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Predicting Response to Switching From Phosphodiesterase Type 5 Inhibitor to Riociguat in Patients With Pulmonary Arterial Hypertension: Biomarker and Responder Analysis of the RESPITE and REPLACE Studies

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ABSTRACT

This exploratory analysis assessed whether plasma biomarkers predict the response to switching from phosphodiesterase type 5 inhibitors (PDE5is) to the soluble guanylate cyclase stimulator riociguat in patients with pulmonary arterial hypertension.

Abbreviations: 6MWD, 6-min walk distance; ADMA, asymmetric dimethylarginine; AUC, area under the curve; BMI, body mass index; BNP, brain natriuretic peptide; cGMP, cyclic guanosine monophosphate; CHD, congenital heart disease; CTD, connective tissue disease; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; ERA, endothelin receptor antagonist; FC, functional class; GDF-15, growth/differentiation factor 15; IQR, interquartile range; mPAP, mean pulmonary artery pressure; NO, nitric oxide; NT-proBNP, *N*-terminal prohormone of brain natriuretic peptide; OR, odds ratio; PAH, pulmonary arterial hypertension; PAWP, pulmonary artery wedge pressure; PCWP, pulmonary capillary wedge pressure; PDE5i, phosphodiesterase type 5 inhibitor; PH, pulmonary hypertension; PVR, pulmonary vascular resistance; Q, quartile; RAP, right atrial pressure; REPLACE, Riociguat rEplacing PDE5i therapy evaLuated Against Continued PDE5i thErapy; RESPITE, Riociguat clinical Effects Studied in Patients with Insufficient Treatment response to PDE5 inhibitors; RHC, right heart catheterization; SBP, systolic blood pressure; SD, standard deviation; sGC, soluble guanylate cyclase; ST-2, suppression of tumorigenicity 2; SvO_2, mixed venous oxygen saturation; SVR, systemic vascular resistance; WHO, World Health Organization.

Tweet: Biomarker analyses of the RESPITE and REPLACE trials of switching from PDE5is to riociguat in pulmonary arterial hypertension did not find markers that identified patients more likely to respond.

Selected biomarkers at baseline and their changes to Week 24 were evaluated in patients with and without a favorable response to riociguat in two trials: RESPITE, in which patients with an inadequate response to PDE5i were switched to riociguat; and REPLACE, in which patients at intermediate risk of 1-year mortality despite a PDE5i were randomized to remain on PDE5i or were switched to riociguat. A response was defined as absence of clinical worsening and at least two of the following criteria: 6-min walk distance increase by 10% or ≥ 30 m, World Health Organization functional class I/II, or *N*-terminal prohormone of brain natriuretic peptide reduction of ≥ 30% at Week 24. In REPLACE, responders had significantly higher baseline cyclic guanosine monophosphate (cGMP) and significantly lower baseline asymmetric dimethylarginine, and growth/differentiation factor 15 (GDF-15) than nonresponders. In RESPITE, responders had lower baseline GDF-15 than nonresponders, and nonresponders showed a significantly greater decrease in cGMP than responders. No baseline threshold value of any biomarker provided a good likelihood of predicting the response to riociguat. Overall, the biomarkers evaluated did not help to identify patients who were more likely to respond to switching from PDE5is to riociguat.

Treatment guidelines for pulmonary arterial hypertension (PAH) recommend achieving or maintaining a low 1-year mortality risk [1]. Initial combination treatment with a phosphodiesterase type 5 inhibitor (PDE5i) and an endothelin receptor antagonist (ERA) is recommended for patients at low or intermediate risk [1]. However, many patients receiving PDE5is do not achieve or maintain low risk, necessitating treatment escalation [1, 2]. Switching from PDE5is (with or without ERA) to the soluble guanylate cyclase (sGC) stimulator riociguat led to clinical improvement in patients at intermediate risk in the RESPITE (NCT02007629) and REPLACE (NCT02891850) studies [3, 4]. As a result, this switch is included as an option in treatment guidelines for patients with idiopathic, drug-associated, or heritable PAH who present at intermediate–low risk of death while receiving ERA/PDE5i therapy [1].

Impairment of nitric oxide−sGC−cyclic guanosine monophosphate (NO−sGC−cGMP) signaling has been implicated in the pathophysiology of many diseases including pulmonary hypertension (PH) [5]. Riociguat stimulates sGC by increasing its sensitivity to NO and by a direct action, increasing intracellular cGMP synthesis [5, 6]. The direct effect of riociguat on sGC may enable it to be effective under conditions of NO deprivation (in which the efficacy of PDE5i might be reduced) [5]. Several biomarkers are of interest as potential predictors of the response to switching from PDE5is to riociguat. These include cGMP as a pharmacodynamic marker of the effect of riociguat on its target enzyme [5, 6]. Asymmetric dimethylarginine (ADMA) inhibits nitric oxide synthase by competing with L-arginine at the active site of the enzyme, resulting in decreased production of NO [7, 8]. Some studies have reported that serum levels of ADMA are elevated in patients with PAH and may correlate with markers of disease severity [9]. Decreased plasma ADMA levels at follow-up are associated with better 3-year and 5-year survival rates in patients with PAH [10]. These results suggest that ADMA may be useful for the evaluation of disease severity, although a targeted therapy for lowering ADMA is yet to be identified.

N-terminal prohormone of brain natriuretic peptide (NT-proBNP) is an established noninvasive biomarker in PAH [11]. Transmural pressure, volume overload, hypoxia, or pro-inflammatory factors stimulate secretion of pre-pro brain natriuretic peptide, which is metabolized to proBNP and then to brain natriuretic peptide (BNP) and NT-proBNP. BNP binds to the natriuretic peptide receptor-A, leading to increases in intracellular cGMP. NT-proBNP has no known function, but its longer half-life and greater stability in storage than BNP confer advantages as a biomarker. Elevated levels of NT-proBNP correlate with pulmonary hemodynamic measures associated with shortened survival, as well as parameters of impaired cardiac function on imaging [1, 11]. NT-proBNP levels are widely used and recommended by guidelines in screening algorithms, risk assessments, and patient follow-up in PAH [1]. Growth/differentiation factor-15 (GDF-15) is a member of the transforming growth factor-B cytokine superfamily and is induced in animal models of cardiac pressure overload, ischemia, oxidative stress, and reperfusion injury [12]. GDF-15 expression is increased in plexiform lesions in patients with PAH [13]. Serum levels of GDF-15 are elevated in patients with PAH, and are associated with increased risks of death or heart transplantation and with increased right atrial and pulmonary capillary wedge pressures [12, 14]. One study reported that GDF-15 levels in pulmonary arterial blood were higher in patients with PAH who were hospitalized for heart failure compared with those who were not [15].

Suppression of tumorigenicity 2 (ST2) is a member of the interleukin 1 receptor family occurring in a transmembrane or cellular isoform (ST2L) and a soluble or circulating isoform (sST2) [16]. In vitro studies have shown that sST2 is elevated by mechanical stress to cardiomyocytes [17]. Some studies have suggested that ST2 levels are elevated in patients with PAH and are associated with worse prognosis [18–20]. A subsequent meta-analysis supported this conclusion [21], although only the three studies cited above were included.

These results suggest that the biomarkers discussed above are worthy of further investigation as markers of prognosis or response to therapy in patients with PAH.

The current study analyzed plasma cGMP, ADMA, GDF-15, ST-2, and NT-proBNP from blood samples collected at baseline and Week 24 in RESPITE [3] and REPLACE [4] to determine whether these markers could identify patients who would have a favorable response after switching from PDE5is to riociguat, and whether a favorable clinical response after switching was associated with significant changes in these biomarkers.

1 | Methods

1.1 | RESPITE and REPLACE Study Designs

RESPITE (started February 18, 2014; completed December 29, 2016) was a 24-week, open-label, uncontrolled study in 61 patients with PAH with inadequate response to a PDE5i who were switched to riociguat (adjusted up to 2.5 mg three times daily [tid]) [3, 22]. Eligible patients were defined as having an inadequate response to a PDE5i if they were in World Health Organization (WHO) functional class (FC) III, had a 6-min walk distance (6MWD) of 165–440 m, a cardiac index (CI) < 3.0 L/min/m^2, mean pulmonary artery pressure (mPAP) > 30 mmHg, pulmonary capillary wedge pressure (PCWP) ≤ 15 mmHg, and pulmonary vascular resistance (PVR) > 400 dyn·s·cm^{-5} after at least 90 days of treatment with a PDE5i either alone or in combination with an ERA. The first 30 patients were recruited with narrower hemodynamic inclusion criteria (CI < 2.5 L/min/m^2 and PVR > 480 dyn·s·cm^{-5}), which were later amended to enhance the feasibility of the trial. All patients underwent right heart catheterization (RHC) at baseline and at 24 weeks [3].

REPLACE (started January 11, 2017; screening completed July 31, 2019; study completed March 3, 2020) was a phase 4, randomized, controlled, open-label study of patients with symptomatic PAH with PVR > 400 dyn·s·cm^{-5}, mPAP ≥ 25 mmHg, and PCWP ≤ 15 mmHg as assessed by the most recent RHC from medical history before screening to confirm the diagnosis [4, 23]. Alternatively, pulmonary artery wedge pressure could be replaced by left ventricular end-diastolic pressure ≤ 15 mmHg. Patients had to be at intermediate risk of 1-year mortality despite treatment with a PDE5i with or without an ERA. Intermediate risk was defined as WHO FC III, with a 6MWD of 165–440 m at screening and randomization, based on the thresholds from the European Society of Cardiology/European Respiratory Society treatment guidelines in place when the study was designed [24]. Eligible patients were randomized to remain on PDE5i ($n = 115$) or were switched to riociguat (adjusted up to 2.5 mg tid; $n = 111$) [4].

For both studies, the institutional review board at each participating center approved the protocol. RESPITE and REPLACE were carried out in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. All patients provided written informed consent. In the RESPITE study, all endpoints were exploratory and included change from baseline to Week 24 in 6MWD, WHO FC, and NT-proBNP, and safety [3, 22].

The composite primary endpoint in the REPLACE study was clinical improvement (absence of clinical worsening and at least two of the following criteria: 6MWD increase by 10% or ≥ 30 m, WHO FC I/II, or NT-proBNP reduction of ≥ 30% at Week 24. Secondary endpoints included 6MWD, WHO FC, time to clinical worsening, and NT-proBNP [4]. Clinical worsening was defined as death from any cause, hospitalization for worsening PAH (nonelective hospitalization due to PAH or initiation of parenteral prostanoid therapy), or disease progression (decrease in 6MWD ≥ 15% on two separate days, plus either worsening WHO functional class, need for new PAH-targeted medication, or decompensated right-sided heart failure). The current study applied this definition to both study populations.

1.2 | Biomarker Analysis

Consistent assay conditions were used for all patients (via a central laboratory), and the study employed strict protocols for collection, freezing, labeling, transport, and analysis of all biomarker samples. For analysis of NT-proBNP, blood was collected in separation tubes at the study sites, thoroughly mixed, and allowed to clot for 30–60 min. Samples were centrifuged at 1500−2000 *g* for ≥ 15 min until clot and serum were separated. For analysis of cGMP, ADMA, GDF-15, and ST-2, blood was collected in ethylenediamine tetra-acetate tubes at the study sites, mixed immediately, and centrifuged at 1500−2000 *g* for ≥ 15 min until cells and plasma were separated. Samples were frozen immediately at −20°C and

TABLE 1 | Reference ranges for biomarkers.

Marker	Reference range	Low alert level	High alert level
cGMP, pmol/mL	None	None	None
GDF-15, pg/mL	None	None	None
ST-2, ng/mL	4.04−20.36	None	> 20.36
ADMA, ng/mL	63−137	< 63	> 137
NT-proBNP, pmol/L			
Age 18−44 years	≤ 11.51	None	> 11.51
Age 45−54 years	≤ 14.31	None	> 14.31
Age 55−64 years	≤ 23.41	None	> 23.41
Age 65−74 years	≤ 33.70	None	> 33.70
Age ≥ 75 years	≤ 62.20	None	> 62.20

Abbreviations: ADMA, asymmetric dimethylarginine; cGMP, cyclic guanosine monophosphate; GDF-15, growth/differentiation factor 15; NT-proBNP, *N*-terminal prohormone of brain natriuretic peptide; ST-2, suppression of tumorigenicity 2.

shipped on dry ice to an accredited central laboratory (Covance Central Laboratory Services, 1217 Meyrin/Geneva, Switzerland) for analysis. Samples were analyzed using the reference ranges in Table 1.

1.3 | Statistical Techniques

Changes in biomarkers from baseline were prospectively assessed according to the statistical analysis plans. The responder data were analysed post hoc to the original investigation. All analyses were exploratory. Descriptive statistics are presented; all p values are nominal and not adjusted for multiplicity or for changes in biomarker levels from baseline to Week 24. In the responder analyses, the Wilcoxon rank-sum test was used to compare parameters in responder vs. nonresponder groups; a paired t-test was used to compare the mean change from baseline vs. Week 24 within treatment groups. In a receiver operating characteristic analysis for baseline NT-proBNP, GDF-15, and ST-2, achievement of the clinical improvement at Week 24 was assessed, with thresholds identified by the Youden index.

Multivariate logistic regression analyses were performed to estimate the probability of responders in the simultaneous presence of these three biomarkers, categorized in quartiles at baseline, using the lowest quartile as the reference. The Wald test was applied to test the null hypothesis that the proportion of responders was equal across the four quartiles of each parameter.

2 | Results

Tables 2 and 3 summarize patient demographics and disease characteristics at baseline for RESPITE [3] and REPLACE [4], respectively. Baseline demographics and disease characteristics in REPLACE were generally well balanced between the riociguat and PDE5i groups. Hemodynamic data were not available for REPLACE because RHC was not required by the study protocol. Baseline biomarker levels and changes to Week 24 are summarized in Table 4. Baseline levels were similar across the riociguat and PDE5i groups in REPLACE, except that NT-proBNP levels were higher in the PDE5i group. Baseline levels of ADMA and ST-2 were similar in RESPITE and REPLACE; however, cGMP levels were lower and GDF-15 levels were higher in RESPITE compared with REPLACE. In RESPITE, there was a significant decrease in NT-proBNP and a significant increase in plasma cGMP from baseline to Week 24 (nominal $p < 0.05$ for both; Table 4). In REPLACE, at Week 24 only ST-2 showed a significant difference between treatment arms.

In REPLACE, baseline cGMP was significantly higher for responders vs. nonresponders, while baseline ADMA and GDF-15 were significantly lower (nominal $p = 0.03$; Table 5). At-Week 24, the only significant difference in biomarker levels between responders and nonresponders was the lower GDF-15 level in the latter. No other statistically significant differences were seen between responders and nonresponders, or in the mean changes from baseline between responders and nonresponders (Table 5).

TABLE 2 | Baseline patient demographics and disease characteristics in RESPITE [3].

Characteristic	Riociguat ($n = 61$)
Age, years	53.9 (13.8)
Age	
< 65 years	46 (75)
≥ 65 years	15 (25)
Female	45 (74)
Race	
Caucasian	56 (92)
Asian	3 (5)
Not reported	2 (3)
Ethnicity	
Not Hispanic or Latino	56 (92)
Not reported	5 (8)
BMI, kg/m^2	28 ± 5
Dana Point classification of PH	
1.1 Idiopathic PAH	56 (92)
1.2 Heritable PAH	1 (2)
1.3 Drug- or toxin-induced PAH	1 (2)
1.4 PAH associated with CHD	2 (3)
1.4 PAH associated with CTD[a]	1 (2)
Concomitant treatment with ERA	50 (82)
Pretreated with sildenafil	40 (66)
Pretreated with tadalafil	21 (34)
Concomitant diuretic use	45 (74)
Time since first PH diagnosis, years	4 ± 4
6MWD, m	357 ± 81
WHO FC III	61 (100)
NT-proBNP, pg/mL[b]	1190 ± 1828
eGFR, mL/min/1.73 m^2	73 ± 22
PVR, dyn•s•cm^{-5}	835 ± 272
Cardiac output, L/min	4.2 ± 0.8
Cardiac index, L/min/m^2	2.3 ± 0.4
mPAP, mmHg	52 ± 12
RAP, mmHg	8.3 ± 4.9
SvO_2, %[b]	64.8 ± 6.9
SVR, dyn•s•cm^{-5}[c]	1624 ± 452
PAWP, mmHg	9.4 ± 3.2
SBP, mmHg	118 ± 15
DBP, mmHg	72 ± 9
Heart rate, beats per min	75 ± 11

Note: Data are n (%) or mean ± SD, unless otherwise stated. $N = 61$ unless otherwise stated.
Abbreviations: 6MWD, 6-min walk distance; BMI, body mass index; CHD, congenital heart disease; CTD, connective tissue disease; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; ERA, endothelin receptor antagonist; mPAP, mean pulmonary arterial pressure; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; PAH, pulmonary arterial hypertension; PAWP, pulmonary artery wedge pressure; PH, pulmonary hypertension; PVR, pulmonary vascular resistance; RAP, right atrial pressure; SBP, systolic blood pressure; SD, standard deviation; SvO_2, mixed venous oxygen saturation; SVR, systemic vascular resistance; WHO FC, World Health Organization functional class.
[a]Protocol violation; patient randomized due to investigator error.
[b]$n = 58$.
[c]$n = 60$.

TABLE 3 | Baseline patient demographics and disease characteristics in REPLACE [4].

Characteristic	Riociguat ($n = 111$)	PDE5i ($n = 113$)
Age, years	49.4 (16.2)	49.1 (15.7)
Age		
< 65 years	81 (73)	91 (81)
≥ 65 years	30 (27)	22 (19)
Female	82 (74)	94 (83)
Race		
White	86 (77)	88 (78)
Black or African American	4 (4)	5 (4)
Asian	17 (15)	19 (17)
Other	1 (1)	0
Not reported	3 (3)	1 (1)
Ethnicity		
Hispanic or Latino	32 (29)	31 (27)
Not Hispanic or Latino	75 (68)	79 (70)
Not reported	4 (4)	3 (3)
BMI, kg/m^2	26 ± 5	27 ± 5
Dana Point classification of PH		
1.1 Idiopathic PAH	69 (62)	73 (65)
1.2 Heritable PAH	4 (4)	4 (4)
1.3 Drug- or toxin-induced PAH	1 (1)	4 (4)
1.4 PAH associated with CHD	6 (5)	7 (6)
1.4 PAH associated with CTD	24 (22)	19 (17)
1.4 Portopulmonary hypertension	7 (6)	6 (5)
Time from first diagnosis to randomization, years	3 (1−7)	4 (1−10)
Monotherapy and combination therapy		
PDE5i monotherapy	32 (29)	32 (28)
PDE5i plus ERA combination therapy	79 (71)	81 (72)
PDE5i pretreatment		
Tadalafil	33 (30)	33 (29)
Sildenafil	78 (70)	80 (71)
Pretreatment with ERA		
Bosentan	19 (17)	20 (18)
Ambrisentan	30 (27)	29 (26)
Macitentan	30 (27)	32 (28)
6MWD, m	374 ± 60	367 ± 62
WHO FC III	111 (100)	113 (100)
NT-proBNP, pg/mL	290 (138−863)[a]	395 (166−1068)

Note: Data are *n* (%), mean ± SD, or median (IQR).
Abbreviations: 6MWD, 6-min walk distance; BMI, body mass index; CHD, congenital heart disease; CTD, connective tissue disease; ERA, endothelin receptor antagonist; IQR, interquartile range; NT-proBNP, *N*-terminal prohormone of brain natriuretic peptide; PAH, pulmonary arterial hypertension; PDE5i, phosphodiesterase type 5 inhibitor; PH, pulmonary hypertension; SD, standard deviation; WHO FC, World Health Organization functional class.
[a] $n = 108$.

In RESPITE, baseline GDF-15 was significantly lower in responders than nonresponders (nominal $p = 0.005$; Table 6), and this difference was maintained at Week 24 (nominal $p = 0.001$; Table 6). At Week 24, levels of ST-2, cGMP, and NT-proBNP were significantly lower in responders than non-responders (nominal $p \leq 0.005$; Table 6). From baseline to Week 24, responders showed a significantly (nominal $p = 0.05$) greater reduction in ST-2 than nonresponders, and a

TABLE 4 | Baseline levels and changes from baseline at Week 24 in biomarkers in the RESPITE and REPLACE studies.

	REPLACE								RESPITE			
	Riociguat ($n = 111$)				PDE5i ($n = 113$)				Riociguat ($n = 61$)			
Parameter, mean (SD)	Baseline		Change from baseline at Week 24		Baseline		Change from baseline at Week 24		Baseline		Change from baseline at Week 24	
ADMA (plasma), μmol/L	$n = 94$	0.53 (0.11)	$n = 75$	−0.02 (0.12)	$n = 91$	0.55 (0.11)	$n = 71$	0.02 (0.11)	$n = 52$	0.56 (0.13)	$n = 49$	−0.02 (0.12)
ST-2, ng/mL	$n = 100$	19.4 (8.0)	$n = 86$	1.3 (8.7)	$n = 98$	18.3 (8.4)	$n = 88$	4.8 (18.8)*,[a]	$n = 53$	21.1 (15.2)	$n = 51$	−2.3 (9.8)
cGMP (plasma), pmol/mL	$n = 101$	216.3 (55.9)	$n = 85$	−4.9 (76.8)	$n = 100$	210.7 (54.8)	$n = 88$	8.7 (77.1)	$n = 53$	16.2 (11.2)	$n = 51$	2.6 (8.0)*,[a]
NT-proBNP, pg/mL	$n = 108$	689 (929)	$n = 108$	−88 (534)	$n = 113$	1037 (1725)	$n = 113$	81 (1268)	$n = 60$	1190 (1828)	$n = 51$	−347 (1235)*,[a]
GDF-15, pg/mL	$n = 101$	1124 (849)	$n = 90$	81 (395)	$n = 102$	1294 (1304)	$n = 93$	347 (2366)	$n = 53$	4633 (4525)	$n = 51$	−669 (2977)

Abbreviations: ADMA, asymmetric dimethylarginine; cGMP, cyclic guanosine monophosphate; GDF-15, growth/differentiation factor 15; NT-proBNP, *N*-terminal prohormone of brain natriuretic peptide; PDE5i, phosphodiesterase type 5 inhibitor; SD, standard deviation; ST-2, suppression of tumorigenicity 2.

*In REPLACE, the *p* values denote a significant difference in change from baseline values between the riociguat and the PDE5i treatment groups at Week 24. In RESPITE, the *p* values denote significant change from baseline to Week 24. Baseline = the last documented value while still receiving PDE5i.

[a]$p < 0.05$. All *p* values are nominal.

TABLE 5 | Biomarker results for responders and nonresponders to riociguat in REPLACE.

Parameter, mean (SD)	Baseline		Week 24		Mean change from baseline at Week 24	
	Responders	Nonresponders	Responders	Nonresponders	Responders	Nonresponders
ADMA (plasma), μmol/L	0.51 (0.07) ($n = 38$)	0.55 (0.13) ($n = 56$)	0.49 (0.12) ($n = 36$)	0.53 (0.11) ($n = 48$)	−0.035 (0.11) ($n = 32$); $p = 0.09$	−0.005 (0.13) ($n = 43$); $p = 0.79$
	$p = 0.03$		**$p = 0.12$**		**$p = 0.21$**	
ST-2, ng/mL	19.1 (8.6) ($n = 40$)	19.7 (7.7) ($n = 60$)	18.0 (7.2) ($n = 40$)	21.3 (12.2) ($n = 51$)	−0.4 (5.3) ($n = 38$); $p = 0.64$	2.6 (10.5) ($n = 48$); $p = 0.09$
	$p = 0.59$		**$p = 0.23$**		**$p = 0.11$**	
cGMP (plasma), pmol/mL	232.1 (58.0) ($n = 41$)	205.5 (52.2) ($n = 60$)	202.4 (67.7) ($n = 39$)	205.1 (73.9) ($n = 52$)	−18.4 (88.0) ($n = 36$); $p = 0.22$	5.0 (66.7) ($n = 49$); $p = 0.60$
	$p = 0.03$		**$p = 0.89$**		**$p = 0.17$**	
NT-proBNP, pg/mL[a]	694 (793) ($n = 43$)	686 (1016) ($n = 65$)	356 (416) ($n = 43$)	737 (1105) ($n = 57$)	−302 (499) ($n = 43$); $p = 0.0007$	53 (512) ($n = 65$); $p = 0.44$
	$p = 0.21$		**$p = 0.15$**		**$p < 0.0001$**	
GDF-15, pg/mL	870 (571) ($n = 41$)	1298 (961) ($n = 60$)	903 (624) ($n = 42$)	1283 (970) ($n = 54$)	35 (339) ($n = 39$); $p = 0.52$	116 (433) ($n = 51$); $p = 0.06$
	$p = 0.03$		**$p = 0.03$**		**$p = 0.35$**	

Note: All p values are nominal. p values to the right of the changes from baseline indicate comparisons within responder or nonresponder groups. p values in bold indicate comparisons between responders and nonresponders for baseline levels, levels at Week 24, and mean changes from baseline at Week 24 for the biomarker indicated.

Abbreviations: ADMA, asymmetric dimethylarginine; cGMP, cyclic guanosine monophosphate; GDF-15, growth/differentiation factor 15; NT-proBNP, *N*-terminal prohormone of brain natriuretic peptide; PDE5i, phosphodiesterase type 5 inhibitor; SD, standard deviation; ST-2, suppression of tumorigenicity 2.

[a]A reduction in NT-proBNP was part of the response definition. This may have confounded these results.

TABLE 6 | Biomarker results for responders and nonresponders to riociguat in RESPITE.

Parameter, mean (SD)	Baseline		Week 24		Mean change from baseline at Week 24	
	Responders	Nonresponders	Responders	Nonresponders	Responders	Nonresponders
ADMA (plasma), μmol/L	0.55 (0.16) ($n = 22$)	0.58 (0.11) ($n = 28$)	0.55 (0.12) ($n = 21$)	0.54 (0.11) ($n = 28$)	−0.001 (0.14) ($n = 21$); $p = 0.96$	−0.036 (0.11) ($n = 28$); $p = 0.09$
	$p = 0.29$		**$p = 0.74$**		**$p = 0.28$**	
ST-2, ng/mL	19.4 (17.6) ($n = 23$)	22.3 (13.2) ($n = 28$)	15.9 (11.4) ($n = 23$)	20.9 (8.7) ($n = 28$)	−3.5 (7.7) ($n = 23$); $p = 0.04$	−1.4 (11.3) ($n = 28$); $p = 0.04$
	$p = 0.23$		**$p = 0.009$**		**$p = 0.05$**	
cGMP (plasma), pmol/mL	14.1 (9.0) ($n = 23$)	17.2 (12.7) ($n = 28$)	14.8 (6.3) ($n = 23$)	21.5 (7.5) ($n = 28$)	0.6 (5.8) ($n = 23$); $p = 0.62$	4.2 (9.2) ($n = 28$); $p = 0.02$
	$p = 0.16$		**$p = 0.0007$**		**$p = 0.01$**	
NT-proBNP, pg/mL[a]	980 (1979) ($n = 23$)	1172 (1671) ($n = 28$)	522 (1061) ($n = 23$)	915 (1146) ($n = 28$)	−458 (1002) ($n = 23$); $p = 0.04$	−256 (1410) ($n = 28$); $p = 0.34$
	$p = 0.17$		**$p = 0.005$**		**$p = 0.05$**	
GDF-15, pg/mL	2656 (2673) ($n = 23$)	5380 (5107) ($n = 28$)	2422 (1813) ($n = 23$)	4803 (2866) ($n = 28$)	−233 (1058) ($n = 23$); $p = 0.30$	−1027 (3900) ($n = 28$); $p = 0.17$
	$p = 0.005$		**$p = 0.001$**		**$p = 1.00$**	

Note: All p values are nominal. p values to the right of the changes from baseline indicate comparisons within responder or nonresponder groups. p values in bold indicate comparisons between responders and non-responders for baseline levels, levels at Week 24, and mean changes from baseline at Week 24 for the biomarker indicated.

Abbreviations: ADMA, asymmetric dimethylarginine; cGMP, cyclic guanosine monophosphate; GDF-15, growth/differentiation factor 15; NT-proBNP, *N*-terminal prohormone of brain natriuretic peptide; PDE5i, phosphodiesterase type 5 inhibitor; SD, standard deviation; ST-2, suppression of tumorigenicity 2.

[a]A reduction in NT-proBNP was part of the response definition. This may have confounded these results.

TABLE 7 | Threshold values for baseline NT-proBNP, GDF-15, and ST-2 predictive of response to riociguat in REPLACE and RESPITE and corresponding response rates (receiver operating characteristic curve analysis).

	REPLACE			RESPITE		
	AUC	Threshold[a]	Responder rate below or above threshold	AUC	Threshold[a]	Responder rate below or above threshold
NT-proBNP, pg/mL	0.571	300	< 300: 18/57 = 32% ≥ 300: 25/51 = 49%	0.631	593	< 593: 18/34 = 53% ≥ 593: 5/26 = 19%
GDF-15, pg/mL	0.629	985	< 985: 33/63 = 52% ≥ 985: 8/38 = 21%	0.748	3604	< 3604: 20/30 = 67% ≥ 3604: 3/23 = 13%
ST-2, μg/L	0.553	14	< 14: 14/30 = 47% ≥ 14: 26/70 = 37%	0.598	19	< 19: 15/28 = 54% ≥ 19: 8/25 = 32%

Abbreviations: AUC, area under the curve; GDF-15, growth/differentiation factor 15; NT-proBNP, *N*-terminal prohormone of brain natriuretic peptide; ST-2, suppression of tumorigenicity 2.
[a]Threshold identified by Youden index.

significantly (nominal $p = 0.05$) smaller increase in cGMP (Table 6). In both studies, NT-proBNP decreased from baseline to Week 24 in responders; however, a reduction in NT-proBNP was part of the response definition.

In the receiver operating characteristic analysis for GDF-15, ST-2, and NT-proBNP, no baseline threshold value was found that had a good likelihood of predicting the response to riociguat, although patients with baseline GDF-15 or ST-2 levels below the threshold tended to show higher response rates (Table 7).

The multivariate logistic regressions for baseline GDF-15, ST-2, and NT-proBNP showed different influences on response between the two studies (Table 8). In RESPITE, the likelihood of response decreased across quartiles of baseline GDF-15 (overall p value 0.0374), while no significant interaction was observed for NT-proBNP or ST-2. In REPLACE, the likelihood of response decreased across quartiles of baseline ST-2 (overall p value 0.0324) and increased across quartiles of NT-proBNP (overall p value 0.0095), with no significant interaction seen for GDF-15. In view of these discordant results, the authors felt that further analyses were unlikely to provide useful information.

3 | Discussion

There is considerable heterogeneity in the clinical phenotype of patients with PAH, which, along with the large number of currently available treatments, has created the need for diagnostic tools that help determine which patients are more likely to respond to a given therapy. In the RESPITE and REPLACE studies, we explored the hypothesis that patients with impaired NO/cGMP signaling would be more likely to respond to stimulation of sGC with riociguat than inhibition of PDE5. We anticipated that higher ADMA levels may impair NO production and lower cGMP synthesis. If so, we expected that lower cGMP levels or higher ADMA levels at baseline would identify patients with impaired cGMP synthesis who were more likely to respond to riociguat. We also expected that a positive clinical response to riociguat would be associated with a greater increase in plasma cGMP. However, in REPLACE, baseline cGMP levels were higher and baseline levels of ADMA were lower in responders than in nonresponders, and no significant differences in baseline levels of these markers were seen between responders and nonresponders in RESPITE. There was also no greater increase in cGMP in responders than in nonresponders in either study. The higher cGMP level at baseline in responders than nonresponders in REPLACE may have indicated a more active NO–sGC–cGMP system, and an increased capacity to respond to riociguat. Lower baseline levels of GDF-15, possibly reflecting less severe PAH, were seen in responders in both studies. However, we found no level of GDF-15 that could discriminate between responders and nonresponders at baseline. Conversely, no differences in baseline ST-2 levels were seen between responders and nonresponders in either study. The multivariate logistic regressions for baseline GDF-15, ST-2, and NT-proBNP showed inconsistent results between RESPITE and REPLACE.

The inability of plasma biomarkers to predict response to riociguat in the current study may be due to several factors. First, circulating levels of biomarkers may not reflect intracellular levels in pulmonary endothelial and vascular smooth muscle cells. Second, some biomarkers are prone to enzymatic degradation and may be affected by how samples are collected, stored, and analyzed. This may have contributed to the large variability in the biomarker levels measured, and discrepancies between RESPITE and REPLACE in baseline biomarker levels, particularly cGMP and GDF-15. Moreover, the within-person change between biomarkers could also be influenced by the natural history of PAH and regression toward the mean, in addition to individual treatment response and measurement errors. The current study used the response definition predefined in the REPLACE trial. This consisted of components of standardized risk assessments, but it is likely to be affected by many variables in addition to the NO−sGC−cGMP pathway. The inclusion of NT-proBNP reduction in the definition of treatment response confounded the analyses of this marker. Finally, this was a post hoc study; all analyses were exploratory, sample sizes were small, biomarker results were not available for all patients, and all p values were nominal and not adjusted for multiplicity or for changes in biomarker levels from baseline to Week 24. Other than NT-proBNP, the biomarkers

TABLE 8 | Logistic regression analyses of biomarkers in REPLACE and RESPITE.

REPLACE				RESPITE			
Biomarker	**OR (95% CI)**	***p* value**[a]	**Overall *p* value**[b]	**Biomarker**	**OR (95% CI)**	***p* value**[a]	**Overall *p* value**[b]
NT-proBNP, pg/mL			0.0095	NT-proBNP, pg/mL			0.4801
Q1 (50.9, 150.3)				Q1 (18.0, 162.0)			
Q2 (150.3, 351.7)	1.117 (0.389, 3.204)	0.8374		Q2 (162.0, 457.5)	2.884 (0.410, 20.286)	0.2871	
Q3 (351.7, 887.9)	4.402 (1.640, 11.812)	0.0033		Q3 (457.5, 1007.5)	0.637 (0.090, 4.535)	0.6529	
Q4 (887.9, 9634.9)	1.595 (0.562, 4.530)	0.3807		Q4 (1007.5, 8,402.0)	3.113 (0.160, 60.639)	0.4535	
GDF-15, pg/mL			0.2087	GDF-15, pg/mL			0.0374
Q1 (191.5, 523.7)				Q1 (482, 1752)			
Q2 (523.7, 807.5)	0.813 (0.325, 2.036)	0.6587		Q2 (1752, 2520)	4.373 (0.653, 29.261)	0.1282	
Q3 (807.5, 1441.9)	0.489 (0.192, 1.248)	0.1345		Q3 (2520, 6078)	0.473 (0.062, 3.602)	0.4696	
Q4 (1441.9, 8187.1)	0.362 (0.123, 1.066)	0.0652		Q4 (6078, 22,092)	0.024 (< 0.001, 0.749)	0.0337	
ST-2, mg/L			0.0324	ST-2, mg/L			0.4407
Q1 (6.0, 12.6)				Q1 (3.8, 11.9)			
Q2 (12.6, 16.9)	0.300 (0.116, 0.776)	0.0131		Q2 (11.9, 16.3)	0.720 (0.107, 4.830)	0.7349	
Q3 (16.9, 23.7)	0.275 (0.099, 0.760)	0.0129		Q3 (16.3, 25.0)	0.294 (0.030, 2.853)	0.2912	
Q4 (23.7, 43.1)	0.336 (0.116, 0.978)	0.0455		Q4 (25.0, 92.3)	2.098 (0.202, 21.772)	0.5347	

Note: OR is the odds ratio of responder/nonresponder for each quartile, with Q1 as the reference quartile. The values in parentheses after Q1, Q2, Q3, and Q4 are the ranges of the quartiles.
Abbreviations: CI, confidence interval; GDF-15, growth/differentiation factor 15; NT-proBNP, *N*-terminal prohormone of brain natriuretic peptide; OR, odds ratio; Q, quartile; ST-2, suppression of tumorigenicity 2.
[a]*p* value is based on Wald test.
[b]Test of equal proportions between categories of biomarkers.

evaluated in the current study are not validated as diagnostic or prognostic markers in PAH, and they may be influenced by concomitant diseases.

Overall, our results do not support a predictive role for baseline levels of the evaluated biomarkers in identifying patients with PAH who are likely to respond to switching from PDE5i to riociguat. However, future studies should continue to investigate the potential of biomarkers to guide treatment decisions for patients who do not achieve a low 1-year risk of mortality following initial PAH therapy. These studies will likely require examination of a greater number of patients and more extensive choice of biomarkers or the use of proteomic or transcriptomic approaches. Proteomic analyses have been applied to the diagnosis, risk stratification, and assessment of disease severity in patients with PAH [25–29]. Another possibility is to derive a composite biomarker score, as has been applied in interstitial lung disease associated with systemic sclerosis [30]. The authors examined 28 biomarkers, and defined important biomarkers as those with univariate area under the curve (AUC) > 0.6. They then performed multivariate analyses of markers with AUC > 0.6, which also satisfied further statistical criteria. In the current study, the only biomarkers with AUC > 0.6 were GDF-15 and NT-proBNP in RESPITE and GDF-15 in REPLACE; therefore, multivariate analyses would not be feasible. Alternatively, it may be necessary to examine cellular markers, rather than serum or plasma protein biomarkers that were examined in the present study. Recent studies have shown the potential of examining pulmonary endothelial cells obtained during right heart catheterization [31] or pluripotent cells derived from skin biopsy [32].

Author Contributions

M.M.H., H.-A.G., J.R.K., G.S., and C.M. contributed to the design of the RESPITE and REPLACE studies. J.R.K., H.A.-H., S.-A.C., H.J.C., H.-A.G., E.G., M.M.H., P.J., J.O.-A., T.P., G.S., C.D.V., and G.M. collected and interpreted the data for the REPLACE study. J.R.K., H.-A.G., E.G., M.M.H., P.J., G.S., and C.D.V. collected and interpreted the data for the RESPITE study. M.M.H., J.R.K., C.D.V., and C.M. accessed and verified all data. C.R. performed the statistical analyses. All authors critically reviewed and revised the manuscript and approved the final version for publication. All authors had full access to the data in the manuscript and had final responsibility for the decision to submit for publication.

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Ethics Statement

The institutional review board at each participating centre approved the protocol for RESPITE, and the study was carried out in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. All patients provided written informed consent. The institutional review board at each participating centre approved the protocol for REPLACE, and the study was carried out in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. All patients provided written informed consent. The biomarker analyses reported in the current paper were performed on samples obtained during the trials. No further samples were taken and no further patient interventions or examinations were performed.

Conflicts of Interest

James R. Klinger has no conflicts of interest to declare. Hikmet Al-Hiti has no conflicts of interest to declare. Sung-A. Chang reports consultancy from Bayer AG, Daewoong, Janssen, and Samjin; honoraria from Antregen and Janssen; and membership of an advisory board for Janssen. Hyuk J. Chang has no conflicts of interest to declare. Hossein-Ardeschir Ghofrani reports grants from Actelion, Bayer AG, Ergonex, and Pfizer; personal fees from Actelion, Bayer AG, Ergonex, Gilead, GSK, Merck, Novartis, and Pfizer; and is currently on the independent data monitoring committee for two studies funded by Actelion. Ekkehard Grünig reports research grants from Actelion, Bayer AG, Ferrer, Janssen, Merck, and MSD; consultancy fees from Actelion, Bayer AG, Ferrer, Janssen, Merck, and MSD; speaker honoraria from Actelion, AOP, Bayer AG/MSD, Ferrer, GEBRO, GSK, GWT, Janssen, OMT, and Pulmonale Hypertenie e.V.; support for attending meetings from Janssen; and membership of advisory boards for Ferrer and MSD. Marius M. Hoeper reports personal fees from Acceleron, Actelion, AOP, Bayer AG, Ferrer, Janssen, and MSD. Pavel Jansa reports grants from Actelion-Janssen and AOP Orphan; consultancy fees from Actelion-Janssen, AOP Orphan, Bayer HealthCare, and MSD; honoraria from Actelion-Janssen, AOP Orphan, and MSD; meeting attendance for AOP Orphan; and membership of advisory boards for Actelion-Janssen and AOP Orphan. Jacquelina Ota-Arakaki has no conflicts of interest to declare. Tomas Pulido reports grants and personal fees from Actelion–Janssen; grants from United Therapeutics, MSD Pharmaceuticals, and Bayer AG, and personal fees from Bayer AG and Pfizer. Gérald Simonneau reports personal fees and nonfinancial support from Actelion, Bayer AG, and MSD. Carmine Dario Vizza has no conflicts of interest to declare. Claudia Rahner was an external employee of Bayer AG at the time the studies were conducted and analyzed. Christian Meier is an employee of Bayer AG. Gisela Meyer reports lecture and consultation fees from Bayer AG, Eli Lilly, and GSK.

Data Availability Statement

Availability of the data underlying this publication will be determined according to Bayer's commitment to the European Federation of Pharmaceutical Industries and Associations and Pharmaceutical Research and Manufacturers of America principles for responsible clinical trial data sharing, pertaining to scope, timepoint, and process of data access. Bayer commits to sharing upon request from qualified scientific and medical researchers patient-level clinical trial data, study-level clinical trial data, and protocols from clinical trials in patients for medicines and indications approved in the USA and European Union as necessary for doing legitimate research. This commitment applies to data on new medicines and indications that have been approved by the European Union and US regulatory agencies on or after January 1, 2014. Interested researchers can use www.clinicalstudydatarequest.com to request access to anonymized patient-level data and supporting documents from clinical studies to do further research that can help advance medical science or improve patient care. Information on the Bayer criteria for listing studies and other relevant information is provided in the study sponsors section of the portal. Data access will be granted to anonymized patient-level data, protocols, and clinical study reports after approval by an independent scientific review panel. Bayer is not involved in the decisions made by the independent review panel. Bayer will take all necessary measures to ensure that patient privacy is safeguarded.

Guarantor

James R. Klinger is the guarantor of this paper and takes responsibility for the integrity of the work as a whole.

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