The utility of cardiac biomarkers and echocardiography for the early detection of bevacizumab- and sunitinib-mediated cardiotoxicity

Kimberly-Ann Bordun,¹ Sheena Premezec,¹ Megan daSilva,¹ Soma Mandal,¹ Vineet Goyal,¹ Tamara Glavinovic,¹ Matthew Cheung,¹ David Cheung,¹ Christopher W. White,¹ Rakesh Chaudhary,¹ Darren H. Freed,¹ Hector R. Villarraga,² Joerg Herrmann,² Manish Kohli,³ Amir Ravandi,⁴ James Thiliveris,⁵ Marshall Pitz,⁶ Pawan K. Singal,⁷ Sharon Mulvagh,² and Davinder S. Jassal¹,⁴,⁶,⁷

¹Institute of Cardiovascular Sciences, St. Boniface Research Centre, University of Manitoba, Winnipeg, Manitoba, Canada; ²Department of Internal Medicine, Division of Cardiovascular Diseases, Mayo Clinic, Rochester, Minnesota; ³Division of Medical Oncology, Department of Oncology, Mayo Clinic, Rochester, Minnesota; ⁴Section of Cardiology, Department of Internal Medicine, University of Manitoba, Winnipeg, Manitoba, Canada; ⁵Department of Human Anatomy and Cell Science, University of Manitoba, Winnipeg, Manitoba, Canada; ⁶Department of Oncology, Division of Cardiovascular Diseases, Mayo Clinic, Rochester, Minnesota; and ⁷Department of Radiology, University of Manitoba, Winnipeg, Manitoba, Canada

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Bordun K, Premezeck S, daSilva M, Mandal S, Goyal V, Glavinovic T, Cheung M, Cheung D, White CW, Chaudhary R, Freed DH, Villarraga HR, Herrmann J, Kohli M, Ravandi A, Thiliveris J, Pitz M, Singal PK, Mulvagh S, Jassal DS. The utility of cardiac biomarkers and echocardiography for the early detection of bevacizumab- and sunitinib-mediated cardiotoxicity. Am J Physiol Heart Circ Physiol 309: H692–H701, 2015. First published June 19, 2015; doi:10.1152/ajpheart.00172.2015.—The recent introduction of novel anticancer therapies, including bevacizumab (BVZ) and sunitinib (SNT), is associated with an increased risk of cardiotoxicity. However, early identification of left ventricular (LV) systolic dysfunction may facilitate dose modification and avoid the development of advanced heart failure. Using a murine model of BVZ- and SNT-mediated cardiotoxicity, we investigated whether cardiac biomarkers and/or tissue velocity imaging (TVI) using echocardiography can detect early changes in cardiac function, before a decrease in LV ejection fraction is identified. A total of 75 wild-type C57BL/6 male mice were treated with either 0.9% saline, BVZ, or SNT. Serial monitoring of blood pressure, high-sensitivity troponin I, and echocardiographic indexes were performed over a 14-day study period, after which the mice were euthanized for histological and biochemical analyses. Mice treated with either BVZ or SNT developed systemic hypertension as early as day 7, which increased by day 14. Cardiac biomarkers, specifically high-sensitivity troponin I, were not predictive of early LV systolic dysfunction. Although conventional LV ejection fraction values decreased at day 13 in mice treated with either BVZ or SNT, TVI confirmed early LV systolic dysfunction at day 8. Histological and biochemical analysis demonstrated loss of cellular integrity, increased oxidative stress, and increased cardiac apoptosis in mice treated with BVZ or SNT therapy at day 14. In a murine model of BVZ- or SNT-mediated cardiomyopathy, noninvasive assessment by TVI detected early LV systolic dysfunction before alterations in conventional echocardiographic indexes.

NEW & NOTEWORTHY

The current basic science study evaluates the utility of cardiac biomarkers and echocardiography using tissue velocity imaging and strain rate imaging for the early detection of bevacizumab- and sunitinib-mediated cardiotoxicity.

CARDIO-ONCOLOGY IS A NOVEL discipline that focuses on the prevention, diagnosis, and management of cancer patients who are at risk of developing cardiovascular complications as a result of their anticancer treatment. Despite the beneficial effects of chemotherapy agents in increasing overall survival of cancer patients, cardiotoxicity remains a serious complication of many systemic anticancer therapies (4, 5, 26, 32, 36, 47, 49, 58). The treatment strategy for each patient is individualized, involving a combination of surgical intervention, radiation, chemotherapy, and novel targeted biological therapy. Two types of targeted therapies widely used for treating metastatic colorectal (CRC) and renal cell cancer (RCC), respectively, are the monoclonal antibody bevacizumab (BVZ; Avastin) and the tyrosine kinase inhibitor sunitinib (SNT; Sutent) (18, 50). In both normal and neoplastic tissues, including CRC, vascular endothelial growth factor (VEGF) plays an important role in angiogenesis, chemotaxis, and regulation of vascular tone (19, 33). By binding specifically to VEGF-A and inhibiting its interaction with the VEGF receptor (VEGFR), BVZ prevents the proliferation of endothelial cells and formation of new blood vessel cells, thus inhibiting tumor growth (18). In combination with non-anthracycline-based chemotherapy, BVZ is approved for the treatment of metastatic CRC by decreasing tumor progression and improving overall survival (22, 29). Additionally, solid tumors, including RCC, are dependent on the development and expansion of a vascular network to support their growth. As opposed to BVZ, which inhibits only VEGF-A and its interaction with VEGFR, SNT is an oral tyrosine kinase inhibitor approved for the treatment of metastatic RCC that blocks the activity of multiple receptors, including VEGFR 1–3, platelet-derived growth factor receptors-α and -β, and AMP-activated protein kinase (AMPK) (10, 21, 26). Despite the beneficial effects of both BVZ and SNT in improving overall survival in the CRC and RCC populations, an unexpected side effect of both anticancer drugs is the risk of...
developing cardiotoxicity in nearly one in four individuals (6, 10, 24, 47, 50, 58).

In clinical practice, serial monitoring of cardiac biomarkers and left ventricular (LV) ejection fraction (LVEF) using noninvasive cardiac imaging are important diagnostic tools in the detection of cardiac dysfunction among cancer patients (3, 7, 8, 14, 15, 35, 52). In the last decade, the use of cardiac biomarkers, including troponin I (TnI), C-reactive protein, and brain natriuretic peptide have emerged as a more sensitive and specific tool for the early identification, assessment, and monitoring of cardiotoxicity due to anticancer drugs (1, 7, 8, 34, 35, 42, 52). Recent studies have demonstrated that frequent sampling of TnI and C-reactive protein was able to identify a subset of women with breast cancer at high risk of doxorubicin-and-trastuzumab- (DOX+TRZ) mediated cardiac dysfunction before a decrease in LVEF (7, 44). Whether these cardiac biomarkers, including high-sensitivity troponins (37), can similarly detect early evidence of BVZ- and SNT-mediated cardiac dysfunction, requires further study.

Noninvasive assessment of LVEF using multiple-gated acquisition scintigraphy and transthoracic echocardiography (TTE) continue to be the most common methods for monitoring cardiac dysfunction in the cancer setting (3, 13, 15, 44, 51, 54). Despite the established use of LVEF as a measure of cardiac function, compensatory myocardial reserve enables adequate ventricular output in the presence of dysfunctional cardiomyocytes, such that the extent of cardiac injury is often not recognized and/or underestimated at an early time point in treatment (14). Therefore, novel echocardiographic parameters, including tissue velocity imaging (TVI) and strain rate imaging (SRI) have been developed to improve the diagnostic value of noninvasive echocardiography (13, 14, 16, 28, 41, 59). A number of basic science and clinical studies have confirmed the role of TVI and SRI for the early detection of DOX+TRZ-mediated cardiac dysfunction in the breast cancer setting (13, 14, 16, 30, 44, 51). However, little is known about whether these novel echocardiographic parameters can be applied in the early detection of BVZ- or SNT-mediated cardiotoxicity.

The objective of this study is to evaluate whether cardiac biomarkers and/or novel echocardiographic techniques can detect early manifestations of cardiac dysfunction before a reduction in LVEF is identified, in a murine model of BVZ- and SNT-mediated cardiac dysfunction, requires further study.

**METHODS**

**Experimental animal model.** All animal procedures were conducted in accordance with guidelines published by the Canadian Council on Animal Care. All procedures, including drug administration and longitudinal echocardiographic studies, were approved by the Animal Protocol Review Committee at the University of Manitoba. A total of 75 C57Bl/6 male mice (8–10 wk old; Jackson Laboratories) were randomized into one of three treatment groups, including the following: 1) 0.9% saline [intraperitoneal (IP), n = 51]; 2) BVZ [10 mg/kg, intravenously (IV) n = 35]; or 3) SNT [40 mg·kg⁻¹·day⁻¹, orally, n = 35] (9, 11, 21, 23). Each animal underwent baseline TTE before administration of the targeted biological agent. A single IP injection of saline or IV injection of BVZ (10 mg/kg; Hoffman-La Roche) was administered following baseline data acquisition. BVZ is a recombinant monoclonal antibody that targets VEGF-A with one additional murine complementary determining region, which provides cross-reactivity with murine receptors. SNT [40 mg·kg⁻¹·day⁻¹, Pfizer Canada] dissolved in saline was administered via daily oral gavage for a total of 14 days. As validated by Chu et al. (11) and Chen et al. (9), the BVZ and SNT dosages used in the our murine model produced blood concentrations comparable to those observed in the clinical settings of CRC and RCC. Serial TTE was performed daily for 14 days, at which time all surviving mice were euthanized (150 mg/kg pentobarbital buffered with 2% lidocaine IP), and the hearts were preserved for histological and biochemical analyses.

**Murine echocardiography.** Echocardiographic data were collected using a 13-MHz probe (Vivid 7, version 11.2, GE Medical Systems, Milwaukee, WI) with TVI capability. All 75 C57Bl/6 mice were awake and underwent TTE at baseline and were followed daily thereafter for 14 days, as previously described (2, 30, 40, 41, 55, 56, 60). To assess the viability of Vendo and radial SR, a total of 30 mice were randomly chosen from the various treatment groups. Both Vendo and radial SR measurements were performed independently by a single observer (D.S.J.), 2 wk apart, to determine intraobserver variability. Interobserver variability was determined from echocardiographic measurements that were processed separately by two independent observers (K.A.B. and D.S.J.).

**Hemodynamics.** Noninvasive measurements of heart rate (HR) and blood pressure (BP) were performed in all 75 conscious, restrained mice via a tail-cuff method (CODA system, High Throughput, Kent Scientific, Torrington, CT), as previously described (2, 17). Briefly, the holding platform was heated to 30°C, at which time five BP readings were recorded with 1-min rest intervals between readings. At baseline, day 7, and day 14, BP measurements were collected, from which average values for mean arterial pressure (MAP) were calculated.

**Cardiac biomarkers: high-sensitivity TnI.** Blood was collected via the internal jugular vein in all 75 mice at baseline, day 7, and day 10. Upon euthanasia at day 14, the heart was removed, and all blood remaining in the thoracic cavity was immediately collected through a pipette. Serum high-sensitivity TnI (hsTnI) was quantified using a mouse-specific enzyme-linked immunosorbent assays (Life Diagnostics, cat. no. 2010-1-HS), and the absorbance was read at 450 nm using a microplate reader (MRX Microplate Reader, Dynex Technologies ICXD-4588, Chantilly, VA).

**Histological analysis.** A total of 35 mice (n = 5 controls; n = 15 for BVZ; and n = 15 for SNT) were euthanized at day 8 and day 14 for electron microscopy (EM) studies. After the chest cavity was rapidly opened and the major blood vessels and connective tissue were removed, the heart was blotted dry and weighed, and the heart weight-to-body weight ratio was calculated. One-half of the LV was sectioned for histological analysis via EM, as previously described (30, 55, 60). Samples were dehydrated in ascending concentrations of ethanol and embedded in Epon 812 using standard techniques. Thin sections were stained with uranyl acetate and lead citrate, viewed, and
Fig. 1. Echocardiography. Left ventricular end-diastolic diameter [LVEDD (mm)] values in mice treated with saline, bevacizumab (BVZ), and sunitinib (SNT). LVEDD increased at day 13 in BVZ- and SNT-treated mice. Values are means ± SD. *P < 0.05 compared with baseline.

Fig. 2. Echocardiography. Left ventricular ejection fraction [LVEF (%)] of saline and BVZ- and SNT-treated mice, as determined by M-mode echocardiography. LVEF decreased at day 13 in BVZ- and SNT-treated mice. Values are means ± SD. *P < 0.05 compared with baseline.

Fig. 3. Echocardiography. Peak endocardial systolic velocity [Vendo (cm/s)] in mice treated with saline, BVZ, and SNT. Vendo decreased in BVZ and SNT mice 8 days posttreatment. Values are means ± SD. *P < 0.05 compared with baseline.

Oxolipidomics analysis was carried on a reverse-phase high-performance liquid chromatography using an Ascentis Express C18 column (15 cm × 2.1 mm, 2.7 μm; Supelco Analytical, Bellefonte, PA). Elution was performed using a linear gradient of solvent A (acetonitrile-water, 60:40 vol/vol) and solvent B (isopropanol-acetonitrile, 90:10 vol/vol). Both of the solvents contained 10 mM ammonium formate and 0.1% formic acid with a flow rate of mobile phase at 0.260 ml/min. The time program used was 0.01 min, 32% B; 1.50 min, 32% B; 4.00 min, 45% B; 5.00 min, 52% B; 8.00 min, 58% B; 11.00 min, 66% B; 14.00 min, 70% B; 18.00 min, 75% B; 21.00 min, 97% B; 25.00 min, 97% B; 25.10 min, 32% B; and 30.00 min, 32% B. The elution was stopped at 30.10 min. Autooxidized 16:0–18:2 phosphatidylcholine and 16:0–20:4 phosphatidylcholine were used to generate an assigned theoretical structure corresponding to the molecular weight. Oxidation products were separated from non-OxPC, allowing for correct determination of OxPC molecules in samples. Data were collected using Analyst 1.6 software (Applied Biosystems, Ontario, Canada) and quantified using MultiQuant 2.1 (AbSciex, Ontario, Canada).

Table 1. Morphological and echocardiographic data at baseline and day 14 in mice receiving either 0.9% saline, BVZ, or SNT treatment

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group</th>
<th>Baseline</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>Saline</td>
<td>24.4 ± 1.2</td>
<td>24.2 ± 1.4</td>
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<tr>
<td></td>
<td>BVZ</td>
<td>24.5 ± 0.9</td>
<td>24.7 ± 1.1</td>
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<tr>
<td></td>
<td>SNT</td>
<td>24.2 ± 1.4</td>
<td>24.4 ± 1.6</td>
</tr>
<tr>
<td>Heart weight/body weight, mg/g</td>
<td>Saline</td>
<td>5.3 ± 0.3</td>
<td>5.2 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>BVZ</td>
<td>5.4 ± 0.5</td>
<td>5.5 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>SNT</td>
<td>5.3 ± 0.4</td>
<td>5.4 ± 0.3</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>Saline</td>
<td>623 ± 9</td>
<td>630 ± 14</td>
</tr>
<tr>
<td></td>
<td>BVZ</td>
<td>615 ± 14</td>
<td>642 ± 14</td>
</tr>
<tr>
<td></td>
<td>SNT</td>
<td>615 ± 14</td>
<td>623 ± 11</td>
</tr>
<tr>
<td>PWT, mm</td>
<td>Saline</td>
<td>0.72 ± 0.02</td>
<td>0.71 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>BVZ</td>
<td>0.72 ± 0.02</td>
<td>0.73 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>SNT</td>
<td>0.73 ± 0.02</td>
<td>0.74 ± 0.02</td>
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<tr>
<td>LVEDD, mm</td>
<td>Saline</td>
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<tr>
<td></td>
<td>BVZ</td>
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<td>3.9 ± 0.2*</td>
</tr>
<tr>
<td></td>
<td>SNT</td>
<td>3.1 ± 0.2</td>
<td>3.9 ± 0.2*</td>
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<tr>
<td>LVEF, %</td>
<td>Saline</td>
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<td>74 ± 2</td>
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<td>BVZ</td>
<td>75 ± 3</td>
<td>48 ± 2*</td>
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<tr>
<td></td>
<td>SNT</td>
<td>74 ± 3</td>
<td>47 ± 3*</td>
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<tr>
<td>Vendo, cm/s</td>
<td>Saline</td>
<td>3.4 ± 0.2</td>
<td>3.1 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>BVZ</td>
<td>3.5 ± 0.3</td>
<td>1.3 ± 0.1*</td>
</tr>
<tr>
<td></td>
<td>SNT</td>
<td>3.4 ± 0.2</td>
<td>1.2 ± 0.2*</td>
</tr>
<tr>
<td>SR, s⁻¹</td>
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<td>22 ± 2</td>
</tr>
<tr>
<td></td>
<td>BVZ</td>
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<td>9 ± 1*</td>
</tr>
<tr>
<td></td>
<td>SNT</td>
<td>21 ± 2</td>
<td>10 ± 2*</td>
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</table>

Values are means ± SD. BVZ, bevacizumab; SNT, sunitinib; HR, heart rate; PWT, posterior wall thickness; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; Vendo, peak endocardial systolic velocity; SR, strain rate. *P < 0.05 compared with baseline.

photographed on a Philips CM12 electron microscope to determine the extent of cell degradation.

Oxolipidomic analysis. A total of 35 mice (n = 5 controls; n = 15 for BVZ; and n = 15 for SNT) were euthanized at day 8 and day 14 for oxidized phosphatidylcholine (OxPC) studies. OxPC, a marker of inflammation and oxidative stress (OS), was quantified using liquid chromatography and electrospray ionization tandem mass spectrometry (20). Briefly, lipid was extracted from LV myocardial tissue in congruence with the protocol adapted from Folch et al. (20) in the presence of 9:0–9:0 phosphatidylcholine as an internal standard.
Values are means with saline, BVZ, and SNT. SR decreases in BVZ and SNT mice after 8 days.

**RESULTS**

Conventional echocardiographic, TVI, and SR imaging parameters. HR, LV cavity dimensions, and systolic function were similar at baseline between all treatment groups. In mice receiving either BVZ or SNT, there were no overt visible behavioral changes nor weight loss during the 14-day study. Heart weight-to-body weight ratio, HR, and posterior wall thickness remained within normal limits throughout the duration of the 14-day study for all treatment groups (Table 1). In both BVZ- and SNT-treated mice, conventional echocardiographic indexes showed a significant increase in LVEDD and a decrease in LVEF beginning at day 13 (Figs. 1 and 2). At day 13, in the BVZ treatment group, LVEDD initially increased from 3.1 ± 0.2 mm at baseline to 3.9 ± 0.2 mm at day 14. Similarly, in the SNT treatment group, LVEDD increased from 3.1 ± 0.2 mm at baseline to 3.9 ± 0.3 mm at day 14 (Fig. 1, Table 1). Both BVZ and SNT treatment groups demonstrated a decrease in conventional LVEF by day 13 of the study (Fig. 2).

Both BVZ and SNT treatment groups demonstrated normal $V_{endo}$ and SR values at baseline. TVI parameters, including $V_{endo}$ and SR, decreased at day 8 in both BVZ and SNT treatment groups (Figs. 3 and 4). In mice treated with BVZ, $V_{endo}$ decreased from 3.5 ± 0.3 cm/s at baseline to 2.4 ± 0.1 cm/s as early as day 8 and continued to decrease to 1.3 ± 0.1 cm/s at day 14. Radial SR decreased from 21 ± 1 s⁻¹ at baseline to 14 ± 2 s⁻¹ at day 8 and continued to decrease to 9 ± 1 s⁻¹ at day 14 (Figs. 3 and 4). Similarly, in SNT-treated mice, $V_{endo}$ decreased from 3.4 ± 0.2 cm/s at baseline to 2.5 ± 0.2 cm/s at day 8 and continued to decrease to a final value of 1.2 ± 0.2 cm/s at day 14. In addition, radial SR decreased from 21 ± 2 s⁻¹ at baseline to 15 ± 2 s⁻¹ at day 8 and continued to decrease to 10 ± 2 s⁻¹ at day 14 (Figs. 3 and 4).

The intra- and interobserver agreement rates for $V_{endo}$ were 0.1 ± 0.05 and 0.2 ± 0.05 cm/s, respectively. The intra- and interobserver agreement rates for SR were 0.8 ± 0.4 and 0.9 ± 0.3 s⁻¹, respectively.

**Hemodynamics.** Baseline measurements of MAP were within normal limits for all treatment groups. At days 7 and 14, the MAP of saline-treated mice remained unchanged, compared with baseline. Mice treated with BVZ demonstrated a decrease in MAP beginning at day 3 and continued to decrease to a final value of 100 ± 20 mmHg at day 14 (Fig. 5).

**Protein analysis: apoptotic markers.** A total of 65 mice (n = 5 controls; n = 20 for DOX; n = 20 for BVZ; and n = 20 for SNT) were used for Western analysis of apoptotic markers at day 14. Mice treated with a single IP injection of DOX (20 mg/kg) were used as a positive control for increased cardiac apoptosis, as previously described (30, 60). Frozen heart tissue was ground in liquid nitrogen, and proteins were extracted in the radioimmunoprecipitation buffer containing protease and phosphatase inhibitors (Thermo Scientific). A total of 30 μg of protein were loaded and electrophoresed in 12% sodium dodecyl sulfate polyacrylamide gels and transferred to a polyvinylidene fluoride membrane (Roche Diagnostics) (30, 38, 39, 55, 60). Membranes were incubated with the primary antibody to caspase-3, Bax, and poly(ADP-ribose) polymerase (PARP) (Cell Signaling Technology) overnight at 4°C. Anti-rabbit (Cell Signaling Technology) secondary antibody was used to detect the primary antibody and was accomplished using the ECL Plus detection reagent (Western Lightning Plus-ECL, Amersham). For the loading control, a polyclonal antibody to GAPDH (Sigma) was used. Band intensities were quantified using image analysis software (Quantity One; BioRad Laboratories) (30, 38, 39, 55, 60).

**Statistical analysis.** All data are expressed as means ± SD. Statistical significance between echocardiographic measurements was determined using a 1 (genotype) × 2 (time) mixed factorial design with repeated measures on the time factor. For post hoc analysis, repeated-measures ANOVA was used to evaluate for significance between independent factors. In post hoc between-group analysis, Levene’s test was used to check for homogeneity of group variances. P values for main effects and interactions were also recorded where appropriate. Histological analyses involved nonparametric comparison of scores, ranging from 1 to 4, which was calculated using the Kruskall-Wallis test, with 4 representing severe damage. A P value of <0.05 was considered significant. For biochemical and western analyses, a Student t-test was performed. A P value of <0.05 was considered significant. The statistical analysis package SPSS 15.0 and Graphpad Prism 5 were used to perform the analysis.
significant increase in MAP from 90 ± 2 mmHg at baseline, to 120 ± 5 at day 7, to 141 ± 5 mmHg at day 14 (P < 0.05) (Fig. 5). Similarly, mice treated with SNT demonstrated a significant increase in MAP from 89 ± 3 mmHg at baseline, to 115 ± 6 mmHg at day 7, to 135 ± 4 mmHg at day 14 (P < 0.05) (Fig. 5).

Cardiac biomarkers: hsTnI. All mice demonstrated non-detectable hsTnI values at baseline, day 7, and day 10. In animals treated with either BVZ or SNT, the hsTnI levels increased to 1.8 ± 0.3 and 2.3 ± 0.4 ng/ml (P < 0.05), respectively, at day 14 (Table 2).

EM. Approximately 15,000 cells were scanned from three randomly derived blocks of myocardial tissue and evaluated for dilation of the sarcoplasmic reticulum and loss of cell integrity. At day 8, there was no evidence of cellular injury in mice treated with BVZ or SNT compared with controls. However, at day 14, BVZ- and SNT-treated animals demonstrated an increased loss of cellular integrity and myofibril disarray (Fig. 6). There was no statistical difference, however, between the BVZ and SNT treatment groups at day 14.

OS and apoptotic markers. A total of 82 distinct OxPC studies were evaluated in each experimental group at days 8 and 14. There was no discernable change in OxPC in mice treated with either BVZ or SNT at day 8 compared with controls. At day 14, however, the heat map generated for the most abundant OxPC molecular demonstrated a 10-fold increase in OxPC molecules in mice treated with either BVZ or SNT compared with controls (Fig. 7). Western blot analysis demonstrated evidence of apoptotic cell death and caspase-3 cleavage among BVZ- and SNT-treated animals at day 14 (P < 0.05) (Fig. 8). Mice treated with either BVZ or SNT demonstrated 2.5- and 2-fold increases in caspase-3 levels, respectively (P < 0.05). Compared with saline-treated animals, there was no significant evidence of an increase in either Bax or PARP expression at day 14 in mice treated with BVZ or SNT (data not shown).

DISCUSSION

An increased understanding of the biology of cancer has allowed for the development of new effective therapies that utilize various pathways to suppress tumor growth, including the use of the targeted agents BVZ and SNT. Although effective against CRC and RCC in reducing overall morbidity and mortality, these novel anticancer drugs are associated with an increased risk of developing cardiotoxicity (6, 11, 18, 22, 24, 25, 43, 45, 50, 53). The aim of the present study was to determine the utility of cardiac biomarkers and TVI parameters for the early detection of BVZ- and SNT-mediated cardiotoxicity, potentially avoiding the development of advanced heart failure. In a murine model of BVZ- and SNT-mediated cardiac dysfunction, we demonstrated the following: 1) development of systemic hypertension; 2) serial hsTnI was unable to detect early myocardial dysfunction; 3) TVI and SRI parameters were able to detect LV systolic dysfunction 5 days earlier than traditional LVEF parameters; and 4) evidence of loss of cellular integrity with increased OS and apoptosis.

BVZ- and SNT-mediated hypertension. The use of novel antiangiogenic drugs, including BVZ and SNT, result in the development of systemic hypertension as outlined in several basic science and clinical studies (10, 12, 18, 31, 43, 45, 50, 53). Both agents inhibit the VEGF pathway, which causes downregulation of endothelial nitric oxide synthase expression and decreased synthesis of endothelial nitric oxide. This results in systemic vasculature constriction and development of hypertension (27, 31, 48). In an acute murine model using Swiss-Webster male mice, Chu et al. (11) observed no change in BP over a 12-day period among animals administered SNT. Conversely, Kappers et al. (31) observed an increase in MAP by day 6 in male Wistar-Kyoto rats treated with SNT, and Curwen et al. (12) observed an increased in diastolic BP at day 4 in male Wistar rats treated with Cediranib, a VEGF inhibitor. Similarly, in our present study, C57Bl/6 male mice treated with either BVZ or SNT developed an increase in MAP as early as day 7, which continued to increase at day 14 by nearly 50% compared with baseline. Although increased LV wall thickness is often associated with increased afterload, our study demons-
Stratified no change in LV wall dimensions throughout the study. This may be due to the acute nature of our 14-day model, as hypertrophy is not typically observed until 4–6 wk following the onset of hypertension (2). Additionally, as there was no overall change in heart weight-to-body weight ratio in mice treated with either BVZ or SNT, it is plausible that these two anticancer drugs may interfere with the adaptive cardiac hypertrophy that occurs in the setting of increased afterload.

Fig. 7. Oxidized phospholipids. A: heat map graphic demonstrating changes of each oxidized phosphatidylcholine (OxPC) species in animals treated with saline (n = 5), BVZ (n = 10), or SNT (n = 10) at day 14. B: total mass of OxPC per milligram of heart tissue extracted from male mice treated with either BVZ or SNT, compared with controls at day 14. Values are means ± SD. *P < 0.05 comparing BVZ with control. **P < 0.05 comparing SNT with control.
mediated cardiotoxicity in the clinical setting. Further investigations are warranted to evaluate the role of intra- and interobserver variability that often accompanies monitoring of cancer patients receiving anticancer therapy as the cardiac biomarker was undetectable at an early predictive marker of cardiotoxicity was not validated, either BVZ or SNT at confirmed evidence of myocardial necrosis in mice receiving and SNT-mediated cardiotoxicity. Although we have confirmed evidence of myocardial necrosis in mice receiving either BVZ or SNT at day 14, the ability of hsTnI to serve as an early predictive marker of cardiotoxicity was not validated, as the cardiac biomarker was undetectable at days 7 and 10. Further investigations are warranted to evaluate the role of cardiac biomarkers for the early detection of BVZ- and SNT-mediated cardiotoxicity in the clinical setting.

Early detection of cardiotoxicity using TVI. Traditional monitoring of cancer patients receiving anticancer therapy involves the measurement of LVEF through the use of serial multigated acquisition scans or TTE throughout the treatment cycle (3, 13–16, 44, 51, 54). In addition to the high degree of intra- and interobserver variability that often accompanies conventional echocardiographic analyses (13), LVEF measure-ment is a relatively insensitive parameter for the early detection of drug-mediated cardiotoxicity (4). Once LVEF falls below 40% in a cancer patient, irreversible cardiac injury may have already occurred, thereby precluding any chance of prevention (4). The novel use of TVI has recently been established to supplement conventional echocardiography in the evaluation of myocardial dysfunction in the cardio-oncology setting (13–16, 28, 44, 51, 59). TVI-derived parameters are proven to be less influenced by hemodynamic variables and provide more precise and reproducible analysis of both systolic and diastolic function (13–16, 28, 44, 51, 59). In an animal-based model of DOX-mediated cardiac dysfunction, Neilan et al. (41) demonstrated that early changes in TVI-derived parameters were predictive of the late development of cardiac dysfunction and increased mortality. In an acute murine model of DOX+TRZ-mediated cardiac dysfunction, our laboratory previously demonstrated that, although LVEF decreased at day 5, TVI was significantly decreased in both Vends and radial SR as early as 24 h following treatment (30). However, the role of TVI for the early detection of BVZ- and SNT-induced cardiotoxicity in an animal model has not been previously explored. In the present study, mice treated with BVZ or SNT demonstrated an increase in LV cavity dimensions and a decrease in conventional echo parameters, including LVEF, at day 13. In contrast, Vends and radial SR decreased 5 days earlier in mice receiving either BVZ or SNT, confirming that TVI is a sensitive and reproducible measure of early cardiac dysfunction. We hypothesize as BVZ and SNT both interrupt the VEGF pathway (9, 11, 19, 21, 23, 43, 61); this common feature may explain why there was a similar decline in cardiac function at the same time point. Considering the lower variability of these novel imaging techniques, TVI may be a more feasible imaging modality for the early detection of subclinical LV systolic dysfunction. However, these results require validation in the clinical setting of CRC and RCC patients treated with BVZ and SNT, respectively.

Mechanisms of BVZ- and SNT-mediated cardiotoxicity. The precise underlying mechanisms of BVZ- and SNT-mediated cardiotoxicity have yet to be fully elucidated. In an animal model of age-matched, wild-type Swiss-Webster mice treated with 40 mg·kg⁻¹·day⁻¹ of SNT, Chu et al. (11) demonstrated mitochondrial swelling and degenerative changes in cardiomyocytes using transmission EM at day 12. Our EM findings corroborate this previous study, as changes in cellular integrity do not occur early at day 8, but rather later at day 14. Additionally, in a chronic 6-mo model involving mice treated with BVZ 10 mg/kg IV every 2 wk, there was evidence of increased cardiac fibrosis as measured by hydroxyproline content, compared with control EndoCD/5-FC treatment groups (9). Our study validates the significant loss and disruption of cardiac myofibrils in BVZ- and SNT-treated animals. These findings may be indicative of cellular damage caused by impaired ATP generation due to mitochondrial dysfunction. An important finding from our present study is the absence of cardiac fibrosis, suggesting that the dysfunction observed may be reversible.

Although BVZ inhibits VEGF-A alone and SNT inhibits multiple receptors, including VEGFR 1–3, platelet-derived growth factor receptors-α and -β, and AMPK (10, 18, 21, 26), it is plausible that the cardiotoxic side effects of these two different anticancer agents may result in the activation of
common downstream apoptotic and OS pathways. Although a few studies have previously evaluated the relationship between SNT-mediated cardiotoxicity and increased apoptosis (11, 27), there is a paucity of data on BVZ-induced cardiac dysfunction and this pathway. Apoptosis occurs through inhibition of AMPK signaling (primarily affected by SNT), which cause the JNK and p38 pathways to be activated, resulting in the enhanced expression of several proapoptotic genes, such as Bax, caspases, and poly ADP ribose polymerase (PARP) (30, 38, 39, 55, 60). In an animal model of SNT-induced cardiomyopathy, Chu et al. (11) demonstrated the direct targeting of myocardial mitochondria, resulting in cytochrome c release and caspase-9 activation. Corroborating these findings, although there was no increase in Bax and PARP expression in our study, there was a significant increase in caspase-3 protein and OxPC levels among BVZ- and SNT-treated animals. It is plausible to propose that the apoptotic events induced by BVZ and SNT are not solely mediated through the Bax/Bcl-xL pathway. In support of this hypothesis, Hasinoff et al. (27) demonstrated that, in ventricular myocytes treated with SNT, Bax levels were not significantly changed, indicative of the inactivity of this pathway in the induction of tyrosine kinase inhibitor-induced apoptosis. However, this group also demonstrated that levels of caspase-3 and caspase-7 rapidly increased following SNT treatment, indicative of the major contribution of caspase in the development of cardiotoxicity (27). In the future, additional basic science and clinical trials are needed to thoroughly elucidate the underlying mechanisms involved in the myocardial dysregulation, OS, and apoptosis observed in this unique population of BVZ- and SNT-mediated heart failure.

Limitations. There are a few limitations to our study. The present study characterized drug-induced cardiac dysfunction in an acute murine model of BVZ- and SNT-mediated heart failure. As these drugs are administered over a period of several months in patients with metastatic CRC or RCC, it would be useful to design a chronic murine model of BVZ- and SNT-mediated cardiac dysfunction, to more closely mimic the clinical setting. Second, we exclusively evaluated changes in cardiac hsTnI. In future studies, the evaluation of various cardiac biomarkers, including cardiac hsTnT and NT pro-brain natriuretic peptide should be investigated for their predictive role of detecting early cardiac dysfunction. Finally, there may be significant sex-based differences in the cardiotoxic side effects of BVZ and SNT. As the present study focused on C57Bl/6 male mice, future studies evaluating BVZ- and SNT-mediated cardiotoxicity in female mice are warranted.

Conclusion. This novel study demonstrated that TVI can detect early LV systolic dysfunction before alterations in conventional echocardiographic parameters in an acute murine model of BVZ- and SNT-induced cardiomyopathy. Future clinical studies are required to investigate the potential use of cardiac biomarkers and TVI parameters for the early detection of subclinical alterations in cardiac function among CRC and RCC patients treated with either BVZ or SNT, potentially avoiding the development of advanced heart failure.

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AUTHOR CONTRIBUTIONS

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