Novel Mechanisms and Predictors of VEGF Receptor Inhibitor- or Immune Checkpoint Inhibitor-Associated Hypertension and Cardiovascular Disease

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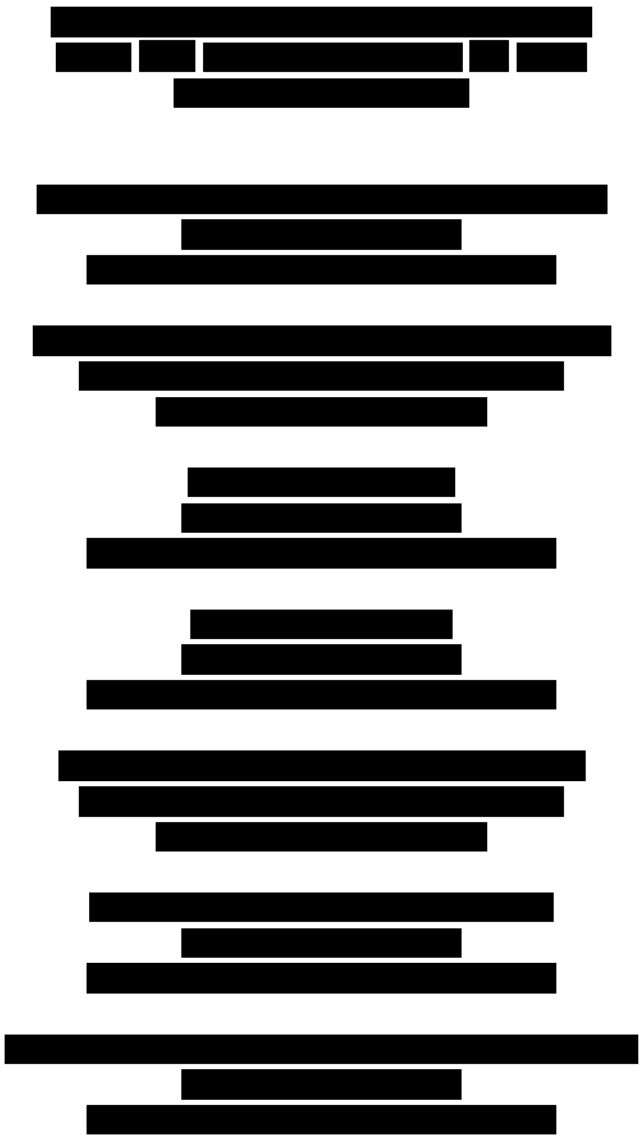

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1.0 Background

The status quo for the prediction, diagnosis and treatment of VEGFR-TKI and immune checkpoint-inhibitor (ICI) hypertension and vascular disease is application of the consensus guidelines developed for essential hypertension in the general population. In particular, the active surveillance for hypertension and use of common anti-hypertensive medications in cardio-oncology patients is derived from randomized controlled trials of hypertension in adults with the common comorbidities of aging. Notably, cancer patients have been systematically excluded from these trials. As a result, the current approach belies the distinct oncologic context of VEGFR-TKI or ICI use and their toxicities and inherently limits the evidence for applying these guidelines to this unique patient group. Overall, it remains unclear whether prevailing mechanistic concepts to explain essential hypertension or the algorithms for anti-hypertensive medications should be extrapolated to patients with malignancies who are on treatment with anti-angiogenic therapies. For example, human studies of VEGFR-TKI-mediated hypertension have described correlations between specific EC metabolites implicated in blood pressure homeostasis such as nitric oxide or ET-1, but the impact of targeted therapies to interdict these pathways remain untested. Our research disrupts this paradigm by constructing a new precision medicine framework for the clinical approach to this at-risk oncology patient population. *The central innovation in this proposal is that VEGFR-TKI-mediated hypertension stems from context-specific biologic mechanisms determined by medication- and patient-specific variables that can be leveraged for risk prediction and implementation of rational therapies.* An additional advance of this proposal is the establishment of an interdisciplinary team focused on cardio-oncology patients at VUMC to enable an innovative, multi-dimensional research approach spanning basic, clinical/translational and population science.

2.0 Rationale and Specific Aim

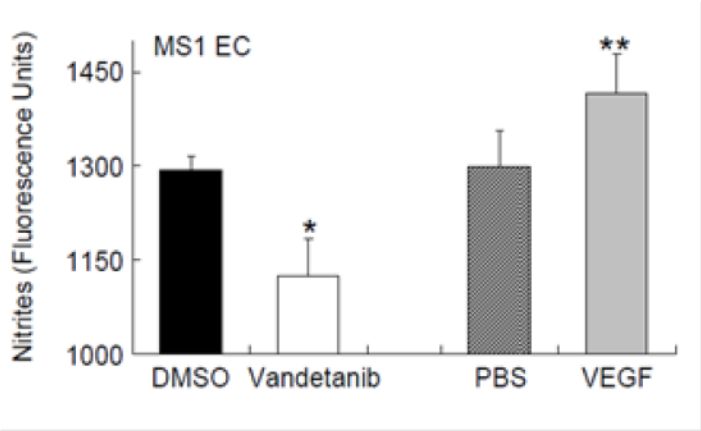
We hypothesize that VEGFR-TKIs, ICIs, and combination treatment cause hypertension and vascular dysfunction through ET-1 induction and this pathway is a target for precision therapeutic interventions and novel risk stratification.

Aim

VEGFR-TKIs, ICIs, and the combination-mediated increases in ETA receptor activation will cause hypertension and reduce the bioavailability of endothelium-derived nitric oxide. We have previously demonstrated that two structurally distinct VEGFR TKIs reduce the bioavailability of endothelium-derived nitric oxide. We hypothesize that VEGFR TKIs antagonism will reduce digital microvessel hyperemia. As hypertension is mediated primarily in the microvasculature, we anticipate an enhanced response in this vascular bed.

3.0 Animal Studies and Previous Human Studies

VEGFR2 inhibition decreases constitutive nitric oxide bioavailability
We have studied seventeen patients with stage IV breast cancer received who received the VEGFR-TKI vandetanib as part of a phase 1 protocol. We determined blood pressure and systemic NOx concentrations. We further examined endothelial function by measuring the effects of vandetanib on endothelial cell nitric oxide production and Akt473 phosphorylation were examined in vitro.



Vandetanib treatment for 6 weeks significantly increased blood pressure and decreased plasma nitrites and nitrotyrosine, but not a marker of oxidative stress (8-OHDG) compared to baseline (Table).

Parameter		**Baseline**	**6 Weeks**	**Comparison**
Mean Arterial Pressure	(mm Hg)	91 +/- 8	102 +/- 10	p = 0.001
Heart Rate	(BPM)	74 +/- 13	71 +/- 10	p = 0.28
Nitrates/Nitrites (NOx)	(AU)	48446 ± 1264	46799 ± 1740	p < 0.001
Nitrotyrosine	(AU)	259814 ± 26139	246157 ± 20501	p = 0.03
8-hydroxy-2'-deoxyguanosine	(AU)	1.96 ± 0.09	2.0 ± 0.15	p = 0.5

4.0 Inclusion/Exclusion Criteria

Inclusion criteria

- Male or female, age 40 - 75 years old
- Diagnosis of cancer
- Receiving VEGF inhibitor, ICI, or combination (VEGF inhibitor + ICI or combination ICI) treatment, or not receiving any treatment.
- Normal blood pressure or blood pressure treated to < 140/90 mm Hg with ≤2 antihypertensive medications

Exclusion criteria

- Presence of peripheral artery disease
- History of a heart attack within 1 year
- History of a stroke within 1 year
- Diabetes
- Life expectancy < 3 months
- Women who are pregnant
- Women who are nursing

5.0 Enrollment/Randomization

We will study 80 subjects with cancer who will be treated with VEGF inhibitor, ICI, or combination (VEGF inhibitor plus ICI or combination ICI treatment), or not receiving any of the above treatments and will serve as controls. We will restrict enrollment to age 40 - 75 years. Subjects will be identified by research staff from Vanderbilt Ingram Cancer Center (VICC) Clinics. If appropriate, the physician seeing the patient in clinic will approach the patient about participating in a clinical trial and alert the appropriate research staff for further study discussion with the patient. The patient will be consented or be allowed to take the consent form home to read if they are interested. We will use the VUMC Research Derivative, a fully identified clinical database specialized for research, to scan the population of patients in the VUMC electronic health record and use this information to approach patients with renal cell carcinoma, with the participants attending physicians' prior consent. Research coordinators at the Vanderbilt Translational and Clinical Cardiovascular Research Center (VTRACC) have had success in recruiting several thousand subjects in the last 5 years for a variety of projects. In addition to the VTRACC research staff, the study will also utilize services provided by the Vanderbilt Coordinating Center. The VCC will provide nursing support to assist the research coordinators with study procedures, consenting patients, placing the IV catheter, and collection of the endothelial samples.

6.0 Study Procedures

Baseline. We will recruit subjects who have not yet begun their treatment. The subjects most recent labs will be reviewed by study staff as part of the screening process. Subjects will report to the Preston Research Building after a ≥ 5 hour fast. First an informed consent will be obtained (if not already obtained during the patient's clinic visit), followed by a medical history, an abbreviated physical exam, and taking the subjects weight. After the patient has been sitting for 10 minutes, blood pressure will be measured in triplicate using an automated oscillometric device, baseline and hyperemic velocity time interval (VTI), and digital pulse amplitude tonometry will be performed to measure the reactive hyperemia index. We will draw blood for renal function, sodium and potassium, fasting glucose and insulin, metabolics, markers of inflammation, and markers of oxidative stress. We will measure urinary albumin/creatinine ratio (UACR). We will place an IV catheter for blood sampling and endothelial cell harvest. Endothelial cells (ECs) will be harvested from patients for gene expression (PCR, RNA-sequencing), staining and ATAC assays. Subjects will then have an ambulatory blood pressure monitor (Spacelabs) placed for 24 hours.

Follow-up Visit Approximately 1 month after starting treatment, subjects will return to the Preston Research Building. The procedures will be the same as the baseline visit procedures. We will start by discussing changes to medical history since the beginning of the study, conduct an abbreviated physical exam, and take the subjects weight. After the patient has been sitting for 10 minutes, blood pressure will be measured in triplicate using an automated oscillometric device, baseline and hyperemic velocity time interval (VTI), and digital pulse amplitude tonometry will be performed to measure the reactive hyperemia index. We will draw blood for renal function, sodium and potassium, fasting glucose and insulin, metabolics, markers of inflammation, and markers of oxidative stress. We will measure urinary albumin/creatinine ratio (UACR). We will place an IV catheter for blood sampling and endothelial cell harvest. Endothelial cells (ECs) will be harvested from patients for gene expression (PCR, RNA-sequencing), staining and ATAC assays. Subjects will then have an ambulatory blood pressure monitor (Spacelabs) placed for 24 hours.

Standard Techniques

Peripheral Artery Tonometry Digital pulse amplitude will be measured in the fasting state with a peripheral artery tonometry (PAT) device placed on the tip of each index finger (Endo-PAT2000, Itamar Medical, Caesarea, Israel). The PAT device comprises a pneumatic plethysmograph that applies uniform pressure to the surface of the distal finger, allowing measurement of pulse volume changes in the finger. Throughout the study, the inflation pressure of the digital device is electronically set to 10 mm Hg below diastolic blood pressure or 70 mm Hg (whichever is lower). Baseline pulse amplitude is measured from each fingertip for 2 minutes 20 seconds. Arterial flow is arrested for 5 minutes by a cuff placed on a proximal forearm (Hokanson AG101, D.E. Hokanson Inc, Bellevue, WA) at whichever occlusion pressure would be higher: 200 mm Hg or 60 mm Hg plus systolic blood pressure. Pulse amplitude is recorded electronically in both fingers and analyzed by a computerized, automated algorithm (Itamar Medical) that provides the average pulse amplitude for each 30-second interval after forearm cuff deflation up to 4 minutes. To determine the PAT ratio, we will first calculate the ratio of the post-deflation pulse amplitude to the baseline pulse amplitude in the 90- to 120-second postdeflation time period (ie, Xh90–120/ Xh0: with h denoting hyperemic finger and 0 denoting baseline, X being the pulse amplitude). Then, we divided this result by the corresponding ratio from the contralateral, control hand (ie, Xc90–120/ Xc0: with c denoting the control finger and 0 denoting baseline) to obtain the PAT ratio. Because PAT ratio has a heteroscedastic error structure, we use natural logarithm transformation in all analyses such that PAT ratio=ln[(Xh90–120/ Xh0)/(Xc90–120/ Xc0)].

Endothelial cell harvest The Brown and Beckman Laboratories have developed a novel technique based on a protocol for isolation of ECs and leukocytes directly from the cubital veins of human subjects[1]. This procedure involves gentle scraping of the vein using a 0.014" j-wire followed by selection of ECs (from red blood cells and leukocytes) using VE-cadherin magnetic beads and rapid sorting by gravity flow (MACS). The cell yields using this technique range from 500-1000. Immunofluorescence of fixed cells demonstrates positive staining for EC markers including von Willebrand factor and no staining for pan-leukocyte marker CD45. Unbiased RNA-sequencing confirms the transcriptomes cluster perfectly by cell type (Figure 6). Further, functional enrichment analysis (ToppGene) reveals differential expression of genes associated with cell type specific functions. Importantly for this proposal, these patient-specific ECs express genes of the endothelin-1 and VEGF signaling pathways including EDN1. This novel technique enables us to test the effects of VEGFR-TKI, ICI, and the combination of agents in the vasculature directly from humans for the first time.

For these experiments, we will isolate ECs from patients before and after starting VEGFR-TKI, ICI, and combination therapy (on treatment, approximately 30 days). EC preparations will be divided for the following: a) RNA extraction to measure expression of relevant genes involved in both VEGF and ET-1 pathways including FLT1, FLK1, FLT4, EDN1, ETRB and endothelin converting enzyme (ECE); b) isolation of nuclei to perform ATAC-seq with the goal of mapping the regulatory elements in patient-derived ECs. ATAC data are strongly correlated with enhancer maps generated with ChIP datasets[2]. However, the lower cell yield renders conventional ChIP assays challenging. As such, ATAC assays will be used to provide specific insight into enhancer architecture. We will integrate ATAC data with gene expression data from

patient ECs to determine the effects of VEGFR-TKI, ICI, or the combination of agents on the EC epigenome in vivo in humans.

BP Measurements At baseline and the follow-up visit outpatient BP will be measured with an aneroid sphygmomanometer (Welch Allyn, Skaneateles Falls, NY), using the appearance and complete disappearance of the Korotkoff sounds (K1 and K5) as SBP and DBP. The mean of three seated measurements, performed two minutes apart, will be used. During study days, BP will be measured using an automated oscillometric recording device (Dinamap, Critikon, Carlsbad, CA) and will be completed with the subjects sitting. Ambulatory BP monitoring will be performed with a portable noninvasive recorder (SpaceLabs Inc., Redmond, WA) for 24-consecutive hours at the end of the baseline and follow up study visits.

Laboratory Testing The following biomarker measurements will be done in collaboration with Dr. Yan Ru Su in the Vanderbilt Cardiology Core lab. Her lab has extensive experience working with ELISA-based biomarker detection, and offers these tests as routine service. Plasma aldosterone level will be tested using Aldosterone ELISA kit (Eagle Biosciences, Cat# DCM053-8). Based on the manufacturer's data, the intraassay variation is ≤ 9.7% and the inter assay variation is ≤ 11%, respectively. The Vascular Endothelial Growth Factor 165B (VEGF-165B) will be tested using quantitative sandwich ELISA kit from MyBiosource (Cat# MBS109074). The sensitivity of this kit is 5.0 pg/ml and both Intra-assay CV (%) and Inter-assay CV (%) is less than 15%. Human Serum Adipokines (HGF, Leptin, IL-1β, IL-6 IL-8/CXCL8, Insulin, MCP-1/CCL2, NGF and TNF-α) will be measured using EMD Millipore/Sigma Milliplex Map Human Adipokine Magnetic Bead Panel 1 (Millipore/Sigma, Cat# HADK1MAG-61K) according to manufacturer's instructions. The Cardiology Core lab has Luminex 200 and is well set up to run these types of assays as their routine service. We will run F2-isoprostanes using mass spectrometric methods developed at Vanderbilt.

Type and Amount Of Sample Needed: About 40 mL and about 10 mL of urine at each of the study visits.

7.0 Risks

Phlebotomy: drawing blood may cause pain, bruising, lightheadedness and rarely, infection.

Intravenous catheter placement: common risks include pain at the site of the needle stick and mild bruising. Uncommon risks are hematoma, infection, and phlebitis.

Endothelial biopsy: there are not any common risks. An uncommon risk is phlebitis.

8.0 Reporting of Adverse Events or Unanticipated Problems involving Risk to Participants or Others

Adverse events will be reported the IRB per Vanderbilt University IRB Policy.

9.0 Study Withdrawal/Discontinuation

Subjects may withdraw consent for study participation at any time by informing the PI.

10.0 Statistical Considerations

Power: Hypertension: In studies of TKI, ICI, and the combinationuse, increases in blood pressure begin within days and reach steady state over weeks. Pazopanib has been shown to increase mean arterial blood pressure by 15 mm Hg in this time frame[3,4]. Based on the previous studies, we assume the mean changes of BP is 15 mm Hg, with a standard deviation of 10 mm Hg. We first conducted power analysis with ANOVA framework. With will have 100% power to detect a difference of 8 mm Hg. Our power analysis is conservative because the standard deviation of BP should be much less than 10 mm Hg.

11.0 Privacy/Confidentiality Issues

All efforts, within reason, will be made to keep protected health information (PHI) private. Using or sharing ("disclosure") such data will follow federal privacy rules.

As per standard practice at VUMC, we may share the results of the study and/or non-study linked lab tests, as well as parts of your medical record, with the Federal Government Office for Human Research Protections, the Vanderbilt University Institutional Review Board, The Food and Drug Administration, other government agencies throughout the world and insurance companies for billing purposes.

Data will be kept in for at least six years after the study is finished. There is no current intent to place data in the medical record. If subjects specifically request, we will store data indefinitely.

12.0 Follow-up and Record Retention

Subject participation in the study will last for about 35 days. Record retention will be indefinite, as described above.

LITERATURE CITED

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3. Suttle AB, Ball HA, Molimard M, et al. Relationships between pazopanib exposure and clinical safety and efficacy in patients with advanced renal cell carcinoma. Br J Cancer 2014;111:1909-16.
4. Heath EI, Infante J, Lewis LD, et al. A randomized, double-blind, placebo-controlled study to evaluate the effect of repeated oral doses of pazopanib on cardiac conduction in patients with solid tumors. Cancer Chemother Pharmacol 2013;71:565-73.