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The VEGF Receptor Tyrosine Kinase Inhibitor Pazopanib in Combination with the MEK Inhibitor Trametinib in Advanced Solid Tumors and Differentiated Thyroid Cancers

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Running Title: Phase I study of pazopanib+trametinib with expansion in DTC

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Statement of Translational Relevance

Molecularly targeted agents have had meaningful efficacy as single agents in multiple tumor types, but have not resulted in durable benefit due to resistance coming often from increased feedback signaling. We previously demonstrated that combination targeted therapy of vascular endothelial growth factor (VEGF) signaling by signal transduction inhibitors inhibiting at the receptor level and downstream using Mitogen-activated protein kinase kinase (MEK) inhibition was more effective than either drug alone in preclinical models of differentiated thyroid cancer (DTC). We report a phase I clinical trial of VEGF receptor inhibitor pazopanib with MEK inhibitor trametinib with an expansion in DTC showing activity of the combination, with potential increased benefit in NRAS-mutated DTC. Our results support the hypothesis that combination targeted therapy with pazopanib and trametinib should be further explored in this molecular subset of DTC.

<u>Abstract</u>

Purpose: Differentiated thyroid cancer (DTC) responds to vascular endothelial growth factor (VEGF) receptors inhibitors. VEGF signals through RAS/RAF/MEK signaling. We evaluated the safety and efficacy of VEGF receptor inhibitor pazopanib and MEK inhibitor trametinib in advanced solid tumors and DTC.

Experimental Design: Advanced solid tumor patients were enrolled in a phase I, multi-center trial with a DTC expansion cohort. Patients received pazopanib 400-800 mg and trametinib 1-2 mg daily. Efficacy in the expansion cohort was assessed with objective response (OR) at six months of treatment.

Results: Twenty-six patients were enrolled in 5 dose levels. Maximum tolerated dose was not reached; the recommended phase II dose was pazopanib 800 mg po and trametinib 2 mg po qd. There was one dose limiting toxicity on dose level 1 with grade 3 fatigue and muscle weakness. Common grade 3 adverse events were elevated transaminases(19%), diarrhea(15%), hypertension(12%), and fatigue(8%). Thirteen patients were enrolled in the DTC cohort; OR was 33% (95% CI: 9.9, 65.1%) and median progression-free survival was 10.7 months. The cohort was terminated after planned interim analysis suggested insufficiently increased activity against the historical control of pazopanib alone. Reduction in tumor diameter negatively correlated with p-ERK change in tumor (Spearman's rho = -0.71; p = 0.05). *NRAS* mutation was associated with response (Fisher's Exact p=0.008).

Conclusions: Pazopanib+trametinib was tolerable at full single-agent doses with clinical activity in DTC but did not achieve the pre-specified response rate target.

Introduction

The vascular endothelial growth factor (VEGF) pathway is an important signaling cascade responsible for angiogenesis, with secondary effects that include increasing proliferation and metastasis of many cancers including differentiated thyroid cancer [1-3]. VEGF binds to its ligand receptor, VEGF-receptors (R) 1-3, which then activates a downstream signaling cascade leading to pro-survival signaling and transcription of genes involved in angiogenesis. Like many receptor tyrosine kinases, VEGFR1-3 can activate major pro-survival circuits including AKT/PI3 Kinase signaling as well as well as RAS signaling. The RAF/MEK/ERK signaling pathway is involved in the regulation of normal cell proliferation, survival and differentiation, and this pathway is frequently aberrantly upregulated in a wide number of cancers including differentiated thyroid cancer (DTC) [4-5].

DTC is a highly prevalent cancer with >600,000 patients living with the disease in the United States presently [6]. The mainstay of therapy for the 90% of patients found with early or locally advanced disease is surgery and radioiodine therapy [7-8]. Mutations in *BRAF* and *RAS* genes, along with re-arrangements in *RET*, *TRK* and *ALK* genes and others have been found in the majority (70%) of patients [9]. Two approved therapies for advanced, radioiodine-refractory DTC, lenvatinib and sorafenib, are multikinase inhibitors targeting VEGFR2 in addition to other kinases [10-11]. Pazopanib is an orally available multikinase inhibitor of VEGFR, platelet-derived growth factor (PDGF) receptor, KIT, fibroblast growth factor (FGF) receptor, and RAF which is approved for advanced renal cell carcinoma and advanced refractory soft tissue sarcoma [12-13]. Phase II clinical trials of pazopanib in DTC have also shown activity, with 49 percent of patients having RECIST partial responses [14-15].

MEK inhibitors have been tested less extensively in DTC. Selumetinib, a selective MEK 1/2 inhibitor, has been tested as a potential re-differentiating agent prior to radioiodine therapy in a small study of 20 patients, with partial responses in five patients and stable disease in three others. [16]

Our group has previously reported that the combination of trametinib, an orally available highly specific inhibitor of MEK1 and MEK2, and pazopanib in thyroid cancer cell lines and xenograft models results in synergistic inhibition of tumor growth [17]. We hypothesized that inhibition of receptor tyrosine kinases VEGFR and PDGFR with the addition of MEK inhibitor trametinib could improve clinical benefit for DTC patients compared to pazopanib alone. We enrolled a phase I study of the combination of pazopanib and trametinib in all solid tumors with an expansion cohort in DTC at the maximum tolerated dose.

Methods

Study design

This was an open-label, multicenter [Sidney Kimmel Comprehensive Cancer Center (SKCCC) at John Hopkins University (JHU) and The University of Texas MD Anderson Comprehensive Cancer Center (MDACC)] trial funded by the National Comprehensive Cancer Network (NCCN). Patients older than 18 years with advanced solid tumors that were refractory to standard of care treatment options were eligible for the dose-finding cohort of the study, and expansion cohorts for DTC, cholangiocarcinoma, and soft tissue sarcoma were subsequently enrolled at the maximum tolerated dose; here we report the dose-finding and DTC expansion cohorts. Subjects enrolling in the DTC expansion cohort must have had progressive disease within 6 months of enrolling in the study as assessed by successive imaging and disease that was also amenable to biopsy. Patients with DTC must have had radioiodine non-avid lesions or radioiodine avid lesions that have not responded to treatment with radioactive iodine (defined as \geq 600 millicuries (mCi), last dose at least 6 months prior to enrollment). Other eligibility criteria included the presence of Response Evaluation Criteria in Solid Tumors (RECIST) criteria 1.1 measurable disease, an Eastern Cooperative Oncology Group performance status ≤ 1 (or Karnofsky performance status $\geq 60\%$) and adequate organ function as defined by absolute neutrophil count $\geq 1,500$ cells/µL, platelet count $\geq 100,000$ cells/µL, international normalized ratio $\leq 1.2 \times$ upper limit of normal (unless stabilized with anticoagulation therapy and within the recommended range for the desired level of anticoagulation), total bilirubin $\leq 1.5 \times$ upper limit of normal (or, in patients with Gilbert syndrome, total bilirubin >1.5× as long as direct bilirubin is normal), and serum creatinine $\leq 1.5 \times$ upper limit of normal or creatinine clearance \geq 45 mL/minute and urine protein to creatinine ratio <1, or, if >1, 24-hour urine protein <1 g [17].

Evaluation and treatment

The protocol was approved by the Institutional Review Board at both study sites, and complied with the International Ethical Guidelines for Biomedical Research Involving Human Subjects and the Declaration of Helsinki. Eligible patients were enrolled centrally at the SKCCC at JHU. The trial was registered under ClinicalTrials.gov as NCT01438554. All patients provided written informed consent prior to enrollment. The study drugs (trametinib and pazopanib) were provided by GlaxoSmithKline (eventually Novartis).

For the dose-finding cohort, patients received escalating doses of pazopanib [dose levels (DL) 1:400 mg; DL 2: 600 mg; DL 3-5: 800 mg) and trametinib (DL 1-3: 1 mg; DL 4: 1.5 mg; DL5: 2 mg) orally daily every day of a 28-day cycles. Three patients would be treated per cohort in a standard 3+3 dose escalation design. If none of the first three patients experienced a dose-limiting toxicity (DLT), then the next three would be treated using the next higher dose. If one of the first three had a DLT, an additional three patients would be enrolled at that level. If only one of six developed a DLT, then the dose would be de-escalated for the next three patients. If two or more of the six had a DLT, then the dose would be de-escalated to the previous dose (or dose level -1) where an additional three patients would be treated as the highest dose at which 0 or 1 DLTs are observed in six patients. A total of 6 patients would be treated at the MTD. The expansion cohort patients were treated at the MTD established in our initial dose-finding cohort.

The treatment protocol allowed dose delays or reduction if patients experienced unacceptable side effects and adverse reactions related to study drug(s). Patients were evaluated every cycle for trial therapy compliance and monitoring of adverse events. The National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 was implemented for adverse event monitoring. Disease assessments (computed tomography or magnetic resonance imaging) were performed at baseline and then every other cycle. Response was evaluated according to the RECIST,

version 1.1 [18]. Upon progression of disease, patients were monitored for long-term adverse events and survival.

Study Design

For the dose-finding cohort, the 3+3 dose escalation design had a target toxicity of less than 30%. For a dose level to be deemed tolerable, at least 3 patients must have been treated for 4 weeks without the development of a DLT prior to treating a new cohort at a higher dose level. In addition, all patients defining a dose level as tolerable (i.e. no DLT in cycle 1) must have received a minimum of 75% of the intended dose for each study drug during cycle 1.

For the expansion cohort in DTC, the primary endpoint was objective response (OR) rate by six months of treatment. At study entry, each patient was categorized into one of two subtype groups: papillary or follicular/Hurthle cell. Based on the phase II clinical trial with pazopanib, we expected that the papillary subtype would have a response rate of 33% and the follicular/Hurthle cell subtype would have a response rate of 33% and the follicular/Hurthle cell subtype would have a response rate of 75% [14-15]. An interim futility analysis would be conducted after response information was available on the first 12 patients in the expansion cohort. If the futility boundary was crossed, we would consider stopping the study and declaring that the combination was not showing sufficient activity to warrant further investigation (Supplementary methods). The stopping boundary for futility was a guideline, however, since the sample size was small. Specifically, the stopping rule called for a review if there was a 75% probability that either the response rate in the papillary subtype was less than 25% or the response rate in the follicular/Hurthle call subtype was less than 67% (Supplementary Table 1).

Pharmacokinetic analysis

Pazopanib and trametinib pharmacokinetic (PK) analyses were performed in patients in the DTC expansion cohort. A full pazopanib PK profile was obtained after the first dose on Cycle 1 Day 1 through 24 hours. In addition, pazopanib and trametinib trough concentration were obtained on Cycle 1 Day 15 and at the time of second tumor biopsy (i.e., $C2D1 \pm 48$ hours). Plasma levels of total trametinib and pazopanib were measured using a validated LC/MS/MS methods. Briefly, trametinib was extracted from plasma using acetonitrile. Chromatographic separation was achieved with a Waters X-Terra® C18 column (3.5 μ m, 2.1x50mm) and isocratic elution with an acetonitrile-water-formic acid (70:30:0.1, v/v/v) mobile phase over a 3 min total analytical run time. An AB Sciex 5500 triple quadrupole mass spectrometer operated in positive electrospray ionization mode was used for the detection of trametinib. The assay range was 1-200 ng/mL. Briefly, pazopanib was extracted from plasma using acetonitrile. Chromatographic separation was achieved with a Waters X-Terra[®] C18 column (3.5 µm, 2.1x50mm) and isocratic elution with an ammonium acetate-acetonitrile containing 0.1% formic acid (40:60, v/v) mobile phase over a 3 min total analytical run time. An AB Sciex 5500 triple quadrupole mass spectrometer operated in positive electrospray ionization mode was used for the detection of pazopanib. The assay range was 0.010-1 μ g/mL, with a 1:100 dilution allowing for quantitation up to 100 μ g/mL. Quality assurance samples were assayed with each analytic run and were within 15% of the nominal concentration for both assays. Individual pazopanib plasma concentrations were analyzed by standard non-compartmental PK methods using Phoenix WinNonlin v7.0 (Certara LP, Princeton, NJ). Maximum plasma concentration (C_{max}) was the observed maximum value. Area under the plasma concentration time curve (AUC) was calculated using the linear trapezoidal rule. Steady-state trough concentrations (C_{ss,min}) were calculated as an average of samples obtained on Cycle 1 Day 15 and at the time of PD studies.

Pharmacodynamic analysis

Fresh tumor biopsies were obtained just prior to therapy and after 1 cycle of combination therapy in the DTC expansion cohort. Biopsies were processed using standard formalin fixation, paraffin embedding at the appropriate histology core facility for each institution and then stored at room temperature prior to analysis. Immunohistochemistry with pERK was performed using anti-phospho-p44/42 MAPK (ERK1/2) (Thr202/Tyr204) (20G11) (4376S, Cell Signaling) rabbit monocloncal antibody. Staining was performed on an automated staining system (Leica-Bond, Leica Microsystem Inc., IN). Tissue sections (4-5µm thick) from paraffin embedded formalin fixed tissue were deparaffinized and prepared for staining. Sections were incubated with primary antibody (1:400 dilution) for 15 minutes followed by incubation with a biotin free HRP-conjugated secondary antibody. A brown signal was developed with DAB-detection according to the manufacturer's instructions (Leica Microsystems Inc., IN). Staining was scored by pathologist (JAB) who applied a HistoScore (H-score) calculated by the percentage of tumor cells staining multiplied by the intensity of staining (on a scale of 0-3) [19]. Only nuclear staining was regarded as positive.

Mutational analysis

DTC cohort patients had hotspot mutational sequencing on tumor DNA from fresh biopsy specimens or archival tissue (if fresh biopsy tissue was not available) performed by next-generation sequencing (NGS) using AmpliSeq Cancer Hotspot Panel (v2) for targeted multi-gene amplification, as previously described [20-21]. Briefly, we used the Ion AmpliSeq Library Kit 2.0 for library preparation, Ion Personal Genome Machine Hi-Q OT2 Kit and Ion OneTouch 2 Instrument for emulsion PCR and template preparation, and the Ion Personal Genome Machine Hi-Q Sequencing Kit with the Ion 318 Chip and Personal Genome Machine as the sequencing platform (Life Technologies). The DNA input ranged from 1 ng to 30 ng, as measured by Qubit 20 Fluorometer (Life Technologies). Up to 8 specimens were barcoded using Ion Xpress Barcode Adapters (Life Technologies) for each Ion 318 chip. One to three controls (a non-template control, a normal peripheral blood control from a male, and/or positive control specimens) were included in each chip. Positive controls were mixed DNA specimens from several cell lines with known mutations as reported previously.

Statistical Methods:

Proportions are reported with exact 95% binomial confidence intervals. Spearman's correlation coefficient was used to assess the association between percent reduction in tumor diameter by RECIST and change in p-ERK expression. Binomial probabilities were compared with Chi-square or Fisher's exact tests as appropriate. Event time distributions for overall survival (OS) and progression free survival (PFS) were estimated with the method of Kaplan and Meier and confidence intervals calculated using the method of Brookmeyer and Crowley. PK parameters were summarized descriptively. Mann–Whitney U-tests were used to assess correlations between drug exposure and toxicity. All p-values reported are two-sided, and the significance level was set at 0.05 for all analyses. Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc, Cary, North Carolina) and R version 3.4.

Results

Patients

From November 2011 until April 2013, 26 patients with advanced solid tumors were enrolled [MD Anderson Cancer Center (n=14 pts) and Johns Hopkins University (n=12)]. From August 2013 until December 2014, 13 patients with differentiated thyroid cancer were enrolled at MD Anderson Cancer Center (n=11 pts) and Johns Hopkins University (n=2) in this expansion cohort of pazopanib plus trametinib. The demographic and disease characteristics of the patients entered onto this study are shown in Table 1. All patients were treated according to the study protocol, and no patients remain on study at the time of this analysis. In the Phase I cohort of the study, 69% of patients discontinued study therapy for disease progression, 12% for physician or patient-initiated withdrawal, and 19% for toxicity. In the DTC cohort, those numbers were 62%, 31%, and 8% respectively.

Toxicity

Dose-escalation cohort

Treatment-related adverse events are detailed in Table 2. One dose-limiting toxicity of grade 3 fatigue possibly related to study treatment was noted and this dose level was expanded; seven patients were enrolled due to one patient being unevaluable for toxicity due to missing doses in the first cycle of study drug administration. There were no further dose-limiting toxicities in the remainder of the study and the trial enrolled 19 more patients on dose levels 2-5. Any patient who came off study in the first cycle of therapy not due to treatment-related toxicity was replaced as per protocol.

There were no treatment-related deaths and no grade 4 toxicities; the most common grade 3 toxicities were transaminitis (19%), diarrhea (15%), and hypertension (12%). Common low-grade treatment related toxicities were diarrhea, rash, hypertension, nausea/vomiting, fatigue and anorexia.

Rash was managed well with topical steroids and dosage holds as necessary; no patient was dose reduced due to rash. Hypertension was also well managed with anti-hypertensive therapy.

Nine patients were dose reduced; 5 had pazopanib reduced alone, one had trametinib reduced alone and 3 patients had both agents reduced. Diarrhea was the reason for dose reduction of pazopanib 50% of the time, followed by fatigue and hypertension. One patient was diagnosed with glaucoma and trametinib was reduced alone for that subject. Median dose intensity for both agents was 100% (pazopanib range 18%-100%; trametinib range 18%-100%).

Differentiated thyroid cancer expansion cohort

Further characterizing toxicity was a secondary endpoint of the expansion cohort in DTC. Generally, treatment-related adverse events were similar in the DTC expansion cohort compared to the dose escalation cohort. Nausea/vomiting, hypertension, rash and diarrhea were still the most common toxicities and usually low-grade. Four patients discontinued study therapy due to non-protocol mandated adverse events and subsequent impact on quality of life at 1-6 cycles (median 5); low-grade diarrhea and fatigue were the common causes in these cases.

Efficacy

Dose-escalation cohort

Twenty-five patients were evaluable for response. Three patients had partial responses (12%) and 18 (72%) had stable disease for a disease control rate of 84% (Figure 1). The three patients with partial responses were DTC (confirmed), ovarian cancer (unconfirmed) and cholangiocarcinoma (unconfirmed). The only other cholangiocarcinoma patient on the study had a best response of stable disease. Nine patients (35%) were on study >6 cycles (DTC: 3 patients; colorectal: 2 patients; cholangiocarcinoma, melanoma, synovial cell sarcoma, ovarian cancer: 1 patient each).

Differentiated thyroid cancer expansion cohort

The primary endpoint being monitored for this expansion cohort was the six month objective response (OR) rate. Of the 13 patients enrolled on study, 12 (93%) were evaluable for disease response. Four patients had a RECIST criteria response (33%) while 50% had stable disease (Figure 2). PFS and OS were secondary endpoints of the study. The median follow-up, calculated by the reverse Kaplan Meier method, was 37 months. PFS and OS are shown in Figure 3. The median progression free survival was 10.7 months (95% CI: 7.3, NA). The two year PFS was 25% (95% CI: 9%, 67%). Median survival was 29.3 months (95% CI: 18.9, NA).

The interim analysis for this trial was performed after 12 patients were enrolled. At that time, the six month response information was available on the first twelve patients in the expansion cohort. The sample sizes in the papillary and follicular subtypes were 6 and 6 respectively and there had been 2 and 2 ORs in the papillary and follicular subtypes. The posterior probabilities of OR less than 25% in the papillary and less than 67% in the follicular groups were 0.287 and 0.86 respectively. Since there was an 86% probability that the response rate in the follicular group was less than 67%, the study was paused for a futility review, and ultimately halted.

Pharmacokinetics

Pharmacokinetic data was obtained from the 13 patients on the expansion cohort of 800 mg of pazopanib and 2 mg of trametinib. For pazopanib, the C_{max} , AUC_{0-24hr}, and $C_{ss,min}$ were 44.5±31.8 µg/mL 952.2±680.0 µg*hr/mL, and 33.8±17.3 µg/mL, respectively. The $C_{ss,min}$ for trametinib was 18.7±6.6 ng/mL. There were also no statistically significant correlations between the worst grade of toxicity for the most common toxicities during cycle 1 and during treatment and pazopanib or trametinib exposure (p > 0.05). There were also no statistically significant correlations between the responses and pazopanib or trametinib exposure (p > 0.05).

Pharmacodynamics

The correlation of change in p-ERK expression with best RECIST response was assessed. Of the 13 DTC cohort patients, four patients did not have tumor cells present in the post treatment biopsy, and one patient did not have best response assessed, leaving eight patients available for this analysis. The percent reduction in tumor diameter by RECIST was negatively correlated with change in p-ERK expression post treatment, spearmen correlation coefficient: -0.71, p = 0.05. Overall, the median change in p-ERK expression score was -50, with an interquartile range of -65 to -20 with 7/9 patients having reductions in pERK in post-treatment specimens (Figure 4). There were also no statistically significant correlations between the change in p-ERK expression score and pazopanib or trametinib exposure.

Mutational Testing

Eleven patients had tumor tissue available for sequencing, and ten of these patients were evaluable for response. NRAS was present in 3 of 3 (100%) responders, and none of the 7 patients with stable disease or progressive disease (Fisher's exact p = 0.008). One responder did not have available tissue for sequencing. Other mutations included BRAF V600E or TP53 (2 patients) and PIK3CA, SMO, CDKN2A, ALB1, and RB1 (1 patient each).

Discussion

Targeting pro-growth signal transduction circuits with combination molecularly targeted inhibition is a rational therapeutic strategy with notable successes in solid tumors, such as BRAF V600E-mutated metastatic melanoma and colorectal cancer [22-25]. Inhibition at the signalizing receptor level as well as downstream signaling proteins theoretically allows more potent inhibition of a given pathway and the potential to stymie compensatory feedback loops that result in resistance to molecularly targeted agents. We employed this strategy to target VEGF receptor signaling at the receptor level with pazopanib and downstream with the MEK inhibitor trametinib, both in tumor cells and endothelial cells. In preclinical studies in DTC, pazopanib treatment was associated with paradoxical up-regulation of p-ERK, an effect that could be abrogated by concurrent trametinib [17].

We did see activity with this regimen in the expected tumor type of differentiated thyroid cancer, where we focused our primary expansion cohort, as well as cholangiocarcinoma, somewhat less expectedly. Bible et al reported significant activity of single agent pazopanib in progressive radioiodine refractory DTC, with an overall confirmed PR rate of 49% [14-15]. The median PFS in this phase 2 study was 360 days. Patients with follicular thyroid cancer had a 73% PR rate (8 of 11), whereas papillary thyroid cancer patients had PR 33% of the time (5 of 11 patients). Mutational analysis and comparable pharmacodynamics studies are not available. The current study applied a futility monitoring plan that would pause the study if there was a greater than 75% probability that the six month OR rate with the pazopanib-trametinib combination was not superior to pazopanib alone in the Bible study (less than 25% in the papillary subtype or less than 67% in the follicular/Hurthle cell subtype). Both the PR rate and the median PFS were not superior for the combination. Small sample size and patient selection differences in the two studies including prior treatment history could potentially skew this comparison.

MEK inhibitors have had modest activity in cholangiocarcinoma with response rates less than 10%, and have been mostly abandoned in that histology [26-27]. In addition, a single phase II study of bevacizumab and erlotinib in treatment-naïve cholangiocarcinoma showed a response rate of 12%, suggesting the possible benefit of VEGF targeting in this rare tumor, though the relative contribution of VEGF inhibition vs EGFR inhibition is unknown for this non-randomized trial [28]. Our study showed benefit for both of the cholangiocarcinoma patients who were enrolled (one PD and one durable SD greater than 6 months), with the major caveat of a sample size of only 2 patients. Indeed, we added an expansion cohort of cholangiocarcinoma to this trial and previously reported a 4-month PFS of 40% (95% CI: 24.7-64.6%) that showed a trend towards increased 4-month PFS as compared with the prespecified null hypothesized 4-month PFS of 25%, but this difference did not reach statistical significance (P=0.063) [29]. A final expansion cohort in soft tissue sarcoma did not show signs of compelling activity [30].

Our correlative studies yielded some interesting results in our exploratory analyses. Our initial hypothesis was that combination pazopanib and trametinib would result in significant inhibition of the MAP kinase signaling pathway, and that this would correlate with clinical response. Indeed, 7 of 9 patients with paired tumor biopsies had inhibition of p-ERK after treatment, though without single agent comparators, we cannot determine if it is more or less p-ERK inhibition compared to either agent alone. However, inhibition of p-ERK correlated with lack of tumor response to therapy, opposite of our initial hypothesis. Limitation of tissue and the small patient numbers did not allow us to understand the mechanism behind this exploratory finding. A possible explanation is that inhibition of the MAPK pathway may result in compensatory increases in other pro-survival pathways, resulting in resistance to this therapeutic strategy; this phenomenon was observed in a recent models of melanoma and low-grade glioma where MEK inhibition resulted in decreased p-ERK but resistance emerged with upregulation of

PI3K/AKT [31-32]. A second possible explanation is that the higher levels of p-ERK are a direct effect of pazopanib treatment, comparable to effects observed in the cell culture and mouse models.

Arguably, our most interesting correlative study explored the effect of common hotspot mutations on the potential efficacy of the pazopanib/trametinib combination in our DTC patients. Three of 11 patients with available tissue had NRAS mutations, slightly higher than expected possibly due to the patients enrolled being more advanced cases, as RAS mutations have been suggested to represent a more aggressive phenotype [8, 33]. All three patients with NRAS mutations had PRs (with the final PR patient not having available tissue for sequencing). With the necessary caveats regarding the low number of patients in this analysis, the data pose an important question regarding whether NRASmutated tumors have a necessary addition to the MAPK signaling pathway making them uniquely sensitive to this approach. In the phase II study of the MEK inhibitor selumetinib in DTC, patients with NRAS-mutated disease had increased effectiveness of therapy in comparison with BRAF-mutated disease, though again with small numbers [16]. Lenvatinib, another VEGFR/FGFR tyrosine kinase inhibitor, is approved in DTC, and a phase 2 study suggested that RAS-mutated patients had more benefit from lenvatinib than wild-type tumors, although this finding was not substantiated in the subsequent phase 3 study [34].

In conclusion, our data suggest that pazopanib and trametinib are tolerable and active in advanced solid tumor patients, particularly in DTC patients. The lack of single agent comparator arms do not allow for assessment of the activity of the combination over either drug alone. However, the trial did not meet is pre-specified threshold for activity for the follicular thyroid cancer patients, resulting in closing the trial for enrollment. Future study of the combination in NRAS-mutated DTC in a larger, randomized trial to truly validate the findings would be meaningful to assess if this strategy has any role in the care of DTC patients.

Acknowledgements: We thank the patients and their families for participating in this study. Figure Legends

Figure 1: Best RECIST 1.1 criteria responses in dose-escalation cohort

Twenty-five patients were available for response (24 patients represented in figure and patient 25 not represented due to clinical progression without imaging).

<u>Figure 2</u>: Best RECIST 1.1 criteria responses in differentiated thyroid cohort. Eleven patients represented in figure, with the 12th evaluable patient not represented due to clinical progression without imaging. *represents new lesions resulting in RECIST criteria progression.

<u>Figure 3</u>: Kaplan-Meier estimate of a) progression-free survival (PFS) and b) overall survival (OS) in differentiated thyroid cohort. The dotted lines represent 95% confidence intervals.

<u>Figure 4</u>: Change in pERK in relation to change in tumor size. Pre- versus post-treatment biopsies were assessed for pERK. Changes in tumor size were assessed by RECIST 1.1 criteria.

Table 1: Baseline	e Patient	Characteristics
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Characteristic	Phase I	Thyroid		
		Expansion		
Ago	(1N=20)	(N=13)		
Age	50	(0)		
Median	59	60		
Range	33-82	32-74		
Sex (%)				
Male	14 (54)	7 (54)		
Female	12 (46)	6 (46)		
Race (%)				
White	20 (77)	11 (85)		
Black	2 (8)	0		
Asian	2 (8)	1 (8)		
Unknown or other	2 (8)	1 (8)		
Previous regimens	- (*)			
Median	4	1		
Range	0-13	0-5		
Radiation-based therapy	11	13		
Treatment site (%)				
MD Anderson Cancer Center	14 (54)	11 (85)		
Johns Hopkins University	12 (46)	2 (15)		
ECOG performance status (%)				
0	10 (38)	2 (15)		
1	16 (62)	11 (85)		
Tumor Histology (%)				
Thuroid subtype				
Panillary	2 (8)	6 (46)		
Follicular	1 (4)	5 (38)		
Hurthle cell	0	1 (8)		
Poorly differentiated	1 (4)	1 (8)		
Carcinoma	1 (4)			
Basal cell carcinoma	1 (4)			
Cholangiocarcinoma	2 (8)			
Colorectal Cancer	2 (8)			
Endometrial Cancer	1 (4)			
Maxillary sinus cancer	1 (4)			
Melanoma	4 (15)			
Merkel cell carcinoma	1 (4)			
Ovarian cancer	3 (12)			
Pancreatic ductal adenocarcinoma	1 (4)			

Pancreatic islet cell tumor	1 (4)	
Sarcoma	2 (8)	
Serous papillary cystadenocarcinoma	1 (4)	
Squamous cell base of tongue cancer	1 (4)	

Table 2: Treatment-related adverse events occurring two or more participants and all grade 3/4treatment-related adverse events (all cycles)

Toxicity	Grade 1		Gra	Grade 2		Grade 3		Grade 4		Any Grade	
	Dose	Thyroid									
	Escalation	Expansion									
Abdominal Pain	3	0	2	0	2	1	0	0	7 (27%)	1 (8%)	
Alopecia	1	3	0	0	0	0	0	0	1 (4%)	3 (23%)	
Anorexia	8	3	3	3	0	0	0	0	11 (42%)	6 (46%)	
Arthralgia	1	0	1	0	0	0	0	0	2 (8%)	0	
Blurry Vision / vision changes	0	2	0	0	0	0	0	0	0	2 (15%)	
Constipation	3	1	1	1	0	0	0	0	4 (15%)	2 (15%)	
Cracking Skin	3	0	0	0	0	0	0	0	3 (12%)	0	
Diarrhea	10	2	8	6	4	3	0	0	22 (85%)	11 (85%)	
Dizziness	5	3	0	0	0	0	0	0	5 (19%)	3 (23%)	
Dry Eyes/Mouth	4	0	0	0	0	0	0	0	4 (15%)	0	
Dry Skin	4	2	0	0	0	0	0	0	4 (15%)	2 (15%)	
Edema	5	3	1	0	0	0	0	0	6 (23%)	3 (23%)	
Decreased EF	0	0	0	2	0	0	0	0	0	2 (15%)	
Epistaxis	3	0	0	0	0	0	0	0	3 (12%)	0	
Esophageal Ulcer	0	0	0	0	0	1	0	0	0	1 (8%)	
Fatigue	8	6	6	2	2	1	0	0	16 (62%)	9 (69%)	
Flatulence	3	0	0	0	0	0	0	0	3 (12%)	0	
Headache	4	0	1	0	1	0	0	0	6 (23%)	0	
Heartburn	2	0	1	0	0	0	0	0	3 (12%)	0	
Hypertension	4	2	7	3	3	6	0	0	14 (54%)	11 (85%)	
Hypokalemia	2	0	0	0	0	0	0	0	2 (8%)	0	
Hypomagnesemia	3	1	0	0	0	0	0	0	3 (12%)	1 (8%)	
Hypopigmentation	2	3	0	0	0	0	0	0	2 (8%)	3 (23%)	
Mucositis	4	4	0	0	0	0	0	0	4 (15%)	4 (31%)	
Muscle cramping	2	0	1	0	0	0	0	0	3 (12%)	0	
Muscle Weakness	2	0	0	0	1	0	0	0	3 (12%)	0	
Nausea/vomiting	12	7	4	5	0	0	0	0	16 (62%)	12 (92%)	
Neutropenia	1	0	0	1	0	0	0	0	1 (4%)	1 (8%)	
Palmar-Plantar	1	1	0	1	٥	0	٥	0	1 (10/)	0 (150/)	
Erythrodysesthesia	1	1	0	I	0	0	0	0	I (4%)	Z (15%)	
Proteinuria	0	0	1	1	1	0	0	0	2 (8%)	1 (8%)	
Pruritus	2	1	0	0	0	1	0	0	2 (8%)	2 (15%)	
Rash	13	5	4	5	1	3	0	0	18 (69%)	13 (100%)	
Taste changes	5	4	2	1	0	0	0	0	7 (27%)	5 (38%)	
Thrombocytopenia	2	1	1	1	0	0	0	0	3 (12%)	2 (15%)	
Transaminitis	3	1	0	2	5	1	0	0	8 (31%)	4 (31%)	
Weight Loss	3	2	1	0	0	1	0	0	4 (15%)	1 (8%)	

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Figure 1: Best response per RECIST 1.1 for dose escalation cohort



BCC=Basal cell, CCA=Chalangiacarcinoma, CRC=Colorectal, EC=Endometrial, FTC=Follicular thyroid, MM=Melanoma, MSC=Maxillary sinus, OV=Ovarian, PANC=Pancreatic islet cell, PSC=Papillary thyroid, SCC=Squamous cell tongue, SS=Synovial cell sarcoma, THY=Thyroid, ULS=Uterine leiomyosarcoma

Figure 2: Best RECIST 1.1 criteria responses in differentiated thyroid cohort



Figure 3: Kaplan-Meier estimate of a) progression-free survival (PFS) and b) overall survival (OS) in differentiated thyroid cohort



Months





Figure 4: Change in pERK in relation to change in tumor size