2 patients with progressive VS have significant benefit with bevacizumab, but it is not clear if associated peripheral tumors in NF2 have any response to bevacizumab. We will use the technique established by Cai et al. using a 1.5T MR imager and an integrated body coil to assess the whole body tumor burden in patients enrolled on the trial at baseline. The images can be acquired at all three centers (JHU, MGH, NIH) and the image processing will be performed at the JHU. In patients with at least one peripheral tumor >2cm in linear measure at baseline, we will repeat the MRI at 25 and 49 weeks.

Whole body MRI is increasing applied to evaluate patients with widely involved systemic disease more efficiently that individual segmental imaging techniques (94-96). This data will provide preliminary information about the feasibility of applying whole body MRI prospectively in the setting of a phase II trial for neurofibromatoses and the effect of bevacizumab on peripheral tumors in patients NF2 that can assist in the design of future trials assessing peripheral tumors in this population.

Quality of Hearing and Quality of Life Assessments

As discussed, there is evidence that late hearing loss can result in increased perceived disability, decreased social interactions, and increased rates of depression (51, 52). Quality of hearing and the relationship to QOL has not yet been prospectively assessed in NF2 patients. In the setting of this prospective therapeutic trial, we will use a global measure of QOL (SF-36) as well as measures that are designed to assess specific hearing function in daily activities (TRQ and SSQ) to explore these measures in patients with progressive VS at baseline and after therapy. Finally, we will include three questions about the patients' experience of being involved in this clinical trial to explore how involvement in an experimental therapeutic trial may be influencing perceived QOL. These surveys will be given to patients at baseline, 25, 49 weeks and at the off-study visit.

Evaluations for Bony Toxicity in Children

One of the systems affected by angiogenesis inhibitors is the epiphyseal growth plate. Inhibition of VEGF signaling affects bone growth by thickening the epiphyseal growth plate due to expansion of the hypertrophic zone. These effects have been attributed to delayed vascular invasion of the epiphyseal growth plate resulting in a reduced rate of hypertrophic chondrocyte apoptosis (97). The changes in growth plate thickness can be morphometrically quantified revealing a dose dependent increase in the epiphyseal area of up to 481% (98). Inhibition of VEGF also seems to impair trabecular bone formation. Mice treated with a soluble VEGF receptor chimeric protein, mFlt (1-3)-IgG showed a reduced length and number of primary trabeculae. Restoration of normal angiogenesis by discontinuation of mFlt (1-3)-IgG resulted in rapid reversal of all growth plate changes within two weeks (99). Partially reversible physeal dysplasia was observed in growing non-human primates after treatment with bevacizumab for four weeks (98).

In the preclinical studies with angiogenesis inhibitors including sorafenib and bevacizumab, effects on bone structure such as thickening of the growth plates were seen in only developing animals, including rats and dogs. Increased bone formation

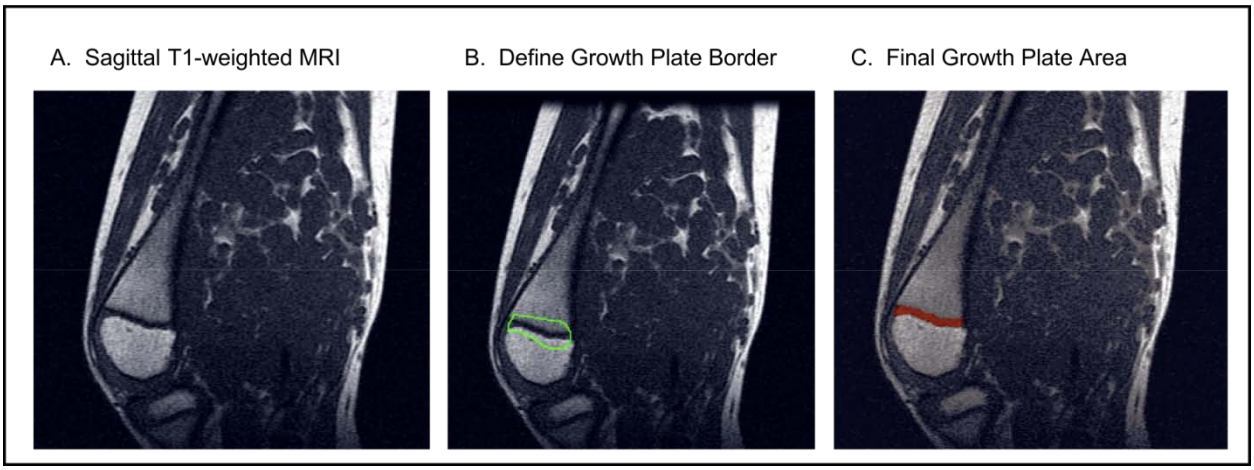
beneath the growth plate and bone malformation or epiphysiolysis was also observed at high doses in rats. These changes in the bone seemed to be reversible after 4 weeks of recovery (100). Since there is a possibility of the bone toxicity from angiogenesis inhibition from the use of bevacizumab in children, we have incorporated careful assessments of skeletal measures as safety and outcome parameters for this protocol. We plan to use the following measures to carefully assess bony changes or toxicity (timing and methods detailed in study implementation):

- Multiple measures of height and growth
- Lower extremity scanogram for femur length measurement and growth plate assessment
- Dual-energy X-ray absorptionmetry (DEXA) of lumbar spine and total body for evaluation of bone mineral density (BMD) and composition
- Serum calcium, phosphorus, bone specific alkaline phosphatase, osteocalcin, PTH, and vitamin D levels (1, 25-dihydroxy vitamin D and 25-hydroxy vitamin D) for evaluation of bone turnover and metabolism
- Knee MRI for patients with open growth plates for evaluation and measurement of the tibial and femoral growth plates

Patient	Age	Observer 1		Observer 2		% mean difference between observers
		volume (mm^3)	CV (%)	volume (mm^3)	CV (%)	
1	13	2493	0.7%	2510	2.0%	-0.71%
2	7	3103	3.0%	2894	2.7%	6.74%
3	8	4477	3.3%	4167	2.1%	6.91%
4	5	3779	6.1%	3622	4.2%	4.15%

Identical monitoring is performed on a CTEP sponsored phase I trial of sorafenib (which also inhibits angiogenesis) for children with neurofibromatosis type 1 and inoperable plexiform neurofibromas.

Growth plate analysis



A method of automated volumetric MRI analysis of growth plate volume has been developed by Jeffrey Solomon, and will be used to centrally monitor growth plate toxicity at the NCI. Standardized sagittal T1 images will be obtained, without the use

of IV contrast, as described in Appendix IV to image the growth plates of the right knee. Growth volume will be determined using the MEDx software platform. The method is based on the pixel intensities within the region of interest and performs a Bayesian probabilistic analysis to classify pixels into three different tissue classes.

The steps of volumetric analysis are outlined in the figure below. A) Sagittal T1-Weighted MRI of the knee. The growth plates appear dark in contrast to adjacent bony structures. B) Region of interest including the low signal intensity growth plate and some higher signal intensity adjacent bone is manually outlined on each MRI slice. C) The program automatically displays the class of interest (i.e. the growth plate) red on each slice and calculates the volume of all the red pixels.

This method is currently undergoing validation at the Pediatric Oncology Branch, including the determination of inter- and intra-observer comparisons, and of the coefficient of variation. Preliminary results from the application of the automated method of volumetric MRI analysis of growth plates are shown in the table below. The table describes average growth plate volumes and coefficient of variation (CV) for three different determinations on three different days by two observers. The percent mean difference between observers is calculated by [(observer 1- observer 2)/observer 1] x 100. Thus far, this method appears to have good inter and intra-observer reproducibility. We will plan on further evaluating with more patients as well as subsequent knee MRI on the same patient at later dates.

3. PATIENT SELECTION

3.1 Eligibility Criteria

1.1.1 Patients must have a confirmed diagnosis of neurofibromatosis 2 by fulfilling National Institute of Health (NIH) criteria or Manchester criteria, or by detection of a causative mutation in the NF2 gene.

The NIH criteria (82) includes presence of:

- Bilateral vestibular schwannomas, OR
- First-degree relative with NF2 and EITHER unilateral eighth nerve mass OR two of the following: neurofibroma, meningioma, glioma, schwannoma, juvenile posterior subcapsular lenticular opacity

The Manchester criteria (101) includes presence of:

- Bilateral vestibular schwannomas, OR
- First-degree relative with NF2 and EITHER unilateral eighth nerve mass OR two of the following: neurofibroma, meningioma, glioma, schwannoma, juvenile posterior subcapsular lenticular opacity, OR
- Unilateral vestibular schwannoma AND any two of: neurofibroma, meningioma, glioma, schwannoma, juvenile posterior subcapsular lenticular opacity, OR
- Multiple meningiomas (two or more) AND unilateral vestibular schwannoma OR any two of: schwannoma, glioma, neurofibroma, cataract

3.1.2 Patients must have measurable disease, defined as at least one VS $\geq$1.5 cm (on longest diameter) as measured by contrast-enhanced cranial MRI scan with fine cuts through the internal auditory canal (3 mm slices, no skip).

3.1.3 Age $\geq$12 years.

3.1.4 Life expectancy of greater than 6 months.

3.1.5 ECOG performance status (Karnofsky $\geq$60% or Lansky Score $\geq$ 60; see Appendix A).

3.1.6 Patients must have normal organ and marrow function as defined below:

• leukocytes	$\geq$3,000/mcL
• absolute neutrophil count	$\geq$1,500/mcL
• platelets	$\geq$150,000/mcL or lower limit of institutional normal
• total bilirubin	$\leq$2 X institutional upper limit of normal

- AST(SGOT)/ALT(SGPT) ≤2.5 X institutional upper limit of normal

3.1.7 Patients must have recovered from acute toxicity of prior treatment to grade 1 or less unless otherwise specified.

3.1.8 Patients must have a creatinine clearance or radioisotope GFR ≥60ml/min/1.73 m^2 or a normal serum creatinine based on age described in the table below.

Age (years)	Maximum Serum Creatinine (mg/dL)
≤5	0.8
5<age≤10	1.0
10<age≤15	1.2
>15	1.5

3.1.9 Subjects must have a VS not amenable to surgery or have refused surgery due to high risk for permanent complications related to surgery (e.g. damage to lower cranial nerve function, facial palsy, risk for cerebrospinal fluid leak, etc.) as determined by a surgeon with experience in management of NF2 associated VS.

3.1.10 Subjects must have had a discussion of all available treatment options and their risks and benefits of these options including surgery, radiation therapy, observation, other clinical trials and expressed their preference for participation in this trial in the informed consent process.

3.1.11 The effects of bevacizumab on the developing human fetus at the recommended therapeutic dose are unknown. For this reason and because anti-angiogenic agents are known to be teratogenic, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.

3.1.12 Ability to understand and the willingness give written informed consent or assent.

3.1.13 Evidence of active disease, defined as progressive hearing loss (with decrease in word recognition score) related to VS (i.e., not due to prior interventions such as surgery or radiation) documented in the preceding 24 months with a word recognition score of <90% in the target ear.

3.1.14 Proteinuria (including albuminuria) should be screened for by either urine analysis for urine protein creatinine (UPC) ratio or by urine dipstick. If the UPC ratio is greater than or equal to 0.5 or if urine dipstick shows 2+ proteinuria, 24-hour urine protein should be obtained and the level should be <1000 mg for patient enrollment.

3.2 Exclusion Criteria

3.2.1 Patients who have had chemotherapy or radiotherapy within 4 weeks (6 weeks for nitrosoureas or mitomycin C) prior to entering the study or those who have not recovered from adverse events due to agents administered more than 4 weeks earlier.

3.2.2 Patients may not be receiving any other investigational agents.

3.2.3 Patients with nervous system tumors associated with NF2 (e.g., schwannomas, meningiomas, ependymomas, or gliomas) will not be excluded from this clinical trial as long as these tumors do not require treatment with radiation, surgery, or medical treatment at the time of enrollment on trial.

3.2.4 Patients with known hypersensitivity of Chinese hamster ovary cell products, other recombinant human antibodies, or compounds of similar chemical or biologic composition to bevacizumab.

3.2.5 Inability to tolerate periodic MRI scans or gadolinium contrast without general anesthesia.

3.2.6 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.7 Clinically significant cardiovascular disease, such as

1. Inadequately controlled HTN (adult subjects: SBP > 160 mmHg and/or DBP > 90 mmHg despite antihypertensive medication, pediatric subjects: Requirement for antihypertensive treatment prior to enrollment, or diastolic blood pressure >95th percentile for age –Appendix F))History of CVA within 12 months
2. Myocardial infarction or unstable angina within 12 months
3. New York heart association grade II or greater congestive heart failure
4. Serious and inadequately controlled cardiac arrhythmia
5. Significant vascular disease (e.g. aortic aneurysm, history of aortic dissection)
6. Clinically significant peripheral vascular disease

3.2.8 Pregnant women (positive pregnancy test) are excluded from this study because bevacizumab is an anti-angiogenic agent with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential

risk for adverse events in nursing infants secondary to treatment of the mother with bevacizumab, breastfeeding should be discontinued if the mother is treated with bevacizumab. Both fertile men and women must agree to use adequate contraceptive measures during study therapy and for at least 6 months after the completion of bevacizumab therapy. Abstinence is considered an adequate contraceptive measure.

In the event that a minor (age 12-17) who undergoes a pregnancy test as part of the screening process receives a positive result, they will be excluded from the study and their parent(s) of record will be notified of this result.

3.2.9 HIV-positive patients or cancer survivors are eligible for this study if they fulfill all other eligibility criteria.

3.2.10 Inability to perform volumetric measurement of target VS (e.g., due to the MRI artifact from auditory brainstem implant or due to presence of collision tumor (two or more tumors abutting each other) in the cerebellopontine angle). Note: questions about the ability to perform volumetric analysis on a baseline MRI scan should be directed to the study radiologist, Dr. Gregory Sorensen.

3.2.11 Concurrent use of anti-coagulant drugs (not including prophylactic doses), history of coagulopathy, or evidence of bleeding diathesis or coagulopathy.

3.2.12 Imaging (CT or MRI) evidence of newly identified hemorrhage (new within the last in the 6 months prior to enrollment), any history of symptomatic intracranial hemorrhage, or any history of spontaneous intracranial hemorrhage.

3.2.13 Serious or non-healing wound, ulcer or bone fracture.

3.2.14 History of abdominal fistula, gastrointestinal perforation or intra-abdominal abscess within 6 months prior to day 1.

3.2.15 Invasive procedures defined as follows:

* Major surgical procedure, open biopsy or significant traumatic injury within 28 days prior to Day 1 therapy
* Brain biopsy within 28 days prior to day 1 of therapy (wounds must be fully healed from brain biopsies performed more than 28 days prior to day 1 of therapy)
* Anticipation of need for major surgical procedures during the course of the study
* Core biopsy within 7 days prior to D1 therapy

3.2.16 Prior treatment with bevacizumab or other VEGF targeting therapies.

3.2.17 Personal history of autoimmune coagulopathy, including idiopathic thrombocytopenia purpura (ITP).

1.3 Inclusion of Women and Minorities

Both men and women, and members of all races and ethnic groups are eligible for this trial.

4. REGISTRATION PROCEDURES

4.1 General Guidelines

Eligible patients will be registered on study centrally at the Johns Hopkins Comprehensive Neurofibromatosis Center by the Study Coordinator, Amanda Bergner. All sites should call Ms. Bergner at 410-955-2509 to verify agent availability.

Following registration, patients should begin protocol treatment within 28 days. Appropriate clinical evaluation will be done (including MRI, audiometry or other indicated studies) for any reported change in clinical status in the interval between registration and treatment. Any new or changing medical issues that would cause treatment delays should be discussed with the Principal Investigator. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. Ms. Bergner should be notified of cancellations as soon as possible.

Except in very unusual circumstances, each participating institution will order DCTD-supplied agents directly from CTEP. Agents may be ordered by a participating site only after the initial IRB approval for the site has been forwarded by the Coordinating Center to the CTEP PIO (PIO@ctep.nci.nih.gov).

4.2 Registration Process

To register a patient, the following documents should be completed by the research nurse or data manager and submitted to the Amanda Bergner by fax at 410-614-0845 or email at abergne1@jhmi.edu:

- Signed patient consent form
- HIPAA authorization form (not applicable to patients enrolled at the NCI, which is not a covered entity)
- Completed Patient Eligibility Checklist (Appendix M)

The research nurse or data manager at the participating site will then contact Amanda Bergner, the Central Study Coordinator by phone at 410-502-6732 or email at abergne1@jhmi.edu to verify eligibility. To complete the registration process, the Coordinator will:

- Assign a patient study number
- Register the patient on the study
- E-mail the patient study number to the participating site and require confirmation of receipt of this email

5. TREATMENT PLAN

5.1 Bevacizumab Administration

Treatment can be administered on an outpatient basis. Reported adverse events and potential risks are described in Sections 6 and 7. Appropriate responses to adverse events are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's tumors.

Bevacizumab is administered by IV infusion at a dose of 7.5 mg/kg every 3 weeks (see Schema page 3). One cycle lasts 6 weeks and includes two infusions of bevacizumab. The dose should be based on the patient's actual body weight. If the institutional standard is to perform weight-based dose calculations on the day of the infusion, this will be the plan for administration. Otherwise, the dose will be recalculated only if there is a weight change of >10% from baseline. The planned treatment duration will be 48 weeks.

Patients will stop bevacizumab treatment at the completion of 48 weeks, but will remain on trial for two additional observations time points (3 and 6 months off of bevacizumab). The consequence of treatment interruption/discontinuation in subjects who respond to treatment is not known. For example, discontinuation of VEGF receptor inhibitors has been associated with rebound edema in some patients with recurrent glioblastoma (19). However, there is speculation that this is related to persistent, and possibly increased, tumor proliferation despite blood brain barrier normalization (REF). In contrast, VS are non-malignant tumors and it is unknown if VEGF inhibition can result in long term tumor control. Our limited experience to date in patients with NF2 treated with bevacizumab that had to stop therapy (3 patients) is that there is not rapid progression of symptoms. Given that patients with NF2 will often require surgery for other tumor types (i.e. ependymoma, spinal schwannoma) which will necessitate stopping bevacizumab for a minimum of 2-3 months peri-operatively, and given the unknown consequences of long-term (i.e. many years) VEGF blockade, it is optimal to determine the minimal amount of VEGF inhibition required to result in clinical improvement in patients with NF2. In addition, the retrospective data that this protocol is seeking to confirm the median treatment duration was 12 months. Hence, the treatment interval for this trial is 12 months (48 weeks).

Patients will be observed for 6 months after stopping bevacizumab. If patients exhibit a decline in hearing to <85% word recognition or lower in the 6 months after stopping bevacizumab, they can be considered for application to CTEP for a compassionate use protocol. In general, this mechanism may allow use of bevacizumab via approval for compassionate use once it has been demonstrated that a patient needs immediate treatment, that no effective alternative treatment exists, and that there is data showing benefit of the agent for the tumor type. The application for special consideration for single patient treatment can be considered in select patients who had demonstrated benefit on bevacizumab and subsequent decline in the 6 months of observation after bevacizumab is stopped. This will be considered on a case by case basis at the discretion of the treating physician, the study PI and the patient (and parent/guardian).

After 18 months (12 months on treatment and 6 months off treatment), all patients will be off study. All treatment decisions at that time will be at the discretion of the treating physician and patient (and parent/guardian).

The first dose of bevacizumab should be given over 90 minutes. If well tolerated, the second dose can be given over 60 minutes. If this dose is well tolerated, then all subsequent infusions can be administered over 30 minutes. If an infusion reaction occurs, subsequent doses of bevacizumab should be administered over the shortest period that was well tolerated.

To insure complete delivery of bevacizumab, flush the IV infusion line with 0.9% sodium chloride. The following are two recommended methods for flushing the bevacizumab IV infusion line:

1. When the bevacizumab infusion is complete, add an additional 50mL of 0.9% sodium chloride for injection to the bevacizumab infusion bag. Continue the infusion until a volume equal to that of the volume contained in the tubing has been administered.

2. Replace the empty bevacizumab infusion bag with a 50mL bag of 0.9% sodium chloride for injection and infuse a volume equal to the volume contained in the tubing. Note: the flush is not included in the total recommended infusion times.

Special Precautions/Safety Issues

Prior to each treatment, the patient should be carefully assessed with special attention to blood pressure, proteinuria, bleeding and cardiovascular events, as well as symptoms or signs of bowel perforation and RPLS. Decisions for retreatment or dose modification/interruption should follow the dose modification guidelines in section 6.

Patients who have an ongoing study agent-related serious adverse event upon study completion or at discontinuation from the study will be contacted by the investigator or his/her designee periodically until the event is resolved or determined to be irreversible.

Special attention will be paid to patients 12-17 years for the following measures:

1. Confirmed >6% bone mineral density decrease relative to baseline and who have a BMD Z score <-2.5
2. Femoral growth plate expansion 2 times the volume from baseline measurements
3. Diastolic blood pressure greater than 25 mmHg above the 95th% for age and gender (Appendix F) confirmed by repeated measurements is dose limiting and will result in discontinuation of bevacizumab

It is anticipated that roughly 5 of the 14 patients enrolled on protocol will be <18 years old. If greater than 33% of the total number of enrolled patients aged 12-17 develop grade 3 or 4 toxicities of any type at any point that requiring them to stop bevacizumab, including the bone toxicity reported above, hypertension, bleeding,

thrombosis and all other potential toxicities, enrollment will be held for all additional patients <18 years old. Patients <18 years old already enrolled without grade 3 or 4 toxicity will remain on study.

Infusional reactions. Routine premedication is not required for the first dose of bevacizumab. If infusional reactions occur, acetaminophen, diphenhydramine, steroids or other medications may be given for symptom control and for premedication as needed. Anaphylactic precautions should be observed during bevacizumab administration.

Hypertension. Patients should have BP monitored prior to each infusion of bevacizumab. Patients ages 12 through 17 will also have weekly BP monitoring for the first six weeks. Hypertensive mediation should be initiated or increased for optimal BP control according to standard public heath guidelines. Specific guidelines for the management of hypertension are provided for children 12 through 17 years old enrolled on this trial, similar to other CTEP sponsored protocols (Sections 2.8, 6, 9.2, 10, and Appendix F).

Proteinuria. Proteinuria (including albuminuria) should be monitored prior to every other infusion by either urine analysis for urine protein creatinine (UPC) ratio or by urine dipstick and dose adjusted per the chart below.

Proteinuria Proteinuria will be monitored by urine analysis dipstick. If Dipstick ≥2+ proteinuria, 24-hour urine protein should be obtained	Dipstick ≥ 2+ or UPC ≥0.5	Hold bevacizumab and obtain 24 hour urine protein
	If 24-h urine protein <2g	Continue bevacizumab
	If 24-h urine protein ≥2 g	· Hold bevacizumab until 24-hour urine protein <2.0 g · Discontinue bevacizumab if urine protein does not recover to < 2.0 g after 8 weeks of bevacizumab interruption

Surgery and wound complication issues and surgery. The appropriate interval from discontinuation of bevacizumab to subsequent elective surgery required to reduce the risk of impaired wound healing has not been determined. Decision on such an interval should take into consideration the half-life of bevacizumab. It is generally recommended that bevacizumab should be discontinued at least 4-8 weeks prior to major elective surgery. In addition, bevacizumab should not be restarted until at least 4 weeks after major surgery provided that the wound has adequately healed; in cases of high-risk procedures such as liver resection, thoracotomy or neurosurgery, it is recommended that bevacizumab be resumed no earlier than 8 weeks after surgery.

Bony toxicity. Children 12 through 17 years old will be carefully monitored for the development of bony toxicity (Sections 2.8, 6, 9.2, 10, and Appendix F).

Ovarian Failure/Irregular Menstruation:
Ovarian failure, defined as amenorrhea lasting 3 or more months with follicle-stimulating hormone (FSH) elevation (≥30 mIU/mL), has recently been shown to be associated with the use of bevacizumab in patients with various solid tumor receiving bevaciumab in combination with cytotoxic chemotherapies. Specifically, the inceidence of ovarian failure was increased

in patients receiving adjuvant bevacizumab plus mFOLFOX compared to mFOLFOX alone (34% vs. 2%). After discontinuation of bevacizumab, resumption of menses and an FSH level <30 mIU/mL was demonstrated in 22% (7/32) of these women.

The CTCAE grading of irregular menstruation is grade 1: intermittent menses with skipped menses for no more than 1-3 months, grade 2: intermittent menses with skipped menses for more than 4-6 months and grade 3: peristsent ammenorrheas for more than 6 months. All patients enrolled on this study of childbearing potential are notified of the risk associated with bevaicumab and the fact that the long term effects of bevacizumab exposure on fertility are unknown. If patients develop ≥grade 2 irregular menstruation that is ≥possible in its attribution to bevacizumab, they are permitted to stay on drug despite this adverse event as long as the local investigator and study PI have confirmed with the patient the potential short and long term risks to fertility by continuing bevacizumab and that the patient wishes to stay on study drug despite these risks.

5.2 General Concomitant Medication and Supportive Care Guidelines

There is no known interaction of bevacizumab with other concomitantly administered drugs. Prophylactic low-dose (81 mg or 325 mg/day) acetylsalicylic acid and systemic anticoagulation is allowed (LMWH preferred relative to warfarin). Other medication considered necessary for the subject's safety and well being may be given at the discretion of the investigator.

5.3 Duration of Therapy

In the absence of treatment delays due to adverse event(s), treatment may continue for 8 cycles (48 weeks) or until one of the following criteria applies (whichever occurs first):

- Decline in word recognition score below the 95% critical difference interval from baseline score in the target ear (Appendix C)
- Radiographic progression of the target VS ≥20% increase in volume from baseline AND neurologically symptomatic or deemed by the treating physician or PI to be of medical risk requiring alternative therapies
- Progression of other NF2-associated tumors (contralateral VS, meningiomas, or ependymomas) that require additional or alternate therapies. Note that growth of other NF2-associated tumors that is consistent with the natural history of the disease, is not symptomatic, and does not require treatment is not a criterion for discontinuing protocol therapy
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Patient (and parent/guardian) decide to withdraw from the study
- General or specific changes in the patient's condition rendering the patient unacceptable for further treatment in the judgment of the investigator

As mentioned, there is a theoretical concern about the risk of rebound edema after discontinuation of therapy. Patients will undergo evaluations at 3 and 6 months after stopping bevacizumab. As detailed above, patients with a decline in word recognition score within 6 months after discontinuation of study drug can be considered for application for compassionate use. In addition, patients who report acute hearing loss (defined as hearing loss with onset over a period of less than 72 hours) at any time on study can be considered for a course of high dose glucocorticoids (typically prednisone 60 mg daily for 10 days followed by a taper of 10 mg/ every 3 days until off) at the discretion of the treating physician. Glucocorticoids should not be used for gradual hearing decline or as a concurrent therapy with bevacizumab.

5.4 Duration of Follow Up

Patients without progressive hearing loss or removal from study for any of the reasons listed in section 5.3 will be followed for 6 months on study after they have completed treatment. Thereafter, patients will be asked to submit standard clinical parameters including: MRI brain, audiometry reports, tumor related treatments or procedures, and adverse events at 3 month intervals for a total of one year. Hence, the total duration of follow-up will be 6 months after stopping bevacizumab with formal evaluations at 3 and 6 months and 12 months thereafter with standard clinical assessments (total of 18 months) or until removal from study or death. Patients removed from treatment for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

5.5 Criteria for Removal from Study

Patients will be removed from study when any of the criteria listed in Section 5.3 applies. The reason for study removal and the date the patient was removed must be documented in the Case Report Form. Patients who discontinue treatment with bevacizumab due to bevacizumab related toxicity will be monitored on protocol until resolution of the toxicities.

6. DOSING DELAYS/DOSE MODIFICATIONS

Note1: There will be no dose reduction for bevacizumab. Treatment should be interrupted or discontinued for certain adverse events, as described below

Note 2: <u>If bevacizumab is interrupted for ANY reasons for > 4 weeks (unless otherwise specified),</u> the patient should discontinue bevacizumab therapy on protocol.

Treatment Modification for Bevacizumab-Related Adverse Events

Event	CTCAE. v4.0 Grade	Action to be Taken

Event	CTCAE. v4.0 Grade	Action to be Taken
Allergic reactions or Infusion-related reactions Or Anaphylaxis	Grade 1-2	Infusion of bevacizumab should be interrupted for subjects who develop dyspnea or clinically significant hypotension. For infusion-associated symptoms not specified above, infusion should be slowed to 50% or less or interrupted. Upon complete resolution of the symptoms, infusion may be continued at no more than 50% of the rate prior to the reaction and increased in 50% increments every 30 minutes if well tolerated. Infusions may be restarted at the full rate during the next cycle. Subjects who experience bronchospasm (regardless of grade) should discontinue bevacizumab.
	G3-4	Discontinue bevacizumab
Thromboembolic Event (Arterial); arterial ischemia - Cardiac ischemia - Myocardia infraction - CNS ischemia (TIA, CVA) - any peripheral or visceral arterial ischemia/thrombosis	Grade 2 (new or worsening since bevacizumab	Discontinue bevacizumab.
	Grade 3-4	Discontinue bevacizumab
Thromboembolic Event Venous)	[Note: Patients with lung cancer requiring therapeutic anticoagulation should discontinue bevacizumab]	
	Grade 3 OR asymptomatic Grade 4	▪Hold bevacizumab treatment. If the planned duration of full-dose anticoagulation is <2weeks, bevacizumab should be held until the full-dose anticoagulation period is over. ▪If the planned duration of full-dose anticoagulation is >2 weeks, bevacizumab may be resumed during full-dose anticoagulation IF <u>all</u> of the criteria below are met: - The subject must not have pathological conditions that carry high risk of bleeding (e.g. tumor involving major vessels or other conditions) - The subject must not have had hemorrhagic events while on study - The subject must on stable dose of heparin or have an in-range INR (usually 2-3) on a stable dose of warfarin prior to restarting bevacizumab. ▪If thromboemboli worsen/recur upon resumption of study therapy, discontinue bevacizumab
	Grade 4 (symptomatic)	Discontinue bevacizumab
Hypertension*	[Treat with anti-hypertensive medication as needed. The goal of BP control should be consistent with general medical practice, including confirming that BP is elevated across three separate measurements on three separate days and all other contributing factors have been addressed (i.e. pain, anxiety).]]	
	Grade 1 (SBP 120-139 mmHg or DBP80-89 mm Hg)	Consider increased BP monitoring; start anti-hypertensive medication if appropriate
	Grade 2 asymptomatic (SBP 140-159 mmHg or DBP 90-99 mm Hg)	Begin anti-hypertensive therapy and continue bevacizumab

Event	CTCAE. v4.0 Grade	Action to be Taken
	· Grade 2 symptomatic (SBP 140-160 mmHg or DBP 90-100 mm Hg) - Grade 3 (≥ SBP 160 mmHg or ≥ DBP 100 mmHg	· Start or adjust anti-hypertensive medication · Hold bevacizumab until symptoms resolve **AND** BP < 160/90mmHg*
	Grade 4	Discontinue bevacizumab.
Heart Failure or LV dysfunction	Grade 3	Discontinue bevacizumab
	Grade 4	Discontinue bevacizumab
Proteinuria	[Proteinuria should be monitored by urine analysis for urine protein creatinine (UPC) ratio, or dipstick prior to every other dose of bevacizumab. If dipstick shows 2+ proteinuria, 24-hour urine protein should be obtained]	
	UPC ratio < 3.5 or 24-h urine protein < 3.5 gm	Continue bevacizumab.
	UPC ratio ≥ 3.5 or 24-h urine protein ≥ 3.5 gm	Hold bevacizumab until it UPC recovers to < 3.5, or 24-h urine protein < 3.5 gm. Discontinue bevacizumab if urine protein does not recover to < 3.5 after 8 weeks or bevacizumab interruption
	Nephrotic syndrome	Discontinue bevacizumab.
Hemorrhage (intracranial or pulmonary)	Grade 2-4	· Discontinue bevacizumab
	Grade 1	· Patients receiving full-dose anticoagulation should discontinue bevacizumab. · For patients not on full-dose anticoagulation, hold bevacizumab until ALL of the following criteria are met: - the bleeding has resolved and Hb is stable - there is no bleeding diathesis that would increase the risk of therapy · there is no anatomic or pathologic condition that could increase the risk of hemorrhage recurrence
Hemorrhage (any other organ systems)	Grade 3	· Patients receiving full-dose anticoagulation should discontinue bevacizumab. · For patients not on full-dose anticoagulation, hold bevacizumab until ALL of the following criteria are met: - the bleeding has resolved and Hb is stable - there is no bleeding diathesis that would increase the risk of therapy - there is no anatomic or pathologic condition that could increase the risk of hemorrhage recurrence. · Patients who experience recurrence of grade 3 hemorrhage should discontinue study therapy.
	Grade 4	Discontinue bevacizumab
Platelet count decreased	Grade 1-2 Less than lower limit of institutional normal down to 50,000	· Hold bevacizumab until resolution of platelet count to greater than or equal to lower limit of institutional normal · Monitor for cleeding episodes · Weekly platelet evaluation until platelet count is greater than 100,000

Event	CTCAE. v4.0 Grade	Action to be Taken
	G3-4 Less than 50,000	· Discontinue bevacizumabif low platelet count is attributed as possibly, probably, or definitely related to study drug · Discontinue bevacizumab until recovery to greater than or equal to institutional normal if attributed as not related or unlikely to be related
RPLS (Reversible Posterior Leukoencephalopathy syndrome or PRES (Posterior Reversible Encephalopathy Syndrome)		·Discontinue bevacizumab upon diagnosis of RPLS.
Wound dehiscence requiring medical or surgical intervention		·Discontinue bevacizumab
Perforation (GI, or any other organ)		Discontinue bevacizumab
Fistula (GI, pulmonary or any other organ)		Discontinue bevacizumab
Obstruction of GI tract	G2 requiring medical intervention	· Hold bevacizumab until complete resolution
	G3-4	· Hold bevacizumab until complete resolution · If surgery is required, patient may restart bevacizumab after full recovery from surgery, and at investigator's discretion
Other Unspecified bevacizumab-related AEs (except controlled nausea/vomiting).	Grade 3+	· Hold bevacizumab until symptoms resolve to ≤ grade 1
	Grade 4	· Discontinue bevacizumab · Upon consultation with the study chair, resumption of bevacizumab may be considered if a patient is benefiting from therapy, and the G4 toxicity is transient, has recovered to ≤ grade 1 and unlikely to recur with retreatment.

+ Patients of childbearing age whom develop grade ≥2 toxicity based on CTCEA for irregular menstruation attributed to bevacizumab as >possible can remain on treatment with consent of the patient regarding long term risk for fertility and under the discretion of the study PI.

*Specific guidelines for the management of hypertension are provided for children 12 through 17 years (Appendix F). If more specific guidelines for hypertension for adults are preferred by the investigators or required for certain protocols, the following guidelines can be used

Hypertension in adults*	[Treat with anti-hypertensive medication as needed. The goal of BP control should be consistent with general medical practice, including confirming that BP is elevated across three separate measurements on three separate days and all other contributing factors have been addressed (i.e. pain, anxiety).]	
	Grade 1	Consider increased BP monitoring
	Grade 2 asymptomatic but diastolic BP < 100 mmHg	Begin anti-hypertensive therapy and continue bevacizumab
	-Grade 2-3 Symptomatic OR -Diastolic BP > 100 mmHg	Hold bevacizumab should until symptoms resolve AND BP < 160/90mmHg*

	Grade 4	· Discontinue bevacizumab permanently

*Current CTCAE definitions used by CTEP:

- Grade 1: asymptomatic, transient (< 24 hours) increase by > 20 mmHg (diastolic) or to >150/100 if previously WNL; intervention not indicated
- Grade 2: recurrent or persistent (> 24 hours) or symptomatic increase by > 20 mmHg (diastolic) or to > 150/100 if previously WNL; monotherapy may be indicated
- Grade 3: requiring more than one drug or more intensive therapy than previously
- Grade 4: life threatening (e.g. hypertensive crisis)

Dose-limiting hypertension in children 12 through 17 years:

Any patient with a diastolic blood pressure greater than 25 mmHg above the 95th% for age and gender (Appendix F) confirmed by repeated measurements is dose limiting and will result in discontinuation of bevacizumab (see Appendix F for assessment of blood pressure recordings and for management).

In patients on antihypertensive therapy, a diastolic blood pressure ≥25 mmHg above the 95th % for age and gender for >14 days is dose-limiting and will result in discontinuation of bevacizumab (see Appendix F).

Dose limiting bony changes in children 12 through 17 years

Growth plate abnormalities

1D measurement through mid growth plate on sagittal view and volumetric measurements via an automated image analysis adapted from the MEDx software program used for volumetric analysis of PN (section 2.8 and Appendix F) will be used to measure growth plate changes. Growth plate expansion greater than 2 times the volume from baseline to interval measurement will be considered dose limiting and result in discontinuation of bevacizumab. Volumetric analysis will be done centrally at NCI.

Bone Density abnormalities

Patients with a >6% bone mineral density (BMD) decrease on lumbar spine DEXA scan relative from baseline to restaging on therapy AND a BMD Z score at the lumbar spine of <-2.5 will be considered dose limiting and result in discontinuation of bevacizumab (see Appendix F for treatment modification for abnormal DEXA lumbar spine scan). These parameters are based on experience gained from a previous study of tenofovir and impact on bone mineral density (BMD) in HIV-infected children (102, 103) .

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited (via AdEERS) reporting in addition to routine reporting.

7.1 Comprehensive Adverse Events and Potential Risks List (CAEPR)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported

and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with ***bold*** and ***italicized*** text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI via AdEERS (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification.

NOTE: Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

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Adverse Events with Possible Relationship to Bevacizumab (rhuMAb VEGF) (CTCAE 4.0 Term)			**Specific Protocol Exceptions to Expedited Reporting (SPEER)** (formerly known as ASAEL)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia		***Anemia (Gr. 3)***
		Blood and lymphatic system disorders - Other (renal thrombotic microangiopathy)	
	Febrile neutropenia		***Febrile neutropenia (Gr. 3)***
CARDIAC DISORDERS			
		Acute coronary syndrome	
		Heart failure	
		Left ventricular systolic dysfunction	
		Myocardial infarction	
	Supraventricular tachycardia		***Supraventricular tachycardia (Gr. 3)***
		Ventricular arrhythmia	
		Ventricular fibrillation	
EAR AND LABYRINTH DISORDERS			
	Vertigo		
GASTROINTESTINAL DISORDERS			
	Abdominal pain		***Abdominal pain (Gr. 3)***
	Colitis		***Colitis (Gr. 3)***
	Constipation		***Constipation (Gr. 3)***
	Diarrhea		***Diarrhea (Gr. 3)***
	Dyspepsia		***Dyspepsia (Gr. 2)***
		Gastrointestinal fistula[2]	
	Gastrointestinal hemorrhage[3]		***Gastrointestinal hemorrhage[3] (Gr. 2)***
	Gastrointestinal obstruction[4]		
		Gastrointestinal perforation[5]	
		Gastrointestinal ulcer[6]	
	Ileus		
	Mucositis oral		***Mucositis oral (Gr. 3)***
	Nausea		***Nausea (Gr. 3)***
	Vomiting		***Vomiting (Gr. 3)***

GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Fatigue		***Fatigue (Gr. 3)***
	Infusion related reaction		***Infusion related reaction (Gr. 2)***
	Non-cardiac chest pain		***Non-cardiac chest pain (Gr. 3)***
	Pain		***Pain (Gr. 3)***
IMMUNE SYSTEM DISORDERS			
	Allergic reaction		***Allergic reaction (Gr. 2)***
		Anaphylaxis	
INFECTIONS AND INFESTATIONS			
	Infection[7]		***Infection[7] (Gr. 3)***
	Infections and infestations - Other (peri-rectal abscess)		
INJURY, POISONING AND PROCEDURAL COMPLICATIONS			
		Gastrointestinal anastomotic leak	
	Wound dehiscence		***Wound dehiscence (Gr. 2)***
INVESTIGATIONS			
	Alanine aminotransferase increased		***Alanine aminotransferase increased (Gr. 3)***
	Alkaline phosphatase increased		***Alkaline phosphatase increased (Gr. 3)***
	Aspartate aminotransferase increased		***Aspartate aminotransferase increased (Gr. 3)***
	Blood bilirubin increased		***Blood bilirubin increased (Gr. 2)***
	Cardiac troponin I increased		
	Neutrophil count decreased		***Neutrophil count decreased (Gr. 3)***
	Weight loss		***Weight loss (Gr. 3)***
	White blood cell decreased		***White blood cell decreased (Gr. 3)***
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		***Anorexia (Gr. 3)***
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		***Arthralgia (Gr. 3)***
	Musculoskeletal and connective tissue disorder - Other (bone metaphyseal dysplasia)[8]		
	Myalgia		***Myalgia (Gr. 3)***
	Osteonecrosis of jaw[9]		
NERVOUS SYSTEM DISORDERS			
	Dizziness		***Dizziness (Gr. 2)***
	Headache		***Headache (Gr. 3)***
		Intracranial hemorrhage	
		Ischemia cerebrovascular	
	Peripheral sensory neuropathy[10]		
		Reversible posterior leukoencephalopathy syndrome	
	Syncope		
RENAL AND URINARY DISORDERS			
		Acute kidney injury	
	Hematuria		***Hematuria (Gr. 3)***
	Proteinuria		***Proteinuria (Gr. 2)***
		Renal and urinary disorders - Other (Nephrotic Syndrome)	
		Urinary fistula	
REPRODUCTIVE SYSTEM AND BREAST DISORDERS			

Reproductive system and breast disorders - Other (ovarian failure)[11]			
		Vaginal fistula	
	Vaginal hemorrhage		***Vaginal hemorrhage (Gr. 3)***
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Allergic rhinitis		***Allergic rhinitis (Gr. 3)***
		Bronchopleural fistula	
		Bronchopulmonary hemorrhage	
	Cough		***Cough (Gr. 3)***
	Dyspnea		***Dyspnea (Gr. 2)***
	Epistaxis		***Epistaxis (Gr. 3)***
	Hoarseness		***Hoarseness (Gr. 3)***
		Respiratory, thoracic and mediastinal disorders - Other (nasal-septal perforation)	
		Respiratory, thoracic and mediastinal disorders - Other (tracheo-esophageal fistula)	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Pruritus		***Pruritus (Gr. 2)***
	Rash maculo-papular		***Rash maculo-papular (Gr. 2)***
	Urticaria		***Urticaria (Gr. 2)***
VASCULAR DISORDERS			
Hypertension			***Hypertension (Gr. 3)***
	Thromboembolic event		***Thromboembolic event (Gr. 3)***
		Vascular disorders - Other (arterial thromboembolic event)[12]	

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

[2]Gastrointestinal fistula may include: Anal fistula, Colonic fistula, Duodenal fistula, Esophageal fistula, Gastric fistula, Gastrointestinal fistula, Rectal fistula, and other sites under the GASTROINTESTINAL DISORDERS SOC.

[3]Gastrointestinal hemorrhage may include: Colonic hemorrhage, Duodenal hemorrhage, Esophageal hemorrhage, Esophageal varices hemorrhage, Gastric hemorrhage, Hemorrhoidal hemorrhage, Intra-abdominal hemorrhage, Oral hemorrhage, Rectal hemorrhage, and other sites under the GASTROINTESTINAL DISORDERS SOC.

[4]Gastrointestinal obstruction may include: Colonic obstruction, Duodenal obstruction, Esophageal obstruction, Ileal obstruction, Jejunal obstruction, Rectal obstruction, Small intestinal obstruction, and other sites under the GASTROINTESTINAL DISORDERS SOC.

[5]Gastrointestinal perforation may include: Colonic perforation, Duodenal perforation, Esophageal perforation, Gastric perforation, Jejunal perforation, Rectal perforation, Small intestinal perforation, and other sites under the GASTROINTESTINAL DISORDERS SOC.

[6]Gastrointestinal ulcer may include: Duodenal ulcer, Esophageal ulcer, Gastric ulcer, and other sites under the GASTROINTESTINAL DISORDERS SOC.

[7]Infection may include any of the 75 infection sites under the INFECTIONS AND INFESTATIONS SOC.

[8]Metaphyseal dysplasia was observed in young patients who still have active epiphyseal growth plates.

[9]Cases of osteonecrosis of the jaw (ONJ) have been reported in cancer patients in association with bevacizumab treatment, the majority of whom had received prior or concomitant treatment with i.v. bisphosphonates.

[10]Increased rate of peripheral sensory neuropathy has been observed in trials combining bevacizumab and chemotherapy compared to chemotherapy alone.

[11]Ovarian failure, defined as amenorrhea lasting 3 or more months with follicle-stimulating hormone (FSH) elevation (≥30 mIU/mL), was increased in patients receiving adjuvant bevacizumab plus mFOLFOX compared to mFOLFOX alone (34% vs. 2%). After discontinuation of bevacizumab, resumption of menses and an FSH level <30 mIU/mL was demonstrated in 22% (7/32) of these women. Long term effects of bevacizumab exposure on fertility are unknown.

[12]Arterial thromboembolic event includes visceral arterial ischemia, peripheral arterial ischemia, heart attack, and stroke.

Also reported on Bevacizumab (rhuMAb VEGF) trials but with the relationship to Bevacizumab (rhuMAb VEGF) still undetermined:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Blood and lymphatic system disorders - Other (idiopathic thrombocytopenia purpura); Disseminated intravascular coagulation
CARDIAC DISORDERS - Pericardial effusion
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Gait disturbance; Sudden death NOS
HEPATOBILIARY DISORDERS - Hepatic failure
INFECTIONS AND INFESTATIONS - Infections and infestations - Other (aseptic meningitis)
INVESTIGATIONS - Platelet count decreased
METABOLISM AND NUTRITION DISORDERS - Hyponatremia
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Musculoskeletal and connective tissue disorder - Other (aseptic necrotic bone); Musculoskeletal and connective tissue disorder - Other (myasthenia gravis)
NERVOUS SYSTEM DISORDERS - Dysgeusia; Peripheral motor neuropathy; Seizure
PSYCHIATRIC DISORDERS - Confusion
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Adult respiratory distress syndrome; Pneumonitis; Pneumothorax; Pulmonary hypertension
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Palmar-plantar erythrodysesthesia syndrome; Skin ulceration
Note: Bevacizumab (rhuMAb VEGF) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

7.2 Adverse Event Characteristics

CTCAE term (AE description) and grade: The CTEP Active Version of the NCI Common Terminology Criteria for Adverse Events (CTCAE) will be utilized for AE reporting. The CTEP Active Version of the CTCAE is identified and located on the CTEP website at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm. All appropriate treatment areas should have access to a copy of the CTEP Active Version of CTCAE.

"Expectedness": AEs can be 'Unexpected' or 'Expected' (see Section 7.1 above) for expedited reporting purposes only. 'Expected' AEs (the ASAEL) are bold and italicized in the CAEPR (Section 7.1.1).

Attribution of the AE:

- Definite – The AE is clearly related to the study treatment.
- Probable – The AE is likely related to the study treatment.
- Possible – The AE may be related to the study treatment.
- Unlikely – The AE is doubtfully related to the study treatment.
- Unrelated – The AE is clearly NOT related to the study treatment.

7.3 Expedited Adverse Event Reporting

7.3.1 Expedited AE reporting for this study must use AdEERS (Adverse Event Expedited Reporting System), accessed via the CTEP home page (http://ctep.cancer.gov). The reporting procedures to be followed are presented in the "CTEP, NCI Guidelines: Adverse Event Reporting Requirements" which can be downloaded from the CTEP home page (http://ctep.cancer.gov). These requirements are briefly outlined in the table below (Section 7.3.3).

In the rare occurrence when Internet connectivity is lost, an AE report may be submitted using CTEP's Adverse Event Expedited Report-Single Agent or Multiple Agent paper template (available at http://ctep.cancer.gov) and faxed to 301-230-0159. A 24-hour notification is to be made to CTEP by telephone at 301-897-7497, only when Internet connectivity is disrupted. Once Internet connectivity is restored, an AE report submitted on a paper template or a 24-hour notification phoned in must be entered electronically into AdEERS by the original submitter at the site.

7.3.2 AdEERS is programmed for automatic electronic distribution of reports to the following individuals: Study Coordinator of the Lead Organization, Principal Investigator, and the local treating physician. AdEERS provides a copy feature for other e-mail recipients.

7.3.3 AdEERS Reporting Requirements for Adverse Events that occur within 30 Days[1] of the Last Dose of the Investigational Agent on Phase 2 and 3 Trials.

Phase 2 and 3 Trials									
	Grade 1	Grade 2	Grade 2	Grade 3		Grade 3		Grades 4 & 5[2]	Grades 4 & 5[2]
	Unexpected and Expected	Unexpected	Expected	Unexpected		Expected		Unexpected	Expected
				with Hospitalization	without Hospitalization	with Hospitalization	without Hospitalization		

Unrelated Unlikely	Not Required	Not Required	Not Required	10 Calendar Days	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days
Possible Probable Definite	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days	10 Calendar Days

[1] Adverse events with attribution of possible, probable, or definite that occur greater than 30 days after the last dose of treatment with an agent under a CTEP IND require reporting as follows:

AdEERS 24-hour notification followed by complete report within 5 calendar days for:

- Grade 4 and Grade 5 unexpected events

AdEERS 10 calendar day report:

- Grade 3 unexpected events with hospitalization or prolongation of hospitalization
- Grade 5 expected events

[2] Although an AdEERS 24-hour notification is not required for death clearly related to progressive disease, a full report is required as outlined in the table.

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Note: All deaths on study require both routine and expedited reporting regardless of causality. Attribution to treatment or other cause must be provided.

- Expedited AE reporting timelines defined:
  - "24 hours; 5 calendar days" – The investigator must initially report the AE via AdEERS within 24 hours of learning of the event followed by a complete AdEERS report within 5 calendar days of the initial 24-hour report.
  - "10 calendar days" - A complete AdEERS report on the AE must be submitted within 10 calendar days of the investigator learning of the event.

- Any medical event equivalent to CTCAE grade 3, 4, or 5 that precipitates hospitalization (or prolongation of existing hospitalization) must be reported regardless of attribution and designation as expected or unexpected with the exception of any events identified as protocol-specific expedited adverse event reporting exclusions.

- Any event that results in persistent or significant disabilities/incapacities, congenital anomalies, or birth defects must be reported via AdEERS if the event occurs following treatment with an agent under a CTEP IND.

- Use the NCI protocol number and the protocol-specific patient ID assigned during trial registration on all reports.

7.3.4 Protocol-Specific Expedited Adverse Event Reporting Exclusions

- For this protocol only, certain AEs/grades are exceptions to the Expedited Reporting Guidelines and do not require expedited reporting (i.e., AdEERS). The following AEs must be reported through the routine reporting mechanism (Section 7.4):

CTCAE Category	Adverse Event	Grade	Hospitalization/ Prolongation of Hospitalization	Attribution	Comments
Vascular	Venous thromboembolism	3-4	Regardless		
Blood/ bone marrow	Neutropenia/febrile neutropenia	3-4	Regardless		
Gastrointestinal	Diarrhea, Nausea, Vomiting	3-4	Regardless		
Reproductive system and breast disorders	Irregular menstruation	3	Regardless		

7.4 Routine Adverse Event Reporting

All Adverse Events must be reported in routine study data submissions. AEs reported through AdEERS must also be reported in routine study data submissions.

7.5 Secondary AML/MDS

Investigators are required to report cases of secondary AML/MDS occurring on or following treatment on NCI-sponsored chemotherapy protocols using the NCI/CTEP Secondary AML/MDS Report Form. This form can be downloaded from the CTEP web site (http://ctep.cancer.gov). Refer to the "CTEP, NCI Guidelines: Adverse Event Reporting Requirements" (available at http://ctep.cancer.gov) for additional information about secondary AML/MDS reporting.

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with bevacizumab can be found in Section 7.1.

8.1 Bevacizumab (NSC #704865)

Other Names. rhuMAb VEGF, Avastin®

Classification. Recombinant humanized monoclonal antibody

Molecular Weight. Approximate molecular weight is 149,000 daltons

Mode of Action. Bevacizumab blocks the binding of vascular endothelial growth factor (VEGF) to its receptors resulting in inhibition of angiogenesis.

Description. Bevacizumab is a recombinant humanized anti-VEGF monoclonal antibody consisting of 93% human and 7% murine amino acid sequences. The agent is composed of human IgG framework and murine antigen-binding complementarity-

determining regions

How Supplied. Bevacizumab is supplied as a clear to slightly opalescent, sterile liquid for parenteral administration. Each 400 mg (25mg/ml – 16 mL fill) glass vial contains bevacizumab with phosphate, trehalose, polysorbate 20, and Sterile Water for Injection, USP.

Preparation. Vials contain no preservatives and are intended for single use only. Place the calculated dose in 100 mL of 0.9% sodium chloride for injection.

Storage. Upon receipt, refrigerate bevacizumab (2º to 8 º C). Do not freeze. Do not shake.

Stability. Shelf-life studies of rhuMAb VEGF are ongoing. The sterile single use vials contain no antibacterial preservatives. Discard vials 8 hours after initial entry. Once diluted in 0.9% sodium chloride, administer solutions of bevacizumab within 8 hours.

Route of Administration. Intravenous

Method of Administration. Please see section 5.1.

8.2 Availability

Bevacizumab is an investigational agent supplied to investigators by the Division of Cancer Treatment and Diagnosis (DCTD), NCI. Bevacizumab is provided to the NCI under a Collaborative Agreement between Genentech and the DCTD, NCI (see Section 12.3).

8.3 Agent Ordering

NCI supplied agents may be requested by the Principal Investigator (or their authorized designee) at each participating institution. Pharmaceutical Management Branch (PMB) policy requires that agent be shipped directly to the institution where the patient is to be treated. PMB does not permit the transfer of agents between institutions (unless prior approval from PMB is obtained). The CTEP assigned protocol number must be used for ordering all CTEP supplied investigational agents. The responsible investigator at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA form 1572 (Statement of Investigator), Curriculum Vitae, Supplemental Investigator Data Form (IDF), and Financial Disclosure Form (FDF). If there are several participating investigators at one institution, CTEP supplied investigational agents for the study should be ordered under the name of one lead investigator at that institution.

Agent may be requested by completing a Clinical Drug Request (NIH-986) and mailing it to the Pharmaceutical Management Branch, DCTD, NCI, 9000 Rockville Pike, EPN Room 7149, Bethesda, MD 20892-7422 or faxing it to (301) 480-4612. For questions call (301) 496-5725.

8.4 Agent Accountability

Agent Inventory Records

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all agents received from DCTD using the NCI Drug Accountability Record Form (DARF). (See the CTEP home page at http://ctep.cancer.gov for the Procedures for Drug Accountability and Storage and to obtain a copy of the DARF and Clinical Drug Request form.)

9. CORRELATIVE/SPECIAL STUDIES

9.1 Laboratory Correlative Studies

9.1.1 Markers of Angiogenesis and Tumor Growth

Recent development in understanding the molecular basis of cancer has dramatically advanced the field in cancer drug discovery and development. Now it is clear that the translation of molecularly targeted cancer therapy into useful and practical therapeutic approaches is highly complex. The discovery of mechanism-based biomarkers can facilitate the efficient development of new anti-cancer medicines and potentially serve as true intermediate or surrogate end point biomarkers for future clinical trials. Biomarkers can also help guiding rational selection of therapeutic agents for combination therapy. Two recent studies show that collagen IV structure was modified after VEGFR2 blockade. These data together with other data on different biomarkers indicate that it is important to include measurement on the serum levels of biomarkers in future clinical practices.

To measure numerous biomarkers simultaneously with exceptional sensitivity but only small amount of plasma proteins, the MSD (Meso Scale Discovery, Maryland) platform will be used. MSD technology utilizes electrochemiluminescence detection to detect binding events on patterned arrays. By customizing the Multi-Array, multiple serum molecules can be tested. We will collect blood samples from the patients before and during the course of treatment (see study table). Analysis will be performed for VEGF-A, VEGF-C, sVEGFR1, sVEGFR2, sVEGFR3, Col IV, SDF1a, IL-1beta, IL-6, IL-8, TNFalpha, G-CSF, Ang1, Ang2, sTie2, s-cKIT, MMP-1, MMP-2, MMP-3, MMP-9, MMP-10, PlGF, and bFGF using the Meso-Scale Discovery multiplex array reader and custom multiplex plates and R&D Systems ELISA kits for collagen IV and SDF1α. This assay provides a sensitivity of 0.001 ng/mL and low variability. The level of these biomarkers during treatment with bevacizumab will be compared to the baseline level.

9.1.2 Circulating endothelial cells

Targeting angiogenic vessels requires adequate methods for the assessment of the biologic effect of various new drugs developed to control cancer progression. Tumor angiogenesis is evaluated mainly by measuring microvessel density (MVD) in biopsy specimens using immunohistochemistry. Predicting and/or assessing accurately the efficacy of anti-angiogenic therapies by this method is hampered by the heterogeneity of tumors, and by the difficulty to obtain specimens at multiple time points during treatments. On the other hand, the

number of circulating endothelial cells (CECs) – measured by flow cytometry – is significantly increased in the peripheral blood of untreated lymphoma and breast cancer patients (104). Furthermore it has been shown that in lymphoma patients achieving complete remission after chemotherapy, the number of CECs was reduced to the values observed in healthy controls, and activated CECs were found to decrease in breast cancer patients evaluated before and after quadrantectomy.

We have recently shown in humans that this method may be used for evaluation of the early response to VEGF blockade in patients with rectal carcinoma (35). Specifically, a decrease in both CD31+CD45 and CPC number was noted 3 days after administration of the VEGF-specific antibody, bevacizumab ($p<0.05$) but not on day 12 when given alone, and not when combined with chemoradiation in rectal carcinoma patients. CEC, CPC and VEGFR2+ monocyte kinetics have been shown to depend on the type of anti-angiogenic agents and their biomarker value to be differential (38).

There have not been studies of circulating cell biomarkers in patients with neurofibromatosis and our study will provide the first data in this regard. The objective of this analysis in the present trial is to assess the kinetics of circulating endothelial cells (CECs) and progenitor cells prior to and during anti-angiogenic therapy with bevacizumab.

CTC detection/characterization: Blood circulating cells are phenotyped and enumerated by flow cytometric analyses of CD31, CD34, CD45, and CD133 expression using fluorescence-labeled monoclonal antibodies and a standard protocol in fresh sample. Fluorescence-labeled isotype-matched nonspecific immunoglobulin G (IgG) antibodies are used as controls. Flow cytometry is performed on FACSVantage instruments (Becton Dickinson, San Jose, CA), as described.

9.1.3 Collection of Specimen(s)

Sample Collection Time Points

Blood samples will be obtained for protein analysis of potential biomarkers for anti-angiogenic therapy 3 hours prior to bevacizumab infusion (i.e., when baseline labs are drawn) at the following time points:

- Day 1 prior to initiating therapy
- Weeks 25 and 49
- At the off study timepoint

Blood collection (needed for each time point sample)

- Collect 30 ml of blood in 3 polypropylene tubes (10 mL in each tube) containing the anticoagulant EDTA. Tubes should be pre-cooled in an ice bath.
  - SARSTEDT Monovette® EDTA KE (9 ml), Part # 02.1333.001 or

- Becton-Dickinson Vacutainer™ K2E (10 ml), Part # 367525
  or
- Greiner Bio-One Vacuette® K3E EDTA K3 (9 ml), Part 455036

- Blood tubes must be gently inverted several times after collection to ensure thorough mixing of EDTA with the sample to prevent clotting.
- Cool all tubes in an ice bath immediately after collection.
- Glass tubes MUST NOT be used as they may break during transport and freeze-thaw cycles.
  Heparin must not be used as an anticoagulant as it may interfere with downstream genotyping methodology.

9.1.4 Handling of Specimens(s)

9.1.4.1 Angiogenesis and Tumor Growth

Centrifuge two of the three tubes collected at 700 G for 20 minutes at 4C° with no breaks within 30 minutes of collection.

Prepare two red labels (for plasma) each printed with Study-No., patient ID, initials and day/time of sample collection (24-hour clock format, i.e., 6:30 pm = 18:30). Alternatively, red screw caps can be used to color code the vials. A label example is provided below:

Study-No.:	Investigator:
Patient-ID:	Patient Initials:
Date of sampling: (mm/dd/yy)	Time of Sampling: (hh:mm) (24-hr format)
Sample Type: (Serum or Plasma)	

- Plasma is pipetted in 1 ml aliquots into two red-labeled Nalgene cryovials.
- Clearly label tubes as “plasma” and store at -80°C.
- When samples from all time-points have been collected (after the patient goes off trial) the plasma samples should be shipped to the Steele Laboratory at Massachusetts General Hospital on DRY ICE in a Styrofoam box (Thermo Safe shippers, Fisher Scientific, cat# :11-676-14; 12/case; $122.30). If a deep freezer is not available on site, the plasma sample should be kept and shipped on dry ice on the same day.

1.1.1.2 Handling of Specimen(s) for Circulating Endothelial Cells

The remaining tube of blood should be wrapped in bubble wrap (from Staples or any other supply store) twice, secured with tape, and placed ON TOP of 2 frozen COLD PACKS (ThermoSafe Polar packs bricks, from Fisher Scientific, cat# 03-530-010, 72 for $46.25) in a Styrofoam

box (Thermo Safe shippers, Fisher Scientific). This sample will be shipped on the same day that it is collected. DO NOT STORE.

9.1.5 Shipping and Analysis of Specimen(s)

Ship all specimens to the following address. Be aware that packaging specifics are different for each test; be certain to package blood and plasma samples correctly when shipping:

Ms. Sylvie Roberge or Christina Koppel
100 Blossom St.
MGH, Cox-734
Boston, MA 02114
Tel. (617) 724-1353
Fax (617) 724-5841
Pager 14082 (617-726-2000)

All analysis will be performed within the Steele Laboratory under the direction of Dr. Dan Duda. The Steele Laboratory has 4 years experience in analyzing and understanding variation in circulating angiogenic markers in various clinical trials (34-36).

9.2 Special Studies

Studies to Evaluate Children (12 through 17 years) for Hypertension and Bony Toxicity

Physical examination including documentation of blood pressure, height, and weight (Section 10).

Blood pressure. Blood pressure will be recorded as the average of 2 measurements separated by at least 2 minutes. If the second value is more than 5 mmHg different from the first, continued measurements should be made every 2 minutes until a stable value is attained. The recorded value should be the average of the last two measurements obtained. Blood pressure is to be measured preferably in the right arm with an appropriate sized cuff, taken in a seated position after 3 minutes of rest. Oscillometric blood pressure measurements that exceed the 95^{th} percentile should be confirmed by auscultation (Appendix F).

Height. The patients should take off shoes and socks and heels should be placed against the wall with ankles together. Height should be measured in a standing position with a stadiometer. Two additional repeat measurements must be made with the patient stepping off the stadiometer in between each measurement. Height measurements should be taken at approximately the same time of day for each visit. The average of the 3 measurements must be plotted on a standardized growth chart.

Evaluation for bone toxicity.

- Growth measurements: 1) Height (as described above)

- Lower extremity scanogram (bilateral hip and lower extremity plain x-rays for leg length discrepancy, femur length measurement, and growth plate assessment).
- Unilateral Knee MRI (Appendix F): The knee MRI will only be required for patients with open growth plates on scanogram. The imaging protocol is outlined in Appendix F. No IV contrast will be used for the knee MRI studies. The knee MRI will be done at the same time of the radiographic evaluation for disease and will add less than 0.5 hour of scan time.
- Serum calcium, phosphorus, bone specific alkaline phosphatase, osteocalcin, PTH, and vitamin D levels (1, 25-dihydroxy and 25-hydroxy).
- Dual-energy X-ray absorptionmetry (DEXA) for bone mineral density quantification of lumbar spine and total body.

Blood pressure, height, and evaluations for bony toxicity will be performed prior to treatment with bevacizumab, and during treatment as described in Section 10 (Study Calendar).

10. STUDY CALENDAR Pre-study evaluations are to be conducted within 28 days prior to administration of protocol therapy.

	Pre-Study	Wk 1	Wk 4	Wk 7	Wk 10	Wk 13	Wk 16	Wk 19	Wk 22	Wk 25	Wk 28	Wk 31	Wk 34	Wk 37	Wk 40	Wk 43	Wk 46	Wk 49	Wk 60	Off-Study[i]
Bevacizumab [a]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Informed consent	X																			
Eligibility	X																			
Demographics	X																			
Medical history	X																			
Concurrent meds		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse event evaluation		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height	X																			
Weight	X					X				X				X						
Performance status	X			X		X		X		X		X		X		X		X		X
CBC with diff, platelets	X	X[b]		X		X		X		X		X		X		X		X		X
Coagulation studies (PT/PTT/INR)	X									X								X		
Serum chemistry [c]	X	X[b]		X		X		X		X		X		X		X		X		X
Urinalysis [d]	X	X[b]		X		X		X		X		X		X		X		X		X
EKG	X									X								X		
Troponin i	X									X								X		
B-HCG [e]	X					X				X				X						
Brain MRI	X[f]					X				X								X		X
Whole body MRI	X									X[g]								X[g]		
Hearing assessment [h]	X					X				X								X	X	X
Circulating cells, plasma biomarkers	X									X								X		X
QOL assessments	X									X								X		X

a. 7.5mg/kg once every 21 days (-1/+3 days).

b. Labs do not need to be repeated if the pre-study levels were drawn within 7 days of the first infusion of study drug.

c. Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, potassium, total protein, SGOT[AST], SGPT[ALT], sodium.

d. For calculation of urine protein. If dipstick $\geq$2+ proteinuria, 24-hour urine protein should be obtained.

e. Serum pregnancy test (for women of child bearing capacity).

f. Pre-study MRI scans will be performed twice on days -4 (+/- 3 days) and -1 (+/- 1 day) to confirm baseline functional MRI characteristics.

g. Whole body MRI scans will be repeated at weeks 25 and 49 only if non-vestibular tumors were located on the pre-study scan.

h. Vestibular function will be evaluated in subjects enrolled at the NCI only (Appendix K).

i. Patients who have an on-going study agent-related serious adverse event upon study completion or at discontinuation from the study should be contacted by the investigator or his/her designee periodically until the event is resolved or determined to be irreversible.

	Pre-Study	Wk 1	Wk 4	Wk 7	Wk 10	Wk 13	Wk 16	Wk 19	Wk 22	Wk 25	Wk 28	Wk 31	Wk 34	Wk 37	Wk 40	Wk 43	Wk 46	Wk 49	Wk 60	Off-Study[i]
Blood pressure[1]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Fractionated alkaline phosphatase, bone specific alkaline phosphatase, osteocalcin,, serum calcium, phosphorus, PTH, and vitamin D levels (1, 25-dihydroxy and 25-hydroxy)	X					X				X				X				X	X	X
Scanogram of lower extremity	X									X[2]										

Additional Evaluations for Hypertension and Bony Toxicity for Children only

MRI of knee for evaluation of tibial/femoral growth plates[3]	X					X				X				X				X		X
DEXA total body and lumbar spine	X					X				X				X				X		X
Height	X					X				X				X				X	X	X

[1]Weekly for first 6 weeks. Guidelines for grading and management of hypertension are provided in Appendix F. [2]As clinically indicated. Scanogram will be repeated if there are any gross discrepancies between measurements or difficulty obtaining clinically accurate measurements. Scanograms will be compared to baseline study prior to treatment with bevacizumab.
[3] The knee MRI (Appendix F) will only be required for patients with open growth plates on scanogram.

11. MEASUREMENT OF EFFECT

Antitumor Effect – Solid Tumors

For the purposes of this study, patients should be re-evaluated for response every 3 to 6 months. Two baseline scans are required for the DCE-MRI to confirm true imaging baseline characteristics. Thereafter MRIs will be obtained according to the study table.

Radiographic response and progression will be evaluated in this study using the criteria proposed by Widemann and colleagues (29) for neurofibromatosis-associated lesions. Response and progression will not be evaluated using the Response Evaluation Criteria in Solid Tumors (RECIST) (105) or by MacDonald Criteria (106) , since they may underestimate progression in these irregularly shaped tumors. However, linear measurements will be collected as part of the trial for comparison with volumetric measurements.

11.1 Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with bevacizumab.

Evaluable for objective response. Only those patients who have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to week 6 will also be considered evaluable.)

11.2 Disease Parameters

Measurable disease. Measurable lesions are defined as those that can be accurately measured using volumetric analysis of cranial MRI scans. All study MRI scans should include standard brain imaging sequences as well as fine cuts through the internal auditory canal (3 mm slice, no gaps) to image small tumors. In patients who have had surgery for tumors in the cerebellopontine angle, fat-saturation should be performed with the post-contrast sequences to compensate for the possible presence of post-operative fat packing.

Note: Tumor lesions that are situated in a previously irradiated area are considered measurable.

Non-measurable disease. Non-measurable lesions include skull-base lesions that are obscured by artifact from auditory brainstem implants (ABIs) or lesions whose margins are completely obscured by neighboring tumors (i.e., “collision” tumors).

Target lesions. Investigators will identify a single target lesion in all subjects. The target lesion in this study is the progressive VS (e.g., the VS associated with hearing loss) that led to enrollment in the protocol. In cases where subjects have

hearing in both ears, the target lesion should be the tumor associated with progressive decline in hearing in the preceding 24 months leading to word recognition score < 90% on a 100-word list as administered by an audiologist associated with this study (enrollment criteria). In rare cases where both VSs are associated with word recognition < 90% and progressing equally rapidly, the target lesion should be the larger of the two tumors on imaging.

Target lesions should be identified at baseline and measured using volumetric analysis of the baseline MRI scan. The baseline volumetric MRI scan will be used as reference for comparison of all future MRI scans to characterize the objective radiographic tumor response. The baseline word recognition score will be used as reference for comparison of all future hearing assessments.

Non-target lesions. Non-target lesions (when present) in this study include (i) VSs contralateral to the target lesion , (ii) non-vestibular schwannomas, and (iii) intracranial meningiomas. Cervicomedullary junction tumors (including ependymomas) may be included as a non-target lesion if they can be reliably imaged on cranial MRI scans. The volumes for each of the non-target lesions will be collected. Additional extracranial tumor burden will be reported based whole body MRI results.

Whole Body MRI. Whole body MRI will be obtained at baseline for all patients. For patients with measurable spine and peripheral nerve tumors, follow-up whole body MRI will be obtained according to the study table. This is to explore the impact of bevacizumab on systemic tumor burden in patients with NF2 and provide pilot data for possible future clinical trials.

Note: Histologic confirmation of tumor type is not required. Designation of tumor type will be determined by the radiographic appearance by the study radiologist.

11.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation [i.e., cubic centimeters (cm^3) and in millimeters (or decimal fractions of centimeters] for linear measures. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 8 weeks before the beginning of the treatment.

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.

Cranial MRI. These studies should be performed according to the description in Appendix D. Volumetric analysis of MRI scans should be performed on sequences with fine cuts through the internal auditory canal (3 mm slices, no gap).

11.4 Response Criteria

11.4.1 Hearing Response Parameters

Hearing response (Section 11.2 below) will be defined by the change in word recognition scores, taking as reference the baseline word recognition score (Appendix C and section 11.2 below). In order to confirm durability of response, all responses must be maintained through the subsequent evaluation period (3 months) to be considered a true response.

Hearing Response (HR): Improvement in word recognition score above the 95% critical threshold, taking as reference the baseline word recognition score maintained across two sequential evaluation time points (3 months) (Appendix C).

Stable Hearing (SH): Persistence of word recognition score within the 95% critical threshold, taking as reference the baseline word recognition score (Appendix C).

Progressive Hearing Loss (PHL): Decline in word recognition score below the 95% critical threshold, taking as reference the baseline word recognition score (Appendix C). Patients with progressive hearing loss will undergo a confirmatory evaluation of word recognition one week after the study showing hearing loss to confirm progression. If progression is not confirmed with the follow-up study, the patient will stay on study.

11.4.2 Radiographic Response Parameters

Radiographic response will be defined by the change in tumor volume compared to baseline, as previously defined in previous studies in NF1 (29) . Since NF2-related tumors do not undergo spontaneous regression in size, the term “minor response (MR)” will be applied to lesions that decrease in size but do not qualify for a radiographic response. In order to confirm durability of response, all responses must be maintained through the subsequent evaluation period (3 months) to be considered a true response.

Radiographic Response (RR): At least a 20% decrease in the volume of the target lesions, taking as reference the baseline volume. Confirmed on two sequential evaluation periods.

Minor Response (MR): A decrease of 5% to 20% in the volume of the target lesion, taking as reference the baseline volume. Confirmed on two sequential evaluation periods.

Progressive Disease (PD): Either: (1) At least a 20% increase in the volume of the target lesion, taking as

reference the baseline volume, or (2) a decrease in word recognition score beneath the 95% critical threshold.

Stable Disease (SD): Does not meet criteria for radiographic or hearing response or for progressive disease.

1.1.3 Evaluation of Non-Target Lesions

Radiographic evaluations should be calculated separately for non-target lesions (contralateral VS, non-vestibular schwannomas, and meningiomas).

Radiographic Response (RR): At least a 20% decrease in the volume of the identified non- target lesions, taking as reference the baseline volume. Confirmed on two sequential evaluation periods.

Minor Response (MR): A decrease of 5% to 20% in the volume of the identified non- target lesions, taking as reference the baseline volume. Confirmed on two sequential evaluation periods.

Stable Disease (SD): Does not meet criteria for radiographic response or progressive disease.

Progressive Disease (PD): At least a 20% increase in the volume of the identified non-target lesions, taking as reference the baseline volume

Although a clear progression of “non-target” lesions is rare, the opinion of the treating physician should prevail in such circumstances. Progression status should be confirmed at a later time by the review panel (or Principal Investigator). In addition, if there is radiographic progression of the target lesion or any non-target lesion that is asymptomatic the patient should remain on treatment. However, if at any time the treating physician or PI is concerned that there is symptomatic tumor progression of the target lesion or any non-target lesions that requires intervention, the patient should come off study as detailed in section 5.3.

11.4.4 Evaluation of Best Overall Response

The best overall response is the best hearing response recorded from the start of the treatment until disease progression (taking as reference for the hearing measurements recorded at baseline). The patient's best response assignment will depend on the achievement of both initial measurement

and confirmation criteria (response maintained for a minimum of 3 months).

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "symptomatic deterioration". Every effort should be made to document the objective progression even after discontinuation of treatment.

11.5 Duration of Response

Hearing evaluations should be performed for non-target (contralateral) VS if present and if hearing is present in the ipsilateral ear.

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for HR and RR until the first date that progressive disease is objectively documented (taking as reference for progressive disease the measurements recorded at baseline).

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the measurements recorded at baseline.

12. DATA REPORTING / REGULATORY CONSIDERATIONS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

12.1 Data Reporting

12.1.1 Method

This study will be monitored by the Clinical Data Update System (CDUS) version 3.0. Cumulative CDUS data will be submitted quarterly to CTEP by electronic means. Reports are due January 31, April 30, July 31, and October 31. Instructions for submitting data using the CDUS can be found on the CTEP web site (http://ctep.cancer.gov). Note: All adverse events that have occurred on the study, including those reported through AdEERS, must be reported via CDUS.

1.1.2 Responsibility for Submissions

Study participants are responsible for submitting CDUS data and/or data forms to the Coordinating Center quarterly by April 30 (Q1 data), July 31 (Q2 data), October 31 (Q3 data), and Jan 31 (Q4 data) to allow time for Coordinating Center compilation, Principal Investigator review, and timely submission to CTEP (see

Section 12.1.1.). For trials monitored by CTMS, the monthly data submission to CTEP from Theradex should be copied to the Coordinating Center.

The Coordinating Center is responsible for compiling and submitting CDUS data to CTEP for all participants and for providing the data to the Principal Investigator for review.

12.2 CTEP Multicenter Guidelines

This protocol will adhere to the policies and requirements of the CTEP Multicenter Guidelines (presented in Appendix B). Specifically:

•Dr. Jaishri Blakeley will be the single liaison with the CTEP Protocol and Information Office (PIO). She is responsible for the coordination, development, submission, and approval of the protocol as well as its subsequent amendments. She will oversee any revisions or modifications to the protocol with input from her co-investigators, but she will take sole responsibility for ensuring that any revision are submitted to the PIO office and approved before they are activated. She will also assure that all participating institutions are using the most updated and correct version of the protocol.

· Dr. Blakeley and the Johns Hopkins Comprehensive Neurofibromatosis Center are responsible for the review of all IND Action Letters or Safety Reports received from CTEP and the quaretly distribution of a summary of these documents to all participating institutions for submission to their individual IRBs for action as required. These will be sent as digital files via active email accounts to Drs. Plotkin and Widemann. Similarly, Dr. Blakeley will be responsible for the timely review and submission of data for study analysis and the timely review of Adverse Events (AE) to assure safety of the patients.

· Dr. Blakeley the Johns Hopkins Comprehensive Neurofibromatosis Center are responsible for the overall conduct of the study at all participating institutions and for monitoring its progress. All reporting requirements to CTEP are the responsibility of Dr. Blakeley.

· Each participating institution will order DCTD-supplied agents directly from CTEP. Agents may be ordered by a participating site only after the initial IRB approval for the site has been forwarded by Amanda Bergner at Johns Hopkins to the CTEP PIO (PIO@ctep.nci.nih.gov)

The Johns Hopkins Comprehensive Neurofibromatosis Center (JHCNFC) will ensure that:

•Each participating institution has an appropriate assurance on file with the Office for Human Research Protection (OHRP) of the NIH. The JHCNFC will maintain this documentation as well as copies of IRB approvals from each participating site and will ensure that an OHRP form 310 (documentation of IRB approval) is submitted to the CTEP PIO prior to the activation of the protocol (and the first patient registration)

at each participating institution.

- The JHCNFC will also be responsible for central patient registration.
- The JHCNFC is responsible for the preparation of all submitted data for review by the Dr. Blakeley including AE reports. The participating institutions (MGH, JHH, NIH) will report all AEs to the JHCNFC and the JHCNFC will submit the AE reports to DR. Blakeley and CTEP for timely review.
- The JHCNFC will conduct periodic audits via review of source documents and research records for selected patients brought from participating sites to JHCNFC. The JHCNFC will in turn be responsible for organizing all source documents, research records, IRB approval documents, NCI Drug Accountability Record forms, patient registration lists, response assessments scans and reports, etc. for the audit.
- The JHCNFC will design and maintain common format data collection forms (Case Report Forms that will be submitted to the JHCNFC within 1 month of the time point at which they were collected.

12.3 Cooperative Research and Development Agreement (CRADA)/Clinical Trials Agreement (CTA)

The agent supplied by CTEP, DCTD, NCI used in this protocol is provided to the NCI under a Collaborative Agreement (CRADA, CTA, CSA) between the Pharmaceutical Company(ies) (hereinafter referred to as A Collaborator(s)@) and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the Intellectual Property Option to Collaborator@ (http:// ctep.cancer.gov/industry) contained within the terms of award, apply to the use of the Agent(s) in this study:

1. Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing investigational Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient's family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: http://ctep.cancer.gov.

2. For a clinical protocol where there is an investigational Agent used in combination with (an)other investigational Agent(s), each the subject of different collaborative agreements , the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data.@):

   a. NCI will provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NIH, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed

combination protocol.

b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own investigational Agent.

c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own investigational Agent.

3. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available exclusively to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order. Additionally, all Clinical Data and Results and Raw Data will be collected, used, and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects including, if applicable, the Standards for Privacy of Individually Identifiable Health Information set forth in 45 C.F.R. Part 164.

4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.

5. Any data provided to Collaborator(s) for Phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.

6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP by the Group office for Cooperative Group studies or by the principal investigator for non-Cooperative Group studies for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release. Copies of any manuscript, abstract and/or press release/ media presentation should be sent to:

Regulatory Affairs Branch, CTEP, DCTD, NCI

Executive Plaza North, Suite 7111
Bethesda, Maryland 20892
FAX 301-402-1584
Email: anshers@mail.nih.gov

The Regulatory Affairs Branch will then distribute them to Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/ proprietary information.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

Profound hearing loss and lack of effective medical treatment lead to NF2 patients with severe hearing impairment. One study was reported in treating 10 NF2 patients with bevacizumab on a compassionate-use base. There were 4 out of 7 evaluable patients had hearing response after the treatment (57%, 95%CI: 18-90%) (6).

This is a multi-institution, single-arm, open label phase II trial to formally assess and estimate proportion of objective hearing response in NF2 patients with symptomatic vestibular schwannomas (VS) and progressive hearing loss treated by bevacizumab.

The primary endpoint is hearing response. It is defined as increased word recognition score above the 95% critical threshold that is maintained across two sequential evaluation time points (3 months) compared to baseline word recognition score as reference (Appendix C).

Based on the international Natural History of NF2 Study (35) the proportion of patients with spontaneous tumor regression is < 1%. Patients enrolled on this trial will be eligible only if they have progressive hearing loss (as measured by a decrease in word recognition score) related to VS (i.e., not due to prior interventions such as surgery or radiation) documented in the preceding 24 months with a word recognition score of <85% in the target ear. There is no expected hearing response without a treatment.

Using a one-stage design and assuming a null response rate of 0.05, a total of 14 patents will yield above 90% power to detect an alternative response rate of 0.5 at alpha level of 0.05 to be statistically significant.

Precision for potential point estimation under various response rates are tabulated below:

Number of Patients	Number Response	Percent Response	95% CI
14	3	21	5-50%
14	5	36	13-65%

14	8	57	29-82%
14	11	79	49-95%

Possible outcome are: hearing response, stable hearing, and progressive hearing loss (Response criteria, section11.4, page 55).

Proportion of hearing response will be estimated using binomial distribution (exact method) along with 95% confidence interval. The duration of the response will be summarized as mean and confidence interval of the mean.

13.2 Accrual/Patient Replacement

A predicted accrual rate is 2 subjects per month for 7 months. Patients who did not finish the first cycle of the treatment due to reasons other than toxicity or patients who are unevaluable will be replaced to ensure a total of 14 patients are evaluated on this trial.

13.3 Analysis of Secondary Objectives

- To assess the safety and tolerability of bevacizumab in this patient population on an every three week dosing schedule of 7.5mg/kg for 12 months of therapy; the toxicities of bevacizumab have been well studied and described in treating different types of solid tumors including brain tumors. There is only one report from a study in NF2 patients using the same dosage in this trial which no grade 3 or 4 adverse events were reported. The proportion of patients with serious or life threatening toxicities will be estimated along with 95% confidence intervals.

- To assess the rate of radiographic response defined by the change in tumor volume compared to baseline ($\geq$20% reduction in volume, section 11.4.2); the proportion of radiographic response will be estimated using binomial distribution. The duration of the response will be summarized as mean and confidence interval of the mean.

- To assess growth rate of VS using volumetric MRI through the 18 month trial period. Six measurements of tumor volume per patient will be obtained including two at the baseline (to ensure reproducibility of the DCE-MRI parameters pre-treatment). The rate of change over 12 months will be estimated using generalized linear model.

- To assess changes in function of the auditory system during bevacizumab treatment, A 12 dB change in 4-frequency Pure Tone Average will be considered clinically significant. A change in BAER peak latency for Waves I and V greater than 0.5 ms will be considered clinically significant. The primary DPOAE measurement will be treated non-parametrically (present or absent across time) DPOAE's will be considered present at the frequency of F2 when the distortion product is 6dB above the noise floor. Variables will be analyzed for differences

using t-tests if the effects and sample sizes warrant, but this may not be advisable given the small numbers to be accrued.

- To explore imaging of vascular permeability (Ktrans), relative cerebral blood volume/flow, mean transit time, and mean vessel diameter from perfusion-weighted MRI. Due to the small number of patients in the study, statistical analyses for research MRI data will be exploratory in nature, aiming to assess the performance of various vascular MRI measures as a potential predictor of response to therapy. As outlined in the protocol, research MRI scans will be performed at baseline (T0 at day -4 and -1), week 12, 24, 48 and week 72 (off study). The changes in imaging parameters from T0 will be assessed at each time point. The correlations between imaging parameters and hearing response will then be estimated based on the estimated changes. Generalized Estimating Equations (GEE) will be used to estimate association of imaging parameters in hearing responses after treatment. The greatest on-treatment change from the pretreatment baseline value during the course of the study will be computed for each subject. Statistical comparisons between MRI parameters measured on different study time point will be performed with a two-tailed paired exact Wilcoxon test as previously reported (19).

- To explore biological effects blood samples from patients will be collected before and during the course of treatment to measure levels of circulating endothelial cells (CECs), circulating progenitor cells (CPCs), and plasma proteins (VEGF-A, VEGF-C, sVEGFR1, sVEGFR2, sVEGFR3, Col IV, SDF1a, IL-1beta, IL-6, IL-8, TNFalpha, G-CSF, Ang1, Ang2, sTie2, s-cKIT, MMP-1, MMP-2, MMP-3, MMP-9, MMP-10, PlGF, and bFGF). Changes in the serum markers during treatment from baseline – during and after treatment – will be summarized using descriptive statistics. Statistical graphics such as boxplots will be used to present the summary statistics at each time point. The differences before and during treatment will be tested using paired statistics. Logistic regression model will be used to explore associations between changes of serum biomarker levels and hearing response.

- The study will explore whether the treatment could improve the quality of the life of NF2 patients. Three instruments will be used in this study including the Health Survey Short Form-36 (SF-36), Speech and Spatial Qualities questionnaire (SSQ), and Tinnitus Reaction Questionnaire (TRQ). Each instrument includes multiple domains and items. They will be implemented 4 times through the trial (baseline, 6 months, 12 months and off study (18 months). Standard scoring manuals will be used to summarize the each item or domains. A overall score at each time point will be compared with the baseline score two-tailed pared t-test will be used to assess the change form the baseline and MANOVA could be used to assess the association between the quality of life and the change of the hearing score. Each item or domain will be summarized using descriptive statistics. Questionnaires will be scored as recommended in the user manual for the

instrument. Comparisons of on-treatment and post-treatment values with the pre-treatment baseline value will be performed using paired t-tests.

13.4 Safety Monitoring

Special attention will be paid to patients 12-17 years for the following measures:

Confirmed >6% bone mineral density decrease relative to baseline and who have a BMD Z score <-2.5 will stop bevacizumab due to toxicity and this will be considered a DLT. Please see Appendix F for details.

It is anticipated that roughly 3-5 of the 14 patients enrolled on protocol will be <18 years old. If the trial has one patient aged 12-17 years old enrolled who develops grade 3 or 4 toxicities of any type requiring them to stop bevacizumab including the bone toxicity reported above, bleeding, thrombosis and all other potential toxicities at any point, the trial will stop enrollment for anyone<18 years old. These toxicities will be considered dose limiting. HTN in children will be addressed as per the algorithm in Appendix F.

In general, we expect a <30% dose limiting toxicity (DLT) rate across all patients. The safety of the treatment will be monitored continuously throughout the cohort using the Bayesian stopping rule at patient number 5, 8, 11 and 14. We assume approximately a 20% of patients will experience a DLT. The probability of a patient experiencing DLT was assumed to follow a binomial distribution. Given the planned sample size of 14, a stopping boundary would be reached if the proportion of the patients experiencing DLT exceeds the proportion with posterior probability at 0.9. A recommendation to redefine the safe dose and a safety review by the institution DSMC will be implemented if 3 or more out of 5, 4 or more out of 8, 5 or more out of 11, and 7 or more out of 14 patients experience a DLT. The probability of meeting the stopping boundary is 0.02 under the null of a 20% DLT rate. As above 1 patient aged 12-17 years old enrolled who develops grade 3 or 4 toxicities of any type requiring them to stop bevacizumab, will result in the halting of accrual of additional patients <18 years old.

APPENDIX A: Performance Status Criteria

ADULTS

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. 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.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. 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	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

CHILDREN

Lansky Score

100	fully active, normal
90	minor restrictions in strenous physical activity
80	active, but tired more quickly
70	greater restriction of play and less time spent in play activity
60	up and around, but active playminimal; keeps busy by being involved in quieter activities
50	lying around much of the day, but gets dressed; no active playing participates in all quiet play and activities

1	40	mainly in bed; participates in quiet activities
2	30	bedbound; needing assistance even for quiet play
3	20	sleeping often; play entirely limited to very passive activities 10
4	10	doesn't play; does not get out of bed
5	0	unresponsive

APPENDIX B: CTEP Multicenter Guidelines

If an institution wishes to collaborate with other participating institutions in performing a CTEP sponsored research protocol, then the following guidelines must be followed.

Responsibility of the Protocol Chair

- The Protocol Chair will be the single liaison with the CTEP Protocol and Information Office (PIO). The Protocol Chair is responsible for the coordination, development, submission, and approval of the protocol as well as its subsequent amendments. The protocol must not be rewritten or modified by anyone other than the Protocol Chair. There will be only one version of the protocol, and each participating institution will use that document. The Protocol Chair is responsible for assuring that all participating institutions are using the correct version of the protocol.
- The Protocol Chair is responsible for the overall conduct of the study at all participating institutions and for monitoring its progress. All reporting requirements to CTEP are the responsibility of the Protocol Chair.
- The Protocol Chair is responsible for the timely review of Adverse Events (AE) to assure safety of the patients.
- The Protocol Chair will be responsible for the review of and timely submission of data for study analysis.

Responsibilities of the Coordinating Center

- Each participating institution will have an appropriate assurance on file with the Office for Human Research Protection (OHRP), NIH. The Coordinating Center is responsible for assuring that each participating institution has an OHRP assurance and must maintain copies of IRB approvals from each participating site.
- Prior to the activation of the protocol at each participating institution, an OHRP form 310 (documentation of IRB approval) must be submitted to the CTEP PIO.
- The Coordinating Center is responsible for central patient registration. The Coordinating Center is responsible for assuring that IRB approval has been obtained at each participating site prior to the first patient registration from that site.
- The Coordinating Center is responsible for the preparation of all submitted data for review by the Protocol Chair.
- The Coordinating Center will maintain documentation of AE reports. There are two options for AE reporting: (1) participating institutions may report directly to CTEP with a copy to the Coordinating Center, or (2) participating institutions report to the Coordinating Center who in turn report to CTEP. The Coordinating Center will submit AE reports to the Protocol Chair for timely review.

- Audits may be accomplished in one of two ways: (1) source documents and research records for selected patients are brought from participating sites to the Coordinating Center for audit, or (2) selected patient records may be audited on-site at participating sites. If the NCI chooses to have an audit at the Coordinating Center, then the Coordinating Center is responsible for having all source documents, research records, all IRB approval documents, NCI Drug Accountability Record forms, patient registration lists, response assessments scans, x-rays, etc. available for the audit.

<u>Inclusion of Multicenter Guidelines in the Protocol</u>

•The protocol must include the following minimum information:

- ➢The title page must include the name and address of each participating institution and the name, telephone number and e-mail address of the responsible investigator at each participating institution.
- ➢The Coordinating Center must be designated on the title page.
- ➢Central registration of patients is required. The procedures for registration must be stated in the protocol.
- ➢Data collection forms should be of a common format. Sample forms should be submitted with the protocol. The frequency and timing of data submission forms to the Coordinating Center should be stated.
- ➢Describe how AEs will be reported from the participating institutions, either directly to CTEP or through the Coordinating Center.
- ➢Describe how Safety Reports and Action Letters from CTEP will be distributed to participating institutions.

<u>Agent Ordering</u>

•Except in very unusual circumstances, each participating institution will order DCTD-supplied agents directly from CTEP. Agents may be ordered by a participating site only after the initial IRB approval for the site has been forwarded by the Coordinating Center to the CTEP PIO.

APPENDIX C: Hearing Response Guidelines

Clinical criteria for definition of hearing response based on a 100-word hearing test. Upper and lower limits for the 95% critical differences for percentage scores are adapted from Thornton (8).

Clinical Criteria for Definition of Hearing Response Based On a 100-Word Hearing Test			
Baseline Word Recognition Score (%)	95% Critical Difference (%)	Baseline Word Recognition Score (%)	95% Critical Difference (%)
0	0-3	50	37-63
1	0-6	51	38-64
2	0-8	52	39-65
3	0-9	53	40-66
4	1-11	54	41-67
5	1-12	55	42-68
6	1-14	56	43-69
7	2-15	57	44-70
8	2-17	58	45-71
9	3-18	59	46-72
10	4-19	60	47-73
11	4-21	61	48-74
12	5-22	62	49-74
13	6-23	63	50-75
14	6-25	64	51-76
15	7-26	65	52-77
16	8-27	66	53-78
17	8-28	67	54-79
18	9-29	68	55-80
19	10-31	69	56-81
20	11-32	70	57-81
21	11-33	71	58-82
22	12-34	72	59-83
23	13-35	73	60-84
24	14-36	74	61-85
25	14-37	75	63-86
26	15-39	76	64-86
27	16-40	77	65-87
28	17-41	78	66-88
29	18-42	79	67-89
30	19-43	80	68-89
31	19-44	81	69-90
32	20-45	82	71-91
33	21-46	83	72-92

34	22-47	84	73-92
35	23-48	85	74-93
36	24-49	86	75-94
37	25-50	87	77-94
38	26-51	88	78-95
39	26-52	89	79-96
40	27-53	90	81-96
41	28-54	91	82-97
42	29-55	92	83-98
43	30-56	93	85-98
44	31-57	94	86-99
45	32-58	95	88-99
46	33-59	96	89-99
47	34-60	97	91-100
48	35-61	98	92-100
49	36-62	99	94-100
		100	97-100

APPENDIX D: Audiology Procedures

A. AUDIOLOGY PERSONNEL

The primary source of reliability and validity will be the qualifications of the clinical audiologists performing the tests, and their adherence to standard practices. These practices will be specified in this section. Study Audiologists will compile information assuring standard calibration, installation of reference-calibrated equipment, etc. (107).

a. Lead Audiologist

Each Clinical Site will designate a Lead Audiologist who will be the contact for study-related issues with the Senior Study Audiologist. This audiologist will oversee local audiology operations and also communicate with the site PI and CRC. The lead audiologist may train other audiologists at the site for testing.

b. Qualifications

Each evaluation will be performed by a fully qualified audiologist. The precise definition of qualification can vary from state to state depending on licensure laws, etc. For the purposes of this study, full qualification is defined as the highest local level of qualification, certification or licensure. Each of these requires a Master's Degree (or higher) and completion of a clinical fellowship or equivalent. Basic requirements for Lead Audiologist will be no different, but only one audiologist per center will be designated for this duty.

c. Training

Each site's Lead Audiologist will be responsible for local training of any audiologist actually testing. This training will be based on this Appendix, and the Appendix will remain available to all trained audiologists as a resource. The procedures can be refined and changed to better accommodate the needs of the local audiologists for precise guidance. All Lead Audiologists will be contacted in advance of the initiation of participant enrollment at the site, and the Senior Study Audiologist will discuss and demonstrate the procedures contained in this Appendix. These will include personnel, test protocols, data cross checking, procedures for correcting or completing evaluations, and reporting results.

B. CONTACTS

A system of regular contact between the Senior Study Audiologist and each Lead Audiologist will be initiated before any participant's enrollment. This will take place primarily by e-mail, with documents faxed as necessary. As audiologic issues arise, the local audiologists will be asked to contact the Lead Audiologist, who will act as liaison with the Senior Study Audiologist. Other local issues are expected to be addressed by contact between the Clinical Site PIs, their Lead Audiologists, and the CRCs.

C. AUDIOLOGY TESTING PROTOCOL OVERVIEW

Participants will be referred for each evaluation by the Clinical Site's PI or CRC, who will determine the timing of return visits. When the participant arrives for each test, the audiologist will greet the participant and accompanying persons and briefly and privately discuss progress if the participant wishes. If the participant requires language interpretation, this will be provided in the customary manner in place at each site. Only minimal history taking (i.e. otalgia) is required of the audiologist, and, as much as possible, study questions should be referred to the PI or CRC. Audiologists will not be formally blinded to any aspect of the study, but no effort will be made to specify the participant's status to the audiologist, and the previous evaluations will not be reviewed in advance.

The audiologist will seat the participant in a sound-treated room (108). No more than one person will be allowed to accompany the participant and this person will not be allowed to sit in the booth or to be in the line of sight of the participant. As much as possible, light levels in the participant and the tester sides will be adjusted to provide a good view of the participant and a poorer view of the audiologist (i.e. participant side bright, tester side dark). The participant should be seated perpendicular to the audiologist to minimize cues. The room door will be fully closed. The participant will be asked to respond by hand raise or by button push, whichever is customary at the Clinical Site.

The Lead Audiologist at each site will ensure that threshold tests are performed in the standard manner (108) . This includes the Hughson-Westlake bracketing procedure (109, 110) . A 200 ms. ON versus 200 ms. OFF duty cycle for tone presentation is recommended with an opportunity to appreciate 3-4 tones per trial. Narrow band noises or FM modulated tones will not be substituted for standard pure tones. Thresholds will be transcribed on the Clinical Site's standard audiogram, using standard symbols (111) At the conclusion of the evaluation, the audiologist may briefly discuss the result with the participant and accompanying persons, again referring most study questions to the PI or CRC where possible. Discussion of helpful strategies and devices as indicated by the case is expected.

Word recognition scores: Patients will be presented a list of 50 words at a level determined to yield the maximum score.NF2 patients may exhibit "rollover" where the score decreases at a fully audible level so we will do a full 50 word list at the fully audible (high) level, and one additional 50 word list at a level 10-15dB below that level (the low level). An additional 50 words will be added at the level of the highest score; the total of this list and the list on which the patient initially scored highest will generate a 100 word list which will be used and compared across visits. This study will use monosyllable lists and standardized recordings (CID W-22; QMAS V. I).

D. AUDIOLOGY DATA FORM

The study uses repeated audiologic measures designed to capture changes with treatment and with time. Therefore, the NF2 Audiology Data form is designed as a uniform data entry form for every test. This form will be filled out for each eligibility screening, and for participants at each designated evaluation point. The NF2 Audiology Data form will be filled out during or immediately after each evaluation by the testing audiologist.

a. Header Section

The top line of the form is devoted to identification of the site, the participant, the study visit number and the date. The participant's ID number and participant's initials as used in the study will be made available by the local CRC. The CRC will also specify the visit #, indicating the progress of each participant through the study. The date field will be filled out to reflect the date of the evaluation, even if some items result from a subsequent review.

b. Data Section

The date field will be filled out to reflect the date of the evaluation, even if some items result from a subsequent review. The following fields reflect the target ear data. Word recognition will reflect the results of tests described above and will always be an integer from 0-100%. Tests where no speech percept was found will be coded as "0%". PTA4 will reflect the average of the thresholds of the target ear for 500, 1000, 2000 and 4000 Hz divided by 4. Any threshold where there was no response at the limits of the audiometer will be coded as being one (5dB) audiometric step above that level, and entered in to the average. DPOAE will be recorded as present (Y) vs. not present (N). All items will be entered in the same manner for the contralateral ear.

E. DATA CROSS CHECKS

This section will describe data cross check activities performed by the testing audiologist and the Lead Audiologist, as well as the mechanism for reporting unforeseen problems or concerns.

a. Testing Audiologist

The primary data cross checks will be the responsibility of the testing audiologist. Specifically, equipment and training will be maintained which will allow such procedures as masking plateau verification, tympanometry, SRT, Stenger's test, etc. These tests will be applied at the discretion of the testing audiologist to verify results and to rule out functional or retrocochlear hearing loss. None of these tests will be used as data, but will be noted on the NF2 Audiometry Worksheet and attached to that worksheet.

b. Lead Audiologist

The testing audiologist will complete the NF2 Audiology Data form, with the exception of the section for Lead Audiologist Review and Comments. The form will then be given to the Lead Audiologist or placed in the file section for data awaiting review. The Lead Audiologist will verify the audiologic aspects of the data. The Lead Audiologist will verify validity and completeness, or will contact the CRC for rescheduling for other testing if necessary. The completed form will be given to the local CRC for transmission to the Data Management Center.

c. Problems and Concerns

The Senior Study Audiologist will be responsible for resolution of problems in audiology data interpretation. If these arise at the office of the PI, they will be communicated to the Senior Study Audiologist (i.e. not the local Lead Audiologist

directly), who will have discretion as to resolution study-wide or site-specific. If problems arise at an individual site, they will be communicated by the local Lead Audiologist to the Senior Study Audiologist, who will again be responsible for resolution either study-wide or site-specific. The anticipated mechanism for resolution of study-wide issues will be communication with all local Lead Audiologists and changes or additions to this Appendix. The Senior Study Audiologist will be responsible for informing and receiving advice and approval from the Study Chair as appropriate.

F. EQUIPMENT

The following section contains specifications for equipment used in this study.

a. Sound-treated Enclosure

A single- or double-walled sound-treated enclosure that meets American National Standard Criteria for Maximum Permissible Ambient Noise Levels for Audiometric Test Rooms shall be used to conduct pure tone air and bone conduction thresholds and word recognition testing.

An illuminated otoscope is used to examine a participant's ear canals. If any possible contraindications to audiometric testing (such as excess cerumen, eardrum abnormalities, etc.) are detected, the participant must be referred for medical evaluation before audiometric testing can proceed.

b. Acoustic Immittance Equipment

An immittance device that meets the American National Standard Specifications for Instruments to Measure Aural Acoustic Impedance and Admittance is used to conduct tympanometry and acoustic reflex threshold testing. Test results will be printed directly from the immittance device or recorded manually at the conclusion of testing on each ear. Probe tips must be appropriate in size to seal the participant's ear canal tightly during tympanometry and acoustic reflex testing. Clinical centers must have an adequate variety of sizes of probe tips to accommodate ear canals of varying dimensions.

c. Audiometer

Audiometers that meet the American National Standard Specifications for Audiometers [1] and have two channels are used to conduct pure tone air and bone conduction threshold, SRT and word recognition testing. One channel of the audiometer generates and delivers the test signals, either pure-tones or prerecorded speech. The second channel delivers narrow-band or speech-band masking noise simultaneously with the test signal, but to the non-test ear whenever necessary. The audiometer must have an input jack for external equipment such as a compact disc player or tape player, which will be used to present speech stimuli for word recognition testing.

d. Audiometer Transducers

Earphones mounted in supra-aural cushions and calibrated according to the

American National Standard Specification for Audiometers (96) are used to deliver the test material from the audiometer to the participant. The earphones are designated as "right" and "left" and will be placed comfortably over the participant's right and left ears, respectively. Bone vibrators calibrated according to the same standards are used to obtain bone conduction thresholds. During the testing, the bone vibrator is positioned over the mastoid area of the participant's test ear, taking care that it is not in contact with the posterior part of the pinna.

e. Compact Disc Player

A compact disc player must be used to deliver pre-recorded speech material to the audiometer and subsequently to the transducers positioned over the participant's ears. A cable extends between the output jack of the compact disc player and the input jack of the audiometer.

f. Distortion Product Otoacoustic Emissions System

There are no standards for DPOAE equipment. Each study site will use their clinical DPOAE system to accomplish testing as specified in the protocol.

g. Maintenance

Each clinical center is responsible for the proper operation and maintenance of its audiometric equipment. Responsibility for proper maintenance is assumed by the Lead Audiologist, and all staff are instructed to report promptly any real or suspected equipment problems to that person. All checks, inspections, and repairs are documented and recorded by date in a permanent log. The Study Chair and Study Senior Audiologist may review this log at periodic site visits. All study test equipment including audiometers and acoustic immittance devices must be calibrated according to the American National Standards Institute. Listening checks may help to identify problems that could influence participants' test behavior and audiometric results in between scheduled physical calibrations. Study audiologists should perform a listening check on any day when a participant enrolled in the protocol will be tested.

APPENDIX E: MRI Protocols

BRAIN MRI

Image Acquisition

Each patient will be scanned on the same 3 Tesla MRI system. Each scanning session will consist of the following sequences:

1) Scout sequence
2) T2-weighted imaging
3) Fluid-attenuated inversion recovery (FLAIR) imaging
4) T1-weighted pre-contrast imaging
5) Blood oxygenation level dependent (BOLD) and arterial spin labeling (ASL) imaging
6) Dynamic contrast-enhanced imaging
7) Diffusion tensor imaging
8) Dynamic susceptibility contrast imaging
9) Post-contrast T1-weighted imaging

The post-contrast T1 weighted imaging with fine cuts through the internal auditory canal (3 mm slices, no skip) and volumetric analysis will be used to assess the endpoint of radiographic response (defined as ≥ 20% decrease in tumor volume by MRI scan). The total scan time for the required images for the primary endpoint is roughly 10 minutes. The remaining sequences will be assessed as secondary corollary endpoints to evaluate the mechanism by which bevacizumab may affect VS biology. The total scan time for all sequences is roughly 45 minutes.

The details of the imaging sequences to be performed are:

I. Scout. The ‘‘AutoAlign’’ method of producing scout images is used to improve scan-to-scan reproducibility. Briefly, this method acquires two low-resolution whole-head scans (2.5 mm isotropic voxels) at different flip angles within 46 s, and uses a computer algorithm to compare the current location of the head with a predefined atlas. This localization is then used to ensure that the slice prescriptions are identical between scan sessions, even across many months (131, 132). Imaging time: 46 seconds.

II. T2-weighted imaging. A single-slab, three-dimensional, T2- weighted turbo-spin-echo sequence with high sampling efficiency (‘‘SPACE’’) is used at high resolution. Specific imaging parameters: 0.9 mm isotropic, 192 slices, 256 × 256 matrix, 24 cm FOV, TR 3200 ms, effective TE 494 ms. Imaging time: 4:30 (min:sec).

III. Fluid-attenuated inversion recovery (FLAIR) imaging. Axial FLAIR images are acquired with TR 10,000 ms, TE 70 ms, and 5 mm slice thickness, 1 mm interslice gap, and 0.6 mm × 0.45 mm in-plane resolution; 23 slices, 384 × 512 matrix. Imaging time: 3:02 (min:sec).

· T1-weighted pre-contrast imaging. Axial T1-weighted images are obtained prior to the injection of contrast. TR 600 ms, TE 12 ms, 5mm slice thickness, 1 mm interslice gap, 0.6 mm × 0.45 mm in-plane resolution; 23 slices, 384 × 512 matrix. Imaging time: 1:59 (min:sec).

IV. Blood oxygenation level dependent (BOLD) and arterial spin labeling (ASL) imaging. This is performed with the total 10 min sequential supply (the baseline 2 min of room air, 4 min of 100% pure oxygen, and then 4 min of room air), and BOLD /ASL images are acquired through EPI readout. Voxel size: 3.4×3.4×6.0 mm, TR 2000 ms, TE 19 ms. Delay in TR: 284 ms. Imaging time: 10:06 (min:sec).

V. Dynamic contrast-enhanced imaging. This is a series of acquisitions of a 50.6 mm thick slab consisting of 20 slices. All scans are 2.9 mm × 2.0 mm resolution, with a 2.1 mm slice thickness, 0.4 mm interslice gap, using a fast gradient echo technique (TR 5.7 ms, TE 2.73 ms). Data to allow computation of a T1 map of the tissue of interest are initially created using five different flip angles (2°, 5°, 10°, 15°, 30°). Then, the same slab of tissue is sampled with a 10 flip angle every 5.04 s for 252 s (50 time points), and 0.05 - 0.1 mmol/kg of Gd-DTPA is injected 52 s after the beginning of the acquisition at 5 cc/s. Imaging time: 4:12 (min:sec).

VI. Diffusion tensor imaging. 35 slices of twice-refocused echo-planar diffusion-weighted images are acquired with TR 4430 ms, TE 87 ms, and a b-value of 700 s/mm2 in 90 directions as well as 10 low b-value images (b ~0 s/mm2) to allow reconstruction of the diffusion tensor at each voxel. Resolution is 1.2 mm isotropic, with a 160 × 160 matrix. Imaging time: 7:45 (min:sec).

VII. Dynamic susceptibility contrast imaging. A 40 mm slab of tissue is imaged using a dual-echo, combined gradient-echo, and spin-echo echo planar sequence to enable relative vessel size mapping. This sequence acquires two images after each 90 RF excitation: a gradient echo image (TE 31 ms) and a spin echo image (TE 96 ms); each image had 1.2 mm in-plane resolution and 2 mm through-plane resolution (160 ×160 matrix). There is a 0 mm interslice gap and 10 slices. 100 blocks of images are acquired. 0.1 - 0.2 mmol/kg of Gd-DTPA is injected at 5 cc/s after 54 s of imaging. Imaging time: 2:14 (min:sec).

VIII. Post-contrast T1-weighted imaging. Axial T1-weighted images are acquired exactly as pre-contrast, as described above. Moreover, axial and coronal images for internal auditory canal with fat-suppression are acquired respectively with TR 750 ms, 9.8 ms, and 3 mm slice thickness, 0 mm interslice gap, and 0.56 mm in-plane resolution; 11 slices, 320 × 320 matrix. Imaging time: 3:56 (min:sec). In addition, a 3D multi-echo magnetization prepared rapid gradient echo (MPRAGE) volumetric acquisition is performed, with 1 mm isotropic voxels, TR 2530 ms, TE 1.64 ms, 3.5 ms, 5.36 ms, 7.22 ms, 256 × 256 matrix, 176 slices. Imaging time: 6:03 (min:sec).

<u>Image Analysis</u>

I. Volumetrics. Enhancing lesions will be quantitatively analyzed by an experienced neuroradiologist blinded to the order of the scans, patient identity and treatment status of the patients. Bi-dimensional diameters will be created and outlined using electronic calipers in accordance with the Macdonald criteria. The lesions will also be outlined using a volumetric approach described previously that includes outlining each enhancing voxel on post-contrast scans and then summing the voxels to calculate an overall lesion volume. All scans on this trial will be sent to the laboratory of Dr. Gregory Sorensen at MGH for central analysis of tumor volume. A report will be generated and then sent to the principal investigators at each study sites and to the study PI.

II. Map Synthesis. Blood volume, blood flow, and vessel size maps. Relative cerebral blood volume of larger vessels (gradient echo images) and smaller vessels (spin echo images) as well as cerebral blood flow will be calculated using a standard deconvolution technique (133) with blood volume corrected for leakage of the contrast agent across the blood brain barrier (134) . Vessel size maps will be created using the ratio of delta-R2* to delta-R2, according to published approaches (135-137) .

III. Apparent diffusion coefficient (ADC) maps. Maps of ADC will be created from the low and high b value images using custom-written software implementing the standard Steskjal-Tanner diffusion approximation.

IV. Permeability maps. Dynamic contrast enhanced MRI data will be processed using custom-made software written in Matlab (The MathWorks, Natick, Massachusetts), following standard

published approaches, including maps of Ktrans (corresponding roughly to wash-in rates of the contrast agent) (138) and Ve (extracellular-extravascular volume fraction).

V. Simultaneous BOLD and flow maps. Both BOLD and flow response maps to 100% pure oxygen will be created using Neurolens (MGH, Massachusetts, and Neurovascular Imaging Lab, UNF Montréal) software. After the processes of motion correction and spatial smoothing, current oxygen block paradigm is applied to linear modeling process.

VI. Synthetic Map Analysis. The tumor will be outlined on the synthesized maps and median values across the entire lesion will be computed. As all of the values other than ADC will be considered relative, rather than absolute, maps will be normalized to each other using an unaffected area of gray and white matter, typically located distantly, such as in the contralateral hemisphere.

WHOLE BODY MRI

A 3-T MR imager with an integrated body coil will be used to acquire whole-body MR images. No intravenous contrast material is required. The MR unit will be calibrated according to standard operational procedures for a clinical MR system. No special calibration is required.

Each subject will be examined from head to toe in the supine position. The entire body will be examined with a coronal short inversion time inversion- recovery (STIR) sequence: repetition time msec/echo time msec/inversion time msec, 4190/111/150; 10-mm section thickness; no intersection gap; 500-mm field of view; echo train length, 25; 320 240 matrix; and five imaging stations providing craniocaudal coverage with an overlap of at least 40 mm between two adjacent stations.

To optimize consistency between examinations, the initial imaging station will be centered on the patient's chin, and the initial coronal section will be positioned at the level of the table top.

These two steps eliminated the need to perform localizer sequences, reducing imaging time. Total imaging time for the five STIR sequences is estimated at 15 minutes.

Images from the five acquisitions will be saved in the Digital Imaging and Communications in Medicine format. Images will then be fused into a single whole-body series by using software available on the MR workstation (for example, Siemens Syngo, version MR B13 4VB13A; Siemens Medical Solutions).

SENDING IMAGES BETWEEN INSTITUTIONS

If images are to be analyzed at a site other than the site obtaining the imaging, all images are to be loaded onto a disk and labeled with the subject ID number only. Complete the case report form for submitting images; fax a copy to the Central Study Office, keep the original and include a copy of this form with the disk. Ship via courier to the address in the protocol that corresponds with the scan that is being shipped.

APPENDIX F: Grading and management of hypertension and of bony toxicity with volumetric MRI of the growth plates in children 12 through 17 years old.

Grading of hypertension

Diastolic blood pressure levels for BOYS aged 12-17 years

	1	2	3	4
Age (years)	ULN* DBP mmHg	DBP ≤10 mmHg above ULN	DBP >10 or ≤25 mmHg above ULN	DBP>25 mmHg above ULN
12	80	81-90	91-105	106
13	82	83-92	93-107	108
14	83	84-93	94-108	109
15	83	84-93	94108	109
16	84	85-94	95-109	110
17	84	85-94	95-109	110

* ≤95th percentile for age and 50% height percentile

Diastolic blood pressure levels for GIRLS aged 12-17 years

	1	2	3	4
Age (years)	ULN* DBP mmHg	DBP ≤10 mmHg above ULN	DBP>10 or ≤25 mmHg above ULN	DBP>25 mmHg above ULN
12	81	82-91	92-106	107
13	82	83-92	93-107	108
14	82	83-92	93-107	108
15	83	84-93	94-108	109
16	85	86-95	96-110	111
17	87	88-97	98-112	113

* ≤95th percentile for age and 50% height percentile

These Charts list DBP levels within the ULN (1), within 10 mmHg above the ULN (2), within

11-25 mmHg above the ULN (3), and >25 mmHg above the ULN (4).

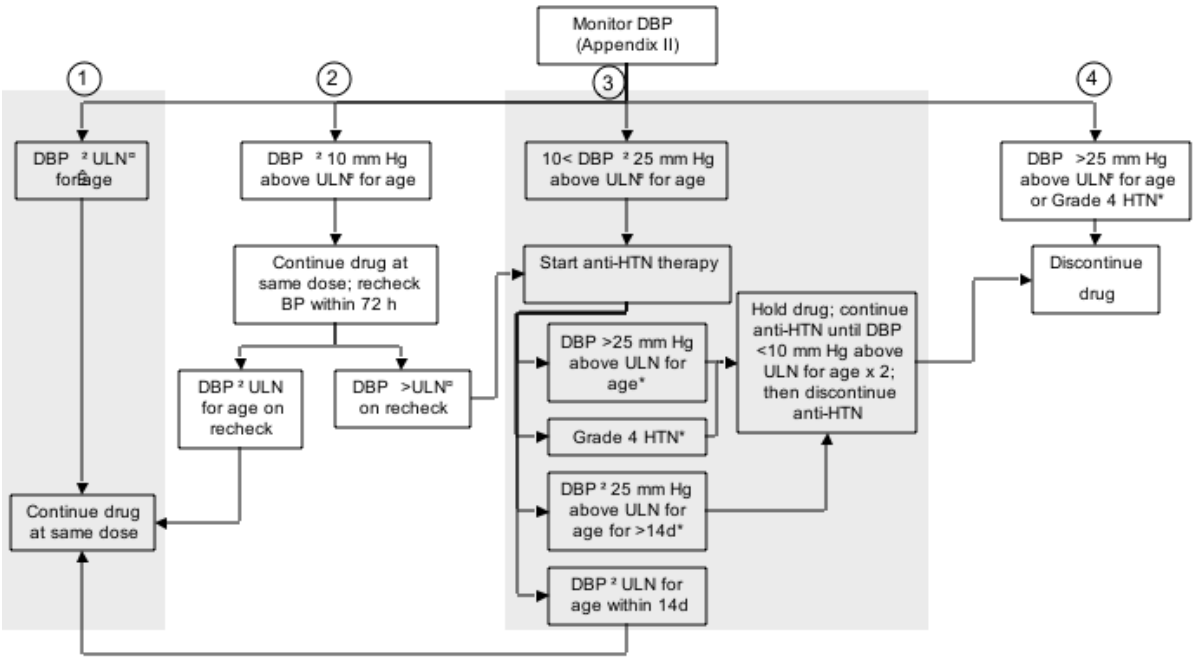
Instructions for using this BP Chart:

1. Measure the patient's blood pressure using an appropriate size cuff.
2. Select appropriate chart for a female or male patient. Age should be rounded to the nearest year.
3. Using the "age" row determine if the DBP is within the ULN (1) or elevated (2, 3, 4).
4. See Section 6 for definition of dose limiting hypertension.

Management of Bevacizumab related Hypertension

The algorithm outline below will be used to grade and manage bevacizumab-related hypertension. A diastolic blood pressure (DBP) equal to the 95th % for age and gender will be defined as the upper limit of normal (ULN).

Patients with elevated DBP at any time should have blood pressure measurements performed twice weekly until DBP is within the ULN.



Elevated diastolic blood pressure (DBP) measurements should be repeated on the same day to confirm the elevation

▫ ULN (Upper Limit of Normal) is a DBP ≤ the 95th percentile from age and gender-appropriate normal values (Appendix G)

* If DBP >25 mm Hg above ULN for age (verified) or grade 4 HTN at any time, discontinue drug. Antihypertensive agents can be used to control hypertension as clinically indicated after study drug is discontinued.

Arm 1 of algorithm:

VII. If DBP ≤95% for age and gender, continue bevacizumab at the same dose.

Arm 2 of algorithm:

5. If DBP ≤10 mm Hg above the ULN for age and gender, continue bevacizumab at same dose.

a. If the DBP ≤ 95% for age and gender on recheck, continue bevacizumab at same dose.

b. If the DBP remains above the ULN for age and gender on recheck, then start single agent antihypertensive therapy (consider a calcium channel blocker such as amlodipine or nifedipine) and follow arm 3 of the algorithm from the point that anti-hypertensive therapy is started.

Arm 3 of algorithm:

VIII. If DBP is 11 to 25 mm Hg above the 95% for age and gender on ≥2 of 3 measurements, start single agent anti-hypertension therapy (consider a calcium channel blocker such as amlodipine or nifedipine), continue bevacizumab at the same dose and monitor blood pressure at least every 3 days.

a. If the DBP remains elevated ≤25 mm Hg above 95% for age and gender for more than 14 days after the institution of single agent anti-hypertensive therapy, discontinue bevacizumab, but continue the antihypertensive agent until the DBP is ≤10 mm Hg above the 95% for age and gender on 2 measurements at least 3 days apart.

o If the DBP increases to ≥25 mm Hg above the 95% for age and gender despite antihypertensive therapy or the participant develops grade 4 hypertension (CTCAE), discontinue bevacizumab permanently, but continue the antihypertensive agent until the DBP is ≤10 mm Hg above the 95% for age and gender on 2 measurements at least 3 days apart.

Arm 4 of algorithm:

- If DBP is >25 mm Hg above the 95% for age and gender or the participant develops a grade 4 hypertension (CTCAE), discontinue bevacizumab permanently and monitor blood pressure at least every 3 days. Antihypertensive agents can be used until the DBP is <10 mm Hg above the 95% for age and gender on 2 measurements at least 3 days apart.
- The cycle remains 42 consecutive days in patients who have dose interruptions.

Protocol for required MRI studies of Knee

Unilateral knee MRI. Right knee preferred over left unless prohibited by contractures, lesions, pain, or hypertrophy. This is done to examine the femoral and tibial growth plates. Only the series outlined below are required for the knee MRI for evaluation of femoral and tibial growth plates and must be performed within protocol specifications as indicated below. Additional series may be obtained as indicated per institutional PI.

T1 Weighted Sagittal	Protocol Specifications	Actual Specifications	Reason For Change
Echo Train Length	72		
TR	500-600		
TE	min full		
Slice Thickness	3		
Bandwidth	20 kHz		
GAP	1.5mm		
FOV	18		
FREQ	512		
Phase	256		
NEX	1		
PHFVO	Full		
Saturation	no FS		
OPTIONS	Fast		

Knee MRI studies requested per protocol will be submitted to the NCI POB within 2 weeks of acquisition for volume analysis. The studies have to be sent on CD in uncompressed DICOM format. For technical support, please contact: Eva Dombi, M.D. (phone 301-451-7023, e-mail: dombie@mail.nih.gov.

Send knee MRI studies to:
Brigitte Widemann, M.D.
NCI, POB
10 Center Drive, Building 10, CRC, Room 1-5750
Bethesda, MD 20892-1101
Phone: 301-496-7387, fax: 301-480-8872, e-mail: widemanb@mail.nih.gov

The growth plate volume will be analyzed at the Pediatric Oncology Branch of the NCI. The NCI Pediatric Oncology Branch will inform participating investigators about the results of the MRI study by written report.

Management of bone related toxicity

Potential bone related toxicity is of great concern for this study based on pre-clinical data and because bevacizumab will be used in a pediatric population (aged 12-17 years). Therefore, we will carefully monitor for it, utilizing multiple serial measurements of height and weight, measurements of serum calcium, phosphorus, PTH, vitamin D levels, osteocalcin, bone specific alkaline phosphatase, lower extremity scanogram, knee MRI for evaluation of femoral and tibial growth plates, and DEXA scans.

Based on experience gained from a previous study of tenofovir and impact on bone mineral density (BMD) in HIV-infected children, (83, 84) the following parameters will be used for management of decreases in lumbar spine BMD based on DEXA scan:

4 Patients with <6% BMD decrease relative to baseline from interval measurement will remain on treatment, and continue follow up as described in section 10.

5 Patients with a confirmed >6% BMD decrease relative to baseline, but BMD Z score >-2.5, will remain on treatment; however, follow up DEXA scan will occur on an every 3 month schedule.

6 Patients with a confirmed >6% BMD decrease relative to baseline and who have a BMD Z score <-2.5 will discontinue bevacizumab.

Growth plate volume: Patients with femoral growth plate expansion 2 times the volume from baseline measurements will be taken off bevacizumab.

For patients with open growth plates, measurements of stature (height) will be measured prior to week 12, 24, 36, and 48.
Bevacizumab will be discontinued if:

- <1 cm growth is noted prior to week 24
- <2.5 cm growth is noted prior to week 48
- Subsequently, <2cm/year annualized growth velocity noted every six months for patients with open growth plates only

If there are any gross discrepancies between measurements or difficulty obtaining accurate measurements, lower extremity scanograms will be obtained as an objective measure to compare to baseline scans when clinically indicated.

APPENDIX G: Data Safety and Monitoring Plan

The JHCNFC and Drs. Blakeley, Plotkin and Widemann are committed to ensuring the safety of patients who participate in this clinical research. The procedures outlined below dealing with the approval of the research protocol, safety evaluation, protocol specific guidelines, data monitoring, audits, and reporting adverse events are the result of review of many clinical research programs including the Adult Brain Tumor Consortium and the Sidney Kimmel Comprehensive Cancer Center. We feel the final plan reflects a comprehensive safety monitoring plan combining the best of previously applied procedures for phase I-II trials for investigative agents. The individuals responsible for designing and implementing the protocol and the safety monitoring plans (Drs. Blakeley, Plotkin and Widemann) will be responsible for safety monitoring for this trial.

Research Protocols: Before the research protocol is opened for accrual, the full document (including monitoring plans and informed consent documents) must be reviewed and approved by the NCI/CTEP Protocol Review Committee. Once this is completed, it must be submitted and approved by the investigational review board (IRB) of each institution that plans to open the clinical trial. IRB approvals and consents from each site must be submitted to the JHCNFC Central Operations Office for regulatory review where it will be kept on file. A site may not enroll a patient until proper IRB documentation is reviewed and approved by the JHCNFC. Additionally each participating centers' IRBs OPRR assurance numbers will have to be confirmed to be on file with the JHCNFC.

Regulatory Documents: The JHCNFC will keep a regulatory file on each site which includes; a copy of each investigators' required CTEP/NCI 1572, their investigator number, investigator(s) CV, medical licensees', site laboratory normal's, site laboratory certificates, and site IRB roster, which will be updated regularly.

Safety evaluations: All patients receiving investigational agents will be evaluated for safety. The safety parameters include all laboratory tests and hematological abnormalities, CNS observations, physical examination findings, and solicited reports of adverse events and spontaneous reports of adverse events reported to the investigator by patients. All toxicities encountered during the study will be evaluated according to the active version of the NCI Common Toxicity Criteria and recorded prior to each course of therapy. Life-threatening toxicities will be reported immediately to Dr. Blakeley, the local Institutional Review Board (IRB), CTEP and the FDA. Additional safety assessments and toxicity monitoring are outlined in the protocol (sections 5 and 6). Dr. Blakeley will evaluate all adverse events weekly and all SAEs within 24 hours.

The data for this protocol will be monitored for safety and toxicity on a week-to-week basis, by the JHCNFC and this will be reported to the internal DSMC (see below).

Data Safety Monitoring Committees: The Data Safety and Monitoring Plan includes both an Internal and an External Data Safety Monitoring Committee (DSMC).

The Internal DSMC (I-DMSC) is made up of Drs. Blakeley, Plotkin, Widemann and the assigned CTEP monitor. Dr. Blakeley will review the submitted adverse event reports weekly and will report any serious adverse events as detailed in section 7. The I-DSMC will be the primary team making critical decisions regarding the ongoing conduct of the trial. However, decisions by this group must be unanimously agreed to by all members reviewing the data and at least three of the four members being present for the review. However, The CTEP Monitor will not have voting power; s/he will only make recommendations to the group. If all present members do not agree, the relevant materials are forwarded by the JHCNFC to the Data Safety Monitoring Committee through the Central Research Office of the Sidney Kimmel Comprehensive Cancer Center which will make a final decision. The DSMC will meet formally after the first 3, 7, 11 and 14 patients are enrolled for a complete review all AEs generated in this study as well as safety reports provided by CTEP. At that time critical appraisal will be made to determine if there are any findings that warrant change to the protocol or to the consent and to ensure that regulatory procedures have been followed appropriately.

The primary goals of the DSMCs are to: 1) ensure that all patients enrolled on this trial receive optimal protection against research risks, and 2) ensure that patients' interests are not made secondary to the interests of the scientific investigation.

Their responsibilities to accomplish these above objectives include:

a) To review interim analyses of outcome data (prepared by the Biostatistician or other responsible person at the time points defined in the protocols approved by the Institutional Review Board) and to recommend, if necessary, whether the study needs to be changed or terminated based on these analyses;
b) To determine whether and to whom outcome results should be released prior to the reporting of study results from this trial at the time specified in the protocol;
c) To review interim toxicity data of all phase II studies, and to review efficacy of treatment data at the completion of each study.
d) To communicate information and recommendations and propose effective resolutions for educational purposes and improved patient care and risk prevention.

JHCNFC Monitoring: The JHCNFC monitors the accrual and immediately suspends a study once the study has reached its enrollment goal. The Data Coordinator, Ms. Bergner, and team monitor toxicities and other events in real time and may temporarily suspend enrollment if unexpected events occur. These events are then reviewed with the I-DSMC to determine if the study may continue. If this team cannot determine a decision the external DSMC may be invoked.

The JHCNFC and Dr. Blakeley will meet weekly to review safety data and other issues that have occurred during the week. This meeting is formally held in the JHCNFC office at Hopkins but members from other institutions may be included by teleconference. Reports are run on the study database, regarding accrual and safety issues and distributed to the team for discussion and management, with specific detail to serious adverse events, i.e. AdEERS, DLTs etc.

Audits: All Consortium Trials are also monitored by the Clinical Data Update System (CDUS)

version 1.9. Cumulative CDUS data is submitted quarterly to CTEP by electronic means. Reports are due January 31, April 30, July 31, and October 31. In addition, the JHCNFC will maintain all study related documents including the current IRB approved protocol, IRB approval from each site, case report forms and source data and be available for audit should the NCI request this.

Reporting Adverse Events: Guidelines for reporting Adverse Events are clearly outlined in section 7. This protocol has adopted and implemented the AdEERS system. All expedited events will be phoned to the JHCNFC at the time the event is known. Dr. Blakeley and Ms. Bergner have 24/7 beepers for this purpose. The JHCNFC will receive copies of all AdEERS and we remind the site at the time the event is discussed with us, that the event must be sent to their IRB according to their local IRB's policies and procedures. A copy of this submission is also on file with the JHCNFC.

Guidelines for Reporting Serious Adverse Events to CTEP:
Investigators are to report toxicities occurring on NCI sponsored protocols according to the guidelines provided by the National Cancer Institute on the web site at http://ctep.info.nih.gov/AdEERS. Serious Adverse Events occurring on CTEP Sponsored studies will be reported via the NCI's electronic Adverse Event Expedited Reporting System (AdEERS) at http://ctep.info.nih.gov/AdEERS. When reporting via AdEERS online, it is mandatory to include the e-mail address of protocol managers at the ABTC Data Management Center in addition to the study chair/PI. Serious Adverse Events (SAE's) are handled according to: 1) whether the toxicities are known or unknown, and 2) the grade of toxicity. The list of known toxicities and specific reporting requirements are included in each protocol. If in doubt, consider the toxicity unknown.

Phase 1 Trials Utilizing an Agent under a CTEP IND: AdEERS Reporting Requirements for Adverse Events That Occur Within 30 Days[1] of the Last Dose of the Investigational Agent

	Grade 1	Grade 2	Grade 2	Grade 3		Grade 3		Grades 4 & 5[2]
				Unexpected		Expected		
	Unexpected and Expected	Unexpected	Expected	with Hospitalization	without Hospitalization	with Hospitalization	without Hospitalization	Unexpected and Expected
Unrelated Unlikely	Not Required	Not Required	Not Required	10 Calendar Days	Not Required	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days
Possible Probable Definite	Not Required	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days	24-Hour; 5 Calendar Days	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days

[1] Adverse events with attribution of possible, probable, or definite that occur greater than 30 days after the last dose of treatment with an agent under a CTEP IND require reporting as follows:
AdEERS 24-hour notification followed by complete report within 5 calendar days for:

- Grade 3 unexpected events with hospitalization or prolongation of hospitalization
- Grade 4 unexpected events
- Grade 5 expected events and unexpected events

[2] Although an AdEERS 24-hour notification is not required for death clearly related to progressive disease, a full report is required as outlined in the table.
December 15, 2004

Note: All deaths on study must be reported using expedited reporting regardless of causality. Attribution to treatment or other cause should be provided.

- Expedited AE reporting timelines defined:
  - "24 hours; 5 calendar days" – The investigator must initially report the AE via AdEERS within 24 hours of learning of the event followed by a complete AdEERS report within 5 calendar days of the initial 24-hour report.
  - "10 calendar days" - A complete AdEERS report on the AE must be submitted within 10 calendar days of the investigator learning of the event.

- Any medical event equivalent to CTCAE grade 3, 4, or 5 that precipitates hospitalization (or prolongation of existing hospitalization) must be reported regardless of attribution and designation as expected or unexpected with the exception of any events identified as protocol-specific expedited adverse event reporting exclusions.

- Any event that results in persistent or significant disabilities/incapacities, congenital anomalies, or birth defects must be reported via AdEERS if the event occurs following treatment with an agent under a CTEP IND.

- Use the NCI protocol number and the protocol-specific patient ID provided during trial registration on all reports.

- A list of agent specific expected adverse events can be found in the protocol.

Phase 2 and 3 Trials Utilizing an Agent under a CTEP IND: AdEERS Reporting Requirements for Adverse Events That Occur Within 30 Days[1] of the Last Dose of the Investigational Agent

	Grade 1	Grade 2	Grade 2	Grade 3		Grade 3		Grades 4 & 5[2]	Grades 4 & 5[2]
				Unexpected		Expected			
	Unexpected and Expected	Unexpected	Expected	with Hospitalization	without Hospitalization	with Hospitalization	without Hospitalization	Unexpected	Expected
Unrelated Unlikely	Not Required	Not Required	Not Required	10 Calendar Days	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days
Possible Probable Definite	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days	10 Calendar Days

[1] Adverse events with attribution of possible, probable, or definite that occur greater than 30 days after the last dose of treatment with an agent under a CTEP IND require reporting as follows:

AdEERS 24-hour notification followed by complete report within 5 calendar days for:

- Grade 4 and Grade 5 unexpected events

AdEERS 10 calendar day report:

- Grade 3 unexpected events with hospitalization or prolongation of hospitalization
- Grade 5 expected events

[2] Although an AdEERS 24-hour notification is not required for death clearly related to progressive disease, a full report is required as outlined in the table.

December 15, 2004

Note: All deaths on study must be reported using expedited reporting regardless of causality. Attribution to treatment or other cause should be provided.

- Expedited AE reporting timelines defined:
  - "24 hours; 5 calendar days" – The investigator must initially report the AE via AdEERS within 24 hours of learning of the event followed by a complete AdEERS report within 5 calendar days of the initial 24-hour report.
  - "10 calendar days" - A complete AdEERS report on the AE must be submitted within 10 calendar days of the investigator learning of the event.

- Any medical event equivalent to CTCAE grade 3, 4, or 5 that precipitates hospitalization (or prolongation of existing hospitalization) must be reported regardless of attribution and designation as expected or unexpected with the exception of any events identified as protocol-specific expedited adverse event reporting exclusions.

- Any event that results in persistent or significant disabilities/incapacities, congenital anomalies, or birth defects must be reported via AdEERS if the event occurs following treatment with an agent under a CTEP IND.

- Use the NCI protocol number and the protocol-specific patient ID provided during trial registration on all reports.

- A list of agent specific expected adverse events can be found in the protocol.

IND Safety Reports: The Consortium takes direction from CTEP for CTEP distributed agents with regards to FDA submission of adverse events. The CTEP monitor is required to review events and determine if it requires FDA submission. Safety Reports are generated by CTEP and sent to the JHCNFC. An email will be sent to alert each site to share any IND Safety Letters and will instruct investigators to file a copy with their protocol file and send a copy to their IRB according to their local IRB's policies and procedures. Only if the Safety Report changes the consent will accrual be stopped for a safety report. If the consent must be amended to include this event, an email will be sent alerting the sites that accrual is suspended for a protocol due to an IND Safety Report and the need to revise the consent. A site may not begin accruing to that protocol until a revised IRB approved consent is received in the JHCNFC.

AdEERS and all other "serious" toxicities will be discussed at our weekly meetings. Weekly meetings are attended by the all members of the JHCNFC and all participants in this trial will be invited to call in as well.

APPENDIX H: Short Form Health Survey-36

SF-36(tm) Health Survey

Instructions for completing the questionnaire: Please answer every question. Some questions may look like others, but each one is different. Please take the time to read and answer each question carefully by filling in the bubble that best represents your response.

Patient Name: _____

SSN#: _____ Date: _____

Person heling to complete this form: _____

1. In general, would you say your health is:

- ❑ Excellent
- ❑ Very good
- ❑ Good
- ❑ Fair
- ❑ Poor

2. Compared to one year ago, how would you rate your health in general now?

- ❑ Much better now than a year ago
- ❑ Somewhat better now than a year ago
- ❑ About the same as one year ago
- ❑ Somewhat worse now than one year ago
- ❑ Much worse now than one year ago

3. The following items are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

a. Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports.
- ❑ Yes, limited a lot.
- ❑ Yes, limited a little.
- ❑ No, not limited at all.

b. Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf?
- ❑ Yes, limited a lot.
- ❑ Yes, limited a little.
- ❑ No, not limited at all.

c. Lifting or carrying groceries.
- ❑ Yes, limited a lot.
- ❑ Yes, limited a little.
- ❑ No, not limited at all.

d. Climbing several flights of stairs.
- ❑ Yes, limited a lot.
- ❑ Yes, limited a little.
- ❑ No, not limited at all.

e. Climbing one flight of stairs.
- ❑ Yes, limited a lot.
- ❑ Yes, limited a little.
- ❑ No, not limited at all.

f. Bending, kneeling or stooping.
- ❑ Yes, limited a lot.
- ❑ Yes, limited a little.
- ❑ No, not limited at all.

g. Walking more than one mile.
- ❑ Yes, limited a lot.
- ❑ Yes, limited a little.
- ❑ No, not limited at all.

h. Walking several blocks.
- ❑ Yes, limited a lot.
- ❑ Yes, limited a little.
- ❑ No, not limited at all.

i. Walking one block.
- ❑ Yes, limited a lot.
- ❑ Yes, limited a little.
- ❑ No, not limited at all.

j. Bathing or dressing yourself.
- ❑ Yes, limited a lot.
- ❑ Yes, limited a little.
- ❑ No, not limited at all.

4. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

a. Cut down the amount of time you spent on work or other activities?
☐ Yes ☐ No

b. Accomplished less than you would like?
☐ Yes ☐ No

c. Were limited in the kind of work or other activities
☐ Yes ☐ No

d. Had difficulty performing the work or other activities (for example, it took extra time)
☐ Yes ☐ No

5. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

a. Cut down the amount of time you spent on work or other activities?
☐ Yes ☐ No

b. Accomplished less than you would like
☐ Yes ☐ No

c. Didn't do work or other activities as carefully as usual
☐ Yes ☐ No

6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?
- ❑ Not at all
- ❑ Slightly
- ❑ Moderately
- ❑ Quite a bit
- ❑ Extremely

7. How much bodily pain have you had during the past 4 weeks?
- ❑ Not at all
- ❑ Slightly
- ❑ Moderately
- ❑ Quite a bit
- ❑ Extremely

8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

- ❑ Not at all
- ❑ Slightly
- ❑ Moderately
- ❑ Quite a bit
- ❑ Extremely

9. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks.

a. did you feel full of pep?
- ❑ All of the time
- ❑ Most of the time
- ❑ A good bit of the time
- ❑ Some of the time
- ❑ A little of the time
- ❑ None of the time

b. have you been a very nervous person?
- ❑ All of the time
- ❑ Most of the time
- ❑ A good bit of the time
- ❑ Some of the time
- ❑ A little of the time
- ❑ None of the time

c. have you felt so down in the dumps nothing could cheer you up?
- ❑ All of the time
- ❑ Most of the time
- ❑ A good bit of the time
- ❑ Some of the time
- ❑ A little of the time
- ❑ None of the time

d. have you felt calm and peaceful?
- ❑ All of the time
- ❑ Most of the time
- ❑ A good bit of the time
- ❑ Some of the time
- ❑ A little of the time
- ❑ None of the time

e. did you have a lot of energy?
- ❑ All of the time
- ❑ Most of the time
- ❑ A good bit of the time
- ❑ Some of the time
- ❑ A little of the time
- ❑ None of the time

f. have you felt downhearted and blue?
- ❑ All of the time
- ❑ Most of the time
- ❑ A good bit of the time
- ❑ Some of the time
- ❑ A little of the time
- ❑ None of the time

g. did you feel worn out?
- ❑ All of the time
- ❑ Most of the time
- ❑ A good bit of the time
- ❑ Some of the time
- ❑ A little of the time
- ❑ None of the time

h. have you been a happy person?
- ❑ All of the time
- ❑ Most of the time
- ❑ A good bit of the time
- ❑ Some of the time
- ❑ A little of the time
- ❑ None of the time

i. did you feel tired?
- ❑ All of the time
- ❑ Most of the time
- ❑ A good bit of the time
- ❑ Some of the time
- ❑ A little of the time
- ❑ None of the time

10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc.)?
- ❑ All of the time
- ❑ Most of the time
- ❑ Some of the time
- ❑ A little of the time
- ❑ None of the time

11. How TRUE or FALSE is each of the following statements for you?

a. I seem to get sick a little easier than other people
- ❑ Definitely true
- ❑ Mostly true
- ❑ Don't know
- ❑ Mostly false
- ❑ Definitely false

b. I am as healthy as anybody I know
- ❑ Definitely true
- ❑ Mostly true
- ❑ Don't know
- ❑ Mostly false
- ❑ Definitely false

c. I expect my health to get worse
- ❑ Definitely true
- ❑ Mostly true
- ❑ Don't know
- ❑ Mostly false
- ❑ Definitely false

d. My health is excellent
- ❑ Definitely true
- ❑ Mostly true
- ❑ Don't know
- ❑ Mostly false
- ❑ Definitely false

APPENDIX I: Speech, Spatial and Qualities of Hearing Scale

Please see attached PDF.

APPENDIX J: Tinnitus Reaction Questionnaire (TRQ) [a]

The study team member administering the TRQ tool to subjects must review responses at the time that the tool is completed. If items numbered 8, 19, 22, and/or 24 are endorsed with a 3 or 4, the local PI should be immediately notified and a psychologist, social worker or psychiatry provider from the hospital should come to talk to the subject prior to them leaving the clinical setting that day.

Number	Item	Scores[b]				
1	My tinnitus has made me unhappy.	0	1	2	3	4
2	My tinnitus has made me feel tense.	0	1	2	3	4
3	My tinnitus has made me feel irritable.	0	1	2	3	4
4	My tinnitus has made me feel angry.	0	1	2	3	4
5	My tinnitus has led me to cry.	0	1	2	3	4
6	My tinnitus has led me to avoid quiet situations.	0	1	2	3	4
7	My tinnitus has made me feel less interested in going out.	0	1	2	3	4
8	My tinnitus has made me feel depressed.	0	1	2	3	4
9	My tinnitus has made me feel annoyed.	0	1	2	3	4
10	My tinnitus has made me feel confused.	0	1	2	3	4
11	My tinnitus “driven me crazy”.	0	1	2	3	4
12	My tinnitus interfered with my enjoyment of life.	0	1	2	3	4
13	My tinnitus made it hard for me to concentrate.	0	1	2	3	4
14	My tinnitus has made it hard for me to relax.	0	1	2	3	4
15	My tinnitus has made feel distressed.	0	1	2	3	4
16	My tinnitus has me feel helpless.	0	1	2	3	4

17	My tinnitus has made me feel frustrated with things.	0	1	2	3	4
18	My tinnitus has interfered with my ability to work.	0	1	2	3	4
19	My tinnitus has led me to despair.	0	1	2	3	4
20	My tinnitus has led me to avoid noisy situations.	0	1	2	3	4
21	My tinnitus has led me to avoid social situations.	0	1	2	3	4
22	My tinnitus has made me feel hopeless about the future.	0	1	2	3	4
23	My tinnitus has interfered with my sleep.	0	1	2	3	4
24	My tinnitus led me to think about suicide.	0	1	2	3	4
25	My tinnitus has made me feel panicky.	0	1	2	3	4
26	My tinnitus has made me feel tormented.	0	1	2	3	4

a	From Wilson et al, 1991
b	0 = not at all, 1 = a little of the time, 2 = some of the time, 3 = a good deal of the time, and 4 = almost all of the time.

APPENDIX K: Evaluation of Vestibular Function (for Subjects Enrolled at NCI only)

Subjects enrolled at the NCI will be evaluated for effects of bevacizumab on vestibular function. As most NF2 VS originate from the vestibular nerve, it is possible that treatment with bevacizumab will result in a change and possibly in improvement of vestibular function. This would be of clinical significance since, until now, loss of vestibular function has been largely left untreated and undermanaged in patients with NF2. Similar to a loss of hearing, a loss of vestibular function can significantly compound a patient's ability to adequately function in daily life, leading to social isolation and underemployment.

Vestibular Research in NF2: Etiology of Vestibular Schwannomas

Most studies investigating the audiological effects of NF2 tumors have focused on hearing sensitivity rather than vestibular function. To date, only a few studies have attempted to characterize the NF2 vestibular phenotype. Sporadic acoustic neuromas are thought to most often originate from the inferior branch of the vestibular nerve (IBVN) rather than the superior branch (SBVN). In contrast, the affinity of vestibular schwannomas associated with NF2 to one particular nerve branch is essentially unknown. Wang et al (2005) provide evidence that the origin of most NF2-related VSs is the SBVN. This finding has been supported by Slattery et al (1998) who documented a 19% occurrence rate for VSs originating from the IBVN in a series of patients who underwent surgical resection of the tumor. Wang et al (2005) proposed that tests dependent upon superior branch vestibular nerve function, such as the caloric test, were often significantly impacted when tumor size was medium or larger (>1cm). However, vestibular tests dependent upon IBVN function were only impacted when tumor size was large (>3cm) and may be able to serve as an indicator of the degree of NF2 infiltration without surgical examination. In light of this data supporting an affinity for NF2 tumors to originate from the SBVN, combined with better clinical data being derived from the IBVN, a combined approach evaluating both branches of the vestibular nerve is warranted.

Vestibular Tests of Inferior Vestibular Nerve Branch Function

Vestibular tests investigating the IBVN are somewhat uncommon and not regularly performed in the clinical setting. The IBVN primarily innervates the posterior semicircular canal and the saccular end organs of the peripheral vestibular system on each side. Until recently, there was not any direct means by which these end organs and the IBVN could effectively and individually be stimulated and subsequently evaluated. The vestibular evoked myogenic potential (VEMP) is an inhibitory sacculocollic reflex recorded from the ipsilateral sternocleidomastoid muscle in response to acoustic stimulation of the saccule (Timmer et al, 2006). Strong evidence supports a saccular stimulation resulting in subsequent IBVN activation during this test. Studies within the past decade have proven the VEMP to be a reliable and effective measure of both saccular and IBVN integrity.

Vestibular Tests of Superior Vestibular Nerve Branch Function

Vestibular tests investigating the SBVN are far more common and regularly performed on a routine basis. In particular, computerized controlled rotational testing primarily investigates the physiologic response of the horizontal semicircular canal and indirectly, the SBVN. In addition, caloric irrigations during videonystagmography also indirectly investigate the SBVN by directly stimulating the horizontal semicircular canal. Between these two measures, caloric testing exhibits significantly less intertest reliability and subsequently is a less desirable measure during repeated study designs. On the other hand, rotational testing offers a high degree of reliability and repeatability, which makes it an excellent test to monitor vestibular function over a period of time.

Purpose of the Study and Research Question

Purpose of the Study

The purpose of the study is to determine whether or not a change in vestibular function is observed in patients with NF2 over the course of treatment with bevacizumab. Vestibular function will be assessed at baseline using measures sensitive to IVBN and SBVN function.

These same measures will be repeated during the course of bevacizumab treatment at the same time as hearing is evaluated (Section 10, study calendar).

Vestibular function will be evaluated using the following procedures:

Rotational Vestibular Testing. Eye movements will be recorded during rotational testing in a lightproof enclosure via binocular infrared video goggles. The patient will be seated in the rotational chair and belted securely in place using a three-point harness. Audio communication between the patient and the tester will occur during the entire test through a two way head set and a video camera will allow the examiner to view the patient throughout the test. Eye movements will be observed and recorded during sinusoidal harmonic acceleration (SHA) of the rotary chair at the following speeds: 0.01, 0.02, 0.04, 0.08, 0.16, 0.32, 0.64, 1.28, and 2.0 Hz. Eye movements will also be observed and recorded during velocity step testing (VST) in which the rotary chair accelerates to a sustained velocity rotation in both the clockwise and counter-clockwise directions at 240 degrees per second. During both SHA and VST testing, eye movements will be recorded in response to the various rotational accelerations of the chair.

Vestibular evoked myogenic potentials (VEMP). VEMP waveforms will be recorded via surface electrodes placed on the sternocleidomastoid (SCM) muscle, sternum, and mastoid. Low frequency, short duration tone bursts at 500 Hz will be delivered to the ear via insert earphones to elicit a vestibular response. Signal levels will not exceed those used in standard audiometric testing and will range from 85-107 dB HL. During signal delivery, patients are instructed to maintain SCM muscle contraction by holding the head slightly elevated from a supine or semi-recumbent position for 1-3 minutes. The level of SCM contraction will be monitored by an EMG monitoring system during administration of the test, providing patients with feedback that will allow them to adjust the amount of contraction to achieve muscle tension within an optimal EMG range. The delivery of the acoustic stimulus and the EMG monitoring of the SCM muscles using surface electrodes adds no additional risk to the patient with the exception of possible slight physical fatigue of the neck muscles while maintaining contraction of the SCM muscle.

Test Protocol

In addition to all hearing measures, vestibular assessment using SHA, VST and VEMP will be conducted on the same day as, and to follow each scheduled follow-up audiology appointment. Furthermore, SHA, VST and VEMP testing will be conducted on two concurrent occasions during the initial appointment (week 0 baseline), mid-study appointment (week 24) and final appointment (48 week) in order to document intrasubject variability of each test.

APPENDIX L: Patient Experience in an Experimental Therapeutic Clinical Trial

To explore how patient involvement in an experimental therapeutic clinical trial might be impacting perceived quality of life, and vice versa, we will pose three open-ended questions to all subjects enrolled in this trial following enrollment and seven open-ended questions after completing 49 weeks of treatment. If the subject is between the ages of 12 and 17 years, the questions will also be posed to one of the parents/guardians of the subject enrolled.

The first set of questions will be asked of participants between the date that they formally consent to participate in the trial and the first pre-study brain MRI (4 days prior to beginning on trial). The second set of questions will be asked of participants between the date of their 49 week visit and the date of their off-study visit. All subjects will be interviewed by the same examiner. Questions will be posed by phone for all participants not available to meet in person with the examiner.

All responses will be taped, transcribed and analyzed for thematic content. The first set of questions are listed here:

1. Why are you participating in this trial?

2. If you were to make a prediction, do you think that you will have a positive response to bevacizumab? Why or why not?

3. How would you describe the impact that NF2 has had on your life so far?

The second set of questions are listed here:

1. Do you feel that you had improvement in your symptoms with bevacizumab? If so, please describe the improvements and the impact of any improvements on your daily life.
2. Did you have any side effects while getting bevacizumab? If so, please describe what side effects you experienced and how troubling they were to you? Did they continue for the length of the study?
3. Did you ever consider not continuing to take bevacizumab because of side effects? Why or why not?
4. Now knowing what it is like to receive bevacizumab every three weeks along with the monitoring studies, would you choose to do it again? Why or why not?
5. What are the most important factors in that decision?
6. How would you describe the amount of time, energy and money that being in this study required from you?
7. Did you experience any times during the trial when you didn't want to continue taking bevaciuzmab? Why or why not?

APPENDIX M: Patient Eligibility Checklist

PATIENT ELIGIBILITY CHECKLIST

Patient Name _____ _____ DOB _____

Medical Record # _____ Site (circle one) JHU MGH NCI

Date eligibility screened: _____ By whom? _____

	American Indian or Alaskan Native	Asian or Pacific Islander	Black, not of Hispanic Origin	Hispanic	White, Not of Hispanic Origin	Other or Unknown
Male						
Female						

Please ask each question to the patient being screened and circle the answer given. If the answer indicates that the patient is not eligible, you may suspend screening at that point in the questionnaire.

1.Is this patient 12 years of age or older?

YES (continue) NO (not eligible)

2.By which mechanism does this patient meet criteria for NF2?

Germline mutation (continue)
NIH criteria (continue)
Manchester criteria (continue)
Does not meet criteria for NF2 (not eligible)

3.Does this patient have at least one vestibular schwannoma (VS) that is $\geq$1.5 cm (on longest diameter) on a contrast-enhanced cranial MRI scan with fine cuts through the internal auditory canal (3 mm slices, no skip)?

YES (continue) NO (not eligible)

4.Does this patient have a history of progressive hearing loss over the past 24 months?

YES (continue) NO (not eligible)

5.Is this patient currently pregnant or breast-feeding?

YES (not eligible) NO (continue)

6.Can this patient provide written informed consent (age 18 or older) or assent (age 12-17) with a parent/guardian willing and able to give written consent?

YES (continue) NO (not eligible)

7. Has this patient ever been treated with bevacizumab or other VEGF targeting therapies?

YES (not eligible) NO (continue)

8.Does this patient have a life expectancy of 6 months or greater?

YES (continue) NO (not eligible)

9.Has this patient had a discussion of all available treatment options, including risks and benefits, for their VS including surgery, radiation therapy, observation, and other clinical trials?

YES (continue) NO (not eligible)

10. Is this patient's VS not amenable to surgery or has this patient refused surgery for their VS?

YES (continue) NO (not eligible)

11. Is this patient currently in need of radiation therapy, surgery or medical treatment for any other NF2-associated tumors (i.e. ependymoma, non-vestibular schwannoma, meningioma)?

YES (not eligible) NO (continue)

12. Does this patient have clinically significant cardiovascular disease, such as:

7 Inadequately controlled hypertension Inadequately controlled HTN (adult subjects: SBP > 160 mmHg and/or DBP > 90 mmHg despite antihypertensive medication, pediatric subjects: Requirement for antihypertensive treatment prior to enrollment, or diastolic blood pressure >95th percentile for age –Appendix G))

8 History of CVA within 12 months

9 Myocardial infarction or unstable angina within 12 months

10 New York heart association grade II or greater congestive heart failure

11 Serious and inadequately controlled cardiac arrhythmia

12 Significant vascular disease (e.g. aortic aneurysm, history of aortic dissection)

13 Clinically significant peripheral vascular disease

YES (not eligible) NO (continue)

13. Does this patient have any uncontrolled intercurrent illness, including but not limited to, ongoing or active infection, or psychiatric illness/social situations that would limit compliance with study requirements?

YES (not eligible) NO (continue)

14. Does this patient currently take anti-coagulant drugs (not including prophylactic doses)?

YES (not eligible) NO (continue)

15. Does this patient have a personal history of coagulopathy (i.e. ITP or other autoimmune hematologic condition) or current evidence of bleeding diathesis or coagulopathy?

YES (not eligible) NO (continue)

16. Does this patient have a history of spontaneous or symptomatic intracranial hemorrhage?
YES (not eligible) NO (continue)

17. Does this patient have any newly identified hemorrhage within the past 6 months?
YES (not eligible) NO (continue)

18. Does this patient have a serious or non-healing wound, ulcer, or bone fracture?
YES (not eligible) NO (continue)

19. Does this patient have a history of abdominal fistula, gastrointestinal perforation or intra-abdominal abscess within the past 6 months?
YES (not eligible) NO (continue)

20. Has this patient had an invasive procedure, including surgery, biopsy, or intra-arterial procedure, within the past 28 days?
YES (not eligible) NO (continue)

21. Has this patient stopped all chemotherapy and anti-cancer therapy for at least 4 weeks?
YES (continue) NO (not eligible)

22. Is this patient taking any other investigational agents currently?
YES (not eligible) NO (continue)

23. Has this patient taken any nitrosoureas or mitomycin C within the past 6 weeks?
YES (not eligible) NO (continue)

24. Does this patient have known sensitivity to Chinese hamster ovary cell products, other recombinant human antibodies, or compounds of similar chemical or biologic composition to bevacizumab?
YES (not eligible) NO (continue)

25. Is this patient able to tolerate periodic MRI scans and gadolinium contrast without the need for general anesthesia?
YES (continue) No (not eligible)

26. Is this patient of childbearing potential?
YES (continue to question 27) NO (continue to question 28)

27. Has this patient agreed to use birth control/contraception for the length of this trial?
YES, method _____ (continue) NO (not eligible)

Note: you must submit a copy of the source documents to verify each of the following results

28. Does this patient have a word recognition score of less than 90% in the target ear related to their VS (i.e. not due to prior interventions such as surgery and radiation) as assessed by an audiologist associated with this study?

YES (continue) NO (not eligible)

29. Does this patient have a Karnofsky or Lansky performance status of $\geq$ 60% (i.e. can the patient care for himself/herself with occasional help from others)?

YES (continue) NO (not eligible)

30. Please provide documentation of the following (if any of these lab results cannot be documented, the patient is not eligible for this study):

1. absolute neutrophil count $\geq$ 1,500/mcL
2. platelet count $\geq$ 100,000/mcL
3. leukocytes $\geq$ 3,000/mcL
4. urine protein creatinine (UPC) ratio < 0.5 OR urine dipstick protein <2+
5. total bilirubin $\leq$ twice the upper limit of institutional normal
6. AST (SGOT)/ALT (SGPT) $\leq$ 2.5 times ULN
7. creatinine clearance or radioisotope GFR $\geq$60ml/min/1.73 m^2 OR a normal serum creatinine based on age described in the table below:

Age (years)	Maximum Serum Creatinine (mg/dL)
$\leq$5	0.8
5<age$\leq$10	1.0
10<age$\leq$15	1.2
>15	1.5

31. Does this patient have any collision tumors that make volumetric measurement of the target VS not possible to perform?

YES (not eligible) No (continue)

32. Does this patient have a documented negative pregnancy test?

NOT APPLICABLE (patient is male OR not of childbearing potential)
YES (eligible)
NO (not eligible)

Eligibility screened by: _____ Date: _____

(signature of person completing screening)

If question 32 indicates that this patient is eligible for the study, please see the Study Manual for directions on registering this patient on the study.

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