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ORIGINAL REPORT

Efficacy and Biomarker Study of Bevacizumab for Hearing Loss Resulting From Neurofibromatosis Type 2–Associated Vestibular Schwannomas

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ABSTRACT

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

Patients and Methods
Bevacizumab 7.5 mg/kg was administered every 3 weeks for 46 weeks, followed by 24 weeks of surveillance after treatment with the drug. The primary end point was hearing response defined by word recognition score (WRS). Secondary end points included toxicity, tolerability, imaging response using volumetric magnetic resonance imaging 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, with a median age of 30 years (range, 14 to 79 years) and progressive hearing loss in the target ear (median baseline WRS, 60%; range 13% to 82%), were enrolled. The primary end point, confirmed hearing response (improvement maintained ≥ 3 months), occurred in five (36%) of 14 patients (95% CI, 13% to 65%; $P < .001$). Eight (57%) of 14 patients had transient hearing improvement above the 95% CI for WRS. No patients experienced hearing decline. Radiographic response was seen in six (43%) of 14 target VSs. 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 vascular endothelial growth factor (not bound to bevacizumab) and increased placental growth factor in plasma. Hearing responses were inversely associated with baseline plasma hepatocyte growth factor ($P = .019$). Imaging responses were associated with high baseline tumor vessel permeability and elevated blood levels of vascular endothelial growth factor D and stromal cell–derived factor 1α ($P = .037$ and .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 (VSs) are histologically benign tumors of the eighth nerve that result in hearing loss, imbalance, and brainstem compression. VSs are common, with roughly 3,000 new cases per year in the United States.[1] Surgery and radiation therapy (RT) achieve sustained control in more than 95% of sporadic, unilateral VSs.[2-4] Germline inactivation of the gene *NF2* results in the rare tumor syndrome neurofibromatosis type 2 (NF2), characterized by a bilateral VS and multiple additional schwannomas, meningiomas, and ependymomas.[5-7] NF2-associated VSs cause higher morbidity because

they are bilateral,[8-10] multilobular,[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 VSs express vascular endothelial growth factor (VEGF; or VEGF-A).[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 immunoglobulin G1 monoclonal blocking antibody specific for VEGF. Anecdotal experience with 31 individuals with NF2-associated VSs treated with bevacizumab showed hearing improvement in 57%, 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 prospectively confirm the hearing response (HR) rate in a well-defined patient population with hearing loss resulting from NF2-associated VSs, define the duration of benefit during and after treatment with the drug, and 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 patients with NF2 and documented VS-associated hearing loss. The primary end point was the proportion of patients with confirmed HR in the target ear. Secondary end points included the durability of HR, HR in nontarget evaluable ears, change in VS volumetric magnetic resonance imaging (MRI) measures compared with 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 National Cancer Institute Cancer Therapy Evaluation Program. Bevacizumab was supplied by Genentech through a Clinical Research and Development Agreement with the Cancer Therapy Evaluation Program. All patients or their legal guardians provided informed consent.

Patients age 12 years or older meeting National Institutes of Health 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) lower than 90%, were eligible. Exclusion criteria included prior antiangiogenesis therapy, medical conditions incompatible with bevacizumab, and tumors not amenable to volumetric MRI analysis (Data Supplement).

Bevacizumab was administered intravenously at 7.5 mg/kg every 3 weeks for 16 doses. Patients were then assessed for 24 weeks after treatment with the drug (Fig 1).

The target tumor was the VS causing documented active, progressive hearing loss. Audiology examinations were performed at baseline, at weeks 13, 25, 49, and 60, and after study treatment. WRS was assessed with a 100-word list of monosyllables delivered via standardized methodology at a sound level determined to yield the optimal score for each participant.[28,29] The 95% (P = .05) critical difference table defined statistically significant increased WRS (HR) or decreased WRS (hearing decline; Appendix Table A1, online only).[14,28,30] Confirmed HR was defined as an increase in WRS exceeding the 95% critical difference referenced to baseline and maintained across two evaluations over 3 months.

MRI of the brain was performed at baseline, at weeks 13, 25, and 49, and after study treatment. Anatomic and functional imaging protocols were standardized across all sites on a Siemens 3T Verio (Siemens Healthcare, Erlangen, Germany) with published protocols.[30,31] Volumetric analysis was performed centrally using the anatomic sequences by independent radiologists blinded to treatment.[31] Enhancing tumor volume was outlined on postcontrast 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 the VS. Change in VS volume compared with baseline was determined for the target and, when feasible, contralateral VSs. RR definitions were as follows: partial response (PR), decrease in tumor volume of 20% or more; minor response (MR), decrease in tumor volume of 5% to 19%; progressive disease (PD), increase in tumor volume of 20% or more; and stable disease (SD) for all others. RR was confirmed at 3 months. Functional MRI sequences, dynamic contrast-enhanced MRI to calculate K^{trans} (a measure of vascular permeability), and apparent diffusion coefficient (ADC) were processed using custom-made software in Matlab (MathWorks, Natick, MA), using published approaches.[32,33]

Adverse events (AEs) were graded and attributed to bevacizumab according the Common Terminology Criteria for Adverse Events (version 4.0) before infusion (every 3 weeks); physical examination was conducted every 6 weeks. Blood pressure was assessed weekly for the first 6 weeks and preinfusion thereafter. For patients younger than 18 years of age, bone toxicity was monitored with laboratory and imaging studies (Data Supplement).

Circulating biomarkers were evaluated in peripheral blood before, during (weeks 25 and 49), and after treatment (week 72). Plasma samples were obtained from fresh blood, aliquots were prepared, 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, sTie-2, interleukin (IL) -1β, IL-6, IL-8, and tumor necrosis factor-α, using multiplex enzyme-linked immunosorbent assay plates from Meso-Scale Discovery (Gaithersburg, MD). Hepatocyte growth factor (HGF), s-cMET, sVEGFR2, stromal cell–derived factor 1α (SDF1α),

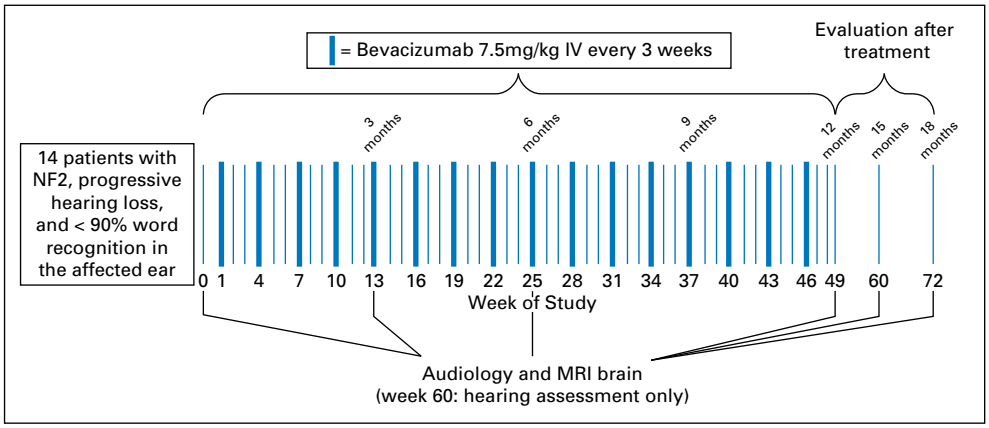

Fig 1. Trial schema. IV, intravenous; MRI, magnetic resonance imaging; NF2, neurofibromatosis type 2.

angiopoietin (Ang) 1 and Ang2, and carbonic anhydrase IX were measured using single-analyte enzyme-linked immunosorbent assay kits from R&D Systems (Minneapolis, MN). All samples were run in duplicate.

The primary end point was HR, defined as increased WRS above the 95% critical threshold and maintained across at least two time points compared with baseline WRS. Using a one-stage design based on a null hypothesis of response rate at 5%, a total of 14 patients with confirmed progressive hearing loss were estimated to yield more than 90% power to detect an alternative response rate of 50% at alpha level of 0.05. The trial required four 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% CIs. 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 two sided. All analyses were conducted using SAS software (version 9.2; SAS Institute, Cary, NC).

The percentage of changes in the blood and imaging biomarkers from before, during, and after 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 signed rank test was used to test the difference between HR and RR groups. Tumor reduction was calculated on the basis of the percentage of 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 patients (10 female), with a median age of 30 years (range, 14 to 79 years), were enrolled between November 2010 and August 2011 (Table 1). Eight participants had undergone prior surgery: six on the nontarget ear and two bilaterally. Three participants had received prior RT (one to target and two to nontarget VS) 15 to 120 months before receiving bevacizumab (Appendix Table A2, online only). All patients were evaluable for response and toxicity. Median baseline target ear WRS was 60% (range, 13% to 82%). Only four (28%) of 14 target ears had serviceable hearing (ie, class A or B) per the American Academy of Otolaryngology-Head and Neck Society Hearing Committee guidelines (Appendix Fig A1, online only).[34] Nine (64%) of 14 patients were anacusic in the nontarget ear.

Five of (36%) 14 patients (95% CI, 13% to 65%; $P < .001$) achieved the primary end point of confirmed HR in the target ear. This was achieved by week 13 in four of five patients and maintained continuously throughout treatment. No patient experienced hearing decline while receiving bevacizumab, despite progressive hearing loss being required for enrollment. Of the five patients evaluable for HR in the nontarget ear, four had confirmed HR (80%; 95% CI, 28% to 99%; Table 2). In total, nine (47%) of 19 evaluable ears (95% CI, 24% to 71%) achieved confirmed HR (Table 2). Pre- and post-treatment hearing scattergrams are presented in Appendix Figure A1.

Bevacizumab was stopped after 12 months to assess durability of response. Three (60%) of five patients with confirmed HR in the target ear maintained HR 6 months after treatment with study drug (Fig 2A). Similarly, two of four patients with confirmed HR in the nontarget ear maintained HR 6 months after treatment with study drug. In total, five of nine (target and nontarget) ears with confirmed HR maintained this for 6 months after treatment with study drug.

The median baseline target VS volume was 3.0 cc (range, 0.7 to 23 cc). The mean difference in volume across the two baseline assessments was 0.02 mL ($P = .83$), confirming the reproducibility of volumetric measurements. A total of 28 VSs (14 target and 14 contralateral) were evaluable for RR. PR at any

Table 1. Baseline Patient Demographics and Clinical Characteristics

Characteristic	No. (%) Total (N = 14)	Confirmed Hearing Response (n = 5)	No Confirmed Hearing Response (n = 9)	*P*
Age, years				.2
Median	30.5	26.0	32.0	
Range	14-79	14-33	14-79	
Sex				.6
Male	4 (29)	1 (20)	3 (33)	
Female	10 (71)	4 (80)	6 (67)	
Race				
White	12 (87)	3 (60)	9 (100)	.1
Karnofsky PS, %				.7
90	7 (50)	3 (60)	4 (44)	
70-80	7 (50)	2 (40)	5 (56)	
WRS in target ear, %				.7
Median	60.5	72.0	56.0	
Range	13-82	20-78	13-82	
Tumor volume in target ear, mL				.9
Median	3.0	2.3	3.4	
Range	0.7-23	1.2-22	0.7-23	

Abbreviations: PS, performance status; WRS, word recognition score.

Table 2. Hearing and Imaging Response Data During 12 Months of Bevacizumab Treatment

Response	Overall Response No.	Overall Response %	Confirmed Response* No.	Confirmed Response* %
Hearing				
Target ear	8 of 14	57	5 of 14	36
Contralateral ear	4 of 5	80	4 of 5	80
All ears	12 of 19	63	9 of 19	47
Imaging				
Target VS				
PR (≥ 20% decrease)	6 of 14	43	2 of 14	14
MR (5%–19% decrease)	4 of 14	29	7 of 14	50
SD	4 of 14	29	5 of 14	36
PD (≥ 20% increase)	0 of 14	0	0 of 14	0
Contralateral VS				
PR (≥ 20% decrease)	6 of 14	43	3 of 14	21
MR (5%–19% decrease)	6 of 14	43	6 of 14	43
SD	2 of 14	14	5 of 14	36
PD (≥ 20% increase)	0 of 14	0	0 of 14	14

Abbreviations: MR, minor response; PD, progressive disease; PR, partial response; SD, stable disease; VS, vestibular schwannoma.
*Confirmed response was defined as maintained across two evaluations at least 3 months apart.

time point was achieved in six (43%) of 14 target and six (43%) of 14 nontarget ears (Table 2). Confirmed PR (imaging response maintained across two evaluation time points) was seen in two (14%) of 14 target VSs (95% CI, 2% to 43%). Maximal reduction was 39.7% at week 49. Confirmed MR occurred in seven (50%) of 14 target VSs (95% CI, 23% to 77%). One person with confirmed MR had received RT to the target ear 10 years earlier, and theoretically, late recovery from RT could have influenced RR (Appendix Table A2). No VS meeting criteria for MR or PR at any time point developed PD while receiving treatment, but VSs with best response of SD developed PD at week 49 (Fig 2B). Regarding nontarget VSs, three (21%) of 14 tumors (95% CI, 5% to 51%) had confirmed PR, and six (43%) of 14 (95% CI, 18% to 71%) had confirmed MR. Of note, two patients in whom PR was achieved in nontarget VSs had received prior RT 15 and 48 months before enrollment, respectively, which could potentially have influenced RR. In total, five (18%) of 28 VSs (95% CI, 6% to 37%) achieved confirmed PR, and 13 (46%) of 28 (95% CI, 28% to 66%) VSs had confirmed MR. Of the 18 of 28 VSs with confirmed PR or MR, nine (50%) maintained durability of RR 6 months off of drug (Fig 2B).

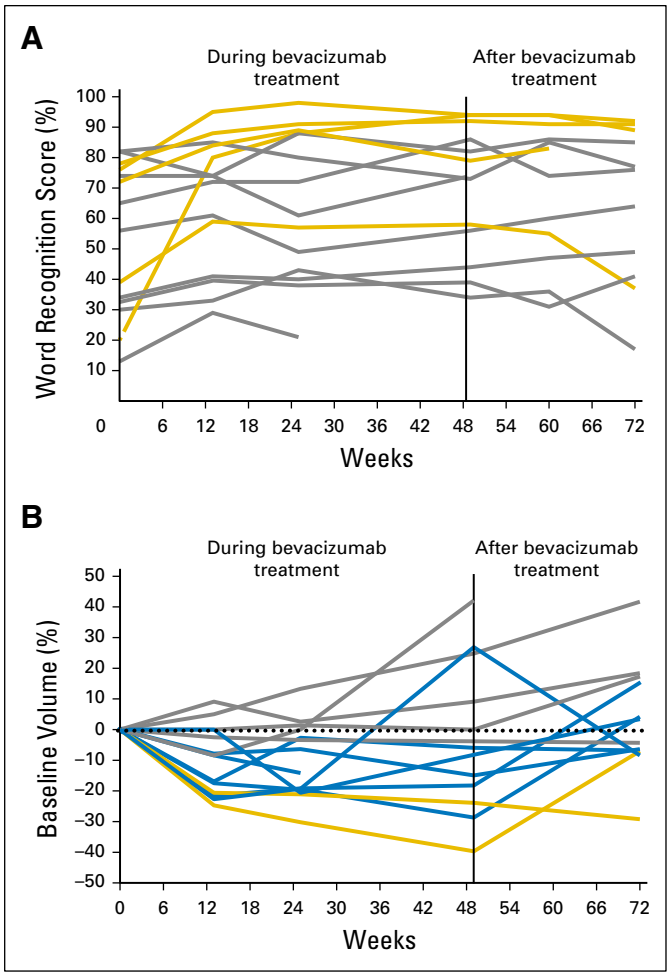

Fig 2. Changes in (A) word recognition score and (B) tumor volume during (through week 49) and after treatment (weeks 60 and 72) for target ears by best confirmed response: (A) yellow line, hearing response; grey line, stable disease; (B) grey line, stable disease; blue line, minor response; yellow line, partial response.

There was no significant correlation between HR and RR when analyzed by patient or by target VS ($r = 0.34$; 95% CI, −0.14 to 0.82; $P = .23$). There was also no significant correlation between WRS and tumor volume over time ($r = 0.287$; 95% CI, −0.29 to 0.71; $P = .32$). Finally, there was no significant relationship between HR and baseline factors including age, sex, or baseline WRS (Table 1).

There were 124 AEs possibly related to bevacizumab. Of these, 121 were classified as grade 1 to 2 (Table 3). The three grade 3 AEs were two episodes of hypertension that responded to monotherapy and one episode of idiopathic thrombocytopenia purpura that required treatment termination at week 16 but resolved after 6 months without the drug. A second patient discontinued treatment after 13 of 16 planned doses because of surgery required for another tumor. No bone toxicity occurred in the two patients age younger than 18 years. Three of seven female patients with normal menstruation at baseline developed grade 1 to 2 irregular menstruation that resolved after stopping treatment. There were 11 additional episodes of grade 1 to 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 VSs and nine contralateral VSs. 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 ($P = .019$), with a median decrease in ADC of 9% in patients 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 (n = 21; $P = .037$), and patients achieving RR in a target VS had higher baseline K^{trans} than nonresponders (0.30 *v* 0.07, respectively; $P = .051$).

Bevacizumab was associated with decreased plasma levels of free VEGF across all time points in all patients. At weeks 25 and 49, this was accompanied by increased levels of total VEGF, which significantly dropped after treatment (Fig 3; Appendix Table A3, online only). Bevacizumab was also associated with increased plasma levels of PlGF at all time points and of VEGF-D and SDF1α at week 49 (Fig 3; Appendix Table A3). Finally, bevacizumab was associated with a transient decrease in Ang2 levels at week 25 (Fig 3; Appendix Table A3).

HR was associated with lower baseline HGF ($P = .019$), decreased plasma carbonic anhydrase IX at weeks 25 and 49 ($P = .010$ and .035, respectively), and increased plasma sVEGFR2 at week 25 ($P = .004$; Appendix Table A4; Appendix Fig A2, online only). RR was associated with higher baseline levels of VEGF-D and SDF1α ($P = .037$ and .025, respectively), decreased s-cKIT at week 25 ($P = .023$), and decreased sTie2 at week 49 ($P = .034$; Appendix Table A5, online only).

DISCUSSION

The most common and universally life-altering consequence of NF2 is hearing loss, with the majority of affected individuals

Table 3. Total AEs Possibly, Probably, or Definitively Related to Bevacizumab in Patients With NF2 and Progressive VSs (N = 14)

AE	No. (%)		
	Grade 1	Grade 2	Grade 3
Abdominal pain	2 (14)		
ALT increased	5 (36)	3 (21)	
Allergic rhinitis	1 (7)		
Anemia	1 (7)		
Anorexia	1 (7)	1 (7)	
AST 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)		

Abbreviations: AE, adverse event; CPK, creatine phosphokinase; VS, vestibular schwannoma.
*Events that could only have occurred among seven female patients. Percentage represents No. of events occurring among seven female patients with baseline normal menstruation.

experiencing progression to deafness in their third decade.[5,6,8,10] Bevacizumab administered 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, much uncertainty remains regarding the optimal patient population, dosing strategy, and long-term durability. The results of this prospective efficacy study have confirmed the proportion of patients with NF2 and symptomatic VS who achieved durable HR with bevacizumab (36%; 95% CI, 13% to 65%; $P < .001$) as well as the durability of response during and after treatment and presented several candidate biomarkers that may ultimately allow rational selection of patients for therapy.

HR was selected as the primary end point because it is clinically meaningful and provides evidence of drug activity, given that durable hearing improvement with NF2-associated VSs 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 overcome concerns about spurious HR and with the awareness that NF2-associated VSs are chronic tumors for which short-term efficacy would have little value. Natural history data show that only 16% of people with NF2 have spontaneous HR if baseline WRS is lower than 90%.[8] The finding of a 36% (95% CI, 13% to 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 the results of 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 three serious AEs, and three of seven female patients who had normal menstruation at baseline developed menstrual irregularities. However, all women recovered baseline menstrual function after stopping treatment with 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 patients who achieved HR in any ear maintained this response for up to 6 months after treatment with study drug. Similar durability was seen with RR. These results suggest that after HR is achieved, multiweek 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 suggested alternative dosing strategies may be more efficacious based on markers of vessel normalization and oxygenation.[35] Analysis of blood markers in our study was also consistent with this hypothesis. Specifically, we saw unexpectedly high baseline VEGF levels, comparable to those in brain cancer.[32,33] Second, 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 nonmalignant tumor syndromes like NF2. Third, 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

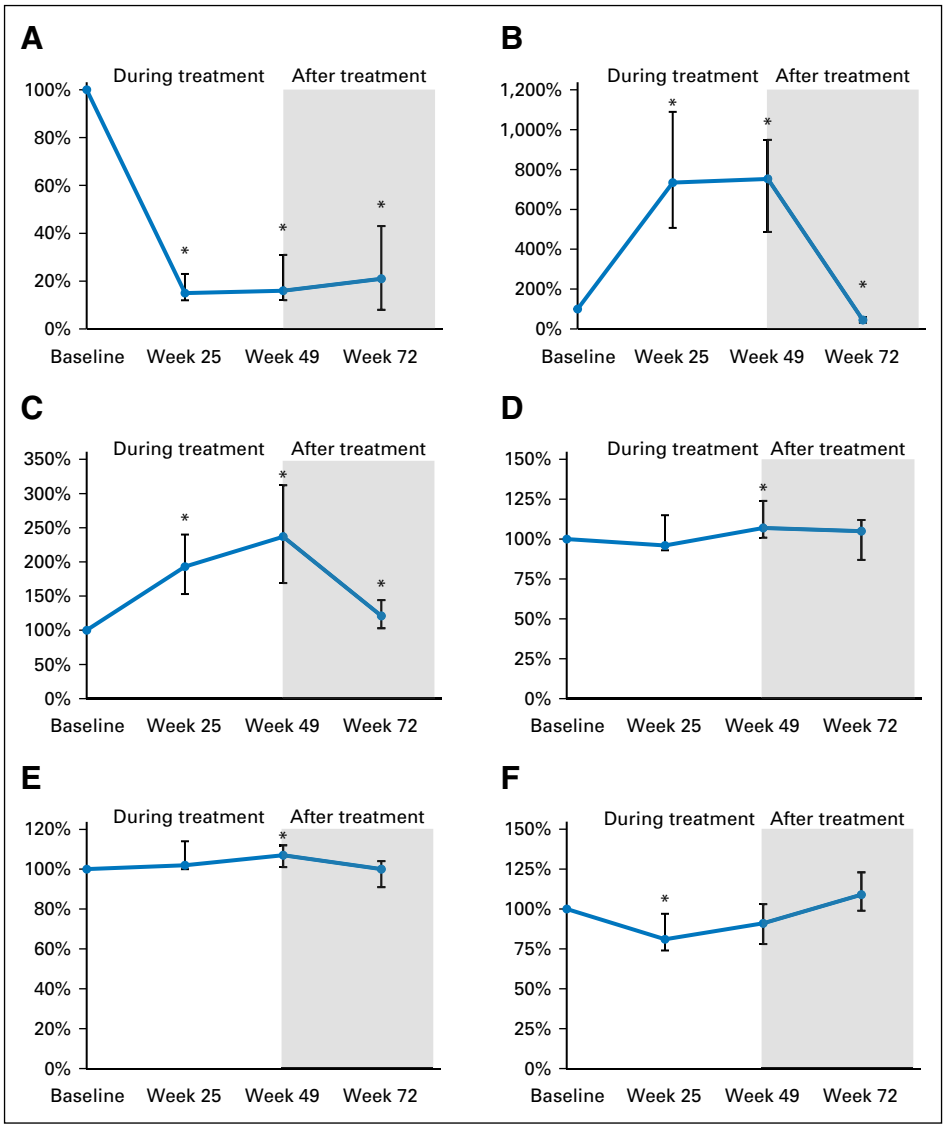

Fig 3. Line graphs showing changes over time in plasma (A) free vascular endothelial growth factor (VEGF), (B) total VEGF (free plus bound), (C) placental growth factor (PlGF), (D) VEGF-D, (E) stromal cell–derived factor 1α (SDF1α), and (F) angiopoietin 2 for all participants (N = 14). Anti-VEGF therapy with bevacizumab decreased the plasma levels of free VEGF and increased the levels of antibody-bound VEGF, PlGF, and SDF1α in patients with neurofibromatosis type 2. Vertical bars indicate interquartile range; (*) indicates $P <$ .05.

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 VSs 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 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 because, first, similar patterns are observed with antiangiogenesis therapy in brain cancer; second, Ang2 and Tie-2 are important factors in proangiogenic pathways in general; and third, 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 VSs. 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 identify 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 resulting from an NF2-associated VS (ClinicalTrials.gov identifier NCT01767792) will further investigate the findings from this study.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Efficacy and Biomarker Study of Bevacizumab for Hearing Loss Resulting From Neurofibromatosis Type 2–Associated Vestibular Schwannomas

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Appendix

Table A1. Clinical Criteria for Definition of Hearing Response on the Basis of 100-Word Hearing Test

		Word Recognition Score During Study (%)	
Baseline Word Recognition Score (%)	95% Critical Difference (%)	Definition of Hearing Improvement	Definition of Hearing Decline
0	**0-3**	**≥ 4**	**NA**
1	**0-6**	**≥ 7**	**NA**
2	**0-8**	**≥ 9**	**NA**
3	**0-9**	**≥ 10**	**NA**
4	**1-11**	**≥ 12**	**0**
5	**1-12**	**≥ 13**	**0**
6	**1-14**	**≥ 15**	**0**
7	2-15	≥ 16	≤ 1
8	2-17	≥ 18	≤ 1
9	3-18	≥ 19	≤ 2
10	4-19	≥ 20	≤ 3
11	4-21	≥ 22	≤ 3
12	5-22	≥ 23	≤ 4
13	6-23	≥ 24	≤ 5
14	6-25	≥ 26	≤ 5
15	7-26	≥ 27	≤ 6
16	8-27	≥ 28	≤ 7
17	8-28	≥ 29	≤ 7
18	9-29	≥ 30	≤ 8
19	10-31	≥ 32	≤ 9
20	11-32	≥ 33	≤ 10
21	11-33	≥ 34	≤ 10
22	12-34	≥ 35	≤ 11
23	13-35	≥ 36	≤ 12
24	14-36	≥ 37	≤ 13
25	14-37	≥ 38	≤ 13
26	15-39	≥ 40	≤ 14
27	16-40	≥ 41	≤ 15
28	17-41	≥ 42	≤ 16
29	18-42	≥ 43	≤ 17
30	19-43	≥ 44	≤ 18
31	19-44	≥ 45	≤ 18
32	20-45	≥ 46	≤ 19
33	21-46	≥ 47	≤ 20
34	22-47	≥ 48	≤ 21
35	23-48	≥ 49	≤ 22
36	24-49	≥ 50	≤ 23
37	25-50	≥ 51	≤ 24
38	26-51	≥ 52	≤ 25
39	26-52	≥ 53	≤ 25
40	27-53	≥ 54	≤ 26
41	28-54	≥ 55	≤ 27
42	29-55	≥ 56	≤ 28
43	30-56	≥ 57	≤ 29
44	31-57	≥ 58	≤ 30
45	32-58	≥ 59	≤ 31
46	33-59	≥ 60	≤ 32
47	34-60	≥ 61	≤ 33
48	35-61	≥ 62	≤ 34
49	36-62	≥ 63	≤ 35
50	37-63	≥ 64	≤ 36
51	38-64	≥ 65	≤ 37
52	39-65	≥ 66	≤ 38
53	40-66	≥ 67	≤ 39
54	41-67	≥ 68	≤ 40
55	42-68	≥ 69	≤ 41
56	43-69	≥ 70	≤ 42
57	44-70	≥ 71	≤ 43
58	45-71	≥ 72	≤ 44
59	46-72	≥ 73	≤ 45
60	47-73	≥ 74	≤ 46
61	48-74	≥ 75	≤ 47
62	49-74	≥ 75	≤ 48

(continued on following page)

Table A1. Clinical Criteria for Definition of Hearing Response on the Basis of 100-Word Hearing Test (continued)

		Word Recognition Score During Study (%)	
Baseline Word Recognition Score (%)	95% Critical Difference (%)	Definition of Hearing Improvement	Definition of Hearing Decline
63	50-75	≥ 76	≤ 49
64	51-76	≥ 77	≤ 50
65	52-77	≥ 78	≤ 51
66	53-78	≥ 79	≤ 52
67	54-79	≥ 80	≤ 53
68	55-80	≥ 81	≤ 54
69	56-81	≥ 82	≤ 55
70	57-81	≥ 82	≤ 56
71	58-82	≥ 83	≤ 57
72	59-83	≥ 84	≤ 58
73	60-84	≥ 85	≤ 59
74	61-85	≥ 86	≤ 60
75	63-86	≥ 87	≤ 62
76	64-86	≥ 87	≤ 63
77	65-87	≥ 88	≤ 64
78	66-88	≥ 89	≤ 65
79	67-89	≥ 90	≤ 66
80	68-89	≥ 90	≤ 67
81	69-90	≥ 91	≤ 68
82	71-91	≥ 92	≤ 70
83	72-92	≥ 93	≤ 71
84	73-92	≥ 93	≤ 72
85	74-93	≥ 94	≤ 73
86	75-94	≥ 95	≤ 74
87	77-94	≥ 95	≤ 76
88	78-95	≥ 96	≤ 77
89	79-96	≥ 97	≤ 78
90	**81-96**	**≥ 97**	**≤ 80**
91	**82-97**	**≥ 98**	**≤ 81**
92	**83-98**	**≥ 99**	**≤ 82**
93	**85-98**	**≥ 99**	**≤ 84**
94	**86-99**	**100**	**≤ 85**
95	**88-99**	**100**	**≤ 87**
96	**89-99**	**100**	**≤ 88**
97	**91-100**	**NA**	**≤ 90**
98	**92-100**	**NA**	**≤ 91**
99	**94-100**	**NA**	**≤ 93**
100	**97-100**	**NA**	**≤ 96**

NOTE. Upper and lower limits for the 95% critical differences for percentage scores are adapted from Thornton and Raffin.[27] Patients with baseline word recognition scores greater than 90% or lower than 6% (bold font) were ineligible for the study because of ceiling and floor effects, respectively.
Abbreviation: NA, not applicable.

Table A2. Timing and Form of Prior Therapy for Participants Undergoing Surgery or RT for Target or Nontarget VS Before Initiating Bevacizumab (n = 8 of 14)

Target Ear Surgery (year)	Time From Surgery to Bevacizumab (months)	RT to Target Ear (year)	Time From RT to Bevacizumab (months)	Nontarget Ear Surgery (year)	Time From Surgery to Bevacizumab (months)	RT to Nontarget Ear (year)	Time to Bevacizumab From RT (months)
				1992	216		
				1972	468		
2006	60			1996	180		
				2010	5	2007 (24 Gy)	48
				2006	58		
						2010 (13 Gy)	15
				2008	36		
1996	180	2001 (40 Gy)	120	1996	180		
				2009	22		

Abbreviations: RT, radiation therapy; VS, vestibular schwannoma.

Table A3. Blood Circulating Biomarker Levels at Baseline and Changes During Treatment in Patients With NF2-Associated VSs

Biomarker	Median (IQR)			
		Percent Change		
		During Treatment		
	Pretreatment Baseline (pg/ml; n = 13)	Week 25 (n = 13)	Week 49 (n = 12)	Post-Treatment Week 72 (n = 11)
Free VEGF	182 (107-237)	**0.15 (0.12-0.23)**	**0.16 (0.12-0.31)**	**0.21 (0.80-0.43)**
P	NA	**< .001**	**< .001**	**< .001**
Total VEGF (free plus bound)	131 (68-197)	**7.34 (5.07-10.89)**	**7.53 (4.87-9.49)**	**0.45 (0.32-0.60)**
P	NA	**< .001**	**< .001**	**< .001**
PlGF	35 (33-41)	**1.93 (1.53-2.40)**	**2.37 (1.69-3.12)**	**1.21 (1.03-1.44)**
P	NA	**.0034**	**< .001**	**.014**
VEGF-C	234 (190-316)	0.88 (0.71-1.35)	1.20 (0.62-1.65)	0.70 (0.41-0.81)
P	NA	.95	.68	.054
VEGF-D	1,019 (879-1,366)	0.96 (0.93-1.15)	**1.07 (1.01-1.24)**	1.05 (0.87-1.12)
P	NA	.68	**.034**	.58
sVEGFR1	79 (64-94)	0.89 (0.80-1.12)	0.92 (0.79-1.31)	0.86 (0.82-0.95)
P	NA	.68	.97	.068
sVEGFR2	11,108 (9,296-12,065)	0.95 (0.93-1.05)	0.92 (0.88-1.05)	0.96 (0.86-1.13)
P	NA	.50	.38	.64
bFGF	79 (43-93)	1.02 (0.48-1.18)	1.28 (0.69-1.57)	0.50 (0.24-0.74)
P	NA	.38	.27	.054
Ang1	5,274 (3,996-6,953)	1.02 (0.48-1.18)	1.28 (0.73-2.26)	0.58 (0.29-0.95)
P	NA	1.00	.23	.067
Ang2	1,654 (1,422-1,938)	**0.81 (0.74-0.97)**	0.91 (0.78-1.03)	1.09 (0.99-1.23)
P	NA	**.0093**	.23	.10
sTie2	6,937 (5,773-8,271)	0.97 (0.87-1.03)	0.89 (0.85-1.03)	1.00 (0.90-1.21)
P	NA	.19	.34	.83
HGF	848 (772-1,016)	1.01 (0.90-1.20)	1.06 (1.01-1.31)	1.09 (0.97-1.17)
P	NA	.45	.052	.24
s-cMet	1,349 (1,186-1,478)	0.97 (0.92-1.06)	0.99 (0.93-1.04)	1.00 (0.96-1.03)
P	NA	.38	.678	.90
CAIX	47 (32-52)	1.84 (0.75-2.16)	1.52 (0.64-2.11)	1.16 (0.64-1.71)
P	NA	.094	.052	.24
IL-1α	1.4 (1.0-2.4)	0.97 (0.84-1.07)	1.15 (0.72-3.02)	1.03 (0.70-1.39)
P	NA	.64	.34	.70
IL-6	2.4 (1.9-3.1)	1.23 (0.97-1.63)	1.01 (0.84-1.30)	1.07 (0.91-1.45)
P	NA	.094	.68	.21
IL-8	4.8 (3.3-5.8)	1.08 (0.88-1.23)	1.07 (0.81-1.41)	0.98 (0.67-1.24)
P	NA	.59	.52	.64
TNF-α	7.8 (7.5-1.0)	1.00 (0.91-1.06)	0.99 (0.85-1.23)	0.88 (0.71-1.30)
P	NA	.89	.68	1.00
SDF1α	1,972 (1,789-2,107)	1.02 (1.00-1.14)	**1.07 (1.01-1.12)**	1.00 (0.91-1.04)
P	NA	.38	**.0093**	.52

NOTE. Bold font indicates statistical significance. *P* values determined from Wilcoxon sign rank test for percentage of change after treatment.

Abbreviations: Ang, angiopoietin; bFGF, basic fibroblast growth factor; CAIX, carbonic anhydrase IX; HGF, hepatocyte growth factor; IL, interleukin; IQR, interquartile range; NA, not applicable; NF2, neurofibromatosis type 2; PlGF, placental growth factor; SDF1α, stromal cell–derived factor 1α; sVEGFR, soluble vascular endothelial growth factor receptor; TNF-α, tumor necrosis factor alpha; VEGF, vascular endothelial growth factor; VS, vestibular schwannoma.

Table A4. Association Between Blood Circulating Biomarker Levels in Hearing Responders (n = 5) Versus Nonresponders (n = 8) Treated With Bevacizumab

	Median (IQR)							
			Percent Change					
	Baseline (pg/ml), n=13		Week 25 (n = 13)		Week 49 (n = 12)		Week 72 (n = 11)	
Biomarker	Responders (n = 5)	Nonresponders (n = 8)	Responders (n = 5)	Nonresponders (n = 8)	Responders (n = 5)	Nonresponders (n = 7)	Responders (n = 4)	Nonresponders (n = 7)
sVEGFR2	10,691 (8,417-11,108)	11,739 (9,911-13,842)	**1.06 (1.05-1.21)**	**0.93 (0.87-0.93)**	1.03 (0.93-1.07)	0.88 (0.85-1.01)	**1.13 (1.01-1.18)**	**0.87 (0.84-0.90)**
P	.19		**.0043**		.074		**.014**	
HGF	**745 (602-821)**	**957 (841-1,054)**	1.11 (1.03-1.20)	0.92 (0.86-1.21)	1.23 (1.05-1.38)	1.03 (0.99-1.22)	1.03 (1.03-1.19)	1.12 (0.85-1.17)
P	**.019**		.092		.42		.65	
CAIX	50 (47-71)	32 (20-51)	**0.72 (0.54-0.75)**	**2.04 (1.40-2.32)**	**0.59 (0.51-0.69)**	**1.81 (1.49-2.63)**	0.95 (0.64-1.16)	1.67 (1.11-2.01)
P	.14		**.010**		**.035**		.12	

NOTE. Bold font indicates statistical significance.
Abbreviations: CAIX, carbonic anhydrase IX; HGF, hepatocyte growth factor; IQR, interquartile range; sVEGFR, soluble vascular endothelial growth factor receptor.

Table A5. Association Between Blood Circulating Biomarker Levels in Radiographic Responders (n = 9) Versus Nonresponders (n = 4) Treated With Bevacizumab

	Median (IQR)							
			Percent Change					
	Baseline (pg/mL; n = 13)		Week 25 (n = 13)		Week 49 (n = 12)		Week 72 (n = 11)	
Biomarker	Responders (n = 9)	Nonresponders (n = 4)	Responders (n = 9)	Nonresponders (n = 4)	Responders (n = 8)	Nonresponders (n = 4)	Responders (n = 8)	Nonresponders (n = 3)
Free VEGF	233 (172-281)	107 (93-149)	**0.12 (0.11-0.15)**	**0.24 (0.22-0.37)**	0.14 (0.09-0.21)	0.31 (0.22-0.40)	0.21 (0.09-0.44)	0.21 (0.06-0.43)
P	.19		**.025**		.075		.76	
Total VEGF (free plus bound)	134 (106-197)	68 (66-139)	**4.98 (1.63-6.95)**	**12.23 (8.93-15.50)**	6.53 (3.80-7.97)	6.91 (3.87-13.63)	0.44 (0.34-0.56)	0.53 (0.17-0.60)
P	.25		**.034**		.67		1.0	
VEGF-D	**1,243 (1,016-1,412)**	**813 (627-949)**	0.96 (0.90-1.04)	1.17 (1.05-1.23)	1.07 (1.0-1.33)	1.13 (1.01-1.24)	1.08 (0.96-1.13)	0.98 (0.83-1.05)
P	**.037**		.054		.93		.13	
SDF1α	**2,040 (1,906-2,185)**	**1,762 (1,707-1,875)**	1.09 (1.00-1.14)	0.92 (0.81-1.07)	1.09 (1.05-1.13)	1.01 (0.99-1.05)	1.0 (0.88-1.03)	0.97 (0.92-1.11)
P	**.025**		.11		.15		.76	
sTie2	8,055 (6,000-8,362)	6,355 (5,416-7,509)	0.89 (0.82-1.01)	1.03 (0.98-1.05)	**0.87 (0.82-0.93)**	**1.12 (0.95-1.47)**	1.01 (0.85-1.14)	1.00 (0.91-1.22)
P	.40		.14		**.034**		.76	
s-cKIT	1,237 (914-1,243)	1,017 (822-1,427)	**0.94 (0.87-1.00)**	**1.16 (1.15-1.18)**	1.05 (0.98-1.53)	1.09 (1.02-1.24)	1.05 (0.87-1.12)	1.01 (0.91-1.14)
P	.70		**.023**		.87		.64	

NOTE. Bold font indicates statistical significance.
Abbreviations: IQR, interquartile range; SDF1α, stromal cell–derived factor 1α; VEGF, vascular endothelial growth factor.

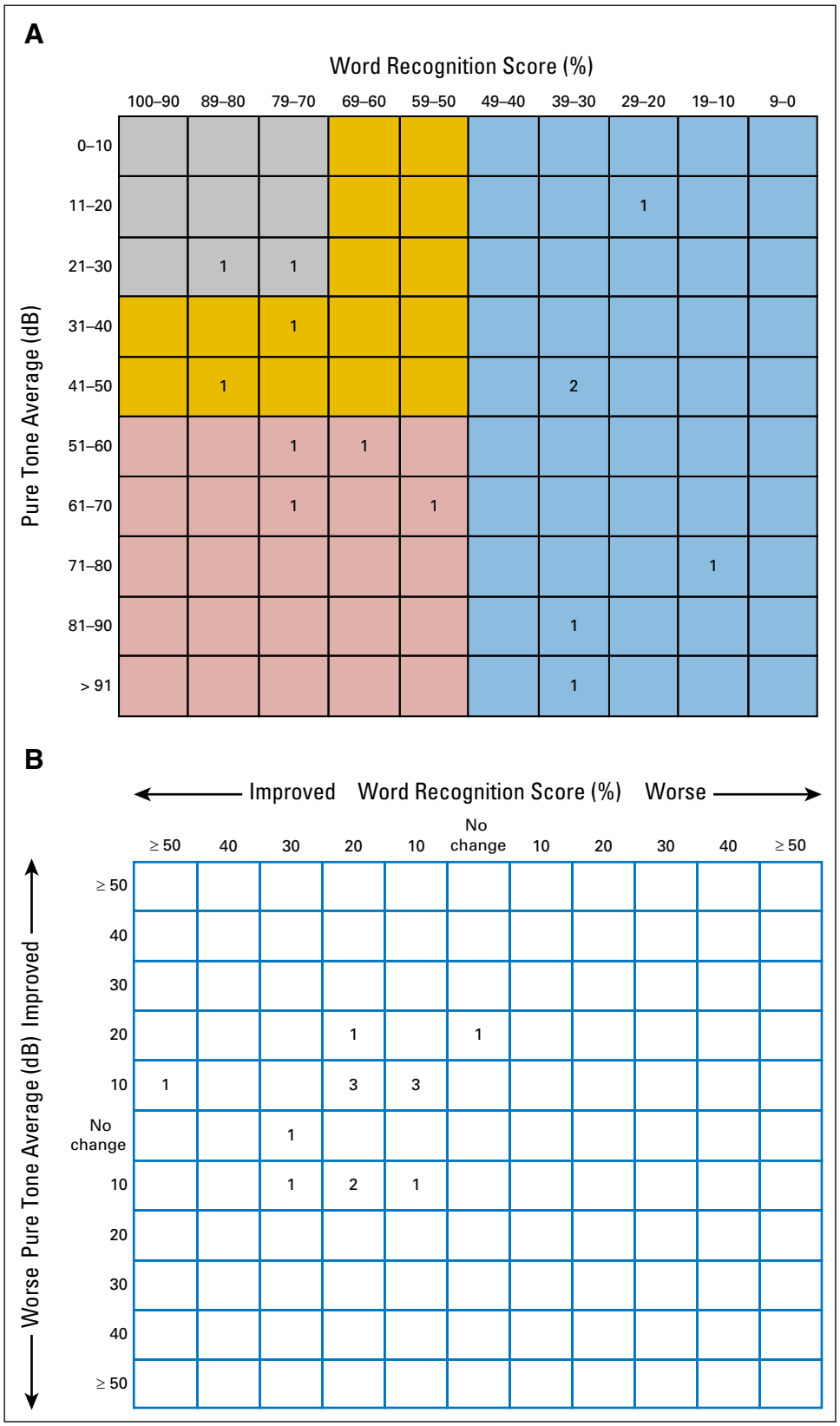

Fig A1. Scattergrams of (A) baseline hearing function for all target ears, as recommended by the Hearing Committee of the American Academy of Otolaryngology-Head and Neck Society, and (B) best change in hearing for all target ears after treatment with bevacizumab. Color code: gray, class A; gold, class B; red, class C; blue, class D. Classes A and B are considered serviceable, and classes C and D are considered unserviceable.[34]

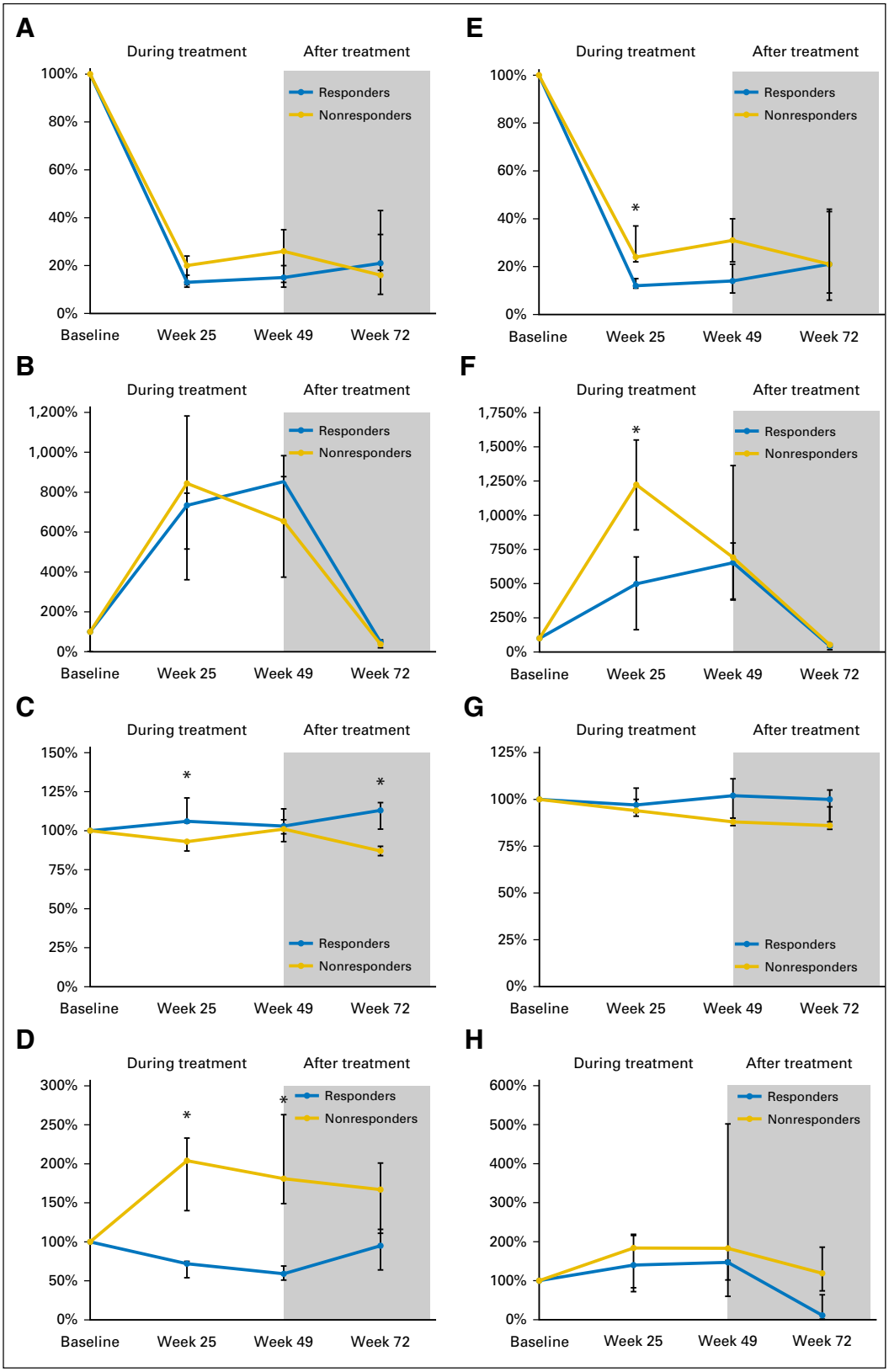

Fig A2. Line graphs for (A to D) patients with confirmed hearing response (HR; n = 5) versus nonresponders (n = 9) and (E to H) confirmed radiographic responders (n = 9) versus nonresponders (n = 5) showing changes over time in (A, E) relative free vascular endothelial growth factor (VEGF), (B, F) total VEGF (free plus bound), (C, G) soluble VEGF receptor 2, and (D, H) carbonic anhydrase IX. Data are presented as median values with interquartile ranges; (*) indicates a significant difference between relative biomarker concentrations in responders and nonresponders ($P < .05$).