P53 Mutations in Advanced Cancers: Clinical Characteristics, Outcomes, and Correlation between Progression-Free Survival and Bevacizumab-Containing Therapy

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ABSTRACT:

Background: Mutations in the p53 gene are amongst the most frequent aberrations seen in human cancer. Our objective was to characterize the clinical characteristics associated with p53 mutation in patients with advanced cancer.

Methods: We retrospectively reviewed and analyzed the clinical features and response to standard systemic therapy of 145 patients with documented tumor p53 mutational status (mutant-type [mtp53] vs. wild-type [wtp53]) referred to the Clinical Center for Targeted Therapy.

Results: Sixty-six (45.5%) patients had mtp53. Mutations in p53 occurred more frequently in older patients (p= 0.015) and in Caucasians (p=0.024). The incidence of liver metastases was 69.2% vs. 43%, p=0.002 in mtp53 and wtp53, respectively. PTEN loss by immunohistochemistry was found more frequently in mtp53-bearing tumors compared to wtp53 (33.3% vs. 10%, p=0.007). The best progression-free survival (PFS) on standard systemic therapy was significantly longer with bevacizumab-containing regimens as compared to non-bevacizumab containing regimen in patients with mtp53 (median 11.0 [95% CI 5.9-16.0], n=22 vs. 4.0 months [95% CI 3.6-5.7], n=35, p<0.0001) but not those with wtp53 (median 5.0 [95% CI 2.0-7.6] vs. 6.0 [95% CI 4.0-7.5] months, p=0.318. The median overall survival from diagnosis in patients with mtp53 and wtp53 was 7.4 [95% CI 6.3-9.8] vs. 11.8 [95% CI 2.9-21.5] years, respectively (p=0.365).

Conclusion: Patients with mtp53 tumors were older at diagnosis, had more incidence of liver metastasis, and more frequent PTEN loss. The best PFS on standard systemic therapy was significantly longer with bevacizumab-containing regimens in patients with mutant p53 tumors but not in those with wtp53.

INTRODUCTION

Mutated p53 is one of the most common genetic abnormalities detected in human cancers (1). The first alteration of the p53 gene in human cancer was reported in 1989 (2). Since then, the p53 gene has been a major research target in cancer biology and drug development, leading to the discovery of more than 30,000 reported mutations so far (3). Most p53 mutations have been reported within centrally located hot spots in the DNA-binding domain (4).

As a major tumor suppressor gene, p53 regulates
the cell cycle, controls DNA repair mechanisms and activates apoptotic pathways (5). In addition, \textit{p53} protein plays a role in regulating angiogenesis at least in part through direct binding to the hypoxia-induced factor-\(\alpha\) (HIF-\(\alpha\)) subunit, leading to HIF-\(\alpha\) destruction (6). Under physiologic conditions, intracellular levels of \textit{p53} protein

| Characteristics | Total Patients, N=145 | mtp53, N=66 (%) | wtp53, N=79 (%) | * P-value |
|-----------------|-----------------------|----------------|----------------|-----------|
| Age at diagnosis (median, range) | 53.4 (14.5-75.3) | 56.1 (22.2-72.6) | 51.0 (14.5-75.3) | 0.015     |
| Age at diagnosis ≥60 years | 45 | 29 (43.9%) | 16 (20.3%) | 0.004     |
| Gender | | | | 0.740 |
| Male | 72 | 34 (47.2) | 38 (52.8) |
| Female | 73 | 32 (43.8) | 41 (56.2) |
| Race | | | | 0.024 |
| White | 115 | 58 (50.4) | 57 (49.6) |
| Non-White | 30 | 8 (26.7) | 22 (73.3) |
| Type of cancer | | | | |
| Breast | 3 | 1 (33.3) | 2 (66.7) |
| Colorectal | 31 | 20 (64.5) | 11 (35.5) |
| Endometrial | 5 | 3 (60.0) | 2 (40.0) |
| Head and Neck | 12 | 5 (41.7) | 7 (58.3) |
| Lung | 8 | 6 (75.0) | 2 (25.0) |
| Melanoma | 13 | 3 (23.1) | 10 (76.9) |
| Ovarian | 13 | 7 (53.9) | 6 (46.1) |
| Pancreatic | 3 | 3 (100.0) | 0 (0.00) |
| Renal | 3 | 0 (0.0) | 3 (100.0) |
| Sarcoma | 23 | 8 (34.8) | 15 (65.2) |
| Other GI | 9 | 5 (55.6) | 4 (44.4) |
| Other GU | 9 | 3 (33.3) | 6 (66.7) |
| Others | 13 | 2 (15.4) | 11 (84.6) |
| Site of metastasis** | | | | |
| Brain | 17 | 8 (12.1) | 9 (11.4) |
| Liver | 79 | 45 (59.2) | 34 (43.0) |
| Lung | 103 | 49 (75.4) | 54 (68.4) |
| Retroperitoneum | 34 | 20 (30.8) | 14 (17.7) |
| Bone | 51 | 22 (33.9) | 29 (36.7) |
| Lymph node | 110 | 51 (78.5) | 59 (74.7) |
| Soft tissue | 30 | 10 (15.4) | 20 (25.3) |
| Peritoneal | 48 | 24 (36.9) | 24 (30.4) |
| Adrenal | 15 | 7 (10.8) | 8 (10.1) |
| Ovary | 19 | 9 (28.1) | 10 (25.0) |
| Skin | 16 | 4 (6.2) | 12 (15.2) |
| Median time from diagnosis to metastases (months) | 8.9 (0 – 361.7) | 7.6 (0 – 361.7) | 9.9 (0 – 235.2) | 0.536 |
| Time from diagnosis to metastases ≥2 years | 32 (22) | 15 (22.7) | 17 (21.5) | 1.000 |
| Median number of phase 1 therapies (range) | 1 (0 – 4) | 1 (0 – 4) | 1 (0 – 4) | 1.000 |
| Median number of prior cancer therapies | 3 (0 – 12) | 3.5 (0 – 10) | 3 (0 – 12) | 0.080 |
| Median number of metastases (range)** | 3 (0 – 9) | 4 (0 – 9) | 3 (0 – 8) | 0.080 |

*P-values are from Fisher’s exact test or Kruskal-Wallis test, as appropriate.

** Patients may have multiple sites of metastasis and some patients may have unavailable data on their sites of metastasis.

The P-value in each row is computed for testing the association of each metastasis site and \textit{p53} mutation.
are maintained at low levels by a complex network of proteins that include murine double minute 2 (Mdm2) (7). Targeting aberrant p53 has proven challenging. Mdm2 inhibitor molecules have demonstrated preclinical promise in a wide variety of tumors with wild-type p53 (8-10). Importantly, MK-1775, a potent and selective small molecule inhibitor of Wee-1 kinase (a tyrosine kinase that phosphorylates and inactivates CDC2 and is involved in G2 checkpoint signaling) selectively sensitizes tumors to DNA damaging agents, probably because p53 is a key regulator in the G1 checkpoint and p53-deficient tumors rely only on the G2 checkpoint after DNA damage (11). It is currently being studied in phase II trials of ovarian cancer with mtp53 (NCT01164995 and NCT01357161) (http://Clinicaltrials.gov). Overall, however, there is a paucity of molecules targeting p53 mutations, and, because these mutations are found in over 50% of cancer, identifying ways to counteract them is important.

The clinical correlates of p53 mutations malignancies have not been fully delineated. Preclinical data have shown that p53 mutation accelerates cancer progression and increases tumor invasiveness and metastasis, which is not, however, the entire picture (12, 13). Here, we studied p53 mutational status in patients with advanced malignancies referred to the Phase I Clinical Trials Program in The University of Texas MD Anderson Cancer Center. Our objectives were to identify clinical, prognostic and predictive characteristics associated with p53 mutational status in advanced solid tumors.

**RESULTS**

**Patient characteristics**

The clinical and demographic characteristics of our patient population are summarized in Table 1. Overall, starting in May 2010, the p53 mutational status of tumors was identified in 145 patients. The median age of the patients at diagnosis was 53.3 years (range, 14.5 to 75.3 years). Women comprised 50.3% (n=73) of the study population. Most patients were Caucasian (n = 115, 79.3%) and the remaining 30 patients were African American (n=15, 10.3%), non-white Hispanic (n= 11, 7.6%), or Asian (n= 4, 2.8%). The type of primary cancer varied among patients. The most common histologic subtypes were colorectal carcinoma (n=31, 21.4%), sarcoma (n=23, 15.9%), ovarian carcinoma (n=13, 9.0%) and melanoma (n=13, 9.0%). The histologic subtypes of patients reflected the pattern of referrals to the Phase I Clinic.

The median time from diagnosis to metastasis/recurrence was 8.9 months (range 0 – 361.7, months). Forty-eight (33.1%) patients had metastatic disease at diagnosis. The most common metastatic sites were the lymphatic system (n=110, 76.4%), lungs (n=103, 71.5%) and liver (n=79, 54.9%). The number of metastatic sites, as reported by the last available imaging studies, ranged from 0 to 9 (median 3). The total number of prior standard systemic therapies before referral to the Phase I Clinical Trials Program ranged from 0 to 9 (median 3) and the total

| Molecular Aberrations | mtp53, N=66 | wtp53, N=79 | *P-value |
|-----------------------|-------------|-------------|----------|
| PTEN loss             | 51          | 17 (33.3)   | 50       | 5 (10.0)   | 0.007    |
| BRAF mutation         | 44          | 1 (2.3)     | 56       | 3 (5.4)    | 0.629    |
| PIK3CA mutation       | 56          | 3 (5.4)     | 66       | 9 (13.6)   | 0.221    |
| KRAS mutation         | 48          | 7 (14.6)    | 56       | 9 (16.1)   | 1.000    |
| EGFR mutation         | 40          | 1 (2.5)     | 46       | 0          | 0.465    |
| c-KIT mutation        | 36          | 0           | 46       | 0          | NA       |
| PTEN mutation         | 19          | 3 (15.8)    | 23       | 0          | 0.084    |
| NRAS mutation         | 29          | 4 (13.8)    | 33       | 1 (3.0)    | 0.176    |
| GNAQ mutation         | 20          | 0           | 25       | 2 (8.0)    | 0.495    |
| MET mutation          | 23          | 1 (4.4)     | 25       | 0          | 0.479    |

*P-values are from Fisher’s exact test
number of phase I clinical trials that patients enrolled on ranged from 0 to 4 (median 1).

**P53 mutational status and clinical features**

Of 145 patients, 66 (45.5%) had tumors harboring the p53 mutation. The presence of a p53 mutation was significantly associated with patient age at diagnosis. Cancer diagnosed at an older age was associated with a greater number of p53 mutations than was cancer diagnosed at a younger age. At diagnosis, the median age of patients with p53 mutated tumors was 56.1 years (22.2 - 72.6 years) versus 51.0 years (14.5 - 75.3 years) for patients with wtp53 tumors (p = 0.0145). In addition, each year’s increase in age at diagnosis was associated with a 4% increase in the odds of having a p53 mutation (OR = 1.04, 95% CI 1.01 to 1.07, p = 0.019).

The percentage of p53 mutations varied with tumor type, as expected (Table 1). Of note, all three patients with pancreatic cancer were found to have mtp53 tumors, and all three patients with renal cell carcinoma were found to have wtp53. Mutant p53 tumors metastasized more frequently to the liver than did wtp53 bearing tumors (69.2% vs. 43.0%) (p = 0.002). In addition, mtp53 tumors tended toward retroperitoneal metastasis compared to wtp53 tumors (30.8% vs. 17.7%, p = 0.078). There was no statistically significant difference in the percentage of metastases between mtp53 and wtp53 tumors to the lung, brain, lymph nodes or to any other metastatic sites.

Univariate analysis showed that mtp53 tumors occurred more frequently in Caucasian patients (50.4%) compared to non-Caucasian patients (26.7%; p = 0.024). However, there was no correlation between p53 mutational status and gender, number of metastatic sites and time from diagnosis to metastasis.

The family history of cancer in patients with mtp53 tumors was reviewed in patients’ electronic medical records. Three of 66 (4.5%) patients with mtp53 met the criteria for Li-Fraumeni-like syndrome (14, 15). Among those three patients, two had sarcoma (ages at diagnosis 27 and 44) with multiple family members (first, 2nd and 3rd degree) having breast cancer, brain tumors, lung cancer and other malignancies. One patient with ovarian cancer (negative BRCA1/2 mutation) diagnosed at age 47 had multiple family members with breast cancer at a young age (42 and 48 years old), leukemia (20 years old), brain tumor and other malignancies.

**Types of p53 mutations and the co-existing molecular aberrations**

Five (7.6%) patients were found to have two molecular abnormalities of the p53 gene in two different exons and two (3.0%) patients were found to have two molecular abnormalities of the p53 gene in the same exons. Insertion or deletion (indels) was seen in 7 (10.9%) patients. The most common p53 mutations were seen in exon 5 (n=23, 33.3%), followed by exon 6 (n=14, 20.3%), exon 7 (n=12, 17.4%), exon 4 (n=9, 13.0%), exon 8 (n=9, 13.0%) and exon 9 (n=2, 3.0%).

The number of other molecular aberrations tested ranged from 0 to 3 (median 1). Insufficient tissue availability for testing was the main reason for the inability to test for other aberrations. However, some data on PI3KCA, KRAS, BRAF, c-KIT and EGFR mutations, and PTEN status assessed by IHC, was available for more than 50% of patients’ specimens. The number and proportions of all molecular aberrations and their relationships to p53 mutational status are summarized in Table 2. PTEN loss by IHC, but not the other aberrations, was statistically correlated with mtp53 (33%, 17/51 mtp53 vs. 10%, 5/50 wtp53).
**P53 mutational status and the longest PFS on conventional systemic treatment**

We analyzed the longest PFS achieved on conventional systemic treatment (before referral for phase I treatment) for metastatic/recurrent disease according to P53 mutational status and types of treatment regimens. Data on the longest times of PFS were available for 112 (77.2%) patients (57 patients with mtp53 tumors and 55 patients with wtp53 tumors). The median duration of the longest PFS for all patients was 5.9 months (range 0.6-49.9 months). The anti-angiogenic agent bevacizumab was included in the systemic treatment regimen in 25.9% (n= 29 out of 112) of patients with available data on the longest PFS. The inclusion of bevacizumab in the treatment regimen that had the longest PFS was significantly associated with P53 mutation status (38.6% (n=22 out of 57) of those in the mutation group versus 12.7% (n=7 out of 55) of those in the wild-type group, p=0.0023).

Among patients with mtp53 tumors, the longest median PFS was 11.0 months (range 0.9 – 29.0 months) when the treatment regimen included bevacizumab (n=22) and 4.0 months (range 0.9 – 15.0 months) when the treatment did not include bevacizumab (n=35) (p< 0.0001) (Figure 1a). On the other hand, among patients with wtp53, there was no significant difference in PFS between bevacizumab-containing regimens (n=7) and regimens without bevacizumab (n=48) with a median of longest PFS being 5.0 (range 2.0-13.0) months vs. 6.0 (range 0.6-49.9) months respectively, p= 0.32 (Figure 1b).

In multivariate analysis, in patients with mtp53, significant predictors of a longer PFS included younger age (p=0.0096) and bevacizumab-containing regimen (p< 0.0001). In the wtp53 group, none of the independent variables considered was predictive of a longer PFS (Table 3). Variables included in the multivariate model were patient age, race, and P53 mutation status, whether the FDA recommended bevacizumab for the patient’s tumor histology and whether the longest PFS was on a bevacizumab-containing regimen. In addition, we included the interaction term for P53 mutation status and whether the patient was on a Bevacizumab containing regimen during longest PFS. We found that the interaction was statistically significant at p = 0.0005 (Table 4).

The bootstrap analysis and permutation test confirmed the independent prognostic value of interaction between P53 mutational status and bevacizumab-containing treatment in predicting PFS in this data set (Figure 2). The 95% bootstrap confidence interval for the coefficient of the interaction between P53 mutation and the bevacizumab-containing treatment is (-3.02, -1.09). The P-value for testing zero interaction was 0.001 based on the permutation test.

Within the subgroup of patients who had colorectal cancer primary tumors, the longest PFS was available for 29 (n=20 with mtp53 and n=9 with wtp53) patients. Among mtp53, the median longest PFS was 11.0 (95% CI: 7.0 -116.0) vs. 7.9 (95% CI: 3.3-15.0) months (p=0.16), if the patient received a bevacizumab-containing regimen or not, respectively. Among wtp53, the median longest PFS was 5.5 (95% CI: 2.0-13.0) vs. 8.0 (95% CI: 4.9-22.0) months, by receipt of a bevacizumab-containing regimen or not, respectively (p=0.26).

**P53 mutational status and survival**

Our patient population was heavily pretreated, with as many as 9 different systemic treatment regimens before referral to our Phase I Clinic and with as many as 5 different phase I clinical trials after referral. The difference between median overall survival from the time of diagnosis for patients with mtp53 (n=66) and wtp53 (n=79) was 4.4 years, but this difference was not statistically significant (7.4 (95% CI 6.3, 9.8 years) vs. 11.8 (95% CI 6.9, 21.5) years respectively, p=0.365). The

| Clinical Feature               | mtp53          | wtp53          |
|-------------------------------|----------------|----------------|
|                               | Hazard Ratio  | 95% CI         | p-value | Hazard Ratio  | 95% CI         | p-value |
| Age*                          | 0.96          | 0.94-0.99      | 0.010   | 0.99          | 0.97-1.02      | 0.651   |
| Race (Non-Caucasian vs. Caucasian) | 0.74          | 0.34-1.62      | 0.456   | 1.54          | 0.84-2.82      | 0.167   |
| Tumor histology group§(Recommended vs. not recommended) | 0.56          | 0.29-1.09      | 0.086   | 0.84          | 0.38-1.84      | 0.657   |
| Bevacizumab-containing regimen (Yes vs. no) | 0.21          | 0.09-0.454     | <0.0001 | 1.82          | 0.661-5.02     | 0.247   |

* Age as continuous variable, § Recommended versus not recommended means dichotomized into tumor histologies for which bevacizumab is or is not FDA-approved
median overall survival from phase I referral was 1.3 years (95% CI 0.9, 1.9 years) for patients with mtp53 tumors and 2.6 years (95% CI 1.1, not attained) for wtp53 patients, \( p=0.190 \).

**DISCUSSION**

P53 aberrations are frequent in cancer. The percentage of p53 mutation varies by tumor type and ranges from 10% to 80% (http://p53.free.fr). Our patients had a 45.5% rate of p53 mutation. We found that mtp53 status was associated with more advanced age, results that are consistent with a previous study showing that mtp53 occurred in older patients with rectal cancer (16). However, in contrast to our data, a previous study showed that mtp53 breast cancer is three times more likely to occur in young women (age ≤40 years at diagnosis) as compared to wtp53 breast cancer (17).

Our analysis showed that p53 mutations occurred more frequently in Caucasian patients in contrast to previously reported data which showed that p53 mutation status did not differ between Caucasian and African-American patients (18, 19). However, the limited number of patients in our series precludes making definitive comparisons.

A role of p53 mutation in cell migration and invasion (20) has been demonstrated in preclinical models (12, 13). These data could explain the higher percentage of patients with liver metastasis and the trend toward a higher percentage of patients with retroperitoneal metastasis seen in our mtp53 tumors compared to those with wtp53 tumors. In fact, Tullo et al. previously showed that mtp53 colorectal cancer had a greater number of metastatic liver lesions than wtp53 colorectal cancer (21).

Previously reported data showed that p53 mutation is correlated with lymph node metastasis in pancreatic and prostate carcinoma (22, 23) which was not confirmed in our analyses.

Li-Fraumeni syndrome, characterized by heterozygous germline p53 mutations and the occurrence of sarcoma, breast carcinoma, adrenocortical carcinoma, brain tumors and leukemia at a young age, is considered a very rare hereditary cancer syndrome and its characterization was initially based on a study of 12 families (24). The initial classical criteria to suspect Li-Fraumeni syndrome are very specific but have only moderate sensitivity (25). Since that seminal study, many other criteria have been uncovered, which increased the likelihood of detecting affected patients and families (25, 26). Gonzalez et al. showed that a germline mutation of p53 resulted in a wider spectrum of tumors, as detected in 17% of the blood of cancer patients tested, and that 95% of patients in that study met the criteria for Li-Fraumeni or Li-Fraumeni-like syndrome (26). The family history of our patients with mtp53 tumors showed that 3 of 66 patients (4.5%) met the criteria of Li-Fraumeni-like syndrome. Testing for germline mutations in this patient population might shed further light on this issue.

The role of the TP53 gene in maintaining genomic integrity in mammalian cells has been previously described (27). Therefore, a greater number of molecular abnormalities would be expected to be associated with mtp53, which was difficult to confirm using our data due to the lack of available tissue for testing from a relatively large number of patients. Even so, our data showed a correlation between mtp53 and PTEN loss by IHC. PTEN

![Figure 2: Distribution of the parameter estimate (-1.89, 95% CI -3.06, -1.06) for the interaction between P53 and bevacizumab using 1,000 bootstrap resamples with replacement](http://impactjournals.com/oncotarget)
is an important inhibitor of the PI3K/Akt/mTOR pathway and loss of PTEN is associated with its activation (28). These findings are consistent with previously reported preclinical data showing coordinate regulation of the p53 and mTOR pathway (29). Stambolic et al. also showed that PTEN transcription is regulated by p53 (30).

Of interest, our analysis demonstrated that the median longest PFS for patients with mtp53 was 11 (95% CI 5.9, 16.0) months if bevacizumab was included in the treatment regimen versus 4 (95% CI 3.6, 5.7) months if it was not (p<.0001). These data complement previously published preclinical data demonstrating a correlation between mtp3 and increased VEGF expression and vessel density in head and neck tumors (31), breast carcinoma (32, 33) and stromal cell of the bone marrow in leukemic patients (34). Other authors also reported the role of p53 protein in inhibiting angiogenesis and neovascularization (35, 36). Additionally, the correlation between p53 mutation and neovascularization has been observed in various tumor cell lines and xenograft tumor models (6). On the other hand, Ince et al. showed that, in patients with colorectal cancer treated with bevacizumab, p53 mutational status was not associated with survival (37). However, our multivariate analysis showed that within the group of patients with mtp53, treatment with a bevacizumab-containing regimen was an independent factor associated with longer PFS. Whether or not bevacizumab was associated with prolonged PFS in the wtp53 group could not be accurately assessed because of the small number of patients. There are methodology differences between the study of Ince et al. and ours that may account for the appearance of discrepant results. The most important difference is that we focused on analyses of PFS while Ince et al. analyzed the overall survival. Hence, two studies cannot be compared.

Our analysis has several important limitations. First, diverse histologies were analyzed with small numbers of patients in each diagnostic category, precluding our ability to determine the relationship between p53 and other factors within disease subgroups. On the other hand, our findings could imply that the impact of bevacizumab on PFS in mtp53 tumors is not dependent on histology. Studies with larger numbers of patients are needed for confirmation. The retrospective nature of the study is also a limitation. There may also be a selection bias, because we analyzed only patients with metastatic disease and cannot ascertain the clinical behavior of tumors in patients whose disease never metastasized. The absence of randomization in regard to PFS also limits the firmness with which conclusions can be made, even in the presence of a multivariate analysis. Because of these limitations, this study is best viewed as exploratory and further studies are warranted to validate our findings.

In conclusion, mtp53 tumors appear to have distinct biological associations, including older age at diagnosis, increased liver metastasis and association with PTEN loss as compared to wtp53 tumors. When analyzed retrospectively, patients with mtp53 had significantly longer PFS if bevacizumab was included in their therapeutic regimens than if it was not. Prospective studies are warranted to further investigate these observations.

**METHODS**

**Patients**

We investigated the p53 mutation status of consecutive patients with advanced tumors with available tissue referred to the Clinical Center for Targeted Therapy in the Department of Investigational Cancer Therapeutics (Phase I Clinical Trials Program) at The University of Texas MD Anderson Cancer Center starting from May 2010 through March 2011. Out of 832 patients, 145 were tested for p53 mutations. Other patients were not tested because of lack tissue availability. Patients referred to the Phase I Clinic were of various ages and histology types. Upon presenting to the Phase I Clinic, all patients had either metastatic or locally recurrent/advanced disease.

**Table 4: Multivariate Cox proportional hazards regression model predicting duration of longest PFS**

| Clinical Feature                                      | Hazard Ratio | 95% CI     | P-value |
|------------------------------------------------------|--------------|------------|---------|
| Age*                                                 | 0.99         | 0.97-1.01  | 0.179   |
| Race (Non-Caucasian vs. Caucasian)                    | 1.32         | 0.83-2.09  | 0.243   |
| Tumor histology group§ (Recommended vs. not recommended) | 0.72         | 0.43-1.21  | 0.215   |
| Bevacizumab -containing regimen (Yes vs. no)          | 2.50         | 1.00-6.29  | 0.051   |
| P53 Status (Mutation vs. wild type)                   | 2.65         | 1.57-4.47  | <0.001  |
| P53 mutation status and bevacizumab therapy interaction | 0.15         | 0.05-0.44  | <0.001  |

* Age as continuous variable, § Recommended versus not recommended means dichotomized into tumor histologies for which bevacizumab is or is not FDA-approved
and were not candidates for treatment that was associated with a clinically significant improvement in survival. In general, no therapy that prolonged survival by more than three months was available. To identify the clinical impact of \(p53\) on cancer behavior and response to treatment, we compared the demographics, clinical characteristics, and responses of patients to their best systemic treatment before referral to the Phase I Clinic based on their \(p53\) mutational status. The study was performed in accordance with MD Anderson Institutional Review Board guidelines.

**Tissue samples and mutational analysis**

Available tissue from diagnostic and therapeutic procedures was used to assess for molecular aberrations including \(p53\) mutational status. Tissue includes paraffin-embedded tissue blocks, formalin-fixed archived specimens, and/or fine needle biopsy aspiration. All pathology was centrally confirmed at MD Anderson. Testing for all molecular aberrations including \(p53\) mutations was performed in a Clinical Laboratory Improvement Amendment (CLIA)–certified Molecular Diagnostic Laboratory within the Division of Pathology and Laboratory Medicine at MD Anderson, and was done on any available sample with adequate tissue for testing.

DNA was extracted from microdissected tumor sections and analyzed using a polymerase chain reaction (PCR)-based DNA sequencing method for \(p53\) mutation within the most common area (exons 4-9) (38).

Whenever tissue was available, other mutations such as \(EGFR\) (exons 18 - 21) (39), \(KIT\) (exons 11, 13 and 17) (40), \(PIK3CA\) (exons 9 and 20) (41), \(NRAS\) and \(KRAS\) (exon 1 and 2) (42, 43), \(BRAF\) (exon 15) (42), \(GNAQ\) (44) and \(MET\) mutations were also tested (45). \(PTEN\) loss was assessed using immunohistochemistry (46) (monoclonal mouse anti-human \(PTEN\), clone 6H2.1; Dako®, Denmark).

**Statistical analysis**

Statistical analysis was carried out by our statisticians (CLW & J JL). Clinical variables were assessed by reviewing patient electronic medical records. The variables analyzed were age, gender, race, site and number of metastases, site of mutation, presence of other aberrations (\(PIK3CA\), \(NRAS\), \(KRAS\), \(EGFR\), \(BRAF\), \(PTEN\), \(GNAQ\) and \(c-KIT\) mutations and \(PTEN\) loss by immunohistochemistry [IHC]), the systemic therapy with the longest progression-free survival (PFS) before referral to the phase I clinic; and survival from date of diagnosis. The family history of each patient with mutated \(p53\) tumors was reviewed and analyzed to determine if patients met Li-Fraumeni or Li-Fraumeni-like syndrome criteria (25).

Patient demographics and clinical characteristics were summarized using simple descriptive statistics including frequencies, percents, and medians. Associations between \(p53\) mutation status and patient characteristics were assessed using Fisher’s exact test and the Kruskal-Wallis test, as appropriate. Odds ratios and 95% confidence intervals were computed using univariate logistic regression models. PFS and overall survival were analyzed by the method of Kaplan and Meier. PFS while on systemic therapy before referral to the Phase I Clinic was the time interval between the start of therapy to the first observation of disease progression (as determined by clinical or radiological findings) or death whichever came first. For the overall survival, patients who were alive at the end of follow-up were censored at the date of their last follow-up. Survival probabilities were compared among subgroups of patients using the log-rank test. Univariate and multivariate Cox proportional hazards regression models were fit to assess the association between the time-to-event endpoints and patient characteristics, including \(p53\) mutation status. A subanalysis was conducted including only patients with colorectal cancer primaries. This subanalysis was performed because colorectal cancer was the largest histologic subgroup, and in addition, bevacizumab is approved for this indication. A post-hoc bootstrap procedure was used to verify the results obtained with the Cox proportional hazards model. From the original data set, we constructed 1000 replicate data sets using resampling with replacement. Thus, the size of the replicate data sets remained the same as in the original cohort, but the composition differed. The distribution of regression parameters in the bootstrap samples was calculated to form the 95% confidence interval. In addition, the P-value based on the permutation test was computed by comparing the model estimate to the estimates under the null hypothesis by shuffling the mutation status 100,000 times in the original data set to create the null distribution. P-values less than 0.05 were considered statistically significant. All statistical analyses were conducted using SAS software (v 9.2).

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