CLINICAL STUDY REPORT

1 Title Page

Study title:	Efficacy of belimumab to improve subclinical cardiovascular abnormalities using imaging endpoints with cardiac magnetic resonance in patients with systemic lupus erythematosus (BeCarma)
Investigational product:	Belimumab (Benlysta®), 200 mg, once weekly, s.c.
Indication studied / study population:	Patients with systemic lupus erythematosus (SLE) and subclinical cardiovascular abnormalities
Study Design:	Randomized, open-label, proof-of-concept clinical study
Sponsor:	Fraunhofer Society for its Fraunhofer Institute for Translational Medicine and Pharmacology ITMP, Director Prof. Dr. med. Dr. rer. nat. Gerd Geißlinger Theodor-Stern-Kai 7, 60596 Frankfurt a.M., Germany
Representative of the Sponsor:	Prof. Dr. med. Frank Behrens, Fraunhofer Institute for Translational Medicine and Pharmacology ITMP Theodor-Stern-Kai 7, 60596 Frankfurt a.M., Germany
Principle investigator:	PD Dr. med. Michaela Köhm, Fraunhofer Institute for Translational Medicine and Pharmacology ITMP Theodor-Stern-Kai 7, 60590 Frankfurt a.M., Germany
Protocol code:	TMP-3001-2020-30
EudraCT-No.:	2021-001802-30
Phase:	IV
Study initiation date:	First patient 1st visit: 14.09.2022
Study completion date:	Last patient last visit: 24.06.2024 Termination of study for further recruitment/study conduct: 09.02.2024 Database hard lock: 07.11.2024 Study end per protocol: Last site closure 26.11.2024
Responsible medical officer:	Dr. med. Stephan Schäfer, Fraunhofer Institute for Translational Medicine and Pharmacology ITMP Theodor-Stern-Kai 7, 60596 Frankfurt a.M., Germany
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Report date:	31.03.2025

The clinical trial was carried out in compliance with the principles of the Declaration of Helsinki, Good Clinical Practice (GCP) and the applicable laws.

2 Synopsis

Name of Sponsor	Fraunhofer Gesellschaft for its Fraunhofer Institute for Translational Medicine and Pharmacology ITMP, Director Prof. Dr. med. Dr. rer. nat. Gerd Geißlinger Theodor-Stern-Kai 7 60596 Frankfurt a.M., Germany
Representative of the sponsor	Prof. Dr. med. Frank Behrens, Fraunhofer Institute for Translational Medicine and Pharmacology ITMP Theodor-Stern-Kai 7 60596 Frankfurt a.M., Germany
Investigational product (IMP)	Belimumab (Benlysta®), 200 mg, once weekly, s.c.
Title of Study	Efficacy of belimumab to improve subclinical cardiovascular abnormalities using imaging endpoints with cardiac magnetic resonance in patients with systemic lupus erythematosus (BeCarma)
Design	Randomized, open-label, proof-of-concept clinical study
Principal Investigator	Dr. med. Michaela Köhm, Fraunhofer Institute for Translational Medicine and Pharmacology ITMP Theodor-Stern-Kai 7, 60590 Frankfurt a.M., Germany
Study sites and location (multicentric)	**PD Dr. med. Michaela Köhm** Phase 1 Station Fraunhofer Institut für Translational Medizin & Pharmakologie ITMP Theodor-Stern-Kai 7 D-60596 Frankfurt a. M. **Prof. Dr. med. Ulf Müller-Ladner** Kerckhoff-Klinik Forschungsgesellschaft mbH Abteilung für Rheumatologie und klinische Immunologie Küchlerstraße 10 D-61231 Bad Nauheim
Publication (reference)	Not yet published
Studied Period	4 years (Q2/2022 – Q4/2026) Recruitment phase: Q2/2022 - Q2/2024 First patient in: 14.09.2022 (FPFV) Last patient out: 24.06.2024 (LPLV) Study Termination for recruitment/study conduct: 09.02.2024 Database hard lock: 07.11.2024 Study end: Study end per protocol: Last site closure 26.11.2024
Phase of study	IV
Primary objective	To determine myocardial changes due to diffuse inflammatory involvement measured by T1 and T2 mapping and first pass perfusion imaging under maximal vasodilation in SLE patients with new initiation of belimumab or continued standard-of care therapy at W24 compared to BL
Secondary objectives	Assessment and treatment comparison of the following endpoints: • Assessment of cardiovascular risk factors and function (deformation and cardiac function (longitudinal strain, left ventricular (LV) volume and mass, ejection fraction, late gadolinium enhancement (LGE), ECG, echocardiography), aortic stiffness (pulse wave velocity), myocardial triglyceride and creatine content (only in a subgroup of patients) • Assessment of cardiovascular risk factors and cardiac biomarkers measured in blood (lipids, triglycerides, VLDL, cholesterine, HDL, LDL, glucose, HbA1c, (apo)lipoproteine levels, hs-troponin T, NT-BNP, hsCRP) • Assessment of antiphospholipid profile (lupus anticoagulant, anticardiolipin and anti-beta 2 GPI) • Measurement of vital signs (blood pressure, heart rate) • Assessment of cardiac echocardiography

	• Determination of SLE disease activity and damage (SLEDAI 2k, S2k Responder Index-50, SLICC damage index, CRP/ESR, PGA) • Incidence of cardiovascular abnormalities • Patient global impression of change (PGIC) • Quality of Life (LupusQoL) • Fatigue (Facit-Fatigue subscale) • Suicidal behaviour (CSSRS) • Compliance of belimumab intake and SLE therapy • Assessment of treatment changes • Safety (Frequency, seriousness and number of adverse events) • Rise in cardiac biomarkers and frequency of worsening of disease • Subgroup analysis depending on previous SLE therapies
Methodology	**Open-label, interventional proof-of-concept study** SLE patients with stable disease activity (no worsening of disease within the last 12 weeks as defined in inclusion criteria) with no clinically manifest cardiovascular symptoms, but cardiac abnormalities in CMR were included. Patients were randomised 1:1 to belimumab or continuation of current standard-of-care SLE therapy. If a pericarditis was diagnosed at BL (or during the study) colchicine was administered as indicated (max. 0,5 mg once daily) in both groups. Patients who changed SLE therapy after W24 have switched to rescue arm. These patients performed all further planned study visits. Patients were planned to be assessed over a 2-year period. CMR was performed at BL, W24, W52, W104. SLE disease activity was assessed at every study visit.
Number of patients (planned and analyzed)	Planned sample size: n=56, 28 pts. in each treatment group Screened patients: 8 Randomized patients: 4 Analyzed: 8 (screened patients); 4 (FAS and safety set) Drop-outs: 0 Screening failures: 4
Diagnosis and main criteria for inclusion	Main inclusion criteria: 1. Established diagnosis of SLE as per American College of Rheumatology revised classification criteria 2. Stable disease (SLEDAI < 6 and no treatment change) over the last 12 weeks 3. Cardiac abnormalities of recovery rate of T1 or T2 relaxation detected in CMR, measured in a midventricular SAX slice conservatively within the septal myocardium defined as follows: 4. Native T1 ≥ 4SD: 1.5T 1050ms, 3.0T 1150 ms or 5. Native T2 ≥ 4SD: 1.5T 50ms, 3.0T 40 ms 6. If pericarditis is diagnosed: 7. colchicine therapy will be started with a maximum of 0,5 mg once daily as indicated 8. If concomitant cortisone treatment: stable dose (≤ 10 mg/day) within the last 2 weeks 9. Patients with a BMI of 18-35 kg/m² 10. Patients aged 18 - 75 years 11. Written informed consent obtained prior to the initiation of any protocol-required procedures by the patient 12. Willingness to comply to study procedures and study protocol
Test Product, dose and mode of administration, batch number, duration of treatment	Investigational medicinal product: Benlysta® Active substance: Belimumab Dosage form: injection Single dose: 200 mg once weekly Duration of treatment: once weekly/ 24 month Mode of application: subcutaneous Batch number: BECARMA-202216/BECARMA-202217
Criteria for evaluation	**Primary efficacy analysis:**

	• Absolute change in diffuse myocardial inflammation and myocardial blood flow using T1 and T2 mapping and first pass perfusion imaging at W24 compared to baseline between the treatment groups.
	
	Secondary efficacy analysis:
	Secondary variables will be analysed at all available visits for both treatment groups.
	
	For all continuous endpoints, "changes" refer to the difference between the visit measurement and baseline (absolute and in %):
	
	• T1 and T2 (native) values and change thereof compared to BL
	• Longitudinal strain and change thereof compared to BL
	• Left ventricular (LV) volume and mass and change thereof compared to BL
	• Ejection fraction and change thereof compared to BL
	• Late gadolinium enhancement (LGE) and change thereof compared to BL
	• Pulse wave velocity and change thereof compared to BL
	• Changes in values in cardiac echocardiography and ECG
	• Presence and extent of coronary artery calcification and soft plaques
	• SLEDAI score and change to BL
	• Sledai-2k responder index-50 (S2k RI-50) and change to BL
	• Proportion of patients with partial and complete recovery according to Sledai-2k and S2k RI-50
	• SLICC damage index score and change to BL
	  o Proportion of patients that fulfil SLICC criteria at BL. The SLICC criteria for SLE classification require: 1) Fulfillment of at least four criteria, with at least one clinical criterion AND one immunologic criterion, OR
	  o 2) Lupus nephritis as the sole clinical criterion in the presence of ANA or anti-dsDNA antibodies)
	• Physicians' global assessment (PGA) and change to BL
	• Changes in cardiovascular risk factors and cardiac biomarkers in blood (lipids, triglycerides, VLDL, cholesterine, HDL, LDL, glucose, HbA1c, (apo)lipoproteine levels, hs-troponin T, hs-CRP, NT-BNP) and CRP and ESR compared to BL
	• Changes in antiphospholipid profile compared to BL
	• Blood pressure, heart rate and change thereof compared to BL
	• Patient global impression of change (PGIC) score
	• LupusQoL Score and change to BL
	• Facit-Fatigue subscale and change to BL
	• CSSRS score and change to BL
	• Oral corticosteroid dose
	• Benlysta®/ SOC intake (empty syringes/pens) and patient diary information for drug accountability / compliance
	• Proportion of patients with a treatment change and proportion of patients with switch to rescue arm; reasons for change /switch
	• Previous therapies for subgroup analyses using primary and other secondary endpoints
	• Analysis of myocardial triglyceride and creatine content through proton magnetic resonance spectroscopy (1H-MRS) (only for a subgroup of patients)
	
	Safety:
	• Frequency, seriousness and number of adverse events and adverse events of special interest
	• Frequency of worsening of disease (defined as a decreased PGA score) and proportion of patients with worsening of disease
	• Number of cardiovascular events and proportion of patients with at least one event
	• Incidence of cardiac abnormalities
	• Rise in cardiac biomarkers

Statistical methods	**<u>Primary Analysis:</u>**

	• Objective: To determine changes in diffuse myocardial diffusion measured by T1 and T2 mapping and first pass perfusion imaging under maximal vasodilation in SLE patients with new initiation of belimumab or continued standard-of care therapy at W24 compared to BL ○ Endpoint: Absolute change in diffuse myocardial inflammation and myocardial blood flow using T1 and T2 mapping (native) and first pass perfusion imaging at W24 compared to baseline between treatment groups (Belimumab (Benlysta®) vs. SOC). For primary analysis, the following was planned to analyse the change in diffuse myocardial inflammation and myocardial blood flow using native T1 and T2 mappings and first pass perfusion imaging at W24 compared to baseline between the treatment groups. For T1 and T2 mappings, univariable linear mixed models with repeated measures was planned to be used for analyses, including time, treatment and time-by treatment interaction. The hypothesis of H0: $\mu_{BEL} = \mu_{Soc}$; H1: $\mu_{BEL} \neq \mu_{Soc}$ with μ representing the expectation value of the T1 respectively T2 change from BL at W24 will be tested confirmatively at the 2.5% significance level in a two-sided manner using linear contrasts representing the treatment difference of the model at W24. Center effect was planned to be modelled as random effects and covariables such as demographics would have been considered as fixed effects if it benefits the model (backward selection, AIC criteria). Kenward-Roger's adjustment would have been considered due to the reduced sample size. Covariance structure was planned to be chosen based on best model fit (CS, AR (1), unstructured). Patients were planned to be evaluated "as randomized" (Treatment Policy). A sensitivity analyses was planned to be performed excluding the observations of patients after they are referred to the rescue arm. A descriptive summary of treatment differences in T1/T2 change within the centres was planned to be given as part of the sensitivity analysis and to assess possible centre-wise differences. Since in February 2024 it was decided that recruitment and further study conduct will be terminated and at this time point, only 8 patients had been screened (planned 112), 4 patients had been randomised and treated (planned 56) statistical assessment was adapted. Due to the limited number of subjects in the database, for the majority of parameters comprehensive evaluations as described above will not be conducted. This includes also evaluations for derived parameters (changes from baseline, responder/incidences) unless these are directly available from the raw data and therefore, do not require a derivation. For selected parameter evaluation will be limited to basic descriptive statistic: • Administrative data • Demographics/anthropometrics • Safety based on Adverse Events The SAP describes the approaches for those data that are intended to be analyzed. Generally, no inferential statistics will be provided. All available data will be listed in the report. **<u>Secondary analysis:</u>** Originally planned secondary analysis was adapted due to severely reduced patient number. Changes are described in the Statistical Analysis Plan. The CMR-diagnostic endpoints are based on the previously published sequence-specific normal ranges in healthy volunteers using multiples of standard deviations (SD) above the mean of the normal range at respective field strength. The predefined CMR endpoints are: 1. Myocardial inflammation by CMR: myocardial oedema by raised native T2 (≥2SD, T2-Flash: @3.0T ≥40 ms 2. Diffuse fibrotic remodelling by CMR: raised native T1 (T1+ve; ≥4SD: @3.0T T1≥1150 ms) 3. Myocardial scar by late gadolinium enhancement.

	A normal CMR result was defined as normal T1 and T2, no evidence of cardiac dysfunction, including LVEF less than 55% and/or dilated LV and/or LGE.
	• Cardiac abnormalities (as detected in CMR (e.g., abnormal T1 and/or T2 values)
	  ○ Cardiac events
	  ○ T1 and T2 values and change thereof compared to BL
	All T1 and T2 measurements and their changes to BL will be listed descriptively.

Efficacy Conclusion	No statistical evaluation was done due to the low amount of available patient data. One subject received the Belimumab treatment, 3 subjects were treated according to standard of care. Only one subject performed a visit at week 52. No conclusion regarding the efficacy and hypothesis can be drawn.
Safety Conclusions	A total of 21 adverse events were experienced by 3 subjects. The subject treated with Benlysta experienced no AE. Therefore, only subjects randomised to the Standard of Care (SOC) arm experienced at least one AE. None of the experienced AE were graded as severe. 14 AEs were classified as moderate, 7 as mild. For 20 AEs no relatedness to SoC treatment was reported. For one AE (the observed worsening of restless legs for subject 003) the relatedness regarding treatment could not be classified unequivocally. No AE was assessed as serious adverse event. No AE of special interest (AEsi) was observed. For 13 AEs no action was taken, for 8 AEs pharmacological treatment was initiated. At the end of the study 13 AEs were resolved, 3 were classified as "recovering/resolving and 5 were classified as not resolved.
Overall Conclusion	No statistical evaluation was done due to the low amount of available patient data. One subject received Belimumab treatment, 3 subjects were treated according to standard of care. Only one subject performed a visit at week 52. No conclusion regarding the efficacy or safety can be drawn. A total of 21 adverse events were experienced by 3 subjects**.** Only subjects randomised to the SOC arm experienced AEs.
Report date:	31.03.2025