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Synergy Between VEGF/VEGFR Inhibitors and Chemotherapy Agents in the Phase I Clinic

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Abstract

Purpose: We hypothesized that chemotherapy synergizes with VEGF/VEGFR (VEGF/R) inhibitors in patients with advanced solid malignancies.

Experimental Design: Patients treated on phase I protocols between December 2004 and July 2013 ($n = 1{,}498$) were included in this analysis. The primary outcome was clinical benefit, defined as stable disease $\geq$6 months, complete response, or partial response. Two odds ratios (OR) for achieving clinical benefit were calculated: one for patients treated with VEGF/R inhibitors (OR with VEGF/R) and another for patients treated without (OR without VEGF/R). To compare these two ORs, an interaction term was included in the multivariate model: (chemotherapy/factor of interest)$\times$(VEGF/R). We took significant interaction terms ($P_{\text{interaction}} < 0.05$) to suggest effect modification (either synergy or antagonism) with VEGF/R inhibitors.

Results: All patients treated with VEGF/R inhibitors exhibited higher OR for clinical benefit than those who were not [OR = 1.9; 95% confidence interval (CI), 1.5–2.4; $P < 0.0001$]. Use of chemotherapy agents concomitant with VEGF/R inhibitors was associated with significantly higher OR for clinical benefit compared with chemotherapy use without VEGF/R inhibitors [OR with VEGF/R = 1.6 (95% CI, 1.1–2.5) vs. OR without VEGF/R = 0.4 (95% CI, 0.3–0.6), $P_{\text{interaction}} = 0.02$]. Specifically, the antimetabolite class was associated with the greatest increase in OR for clinical benefit [OR with VEGF/R = 2.7 (95% CI, 1.5–4.7) vs. OR without VEGF/R = 0.2 (95% CI 0.1–0.3), $P_{\text{interaction}} = 0.004$].

Conclusions: VEGF/R inhibitor was found to synergize with chemotherapeutics. This effect was most pronounced with the antimetabolite class. *Clin Cancer Res; 20(23); 5956–63. ©2014 AACR.*

Introduction

Inhibitors of VEGF and their receptors (VEGFR) have been extensively studied in numerous cancer types. Bevacizumab was one of the first VEGF inhibitors and was found to be efficacious in metastatic non–small cell lung cancer and colorectal cancer through the pivotal Eastern Cooperative Oncology Group studies E4599 (1, 2) and E3200 (3), respectively. Conversely, administration of bevacizumab failed to extend overall survival in other cancer types including breast (4) and pancreatic (5). Following these mixed signals, the FDA approved a number of more recent drugs with anti-VEGFR activity, including sorafenib in hepatocellular (6) and renal (7), sunitinib in renal (8) and neuroendocrine (9), and pazopanib for renal (10) cancer. However, despite the growing number of VEGF and VEGFR (VEGF/R) inhibitors, there remains little consensus on the optimal patient subgroups and therapy combinations.

To improve clinical efficacy, bench-top and clinical research has focused on combining these agents with other therapeutics, most notably chemotherapy. Numerous hypotheses have arisen to support synergistic interactions between these two agents. One such hypothesis is that inhibition of angiogenesis produces transient and long-term hemodynamic phenomena by modifying vasculature architecture resulting in a net improvement in chemotherapy mass transport (11, 12). Chemotherapy cytotoxicity directed toward cancer vasculature has also been proposed to synergize with VEGF/R inhibitors (13–15). However, the proposed synergy between these agents has not been extensively validated. Although many phase III trials have combined VEGF/R inhibitors with chemotherapy, most of these

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Translational Relevance

It is controversial whether inhibitors of VEGF and its receptor (collectively termed VEGF/R inhibitors) interact synergistically with chemotherapy. To address this question, we tested for significant therapy and patient subgroup interactions with VEGF/R inhibitors among patients with advanced solid disease treated on phase I protocols within a single department. This analysis identified a synergistic interaction between chemotherapy agents, most notably with the antimetabolite class. These results provide an actionable hypothesis for future clinical and bench-top research. In addition, future analyses of aggregated phase I trials may serve as a useful adjunct in the increasingly rapid development of targeted agents to identify promising therapy combinations and patient subgroups to test in phase III trials.

trials were conducted with disparate chemotherapy classes on different patient subgroups, thereby complicating a fair assessment of these factors (5, 16–18).

We sought to test the hypothesis that chemotherapy agents synergize with VEGF/R inhibitors by assessing the probability of clinical benefit in patients enrolled in phase I trials at the Department of Investigational Therapeutics at MD Anderson Cancer Center (Houston, TX). In addition, we further tested whether patient diagnosis or mutational status predicted for response with VEGF/R inhibitors.

Patients and Methods

Patient characteristics

Following Institutional Review Board approval, 1,498 patients were included in this study. Inclusion criteria were recurrent and/or metastatic disease on a phase I clinical trial within the Department of Investigational Therapeutics at the MD Anderson Cancer Center from December 2004 to July 2013. Exclusion criteria were lack of clinical or objective evaluation on protocol, treatment for nonmalignant conditions, or treatment for lymphoma or leukemia. ClinicalTrials.gov identifiers (NCT numbers) are listed for all trials included in this analysis (Supplementary Appendix S1).

Treatment and evaluation

After meeting individual trial inclusion criteria, patients were initiated on phase I trials judged to be clinically appropriate by the attending physician. Treatment was continued until patient withdrawal of consent or the development of unacceptable toxicity, progressive disease, or death. Clinical assessments generally consisted of baseline measurements followed by interim evaluation before each treatment cycle. Imaging was conducted at baseline and with every other treatment cycle (~6–8 weeks). Imaging evaluation entailed CT scan, MRI, and/or PET. Objective responses were categorized via response evaluation criteria in solid tumor (RECIST) criteria (19, 20) and reported as the best achieved response. All patients receiving PET imaging also received a coregistered CT scan, from which RECIST criteria measurements were obtained. In the event that a patient was treated on more than one phase I trial, the protocol that yielded the best response was analyzed.

Tissue samples and molecular analyses

All pathology analyses were conducted on archival formalin-fixed, paraffin-embedded tissue blocks or materials from fine-needle aspirates obtained from clinical diagnostic or therapeutic procedures. Histologies were centrally reviewed and confirmed at MD Anderson Cancer Center (Houston, TX) before protocol initiation. *MET* and *HER2* amplification status were assessed via FISH and expressed as a ratio with a centromeric control gene. Samples were considered amplified at signal ratios $\geq$2. Mutational analyses were performed in Clinical Laboratory Improvement Amendment (CLIA)-certified laboratories as part of a gene panel or as a single test.

Statistical analysis

The outcome of interest was clinical benefit, which was defined as stable disease $\geq$6 months or objective evidence of partial or complete response via RECIST criteria (19, 20). Multivariate ORs were generated via logistic regression. First, an overall OR for clinical benefit was calculated associating each factor of interest/chemotherapy with frequency of clinical benefit among the entire cohort. Then for each factor of interest/chemotherapy, two ORs for achieving clinical benefit were calculated within a multivariate model: one for patients treated with VEGF/R inhibitors (OR with VEGF/R) and another for patients who did not receive these inhibitors (OR without VEGF/R). To compare these two ORs, an interaction term was included in the multivariate model: (chemotherapy/factor of interest)$\times$(VEGF/R). We took significant interaction terms ($P_{\text{interaction}} < 0.05$) to suggest effect modification (either synergy or antagonism) with VEGF/R inhibitor use. Supplementary Appendix S2 illustrates example calculations for synergistic and antagonistic interactions. Other adjusted covariates included: number of prior systemic therapies, number of agents utilized during the best phase I protocol, age, gender, race, favorable/unfavorable diagnoses, year of treatment initiation, and chemotherapy class. Favorable and unfavorable diagnoses were defined as $\geq$40% or <40% proportion of clinical benefit among all patients, respectively (as listed in Table 2, footnote).

Continuous variables were compared via Student *t* test, whereas categorical variables were compared using χ^2 and Fisher exact tests. Recursive partitioning analysis was conducted to separate patients treated with VEGF/R inhibitors into strata based on frequency of clinical benefit. All *P* values were two sided when appropriate with significance considered at the $P < 0.05$ level. Analyses were conducted using SAS ver. 9.3 and JMP ver. 10 (both SAS Institute Inc.).

Table 1. Baseline patient and treatment characteristics

Characteristics	Frequency of clinical benefit without VEGF/R ($n = 757$)	Frequency of clinical benefit with VEGF/R ($n = 741$)
Sex		
Female	414 (55%)	412 (56%)
Male	343 (45%)	329 (44%)
Age at phase I (median, range)	59 (19–90)	58 (4–88)
Number of agents used in best phase I (median, range)	2 (1–3)	2 (1–4)
Number of prior systemic treatments (median, range)	3 (0–14)	3 (0–15)
Ethnicity		
White	619 (82%)	570 (77%)
Black	56 (7%)	80 (11%)
Hispanic	54 (7%)	66 (9%)
Asian	28 (4%)	25 (3%)
Chemotherapy		
Any chemotherapy use	183 (24%)	168 (23%)
Alkylating	3 (<1%)	19 (3%)
Taxanes	85 (11%)	87 (12%)
Antimetabolite	49 (6%)	66 (9%)
Platinum	55 (7%)	67 (9%)
Anthracycline	10 (1%)	96 (13%)
Others	35 (5%)	26 (4%)
Mutations/amplifications[a]		
PTEN expression loss	97/325 (22%)	71/320 (22%)
TP53 mutation	66/176 (37%)	61/151 (40%)
KRAS mutation	79/500 (16%)	77/465 (17%)
PIK3CA mutation	73/561 (13%)	46/514 (9%)
HER2 amplification	17/248 (7%)	20/253 (8%)
MET mutation	26/256 (10%)	16/202 (8%)
NRAS mutation	13/353 (4%)	16/277 (6%)
BRAF mutation	79/515 (15%)	24/456 (5%)
EGFR mutation	27/414 (7%)	10/386 (3%)
MET amplification	14/379 (4%)	6/324 (2%)
KIT mutation	4/287 (1%)	5/245 (2%)

NOTE: Use of chemotherapy and the presence of mutations/amplifications do not represent mutually exclusive categories. Frequency (percent proportions) displayed for all categorical variables and median (range) displayed for all continuous variables.
[a]The frequency of mutations/amplifications is displayed over the number of patients tested.

Results

Patient characteristics

Baseline and treatment characteristics stratified by VEGF/R inhibitor treatment are summarized in Table 1. Almost equal numbers of patients were treated on protocol with ($n = 741$) and without ($n = 757$) VEGF/R inhibitors. No baseline demographic variables were significantly different when stratified by VEGF/R inhibitor use (all $P > 0.05$). However, patients treated with a VEGF/R inhibitor exhibited lower proportions of *PIK3CA*, *EGFR*, or *BRAF* mutation and were treated on protocols with a greater number of simultaneous agents in addition to a higher frequency of alkylating or anthracycline chemotherapeutics (all $P < 0.05$).

Overall response

Among the entire cohort, 469 (31%) patients achieved clinical benefit on their best phase I protocol. This included 160 partial responses (11%), 8 complete responses (0.5%), and 301 instances of stable disease ≥6 months (20%). Time-to-failure (TTF) was defined as duration on the best phase I trial. Median TTF was 3.4 months (range 0.1–95.5 months) and was significantly longer ($P < 0.0001$) in patients treated with VEGF/R inhibitors (median 4.0 months; range 0.1–72.7 months) compared with those who were not (median 2.7 months; range 0.1–95.5 months). Assessing the entire cohort, VEGF/R inhibitor was associated with a higher frequency of clinical benefit [OR = 1.9; 95% confidence intervals (CI), 1.5–2.4; $P < 0.0001$]. Significance held on

Table 2. Rate of clinical benefit with VEGF/VEGFR inhibition

Diagnoses	Frequency without VEGF/R ($n = 757$)	Frequency with VEGF/R ($n = 741$)
Thyroid[a]	11/21 (52%)	43/56 (77%)
Thymic[a]	1/1 (100%)	5/7 (71%)
Adrenal[a]	5/9 (56%)	9/13 (69%)
Head and neck (nonsquamous histology)[a]	12/32 (38%)	12/22 (55%)
Cervix	3/14 (21%)	11/22 (50%)
Renal	6/25 (24%)	8/16 (50%)
Small bowel[a]	2/7 (29%)	4/8 (50%)
Mesothelioma[b]	0/4 (0%)	1/2 (50%)
Ovarian	21/66 (32%)	37/84 (44%)
Prostate	7/20 (35%)	8/18 (44%)
Hepatobiliary	4/18 (22%)	13/30 (43%)
Breast	19/65 (29%)	26/64 (41%)
Unknown primary[a]	4/8 (50%)	4/10 (40%)
Bladder	2/11 (18%)	5/13 (38%)
Sarcoma	7/37 (19%)	23/63 (37%)
Head and neck (squamous histology)	7/35 (20%)	5/15 (33%)
Genitourinary (other than prostate)[b]	0/5 (0%)	1/3 (33%)
Lower bowel[b]	13/128 (10%)	38/135 (28%)
Lung[b]	9/66 (14%)	9/32 (28%)
Gastroesophageal[b]	3/31 (10%)	6/23 (26%)
Melanoma	39/94 (41%)	12/55 (22%)
Cutaneous (other than melanoma)[b]	2/13 (15%)	2/9 (22%)
Uterus[b]	2/22 (9%)	6/26 (20%)
Pancreas	6/20 (30%)	3/20 (15%)
Central nervous system[b]	1/5 (20%)	0/4 (0%)

NOTE: Frequency (percent proportions) displayed for all variables.

[a]Defined as favorable histology in multivariate analyses (≥40% rate of clinical benefit) within entire cohort, which includes both VEGF/VEGFR inhibited and noninhibited.

[b]Defined as unfavorable histology in multivariate analyses (≤20% rate of clinical benefit) within entire cohort, which includes both VEGF/VEGFR inhibited and noninhibited.

multivariate analysis (OR = 1.7; 95% CI, 1.3–2.2; $P = 0.0001$).

VEGF/R inhibitors were classified into three mutually exclusive categories: bevacizumab alone ($n = 443$), multikinase inhibitors ($n = 243$), or simultaneous bevacizumab and multikinase inhibitors ($n = 65$). Of note, the only VEGF inhibitor (antibody or otherwise) assessed in our pooled analysis of phase I protocols was bevacizumab, while numerous multikinase VEGFR inhibitors were utilized. On multivariate analysis, no differences in the OR for clinical benefit were observed when comparing between VEGF/R inhibitor classes: bevacizumab alone (OR = 1 reference), multikinase inhibitors (OR = 0.9 reference to bevacizumab; 95% CI, 0.6–1.3; $P = 0.5$), and simultaneous multikinase-inhibitors and bevacizumab (OR = 1.0 reference to bevacizumab; 95% CI, 0.5–1.8; $P = 0.97$).

Chemotherapy and VEGF/R response

The frequency of clinical benefit among all patients within this study who received chemotherapy was 23%. Among the entire cohort, chemotherapy use was not associated with a higher probability of clinical benefit (OR = 1.0; 95% CI, 0.8–1.3; $P = 0.99$). Assessing individual chemotherapy classes, only anthracyclines were associated with higher OR for clinical benefit among the entire cohort (OR = 1.6; 95% CI, 1.1–2.4; $P = 0.02$). Of note, the only anthracycline utilized in this study was doxorubicin.

VEGF/R inhibitor use was found to modify the effects of chemotherapy on multivariate analysis. Use of any chemotherapy agent concomitant with VEGF/R inhibitors was associated with a significantly higher OR for clinical benefit compared with chemotherapy use without VEGF/R inhibitors, suggesting a synergistic interaction [OR with VEGF/R = 1.6 (95% CI, 1.1–2.5) vs. OR without VEGF/R = 0.4 (95% CI, 0.3–0.6), $P_{interaction} = 0.02$]. All chemotherapy classes were associated with improved OR for clinical benefit in conjunction with a VEGF/R inhibitor; however, wide differences were noted between classes. The multivariate OR for clinical benefit in patients receiving a VEGF/R inhibitor (γ-axis) versus the OR for clinical benefit in patients who did

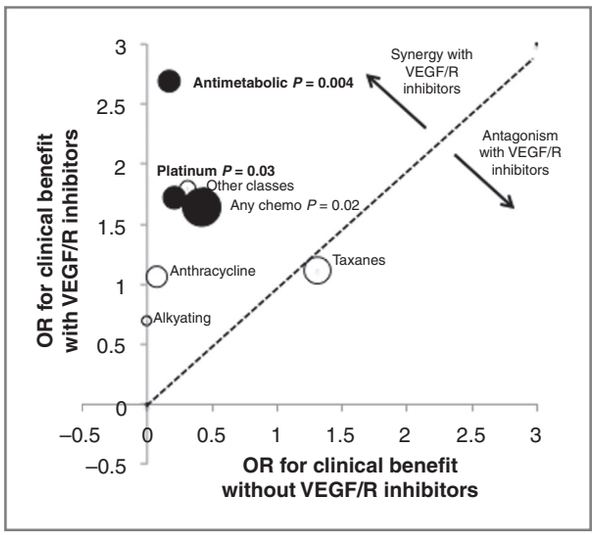

Figure 1. ORs for clinical benefit among different chemotherapy classes in patients treated with concomitant VEGF/R inhibitors (*y*-axis: OR with VEGF/R inhibitors) plotted against the ORs for clinical benefit in patients who did not receive VEGF/R inhibitors (*x*-axis: OR without VEGF/R inhibitors). Dashed line indicates equivalent ORs with and without VEGF/R inhibitor use. Points above this line indicate potential synergistic interactions, whereas those below indicate antagonistic interactions. Points are scaled to the relative number of patients within each plot. In instances of significant interaction ($P_{interaction} < 0.05$), the point is filled and *P* value displayed.

not (*x*-axis) is presented in Fig. 1. Synergy was identified when the observed joint effect of chemotherapy in conjunction with VEGF/R inhibitors was significantly larger than the product of the observed individual effects of chemotherapy and VEGF/R inhibitors. To aid interpretation, chemotherapy classes exhibiting significant synergy are presented in bold with the corresponding $P_{interaction}$.

Among specific classes of chemotherapy agents, antimetabolic chemotherapeutics (gemcitabine, capecitabine, 5-FU, 5-Azacytidine, TAS-106, pemetrexed, and pralatrexate) exhibited the greatest synergy with VEGF/R inhibitors [OR with VEGF/R = 2.7 (95% CI, 1.5–4.7) vs. OR without VEGF/R = 0.2 (95% CI, 0.1–0.3), $P_{interaction} = 0.004$). In addition, platinum agent use (carboplatin, cisplatin, or oxaliplatin) was also associated with higher OR for clinical benefit in conjunction with a VEGF/R inhibitor [OR with VEGF/R = 1.7 (95% CI, 1.0–3.0) vs. OR without VEGF/R = 0.2 (95% CI, 0.1–0.4), $P_{interaction} = 0.03$]. Illustrative calculation of the univariate OR for clinical benefit associated with antimetabolite chemotherapy is presented in Supplementary Appendix A2.

Mutation status and VEGF/R response

The most common genetic mutation detected among the entire cohort was in *TP53* (39%) followed by *KRAS* (16%). *KRAS* mutations were associated with significantly lower OR for clinical benefit among all patients (OR = 0.5; 95% CI, 0.3–0.7; $P = 0.0004$). However, no significant differences in OR for clinical benefit were associated with *KRAS* mutations with VEGF/R inhibitor use compared to without [OR with VEGF/R = 0.5 (95% CI, 0.3–0.9) vs. OR without VEGF/R = 1.0 (95% CI, 0.5–1.8), $P_{interaction} = 0.4$; Fig. 2). Analysis of specific KRAS mutations revealed point mutations at G12 ($n = 64$, 81% of tested) and G13 ($n = 10$, 13%) to be most prevalent. There was no significant difference in the frequency of known activating *KRAS* mutations in patients who received VEGF/R inhibitors ($n = 69$, 87%) compared with those who did not ($n = 70$, 91%; $P = 0.6$). A description of *MET*, *KRAS*, and *NRAS* mutations in relation to their functional significance and VEGF/R inhibitor use is presented in Supplementary Table S1.

In contrast, among the entire cohort, *BRAF* mutation was associated with significantly higher OR for clinical benefit (OR = 2.3; 95% CI, 1.5–3.4; $P < 0.0001$). Interestingly, the OR for clinical benefit was significantly greater in patients who did not receive VEGF/R inhibitors compared with those who did, suggesting an antagonistic interaction [OR with VEGF/R = 0.6 (95% CI, 0.3–1.6) vs. OR without VEGF/R = 25 (95% CI, 10–63), $P_{interaction} = 0.0006$). In contrast, patients with *TP53* mutations treated with VEGF/R inhibitors exhibited significantly higher OR for clinical benefit compared with patients who were not, suggesting a synergistic interaction [OR with VEGF/R = 1.3 (95% CI, 0.6–2.7) vs. OR without VEGF/R = 0.1 (95% CI 0.06–0.3), $P_{interaction} = 0.003$).

As the prognosis of *BRAF* mutations is variable depending on cancer type, stratification of BRAF mutants by diagnoses is presented in Supplementary Table S2. *BRAF* mutation was most frequently observed in melanoma (66 of 129 tested, 51%) and thyroid (15 of 47 tested, 32%) cancer. To test whether the observed antagonism between BRAF mutations

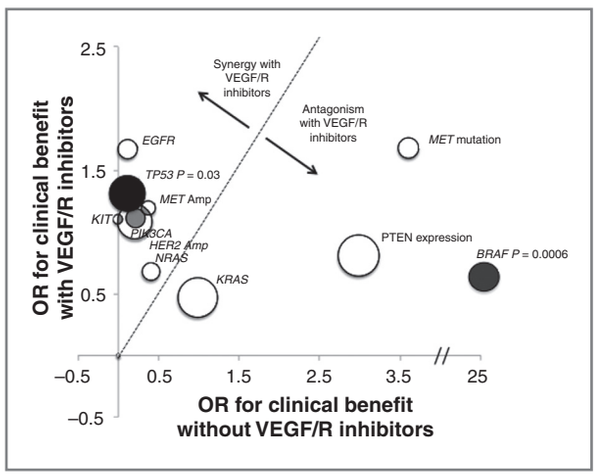

Figure 2. OR for clinical benefit among different mutations in patients treated with concomitant VEGF/R inhibitors (*y*-axis: OR with VEGF/R inhibitors) plotted against the ORs for clinical benefit in patients who did not receive VEGF/R inhibitors (*x*-axis: OR without VEGF/R inhibitors). Dashed line indicates equivalent ORs with and without VEGF/R inhibitor use. Points above this line indicate potential synergistic interactions, whereas those below indicate antagonistic interactions. Points are scaled to the relative number of patients within each plot. In instances of significant interaction ($P_{interaction} < 0.05$), the point is filled and *P* value displayed.

and VEGF/R inhibition results not from biologic synergy but instead from not receiving a highly efficacious therapy, patients with melanoma treated with *BRAF* inhibitors (21) were removed from the regression model ($n = 43$). In this sensitivity analysis, BRAF mutation did not significantly interact with VEGF/R inhibitor use [OR with VEGF/R = 0.7 (95% CI, 0.3–1.7) vs. OR without VEGF/R = 4.6 (95% CI, 1.8–11.7), $P_{interaction} = 0.11$).

Diagnosis and VEGF/R response

Frequency of overall response varied greatly among diagnoses (Table 2). Clinically favorable diagnoses within this study (≥40% clinical benefit among all patients) were thymic (75% response), thyroid (70%), adrenal (46%), nonsquamous head and neck cancer (44%), unknown primaries (44%), and small bowel (40%). In contrast, clinically unfavorable diagnoses (<20% clinical benefit among all patients) were lower bowel (19%), lung (18%), cutaneous (18%; other than melanoma), mesothelioma (17%), gastroesophageal (17%), uterine (17%), and central nervous system (11%).

On multivariate analysis, diagnoses of melanoma [OR with VEGF/R = 0.5 (95% CI, 0.2–0.9) vs. OR without VEGF/R = 13 (95% CI, 6.7–26), $P_{interaction} < 0.0001$) and pancreatic cancer [OR with VEGF/R = 0.2 (95% CI, 0.06–0.8) vs. OR without VEGF/R = 7.0 (95% CI, 1.9–25), $P_{interaction} = 0.03$] were associated with lower OR for clinical benefit with VEGF/R inhibitors compared with without (Fig. 3). Illustrative calculation of the univariate OR for clinical benefit associated with melanoma diagnosis is presented in Supplementary Appendix S2. A sensitivity analysis was conducted once again removing patients with melanoma who received BRAF inhibitors ($n = 43$). This analysis revealed no significant interactions between melanoma diagnosis and VEGF/R use [OR with VEGF/R = 0.5 (95% CI 0.2–0.9) vs. OR without VEGF/R = 0.9 (95% CI, 0.5–1.9), $P_{interaction} = 0.48$].

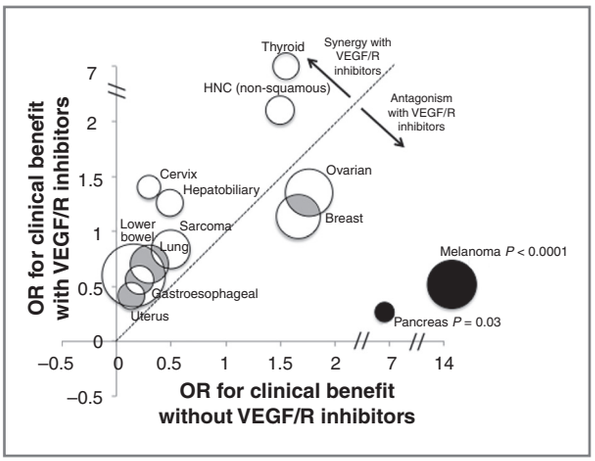

Figure 3. OR for clinical benefit among different diagnoses in patients treated with concomitant VEGF/R inhibitors (*y*-axis: OR with VEGF/R inhibitors) plotted against the ORs for clinical benefit in patients who did not receive VEGF/R inhibitors (*x*-axis: OR without VEGF/R inhibitors). Dashed line indicates equivalent ORs with and without VEGF/R inhibitor use. Points above this line indicate potential synergistic interactions, whereas those below indicate antagonistic interactions. Points are scaled to the relative number of patients within each plot. In instances of significant interaction ($P_{interaction} < 0.05$), the point is filled and *P* value displayed.

Discussion

This is to our knowledge the first study to aggregate patient-level data from multiple phase I trials to explore the interaction of chemotherapy with VEGF/R inhibitors. Significant findings include the association between VEGF/R inhibitors with higher frequency of clinical benefit among all patients, a finding that held on multivariate analyses and remained consistent across different VEGF/R inhibitor categories. Chemotherapy use was not associated with higher probability for clinical benefit among all patients; however, concomitant chemotherapy use with a VEGF/R inhibitor was associated with significantly higher OR for clinical benefit, suggesting a synergistic interaction. Among chemotherapy classes, the highest degree of synergy with VEGF/R inhibitors was exhibited by antimetabolites followed by platinum agents. About mutational status and cancer diagnoses, *BRAF* mutations or diagnosis of melanoma or pancreatic cancer were associated with significantly lower OR for clinical benefit with VEGF/R inhibitor use compared to without. On the other hand, TP53 mutations were associated with significantly higher OR for clinical benefit. Finally, although KRAS mutations were associated with significantly lower OR for clinical benefit among the entire cohort, ORs were not significantly modified by VEGF/R inhibitor use.

A consistent finding was the higher OR for clinical benefit with the combination of chemotherapy and VEGF/R inhibitors, suggesting synergy between these systemic agents (Fig. 1). Among chemotherapy agents, this effect was most pronounced with the antimetabolite class. One hypothesis is that chemotherapeutics, especially antimetabolites, exhibit potent cytotoxic antivasculature effects that synergize with VEGF/R inhibitors (13–15). Another hypothesis is that angiogenesis inhibitors might "normalize" leaky tumor vasculature, resulting in a transient "burst" of mitotic activity that renders tumor cells more susceptible to cell-cycle–dependent chemotherapy such as antimetabolites. Furthermore, such normalization in tumor vasculature and corresponding decrease in tumor interstitial fluid pressure have been hypothesized to facilitate chemotherapy delivery to tumors (11, 12).

Another finding in this study was that pancreatic cancer and melanoma were associated with significantly lower OR for clinical response with VEGF/R inhibitor use compared to without VEGF/R inhibitor. This finding is consistent with phase III trials that did not show a survival benefit with the addition of bevacizumab in these cancers (5, 18). Resistance to these inhibitors may reflect an underlying hypoxic drive, which is either not affected by or produces significant compensatory mechanisms when treated with VEGF/R inhibitors (22, 23). Specific to pancreatic cancer is the hypoxic desmoplastic stroma, which may represent a microenvironment less sensitive to

angiogenesis inhibition (24, 25). However, it is important to note that reduced OR for clinical benefit with VEGF/R inhibitor use may not reflect an antagonistic effect, but may instead result from exclusion of more efficacious therapies. For example, melanoma diagnosis and *BRAF* mutations were associated with lower OR for clinical benefit with VEGF/R inhibitors. This finding may reflect the opportunity cost of *BRAF*-mutated melanomas patients not enrolled in *BRAF* inhibitor trials due to their enrollment in trials with VEGF/R inhibitors (21, 26). This hypothesis is supported by sensitivity analyses removing patients with melanoma treated with BRAF inhibitors from the regression model. In these analyses, both the diagnosis of melanoma and BRAF mutation status did not significantly interact with VEGF/R inhibitor use (both $P > 0.05$).

In contrast, patients with *TP53* mutations exhibited a higher rate of clinical benefit with VEGF/R inhibitors, a finding observed in our previous work (27). Possible explanations include a role of *TP53* mutations in promoting vascularization. This hypothesis is supported by clinical studies that associate this mutation with increased vessel density (28) and neovascularization in xenograft models (29). With regard to other mutations, patients exhibiting MET mutation and amplifications seemed to have opposite albeit nonsignificant interactions with VEGF/R inhibitors (Fig. 2). Analyzing specific mutation sites revealed the most common *MET* alteration to be N375S (60%), which has been suggested to be an inactivating mutation/polymorphism (30). As such, the observed differences in clinical benefit between *MET* mutations and alterations may be explained by the function of this predominant alteration (Fig. 2). Similarly, *NRAS* and *KRAS* exhibit analogous functions but seem to have different (once again, nonsignificant) interactions with VEGF/R inhibitors. Unfortunately, given the unknown activity of *NRAS* mutations presented here, it is difficult to interpret this finding. Given the limited number of patients exhibiting specific gene mutations, further analysis of mutation interaction with VEGF/R inhibitors is warranted.

There are several limitations that must be considered. First, many VEGFR inhibitors target multiple receptor tyrosine kinases (31, 32). Although our analysis stratifying by VEGF/R inhibitor class did not reveal significant differences among classes, we cannot exclude the contributions of off-target effects. We regret to report that due to the ongoing nature of many of these trials, we are unable to disclose investigational agent identity. However, we present a list of NCT numbers of all trials included (Supplementary Appendix S1). Second, as specific attending physicians selected the most appropriate trial for each patient, there was nonrandom pairing of patients and systemic agents. Although we attempted to control for baseline differences through multivariate analyses, statistical manipulations must be considered a poor substitute for adequate randomization. Also, as patients were treated on different phase I protocols, often in the setting of a dose-finding design, efficacious doses may not have been achieved. Drug toxicity data are also not available in this analysis. Presentation of such data would provide the current efficacy analysis with greater clinical context. Finally, although we control for the number of prior systemic therapies, our database lacks information on prior VEGF/R inhibitor use. It should be noted that we view these results as exploratory and therefore did not adjust for multiple testing.

Despite these limitations, numerous strengths of our analysis deserve mention. First, this analysis included a large number of patients that were subjected to regular and stringent radiographic monitoring per protocol stipulations. Furthermore, as the development pipeline for new targeted therapies often transitions rapidly between phase I to III trials, it is often difficult to generate effective subgroup analyses or predict optimal therapy combinations before phase III trial initiation. To our knowledge, this study is the first patient-level analysis of this size assessing interactions within combination therapies with VEGF/VEGFR inhibitors.

In conclusion, we believe that our results generate a number of actionable hypotheses with respect to VEGF/R inhibitors. Perhaps most prominent among these is the discovery of VEGF/R inhibitors' synergy with chemotherapy use, specifically with antimetabolites. If validated, these findings offer interesting insight into the biology of clinical angiogenesis inhibition and may guide future trial design.

Disclosure of Potential Conflicts of Interest

L.M. Ellis is a consultant/advisory board member for Genentech/Roche and Lilly/ImClone. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

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