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TITLE PAGE

Pharmacology of ranolazine versus common cardiovascular drugs in patients with early diastolic dysfunction induced by anthracyclines or nonanthracycline chemotherapeutics: A phase 2b minitrial

Giorgio Minotti, Pierantonio Menna, Vito Calabrese, Carlo Greco, Grazia Armento, Ombretta Annibali, Francesco Marchesi, Emanuela Salvatorelli, and Giorgio Reggiardo

Clinical Pharmacology Unit (G.M., P.M.) and Cardio Center (V.C.), Campus Bio-Medico University Hospital, Rome; Units of Drug Sciences (G.M., E.S.), Radiation Oncology (C.G.), Oncology (G.A.), Hematology (O.A.), Department of Medicine and Center for Integrated Research, University Campus Bio-Medico, Rome; Hematology and Stem Cell Transplant Unit, IRCCS Regina Elena National Cancer Institute, Rome (F.M.); Mediservice S.r.l., Agrate Brianza (Monza) (G.R.); Italy.

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CORRESPONDING AUTHOR ADDRESS:

Giorgio Minotti
Department of Medicine
University Campus Bio-Medico
Via Alvaro del Portillo, 21
00128 Rome - ITALY

TELEPHONE: 011-39-06-225419109
FAX: 011-39-06-22541456
E-MAIL: g.minotti@unicampus.it

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NONSTANDARD ABBREVIATIONS: ACEI, angiotensin converting enzyme inhibitors; AE, adverse events; ARB, angiotensin receptor blockers; BMI, body mass index; BNP, B-type natriuretic peptide (Nt-proBNP, aminoterminal fragment of B-type natriuretic peptide pro-hormone); BST, best standard therapy; cTnI, cardiac troponin isoform I; DBP, diastolic blood pressure; DT, mean deceleration time of early filling velocity; E/A, mean ratio of peak early filling (E wave) to late diastolic filling (A wave); Hb, hemoglobin; HF, heart failure; HR, heart rate; late I_{Na}, late inward sodium current; LVEF, left ventricular ejection fraction; QTc, heart-corrected QT interval; SBP, systolic blood pressure; ULN, upper limit of normal.

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ABSTRACT

We have reported that anthracyclines and nonanthracycline chemotherapeutics caused diastolic dysfunction in cancer patients without cardiovascular risk factors. Diastolic dysfunction occurred as early as one week after the last chemotherapy cycle and manifested as impaired myocardial relaxation at echocardiography or persistent elevations of B-type natriuretic peptide (BNP) or troponin. The antianginal drug, ranolazine, shows cardiac relaxant effects that we considered of value to treat early diastolic dysfunction induced by cancer drugs; therefore, 24 low risk patients with post-chemotherapy diastolic dysfunction were randomized (1:1) to ranolazine or investigator's choice of common cardiovascular drugs, like β blockers and/or angiotensin converting enzyme inhibitors or loop diuretics (best standard therapy, BST). After 5-weeks 12 of 12 patients on ranolazine recovered from diastolic dysfunction, while 3 of 12 patients on BST failed to improve; however, not serious adverse events were apparently more frequent for ranolazine than BST (4/12 versus 1/12). Ranolazine did not lower blood pressure while BST reduced systolic pressure and caused a trend toward a reduced diastolic pressure. The majority of patients presented at randomization with tachycardia due to chemotherapy-related anemia. Hemoglobin recovery contributed to normalizing heart rate in these patients; however, some patients in ranolazine arm developed tachycardia through chronotropic effects of high BNP levels and returned to a normal heart rate through the effects of ranolazine on decreasing BNP levels. This minitrial describes potential effects of ranolazine on relieving chemotherapy-related diastolic dysfunction; however, clinical implications of these findings need to be characterized by studies with an adequate sample size.

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SIGNIFICANCE STATEMENT

The antianginal drug, ranolazine, causes cardiac relaxant effects that might relieve diastolic dysfunction. We report on a clinical pharmacology study in which 24 patients were randomized (1:1) to receive ranolazine or common cardiovascular drugs for treatment of early diastolic dysfunction induced by anthracycline-based or nonanthracycline chemotherapy. Ranolazine showed effects on relieving diastolic dysfunction in these patients. The safety profile of ranolazine in cancer patients was similar to that characterized for the general population. Compared to common cardiovascular drugs, ranolazine relieved diastolic dysfunction without lowering blood pressure. The sample size of this study was nonetheless too small to permit considerations about the potential clinical value of ranolazine for oncologic patients with early diastolic dysfunction induced by anthracyclines or nonanthracycline chemotherapeutics. This information should be obtained by studies with an adequate sample size.

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INTRODUCTION

Many cancer drugs can cause cardiotoxicity. Doxorubicin and other anthracyclines inflict a multifactorial damage to cardiomyocytes, while nonanthracycline chemotherapeutics (alkylators, antimetabolites, tubuline-active agents) act primarily, but not exclusively, by inducing microvascular dysfunction (Menna et al., 2008). Dose reductions have limited the incidence of cardiac events during the course of chemotherapy; however, heart failure (HF) and/or ischemic disease may occur long after cancer patients were treated by chemotherapy (Armenian et al., 2017, Carver et al., 2007).

In patients treated by anthracycline-based or nonanthracycline chemotherapies, mild diastolic dysfunction (grade 1, impaired relaxation) was detected at 4 or 12 months follow-up, often before systolic dysfunction occurred (Altena et al, 2009; Klein et al., 2019; Serrano et al., 2015). In long term cancer survivors, cardiac events such as HF or myocardial infarction were preceded or accompanied by worsening of diastolic dysfunction (grade 2-3, pseudonormal-restrictive patterns) (Armstrong GT et al., 2015). These notions describe cardiotoxicity as a continuum that begins with mild diastolic dysfunction and then progresses toward more serious events.

Cause-and-effect relations between cancer drugs and diastolic dysfunction should nonetheless be disentangled from risk factors that predispose to diastolic dysfunction (e.g., hypertension, diabetes, overweight) (Borlaug et al., 2011). Recently we conducted a pilot study of the incidence of diastolic dysfunction in comorbid-free cancer patients treated by anthracycline-based or nonanthracycline chemotherapy. We observed that asymptomatic diastolic dysfunction occurred in 36% of such low risk patients and could be detected at one week after the last chemotherapy cycle. Of note, all patients showed a normal systolic function, as evidenced by a preserved left ventricular ejection fraction (LVEF) (Calabrese et al., 2018). These findings highlighted diastolic dysfunction as a very early manifestation of cardiotoxicity, regardless of the influence of competing risk factors.

Diastolic dysfunction was detected as impaired relaxation at echocardiography but occurred also in the form of high circulating levels of B-type natriuretic peptide (BNP) (Calabrese et al., 2018). We in fact demonstrated that high BNP levels caused cardiac relaxant effects (“positive lusitropic

effects") that compensated for impaired relaxation before this could be detected by echocardiography. High BNP levels and impaired relaxation were therefore characterized as mutually exclusive manifestations of diastolic dysfunction (Menna, Calabrese et al., 2018). BNP lusitropy develops through the activation of receptor-coupled guanylyl cyclase, followed by cGMP formation and beneficial effects of protein kinase G on reducing myofilament tension in diastole (Bishu et al., 2011).

Diastolic dysfunction also occurred in the form of increased circulating levels of troponin, a marker of cardiomyocyte necrosis. We suggested that impaired relaxation, by causing energy dissipation in diastole, could synergize with an otherwise reversible damage induced by anthracyclines, eventually causing some cardiomyocytes to die. Accordingly, BNP mitigation of impaired relaxation was shown to diminish cardiac troponin release (Menna, Salvatorelli et al., 2018).

Common cardiovascular drugs, like β blockers or inhibitors of renin-angiotensin system, cannot be considered as specific curative agents of diastolic dysfunction (Paulus et al., 2010). On the other hand, the possible cause-and-effect relations between early diastolic dysfunction and late cardiac sequelae of cancer treatment call for studies that characterize drugs of potential value to treat asymptomatic diastolic dysfunction before it progresses toward more serious cardiac events. The orally available piperazine, ranolazine, might be considered as a valuable option in these settings.

Ranolazine was approved by the US Food and Drug Administration as a first-line or top-on-therapy agent to treat chronic angina. In Europe ranolazine was approved for the treatment of chronic angina in patients who are inadequately controlled by, or intolerant to other antianginal drugs. Unlike most common cardiovascular drugs, ranolazine lacks significant hemodynamic effects. Ranolazine acts through lusitropic effects that reduce myocardial interstitial pressure and eventually improve coronary conductance (Stone, 2008). Such effects depend on ranolazine inhibiting the late inward sodium current (late I_{Na}). If persistently activated, as is in the repolarizing ischemic myocardium, late I_{Na} causes inward Na^+ fluxes that force the Na^+-Ca^{2+} exchanger to work in a reverse manner to extrude Na^+. Calcium entry then occurs, activating myofilaments and causing diastolic wall tension (Stone, 2008). Ranolazine inhibition of late I_{Na} therefore relieves diastolic tension.

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We considered that also cancer drugs could activate late I_{Na} through e.g., metabolic hypoxia due to continued oxyradical formation by anthracyclines or subclinical ischemia due to microvascular dysfunction induced by nonanthracycline chemotherapeutics (Minotti et al., 2013). This offered a rationale to probe ranolazine in patients with an early diastolic dysfunction induced by cancer drugs.

Here we present the results of INTERACT, a phase 2b study that compared single-agent ranolazine to common cardiovascular drugs to treat early diastolic dysfunction induced by cancer drugs.

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EXPERIMENTAL PROCEDURES

Study design

INTERACT (ranolazINe to Treat EaRly cArdiotoxiCity induced by antiTumor drugs) was an open label, multicentre, real life phase 2b study. It recruited patients from our previous pilot, prospective, multicenter real life study in which the incidence of diastolic dysfunction at one week after the end of chemotherapy was evaluated. The pilot study population consisted of 80 evaluable comorbid-free patients, aged 18 to 70 years, and exposed to anthracycline-based adjuvant treatment of breast cancer, anthracycline-based frontline treatment of non-Hodgkin lymphoma, or fluoropyrimidine/platinum-based adjuvant treatment of colorectal cancer. Prior to chemotherapy (T0), all patients showed a normal diastolic function, an LVEF >50%, BNP and troponin levels below the upper limit of normal (ULN). One week after the last chemotherapy cycle (T1), all patients showed an LVEF >50%, but 29 patients showed diastolic dysfunction as defined by echocardiographic or biomarkers abnormalities (Calabrese et al., 2018). Twenty-four of such 29 patients were available for recruitment in INTERACT and were randomized (1:1) to ranolazine or investigator's choice of common cardiovascular drugs (best standard therapy, BST). Randomization was operated centrally by SAS® software. Efficacy, safety and cardiovascular pharmacology endpoints were evaluated 5 weeks after randomization (T5) (**Figure 1**).

The pilot study and INTERACT were designed to elucidate only the incidence and pharmacologic treatability of diastolic dysfunction induced by frontline chemotherapy. Patients candidate for sequential treatment with other potentially cardiotoxic drugs, like the anti epidermal growth factor receptor 2 antibody, trastuzumab, were excluded from the pilot study. Likewise, no patient received mediastinal irradiation or began left thorax irradiation during the 5-weeks treatment with ranolazine or BST.

The pilot study and INTERACT conformed with the principles outlined in the Declaration of Helsinki and were approved by the Institutional Review Board of each participating center. Written informed consent was obtained from all patients.

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Patient characteristics

Patients recruited in INTERACT ("cases") showed the same demographic, oncologic and cardiovascular characteristics as those of patients without diastolic dysfunction at the end of the pilot study ("controls"). Cases and controls were balanced for gender and distribution in age groups (<40, 40-60, >60 years) for which ranges of normality of diastolic function at echocardiography were characterized (Nagueh et al., 2009); moreover, the two groups were balanced for oncologic disease, chemotherapy type, cumulative exposure to anthracyclines (**Table 1**). Cases and controls were balanced for systolic blood pressure (SBP) and diastolic blood pressure (DBP), which was normal at T0 and T1; moreover, the two groups were balanced for body mass index (BMI), which averaged well below the value of 29.6 kg/m^2 that associated with diastolic dysfunction at 9-12 months after anthracycline-based chemotherapy (Serrano et al., 2015). Cases and controls were balanced for heart rate (HR) at T0 and T1; at the latter time point, however, both groups presented with a significant increased HR (**Table 2**). Cases and controls were balanced for LVEF at T0 but not at T1, cases showing a marginal but significant lower LVEF as compared to controls at T1. Both cases and controls nonetheless showed an LVEF that was ≥10 percentage points higher than the predefined cut off of preserved LVEF (≥50 percentage points); moreover, longitudinal analyses showed that cases did not develop a net decrement of LVEF from T0 to T1 (see also Table 2). Thus, cases were fully representative of the source population of the pilot study and developed diastolic dysfunction with a preserved LVEF as per protocol definition.

Study procedures

a) Echocardiography

Impaired relaxation was detected by abnormalities of transmitral flow indices at 2D echocardiography and was defined as borderline or low-for-age ratio of peak early filling (E wave) to late diastolic filling (A-wave) (E/A ratio), and concomitant borderline or long-for-age deceleration time of early filling velocity (DT). The following age-adjusted ranges of normality were adopted: i) 0.73-2.33 (20-40 years), 0.78-1.78 (40-60 years), 0.6-1.32 (>60 years) for E/A ratio and ii), 138-194 msec (20-40 years), 143-219 msec (40-60 years), 142-258 msec (>60 years) for DT (Nagueh et al., 2009).

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Absolute E/A and DT values were then expressed as percentages of normal to permit comparisons between patients of different age. This was done by the formula

E/A (or DT) = 100 x [(absolute value-lower limit of range)]/range.

LVEF was calculated by the modified biplane Simpson's rule (Calabrese et al., 2018).

b) Biomarkers

BNP was measured by the circulating levels of the long lived aminoterminal fragment of its prohormone (Nt-proBNP). Troponin was measured by the circulating levels of its cardiac-specific isoform I (cTnI). Both Nt-proBNP and cTnI were measured in heparin-litium plasma by Siemens immuno-chemiluminescence assays for Dimension Vista® System (luminescent oxygen channeling immunoassay technology, LOCI®). All assays were centralized at the Clinical Pharmacology Unit of University Campus Bio-Medico of Rome. For patients 18-70 years of age, BNP ULN was set at 125 pg/ml (Siemens Dimension Vista®, technical note, 2012). For cTnI, the manufacturer recommended ULN was rounded from 0.045 to 0.05 ng/ml (Calabrese et al., 2018). Further details have been given elsewhere (Menna, Calabrese et al., 2018; Menna, Salvatorelli et al., 2018).

c) Study treatments

Given that the optimal dosage of ranolazine to treat diastolic dysfunction from cancer drugs was unknown, ranolazine was titrated from 375 to 750 mg bid as recommended by the European Medicines Agency for the treatment of chronic angina (European Medicines Agency, 2016). Ranolazine was started at the dose of 375 mg bid for two weeks, followed by up-titration to 500 mg bid for ten days and 750 mg bid for ten more days. For patients randomized to BST, angiotensin converting enzyme inhibitors (ACEI) and β blockers were the main investigator's choices. BST dosage was left at the investigator's discretion (**Table 3**). Dose reductions were allowed anytime for patients with signs or symptoms of intolerance to ranolazine or BST.

Study endpoints

a) Efficacy

Ranolazine or BST efficacy was defined by the number of patients showing normalization or improvement of impaired relaxation or biomarker elevations. This was a descriptive endpoint;

superiority analysis was not in the scope of this study. Normalization denoted that echocardiographic indices of relaxation or biomarkers returned into the ranges of normality; improvement denoted that deviations from the range of normality decreased to an extent that the investigators deemed as clinically significant. Treatment failure was defined by the lack of any improvement or by abnormalities that were not detected at T1 but occurred at T5.

b) Safety

Safety was defined by the number of patients showing treatment emergent adverse events (AE). Event severity was graded according to Common Terminology Criteria for Adverse Events, version 5.0 (U.S. Department of Health and Human Services, 2017).

b) Cardiovascular pharmacology endpoints

These endpoints were defined as any effect that ranolazine or BST caused on cardiovascular parameters other than myocardial relaxation or biomarkers.

Other conditions

Heart rate (HR) was determined by 12-lead ECG. Where indicated, changes of HR from T0 to T1, and from T1 to T5, were normalized to concomitant changes of hemoglobin (Hb). Δbpm/ΔHb ratios were then compared to a range of normality, defined as the interquartile range for Δbpm/ΔHb ratios of all 24 cases. Rate-corrected QT interval (QTc) was calculated by the Bazett formula (Yap and Camm, 2004). Doxorubicin myelotoxic equivalents were calculated by assuming an epirubicin:doxorubicin conversion factor of 0.66 (Ewer, 2013). Data were expressed as medians with interquartile ranges or absolute number and percentages. Data from a same arm were compared by two-tailed Wilcoxon Signed Rank test or one-way repeated measures analysis of variance with Bonferroni's post hoc test, as appropriate. Data from different arms were compared by two-tailed Mann-Whitney test or one-way Kruskall-Wallis analysis of variance with Dunn's post hoc test, as appropriate. Categorical variables were analyzed by χ^2 test or Fisher's Exact test. Differences were considered as significant when the P value was <0.05.

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RESULTS

Efficacy endpoint

INTERACT recruited and randomized 24 patients showing a total 26 protocol-defined indices of diastolic dysfunction (impaired relaxation, n=11; BNP>ULN n=12; cTnI>ULN n=3). No patient presented with impaired relaxation and a concomitant BNP >ULN (**Table 4**). Impaired relaxation and biomarker levels were normalized or improved in all patients randomized to ranolazine. In BST arm, 2 patients with impaired relaxation failed to improve, while a third patient showed normalization of impaired relaxation but developed BNP levels >ULN at T5. Thus, there were 3 treatment failures in BST arm versus none in ranolazine arm (see also Table 4). Treatment failures occurred in patients randomized to β blocker and/or ACEI. Patterns of treatment efficacy or failure are shown in **Figure 2**.

Safety endpoint

Adverse events of mild to severe intensity occurred in 4 of 12 patients randomized to ranolazine and in 1 of 12 patients randomized to BST (**Table 5**). All AEs were judged as not serious, i.e., they did not result in any of the life-threatening or disabling outcomes that US Food and Drug Administration identified for defining the seriousness of AEs (U.S. Department of Health and Human Services, 2012). All AEs were judged as certainly or probably related to study treatment. For one patient with ranolazine-related constipation and tinnitus, uptitration from 375 to 500 mg of ranolazine bid was stopped and the patient was maintained on 375 mg bid until evaluation at T5. In BST arm, one patient complained about asthenia induced by the ACEI, ramipril, which was managed by reducing ramipril from 2.5 to 1.25 mg/day. Dose reductions did not interfere with normalization or improvement of diastolic dysfunction by ranolazine or BST (see also Table 5).

Cardiovascular pharmacology endpoints

Ranolazine or BST did not cause effects on LVEF or BMI, which remained unchanged both between and within arms. Previous studies showed that ranolazine caused a modest prolongation of QTc, probably reflecting a moderate inhibition of delayed rectifier K^+ current (Antzelevitch et al., 2004). Here, 5-weels ranolazine did not prolong QTc in cancer patients. Moreover, ranolazine and

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BST arms were balanced for QTc at T1 and T5, and no patient presented at T5 with QTc values higher than gender-related pathologic thresholds (450 msec for males, 470 msec for females) (**Table 6**).

BST, but not ranolazine, caused a significant reduced SBP, which was also borderline lower as compared to ranolazine arm; moreover, BST caused an insignificant decrease of DBP (P=0.160) (see also Table 6). Individual changes of blood pressure, measured as Δ mm Hg from T1 to T5, were characterized. Cases in BST arm showed a significant trend toward a lowered SBP as compared to cases in ranolazine arm. This was not observed when all cases in BST and ranolazine arms were compared for DBP; however, a significant difference occurred when only cases with Δ mm Hg <0 were considered, the patients in BST arm showing greater decrements of DBP (**Figure 3**). Collectively, data in Table 6 and Figure 3 showed that BST lowered blood pressure while ranolazine lacked significant effects.

BST and ranolazine arms were balanced for HR at T0, T1 and T5. At the latter time point all cases showed essentially the same HR they showed at T0. Patients in ranolazine arm were nonetheless characterized by a trend toward tachycardia at T1. In recovering a normal HR at T5, ranolazine cases therefore lost more bpm as compared with BST cases (**Table 7**).

Loss or gain of hemoglobin is known to increase or decrease HR in cancer patients (Menna, Calabrese et al., 2018). Hemoglobin effects on HR probably occur through a modulation of sympathetic reflexes from carotid body (Lahiri et al., 2006). We therefore normalized individual changes of HR (Δbpm) to concomitant changes of Hb (ΔHb). At T1 and T5 all cases of BST arm showed Δbpm/ΔHb ratios within the range of normality, i.e., they gained or lost bpm in response to Hb decrements at T1 or Hb recovery at T5. In contrast, some cases of ranolazine arm showed Δbpm/ΔHb ratios outside the range of normality, both at T1 and T5. These patients gained or lost bpm in response to factors other than Hb changes; of note, these patients were randomized to ranolazine because of BNP levels >ULN at T1 (denoted by asterisks in **Figure 4A**).

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We next characterized how patients randomized to ranolazine or BST compared with respect to the magnitude of BNP increases above ULN at T1 or BNP decrements toward ULN at T5. Both changes were remarkably more evident for cases of ranolazine arm as compared to cases of BST arm (**Figure 4B**). For the patient that developed BNP >ULN after an ACEI normalization of impaired relaxation, ΔBNP was of a lower magnitude as compared to cases in ranolazine arm, and Δbpm/ΔHb ratio at T5 was within the range of normality (see also Figure 3/A-B, columns with arrow).

When examined in aggregate, all cases randomized to BST or ranolazine because of BNP >ULN at T1 showed Δbpm/ΔHb ratios that correlated with BNP deviations above ULN at T1 or toward ULN at T5; however, separate analyses of the two arms showed that such correlation was contributed only by patients of ranolazine arm (**Figure 5**).

Collectively, data in Figures 4 and 5 suggested that some patients of ranolazine arm showed HR changes that were influenced by BNP rather than Hb.

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DISCUSSION

General considerations on ranolazine efficacy and safety in cancer patients

We probed the lusitropic effects of ranolazine in patients with an early diastolic dysfunction induced by cancer drugs. Ranolazine normalized or improved diastolic dysfunction in each of 12 evaluable patients. Also BST was effective at relieving diastolic dysfunction but treatment failure occurred for 3 of 12 patients (see Figure 2 and Table 4).

Treatment emergent AE were apparently more frequent for patients in ranolazine arm (see Table 5). Ranolazine was titrated as recommended for patients with chronic angina but we cannot exclude this was too high a dose to treat an early and asymptomatic manifestation of cardiotoxicity. Accordingly, reducing ranolazine dosage could resolve AE in one patient without interfering with normalization of diastolic function. On the other hand, we cannot exclude that AE from common cardiovascular drugs were taken for granted in a real life study and were therefore underreported by the investigators. Ranolazine AE, such as constipation and nausea or tinnitus, were nonetheless consistent with the known characteristics of ranolazine (Keating, 2013). Moreover, ranolazine did not prolong QTc. The safety profile of ranolazine in oncologic patients was therefore satisfactory.

Cardiovascular pharmacology endpoints

BST, but not ranolazine, reduced SBP, showed a trend toward reducing DBP, and caused asthenia of a severe intensity in one patient (see Table 6 and Figure 3). These findings anticipate potential advantages for ranolazine over common cardiovascular drugs; in fact, medication of early asymptomatic cardiotoxicity is uncommon in real life oncology and many doctors refrain from prescribing cardiovascular drugs that could expose cancer patients to hypotension or asthenia (Minotti et al., 2013).

Cancer patients' compliance to an early medication of cardiotoxicity might be limited also by HR-lowering effects of most common cardiovascular drugs. Here we have shown that all cases randomized to BST or ranolazine presented at T5 with the same HR they had prior to starting chemotherapy. The HR-lowering effects of BST could not be distinguished from concomitant HR-

lowering effects due to Hb recovery, which likely served as the major determinant of HR normalization. In contrast, ranolazine seemed to normalize HR through an Hb-independent negative chronotropic effect. Our data show that such an effect was apparent but actually reflected an ability of ranolazine to improve or normalize BNP elevations, which were of a greater magnitude as compared to BNP elevations in BST cases. Three lines of evidence support this notion: i) ranolazine caused Hb-independent HR decrements in patients with BNP >ULN and Hb-independent tachycardia at T1 ii), ranolazine lacked HR lowering effects in patients with BNP <ULN iii), HR changes correlated with BNP levels (see Figures 4 and 5).

The effects of ranolazine on HR must be reconciled with the multifaceted role BNP may play in cancer patients. In addition to relieving impaired relaxation, BNP elevations can in fact increase HR in a concentration-related manner (Menna, Calabrese et al., 2018; Menna, Salvatorelli et al., 2018). This probably occurs via BNP stimulation of cGMP formation in sinoatrial node, followed by activation of L-type Ca^{2+} current and higher conduction velocity within the node and toward the surrounding cardiomyocytes (Springer et al., 2012). Ranolazine, by inducing positive lusitropic effects, relieved the diastolic tension that stimulated BNP elevations and blunted the positive chronotropism induced by high BNP levels.

The lack of an authentic negative chronotropism of ranolazine was well consistent with previous findings of preclinical and clinical studies. Ranolazine lacked significant β blocking and HR-lowering effects in laboratory animals (Zhao et al., 2011) but induced bradycardia only in limited experiments that adopted suprapharmacological doses of ranolazine (Letienne et al., 2001; Dhalla et al., 2009). Moreover, ranolazine did not significantly lower HR in patients with chronic angina, regardless of whether the patients were also taking β blockers as part of the anti-ischemic therapy (Stone, 2008; Rousseau et al., 2005).

Study limitations and strengths

The main limitation of this study is sample size. The pilot study was designed to recruit 100 patients and to intercept 40 patients with diastolic dysfunction at T1 (Minotti, 2013); however, only 80 patients were evaluable at T1 and only 29 of them showed diastolic dysfunction. The incidence

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of diastolic dysfunction in the pilot study was in fact congruent with that anticipated by the study protocol (36% versus 40%) but the number of patients to recruit in INTERACT was significantly reduced. Moreover, only 24 of 29 randomizable patients were eventually assigned to study treatment (see Figure 1). From T0 to T1, and from T1 to randomization, sample size reduction was primarily due to patient's refusal to participate, consent withdrawal, or failure to attend study visits. These facts are not uncommon in cardio-oncology trials (Pituskin et al., 2017). They denote that much has to be done for improving patients awareness of cardiovascular consequences of cancer treatment.

Further limitations pertain to the role of late I_{Na}. Ranolazine improved diastolic indices in murine models of anthracycline-related cardiomyopathy but whether this depended on a canonical inhibition of late I_{Na} was not demonstrated (Cappetta et al., 2017). More in general, some reports challenged cause-and-effect relations between late I_{Na} activation, Ca^{2+} overload, and diastolic dysfunction (Papp et al., 2014; Runte et al., 2017). Here it is worth noting that both anthracyclines and nonanthracycline chemotherapeutics might increase diastolic $[Ca^{2+}]_I$ by mechanisms that do not necessitate an activation of late I_{Na} but either stimulate Ca^{2+} release from sarcoplasmic reticulum or inhibit Ca^{2+} reuptake in it (Minotti et al, 2004; Zhang et al., 2010). On a different note, ranolazine may improve diastolic relaxation by mechanisms that do not necessitate an inhibition of late I_{Na} but reduce myofilament sensitivity to Ca^{2+} (Lovelock et al., 2012). Ranolazine could therefore improve diastolic dysfunction even if late I_{Na} was not involved.

In spite of limitations and uncertainties INTERACT shows some important strengths. All patients randomized to study treatment were characterized by impaired relaxation or biomarkers abnormalities that either compensated for impaired relaxation (BNP >ULN) or associated with it (cTnI >ULN). No patient presented with LVEF <50% or grade ≥2 diastolic dysfunction. Thus, ranolazine or BST was probed in patients showing a very early manifestation of diastolic dysfunction (Nagueh et al., 2006; Nagueh et al., 2016). This precisely represents the target of pharmacologic interventions aimed at preventing a progression of cardiotoxicity toward more serious sequelae.

On a different note, INTERACT confirmed that impaired relaxation and BNP elevations were mutually exclusive manifestations of diastolic dysfunction, not only because no patient presented at

T1 with impaired relaxation and a concomitant BNP >ULN but also because one patient in BST arm responded to an ACEI by switching from impaired relaxation to BNP >ULN. Given that BNP serves as an endogenous lusitropic agent, this limited observation suggests that BNP increased to compensate for a lack of efficacy of the ACEI at relieving impaired relaxation in that patient. We acknowledge that in non oncologic patients BNP levels actually increased with the severity of diastolic dysfunction at echocardiography, as if BNP lacked lusitropic effects and failed to mitigate diastolic abnormalities (Lubien et al., 2002). We nonetheless observe that ≥50% of such patients presented with risk factors, like hypertension and/or diabetes or coronary artery disease, that probably caused different mechanisms of diastolic dysfunction as compared to the action of cancer drugs in comorbid-free patients.

We mentioned that some treatment failures occurred in BST arm but not in ranolazine arm. This by no means imply that ranolazine was superior to BST. Superiority analyses were not in the scope of INTERACT and would have not been in the light of the limited sample size of this study. Treatment failures only denoted that even a mild diastolic dysfunction can be difficult to treat, which in principle would be consistent with the liability of mild dysfunction to progress toward moderate-severe dysfunction. With that said, we noted how effectively 5-weeks ranolazine relieved diastolic dysfunction in cancer patients, which was reminiscent of the efficacy with which 4-weeks ranolazine improved myocardial relaxation and perfusion in patients with chronic angina (Venkataraman et al., 2009). Moreover, the efficacy with which ranolazine normalized HR in patients with chronotropic levels of BNP was reminiscent of the higher protective efficacy ranolazine showed in patients with acute coronary syndrome and increased BNP levels as compared to patients with low BNP levels (Morrow et al., 2010).

Conclusions

We have shown that ranolazine, being a lusitropic agent, is potentially able to relieve an early diastolic dysfunction induced by anthracyclines or nonanthracycline chemotherapeutics. The safety profile of ranolazine in oncologic patients seems to be similar to that characterized for the general population. The sample size of this exploratory study is nonetheless too small to permit

considerations about the clinical use of ranolazine for patients with chemotherapy-related diastolic dysfunction. An exploration of potential different mechanisms of action of ranolazine in patients with impaired relaxation or BNP >ULN is also precluded at this point in time. The efficacy, safety and pharmacodynamics of ranolazine in oncologic patients with diastolic dysfunction should be characterized by studies with an adequate sample size.

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AUTHORSHIP CONTRIBUTIONS

Designed the study protocol: Minotti

Conducted experiments: Menna, Calabrese, Armento, Annibali, Greco, Marchesi

Contributed new reagents or analytic tools: Menna

Performed data analysis: Reggiardo, Minotti, Salvatorelli

Wrote the manuscript: Minotti

Reviewed and approved the manuscript: All authors

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FOOTNOTES

This study was promoted by Menarini International Operations Luxembourg S.A. and was registered at the European Clinical Trials Database (EUDRACT 2009-016930-29).

Reprints request to:

Giorgio Minotti, MD
Department of Medicine, Center for Integrated Research, and Unit of Drug Sciences
University Campus Bio-Medico
Via Alvaro del Portillo 21
00128 Rome-ITALY
Phone: 011-39-06-225419109
FAX 011-39-06-22541456
g.minotti@unicampus.it

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LEGENDS FOR FIGURES

Figure 1 Flow chart of pilot study and INTERACT from T1 to T5

T1, one week after chemotherapy; T5, five weeks after randomization.

BST, best standard therapy.

Figure 2 Patterns of treatment efficacy or failure in patients with impaired relaxation or BNP or cTnI >ULN

For impaired relaxation, treatment efficacy was denoted by normalization of E/A decrements and DT prolongations. For biomarkers, treatment efficacy was denoted by normalization or improvements of BNP or cTnI >ULN. Shaded areas denote ranges of normality.

Panel A shows E/A ratio and DT at T0 (baseline), T1 (one week after chemotherapy), T5 (five weeks after randomization to ranolazine or BST). Values are expressed as percentages of normal. Panel B and the inset show changes of BNP and cTnI at T0, T1, T5.

Bold red lines denote patients who failed to normalize E/A and DT, or developed BNP>ULN, after randomization to BST. BNP was measured as Nt-proBNP.

ULN, upper limit of normal.

Figure 3 Individual changes of systolic or diastolic blood pressure after five weeks ranolazine or BST arms

Individual blood pressure changes were measured as Δ mm Hg from T1 (one week after chemotherapy) to T5 (five weeks after randomization to ranolazine or BST). Data were analyzed by two-tailed Mann Whitney test. Where indicated, differences in DBP changes were analyzed only for ranolazine or BST cases that showed negative Δ mm Hg values.

SBP, systolic blood pressure; DBP, diastolic blood pressure.

Figure 4 Hemoglobin-adjusted heart rate and BNP elevations in ranolazine or BST arms

Panel A shows individual increases or decreases in bpm (Δbpm) at T1 or T5 in ranolazine and BST arms. All values were normalized to concomitant increases or decreases of Hb (ΔHb). The shaded

area denotes ranges of normality for ΔbpmΔHb ratios at T1 or T5, calculated as the interquartile range of ΔbpmΔHb ratios in the complete cohort of 24 cases at each time point. Columns with asterisks identify cases of ranolazine arm that presented at T1 with BNP >ULN.

Panel B shows net excursions of BNP from ULN at T1 and toward ULN at T5. Differences were calculated by two-tailed unpaired Mann-Whitney test.

The arrows denote the patient in BST arm who developed BNP>ULN at T5.

BNP was measured as Nt-proBNP, ΔHb was expressed as grams/dL.

ULN, upper limit of normal.

Figure 5 Correlations between hemoglobin-adjusted changes of heart rate and BNP levels.

The upper panel shows the correlation between ΔbpmΔHb ratios and net excursions of BNP from ULN at T1 or toward ULN at T5 for all cases randomized to study treatments. Lower panels show that ΔbpmΔHb ratios correlated with BNP excursions for cases of ranolazine arm but not for cases of BST arm. Shaded areas denote 95% confidence intervals of linear regressions.

BNP was measured as Nt-proBNP, ΔHb was expressed as grams/dL.

ULN, upper limit of normal.

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Table 1

Demographic and oncologic characteristics of controls and cases

Characteristics	**Controls** (n=51)	**Cases** (n=24)	**P**
Age (n,%)			
<40	9 (18%)	2 (8%)	
40-60	36 (70%)	18 (75%)	0.090
>60	6 (12%)	4 (17%)	
Gender			
male	9 (18%)	3 (13%)	0.480
female	42 (82%)	21 (87%)	
Oncologic disease			
breast cancer	35 (69%)	16 (67%)	
non Hodgkin lymphoma	10 (20%)	6 (25%)	0.480
colorectal cancer	6 (11%)	2 (8%)	
Chemotherapy			
anthracycline-based[a),b)]	45 (88%)	22 (92%)	0.844
nonanthracycline[c)]	6 (12%)	2 (8%)	
anthracycline dose (mg/m^2)[d)]	240 (240-267)	240 (240-291)	0.844

Data are medians with interquartile ranges or percentages. Differences were analyzed by two-tailed Mann Whitney test, Fisher exact test or two-tailed χ^2 test, as appropriate.

[a)]For breast cancer: doxorubicin(or epirubicin)-cyclophosphamide followed by a taxane, or epirubicin-cyclophosphamide-taxane, or fluorouracil-epirubicin-cyclophosphamide with or without a subsequent taxane.

[b)]For non Hodgkin lymphoma: rituximab-cyclophosphamide-doxorubicin-vincristine-prednisone.

[c)]For colorectal cancer: fluorouracil-folinate-oxaliplatinum, or capecitabine-oxaliplatinum.

[d)]Expressed as doxorubicin myelotoxic equivalents.

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Table 2

Cardiovascular characteristics of controls and cases

Characteristics	Controls (n=51)	Cases (n=24)	P
LVEF (%)			
T0	61 (60-65)	60 (60-65)	0.276
T1	62 (60-65)*	60 (58-62)**	0.005
HR (bpm)			
T0	76 (70-81)	78 (70-85)	0.815
T1	83 (75-99)†	85 (77-87)††	0.666
SBP (mm Hg)			
T0	120 (120-130)	120 (110-124)	0.233
T1	120 (115-130)	120 (110-128)	0.446
DBP (mm Hg)			
T0	80 (70-80)	80 (70-80)	0.766
T1	80 (70-84)	80 (70-80)	0.855
BMI (kg/m^2)			
T0	24 (22-25)	23 (20-25)	0.297
T1	25 (22-28)	23 (19-27)	0.100

Data are medians with interquartile ranges. Differences were analyzed by two-tailed Mann Whitney test or Wilcoxon Signed Rank test, as appropriate.

*P=0.820 and **P=0.170 for T1 versus T0.

†P<0.001 and ††P<0.01 for T1 versus T0.

Table 3

Investigator's choice of BST

Class	Drug(s)	mg/day
ACEI (n=5)	ramipril	1.25 (n=1) 2.5 (n=4)
β blocker (n=2)	bisoprolol	1.25
ACEI + β blocker (n=2)	ramipril-carvedilol ramipril-bisoprolol	1.25 + 6.25 5 + 2.5
ARB (n=1)	valsartan	80 mg
ACEI + β blocker + loop diuretic (n=1)	ramipril-bisoprolol-furosemide	5 + 1.25 + 25*
loop diuretic (n=1)	furosemide	25

*once every two days

ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker.

Table 4

Efficacy of 5 weeks of ranolazine or BST on chemotherapy-induced diastolic dysfunction

Arm	Event at T1	Event outcome at T5	Post-randomization events	Treatment failures
Ranolazine (n=12)	Impaired relaxation (n=5) impaired relaxation + cTnI >ULN cTnI >ULN BNP >ULN (n=4) BNP >ULN + cTnI >ULN	normalized normalized/normalized normalized normalized (n=3), improved (n=1) improved-normalized	none none none none none	none
BST (n=12)	Impaired relaxation (n=2) Impaired relaxation* Impaired relaxation† Impaired relaxation†† BNP >ULN (n=7)	normalized normalized failure failure normalized (n=4), improved (n=3)	none BNP >ULN* none none none	3

*Patient treated by ramipril (2.5 mg/day)

†Patient treated by bisoprolol (1.25 mg/day)

††Patient treated by bisoprolol + ramipril (2.5 mg/day + 5 mg/day)

T1, one week after chemotherapy; T5, five weeks after randomization

Table 5

Ranolazine- or BST-related adverse events from T1 to T5

Patients with AE	AE	AE intensity	AE seriousness	AE management	Diastolic dysfunction event at T1	Event outcome at T5
Ranolazine arm (n=4)	constipation constipation nausea constipation-tinnitus	moderate severe mild-mild severe-moderate	not serious not serious not serious not serious	none none none dose reduction[a)]	BNP >ULN BNP >ULN impaired relaxation cTnI >ULN	normalized normalized normalized normalized
BST arm (n=1)	asthenia	Severe	not serious	dose reduction[b)]	BNP >ULN	improved

[a)]From 500 to 375 mg bid.

[b)]Ramipril reduced from 2.5 to 1.25 mg/day.

AE, adverse event; T!, one week after chemotherapy; T5, five weeks after randomization.

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Table 6

Cardiovascular parameters in ranolazine or BST arms from T1 to T5.

Parameters	**Ranolazine** (n=12)	**BST** (n=12)	**P**
LVEF (%)			
T1	60 (56-61)	60 (59-62)	0.610
T5	60 (58-65)	60 (58-64)	0.837
P (T1 vs T5)	0.324	0.572	
SBP (mm Hg)			
T1	120 (110-130)	120 (113-120)	0.845
T5	120 (111-129)	110 (110-115)	0.051
P (T1 vs T5)	0.887	0.046	
DBP (mm Hg)			
T1	78 (70-80)	80 (70-85)	0.613
T5	78 (70-80)	70 (70-79)	0.364
P (T1 vs T5)	0.665	0.160	
QTc (mm Hg)			
T1	423 (405-430)	405 (390-420)	0.220
T5	419 (400-430)	417 (410-439)	0.772
P (T1 vs T5)	0.689	0.220	
BMI (kg/m^2)			
T1	21 (19-26)	25 (20-27)	0.338
T5	22 (20-26)	25 (20-28)	0.400
P (T1 vs T5)	0.102	0.824	

Data are medians with interquartile ranges and were analyzed by two-tailed Mann Whitney test (for comparisons between arms) or two-tailed Wilcoxon Signed Rank test for comparisons within arms.

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Table 7

Heart rate in patients of ranolazine or BST arms

	Ranolazine (n=12)	**BST (n=12)**	**P**
HR (bpm)			
T0	76 (70-80)	78 (70-83)	0.966
T1	95 (79-101)	78 (75-94)	0.088
T5	75 (69-79)	73 (64-80)	0.728
P (T0-T1)	<0.01	>0.05	
P (T1-T5)	<0.01	>0.05	
P (T0-T5)	>0.05	>0.05	

Data are medians with interquartile ranges and were analyzed by two-tailed Mann Whitney test for comparisons between arms, and by one-way repeated measures analysis of variance with Bonferroni's post hoc test for comparisons within arms.

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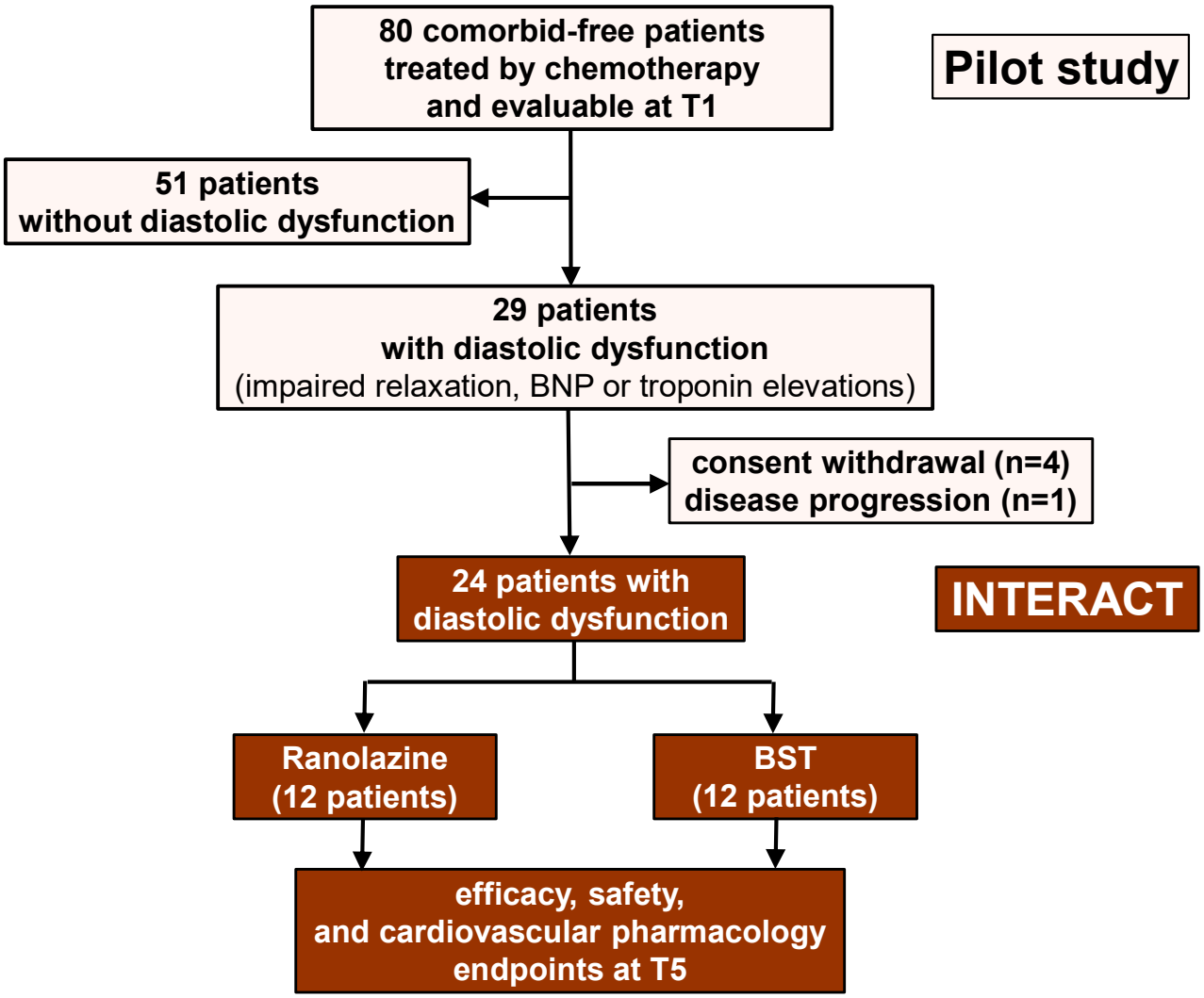

FIGURE 1

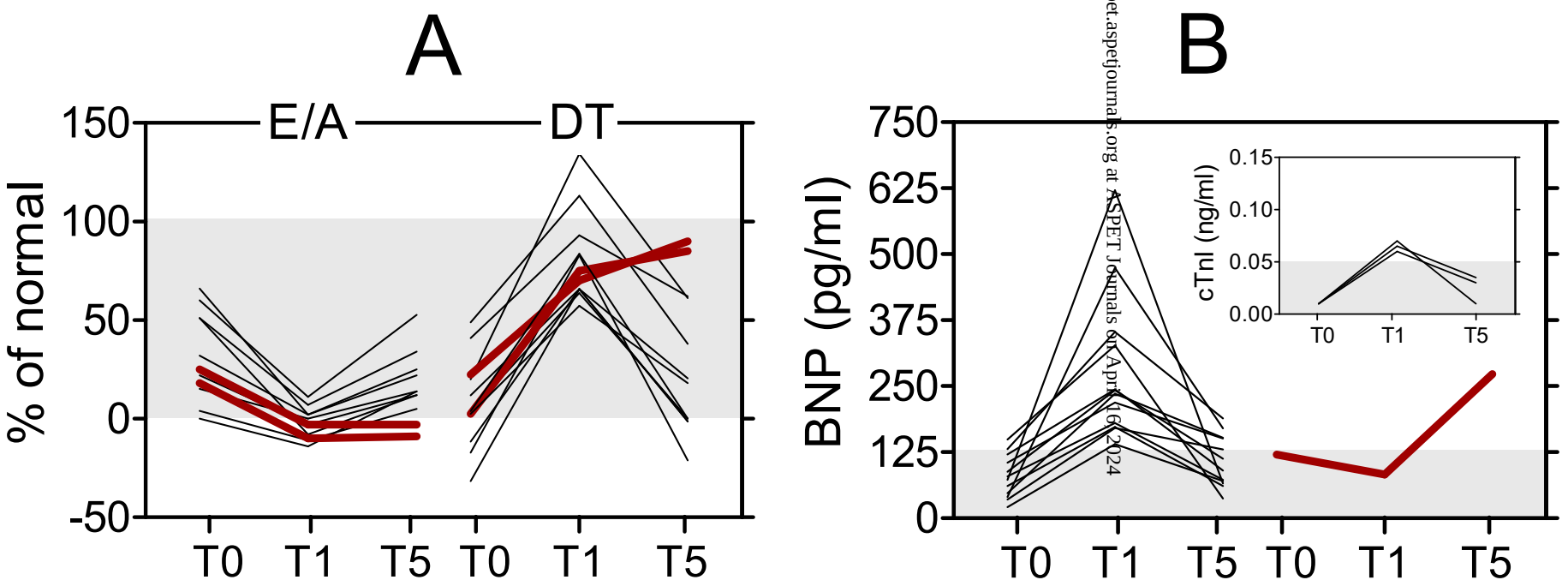

FIGURE 2

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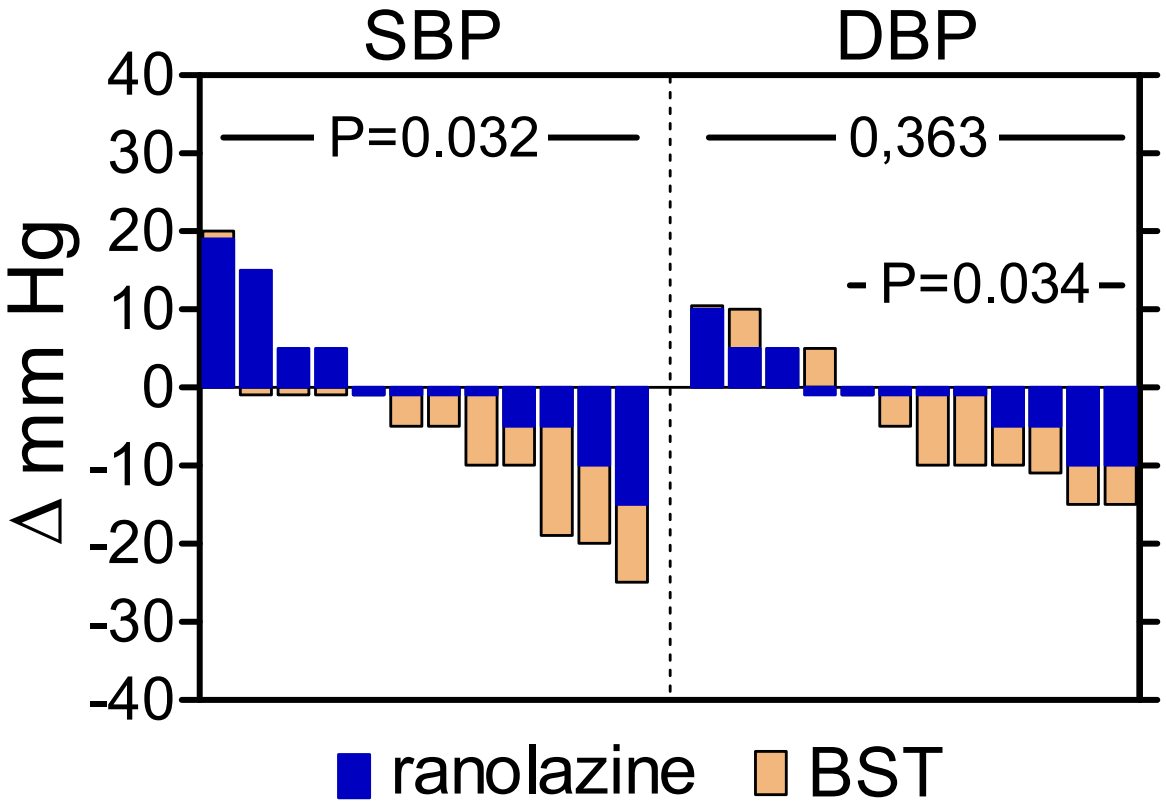

FIGURE 3

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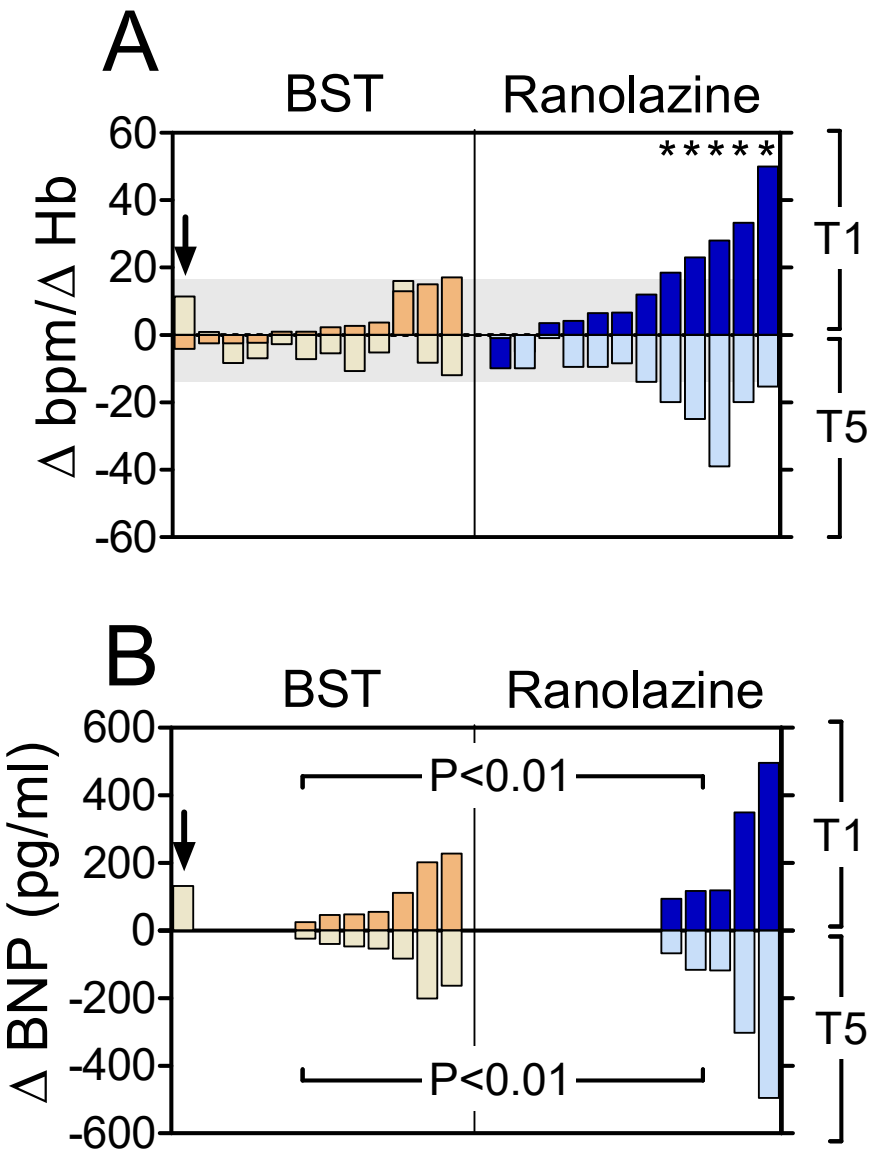

FIGURE 4

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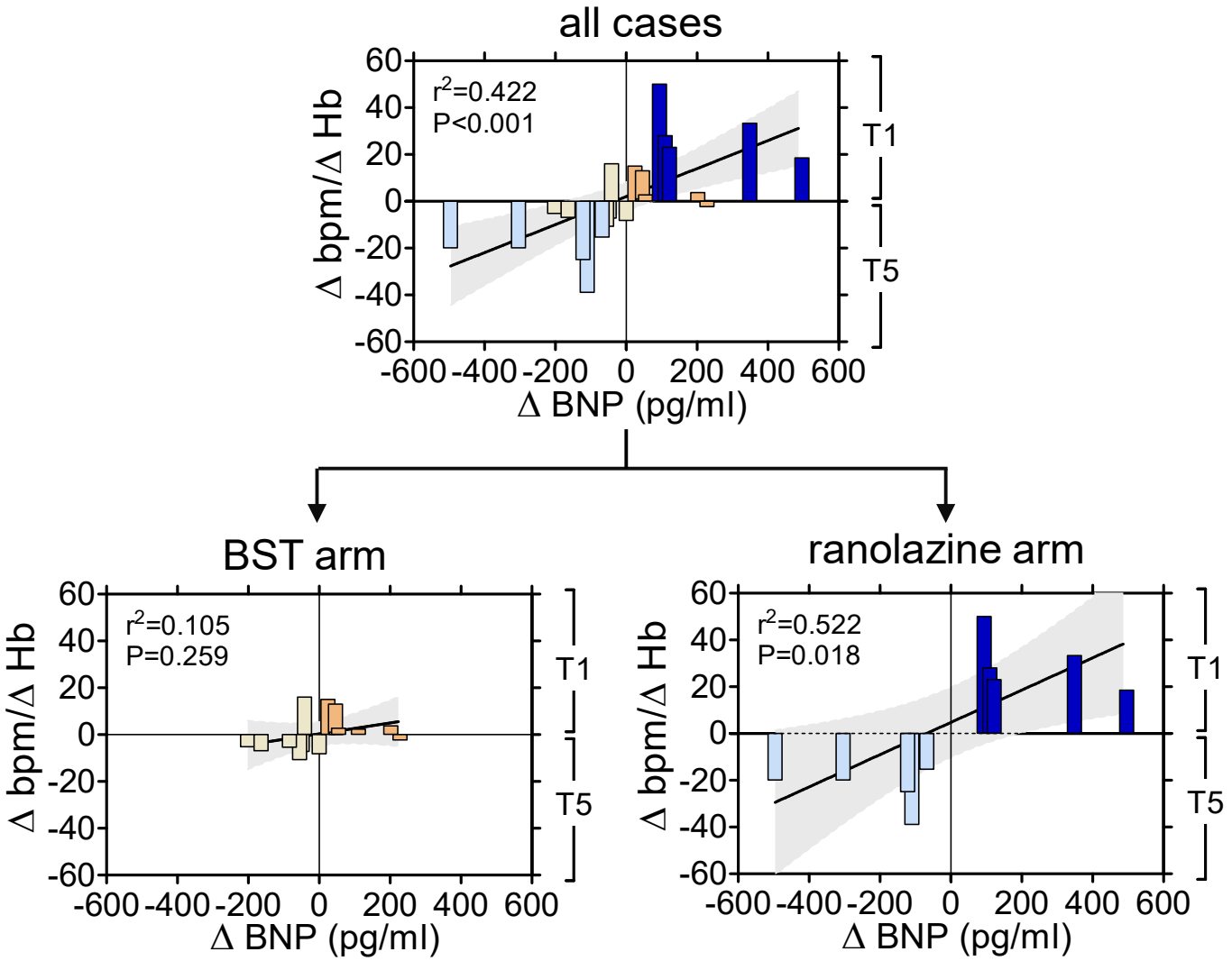

FIGURE 5