Pharmacogenetic analysis of belimumab fails to identify robust genetic predictors of efficacy in lupus

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GlaxoSmithKline (GSK) conducted pharmacogenetic (PGx) analyses to determine whether genetic variants influence response to belimumab treatment in patients with systemic lupus erythematosus (SLE). We conducted an exploratory genome-wide meta-analysis (GWAS) of 10.9 million genetic variants and the efficacy data from 816 belimumab-treated SLE patients in three phase 3 belimumab clinical studies. Two highly correlated variants, rs293983 and rs364370, in the *ANO3* (anoctamin 3) gene region were significantly associated with efficacy as measured by the SLE Response Index (SRI4) with a per-T-allele odds ratio (OR) of 2.15 [95% confidence interval (CI): 1.66–2.79, $P = 8.0 \times 10^{-9}$]. In contrast, there was no association with SRI4 response in 577 placebo-treated patients (per-T-allele OR: 0.98; 95% CI: 0.74–1.29, $P = 0.87$). A post-hoc analysis by geographic region revealed a strong SRI4 response signal in 157 belimumab-treated patients from Asia (per-T-allele OR = 2.85, 95% CI: 1.41–5.74, $P = 0.0021$). On the basis of this encouraging finding in Asian patients, we conducted a confirmatory analysis of the SRI4 end point in an independent phase 3 study of SLE patients from northeast Asia. We found no evidence of an association between rs293983 and SRI4 response in 204 belimumab-treated patients (per-T-allele OR: 0.90, 95% CI: 0.52–1.57, $P = 0.64$). The inability to replicate the observed GWAS effect suggests this was a false positive result; hence, we failed to identify any genetic variants significantly associated with belimumab efficacy. *Pharmacogenetics and Genomics* 29:132–135 Copyright © 2019 The Author(s). Published by Wolters Kluwer Health, Inc.

Introduction

Systemic lupus erythematosus (SLE or lupus) is a chronic autoimmune disorder characterized by autoantibody production and abnormal B-lymphocyte function [1]. Genetics, sex hormones, and environmental conditions are believed to contribute to lupus [1–3]. Lupus is more prevalent in women (∼90% of patients) than men [4] and the incidence and prevalence vary across racial populations. The lowest prevalence is observed in Europeans and the highest prevalence in African populations. African and South Asian populations, respectively, have a 5–9- and 1.2–6-fold increased incidence and 2–3- and 2–2.4-fold increased prevalence compared with Europeans [5]. Lupus manifestations vary among patients and the American College of Rheumatology criteria for lupus diagnosis requires that four of 11 criteria be met.

BENLYSTA (belimumab) is a B-lymphocyte stimulator (BLyS)/B-cell activating factor (BAFF)-specific inhibitor indicated for the treatment of adult patients with active, auto-antibody-positive SLE who receive standard therapy. Belimumab does not directly bind B cells but, by binding BLyS, inhibits the survival of B cells, including autoreactive B cells, and reduces their differentiation into immunoglobulin-producing plasma cells. An intravenous formulation of belimumab is approved in over 65 countries; as of January 2018, a subcutaneous formulation is approved in the USA, EU, Japan, and Canada and has been submitted in other countries.

Precision medicine, which includes the integration of clinical, genomic, and molecular profiling into medical practice, aims to increase the cost-effectiveness of healthcare and improve patient outcomes. The presence of antinuclear autoantibodies and low complement C3 and C4 levels are established clinical criteria that differentiate lupus from other autoimmune diseases. We conducted pharmacogenetic (PGx) analyses to determine whether genetic variants identify lupus patients who preferentially respond to belimumab. This paper describes an exploratory belimumab PGx efficacy analysis, identification of two significantly associated and completely correlated genetic variants in the *ANO3* (anoctamin 3) gene region, and an unsuccessful attempt to confirm the association in an independent study.

Patients and methods

Patients in the GSK phase 3 Benlysta (belimumab; GlaxoSmithKline, Brentford, UK) clinical program met American College of Rheumatology criteria and entered the studies on stable lupus therapy. The primary efficacy end point, the SLE Response Index (SRI4) integrates three validated lupus instruments measuring disease activity (SELENA SLEDAI), organ worsening (British Isles Lupus Assessment Group or BILAG) and overall patient condition (Physician's Global Assessment) [6]. SRI4 defines efficacy as at least a four-point reduction in the SELENA SLEDAI disease activity rating, no new BILAG A organ domain or two new BILAG B organ domain scores, and no worsening (<0.30 increase) in the Physician's Global Assessment compared with baseline. In accordance with the Declaration of Helsinki [7] and following ethics committee approval, randomized patients were invited to participate in genetic research. Participation was optional and required written informed consent and collection of a 6 ml blood (DNA) sample. Patients were permitted to withdraw consent at any time. The composition of the analysis populations is summarized in Table 1. Genotyping and imputation details for the exploratory analyses, including Manhattan, Q–Q, and principal component analysis plots are summarized in the Supplementary Figs 1–3, respectively (Supplemental digital content 1, *http://links.lww.com/FPC/B352*).

Analysis
Exploratory analysis
The genetic analysis of belimumab efficacy was conducted using the 10 mg/kg intravenous [BEL110751 (*https://clinicaltrials.gov*, NCT00410384) and BEL110752 (*https://clinicaltrials.gov*, NCT00424476)] belimumab treatment groups and 200 mg subcutaneous [BEL112341 (*https://clinicaltrials.gov*, NCT01484496)] belimumab treatment group. The 1 mg/kg intravenous arm was not included as this dose did not meet the primary efficacy objective in one phase 3 intravenous study. Because two genotyping platforms were used, analyses were conducted separately in the combined BEL110751 and BEL110752 studies and the BEL112341 study to control for technical batch effects. A meta-analysis was then conducted using effect estimates from the two aforementioned analyses. Five efficacy end points were evaluated as described on the GSK Clinical Study Register (*https://www.gsk-clinicalstudyregister.com/study/204901*). This paper focuses on the primary SRI4 end point that had the strongest statistical association. The association of genotype with the binary SRI4 efficacy outcome was assessed in the combined BEL110751 and BEL110752 analysis and BEL112341 by logistic regression, assuming an additive genetic model and adjusting for 10 genetic ancestry principal components, baseline SELENA SLEDAI scores and baseline C3 and C4 complement levels. Genomic control adjustments corrected for test statistic inflation. Fifteen candidate variants in nine genes were included on the basis of functional significance or suggestive trends from previous exploratory PGx investigations. Additional variants in these genes were included in the GWAS. Genome-wide variants with minor allele frequencies of at least 1% were analyzed. A fixed effect variance weighted meta-analysis was then conducted using the effect estimates from the by-study analyses. Prespecified statistical significance thresholds for the meta-analysis results were as follows: P values of less than or equal to 0.0017 for candidate variants and P values

Table 1 Composition of the PGx analysis populations and SRI4 response rates

	ITT population	PGx population as % of ITT	PGx sample size	PGx population by racial population and percent representation in the analysis population
Exploratory meta-analysis comprised of the following studies: Two phase 3 belimumab intravenous (i.v.) clinical studies (BEL110751 and BEL110752) Phase 3 clinical study of belimumab subcutaneous (s.c.) (BEL112341)	2499	71	1774[a]	927 (52%) European Caucasian 354 (20%) Asian 339 (19%) American Indian or Alaskan native (primarily from Central and South America) 143 (8%) African American 11 (<1%) Other
Confirmatory study: BEL113750 randomized 2:1 to belimumab or placebo i.v.	677	43	291[a]	215 (74%) Chinese 44 (15%) Korean 32 (11%) Japanese

SRI4 response rate in the PGx ITT population across the four clinical studies	SRI4 placebo % (95% CI)	SRI4 belimumab % (95% CI)	Difference
BEL110751 i.v.	34.2 (27.3–41.2)	41.8 (34.7–49.0)	7.6
BEL110752 i.v.	42.6 (35.4–50.0)	55.0 (48.0–62.0)	12.4
BEL112341 s.c.	53.8 (47.0–60.6)	63.4 (58.9–68.0)	9.6
BEL113750 s.c.	37.9 (27.5–48.3)	49.5 (42.6–56.4)	11.6
Average response rate	43.2 (39.4–47.0)	55.1 (52.0–58.2)	11.9

BEL110751 included SLE patients from Austria, Belgium, Canada, Costa Rica, Czech Republic, France, Germany, Israel, Italy, Mexico, Netherlands, Poland, Puerto Rico, Romania, Slovakia, Spain, Sweden, UK, and the USA.
BEL110752 included SLE patients from Argentina, Australia, Brazil, Chile, Colombia, Hong Kong, India, Peru, Philippines, Romania, Russia, South Korea, and Taiwan.
BEL112341 included SLE patients from Argentina, Austria, Belgium, Brazil, Bulgaria, Chile, Columbia, Croatia, Czech Republic, Denmark, France, Germany, Hungary, Italy, Japan, Malaysia, Mexico, Philippines, Poland, Portugal, Romania, Russian Federation, Serbia, Spain, Sweden, Taiwan, Thailand, Ukraine, UK, and the USA.
BEL113750 included SLE patients from China, Japan, and South Korea.
CI, confidence interval; ITT, intent-to-treat; i.v., intravenous; PGx, pharmacogenetic; s.c., subcutaneous; SRI4, SLE Responder Index.
[a]Received at least one dose of belimumab or placebo in addition to standard of care and were successfully genotyped.

of less than or equal to 2.50×10^{-8} for genome-wide variants to control for the family-wise error rate of 0.05 per end point. Per-allele odds ratios (ORs) with 95% confidence intervals (CIs) were generated. To aid in results interpretation, the placebo groups were analyzed in the same manner as the belimumab treatment groups (single-study analyses followed by a meta-analysis of the effect estimates).

Confirmatory analysis (https://www.gsk-clinicalstudyregister.com/study/207521)

The rs293983 variant was modeled as T-allele dose (0, 1, or 2 T alleles corresponding to genotypes CC, CT, or TT, respectively) or T-allele carriage (CT/TT vs. CC). Association of rs293983 with SRI4 response in belimumab-treated patients in BEL113750 was tested using logistic regression adjusting for the country, baseline SELENA SLEDAI scores, and baseline complement levels. A one-tailed test, with a prespecified significance threshold of P less than or equal to 0.05 was used. The placebo group was analyzed to aid interpretation.

Results and discussion

In the exploratory meta-analysis, 10.9 million genetic variants were analyzed and two completely correlated ($r^2 = 1.0$) genetic variants in the *ANO3* gene region, rs293983 and rs364370, were associated with belimumab efficacy as measured by SRI4 with the T-allele of both variants being associated with improved response (Table 2). These variants were not associated with placebo response, suggesting the variants might be predictive of drug response rather than prognostic of lupus progression [8]. No candidate variants were associated with belimumab efficacy (Supplementary Table 1, Supplemental digital content 1,

http://links.lww.com/FPC/B352), including four variants in the *TNFSF13B/BAFF/BLYS* gene. Recently, genetic variants in the *BAFF* gene, rs12874404 and *BAFF-var* [a combination of rs374039502 and an insertion-deletion variant GCTGT→A (rs200748895)], were associated with increased levels of soluble BAFF, B lymphocytes, and immunoglobulins in a predominantly Sardinian SLE population [9]. The OR for the carriage of *BAFF-var* and SLE risk was ~1.4. The authors hypothesized that these variants may predict response to B-cell depleting therapies, including belimumab. The *BAFF* genetic variants are more frequent (33%) among Sardinians than other populations (Europeans ~3–5%). While we did not directly genotype these variants in our 2015 GWAS analysis, rs200748895 was imputed (imputation $r^2 = 0.73$). This variant was not associated with belimumab efficacy as measured by the SRI4 end point ($P = 0.84$), although we had less than 10% power to detect an association for an additive per-allele OR of 2 for variants with a minor allele frequency of 5% or less.

Post-hoc analyses of BEL110751 and BEL110752 identified baseline disease activity characteristics that predict moderate to severe SLE flare, including baseline BLyS levels of at least 2 ng/ml [10]. Treatment differences for SRI responses at week 52 between belimumab 10 mg/kg and placebo were greater in the group with BLyS of at least 2 ng/ml (24.1%, $P < 0.0001$) compared with the group with BLyS of less than 2 ng/ml (8.2%, $P = 0.0158$) and the risk of severe flare was reduced with belimumab versus placebo in the group with BLyS of at least 2 ng/ml ($P = 0.0002$). Patients with baseline BLyS levels of at least 2 ng/ml had a poorer response to standard background therapy and benefited from belimumab treatment, presumably through belimumab's inhibition of BLyS. Taken

Table 2 Analysis of rs293983 *ANO3* gene region variant and belimumab efficacy as measured by change in the SRI4 response index from baseline to the week 52 end point

Study	Treatment added to standard of care	rs293983 genotype (number of patients)	Percentage of patients who Met SRI4 responder criteria (95% CI)	Per-T-allele odds ratio (95% CI)	P value
Exploratory meta-analysis[a]	Belimumab 10 mg/kg i.v. or 200 mg s.c. ($N = 816$[a])	CC (382)	47.4 (42.4–52.4)	2.15 (1.66–2.79)	8.0×10^{-9}
		CT (328)	61.6 (56.3–66.9)		
		TT (73)	82.2 (73.2–91.2)		
	Placebo ($N = 577$)	CC (281)	44.1 (38.3–50.0)	0.98 (0.74–1.29)	0.87
		CT (232)	42.2 (35.8–48.6)		
		TT (50)	44.0 (29.7–58.3)		
Exploratory post-hoc analysis (Asian subset)	Belimumab 10 mg/kg i.v. or 200 mg s.c. ($N = 153$)	CC (92)	42.4 (32.8–52.6)	3.15 (1.52–6.51)	0.0019
		CT (54)	66.7 (53.4–77.8)		
		TT (7)	85.7 (48.7–97.4)		
Confirmatory analysis	Belimumab ($N = 204$)	CC (137)	48.9 (40.4–57.4)	0.90 (0.52–1.57)	0.64
		CT (62)	51.6 (38.8–64.4)		
		TT (5)	40.0 (0–100)		
	Placebo ($N = 87$)	CC (54)	40.7 (27.2–54.3)	0.75 (0.33–1.69)	0.76
		CT (29)	34.5 (16.1–52.9)		
		TT (4)	25.0 (0–100)		

Results for rs364370 (not shown) are comparable because of near complete linkage disequilibrium between rs293983 and rs364370.
For the SRI4 response by genotype, dosage data was translated to genotype as follows:
If dosage was ≤ 0.2 then genotype was 'CC'; if 0.9 ≤ dosage ≤ 1.1 then genotype = 'CT'; if dosage was ≥ 1.8 then genotype = 'TT'. For the belimumab and placebo arms, 33 of 816 (4%) and 14 of 577 (2%) dosages, respectively, were not translatable to genotype.
CI, confidence interval; i.v., intravenous; s.c., subcutaneous; SRI4, SLE Responder Index.
[a]In the exploratory analysis, the odds ratio and P value are based on the imputed dosage data.

Table 3 SRI4 response rates by clinical study

Analysis population	rs293983 odds ratio (95% CI)	*P* value	T allele frequency (based on dosage)
BEL110751/BEL110752 i.v. (*N* = 384)	2.25 (1.56–3.23)	5.79E − 6	0.296
BEL112341 s.c. (*N* = 432)	2.06 (1.43–2.97)	6.65E − 5	0.317
Meta-analysis (*N* = 816)	2.15 (1.66–2.79)	8.00E − 9	0.306

While not meeting genome-wide significance thresholds, the odds ratios and direction of effect for rs293983 were similar between BEL110751/BEL110752 that used the intravenous formulation of belimumab and BEL112341 that used the subcutaneous formulation of belimumab.
CI, confidence interval; i.v., intravenous; s.c., subcutaneous.

together, these data are consistent with belimumab efficacy in patients with elevated BLyS/BAFF levels.

Because the GWAS meta-analysis is an exploratory analysis of millions of genetic variants, subject to many potential sources of bias and technical artifacts, it is important to confirm any novel associations in an independent dataset. We determined that Study BEL113750 (*http://www.clinicaltrials.gov identifier NCT01345253*), a 52-week study of belimumab versus placebo added to standard of care in Northeast Asian lupus patients, provided the best opportunity to evaluate the preliminary association as this study included the SRI4 composite efficacy end point. In the meta-analysis, the SRI4 belimumab response rate was 54.1% in Asians (10 mg/kg intravenous or 200 mg subcutaneous) and 57.0% in non-Asians. The preliminary association between rs293983 and belimumab efficacy was not confirmed in BEL113750 (Table 2). These negative results are unlikely to be due to a lack of generalizability of the preliminary association to an Asian population; a post-hoc analysis of the exploratory meta-analysis revealed the preliminary genetic association was observed in the subset of Asian patients treated with belimumab (*n* = 157, ~19% of the meta-analysis study population) (per-T-allele OR = 2.85, 95% CI: 1.41–5.74, *P* = 0.0021) was slightly stronger than in the overall exploratory meta-analysis. Furthermore, while the preliminary association did not meet genome-wide significance criteria in the underlying studies (BEL110751/BEL110752 that included belimumab intravenous and BEL112341 that included belimumab subcutaneous), carriage of the rs293983 T allele was enriched among the SRI4 responders (Table 3). The sample size of the confirmatory study, BEL113750, had 85% power to confirm the original OR of 2.15. The BEL113750 PGx analysis group was very similar to the GWAS discovery population with respect to age, sex, SLE duration, and baseline SELENA SLEDAI scores. The most likely explanation is that the preliminary PGx associations were false positives. This was not entirely surprising as investigation of the *ANO3* gene region did not identify plausible biological mechanisms that might explain improved belimumab efficacy in carriers of *ANO3* gene variants. Our

failure to replicate GWAS findings emphasizes the importance of replication cohorts to confirm or refute preliminary associations. The failure to identify statistically significant associations does not imply that there are no genetic influences on belimumab efficacy, but that they are unlikely to be common enough or large enough to be identified in a study of this size. Future studies with larger sample sizes may support further explorations of genetic contributions to belimumab response.

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