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Mutated TP53 is a marker of increased VEGF expression: analysis of 7,525 pan-cancer tissues

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ABSTRACT
Anti-angiogenic therapies are an important class of anti-cancer treatment drugs. However, their efficacy is limited to certain tumors and would benefit from identifying a biomarker predictive of therapeutic response. TP53 (tumor protein p53) is a tumor suppressor gene frequently mutated in cancer and implicated in cell-cycle regulation, apoptosis, and angiogenesis. Data from 7,525 unique tumor samples (representing 30 tumor cohorts) were retrieved from the TCGA database to analyze the relationship between TP53-mutation status and VEGFA (vascular endothelial growth factor A) expression. Univariate analyses were done using a Mann-Whitney univariate test or Fisher’s exact test. Parameters with a p-value (p) ≤ 0.1 in univariate analysis were selected for follow-up multivariate analyses, including TP53-mutation status, cancer cohorts, cancer subtypes, and VEGFA expression. Our analysis demonstrates statistically significant increases in VEGFA mRNA tissue expression in TP53-mutated adenocarcinomas (but not in squamous cancers) compared to TP53 wild-type tumors. This association holds true in multivariate analyses and remains independent of HIF-1α and MDM2 overexpression. Our findings provide additional evidence that TP53 mutations are linked to the VEGF pathway, potentially offering insight into the mechanism behind increased sensitivity to anti-angiogenic therapies observed in some TP53-mutant tumors.

Introduction
Personalizing therapy for cancer patients by pairing their tumor molecular profiles with “matched” treatments has demonstrated success in several clinical contexts. However, continuing to identify biomarkers that predict therapeutic response is necessary to expand this approach. In particular, anti-angiogenic therapies (drugs that target the neo-vascularization process allowing tumors to self-sustain) would benefit from identification of a specific biomarker. This pharmacology class encompasses over ten approved antibodies or small molecule inhibitors that target the vascular endothelial growth factor/vascular endothelial growth factor receptor (VEGF/VEGFR) axis (Supplementary Figure S1). Indeed, one of the best-selling drugs in oncology is bevacizumab, a VEGF-A monoclonal antibody. Though indicated for use in a variety of cancers, such as renal cancer, colon cancer, non-small cell lung cancer (NSCLC) and glioblastoma, the impact on survival in non-selected patients is modest, and bevacizumab’s approval in metastatic breast cancer was revoked in 2011 by the Food and Drug Administration (FDA). Anti-angiogenic therapies are also expensive and have numerous side effects, including gastrointestinal perforation, hypertension, and hemorrhage. Identifying specific parameters that predict response to anti-angiogenesis therapy may be used to separate patients likely to benefit from those that might be transferred to an alternative therapy.

TP53 (tumor protein p53) is a multifunctional tumor suppressor gene that is also intimately involved in the process of neo-vascularization, often through various inhibitory mechanisms. For example, TP53 promotes degradation of the hypoxia-induced factor subunit α (HIF-1α) in the cell. HIF-1 serves as a key transcriptional activator of VEGF-mediated angiogenesis in response to oxygen deprivation. The relationship between TP53 and HIF-1α is not yet fully clear, but TP53 appears to also affect angiogenesis via other pathways. One study demonstrated that TP53 inhibits VEGF expression through a pathway involving tumor protein 21 (p21) and retinoblastoma (Rb) in vitro. Another study characterized the E2F transcription factor 1 (E2F1) and found an apparent relationship with TP53 to directly downregulate VEGF expression in a HIF-1α independent fashion. A role for TP53 in angiogenesis has been established in multiple studies – in bone marrow stromal cells, transfection of mutant TP53 increased synthesis of VEGF and supported leukemia cell growth. Angiogenesis is an area of interest in these disorders, as vascular development has been observed in lymph nodes and bone marrow in patients with various hematologic malignancies; treatment strategies are being explored in this area. In a separate study demonstrating the reverse, the restoration of TP53 expression in

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a population of previously altered-TP53 cells was associated with decreased angiogenesis.\textsuperscript{13}

Somatic mutations in the TP53 gene are found in high rates across multiple cancers, such as colorectal, lung, and head and neck – even ranging up to over 85% in high-grade ovarian serous carcinoma.\textsuperscript{14} Mutations in TP53 are used as markers of clonality, recurrence and cancer prognosis,\textsuperscript{15} but are often considered non-actionable by conventional therapies.

Recently, several studies have suggested that TP53 mutation status may be predictive of clinical sensitivity to VEGF/VEGFR inhibitors in certain tumors. A recent analysis showed that VEGFA transcript expression correlated independently with TP53 mutational status in patients with adenocarcinoma (but not squamous) NSCLC.\textsuperscript{16} Additional studies have demonstrated statistically significant improvements in clinical outcomes (such as response rate, progression-free survival, and overall survival) among TP53-mutant patients treated with anti-VEGF or anti-VEGFR therapies, compared to TP53-wild-type populations.\textsuperscript{17–20} The increased expression of VEGFA may explain the improved response to VEGF/VEGFR inhibitors in TP53-mutant populations, but to date, datasets have only interrogated a relatively small number of patients. Herein, we present an analysis of TP53 mutation status and VEGF/VEGFR expression in a large pan-cancer cohort, using a collection of 7,525 tumor samples from The Cancer Genome Atlas (TCGA).

Results

Somatic TP53 mutations are frequent in human tumors

Of 10,011 samples available through the TCGA database, only 7,525 (30 tumor types) included available data on both TP53 mutation status and VEGF pathway transcript expression estimates (Table 1). Analyses were performed on the full set of 7,525 samples (pan-cancer cohort) and/or by individual tumor types.

TP53 mutations were found in 35% of all samples. The most mutated cohorts, percentage-wise, were uterine carcinoma (50/56 samples, 89%), ovarian serous cystadenocarcinoma (210/245, 86%), and squamous NSCLC (145/178, 81%). In contrast, uveal melanoma (0/80, 0%), pheochromocytoma and paraganglioma (1/161, 1%), and thyroid carcinoma (3/397, 1%) contained the lowest percentage of TP53-mutated samples.

VEGF pathway biomarkers are differentially expressed in several subsets of TP53-mutated tumors

Table 2 presents the analysis of mRNA expression levels of key angiogenesis biomarkers, considering the presence or absence of a somatic TP53 mutation.

When examining specific cancer cohorts, TP53 mutations were associated with a significant increase of VEGFA mRNA expression levels in breast carcinoma, colon adenocarcinoma, and NSCLC adenocarcinoma, compared to TP53 wild-type tumors (univariate analysis, breast carcinoma \( p < .001 \), colon adenocarcinoma \( p = .007 \), NSCLC adenocarcinoma \( p = .024 \) – Table 2). VEGFA expression remained a factor independently associated with TP53 mutation in breast and colon carcinoma in multivariate analysis models including other VEGF ligands (VEGFA, VEGFB and VEGFC) and VEGF receptors (FLT1, KDR and NRP1): \( p < .001 \), odds ratio (OR)[Confidence Interval (CI)] 95%] = 2.10 [1.74–2.54] for breast carcinoma, and \( p = .005 \), OR[95%CI] = 1.48 [1.13–1.95] for colon adenocarcinoma – Supplementary Table S1, Panels B and C. In contrast, squamous NSCLC tumors demonstrated a markedly different biomarker expression profile, with significant decreases in VEGFC, FLT1, and KDR expression levels (VEGFC \( p = .030 \), FLT1 \( p = .035 \), KDR \( p = .007 \) – Table 2), as well as no increase in VEGFA expression (in univariate analysis). However, upon multivariate analysis, no change in expression in any of these factors was observed (Supplementary Table S1, panel E). Glioblastoma showed no significant change in expression in any factor associated with TP53 mutations (Table 2 and Supplementary Table S1, panel F).

Similar results were found when considering the combined tumor set (pan-cancer analysis): angiogenesis ligands demonstrated significant increases of expression in TP53-mutated tumors, compared to their wild-type counterparts (multivariate analysis, VEGFA \( p < .001 \), VEGFB \( p = .007 \), VEGFC \( p = .012 \) – Table 2). Conversely, angiogenesis receptors demonstrated significant decreases of expression in TP53-mutated tumors (multivariate analysis, FLT1 \( p = .049 \), KDR \( p < .001 \) – Table 2).

Table 1. Description of the pan-cancer cohort (\( N = 7,525 \) samples with known TP53 status).

| Cancer Type | Total samples | TP53-mutated samples |
|-------------|---------------|----------------------|
| All cancer cohorts | 7,525 | 2,670 (35%) |
| Adrenocortical carcinoma | 52 | 12 (23%) |
| Bladder Urothelial Carcinoma | 388 | 193 (50%) |
| Breast Invasive Carcinoma | 960 | 292 (30%) |
| Cervical Squamous Cell Carcinoma & Endocervical Adenocarcinoma | 190 | 9 (5%) |
| Cholangiocarcinoma | 35 | 5 (14%) |
| Colon Adenocarcinoma | 360 | 227 (63%) |
| Lymphoid Neoplasm Diffuse Large B-cell Lymphoma | 41 | 5 (12%) |
| Glioblastoma Multiforme | 136 | 44 (32%) |
| Head and Neck Squamous Cell Carcinoma | 488 | 343 (70%) |
| Kidney Chromophobe | 66 | 22 (33%) |
| Kidney Renal Clear Cell Carcinoma | 431 | 9 (2%) |
| Kidney Renal Papillary Cell Carcinoma | 161 | 5 (3%) |
| Acute Myeloid Leukemia | 160 | 13 (8%) |
| Brain Lower Grade Glioma | 509 | 247 (49%) |
| Liver Hepatocellular Carcinoma | 190 | 60 (32%) |
| Non-small Cell Lung Adenocarcinoma | 487 | 253 (52%) |
| Non-small Cell Lung Squamous Cell | 178 | 145 (81%) |
| Carcinoma | 245 | 210 (86%) |
| Pancreatic Adenocarcinoma | 113 | 72 (64%) |
| Pheochromocytoma and | 161 | 1 (1%) |
| Paraganglioma | 332 | 41 (12%) |
| Prostate Adenocarcinoma | 119 | 88 (74%) |
| Rectum Adenocarcinoma | 239 | 80 (33%) |
| Melanoma | 294 | 48 (16%) |
| Uveal | 80 | 0 (0%) |
| Stomach Adenocarcinoma | 269 | 123 (46%) |
| Testicular Germ Cell Tumors | 147 | 2 (1%) |
| Thyroid Carcinoma | 397 | 3 (1%) |
| Uterine Corpus Endometrial Carcinoma | 241 | 68 (28%) |
| Uterine Carinosarcoma | 56 | 50 (89%) |

Abbreviations: \( N = \) number.
5,752 tumors presenting a TP53 mutation were assessed – tumors with a TP53 mutation were compared to those without a TP53 mutation, within individual cohorts. All p-values ≤0.05 were considered significant (Mann Whitney U Test) and are presented. *NRP1 is a co-receptor interacting with VEGFR1 and VEGFR2. **p-values ≤0.1 in univariate were selected for multivariate analysis. Detailed calculations are given in Supplementary Table S1.

Abbreviations: * = non-significant change; 95% CI = 95% confidence interval; N = number; OR = odds ratio.

### Table 2. Angiogenesis factors associated with TP53 mutations in selected and non-selected cancer cohorts.

|                | Breast carcinoma | Colon carcinoma | Adenocarcinoma | Squamous carcinoma | Glioblastoma | Pan-cancer |
|----------------|------------------|-----------------|----------------|--------------------|--------------|------------|
| Number of TP53-mutated samples (N) | 292              | 227             | 253            | 145                | 44           | 2,670      |
| LIGANDS        |                  |                 |                |                    |              |            |
| VEGFA expression | Increased        | Increased       | Increased      | Decreased          | Decreased    | Increased  |
| p-value        | p < .001         | p = .007        | p = .024       | p = .030           | p = .038     | p < .001   |
| VEGFR expression | -                | -               | -              | -                  | -            | -          |
| p-value        |                  |                 |                |                    |              |            |
| VEGFC expression | Decreased        | -               | -              | Decreased          | Decreased    | Decreased  |
| p-value        | p = .035         |                  |                | p = .007           | p < .001     | p < .001   |
| RECEPTORS      |                  |                 |                |                    |              |            |
| FLT1 [VEGFR1] expression | -     | -               | -              | -                  | -            | -          |
| p-value        |                  |                 |                |                    |              |            |
| KDR [VEGFR2] expression | Decreased | -               | -              | Decreased          | Decreased    | Decreased  |
| p-value        | p = .003         |                  |                | p = .007           | p < .001     | p < .001   |
| NRP1 expression* | -                | -               | -              | -                  | -            | -          |
| p-value        |                  |                 |                |                    |              |            |

* VEGFA, HIF1A, MDM2 over-expression were first considered as categorical variables, defined: yes if Z-score for mRNA expression ≥ 1.645 (i.e. biomarker is significantly overexpressed, compared to pan-cancer expression levels); no if Z-score for mRNA expression < 1.645 (i.e. biomarker is not significantly overexpressed, compared to pan-cancer expression levels).

**Several multivariate models were built, selecting independent variables with p-values ≤0.1 from univariate analysis. These include TP53 mutation status, cancer type (adenocarcinoma vs squamous), selected cancer cohorts (breast, colon, lung and brain tumors), and HIF1A and MDM2 over-expression.

Abbreviations: * = non-significant change; 95% CI = 95% confidence interval; NSCLC = non-small cell lung cancer; OR = odds ratio

### Table 3. Analysis of factors associated with VEGFA mRNA expression in the pan-cancer cohort (N = 5,752 samples).

|                  | Univariate       | Multivariate** |
|------------------|------------------|----------------|
| VEGFA expression (categorical variable) |                  |                |
| OR [95% CI]      | P-value          | OR [95% CI]    | P-value        |
| TP53 mutation    | 1.26 [1.04–1.54] | .022           | 1.26 [1.04–1.54]| .022 | <.001 | <.001 |
| Adenocarcinoma   | 0.90 [0.74–1.10] | .313           | -              | -    | .448  | -     |
| Squamous         | 1.06 [0.78–1.44] | .690           | -              | -    | .009  | -     |
| TP53 mutation    | 1.26 [1.04–1.54] | .022           | 1.31 [1.07–1.60]| .009 | <.001 | <.001 |
| Breast carcinoma | 0.84 [0.61–1.14] | .296           | -              | -    | .288  | -     |
| Colon adenocarcinoma | 0.61 [0.35–1.07] | .101 | -              | -    | .185  | -     |
| NSCLC adenocarcinoma | 1.49 [1.06–2.10] | .026 | -              | -    | .240  | -     |
| NSCLC squamous   | 0.38 [0.14–1.02] | .047           | 0.33 [0.12–0.90]| .031 | <.001 | <.001 |
| Glioblastoma     | 1.04 [0.50–2.13] | .852           | -              | -    | .074  | -     |
| TP53 mutation    | 1.26 [1.04–1.54] | .022           | 1.30 [1.07–1.59]| .009 | <.001 | <.001 |
| HIF1A overexpression | 2.01 [1.37–2.96] | .001 | 2.01 [1.36–2.95]| <.001| .044  | .002  |
| MDM2 overexpression | 1.50 [0.96–2.34] | .081 | -              | -    | .609  | -     |
| Breast carcinoma | 0.84 [0.61–1.14] | .296           | -              | -    | .288  | -     |
| Colon adenocarcinoma | 0.61 [0.35–1.07] | .101 | -              | -    | .185  | -     |
| NSCLC adenocarcinoma | 1.49 [1.06–2.20] | .026 | -              | -    | .240  | -     |
| NSCLC squamous   | 0.38 [0.14–1.02] | .047           | 0.33 [0.12–0.90]| .031 | <.001 | <.001 |
| Glioblastoma     | 1.04 [0.50–2.13] | .852           | -              | -    | .074  | -     |

* VEGFA, HIF1A, MDM2 over-expression were first considered as categorical variables, defined: yes if Z-score for mRNA expression ≥ 1.645 (i.e. biomarker is significantly overexpressed, compared to pan-cancer expression levels); no if Z-score for mRNA expression < 1.645 (i.e. biomarker is not significantly overexpressed, compared to pan-cancer expression levels).

**Several multivariate models were built, selecting independent variables with p-values ≤0.1 from univariate analysis. These include TP53 mutation status, cancer type (adenocarcinoma vs squamous), selected cancer cohorts (breast, colon, lung and brain tumors), and HIF1A and MDM2 over-expression.

Abbreviations: * = non-significant change; 95% CI = 95% confidence interval; NSCLC = non-small cell lung cancer; OR = odds ratio

### Table 3. Analysis of factors associated with VEGFA mRNA expression in the pan-cancer cohort (N = 5,752 samples).

|                  | Univariate       | Multivariate** |
|------------------|------------------|----------------|
| VEGFA expression (continuous variable) |                  |                |
| OR [95% CI]      | P-value          | OR [95% CI]    | P-value        |
| TP53 mutation    |                  |                |                |                |
| Adenocarcinoma   |                  |                |                |                |
| Squamous         |                  |                |                |                |
| TP53 mutation    |                  |                |                |                |
| Breast carcinoma |                  |                |                |                |
| Colon adenocarcinoma |              |                |                |                |
| NSCLC adenocarcinoma |              |                |                |                |
| NSCLC squamous   |                  |                |                |                |
| Glioblastoma     |                  |                |                |                |
| TP53 mutation    |                  |                |                |                |
| HIF1A overexpression |              |                |                |                |
| MDM2 overexpression |              |                |                |                |
| Breast carcinoma |                  |                |                |                |
| Colon adenocarcinoma |              |                |                |                |
| NSCLC adenocarcinoma |              |                |                |                |
| NSCLC squamous   |                  |                |                |                |
| Glioblastoma     |                  |                |                |                |

In each multivariate, TP53 mutation status appeared independently and significantly associated with an increase in VEGFA expression (p = 0.022 OR [95% CI] = 1.26 [1.04–1.54], p = 0.009 OR [95% CI] = 1.31 [1.07–1.60], p = 0.009 OR [95% CI] = 1.30 [1.07–1.92] – Table 3). This relationship was preserved even in the presence of HIF1A and MDM2 overexpression.

Outside of TP53 mutation status, NSCLC squamous status also demonstrated a relationship with VEGFA expression, albeit a negative one (p = 0.030 OR [95% CI] = 0.86 [0.12–0.90], p = 0.031 OR [95% CI] = 0.83 [0.12–0.90] – Table 3), independent from TP53 mutation status.

Both relationships were preserved when VEGFA expression was evaluated as a continuous variable instead of
a dichotomized categorical variable (TP53 mutation $p < .001$; NSCLC squamous status $p < .001$ – Table 3).

**Relationship between specific TP53 mutation hotspots and VEGF-A expression**

We performed a multi-comparison analysis considering different TP53 mutational hotspots such as variants encompassing codons 175, 220, 245, 248, 273 and all other loci. The level of VEGFA mRNA expression was not significantly different between each of these hotspot mutations (non-parametric ANOVA – Kruskal-Wallis test, $p = 903$, all individual comparisons were non-significant), suggesting that the association between TP53 mutation and VEGFA expression is independent of the type of variant presented by the tumor.

**Discussion**

We provide evidence that somatic TP53 mutation – one of the most frequent genomic alterations found in human tumors – is associated with an increase in expression of VEGFA, the major ligand of the VEGF/VEGFR pathway and a key regulator of angiogenesis. This association, found in a pan-cancer analysis and in selected histological subsets, remains independent from other investigated factors and was preserved in a bidirectional fashion (i.e. TP53-mutated samples are enriched for VEGFA overexpression and VEGFA overexpressed tumors are more likely to present a TP53 mutation, when considering different confounder variables) (Tables 2 and 3). As VEGF-A and its downstream receptors – FLT1 (VEGFR1) and KDR (VEGFR2) – are the targets of multiple FDA-approved therapies, demonstrating a relationship between TP53 mutation and VEGFA expression may help to explain the correlation between TP53 mutations and favorable outcomes observed in clinical studies of patients treated with anti-angiogenesis agents. Though previously published data on the predictive value of circulating VEGF-A did not show a correlation with patient outcome after bevacizumab treatment, it is also known that blood-derived VEGF-A levels do not correlate well with tissue VEGF-A expression.

Interestingly, the relationship between VEGF-A expression and mutant TP53 was found in adenocarcinomas, but not in squamous cell cancers (Supplementary Table S2 and Table 1). This distinction has been previously described in NSCLC. In this study, the association between TP53 mutation and VEGFA expression was established in breast, colon and lung adenocarcinoma, but not in glioblastoma. Furthermore, lung squamous cell carcinomas did not present upregulation of VEGF-A and actually demonstrated downregulation of both receptors VEGFR1 and VEGFR2 (Table 2). VEGFR2 was downregulated across squamous cell cancers regardless of their origin (Supplementary Table S2).

TP53 mutation is a common molecular alteration (35% of all cancers in our study – Table 1) and is found across diverse malignancies, with particularly high mutation rates observed in certain tumor types. High-grade serous ovarian cancers, over 85% of which harbor TP53 mutations, are unusual in that they respond positively to bevacizumab monotherapy. Anti-angiogenic therapies are also important in fields of medicine outside of oncology. For instance, bevacizumab is effective in the treatment and prevention of recurrent pterygium, a benign vascular growth of the eye. Of interest, both TP53 mutations and overexpression of VEGF ligands have been observed in this condition. Additionally, several pre-clinical studies have shown mechanistic associations between TP53 mutations and angiogenesis – in the presence of TP53 mutations, HIF-1α, which is a transcriptional activator of VEGF-A, is increased. Furthermore, transfected mutant TP53 increases VEGF-A levels in bone marrow stromal cells. In regard to HIF-1α, however, our observations suggested that VEGF-A and HIF-1α transcripts are both overexpressed in TP53 mutated tumors, but the higher levels are independently associated with the TP53 mutations (Table 3). The regulation of HIF-1α is mostly at the protein level; however, we did not find a significant association between the presence of TP53 mutation and HIF-1α protein signal detection obtained by reverse-phase protein assay (RPPA) from The Cancer Genome Atlas (TCGA) (mean of protein expression of HIF-1α [95%CI] = 0.20 [−0.46–0.86] vs 0.49 [0.42–0.56], (TP53 mutated versus not) $p = .317$); nor a significant association between VEGFA mRNA overexpression and HIF-1α protein level (mean of HIF-1α protein level [95%CI] = 0.38 [0.15–0.61] vs 0.49 [0.41–0.57], (VEGF-A mRNA high versus low) $p = .350$).

There are limitations to this study. For instance, this study focuses on TP53 mutational status and does not assess other genomic variants. However, a more comprehensive study could possibly highlight additional molecular biomarkers significantly associated with elevated VEGF pathway expression and merits future investigation. Another limitation is that TP53 mutation is being used to predict the expression of another biomarker (VEGF in this case). VEGFA expression or VEGF pathway expression could serve as a biomarker itself, but examining biomarkers based on protein expression (such as by immunohistochemistry) has produced variable results at times. Still, this concept should be explored. Another limitation is that we cannot know, based on the available data, if the TP53 mutations are heterozygous or homozygous. In addition, we cannot state for certain that increased VEGF expression explicitly translates to increased angiogenesis. Another limitation is that many other genes in addition to VEGF are involved in angiogenesis and future studies should address the relationship between this complex network of genes and TP53. Finally, categorization of tumors into adenocarcinomas and squamous cancers across multiple sites of origin represents an oversimplification; however, this represents an attempt to further stratify the analyses completed. Significant further investigation is required to truly elucidate if increased VEGFA mRNA expression in p53-mutated tumors is preserved across multiple types of adenocarcinomas.

Based on this data and the above limitations, it cannot be assumed that all TP53-mutant cancers will respond to anti-angiogenesis agents or that TP53 wild-type tumors will not respond; rather the response rates are expected to be higher in TP53-mutant tumors, consistent with prior studies in the literature. In summary, using a large pan-cancer cohort of 7,525 samples, we demonstrate that increased VEGF-A expression could serve as a biomarker itself, but examining biomarkers based on protein expression (such as by immunohistochemistry) has produced variable results at times. Still, this concept should be explored. Another limitation is that we cannot know, based on the available data, if the TP53 mutations are heterozygous or homozygous. In addition, we cannot state for certain that increased VEGF expression explicitly translates to increased angiogenesis. Another limitation is that many other genes in addition to VEGF are involved in angiogenesis and future studies should address the relationship between this complex network of genes and TP53. Final
organ of origin. This observation may provide a further mechanistic underpinning for the association between TP53 mutations and response to anti-angiogenesis agents and may be of great interest when one considers the high frequency of deleterious TP53 genomic events in human tumors.

Materials and methods

Molecular data retrieval: Data corresponding to 7,525 unique tumor samples, representing 30 different tumor cohorts, were retrieved from the TCGA database (https://cancergenome.nih.gov/): sequencing-based TP53 mutation status and mRNA expression Z-scores (indicating the number of standard deviations away from the mean expression level of the population) of angiogenesis ligands VEGFA, VEGFB, and VEGFC; receptors FMS-like Tyrosine Kinase-1 (FLT1 or VEGFR1) and Kinase Insert Domain Receptor (KDR or VEGFR2); and co-receptor Neuropilin-1 (NRPI, co-receptor to FLT1 and KDR) (Supplementary Figure S1).

TP53 mutation and VEGF biomarker expression association analyses: We analyzed the association between TP53 mutation status and mRNA expression levels of the genes listed above. This analysis was carried out for the entire tumor set as well as for common cancer cohorts in which bevacizumab is commonly used - glioblastoma, NSCLC, and colon cancer. Breast cancer was also included due to the availability of a large number of samples and because bevacizumab was formerly indicated for this disease. We further analyzed the relative associations between factors such as TP53-mutation status, cancer cohorts, cancer subtypes, and VEGFA expression (multivariate analysis). VEGFA expression was characterized as a continuous variable and also dichotomized as a categorical variable, defining mRNA expression Z-scores ≥1.645 as “overexpression” of VEGFA. A Z-score ≥1.645 corresponds to a value in the top 5% of biomarker expression, conferring significance considering a one-tailed p-value of 0.05.

Statistical analysis: Statistical analyses were performed by AB, using SAS University Edition. All univariate analyses were done using a Mann-Whitney univariate test or Fisher’s exact test. Parameters with a p-value (p) ≤0.1 in univariate analysis were selected for follow-up multivariate analyses, in order to further describe the factors independently associated with TP53 mutation or VEGFA overexpression. P-values ≤0.05 were considered statistically significant.

Disclosure statement:

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