Molecular Cancer Therapeutics

TP53 Alterations Correlate with Response to VEGF/VEGFR Inhibitors: Implications for Targeted Therapeutics

Jennifer J. Wheler[1], Filip Janku[1], Aung Naing[1], Yali Li[2], Bettzy Stephen[1], Ralph Zinner[1], Vivek Subbiah[1], Siqing Fu[1], Daniel Karp[1], Gerald S. Falchook[3], Apostolia M. Tsimberidou[1], Sarina Piha-Paul[1], Roosevelt Anderson[1], Danxia Ke[1], Vincent Miller[2], Roman Yelensky[2], J. Jack Lee[4], David Hong[1], and Razelle Kurzrock[5]

Abstract

TP53 tumor-suppressor gene mutations are among the most frequent abnormalities in cancer, affecting approximately 40% of patients. Yet, there is no accepted way to target these alterations in the clinic. At the same time, antagonists of VEGFR or its ligand are best-selling oncology drugs, with multiple, expensive compounds approved. Although only a subset of patients benefit from these antiangiogenesis agents, no relevant biomarker has been identified. Interestingly, *TP53* mutations upregulate VEGF-A and VEGFR2. We prospectively enrolled 500 patients, to be interrogated by comprehensive genomic profiling (CGP) (next-generation sequencing, 236 genes), and to be matched, whenever possible, with targeted agents. Herein, we analyze outcomes based on VEGF/VEGFR inhibitor treatment and presence of *TP53* mutations. Of the 500 patients, 188 (37.6%; with $\geq$1 alteration) were treated; 106 (56% of 188) had tumors that harbored *TP53* mutations. VEGF/VEGFR inhibitor therapy was independently associated with improvement in all outcome parameters [rate of stable disease (SD) $\geq$6 months/partial and complete remission (PR/CR); (31% versus 7%; *TP53*-mutant patients (who received no other molecular-matched agents) treated with versus without VEGF/VEGFR inhibitors), time-to-treatment failure, and overall survival (multivariate analysis: all $P \leq 0.01$)] for the patients harboring *TP53*-mutant cancers, but improvement was not seen in any of these parameters for patients with *TP53* wild-type neoplasms. We conclude that *TP53* mutations predict sensitivity to VEGF/VEGFR inhibitors in the clinic. *TP53* alterations may therefore be a ready biomarker for treatment with antiangiogenesis agents, a finding of seminal importance across the cancer field. *Mol Cancer Ther; 15(10); 2475–85. ©2016 AACR.*

Introduction

Angiogenesis fuels the proliferation and spread of cancer (1). VEGF is probably the most commonly involved proangiogenic factor. FDA-approved drugs now include antibodies against VEGF, as well as numerous small-molecule tyrosine kinase inhibitors (TKI) that target a VEGF receptor (VEGFR). Indeed, bevacizumab, an antibody targeting the VEGF-A ligand, has previously been proclaimed one of the best-selling drugs in oncology. However, for most malignancies in which it is deployed, including colon, lung, and kidney cancer, as well as glioblastoma multiforme, bevacizumab increases survival by only a few weeks. Small-molecule VEGFR inhibitors produce comparable results. Indeed, the FDA revoked its approval of bevacizumab in breast cancer because of the absence of evidence demonstrating a survival advantage, despite prior activity signals (2). It seems plausible that, in relevant neoplasms, a subgroup of patients across tumor types may be responsive to bevacizumab and/or small-molecule VEGFR inhibitors; however, biomarkers to identify and select these subsets remain elusive (3).

TP53 mutations are the most common molecular alterations across cancers, with about 40% of diverse tumors harboring this type of abnormality. Yet, there is no approved therapy that targets p53 (4, 5). We previously reported results of a retrospective study of patients with diverse cancers demonstrating that bevacizumab-containing regimens predicted for longer progression-free survival (PFS) in *TP53*-mutant tumors (multivariate analysis; $P < 0.001$; PFS = 11 vs. 5.0 months; mutant vs. wild-type *TP53*; ref. 6). The mechanism by which this correlation might occur remains uncertain; however, there is evidence to suggest that *TP53* is implicated in angiogenesis. For instance, a multiple regression analysis of the transcriptome in non–small cell lung cancer (NSCLC) revealed that high VEGF-A expression correlated independently with *TP53* mutational status (7). Though early data failed to find a clear association between VEGF expression and outcome after bevacizumab

[1]Department of Investigational Cancer Therapeutics, The University of Texas MD Anderson Cancer Center, Houston, Texas. [2]Foundation Medicine, Cambridge, Massachusetts. [3]Sarah Cannon Research Institute at HealthONE, Denver, Colorado. [4]Department of Biostatistics, The University of Texas MD Anderson Cancer Center, Houston, Texas. [5]Center for Personalized Cancer Therapy, Moores Cancer Center, The University of California, San Diego, La Jolla, California.

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Corresponding Author: Razelle Kurzrock, University of California San Diego, Moores Cancer Center, 3855 Health Sciences Drive, MC #0658, La Jolla, CA 92093. Phone: 858-246-1102; Fax: 858-246-1915; E-mail: rkurzrock@ucsd.edu

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administration, recent observations using improved technology indicate that circulating levels of the short isoform of VEGF-A is a strong biomarker candidate for predicting benefits (3).

Herein, we report results of a sub-analysis of a prospective study evaluating comprehensive genomic profiling (CGP) using a 236 gene next-generation sequencing (NGS) platform for matching patients ($n = 188$) to drugs. These data demonstrate that patients whose tumors harbored the *TP53*-mutant, but not *TP53*–wild-type, gene had improved outcomes when treated with VEGF or VEGFR antagonists. Our current observations provide important support for the concept that *TP53* mutations represent a biomarker that predicts salutary effects of anti-angiogenic agents.

Materials and Methods

Patient population and study design

The parent study enrolled 500 patients with refractory or progressive cancers. Its primary objective was to analyze the outcome of patients with diverse cancers prospectively matched to targeted therapy on the basis of CGP. An analysis of these data has been published (8). The current study represents a complete sub-analysis of the clinical correlates of *TP53* status in all 188 patients exhibiting at least one molecular abnormality who were treated on study (Fig. 1). The purpose of this analysis was determining whether the presence of *TP53* mutations was associated with a better outcome when antiangiogenesis agents were administered. The antiangiogenesis agents administered are shown in Supplementary Table S1, and included antibodies and small-molecule TKIs, which targeted either VEGF or VEGFR (at low IC50s). Supplementary multivariate analyses are shown in Supplementary Table S2. The study included 17 tumor types (Supplementary Table S3).

Study eligibility criteria included ECOG performance status ≤ 1 (9), tumor (archived or fresh) likely available for CGP, a life expectancy of ≥ 3 months, and likely able to qualify for therapy. The study was designed as a navigation trial, where the physician could use the CGP diagnostic, in the context of the patient's other medical conditions and laboratory tests, to choose a therapy, such as an approved medication used on- or off-label or a clinical trial. This protocol (NCT02437617) was approved by the MD Anderson Internal Review Board, and all patients gave informed consent.

CGP using a broad, hybrid capture–based NGS assay

Base substitutions, insertions, deletions, copy-number alterations, and selected gene fusions were detected [Foundation Medicine (Cambridge, MA; clinical laboratory improvements amendment (CLIA) laboratory; ref. 10]. DNA was isolated from 40 μm of formalin-fixed paraffin-embedded tissue (for specimens with $\geq$20% tumor cells) using the Maxwell 16 FFPE Plus LEV DNA Purification kit (Promega) and quantified using a standardized PicoGreen fluorescence assay (Invitrogen). Library construction was performed with the use of a 50 to 200 ng of DNA sheared by sonication to about 100 to 400 bp before end-repair, dA addition and ligation of indexed, Illumina sequencing adaptors. Enrichment of target sequences (all coding exons of 236 cancer-related genes (3,769 exons) plus 47 introns from 19 genes often rearranged or altered in malignancy (Supplementary Table S4) was achieved by solution-based hybrid capture with custom biotinylated oligonucleotide bases. Enriched libraries were sequenced to an average median depth of >500X with 99% of bases covered >100X (IlluminaHiSeq 2000 platform using 49×49 paired-end reads) and mapped to the reference human genome (hg19) using the Burrows–Wheeler Aligner and the publicly available SAM tools, Picard, and Genome Analysis Toolkit. Point mutations were identified by a Bayesian algorithm; short insertions and deletions, determined by local assembly; gene copy-number alterations (amplifications), by comparison with process matched normal controls; and gene fusions/rearrangements, by clustering chimeric reads mapped to targeted introns.

Amplifications were called for $\geq$6 copies (>7 for triploid, >8 for tetraploid samples) except for ErbB2 ($\geq$5 copies). Aberrations, mutations or other alterations in kinases that were presumed to be non-functional based on wet lab experiments or structural modeling, or lack of data were not included.

Antiangiogenic therapies

Antiangiogenic treatments included antibodies and small-molecule kinase inhibitors (Supplementary Table S1). These treatments could be given as single agents or in combination with other drugs. Antibodies are usually specific for angiogenic molecules, whereas small-molecule inhibitors often had targets in addition to VEGFRs.

Outcome assessment

Patients who were lost to follow-up or came off early (before restaging) for payor reasons were considered inevaluable for stable disease (SD) $\geq$6 months/partial and complete remission (PR/CR); patients who came off early for side effects were considered progressors. Patients who had not been restaged at the date of data cutoff (6 months after the date of the last enrollment; August 4, 2014) were considered inevaluable for SD $\geq$6 months/PR/CR (unless they had already achieved a CR or PR). SD, PR, or CR was determined per the assessment by the treating doctor. Time-to-treatment failure (TTF) and overall survival (OS) were assessed by the method of Kaplan and Meier, and the survival function between groups was compared using a two-sided log-rank test. TTF was the interval from the treatment start date to documentation of disease progression, death, or removal from study for any reason (mostly side effects), whichever occurred first. Patients who had not reached any of those endpoints at the date of data cutoff were censored on that date. If they had not reached the endpoints at the time of being lost to follow-up, they were censored at the date of last contact. OS was measured from the time of study enrollment. Patients still alive (for OS) at the date of data cut off, or the date of last contact for patients lost to follow-up, were censored on that date.

The treatment assessed was the first trial/therapy after receiving the CGP result. Local therapy (hepatic arterial infusion, for example) and transplant was considered inevaluable. If the therapy immediately before the on-study treatment was immunotherapy, that patient was inevaluable for all outcome parameters (SD $\geq$6 moths/PR/CR, TTF, OS) because of the potential for delayed responses.

Statistical analysis

Statistical analysis was verified by our statistician J.J. Lee. Patient characteristics were summarized using descriptive statistics. The χ^2 test or Fisher's exact test, as appropriate was used

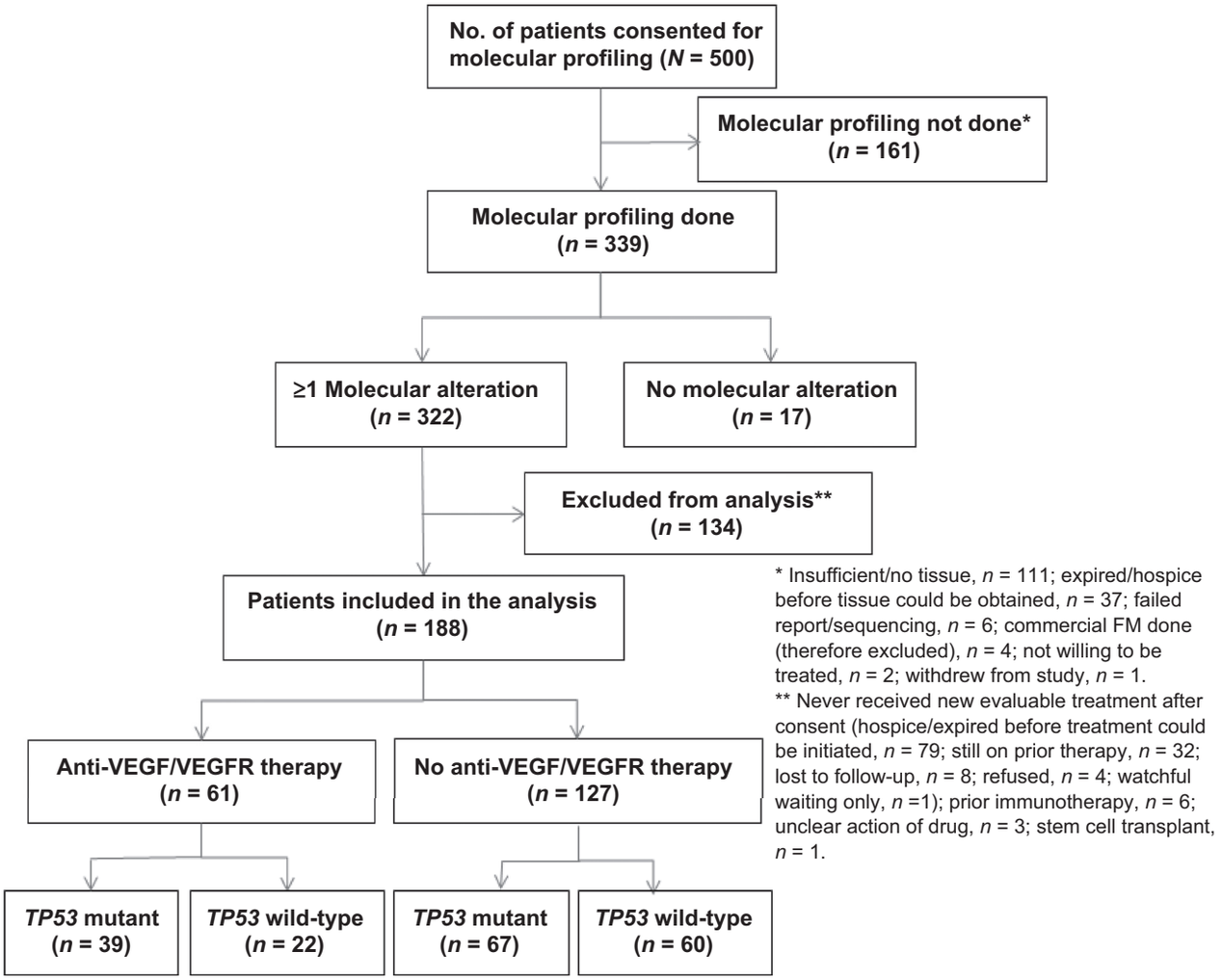

Figure 1.
Consolidated Standards of Reporting Trials (CONSORT) diagram demonstrates the flow of 500 patients who consented for the study.

to evaluate the associations between categorical variables and SD $\geq$6 months/PR/CR. The Mann–Whitney *U* test was applied to compare number of molecular alterations per person between groups (11).

The main endpoints of the study were SD $\geq$6 months/PR/CR as well as TTF, and OS. Kaplan–Meier estimates were computed for the time-to-event data (12). Log-rank test was applied for comparing the survival curves between groups. The multivariable Cox proportional hazards regression model (13) was used to examine risk factors related to TTF and OS, after adjusting for other factors, in particular *TP53* mutation status. Covariates included in the stepwise multivariate analysis were those with a *P* value of <0.2 in univariate analysis. Only variables with $P \leq 0.10$ in the multivariate analysis (and treatment with VEGF/VEGFR inhibitors) were included in the final model. Bootstrapping was performed using random sampling with replacement. The bootstrap method may be superior to approaches relying on the asymptotic distribution of the tests that assume the data come from a normal distribution.

All statistical tests were two-sided, and $P \leq 0.05$ was considered statistically significant. Statistical analyses were carried out using SPSS (version 22.0; SPSS).

Results

Patient characteristics

Of the 500 individuals enrolled, 188 patients (37.6%) with at least one molecular alteration were treated (Fig. 1). One hundred and eighty-two treated patients (97%) were evaluable for assessment of SD $\geq$6 months/PR/CR, and all 188 were evaluable for TTF and OS. Of patients who were not treated or not evaluable for treatment, the most frequent reasons were insufficient tissue, progressive cancer or succumbing to disease. One hundred and six patients (56% of 188) had tumors that harbored *TP53* mutations.

Of 188 treated patients, 55% were 60 years old or younger, and 65% were women. Gastrointestinal malignancies were present in 18% of patients (Table 1). The median number of molecular

Table 1. Patient characteristics by *TP53* mutation status ($n = 188$)

Variable	Group	All patients $n = 188$ (%)	*TP53* mutant $n = 106$ (%)	*TP53* wild-type $n = 82$ (%)	P[a]
Age	≤60 years	103 (55)	57 (54)	46 (56)	0.865
	>60 years	85 (45)	49 (46)	36 (44)	
Sex	Women	122 (65)	73 (69)	49 (60)	0.253
	Men	66 (35)	33 (31)	33 (40)	
Tumor type	Other	154 (82)	87 (82)	67 (82)	1.000
	Gastrointestinal	34 (18)	19 (18)	15 (18)	
Metastatic sites	≤2	103 (55)	62 (58)	41 (50)	0.311
	>2	85 (45)	44 (42)	41 (50)	
No. of prior therapies (before therapy on study)	<3	58 (31)	32 (30)	26 (32)	0.949
	≥3	130 (69)	74 (70)	56 (68)	
ECOG performance status	0	34 (20)	18 (19)	16 (21)	0.969
($n = 170$)	≥1	136 (80)	75 (81)	61 (79)	
Serum platelets	≤440 K/μL	176 (96)	100 (98)	76 (94)	0.244
($n = 183$)	>440 K/μL	7 (4)	2 (2)	5 (6)	
Serum LDH	≤618 U/L	102 (67)	52 (64)	50 (69)	0.606
($n = 153$)	>618 U/L	51 (33)	29 (36)	22 (31)	
Serum albumin	≥3.5 g/dL	143 (80)	82 (84)	61 (76)	0.294
($n = 178$)	<3.5 g/dL	35 (20)	16 (16)	19 (24)	
PI3K pathway alteration	Yes	91 (48)	54 (51)	37 (45)	0.519
	No	97 (52)	52 (49)	45 (55)	
Anti-VEGF/VEGFR therapy	Yes	61 (32)	39 (37)	22 (27)	0.197
	No	127 (68)	67 (63)	60 (73)	
Combination Therapy	Yes	123 (65)	79 (75)	44 (54)	**0.005**
	No	65 (35)	27 (25)	38 (46)	
RMH score[b]	≤1 (low risk)	111 (73)	60 (74)	51 (71)	0.790
($n = 153$)	>1 (high risk)	42 (27)	21 (26)	21 (29)	
MDACC score[c]	≤2 (low risk)	102 (68)	54 (68)	48 (68)	1.000
($n = 150$)	>2 (high risk)	48 (32)	25 (32)	23 (32)	
No. of molecular alterations per person	Median	5	5	3	**<0.00001**
	Range	1–14	1–14	1–13	
No. of matches per patient (excludes VEGF/VEGFR inhibitors matched to *TP53* alterations)	Median	1	0	1	0.163
	Range	0–9	0–6	0–9	
TTF on last prior therapy before protocol enrollment ($n = 140$)	Median (range) months	2.6 (0.4–96.0)	2.8 (0.5–20.9)	2.5 (0.4–96.0)	0.171
SD ≥ 6 months/PR/CR ($n = 182$)	*N* (%)	28 (15)	17 (17)	11 (14)	0.786
TTF	Median (range) months	2.6 (0.1–19.0)	2.6 (0.1–15.2+)[d]	2.7 (0.2–19.0)	0.737
OS	Median (range) months	8.0 (0.3–23.6)	7.6 (0.4–19.0)	9.2 (0.3–23.6)	0.132

NOTE: Bold font indicates statistically significant, $P \leq 0.05$.

Abbreviations: CR, complete response; ECOG, Eastern Cooperative Oncology Group; MDACC, MD Anderson Cancer Center; PR, partial response; RMH, Royal Marsden Hospital; TTF, time-to-treatment failure on protocol therapy.

[a]Fisher or χ^2 test as appropriate; Mann–Whitney *U* test for no. of molecular alterations per person, as well as number of molecular matches excluding VEGF/VEGFR inhibitors; log-rank tests for TTF and OS (Kaplan–Meier).

[b]The Royal Marsden Hospital (RMH) prognostic scoring system included the following factors: LDH and albumin levels and number of sites of metastases (8, 9).

[c]The MD Anderson Cancer Center (MDACC) scoring system includes the following factors: LDH and albumin levels, number of sites of metastases, ECOG performance status, and whether or not patients had gastrointestinal tumors (10).

[d]"+" sign indicates that the patient was still continuing on the study at the time of data cutoff.

alterations was five per person (range, 1–14). Patients were heavily pretreated. The median number of prior therapies in the metastatic setting was 4 (range, 0–17). The median OS for all 188 patients was 8.0 months (range, 0.3–23.6 months). There was a trend for participants with *TP53* wild-type tumors to survive longer than the *TP53*-mutant tumor-bearing patients, but this did not reach statistical significance (9.2 vs. 7.6 months; $P = 0.132$)

Patient characteristics in the *TP53*–wild-type and -mutant groups were generally well balanced (Table 1; refs. 14–16), except that *TP53* mutant versus wild-type individuals generally had a greater number of molecular alterations (median = 5 versus 3, $P < 0.00001$) and received combination therapies more often ($P = 0.005$), variables generally associated with worse and better outcomes, respectively (17, 18). Overall, *TP53* mutant and wild-type groups were equivalent for both canonical composite prognostic scores–the Royal Marsden Hospital Score (RMH) score [based on levels of albumin, and lactate dehydrogenase (LDH), and number of metastatic sites] and the MD Anderson Cancer Center (MDACC) score (based on RMH with tumor type and ECOG performance status as additional variables; Table 1; refs. 14–16).

Univariate analysis of factors associated with outcome

TP53-mutant tumors (n = 106). The only factor associated with a higher rate of SD ≥6 months/PR/CR was treatment with VEGF/VEGFR inhibitors ($P = 0.020$; Supplementary Table S1 for drugs used; Table 2 for univariate analysis).

Lower LDH, higher albumin, lower MDACC prognostic score and treatment with VEGF/VEGFR targeting agents were associated with longer TTF (all $P \leq 0.05$).

Lower number of metastatic sites, low LDH, higher albumin, absence of PI3K pathway aberrations, and lower RMH or MDACC

Table 2. Univariate analysis of factors affecting response, TTF, and, OS for mutant versus wild-type *TP53*

Variable	Group	Evaluable for SD ≥6 months/ PR/CR ($n = 103$)[a]	SD ≥6 months/PR/ CR *n* (%)[a]	*P*	Evaluable for TTF and OS ($n = 106$)	Median TTF (95% CI; months)	*P*	Median OS (95% CI; months)	*P*
Patients with *TP53* molecular alterations ($n = 106$)									
Age (y)	≤60	56	9 (16)	1.000	57	1.9 (1.2–2.6)	0.652	6.9 (4.6–9.2)	0.646
	>60	47	8 (17)		49	2.8 (1.4–4.2)		8.1 (7.1–9.1)	
Sex	Women	72	12 (17)	1.000	73	2.2 (1.6–2.8)	0.545	8.1 (6.1–10.1)	0.051
	Men	31	5 (16)		33	2.7 (1.3–4.1)		4.6 (3.3–5.9)	
Tumor type	Other	85	12 (14)	0.171	87	2.6 (1.9–3.3)	0.559	7.7 (5.7–9.7)	0.665
	Gastrointestinal	18	5 (28)		19	2.7 (0.9–4.5)		6.9 (0.0–14.5)	
Metastatic sites	≤2	59	12 (20)	0.344	62	2.7 (1.6–3.8)	0.195	9.8 (6.7–12.9)	**0.015**
	>2	44	5 (11)		44	2.1 (1.4–2.8)		4.4 (3.6–5.2)	
Prior therapies	<3	30	8 (27)	0.087	32	2.9 (1.4–4.4)	0.160	11.4 (7.8–15.0)	0.206
	≥3	73	9 (12)		74	2.1 (1.4–2.8)		6.9 (4.4–9.4)	
ECOG PS[b]	0	18	4 (22)	0.513	18	1.9 (0.2–3.6)	0.669	12.3 (5.1–19.5)	0.051
	≥1	73	12 (16)		75	2.7 (1.9–3.5)		6.1 (3.6–8.6)	
Platelets[b]	≤440 K/μL	97	17 (18)	1.000	100	2.6 (1.8–3.4)	0.174	7.6 (5.5–9.7)	0.109
	>440 K/μL	2	0 (0)		2	0.7 (-)		2.6 (-)	
Serum LDH[b]	≤618 U/L	50	10 (20)	0.696	52	3.4 (2.4–4.4)	**0.001**	9.6 (7.5–11.7)	**0.001**
	>618 U/L	29	4 (14)		29	1.4 (0.9–1.9)		4.0 (2.2–5.8)	
Serum albumin[b]	≥3.5 g/dL	79	16 (20)	0.065	82	2.6 (1.6–3.6)	**0.016**	8.1 (6.7–9.5)	**0.001**
	<3.5 g/dL	16	0 (0)		16	1.6 (0.8–2.4)		3.1 (1.4–4.9)	
PI3K pathway alteration	Yes	52	5 (10)	0.102	54	1.9 (1.6–2.2)	0.090	4.5 (3.8–5.2)	**0.003**
	No	51	12 (24)		52	3.3 (2.5–4.1)		9.6 (5.8–13.4)	
RMH score[b]	≤1 (low risk)	58	11 (19)	0.749	60	2.8 (2.0–3.6)	0.073	8.3 (5.6–11.0)	**0.033**
	>1 (high risk)	21	3 (14)		21	1.6 (0.8–2.4)		4.0 (2.2–5.8)	
MDACC score[b]	≤2 (low risk)	52	10 (19)	0.529	54	3.2 (2.3–4.1)	**0.011**	8.7 (6.6–10.8)	**0.003**
	>2 (high risk)	25	3 (12)		25	1.6 (1.0–2.2)		3.4 (1.2–5.6)	
Molecular alterations (N)[c]	≤5	58	10 (17)	1.000	59	2.8 (1.7–3.9)	0.815	8.7 (5.0–12.4)	0.289
	>5	45	7 (16)		47	2.2 (1.4–3.0)		7.5 (4.4–10.6)	
Combination therapy	Yes	76	15 (20)		79	2.8 (1.6–4.0)		7.7 (5.3–10.1)	
	No	27	2 (7)	0.226	27	1.9 (1.1–2.7)	0.257	7.5 (3.6–11.4)	0.660
Treatment: VEGF/VEGFR inhibitors	Yes	38	11 (29)	**0.020**	39	3.2 (1.8–4.6)	**0.021**	8.0 (4.1–11.9)	0.163
	No	65	6 (9)		67	2.1 (1.4–2.8)		7.5 (4.2–10.8)	
Patients with *TP53* wild-type ($n = 82$)									
Age (y)	≤60	44	8 (18)	0.329	46	3.3 (1.7–4.9)	0.371	11.3 (1.1–21.5)	**0.020**
	>60	35	3 (9)		36	2.7 (2.1–3.3)		5.1 (2.1–8.1)	
Sex	Women	47	5 (11)	0.338	49	2.7 (2.1–3.3)	0.681	10.2 (5.4–15.0)	0.853
	Men	32	6 (19)		33	2.8 (1.4–4.2)		9.2 (5.4–13.0)	
Tumor type	Other	65	9 (14)	1.000	67	2.5 (1.9–3.1)	0.998	9.9 (7.1–12.7)	0.263
	Gastrointestinal	14	2 (14)		15	3.3 (1.2–5.4)		5.1 (3.4–6.8)	
Metastatic sites	≤2	39	7 (18)	0.487	41	3.1 (2.3–3.9)	0.151	11.1 (8.0–14.2)	0.193
	>2	40	4 (10)		41	2.1 (1.6–2.6)		6.2 (3.3–9.1)	
Prior therapies	<3	25	6 (24)	0.093	26	3.3 (1.4–5.2)	0.173	19.1 (6.2–32.0)	**0.031**
	≥3	54	5 (9)		56	2.5 (1.9–3.1)		7.2 (3.7–10.7)	
ECOG PS[b]	0	16	5 (31)	0.052	16	4.0 (3.2–4.8)	**0.012**	19.1 (11.3–26.9)	**0.004**
	≥1	58	6 (10)		61	2.1 (1.6–2.6)		7.1 (4.4–9.8)	
Platelets[b]	≤440 K/μL	73	10 (14)	0.543	76	2.7 (1.7–3.7)	**0.028**	9.9 (6.3–13.5)	0.170
	>440 K/μL	5	1 (20)		5	1.8 (0.0–4.6)		5.9 (4.0–7.8)	
Serum LDH[b]	≤618 U/L	49	9 (18)	0.490	50	3.1 (2.2–4.0)	0.183	14.0 (6.0–22.0)	**0.002**
	>618 U/L	20	2 (10)		22	1.9 (1.6–2.2)		4.7 (3.1–6.3)	
Serum albumin[b]	≥3.5 g/dL	59	8 (14)	0.712	61	3.1 (2.0–4.2)	0.297	11.3 (7.0–15.6)	**0.00003**
	<3.5 g/dL	18	3 (17)		19	2.1 (1.4–2.8)		4.1 (2.5–5.7)	
PI3K pathway alteration	Yes	36	6 (17)	0.750	37	2.7 (2.0–3.4)	0.281	9.2 (3.6–14.8)	0.461
	No	43	5 (12)		45	2.5 (1.5–3.5)		9.9 (5.6–14.2)	
RMH score[b]	≤1 (low risk)	50	8 (16)	1.000	51	3.4 (2.8–4.0)	0.336	14.0 (6.7–21.3)	**0.002**
	>1 (high risk)	19	3 (16)		21	1.9 (1.4–2.4)		5.1 (2.7–7.5)	
MDACC score[b]	≤2 (low risk)	47	8 (17)	1.000	48	3.7 (2.9–4.5)	0.177	14.0 (6.2–21.8)	**0.002**
	>2 (high risk)	21	3 (14)		23	1.9 (1.4–2.4)		4.1 (2.2–6.0)	
Molecular alterations (N)[c]	≤3	39	7 (18)	0.487	42	2.8 (1.8–3.8)	0.161	11.1 (8.0–14.2)	0.157
	>3	40	4 (10)		40	2.5 (1.3–3.7)		7.2 (4.8–9.6)	
Combination therapy	Yes	43	5 (12)	0.750	44	2.1 (1.2–3.0)	0.593	8.2 (5.2–11.2)	0.733
	No	36	6 (17)		38	2.8 (1.9–3.7)		10.2 (5.9–14.5)	
Treatment: VEGF/VEGFR inhibitors	Yes	20	4 (20)	0.456	22	2.3 (1.5–3.1)	0.804	8.2 (4.6–11.8)	0.818
	No	59	7 (12)		60	2.8 (1.9–3.7)		10.2 (6.3–14.1)	

NOTE: Bold font indicates statistically significant, $P \leq 0.05$.

Abbreviations: CR, complete response; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; MDACC, MD Anderson Cancer Center; PR, partial response; PS, performance status; RMH, Royal Marsden Hospital; TTF, time to treatment failure on protocol therapy; VEGF, vascular endothelial growth factor.

[a]*n* equals total number of patients available for outcome analysis; the numbers of patients available for assessment of each of SD ≥6 months/PR/CR, TTF or OS may differ slightly.

[b]Numbers of patients with available data for different outcome assessments may differ slightly.

[c]Dichotomized at median.

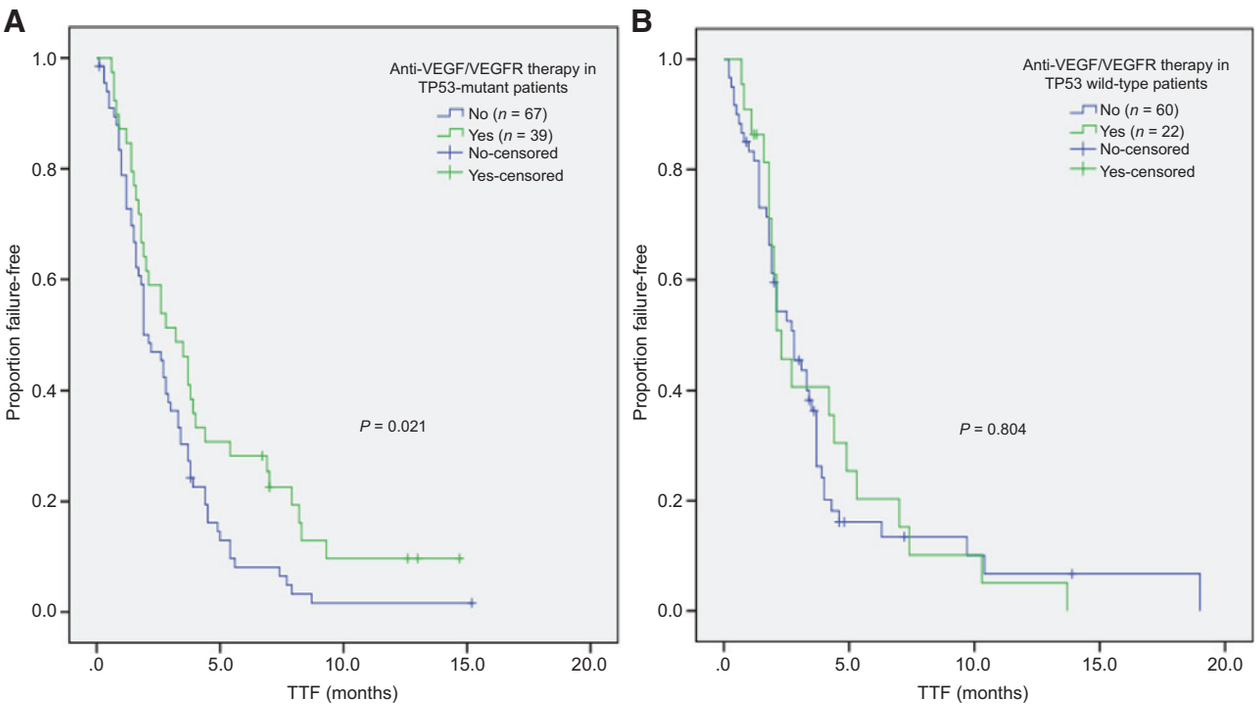

Figure 2.

Kaplan–Meier curves of 188 patients for TTF by type of therapy (anti-VEGF/VEGFR therapy vs. no anti-VEGF/VEGFR therapy). **A,** TTF of *TP53*-mutant patients ($n = 106$). **B,** TTF of *TP53* wild-type patients ($n = 82$). Survival differences were analyzed by the Kaplan–Meier method (log rank test). Overall, patients with *TP53*-mutant cancers (**A**), but not those with wild-type tumors (**B**), demonstrated improved TTF on VEGF/VEGFR inhibitors.

prognostic score were associated with better OS. Treatment with VEGF/VEGFR targeting agents showed a trend toward association with longer survival ($P = 0.163$).

TP53–wild-type tumors (n = 82). No factors were associated with a higher rate of SD ≥6 months/PR/CR. In contrast with the *TP53*-mutant patients, in whom the VEGF/VEGFR inhibitors were significantly associated with a higher rate of SD ≥6 months/PR/CR ($P = 0.02$), for the *TP53* wild-type tumors, the p value was 0.456 (Table 2).

Only better ECOG performance status and lower platelet counts correlated with better TTF. In contrast with the *TP53*-mutant patients, where VEGF/VEGFR inhibitors were significantly associated with longer TTF ($P = 0.021$), for the *TP53* wild-type tumors, the *P* value was 0.804 (and median TTF was actually numerically shorter for the VEGF/VEGFR inhibitor-exposed patients).

For OS, younger age, better ECOG performance status, few number of prior therapies, lower LDH, higher albumin and better RMH or MDACC prognostic scores were correlated with a better outcome (all $P \leq 0.05$). In contrast with the *TP53*-mutant patients, where VEGF/VEGFR inhibitors were associated with a trend toward a longer OS ($P = 0.163$), for the *TP53* wild-type tumors, the *P* value was 0.818 (and median OS was actually numerically shorter for the VEGF/VEGFR inhibitor-exposed patients).

Additional alterations in VEGFR/VEGF family genes

Only two patients with a *TP53* mutation also carried an additional alteration in a VEGF/VEGFR-related gene. These patients both had a *KDR* (VEGFR2) amplification. One achieved SD ≥6 months/PR/CR and the other did not; therefore, the improved response rate for VEGF/VEGFR inhibitors in *TP53*-mutant patients could not be attributed to the presence of another VEGF/VEGFR-related alteration.

Multivariate analysis of factors associated with outcome

The multivariate analysis was performed first by including all variables with a p value of <0.2 in the univariate analyses, and then selecting the variables with a *P* value of <0.1 and repeating the analysis (Supplementary Table S2). Using this methodology, treatment with VEGF/VEGFR inhibitors was independently associated with improvement in all outcome parameters [rate of SD ≥6 months/PR/CR, length of TTF and OS (all $P \leq 0.01$)] for the patients harboring *TP53*-mutant cancers, but improvement was not seen in any of these parameters for the group of patients with *TP53* wild-type neoplasms (Fig. 2A and B, TTF for *TP53*-mutant versus wild type). (In general, the number of patients with each tumor type was too small to sub-analyze. We did examine gastrointestinal versus other tumors. The rate of SD ≥ 6 months/PR/CR for *TP53*-mutant gastrointestinal tumors treated with VEGF/VEGFR inhibitors was 29% (2/7 patients) versus 29% (9/31) for p53-mutant other tumors ($P = 1.000$).

A second analysis was performed similarly, except that the variable of interest—treatment with VEGF/VEGFR inhibitors—was included in the analysis; even if the *P* value for this variable was greater than 0.2 in the univariate analysis. Under these conditions, treatment with VEGF/VEGFR inhibitors was again independently associated with improvement in all outcome

Table 3. Multivariate analysis of factors affecting treatment outcomes in 106 patients with *TP53* molecular alterations versus 82 patients with *TP53* wild-type, by individual factors of MDACC score versus MDACC score (anti-VEGF/VEGFR included in multivariate regardless of univariate *P*)

Treatment outcomes in 106 *TP53*-mutant patients										
SD ≥6 months/PR/CR	**Individual factors**					**MDACC score**				
		Asymptotic design		**Bootstrapping[d]**			**Asymptotic design**		**Bootstrapping**	
Variable[a]	**OR[b]**	**95% CI**	***P***	**95% CI**	***P***	**OR[b]**	**95% CI**	***P***	**95% CI**	***P***
Prior therapy <3 (vs. ≥3)	3.4	1.0–12.3	0.057	0.0–2.8	**0.045**	—	—	—	—	—
Albumin ≥3.5 g/dL (vs. <3.5 g/dL)	[j]			18.6–20.4	**0.001**	—	—	—	—	—
Anti VEGF/VEGFR therapy Yes (vs. No)	7.1	2.0–25.6	**0.003**	0.2–25.8	**0.002**	—	—	—	—	—
TTF	**Individual factors**					**MDACC score**				
		Asymptotic design		**Bootstrapping[e]**			**Asymptotic design**		**Bootstrapping[g]**	
Variable[a]	**HR[c]**	**95% CI**	***P***	**95% CI**	***P***	**HR[c]**	**95% CI**	***P***	**95% CI**	***P***
Prior therapy ≥3 (vs. <3)	2.1	1.2–3.6	**0.007**	0.1–1.5	**0.016**					
Platelets >440 K/μL(≤440 K/μL)	3.4	0.8–14.8	0.107	0.4–2.2	**0.005**	5.4	1.2–24.1	**0.026**	0.9–2.9	**0.001**
LDH >618 IU/L (vs. ≤618 IU/L)	4.3	2.4–7.6	<**0.0001**	0.8–2.3	**0.001**					
Albumin <3.5 g/dL (vs. ≥3.5 g/dL)	2.1	1.1–4.2	**0.033**	(−0.1)–2.1	0.060					
Anti VEGF/VEGFR therapy No (vs. Yes)	3.1	1.8–5.4	**0.00005**	0.4–1.9	**0.002**	2.5	1.4–4.5	**0.001**	0.3–1.6	**0.001**
MDACC score >2 (vs. ≤2)						3.2	1.8–5.8	**0.0001**	0.5–2.0	**0.001**
OS	**Individual factors**					**MDACC score**				
		Asymptotic design		**Bootstrapping[f]**			**Asymptotic design**		**Bootstrapping[h]**	
Variable[a]	**HR[c]**	**95% CI**	***P***	**95% CI**	***P***	**HR[c]**	**95% CI**	***P***	**95% CI**	***P***
Men (vs. Women)	3.1	1.6–5.9	**0.001**	0.4–2.2	**0.002**	1.9	1.1–3.3	**0.023**	(−0.1)–1.7	0.055
Metastatic sites >2 (vs. ≤2)	1.7	0.9–3.1	0.078	(−0.2)–1.5	0.082					
Platelets >440 K/μL(≤440 K/μL)	—	—	—	—	—	3.2	0.7–13.8	0.119	0.3–3.1	0.067
LDH >618 IU/L (vs. ≤618 IU/L)	4.9	2.4–9.9	**0.000009**	0.8–2.8	**0.001**					
Albumin <3.5 g/dL (vs. ≥3.5 g/dL)	2.8	1.3–6.0	**0.009**	0.0–2.7	**0.029**					
PI3K pathway alteration No (vs. Yes)	0.5	0.3–1.0	**0.033**	(−1.4 to −0.1)	0.079	0.4	0.2–0.7	**0.003**	(−1.8 to −0.3)	**0.005**
Anti VEGF/VEGFR therapy No (vs. Yes)	2.6	1.3–5.0	**0.005**	0.2–1.9	**0.012**	2.3	1.2–4.5	**0.013**	0.1–1.9	**0.016**
MDACC score >2 (vs. ≤2)						5.3	2.6–10.6	**0.000003**	0.9–2.8	**0.001**
Treatment outcomes in 82 *TP53* wild-type patients										
SD ≥6 months/PR/CR	**Individual factors**					**MDACC score**				
		Asymptotic design		**Bootstrapping[d]**			**Asymptotic design**		**Bootstrapping**	
Variable[a]	**OR[b]**	**95% CI**	***P***	**95% CI**	***P***	**OR[b]**	**95% CI**	***P***	**95% CI**	***P***
Prior therapy <3 (vs. ≥3)	4.3	0.9–20.7	0.068	(−0.5)–20.7	**0.035**	—	—	—	—	—
ECOG PS 0 (vs. ≥1)	4.3	1.0–19.0	0.058	(−0.8)–20.9	**0.022**	—	—	—	—	—
Anti VEGF/VEGFR therapy Yes (vs. No)	4.7	0.8–25.7	0.076	(−18.4)–21.4	**0.038**	—	—	—	—	—
TTF	**Individual factors**					**MDACC score**				
		Asymptotic design		**Bootstrapping[f]**			**Asymptotic design**		**Bootstrapping[i]**	
Variable[a]	**HR[c]**	**95% CI**	***P***	**95% CI**	***P***	**HR[c]**	**95% CI**	***P***	**95% CI**	***P***
ECOG PS ≥1 (vs. 0)	2.5	1.3–4.9	**0.007**	0.2–1.9	**0.009**					
Platelets >440 K/μL (≤440 K/μL)	3.1	1.2–8.2	**0.020**	0.2–2.8	**0.030**	2.8	1.1–7.0	**0.034**	0.3–3.0	0.056
Molecular alterations >3 (vs. ≤3)	1.8	1.1–3.0	**0.024**	0.0–1.1	**0.023**					
Anti-VEGF/VEGFR therapy No (vs. Yes)	1.6	0.9–2.8	0.30	(−0.3)–1.3	0.52	1.1	0.7–1.9	0.667	(−0.5)–0.8	0.660
MDACC score >2 (vs. ≤2)										
OS	**Individual factors**					**MDACC score**				
		Asymptotic design		**Bootstrapping[d]**			**Asymptotic design**		**Bootstrapping[d]**	
Variable[a]	**HR[c]**	**95% CI**	***P***	**95% CI**	***P***	**HR[c]**	**95% CI**	***P***	**95% CI**	***P***
LDH >618 IU/L (vs. ≤618 IU/L)	2.8	1.4–5.5	**0.004**	0.4–1.8	**0.002**					
Anti-VEGF/VEGFR therapy No (vs. Yes)	0.9	0.4–1.7	0.707	(−0.9–0.6)	0.705	0.8	0.4–1.6	0.527	(−1.0)–0.7	0.528
MDACC score >2 (vs. ≤2)						2.7	1.4–5.3	**0.003**	0.2–2.0	**0.005**

NOTE: Bold font indicates statistically significant, $P \leq 0.05$.

Abbreviations: CI, confidence interval; MDACC, MD Anderson Cancer Center; TTF, time to treatment failure on protocol therapy.

[a]Multivariate included variables with *P* value <0.2 in the univariate analyses and the variable "Anti VEGF/VEGFR therapy"; the analysis was then repeated with variables that had cut off of <0.1.

[b]OR >1 = increases odds for SD ≥6 months/PR/CR.

[c]HR >1 = shorter TTF/OS.

Number of samples generated by boot strapping method:

[d]1,000.

[e]866.

[f]994.

[g]868.

[h]864.

[i]992.

[j]OR is undefined for albumin as one of the cells had a value of "zero."

parameters (SD ≥6 months/PR/CR, TTF and OS) in the *TP53*-mutant group (all *P* values ≤0.012)] (Table 3). For the *TP53* wild-type group, when VEGF/VEGFR treatment was forced into the multivariate analysis despite its insignificance in univariate analysis, it was associated with an improved rate of SD ≥6 months/PR/CR (univariate *P* value = 0.456; multivariate *P* value = 0.038;

Table 4. Univariate analysis of factors affecting SD ≥6 months/PR/CR, TTF, and, OS in 60 *TP53*-mutant and 36 *TP53* wild-type patients with no other matches

	SD ≥6 months/PR/CR *n* (%)		Median TTF (95% CI; months)		Median OS (95% CI; months)	
Variable	***TP53* mutant**	***TP53* wild-type**	***TP53* mutant**	***TP53* wild-type**	***TP53* mutant**	***TP53* wild-type**
VEGF/VEGFR inhibitors (yes)	$n = 29$[a]	$n = 7$[a]	$n = 30$	$n = 8$	$n = 30$	$n = 8$
	9 (31)	1 (14)	2.8 (0.7–4.9)	1.9 (1.6–2.2)	9.6 (4.2–15.0)	8.2 (6.7–9.7)
VEGF/VEGFR inhibitors (no)	$n = 29$[a]	$n = 28$[a]	$n = 30$	$n = 28$	$n = 30$	$n = 28$
	2 (7)	2 (7)	1.6 (1.2–2.0)	2.1 (0.7–3.5)	5.2 (3.3–7.1)	7.2 (3.6–10.8)
P	**0.044**	0.499	**0.005**	0.543	**0.070**	0.607

NOTE: Bold font indicates statistically significant, $P \leq 0.05$.

Abbreviations: CI, confidence interval; TTF, time to treatment failure on protocol therapy.

[a]*n* equals total number of patients available for outcome analysis; the numbers of patients available for assessment of each of SD ≥6 months/PR/CR, TTF, or OS may differ slightly.

bootstrapping method) but the impact on TTF and OS remained insignificant. Patients often received combination regimens and may have been given matched therapy (concomitant with VEGF/VEGFR inhibitors); indeed, 65% of the VEGF/VEGFR-treated wild-type patients received another match whereas only 38% of the *TP53*-mutant patients treated with VEGF/VEGFR inhibitors had other matches ($P = 0.0039$). We therefore analyzed the subgroup of patients whose regimens included no matches to their genomic anomalies (other than VEGF/VEGFR matched to *TP53* alterations; Table 4). Comparing patients who had VEGF/VEGFR inhibitors as part of their regimens versus those who did not, in individuals with *TP53* mutations and no other matches, the rate of SD ≥6 months/PR/CR was 31% versus 7% ($P = 0.04$); in contrast, for the patients whose tumors harbored wild-type *TP53*, the *P* value was 0.49, indicating no difference in response rate between VEGF/VEGFR inhibitor treatment or not (in the absence of other matches). Similarly, in the absence of other matches, TTF was longer for the *TP53*-mutant individuals who were treated with VEGF/VEGFR inhibitors versus those who were not [$P = 0.005$; *TP53*-mutant patients (VEGF/VEGFR inhibitors yes vs. no)], but not for *TP53* wild-type patients ($P = 0.543$; *TP53* wild-type patients treated with VEGF/VEGFR inhibitors, yes versus no; with the *TP53* wild-type patients actually showing numerically shorter TTF in the VEGF/VEGFR–treated patients). Similarly, in the absence of other matches, OS showed a trend toward improvement in the *TP53*-mutant patients who received VEGF/VEGFR inhibitors versus those who did not [9.6 vs. 5.2 months ($P = 0.07$)], whereas this was not seen in the patients whose tumors were wild-type *TP53* ($P = 0.607$). These data confirm that, in the absence of other concomitant matched agents, VEGF/VEGFR inhibitors are associated with improvement in all outcome parameters in *TP53*-mutant, but not in *TP53* wild-type, malignancies.

Discussion

We have previously described, in a retrospective study, a clinical association between *TP53* mutations and better PFS after bevacizumab treatment (6). Furthermore, a comprehensive transcriptomic analysis in NSCLC demonstrated that, in multivariate analysis, *TP53* mutations were an independent predictor of high expression of VEGF-A (7), the primary target of bevacizumab, and the ligand for VEGFR-1 (Flt-1) and VEGFR-2 (KDR/Flk-1). The mechanism underlying the association between *TP53* mutations and angiogenesis through VEGF-A is complicated, but may be in part related to the fact that wild-type *TP53* indirectly represses VEGF expression by interaction with, and inhibition of, transcription factors such as SP1 and E2F (reviewed in ref. 19). Our current study was a prospective navigation trial and provides validation for the concept that *TP53* mutations are a biomarker predicting beneficial effects when VEGF/VEGFR inhibitors are used in the clinic.

In recent years, molecular interrogation of tumors and deployment of matched therapies has shown remarkable responses in a variety of refractory malignancies (4). Aberrant *TP53* has, however, proven to be difficult to target, though several approaches are being attempted. For instance, Mdm2 inhibitor molecules demonstrate preclinical promise in a wide variety of tumors with wild-type *TP53* (20). In addition, AZD-1775, a potent and selective small-molecule inhibitor of Wee-1 kinase (a tyrosine kinase that is involved in G2 checkpoint signaling) selectively sensitizes tumors to DNA damaging agents, probably because *TP53* is a key regulator in the G1 checkpoint, and *TP53*-deficient tumors depend on the G2 checkpoint after DNA damage (21). More recent work has demonstrated that the p53 protein requires the binding of a single zinc ion for proper folding, and impairing zinc binding is a major mechanism for loss of function in missense mutant p53. A new class of drugs termed zinc metallochaperones may reactivate wild-type p53 structure and function by restoring zinc binding in p53-mutant tumors (22). Overall, however, even though *TP53* mutations are among the most common in cancer, there is a dearth of strategies in the clinic to target this frequently altered tumor suppressor. Our current multivariate analysis demonstrates that the administration of VEGF/VEGFR inhibitors is an independent variable significantly associated with improvement in all outcome parameters in patients with *TP53*-mutant, but not *TP53* wild-type, malignancies (Tables 2, 3, and 4, Supplementary Table S2, and Fig. 2).

Other mechanisms, in addition to upregulation of the VEGF-A ligand, may also be operative in the relationship between *TP53* and angiogenesis. Indeed, the role of *TP53* is broad and reaches far beyond its most commonly studied effects on apoptosis (5, 23–27). *TP53* leads to activation of target genes that regulate "healthy" biological processes, including cell-cycle arrest, apoptosis, senescence, energy metabolism, and antioxidant defense to prevent tumorigenesis. In addition to losing tumor suppressive functions of wild-type *TP53*, proteins associated with mutant *TP53* also gain new oncogenic functions, including tumor cell proliferation, antiapoptosis, angiogenesis, metastasis-promotion, and metabolic changes defined as mutant *TP53* gain-of-function. A role for *TP53* in immune response has also been defined, and here angiogenesis also plays a key role. Importantly, VEGF suppresses the maturation

of dendritic cells from hematopoietic progenitors (28). In turn, inadequate function of dendritic cells is one mechanism of tumor escape from "normal" immune system control. Indeed, decreased dendritic cells in tissue result in a dramatically reduced ability of these cells to stimulate allogeneic T cells and antigen-specific primary T-cell response. In a preclinical model, treatment of pulsed dendritic cells in combination with an anti-VEGF antibody led to tumor shrinkage and was associated with significant anti–*p53* T-cell response (29). Hence, *TP53* mutations may lead to attenuation of immune responses, which can be recovered, at least in some models, with dendritic cell treatment together with antiangiogenesis agents.

Expression analyses have demonstrated that VEGFR2 upregulation may be another mechanism by which mutant p53 controls angiogenesis (30). VEGFR2 is a receptor tyrosine kinase activated by binding of the VEGF-A ligand, and it is the main receptor mediating endothelial cell neovascularization. Mutant p53 binds near the VEGFR2 promoter transcriptional initiation site and plays a role in preserving an open conformation at that location. Relatedly, mutant p53 cooperates with the SWI/SNF complex, which is essential for remodeling the VEGFR2 promoter. Given that wild-type *TP53* is a canonical repressor of the VEGF pathway through multiple mechanisms, including transcriptional repression of VEGF-A (7, 19, 31, 32), loss of wild-type p53 function/presence of mutant p53 has several mechanisms by which to turn on the angiogenic "switch." These mechanisms are compatible with increased susceptibility of *TP53*-mutant tumors to the effects of VEGF or VEGFR antagonists (5, 33) as confirmed in the current investigation.

There are several limitations to this study. First, there were insufficient numbers of patients to determine whether specific alterations in *TP53* are more important than others. Preclinical work has also not clarified which of the *TP53* mutations may function to upregulate VEGF-A or VEGFR2, or participate in the immune response. Furthermore, because this study was conducted across tumor types, it remains unclear whether or not there would be differences in significance in specific histologies. Of note, however, in this regard, our patients were well balanced in the number of gastrointestinal versus other tumors (Table 1), and the fact that the study was histology agnostic may suggest generalizability. Another limitation was that a variety of VEGF/VEGFR inhibitors were given. The strength of correlation with benefit by agent could not be analyzed based on the sample size. Still, previous data have suggested that *TP53* mutations may correlate with better outcome after either VEGF antibody (6) or VEGFR small-molecule inhibitor treatment (33), and this concept is further supported by the observation that *TP53* mutations are associated with increased levels of VEGF-A transcripts (7), which in turn interact with VEGFR1 and VEGFR2. Finally, our study sample size was small. Further prospective studies with larger numbers of treated patients are needed.

In summary, there are at least 12 VEGF/VEGFR inhibitors that are approved, and many more in clinical trials; none have a biomarker for patient selection (34–44). Yet, it is known that most of these agents improve survival by only a few weeks, most likely because a subset of patients respond, whereas others do not benefit or may even be harmed by these drugs. The importance of this problem is further underscored by the immense costs of antiangiogenesis agents, which often runs at thousands of dollars per month. At the same time, *TP53* is a tumor-suppressor gene that affects a wealth of cancer-related pathways, and is the most common alteration across diverse tumor types. Yet, to date, this gene has not proven susceptible to targeted agents in the clinic. Our current data support the concept that *TP53* status may represent a ready biomarker for response to anti-angiogenesis agents, with multivariate analysis demonstrating significant improvement in all outcome parameters when individuals with *TP53*-mutant, but not *TP53* wild-type, advanced neoplasms were treated with anti-angiogenesis agents. Recently, *TP53* mutational status was also shown to be predictive of response to the VEGFR inhibitor pazopanib in advanced sarcomas (45). The underlying mechanism of response may relate to *TP53* mutations being associated with upregulation of VEGF-A and VEGFR2 expression (7, 30–32). Of interest in this regard, other tumor suppressors that are not directly druggable may also be actionable based on the upregulation of affected pathways when the suppressor gene is mutated (46). For example, PTEN alterations are not directly targeted; rather they result in upregulation of the PI3K–Akt–mTor pathway, which is actionable (47). In addition, it seems likely that the magnitude of potential benefit in patients with *TP53*-mutant tumors versus *TP53*–wild-type is increased because of a distinct role of anti-VEGF in modulating T-cell response. Treatment of *TP53*-mutant tumors with VEGF/VEGFR inhibitors likely increases dendritic cells, thus promoting innate T-cell response and antitumor activity (29). Overall, our observations indicate that mutant *TP53* may be a pharmacologically tractable alteration that can be prosecuted by VEGF/VEGFR inhibitors. This concept requires validation by prospective randomized trials.

Disclosure of Potential Conflicts of Interest

Y. Li ownership interest (including patents) in Foundation Medicine. A.M. Tsimberidou reports receiving a commercial research grant Foundation Medicine. V. Miller is CMO of and has ownership interest (including patents) in Foundation Medicine. R. Yelensky is VP Biomarkers and CDx and has ownership interest (including patents) in Foundation Medicine. R. Kurzrock is employed by and has ownership interest (including patents) in Novena, Inc. and Curematch, Inc.; reports receiving a commercial research grants from Genentech, Merck Serono, Pfizer, Sequenom, Foundation Medicine, and Guardant; and is a consultant/advisory board member for Sequenom, Actuate Therapeutics, and Xbioetch. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: J.J. Wheler, A. Naing, S. Fu, A.M. Tsimberidou, R. Yelensky, J.J. Lee, D. Hong

Development of methodology: J.J. Wheler, A.M. Tsimberidou, R. Yelensky

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J.J. Wheler, F. Janku, A. Naing, R. Zinner, V. Subbiah, S. Fu, D. Karp, G.S. Falchook, A.M. Tsimberidou, S. Piha-Paul, D. Ke, V. Miller, D. Hong

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J.J. Wheler, B. Stephen, V. Subbiah, S. Fu, R. Yelensky, J.J. Lee, D. Hong, R. Kurzrock

Writing, review, and/or revision of the manuscript: J.J. Wheler, F. Janku, A. Naing, V. Subbiah, S. Fu, D. Karp, G.S. Falchook, A.M. Tsimberidou, S. Piha-Paul, V. Miller, J.J. Lee, R. Kurzrock

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J.J. Wheler, F. Janku, Y. Li, B. Stephen, V. Subbiah, A.M. Tsimberidou, R. Anderson

Study supervision: J.J. Wheler, D. Karp, R. Anderson, R. Kurzrock

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