Safety and activity of vandetanib in combination with everolimus in patients with advanced solid tumors: a phase I study

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Background: Preclinical studies suggest that combining vandetanib (VAN), a multi-tyrosine kinase inhibitor of rearranged during transfection (RET) proto-oncogene, vascular endothelial growth factor receptor (VEGFR), and epidermal growth factor receptor (EGFR), with everolimus (EV), a mammalian target of rapamycin (mTOR) inhibitor, may improve antitumor activity. We determined the safety, maximum tolerated dose (MTD), recommended phase II dose (RP2D), and dose-limiting toxicities (DLTs) of VAN + EV in patients with advanced solid cancers and the effect of combination therapy on cancer cell proliferation and intracellular pathways.

Patients and methods: Patients with refractory solid tumors were enrolled in a phase I dose-escalation trial testing VAN (100-300 mg orally daily) + EV (2.5-10 mg orally daily). Objective responses were evaluated using RECIST v1.1. RET mutant cancer cell lines were used in cell-based studies.

Results: Among 80 patients enrolled, 72 (90%) patients were evaluable: 7 achieved partial response (PR) (10%) and 37 had stable disease (SD) (51%; duration range: 1-27 cycles). Clinical benefit (SD or PR ≥ 6 months) was observed in 26 evaluable patients [36%, 95% confidence intervals (CI) (25% to 49%)]. In 80 patients, median overall survival (OS) was 10.5 months [95% CI (8.5-16.1)] and median progression-free survival (PFS) 4.1 months [95% CI (3.4-7.3)]. Six patients (7.5%) experienced DLTs and 20 (25%) required dose modifications. VAN + EV was safe, with fatigue, rash, diarrhea, and mucositis being the most common toxicities. In cell-based studies, combination therapy was superior to monotherapy at inhibiting cancer cell proliferation and intracellular signaling.

Conclusions: The MTDs and RP2Ds of VAN + EV are 300 mg and 10 mg, respectively. VAN + EV combination is safe and active in refractory solid tumors. Further investigation is warranted in RET pathway aberrant tumors.

Key words: vandetanib, everolimus, receptor tyrosine kinase inhibitors, RET/VEGFR/EGFR, mTOR signaling

INTRODUCTION

Receptor tyrosine kinases (RTKs) play a critical role in maintaining tissue homeostasis by regulating a number of cellular processes, including cell division, migration, differentiation, and survival.1 Oncogenic mutations or structural alterations in RTKs can trigger sustained receptor activation resulting in uncontrolled cell proliferation, oncogenesis, and cancer progression.2 Aberrant RTK signaling has been the focus of considerable drug discovery efforts and a number of antibodies and small molecule drugs that prevent endless receptor activation have been approved for cancer treatment.3 Unfortunately, clinical benefits of RTK inhibitors are short-lived and tumors often develop therapeutic resistance.4,5 There is an urgent need to identify new strategies.
that can overcome resistance and increase clinical benefit of RTK inhibitors.

Results from preclinical investigations indicate that many tumors rely on activation of bypass signaling networks to become refractory to RTK inhibitors and ensure tumor progression.4 Therapeutic strategies that provide simultaneous inhibition of both the primary oncogenic signal and the bypass pathway have been shown to delay emergence of resistance and improve survival in experimental tumor models.5 However, translating combinations of molecular-targeted therapy for clinical use is often challenging, in part because simultaneous inhibition of multiple signaling pathways can alter normal physiology leading to prohibitive clinical ‘off-target’ side-effects.7

Vandetanib (VAN), an oral multi-targeted RTK inhibitor of vascular endothelial growth factor receptor-2 (VEGFR-2), epidermal growth factor receptor (EGFR), and the rearranged during transfection (RET) proto-oncogene, is US Food and Drug Administration (FDA) approved for unresectable metastatic medullary thyroid cancer (MTC).8 Oncogenic RET aberrations can be either activating point mutations or genomic rearrangements that produce RET fusion protein kinases that have transforming and oncogenic properties.9

Everolimus (EV) is an allosteric, small molecule inhibitor of mammalian target of rapamycin (mTOR), a kinase that lies downstream in the phosphatidylinositol 3-kinase (PI3K)-protein kinase B (AKT) pathway.10 The PI3K/AKT/mTOR pathway is constitutively activated in several types of cancers and targeting this pathway represents an important anticancer strategy.11,12 Studies have shown that some cancer cells respond to mTOR inhibitors by increasing signaling through the mitogen-activated protein kinase/rat sarcoma/extracellular signal-regulated kinase (MAPK/RAS/ERK) and PI3K/AKT pathways.13,14 Recent evidence demonstrated that combined inhibition of VEGFR/RET and mTOR kinases achieves increased clinical efficacy and maximally suppresses growth mediated by oncogenic RET mutations.15,16 Here, we sought to determine the safety and maximum tolerated dose (MTD) and recommend phase II dose (RP2D) of VAN plus EV in patients with advanced solid tumors, including those harboring genomic aberrations in study drug targets. We also evaluated the effect of combination therapy on cell proliferation and downstream signaling pathways in RET mutant cancer cell lines.

PATIENTS AND METHODS

Patients

Eligible patients were ≥18-years-old with histologically confirmed advanced/metastatic cancers whose tumors failed to respond to standard therapy and/or had progressed despite initial response to standard therapy. Patients were required to be off systemic therapy for at least 3 weeks (or for a period equivalent to five half-lives of a drug in the case of a biologic or targeted agent) and have an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of ≤3. Palliative radiation therapy was allowed during study treatment, but administration of other standard or investigational anticancer agents was prohibited. Other inclusion or exclusion criteria are detailed in the Supplementary Methods, available at https://doi.org/10.1016/j.esmoop.2021.100079. The study protocol was approved by the MD Anderson Cancer Center institutional review board and all patients gave written informed consent. The study was conducted according to good clinical practice and the Declaration of Helsinki and its amendments and is registered at ClinicalTrials.gov (identifier: NCT01582191).

Study design

This was a single institution (University of Texas MD Anderson Cancer Center), investigator-initiated non-randomized, open-label, dose-escalation phase I clinical trial of VAN and EV. The primary objectives were to determine the safety, MTD, RP2D and dose-limiting toxicities (DLTs) of VAN and EV combination in patients with advanced/refractory solid malignancies, including those harboring molecular aberrations. Patients were enrolled at five dose levels using 100 mg of VAN orally daily and 2.5 mg of EV orally daily for 28 days as starting doses (level 0) in a standard ‘3 + 3’ dose-escalation design. After reaching the MTD and RP2D, the trial was amended to multiple expansion cohorts that included expansion to tumor types that demonstrated a partial response (PR) in escalation phase and expansion based on tumor molecular aberrations in study drug targets. The comcomitant use of cytochrome P450 3A4 (CYP3A4) inhibitors was discouraged. If a patient experienced a new grade (G)3 or higher toxicity, treatment was withheld until the condition recovered to G1 or baseline. Treating physicians were allowed to reduce the dose by up to 50% if the toxicity was attributed to either or both study drugs. Patients continued treatment until they experienced progression of disease (PD), intolerable toxicities, or until the treating physician or patient felt that it was not in the patient’s best interest to continue. All patients enrolled at each dose level were evaluated during the first 28 days for DLTs, defined as any clinically significant G3 or G4 non-hematologic toxicity as described in the National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE) v3.0, expected and believed to be related to the study medications, any G4 hematologic toxicity lasting 2 weeks or longer associated with bleeding and/or sepsis; G3-G4 thrombocytopenia lasting 7 days or thrombocytopenia associated with active bleeding or requiring platelet transfusion; G3 nausea/vomiting lasting >48 h or any G4 nausea/vomiting despite maximum anti-nausea regimens (i.e. excluding G3 nausea or G3-G4 vomiting or diarrhea in patients who had not received optimal antiemetic and anti diarrheal treatment); and any other clinically significant G3 non-hematologic toxicity, including symptoms or signs of vascular leak or cytokine release syndrome; or any severe or life-threatening complications or abnormality not defined in the NCI-CTCAE that is attributable to the therapy. Correctable electrolyte imbalances and alopecia were not
considered DLTs. The MTD was defined by DLTs that occur in the first 28-day cycle (induction phase). Patients were evaluated every 28 days before each cycle. The MTD was defined as the highest dose at which no more than 33% of patients developed DLTs. Tumor molecular aberrations were determined by next-generation sequencing (NGS) using Clinical Laboratory Improvement Amendments (CLIA)-certified panels, either Foundation Medicine and/or MD Anderson gene panels, in 66 patients (83%). Patients were classified as ‘unmatched’ if there were no actionable aberrations in study targets and ‘matched’ if actionable alterations were found in the study drug targets (RET, VEGFR, EGFR, and PI3K/AKT/mTOR signaling pathways). Response to therapy was assessed using RECIST v1.1.\(^{17}\)

### Statistical analysis

Exact 95% confidence intervals (CI) for proportions were computed using the Clopper–Pearson method. Odds ratios with 95% CIs and \(P\) values for comparing proportions were estimated using logistic regression. The Wilcoxon rank-sum test was used to compare interval-scaled variables between groups. The median progression-free survival (PFS) and overall survival (OS) times were determined using the Kaplan–Meier method and statistical significance was defined using the log-rank test. Waterfall plots and event charts were generated. Analyses were carried out using TIBCO S+ 8.2 for Windows.

### Supplementary materials and methods

The materials and methods of *in vitro* studies, including cell lines, proliferation assay, drug combination studies, and western blot analysis, are detailed in Supplementary Materials and Methods, available at https://doi.org/10.1016/j.esmoop.2021.100079.

### RESULTS

#### Patient characteristics

From January 2013 to August 2016, 175 patients were screened and a total of 98 patients were started on treatment in the dose-escalation phase. The results of 80 patients with refractory solid malignancies are described. The results of the non-small-cell lung cancer (NSCLC) patient cohort will be reported separately. Seventy-seven patients (73%) were White and the median age at study enrollment was 54 years (range, 18-82 years). Sarcoma, renal cell carcinoma, thyroid, breast, and neuroendocrine tumors comprised 66% of cases. Sixty patients (75%) discontinued therapy due to disease progression including death, 10 patients (12.5%) due to toxicities, and 5 (6%) due to withdrawal of consent. Five patients remained on treatment at time of analysis.

### Toxicity

All 80 patients were evaluated for toxicities and 6 patients (7.5%) experienced DLTs that led to reduction and/or discontinuation of study drug/s before completing cycle 1 (C1) of therapy (Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2021.100079). Based on the DLTs, we identified the MTD and RP2Ds as 300 mg of VAN and 10 mg of EV. For toxicities, there were 61 patients with G1, 48 patients with G2, 24 patients with G3, and 5 patients with G4 events. Fatigue, rash, diarrhea, and mucositis were the most common G1-G2 toxicities, while thrombocytopenia, diarrhea, hyperglycemia, hypertriglyceridemia, and hypercholesterolemia were the most common G3-G4 toxicities (Table 2).

Twenty patients required dose modifications, of those one patient with G2 diarrhea and mucositis and G3 fatigue never resumed therapy due to intolerance, and in five patients, both drugs were discontinued after dose reduction due to one or multiple prolonged toxicities, including edema (G3, \(n = 1\)), diarrhea (G3, \(n = 2\)), rash (G3, \(n = 1\)), mucositis (G3, \(n = 1\)), weight loss (G3, \(n = 1\)), corrected QTc prolongation (G1, \(n = 1\)), and fatigue (G3, \(n = 1\)).

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**Table 1. Patient demographic and clinical characteristics**

| Characteristic | \(N\) (%)
|---------------|----------------|
| Sex           |               |
| Female        | 43 (54)       |
| Male          | 37 (46)       |
| Median age at study enrollment, years (range) | 54 (18-82) |
| Ethnicity     |               |
| White         | 58 (73)       |
| Hispanic      | 13 (16)       |
| African-American | 4 (5)   |
| Other         | 5 (6)         |
| Number of metastatic sites \(<3\) | 62 (78) |
| \(\geq3\) | 18 (22) |
| Disease type  |               |
| Sarcoma       | 21 (26)       |
| Renal cell carcinoma (RCC) | 14 (18) |
| Thyroid       | 9 (11)        |
| Medullary, papillary, follicular, anaplastic, poorly differentiated | 3, 1, 1, 1 |
| Breast        | 5 (6)         |
| Neuroendocrine | 4 (5)   |
| Others        | 27 (34)       |
| ECOG PS       |               |
| 0             | 15 (19)       |
| 1             | 58 (73)       |
| 2             | 5 (6)         |
| 3             | 2 (2)         |
| Number of prior therapies (range) | 1-11 |
| 1-2           | 31 (39)       |
| \(\geq3\) | 49 (61) |

ECOG: Eastern Cooperative Oncology Group; PS, performance status.

\(^{a}\) Refer to Supplementary Table S7, available at https://doi.org/10.1016/j.esmoop.2021.100079 for list of tumors categorized under ‘Others’.
Clinical activity

Among 80 patients treated on trial, 16 patients (20%) did not have available tumor measurements by RECIST criteria: 1 patient had an improvement of his clinical status and received 16 cycles of the study drugs at time of data analysis but did not have measurable disease (bony involvement) by RECIST; 7 patients experienced clinical deterioration before restaging scans but after receiving at least one cycle of the study drugs (clinical PD). Therefore, these eight patients were included in the efficacy analysis. Of the remaining eight patients, the status of disease response was not evaluable due to toxicities (four), consent withdrawal before restaging (three), and clinical deterioration before completion of one cycle of therapy (one). Therefore, these eight patients were excluded from the efficacy analysis, and a total of 72 patients (90%) were evaluable using RECIST criteria with available percent changes in tumor measurements (n = 64) and/or clinically after receiving one cycle of therapy (n = 8). Of these, 30 patients were found to have tumor molecular aberrations in the study drug targets (matched), 29 patients did not have tumor molecular aberrations in the study drug targets (unmatched), and in 13 patients the tumor molecular status was unknown (Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2021.100079). Molecular aberrations in study drug targets included alterations in molecular components of RET, VEGFR, EGFR, and PI3K/AKT/mTOR signaling pathways. The objective response rate [ORR = PR + complete response (CR)] was 10% (n = 7, all PRs). Among the responders, four PRs were observed in matched patients (4/30, 13%) and two PRs were noted in unmatched patients [2/29, 7%; odds ratio (OR) 2.1, 95% CI = (0.4, 12), P = 0.41]. One PR was noted among 13 patients with unknown tumor molecular status (1/13 = 8%). A waterfall plot showing responses in all patients with available radiographic tumor measurements and based on the tumor molecular aberration status is shown in Figure 1A (n = 64). Tumor response, time of progression, and death for each patient treated on trial from cycle 1 day 1 (C1D1) are shown in Figure 1B.

Clinical benefit, defined as PR or stable disease (SD) for 6 months or longer, was observed in 26 patients included in the efficacy analysis [11/30 in matched patients, 7/29 in unmatched patients; OR = 1.8, 95% CI = (0.6, 5.6), P = 0.29] (Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2021.100079). The median percent change (mPC) in tumor size in 64 patients with available measurements was 0.5%. In matched patients (n = 26), the mPC in tumor size compared with baseline was −6%, which was significantly higher when compared with that of unmatched patients (n = 26, median 8% increase, P = 0.023), suggesting significant antitumor activity of combination therapy in patients with refractory solid tumors harboring molecular alterations in study drug targets. In all 80 treated patients, the median duration of follow-up was 20 months (range, 1-34 months). The median PFS was 4.1 months (95% CI: 3.4-7.3) and the median OS time was 10.5 months (95% CI: 8.5-16.1) (Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2021.100079). At the time of analysis, 57/80 (71%) patients had died.

### Table 2. Non-hematologic and hematologic toxicities by grade

| Adverse event | Grade 1 | Grade 2 | Grade 3 | Grade 4 | Total grade 1-2 (%) | Total grade 3-4 (%) |
|---------------|---------|---------|---------|---------|---------------------|---------------------|
| Fatigue       | 24      | 11      | 3       | 0       | 35 (44)             | 3 (4)               |
| Rash and/or acne | 23      | 11      | 1       | 0       | 34 (43)             | 1 (1)               |
| Diarrhea      | 17      | 11      | 4       | 0       | 28 (35)             | 4 (5)               |
| Mucositis     | 13      | 5       | 1       | 0       | 18 (23)             | 1 (1)               |
| Hyperglycemia and/or hypercholesterolemia and/or hypertriglyceridemia | 13 | 4 | 2 | 2 | 17 (21) | 4 (5) |
| Transaminis and/or hyperbilirubinemia | 12 | 3 | 3 | 0 | 15 (19) | 3 (4) |
| Anorexia      | 12      | 6       | 0       | 0       | 18 (23)             | 0 (0)               |
| Nausea/vomiting | 8       | 5       | 0       | 0       | 13 (16)             | 0 (0)               |
| Elevated creatinine/proteinuria | 7 | 7 | 1 | 0 | 14 (18) | 1 (1) |
| Anemia        | 7       | 3       | 1       | 0       | 10 (13)             | 1 (1)               |
| Cough         | 5       | 1       | 0       | 0       | 6 (8)               | 0 (0)               |
| Dyspnea       | 5       | 2       | 0       | 0       | 7 (9)               | 0 (0)               |
| Constipation  | 5       | 1       | 1       | 0       | 6 (8)               | 1 (1)               |
| Thrombocytopenia | 3   | 4      | 5      | 2       | 7 (9)               | 7 (9)               |
| Hypokalemia and/or hypomagnesemia and/or hypocalcemia | 3 | 2 | 1 | 0 | 5 (6) | 1 (1) |
| QTc prolongation | 3 | 3 | 1 | 1 | 5 (6) | 2 (3) |
| Neuropathy    | 3       | 0       | 0       | 0       | 3 (4)               | 0 (0)               |
| Hemoptysis    | 2       | 0       | 0       | 0       | 2 (3)               | 0 (0)               |
| Hand-foot syndrome | 2 | 0 | 0 | 0 | 2 (3) | 0 (0) |
| Fever         | 2       | 0       | 0       | 0       | 2 (3)               | 0 (0)               |
| Edema         | 1       | 0       | 2       | 0       | 1 (1)               | 2 (3)               |
| Weight loss   | 1       | 1       | 1       | 0       | 2 (3)               | 1 (1)               |
| Hypertension  | 0       | 3       | 0       | 0       | 3 (4)               | 3 (4)               |
| Pleural effusion | 0  | 0 | 0 | 0 | 0 (0) | 0 (0) |
| Myocardial infarction | 0 | 0 | 0 | 0 | 0 (0) | 1 (1) |

* Toxicities consisting of less than two G1-G2 events and no G3-G4 events were not included in this table.
Molecular profiles

Among the 80 patients, 66 underwent molecular sequencing of their tumor with clinical NGS testing using a CLIA-certified assay, either Foundation One and/or a solid tumor genomic DNA assay in the MD Anderson Molecular Diagnostics Laboratory. The most common molecular aberrations in the most frequent tumor types are shown in Supplementary Table S3, available at https://doi.org/10.1016/j.esmoop.2021.100079. The list of molecular aberrations in patients who experienced a PR is shown in Supplementary Table S4, available at https://doi.org/10.1016/j.esmoop.2021.100079. Among the seven patients with a PR to therapy, one patient with a metastatic poorly differentiated thyroid carcinoma with a PIK3CA Q546K mutation had a 37% reduction in tumor size from baseline and remained on therapy for 14 cycles (Figure 2A). Another patient with metastatic salivary duct carcinoma harboring a PIK3CA H1047R mutation experienced a 33% reduction in tumor size compared with baseline and received a total of 12 cycles (Figure 2B). Interestingly, one patient with epithelioid sarcoma harboring single nucleotide polymorphisms (SNPs), kinase insert domain receptor (KDR) Q472H, and KIT M541L aberrations experienced a 74% reduction in tumor size when compared with baseline. A patient with MTC harboring the RET M918T mutation was started on therapy in September 2013 and stopped due to progression in March 2014, as shown in Figure 3A. The patient had multiple nodal and hepatic metastases. Representative measurements for nodal metastases in the left lower neck (solid line) and superior mediastinum (dashed line) are shown in Figure 3B. The waterfall plot depicts percentage change in target lesions (RECIST) in 64 patients with available tumor measurements with advanced cancers treated with VAN and EV in the phase I study (escalation and expansion phases). Molecular aberrations in study drug targets (matched) include aberrations in molecular components of RET, VEGFR, EGFR and PI3K/AKT/mTOR signaling pathways. Denotes the patients that are used as radiographic examples in later figures. (B) Response to therapy, time to progression, and death from cycle 1 day 1 (C1D1) in 80 treated patients on trial.

AKT, Protein kinase B; EGFR, epidermal growth factor receptor; EV, everolimus; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3-kinase; RET, rearranged during transfection; VAN, vandetanib; VEGFR, vascular endothelial growth factor receptor.

Figure 1. Changes in tumor burden in patients treated with combined VAN and EV.

(A) Waterfall plot depicts percentage change in target lesions (RECIST) in 64 patients with available tumor measurements with advanced cancers treated with VAN and EV in the phase I study (escalation and expansion phases). Molecular aberrations in study drug targets (matched) include aberrations in molecular components of RET, VEGFR, EGFR and PI3K/AKT/mTOR signaling pathways. Denotes the patients that are used as radiographic examples in later figures. (B) Response to therapy, time to progression, and death from cycle 1 day 1 (C1D1) in 80 treated patients on trial.

AKT, Protein kinase B; EGFR, epidermal growth factor receptor; EV, everolimus; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3-kinase; RET, rearranged during transfection; VAN, vandetanib; VEGFR, vascular endothelial growth factor receptor.
Baseline computed tomography (CT) scans (first column of CT images) showed nodal metastases in the left lower neck (upper row of CT images) and superior mediastinum (lower row of CT images). First follow-up imaging (second column) in November 2013 showed a decrease in the size of both nodal metastases. This trend continued on the second follow-up scans (third column) in January 2014. However, the third follow-up scans (fourth column) in March 2014 showed tumor progression and therapy was stopped. The patient had an N-of-One™ report that showed interesting insights into the biology of disease. The patient's tumor molecular profile is shown in Figure 3B and reveals additional molecular aberrations, including strong (+3) immunohistochemical expression of mTOR, pAKT, and the anti-apoptotic molecule B-cell lymphoma 2 (Bcl-2).

**Figure 2. Representative radiographic responses in patients with tumors harboring molecular aberrations in PI3K3CA pathway in response to VAN and EV combination therapy.**

Representative radiographic response to treatment of a (A) 31-year-old patient with metastatic poorly differentiated thyroid carcinoma harboring a PIK3CA Q546K mutation, who experienced PR by RECIST and received combination therapy on trial for a total of 14 cycles, and (B) of a 32-year-old patient with metastatic salivary duct carcinoma harboring a PIK3CA H1047R mutation, who experienced PR by RECIST and received combination therapy on trial for a total of 12 cycles. The black arrows indicate the changes in tumor lesion size over time.

EV, everolimus; PR, partial response; VAN, vandetanib.

* Denotes the percent change in tumor size plotted in Figure 1A for the radiographic cases shown in Figures 2A and B.

**VAN and EV combination induces antiproliferative activity and inhibits downstream signaling of RET, AKT/mTOR, and ERK pathways in RET mutant cancer cells**

In preclinical studies testing the antiproliferative activity of VAN, EV, or the combination in two RET mutant MTC cell lines, the addition of EV to VAN decreased cell proliferation in a dose-dependent manner in both cell lines (Supplementary Figure 1A and B, Supplementary Table S5, available at https://doi.org/10.1016/j.esmoop.2021.100079). Even at the highest dose level, EV had only a modest inhibitory effect on cell proliferation, suggesting that mTOR inhibition alone is not sufficient to control cell proliferation. In both cell lines, the administration of escalating doses of EV to higher doses of VAN resulted in a more profound reduction in cell proliferation, suggesting that mTOR inhibition may play a role in counteracting and preventing...
resistance to RET inhibition. However, MZ-CRC-1 cells had a similar decrease in cell proliferation with the highest dose of VAN alone and the highest dose of combination therapy. Drug combination studies carried out to test potential synergistic drug interaction revealed a combination index less than one when both VAN and EV were tested in both cancer cell lines, suggesting overall moderate synergy at these intermediate doses (Supplementary Table S6, available at https://doi.org/10.1016/j.esmoop.2021.100079). The results of these studies are consistent with the findings from western analysis which showed that combined VAN plus EV resulted in a more profound inhibition of phosphorylated RET and AKT signaling pathways as compared with either drug alone (Supplementary Figure 1C, available at https://doi.org/10.1016/j.esmoop.2021.100079). Phosphorylated ERK was equally suppressed by VAN alone or VAN combined with EV. EV alone suppressed pAKT and pS6 kinase, which are components of the mTOR pathway, but had minimal effect on pRET and pERK. Combined VAN plus EV was more effective at suppressing pAKT and pS6 kinase signaling compared with either agent in monotherapy and increased the expression of cleaved poly (ADP-ribose) polymerase (PARP) and phosphorylated histone subtype H2A isoform X (γH2AX), both of which are involved in the DNA repair pathway. Together, these findings illustrate that combination VAN plus EV may be superior at inhibiting potential downstream resistant signaling pathways that could be activated by either agent administered as monotherapy.

DISCUSSION

Accumulating evidence suggests that targeting both the primary oncogenic signal and the secondary escape signaling pathways may be an effective strategy to delay or overcome therapeutic resistance.18-21 Combination of TKIs of VEGFR and mTOR pathways have shown clinical benefit in earlier clinical trials.22,23 In fact, an analogous combination of a VEGFR-2/RET plus mTOR inhibitors lenvatinib and everolimus is FDA-approved for metastatic renal cell carcinoma.16 The current study was motivated, in part, by preclinical results demonstrating that co-inhibition of VEGFR/RET and mTOR kinases provides greater antiproliferative activity than either agent alone in RET mutant cancer cells.15 VAN is approved for use in unresectable MTC and our preclinical data in MTC cell lines provides preliminary insight into the effect of VAN plus EV combination compared with monotherapy in this tumor type, which will serve to guide future investigations in this area. Due to the multi-targeted nature of both VAN and EV, these drugs may also be applicable to a number of different solid tumors with molecular aberrations in the study drug targets. Targeting the mTOR pathway with EV has also been shown to produce antitumor activity in EGFR-resistant cancer cell
lines and experimental tumor models and to resensitize resistant cancer cells to EGFR inhibitors.  

The results of our dose-escalation study demonstrated that 300 mg of VAN can be safely combined with 10 mg EV to produce clinical activity. Twenty (25%) patients required dose modifications due to toxicity. Thirty percent of patients with molecular aberrations in the drug targets (8% increase) or those harboring tumors with an undefined molecular status (3% increase) or other tumors did not have molecular alterations in the drug targets (8% increase) or those harboring tumors with an undefined molecular status (3% increase). We observed tumor regression in patients with renal cell carcinoma, salivary duct carcinoma, soft tissue sarcoma, thyroid carcinoma, ovarian cancer, breast cancer, and epithelioid sarcoma. Of the seven patients whose tumors demonstrated a PR to therapy, two had molecular aberrations in both the KDR and KIT kinases, and one had an MDM2 amplification in addition to a variant of uncertain significance in the tumor suppressor gene tuberous sclerosis complex 1 (TSC1), both of which are components of the PI3K/AKT/mTOR pathway. The aberration identified in KIT has been reported as a benign polymorphism in the Single Nucleotide Polymorphism Database (dbSNP), but it also has been described as a somatic mutation in COSMIC (COSM28206) in association with tumors such as aggressive fibromatosis, meningioma, and chronic myeloid leukemia and some studies suggest that it may confer increased risk of hematologic malignancies. KDR Q472H aberration has been shown to mediate VEGFR-2 phosphorylation and enhanced tumor angiogenesis. No aberrations were noted in two other patients exhibiting a PR and the molecular status of the tumor from the remaining patient with a PR was unknown. Thirty-six percent of patients enrolled received clinical benefit, most of whom had alterations in the drug targets. We observed early signals of antitumor activity of combination therapy in tumors harboring actionable alterations in the study drug targets. While these results are encouraging, they should be viewed as preliminary and further studies are needed to explore the relationship between potentially targetable molecular aberrations and response to therapy.

Since we completed these analyses, at least two next-generation selective RET inhibitors have been described. These drugs were developed with the goal of limiting the toxicity associated with multi-targeted RTK inhibitors by sparing non-RET targets, such as VEGFR-2. In preliminary studies, BLU-667 (pralsetinib) demonstrated activity against wild-type RET and oncogenic RET while maintaining selectivity toward the target. BLU-667 was much more potent (>10-fold increase) and selective over VAN and cabozenatiniib at inhibiting RET signaling and proliferation in RET-driven cancer cell lines. BLU-667 also demonstrated antitumor activity in RET-driven preclinical models and induced clinical responses in patients with RET-altered NSCLC and MTC with notable off-target toxicity. LOXO-292 (selpercatinib) is another selective VEGFR-2-sparing RET kinase inhibitor that was designed to inhibit diverse RET fusions, activating mutations, and acquired resistance. LOXO-292 demonstrated robust antiproliferative activity in RET fusion-positive and RET mutant cancer cells in vitro and in vivo, including an orthotopic model of RET mutant brain metastases. More importantly, LOXO-292 demonstrated antitumor activity in patients with RET-altered tumors. Collectively, these initial studies suggest that inhibition of VEGFR-2 is not necessary for an antitumor response in patients with RET-driven cancers who are treated with RET selective inhibitors. Additional testing in a larger cohort of patients will reveal the benefit of these VEGFR-2 sparing inhibitors on toxicity profiles and later studies will determine how they impact emergence of the resistant phenotype. The combination of a multikinase RET inhibitor with an mTOR inhibitor could be an interesting strategy to address off-target resistance mechanisms from selective RET inhibitors, but further data are warranted to unravel off-target resistance mechanisms and design specific trials.

This single-institution, investigator-initiated clinical trial included patients with heavily pre-treated advanced solid tumors with more flexible schedules with two oral FDA-approved agents. Being more inclusive of ECOG PS and with no restriction to a number of lines of therapy when compared with other sponsored trials may have reduced the clinical efficacy of the trial. In the present study, we found that 300 mg of VAN orally and 10 mg of EV orally (the current FDA-approved dose of either agent) can be combined safely to produce antitumor activity in patients with solid tumors. Our cell-based studies suggest that the multikinase targeting approach effectively inhibits signaling pathways associated with cell division and survival that may be upregulated as potential mechanisms of resistance to either drug administered as monotherapy. Although selective RET inhibitors are in clinical development, tumors may develop resistance mechanisms via alternative signaling pathways that can be on- or off-target, leading to disease progression. Therefore, therapies directed against non-specific drug targets such as VAN and EV may prove beneficial in resistant tumors and warrant further investigation. The overall manageable toxicity profile and antitumor activity of VAN and EV in this study support additional testing in patients with advanced/refractory solid tumors, including those harboring genomic aberrations in the study drug targets.

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DATA SHARING

The data supporting the findings presented in this manuscript are available upon reasonable academic request to the study principal investigator (VS).

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