CLINICAL TRIAL PROTOCOL

A Phase III, Prospective, Multicenter, Randomized, Controlled Clinical Trial to Demonstrate the Efficacy and Safety of Liposomal Cyclosporine A (L-CsA) Inhalation Solution Delivered via the [redacted] plus Standard of Care versus Standard of Care Alone in the Treatment of Chronic Lung Allograft Dysfunction / Bronchiolitis Obliterans Syndrome in Patients post Double Lung Transplantation

Protocol No: BT – L-CsA – 302 – DLT

(BOSTON-2)

Version: 6.0

Date: 7-Apr-2023

Protocol Amendment Number 5

IND Number: 78,854

EUDRACT Number: 2018-003205-25

SPONSOR

Zambon SpA

Via Lillo del Duca, 10

Bresso (MI) 20091

Italy

INVESTIGATIONAL MEDICINAL PRODUCT

Liposomal Cyclosporine A (L-CsA)

[redacted]

1 SIGNATORIES

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Protocol Acknowledgment / Confidentiality

A Phase III, Prospective, Multicenter, Randomized, Controlled Clinical Trial to Demonstrate the Efficacy and Safety of Liposomal Cyclosporine A (L-CsA) Inhalation Solution Delivered via the [redacted] plus Standard of Care versus Standard of Care Alone in the Treatment of Chronic Lung Allograft Dysfunction / Bronchiolitis Obliterans Syndrome in Patients post Double Lung Transplantation

(Protocol No.: BT – L-CsA – 302 – DLT)

By signing this Protocol, the Principal Investigator acknowledges and agrees:

The Protocol contains all necessary details for conducting the clinical trial. The Principal Investigator will conduct this clinical trial as detailed herein, in compliance with Good Clinical Practice (GCP) and the applicable regulatory requirements, and will make every reasonable effort to complete the clinical trial within the time designated.

The Protocol and all relevant information on the drug and device relating to nonclinical and clinical experience, which was furnished by the Sponsor, Zambon SpA, will be made available to all physicians, nurses and other personnel who participate in the conduct of this clinical trial. The Investigator will discuss this material with them to assure that they are fully informed regarding the Investigational Medicinal Product and the conduct of the clinical trial.

This document contains information that is privileged or confidential. As such, it may not be disclosed unless specific prior permission is granted in writing by the Sponsor or such disclosure is required by federal or other laws or regulations. Persons to whom any of this information is to be disclosed must first be informed that the information is confidential. These restrictions on disclosure will apply equally to all future information supplied, which is indicated as privileged or confidential.

Zambon SpA or its designee will have access to any source documents from which Case Report Form information may have been generated. The Case Report Forms and other data pertinent to this clinical trial are the sole property of Zambon SpA which may utilize the data in various ways, such as for submission to government regulatory authorities, or in publication of the results of the clinical trial.

The conduct and results of this clinical trial will be kept confidential. The results of this clinical trial may be published. Upon completion of the clinical trial it is the intention of the parties to prepare a joint publication regarding or describing the clinical trial and all the results there from and both parties shall cooperate in this regard.

I have read and agree to the protocol numbered BT – L-CsA – 302 – DLT. I am aware of my responsibilities as a Principal Investigator and agree to conduct this clinical trial according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonisation guidelines), applicable government regulations, and Institutional research policies and procedures under the guidelines of International Conference on Harmonisation (ICH) Good Clinical Practice (GCP), national regulations, and the protocol. I agree to appropriately direct and assist the staff under my control, who will be involved in the clinical trial.

Principal Investigator's Signature

Name & Title _____ _____

(Print) Date Signature

Center Name _____

(Print)

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3 ABBREVIATIONS

AC	Adjudication Commitee
ADR	Adverse Drug Reaction
AE	Adverse Event
ALT	Alanine Aminotransferase
AMR	Antibody-Mediated Rejection
AST	Aspartate Aminotransferase
ATG	Anti-Tymocyte Globulin
ATS	American Thoracic Society
AZA	Azathioprine
BAL	Bronchoalveolar Lavage
BID, b.i.d.	*Bis in die*, twice daily
BO	Bronchiolitis Obliterans
BOS	Bronchiolitis Obliterans Syndrome
BDRM	Blind Data Review Meeting
BUN	Blood Urea Nitrogen
CFR	Code of Federal Regulation
CLAD	Chronic Lung Allograft Dysfunction
CMV	Cytomegalovirus
CNI	Calcineurin Inhibitor
COPD	Chronic Obstructive Pulmonary Disease
CRF	Case Report Form
CRO	Contract Research Organization
CsA	Cyclosporine A
CsA-PG	Cyclosporine A Propylene Glycol Inhalation Solution
CRS	Clinical Study Report
CT	Computed Tomography
CTC	Common Toxicity Criteria
dL	Deciliter
DLT	Double Lung Transplant

DMC	Data Monitoring Committee
DSA	Donor specific Antibodies
EBV	Epstein Barr Virus
EC	Ethics Committee
ECP	Extracorporeal Photopheresis
eCRF	Electronic Case Report Form
EoS	End of Study
EoT	End of Treatment
EQ-5D-5L	Euro QOL Health Questionnaire
ERS	European Respiratory Society
FAS	Full Analysis Set
FDA	Food and Drug Administration
CCI	[redacted]
FEV_1	Forced Expiratory Volume in One Second
FVC	Forced Vital Capacity
GDPR	General Data Protection Regulation
GERD	Gastro-Esophageal Reflux Disease
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
HLA	Human Leukocyte Antigen
IB	Investigator Brochure
ICH	International Conference on Harmonisation
ICU	Intensive Care Unit
IgM	Immunoglobulin M
IMP	Investigational Medicinal Product (study drug: L-CsA)
IND	Investigational New Drug
IRB	Institutional Review Board
ISHLT	International Society of Heart and Lung Transplantation
IUD	Intrauterine Device
Kg	Kilogram

L	Liter
LABA	Long-Acting Beta Agonist
LAMA	Long-Acting Muscarinic Antagonist
L-CsA	Liposomal Cyclosporine A
[redacted]	[redacted]
LMM	Linear Mixed Model
MAR	Missing At Random
MedDRA	Medical Dictionary for Regulatory Activities
Mg	Milligram
µm	Micrometer
Min	Minute
mL	Milliliter
MMF	Mycophenolate Mofetil
mTOR	Mammalian Target of Rapamycin
N (n)	Number
Ng	Nanogram
NOAEL	No Observable Adverse Effect Level
OB	Obliterative Bronchiolitis
PG	Propylene Glycol
PI	Principal Investigator
Ph Eur	European Pharmacopeia
PK	Pharmacokinetics
PPS	Per Protocol Set
Q	Quartile
RAS	Restrictive Allograft Syndrome
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Statistical Analysis Software
CCI	[redacted]
SLT	Single Lung Transplant

SmPC	Summary of Product Characteristics
SOC	Standard of Care
SUSAR	Suspected Unexpected Serious Adverse Reaction
TAC	Tacrolimus
TDT	Triple Drug Therapy
TEAE	Treatment-Emergent Adverse Event
USA	United States of America
USP	United States Pharmacopeia
WHO-DD	World Health Organization Drug Dictionary

4 PROTOCOL-SPECIFIC DEFINITIONS

Acute Rejection: Acute rejections are classified according to the revised working formulation for classification and grading of pulmonary allograft rejection and will be assessed at any time of occurrence throughout the clinical trial period.

Antibody Mediated Rejection: Diagnosis of antibody mediated rejection is performed according to the International Society of Heart and Lung Transplantation (ISHLT) consensus guidelines and assessed at any time of occurrence throughout the clinical trial period. In this context, clinically stable patients displaying low and stable levels of Donor Specific Antibodies at Screening (as judged by the Investigator) are eligible for the study.

BOS diagnosis: The Investigator will have used the site's clinical spirometer to determine that the patient has clinically defined BOS (CLAD – BOS phenotype) (for definitions, please see below) before referring patients to the study. In this context, FEV_1 values are defined as follows:

> **Personal Best FEV_1**: The highest FEV_1 value following transplant surgery - this can be derived from one single spirometry session or the mean of two best FEV_1 values derived from two spirometry sessions (as per the usual practice by the site; see also Meyer 2014).
>
> **FEV_1 confirming diagnosis of BOS:** The FEV_1 value used by the clinician to confirm the diagnosis of BOS - this can be derived from one single spirometry session or the mean of two best FEV1 values derived from two spirometry sessions (as per the usual practice by the site; see also Meyer 2014).

Baseline FEV_1: For the purpose of this study, the FEV_1 value that is the mean of the best FEV_1 obtained with the study spirometer at the Screening Visit and the best FEV_1 obtained at the Baseline Visit is referred to as "baseline FEV_1".

CLAD (BOS and RAS phenotypes): Following the ISHLT 2019 recommendations [Verleden 2019] for the definition of lung allograft disease following lung transplantation, sufficient diagnostic proof using – amongst others – spirometry, body plethysmography, and/or CT scans should be available to confirm the diagnosis of CLAD and differentiate subtypes (see also Table 1 and Table 2).

For the purpose of this protocol, patients with RAS phenotypes are to be excluded applying the definitions in the consensus report issued by the ISHLT 2019 [Glanville 2019]. In particular, persisting parenchymal opacities on historical High Resolution CT scan or Chest X-Ray should be considered as an important diagnostic feature of CLAD - RAS phenotype.

Table 1. CLAD Staging of Lung Allograft Disease Following Lung Transplantation

CLAD Stage (2019)	Spirometric criteria	BOS Grade (2002)
CLAD 0	FEV_1 > 90% of personal best	0
CLAD 0	FEV_1 81-90% of personal best	0-p
CLAD 1	FEV_1 66-80% of personal best	1
CLAD 2	FEV_1 51-65% of personal best	2
CLAD 3	FEV_1 35-50% of personal best	3
CLAD 4	FEV_1 < 35% of personal best	3

Table 2. Basic Phenotypes of Chronic Lung Allograft Dysfunction

	Obstruction (CCI < 0.7)	**Restriction (TLC decline ≥ 10% from baseline)**	**CT opacities**
BOS	YES	NO	NO
RAS	NO	YES	YES
Mixed	YES	YES	YES
Undefined	YES YES	NO YES	YES NO

For example, the diagnosis and staging according to these recent recommendations would read "CLAD Stage 2, BOS phenotype" for a diagnosis which would have been entitled "BOS Grade 2" following previous recommendations [Glanville 2019].

5 PROTOCOL SYNOPSIS

Protocol Title:	A Phase III, Prospective, Multicenter, Randomized, Controlled Clinical Trial to Demonstrate the Efficacy and Safety of Liposomal Cyclosporine A (L-CsA) Inhalation Solution Delivered via the [redacted] Device plus Standard of Care versus Standard of Care Alone in the Treatment of Chronic Lung Allograft Dysfunction / Bronchiolitis Obliterans Syndrome in Patients post Double Lung Transplantation
Sponsor:	Zambon SpA Via Lillo del Duca, 10 Bresso (MI) 20091 Italy
Protocol Number:	BT – L-CsA – 302 – DLT (BOSTON-2)
IND Number:	78,854
EUDRACT Number:	2018-003205-25
NCT Number:	NCT03656926
Investigational Medicinal Product:	Liposomal Cyclosporine A (L-CsA) Inhalation Solution
Protocol Phase:	Phase III
Protocol Date:	Version 6.0; 7-Apr-2023
Protocol Amendment:	5
Clinical Trial Centers/Countries:	Approximately 43 centers Worldwide.
Planned Clinical Trial Period:	Duration of therapy: 48 weeks Total clinical trial duration per patient: up to 56 weeks
Planned Number of Patients:	The clinical trial plans to stop randomization upon achievement of a total of approximately 220 patients with clinically defined bronchiolitis obliterans syndrome (BOS) in both the present BT – L-CsA – 302 – DLT (BOSTON-2) and the BT – L-CsA – 301 – SLT (BOSTON-1) studies combined. Patients will be randomized 1:1 as follows: ***Treatment Arm A (investigational treatment arm):*** L-CsA inhalation therapy (10 mg bid) plus Standard of Care (SoC). ***Treatment Arm B (control treatment arm):*** SoC alone.

Objective:	The objective of the trial is to assess the efficacy and safety of L-CsA plus SoC in the treatment of BOS in double lung transplant (DLT) recipients.
Clinical Trial Design:	This is a Phase III, prospective, multicenter, randomized, controlled clinical trial of L-CsA for the treatment of bronchiolitis obliterans syndrome in adults diagnosed with BOS following double lung transplant. Patients will receive either L-CsA (10 mg) via the CCI [redacted] twice daily plus SoC treatment or SoC alone, for a period of 48 weeks. All patients will be eligible to continue in an open-label extension trial of L-CsA following completion of BOSTON-2.
Stratification	To assure balance between study treatment arms with regard to some key variables and potential confounders, stratification prior to randomization will be performed for the following variables: • Screening $FEV_1 \geq 81\%$ of personal best FEV_1 value post-transplant versus Screening FEV_1 between 80-51% of personal best FEV_1 value post-transplant • Age at the time of randomization: < 55 years versus ≥ 55 years • Region: North America versus all other countries together

Eligibility Criteria:	***Inclusion Criteria:*** 1. Adult patients ≥ 18 years who received a double lung transplant at least 12 months prior to Screening. 2. Patients with BOS diagnosis defined as CLAD-BOS phenotype with: a) Screening FEV_1 between 85-51% of personal best FEV_1 value post-transplant. OR b) Screening FEV_1 >85% of personal best FEV_1 associated with EITHER a ≥ 200 mL decrease in FEV_1 in the previous 12 months OR according to medical history showing BOS progression. 3. Diagnosis of CLAD-BOS must be made at least 12 months after lung transplantation and a) within 12 months prior to the screening visit OR b) more than 12 months from screening and patient must have shown a decline in FEV1 ≥ 200ml in the previous 12 months before screening, which is not due to acute infection or acute organ rejection 4. Patients in whom the diagnosis of BOS has been confirmed by the elimination of other possible causes of obstructive or restrictive lung disease (CLAD – RAS phenotype, see Protocol Specific Definitions). 5. Patients should be on a maintenance regimen of immunosuppressive agents including tacrolimus, a second agent such as but not limited to MMF or azathioprine, and a systemic corticosteroid such as prednisone as third agent. The regimen must be stable within 4 weeks prior to randomization with respect to the therapeutic agents. In case a patient is also receiving concomitant azithromycin for prophylaxis or treatment of BOS, in addition to the previously described immunosuppressive regimen, azithromycin must be on a stable regimen for at least 4-weeks prior to randomization. 6. Patients capable of understanding the purposes and risks of the clinical trial, who have given written informed consent and agree to comply with the clinical trial requirements/visit schedules, and who are capable of aerosol inhalation. Patients must consent to retrieve prespecified data from the historic

	medical record (e.g., information related to the transplant surgery; spirometry data; medication use). 7. Women of childbearing potential must have a negative serum or urine pregnancy test within 7 days prior to randomization and must agree to use one of the methods of contraception listed in Appendix II through their End of Study Visit. 8. Patients have no concomitant diagnoses that are considered fatal within one year (12 months) of Screening.

	Exclusion Criteria: 1. Patients with confirmed other causes for loss of lung function, such as acute infection, acute rejection, restrictive allograft syndrome (RAS) (CLAD – RAS phenotype, see Protocol Specific Definition), etc. 2. Cystic Fibrosis patients with multi-drug resistant infections not responding to available anti-microbial therapies. 3. Patients with acute antibody-mediated rejection at Screening. In this context, clinically stable patients (as judged by the Investigator) with detectable levels of donor specific antibodies (DSA) at the Screening Visit are eligible for the study. 4. Active acute bacterial, viral, or fungal infection not successfully resolved at least 4 weeks prior to the Screening Visit. Patients with chronic infection or colonization who are clinically stable as per judgement of the Investigator are eligible for the study. 5. Mechanical ventilation (including CPAP) within 12 weeks prior to Randomization. 6. Patients with uncontrolled hypertension. 7. Patient has baseline resting oxygen saturation of < 89% on room air or use of supplemental oxygen at rest. 8. Evidence of functional airway stenosis (e.g., bronchomalacia/tracheomalacia, airway stents, or airways requiring balloon dilatations to maintain patency)with onset after the initial diagnosis of BOS and ongoing at Screening and/or Randomization Visit. 9. Known hypersensitivity to L-CsA or to cyclosporine A. 10. Patients with chronic renal failure, defined as serum creatinine > 2.5 mg/dL at screening, or requiring chronic dialysis. 11. Patients with liver disease and serum bilirubin > 3-fold upper limit of normal range or transaminases > 2.5 upper limit of normal range. 12. Patients with active malignancy within the previous 2 years, including post-transplant lymphoproliferative disorder, with the exception of treated, localized basal and squamous cell carcinomas. 13. Pregnant women or women who are unwilling to use appropriate birth control to avoid pregnancy through their End of Study Visit. 14. Women who are currently breastfeeding. 15. Receipt of an investigational drug as part of a clinical trial within 4 weeks prior to the Screening Visit. This is defined as any treatment that is implemented under an Investigational New Drug (IND) or compassionate use.

	16. Patients who have received extracorporeal photophoresis (ECP) for treatment of BOS within 1 month prior to Randomization. 17. Patients who are currently participating in an interventional clinical trial. 18. Psychiatric disorders or altered mental status precluding understanding of the informed consent process and/or completion of the necessary procedures. 19. Any co-existing medical condition that in the Investigator's judgment will substantially increase the risk associated with the patient's participation in the clinical trial.
Investigational Medical Product: **Mode of Administration/ Dosing Schedule:**	The L-CsA lyophilizate is supplied in CCI containing 10 mg of product. The lyophilizate is reconstituted with 2.4 mL 0.25% NaCl to obtain a ready to use dispersion for inhalation using the CCI . The investigational medicinal product (IMP) will be administered by CCI . At every visit, CCI . The patient must remain in the clinic for at least 2 hours for observation after the first inhalation. CCI provides the investigators with further information on the inhalations performed by the patient. Nebulization time per inhalation dose is approximately CCI . Inhalation will be performed twice daily, approximately CCI through a mouthpiece by slow and deep respiration using the CCI

Treatment Regimen:	***Basic Immunosuppression:*** Regardless of treatment allocation, all participants will continue to receive their Standard of Care (SoC) regimen for maintenance of the lung allograft. See Section 11.3 for additional information regarding use of concurrent medications or treatments prior to and during clinical trial participation. Patients should be on a maintenance regimen of immunosuppressive agents including tacrolimus, a second agent such as but not limited to MMF or azathioprine, and a systemic corticosteroid such as prednisone as third agent. The regimen must be stable within 4 weeks prior to randomization with respect to the therapeutic agents. Patients will be randomized to one of the following treatment Groups: ***Group A (L-CsA treatment plus SoC Therapy):*** • L-CsA 10 mg/2.5 mL twice daily for 48 weeks Plus • Standard of Care Therapy ***Group B (Standard of Care alone):*** • Standard of Care Therapy

Endpoints:	***Primary Endpoint:*** Mean change in FEV_1 (mL) from baseline to Week 48. CCI CCI ***Safety:*** • Adverse events (including infections and malignancies) • Acute tolerability of IMP • Clinical laboratory parameters • Renal Function • Vital signs • Physical examinations

Assessment of Efficacy:	***Clinical Trial Plan:*** A total of 11 study visits (Screening, V1 through V10) will be performed during the clinical trial. After informed consent has been obtained, a Screening Visit will be carried out in order to check general eligibility for participation. At the Baseline Visit (V1, randomization visit), inclusion and exclusion criteria from screening visit will be verified and procedures as per Schedule of Activities will be performed. During the 48-week treatment period, visits are scheduled every 4-8 weeks (V2 – 9). . Visit 10 will be a follow-up visit unless the patient is enrolled into the extension study, in which case visit 10 will not be completed. Every effort will be made to have all planned and unscheduled visits at the study site. Mandatory on site visits are Screening Visit, Visit 1 and Visit 9. However, if one of the visits from Visit 2 to Visit 8 and discontinuation visits cannot be performed at site due to COVID-19, remote visits (e.g. by telephone) are possible. ***Assessments:*** Spirometry (FEV_1, [redacted], and FVC) will be measured at all visits using the provided study on-site spirometer [redacted]) according to ATS/ERS 2005 guidelines. Spirometry measurements on site will be performed by a registered respiratory therapist or certified pulmonary function technician who is blind to the patient's treatment assignment. Spirometry data will be reviewed by a blinded central reader, as described in the Vitalograh site manual. Portable spirometers ([redacted]) will also be introduced at the screening visit for all patients newly enrolled under protocol version 3.0 or later. In addition to the Spirometry performed on the [redacted] patients will perform spirometry using the [redacted] device at every on-site study visit [redacted]. If patients cannot attend visits on site due to COVID-19, remote visits with guided home spirometry will be performed (FEV_1, [redacted], and FVC) using the [redacted]. For the determination of eligibility at Screening, only spirometry data obtained with the study [redacted] spirometer will be used. For the evaluation of of BOS progression during the clinical trial period the further decline of FEV_1 of $\geq$ 10% or $\geq$ 200 mL from baseline, absolute decrease in [redacted] of > 5% must be

	confirmed by measurements taken with CCI spirometer at least 2 weeks apart. Changes to standard of care are allowed at the investigator's discretion (e.g., changes in doses of immunosuppression, azithromycin, ATG, Photopheresis, leukotriene receptor antagonists, cytostatics, irradiation therapy). All changes to SoC must be recorded in the eCRFs (Electronic Case Report Forms). All medically indicated procedures performed during the course of the clinical trial will be documented in the eCRFs. For assessment of patient survival, overall mortality, BOS-related mortality and non-BOS-related mortality will be taken into account at any time throughout the clinical trial period. Cumulative dose of immunosuppressants will be assessed throughout the Study for tacrolimus, MMF/equivalent, and corticosteroids. Quality of Life will be assessed at Visits 1, 4, 6, and 9/End of Treatment (EoT) when patients have to complete the EQ-5D-5L Questionnaire. Absolute and relative change from baseline will be calculated. Hospitalization will be assessed for overall number of days, days on regular ward, days in ICU, and days in ICU on ventilation.

Assessment of Safety:	Safety Assessments at every study visit include physical examination, vital signs, adverse event reporting, and clinical laboratory tests performed in the local labs at each clinical trial site. Acute tolerability of IMP will be assessed measuring spirometry before and CCI after inhalation of L-CsA at initial dosing.
Data Monitoring Committee:	An independent Data Monitoring Committee (DMC) will be established to monitor the safety of Investigational Medicinal Product (IMP) throughout the clinical trial. The DMC will monitor safety by evaluating the safety analyses generated by the unblinded independent statistician during the course of the clinical trial. Details of the safety analyses will be included in the DMC's Charter. The DMC will evaluate treatment groups for possible trends in adverse events, determine whether the basic protocol assumptions remain valid, evaluate whether the overall integrity, scientific merit and conduct of the clinical trial remain acceptable, and make recommendations to the Sponsor. The DMC will also perform comparisons between the rate of observed COVID-19 cases, rate of patients withdrawal, and rate of missing data among trial participants in both treatment arms.
Adjudication Committee:	The AC – consisting of three independent physicians, with documented expertise in the clinical management of lung transplantation and diagnosis and treatment of BOS - will maintain the role of reviewing the BOS progression data in blind condition once each participating subject completes the study period, with the aim to ensure consistent and complete recording of data. If necessary, the AC may query the sites to ask for clarifications. In case there are discrepancies between the assessment of the AC and that of the investigator re: BOS progression events, the CRO will notify the Investigator. In all cases the final decision as to whether or not specific event qualifies as BOS progression stands with the investigator. Details of the AC activities will be included in the AC's Charter

Statistical Considerations:	FEV_1 data collected from the CCI spirometer will be considered primary, while the data collected with the CCI home spirometer will be used for supportive analyses. The primary estimand to be assessed will be the between-treatment-group difference in mean absolute change in FEV_1 between baseline and the end of Week 48, assessed in the patient population defined by the inclusion and exclusion criteria, regardless of whether or not starting additional immunosuppressive medication or stopping of the investigational treatment had occurred (Treatment Policy Strategy, effectiveness). Testing will be performed on the full analysis set (FAS) which will include all randomized patients All available FEV_1 measurements will be entered into a linear mixed model (LMM), with change in FEV_1 between baseline and subsequent visits as the dependent variable, randomized treatment, time, region (North America vs all other countries together), and age (< 55 versus ≥ 55 years), as the independent factors and with baseline FEV_1 and azithromycin use at randomization as covariates. Moreover, the treatment by time interaction and the baseline FEV_1 by time interaction will be included into the model. In case few data are collected at intermediate timepoints between baseline and Week 48 (see section 15.3 on interim analysis), the LMM model will be replaced by an ANCOVA model on the changes in FEV_1 from baseline to Week 48, using the same stratification factors and the same covariates described for the LMM. Sensitivity analyses will be performed in the per-protocol analysis set as well as using an alternative estimand according to which outcome measurements will only be used for the period during which patients were on randomized treatment (i. e., prior to the initiation of additional immunosuppressive therapy or L-CsA discontinuation; While-on-Treatment strategy, efficacy). Moreover, sensitivity analyses will also be performed to assess the validity of the Missing-at-Random assumption for the LMM. In addition to use of LMM to analyze the primary endpoint, simple t-test comparing the difference in mean FEV1 change from baseline to Week 48 between the treatment group in the FAS population including a 95% confidence interval for the treatment difference of the mean FEV1 change between the two treatment groups will be performed. Further sensitivity analyses will be described in the SAP. The overall type I error rate of the clinical trial will be $\alpha = 0.025$ one-sided, corresponding to $\alpha = 0.05$ two-sided. Strong control of the clinical trial-wise type I error rate will be achieved by a-priori

ordering of hypotheses in the indicated order of primary CCI endpoints (see section 15.2.3.3 on type I error level control).

The previous version of the protocol anticipated the following:

CCI

The protocol also anticipated a blinded interim analysis for:

- Re-estimation of the standard deviation, with the purpose of increasing the sample size (bigger SD then assumed) but not of decreasing it Evaluation of the actual amount of FEV_1 data collected at all timepoints using the site spirometer
- Evaluation of the number of drop-outs and the number and pattern of the missing data
- Appropriateness of the analysis based on the anticipated longitudinal model (LMM) vs. the analysis based on the ANCOVA model in the light of the collected data.

CCI

Furthermore, the sample size was judged roughly appropriate for this model but considered small for ANCOVA.

CCI

Since the BOSTON-1 study is experiencing serious enrolment delay, it is anticipated that approximately 160-170 patients will be randomized in the present study and 50-60 patients in BOSTON-1. The sample size for BOSTON-2 will provide a more reasonable power for the planned sensitivity analyses, including ANCOVA.

Details of all evaluations will be provided in the Statistical Analysis Plan.

6 INTRODUCTION

6.1 Bronchiolitis Obliterans and Bronchiolitis Obliterans Syndrome

Lung transplantation is the ultimate treatment option for patients with advanced lung disease or irreversible pulmonary failure. Although refinements and advancements in surgical techniques, lung preservation, immunosuppressive regimen, management of ischemia/reperfusion injury and infections, as well as post-transplant patient care have resulted in improved outcomes, acute and chronic pulmonary allograft rejection continues to be the major obstacle for satisfactory long-term results. Acute rejection after lung transplantation exceeds the incidence and severity compared with other solid organ transplantations. Chronic rejection, commonly denoted bronchiolitis obliterans (BO), obliterative bronchiolitis (OB), or bronchiolitis obliterans syndrome (BOS), is the leading cause of death beyond the first-year after lung transplantation. Whereas the development of BOS is rare within the first year after lung transplantation, annual increments of approximately 10% are recorded in subsequent years, resulting in a cumulative incidence range of 40-50% within the first five years and 70-80% within 10 years of transplantation (Figure 1; ISHLT Registry 2017).

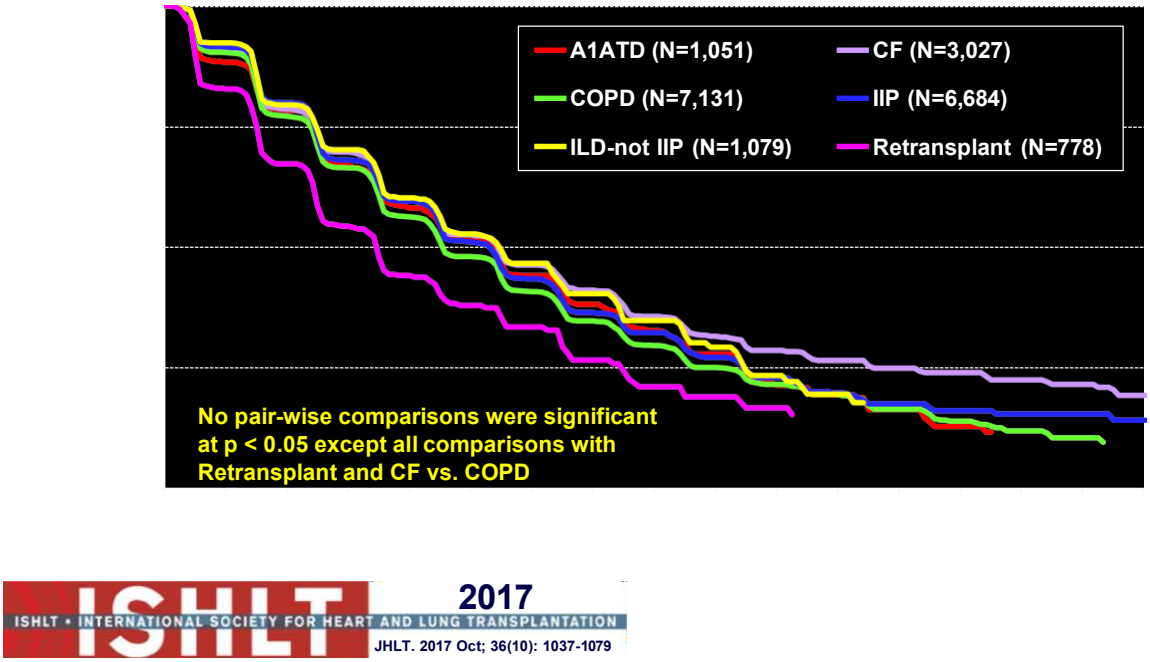

Figure 1: Freedom from bronchiolitis obliterans syndrome by diagnosis (ISHLT Registry 2017)

The main histologic hallmark of BO is scarring with fibrosis of the airway. However, this process is patchy, making the sensitivity of transbronchial biopsy for detection of BO highly dependent on adequacy of sampling and skill of histologic interpretation. Therefore, the clinical manifestation of BO, the bronchiolitis obliterans syndrome (BOS), is defined as irreversible airway obstruction, diagnosed by pulmonary function, in the absence of other causes. This diagnosis does not require histologic confirmation but is considered a reliable surrogate physiologic marker of the presence of BO.

In its most common presentation, BO is characterized by the pathophysiological features of respiratory obstruction. The major findings on spirometry are a reduced forced expiratory volume in 1 second (FEV_1), a normal, or slightly decreased forced vital capacity (FVC), and a reduced ratio of FEV_1 to FVC, with poor response to inhaled bronchodilators. Lung volumes indicate air trapping, with a normal total lung capacity and high residual volume. However, a subset of patients present with a restrictive pattern characterized by a low FVC and a normal CCI ratio, or a mixed pattern of obstruction and restriction [Barker 2014]. BOS is often a diagnosis of exclusion

and other causes of allograft dysfunction, including restrictive allograft syndrome (RAS), acute rejection, and acute infection, need to be excluded before the diagnosis of BOS is made [Glanville 2019].

The 2002 BOS grading system for Grades 0 through 3 (Table 3), which is based solely on FEV_1 and/or CCI is a useful surrogate marker for BO: it is easy to measure by standardized spirometry and is reproducible; it becomes abnormal prior to histologic confirmation [Chacon 2000]; and it displays high sensitivity and specificity when assessed against biopsy- and autopsy-confirmed BO [Heng 1998]. In the following Tables, "baseline" is defined as the patient's personal best FEV_1 following transplant.

Table 3. Bronchiolitis obliterans syndrome (BOS) classification

Original classification (1993)		2002 Classification		2019 ISHLT Classification	
-	-	BOS 0	FEV_1 > 90% of baseline and CCI > 75% of baseline	-	-
BOS 0	FEV_1 80% or more of baseline	BOS 0-p	FEV_1 81% to 90% of baseline and/or CCI ≤ 75% of baseline	CLAD 0	FEV_1 > 80% of baseline
BOS 1	FEV_1 66% to 80% of baseline	BOS 1	FEV_1 66% to 80% of baseline	CLAD 1	FEV_1 66-80% of baseline
BOS 2	FEV_1 51% to 65% of baseline	BOS 2	FEV_1 51% to 65% of baseline	CLAD 2	FEV_1 51-65% of baseline
BOS 3	FEV_1 50% or less of baseline	BOS 3	FEV_1 50% or less of baseline	CLAD 3	FEV_1 35-50% of baseline
-	-	-	-	CLAD 4	FEV_1 < 35% of baseline

BOS: bronchiolitis obliterans syndrome; CCI; FEV_1: forced expiratory volume in 1 second [Estenne 2002]. CLAD: chronic lung allograft dysfunction.

Table 4. Basic Phenotypes of Chronic Lung Allograft Dysfunction

	Obstruction CCI < 0.7)	Restriction (TLC decline ≥ 10% from baseline)	CT opacities
BOS	Yes	No	No
RAS	No	Yes	Yes
Mixed	Yes	Yes	Yes
Undefined	Yes Yes	No Yes	Yes No

BOS represents the major determinant of long-term graft and patient survival. To reduce the rate of rejection (acute as well as chronic forms), immunosuppressive regimens are employed. Although the immunosuppressive protocols vary from center to center, conventional maintenance therapy consists principally of triple drug therapy comprising a calcineurin inhibitor (cyclosporine A [CsA] or tacrolimus [TAC]), an antiproliferative agent (mycophenolate mofetil [MMF], azathioprine [AZA], sirolimus, or everolimus), and corticosteroids. Historically, CsA and AZA were used along with prednisone, but additional agents have become available, including

tacrolimus, mycophenolate, and Mammalian Target of Rapamycin (mTOR) inhibitors like sirolimus and everolimus. Often when BOS is diagnosed in a lung transplant recipient, immunosuppression is augmented but no augmentation therapy has been demonstrated to impact BO reliably. Additional systemic immunosuppression comes with the added burden of additional end-organ toxicity and tolerability issues. Development of BOS is the major confounding factor of long-term successful outcome. BOS is reported as the main cause of death beyond the first post-transplant year over the last three decades (Figure 2; ISHLT Registry 2017).

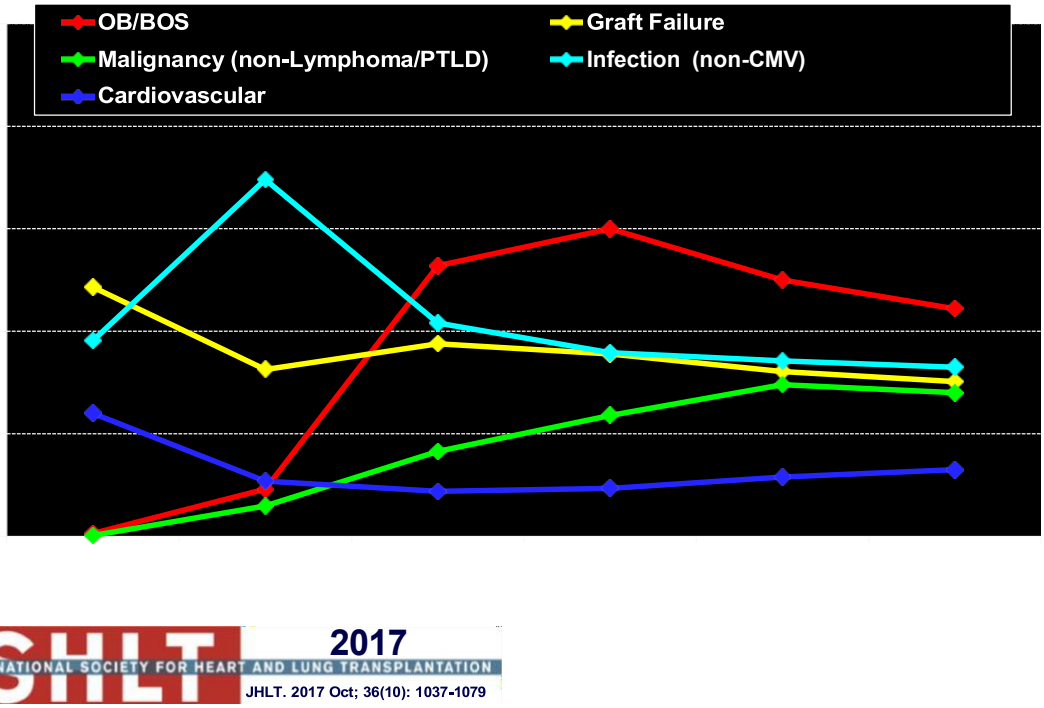

Figure 2: Relative incidence of leading causes of death (ISHLT Registry 2017)

Bronchiolitis obliterans as a disease has been recognized for many decades. However, the need for better treatment options for BOS increased dramatically in the 1990s when lung transplantation became a more acceptable treatment for lung failure, but was also limited by BO as a late-term sequelae. A better understanding of the pathology of the disease as well as the limitations of high-dose systemic immunosuppression, due to their toxic effects, has led to the idea of administering higher doses of drug via alternative routes. BO is uniquely suited to this potential approach as a T-cell mediated disease of the airways of the lungs.

The first studies of an inhaled immunosuppressive for BOS associated with lung transplantation were done using cyclosporine in a formulation that contained propylene glycol, CsA-propylene glycol (CsA-PG). In these studies, CsA-PG showed poor tolerability due to the hyperosmolality of the PG formulation and its propensity to cause airway inflammation. However, efficacy results from clinical trials evaluating prevention of BOS were promising [Iacono 1996; Iacono 2006].

With the goal of increasing tolerability as well as improving airway deposition, a liposomal formulation of CsA (L-CsA) was developed. L-CsA also was formulated specifically for use with the CCI for optimal aerosol dynamics.

7 BACKGROUND INFORMATION

7.1 Investigational Medicinal Product

The Investigational Medicinal Product is a drug/device combination consisting of liposomal cyclosporine A (L-CsA) and the CCI .

7.1.1 Drug component

L-CsA consists of the active substance CsA and excipients as described in the Investigator Brochure (IB). L-CsA is a lyophilisate for reconstitution with 0.25% saline. The formulation is developed for inhalation use and is therefore adjusted to a physiological tolerable value of CCI .

The liposome size in the reconstituted formulation is in the range of 40-100 nm with a polydispersity index of not more than 0.50, which indicates the width of the size distribution. PI values above 0.5 indicate a broad distribution.

The dose of 10 mg L-CsA is reconstituted in a total volume of 2.5 mL. The entire volume of the reconstituted solution is used per aerosol administration.

Additional information can be found in the IB.

7.1.2 Device component

CCI

The CCI to be used in this clinical trial is a modified version of the CCI for an optimized delivery of the investigational drug for the designated patient group. The configuration of the optimized CCI has a high delivered dose, short nebulization time and inhibits contamination of the environment by using an exhalation filter. The device is CE-marked in Europe. Comparable devices are also available on the US market. The CCI was used in the nonclinical and clinical trials of L-CsA. The CCI . The usual nebulization time is CCI for the 10 mg dose.

Additional information can be found in the IB.

7.1.3 Nonclinical Data

For systemic administration of CsA, the nonclinical pharmacologic, pharmacokinetic, and toxicity profiles of systemic CsA are well established [UK SmPC Ciclosporin A CCI].

For aerosolized administration of L-CsA, additional nonclinical studies have been conducted with L-CsA. Importantly, L-CsA inhibited T-cell proliferation in stimulated human peripheral blood mononuclear cells. Across 3 nonclinical toxicology studies in rats, aerosolized L-CsA given as 4-day, 6-week, and 26-week repeated applications of up to 2.6 mg/kg/day, no dose-limiting toxicity occurred. The no-observed-adverse-effect-level (NOAEL) is considered to be 2.0 mg/kg/day via inhalation for chronic application based on the maximum dosage achieved in the 26-week inhalation study in rats.

Nonclinical and clinical data to date with L-CsA show negligible increase in serum levels of calcineurin inhibitors and do not show an increased risk of nephrotoxicity, respiratory infections, or malignancies.

Additional information can be found in the IB.

7.1.4 Clinical Experience

L-CsA has been evaluated in 3 clinical trials in adult patients who received a single-lung or double-lung transplantation: (1) a Phase 1b study of the deposition and pharmacokinetics (PK) of aerosolized L-CsA in patients with lung transplantation (G035.011); (2) a Phase 3 study of the efficacy, safety, and PK of the addition of aerosolized L-CsA to SOC systemic immunosuppression therapy vs aerosolized placebo plus SOC therapy in the prevention of BOS following lung transplantation (12011.201; study was terminated early for business reasons); and (3) an investigator-initiated study of the efficacy, safety, and PK of the addition of aerosolized L-CsA to SOC systemic immunosuppression therapy vs SOC therapy alone in the treatment of BOS following lung transplantation (AI001). Collectively, these studies provided safety, efficacy, and PK data to support further evaluation in 2 Phase III studies of the efficacy and safety of the addition of aerosolized L-CsA to SOC systemic immunosuppression therapy vs SOC therapy alone for the treatment of BOS following single-lung transplantation (BT – L-CsA – 301 – SLT) or double lung transplantation (BT – L-CsA – 302 – DLT). In particular:

- The Phase 1b lung deposition study (G035.011) showed that inhalation of 10 mg and 20 mg L-CsA via the CCI would result in sufficient peripheral lung deposition of L-CsA (≥15 mg L-CsA/week). Aerosol application of 10 mg L-CsA was associated with an acceptable inhalation time of approximately 9 minutes.
- In the Phase 3 study investigating prevention of BOS (12011.201), the primary endpoint for prevention of BOS (BOS-free survival) was not met, although efficacy results may have been confounded by early termination of the trial due to business reasons.
- In the investigator-initiated study investigating treatment of BOS (AI001), the responder analysis of BOS progression-free survival based on Kaplan-Meier estimates revealed a treatment success rate of 81.8% in the L-CsA + SOC group (9 of 11 patients) and 50% in the control group (5 of 10 patients). FEV_1 slope analyses over time resulted in monthly decrements

of -0.008 L in the L-CsA + SOC group and -0.045 L in the control group. This difference translated into an annual preservation of FEV_1 of more than 400 mL in the L-CsA + SOC group, consistent with a meaningful slowing of the progression of the disease. The beneficial effect of L-CsA was also reflected in consistently positive trends favoring the L-CsA + SOC group in other lung function parameters, including CCI, FVC, and FEV_1 % predicted. Five years after the start of Study AI001 (as of September 2017), 5 of 11 patients treated with L-CsA + SOC were alive, compared with 0 of 10 control patients who received SOC alone.

With respect to safety, inhaled L-CsA was generally well tolerated across all clinical trials. The overall analysis of Adverse Events (AEs), Serious Advere Events (SAEs), and deaths showed that these were mostly associated with pulmonary and immunological events, which are expected in a population of lung transplant and immunosuppressed patients and did not differ from the control arms. Neither laboratory values nor vital signs assessments showed any clinically relevant changes over time.

Nonclinical and clinical data to date with L-CsA do not show an increased risk of nephrotoxicity, respiratory infections, or malignancies.

Additional information can be found in the IB.

7.1.5 Summary of Known and Potential Risks and Benefits

Details about specific risks for patients participating in this clinical trial may be found in the IB, including a tabular summary of all adverse reactions reported to date for L-CsA.

Patients in clinical trials generally cannot expect to receive direct benefit from treatment during participation, as clinical trials are designed to provide information about the safety and efficacy of an investigational medicine.

BOS is a known life-limiting complication of lung transplantation without any meaningful therapy. This trial is the first to evaluate the safety and efficacy of L-CsA for the treatment of BOS in patients with BOS following double lung transplantation.

COVID-19 specific considerations: according to the Guidance from the International Society of Heart and Lung Transplantation regarding the SARS CoV-2 pandemic (ISHLT, 2020) "*at this time, it is unknown if specific patient populations are at higher risk of SARS-CoV-2 infection. Published case series and personal reports thus far do not suggest that transplant recipients in particular have a higher risk of acquiring the virus*".

The medical value of this trial remains unchanged.

Furthermore, the medical surveillance of transplanted patients is already based on specific precautions typical to all patients taking immunosuppressive agents.

7.1.6 Justification of Dose Regimen

Although multifactorial in etiology, most evidence suggests that BO is a host-dependent, immune mediated airway injury to bronchiolar cells. It has been postulated that the most important cause of chronic rejection of the lung allograft and BOS is T-lymphocyte activation by major histocompatibility alloantigens- or alloimmune-dependent mechanisms [Halloran 1997; Soubani & Uberti 2007]. Cyclosporine A was selected as the active substance for the development of a liposomal formulation because of its well characterized mode of action and proven immunosuppressive potency.

The liposomal formulation of cyclosporine A (L-CsA) was developed with the intent of improving local tolerability, as shown in studies 12011.201 and AI001. Because lung function is significantly compromised in patients with BOS, an inhalation formulation that is tolerable and facilitates patient use is important for treatment compliance and assessment of safety and efficacy parameters of the IMP. The rationale for the selection of twice-daily 5 mg and 10 mg doses of aerosolized L-CsA, in SLT and DLT patients, respectively, is based on:

- Correlation of drug amount deposition and stabilization of lung function [Corcoran 2004] with CsA-PG
- Clinical trial deposition data from Corcoran [2006] in SLT and DLT patients (CsA-PG)
- Clinical trial deposition data from Behr [2009] in SLT and DLT patients (L-CsA)

During the clinical development of cyclosporine in its propylene glycol formulation, [Corcoran 2004] recorded lung function in correlation with different amounts of CsA-PG deposited in the transplanted lung. A threshold of ≥ 5 mg CsA-PG peripheral dose per inhalation was determined to be required to result in a stabilization of lung function on the basis of FEV_1 measurements. Patients in this clinical trial inhaled CsA-PG three times per week. Hence, the minimal effective peripheral drug dose was calculated to be 15 mg/week.

The same group [Corcoran 2006] investigated the pattern of drug deposition in SLT and DLT patients and discovered that substantial amounts of drug were found only in the transplanted parts of the lung. The native lung of SLT patients was almost free of drug due to its limited ventilation capacity. The same result was achieved when Behr [2009] repeated the investigation of drug deposition in SLT and DLT patients with the L-CsA formulation. Again, it was shown that major deposition of the drug took place only in the ventilated parts of the lung allograft, i.e., almost no drug arrived in the native lung via inhalation.

The combination of the clinical data cited above on drug deposition behavior and the pharmacokinetic data from Behr [2009] showing that approximately 20% of the inhaled drug reaches the periphery of the lung led to the calculation for a dosing regimen of L-CsA. It is

estimated that a dose of L-CsA 10 mg BID will result in 4 mg deposited in the peripheral airways per day, and 28 mg per week.

7.1.7 Statement of Compliance

This study will be conducted in compliance with the protocol approved by the appropriate Institutional Review Boards (IRB) and Ethics Committees (EC), according to International Council for Harmonisation (ICH) and Good Clinical Practice (GCP) standards, and in accordance with the ethical principles that have their origin in the Declaration of Helsinki.

7.1.8 Population

Recipients of a double pulmonary allograft, ≥ 18 years of age, with clinically defined BOS with screening FEV_1 above 51% of personal best FEV_1 value post-transplant during the screening period. The study investigator should confirm the diagnosis of BOS prior to referring the subject for the study.

8 TRIAL OBJECTIVES AND PURPOSE

8.1 Trial Objectives

The objective of this trial is to assess the efficacy and safety of add-on aerosolized L-CsA to Standard of Care therapy as compared to SoC therapy alone in the treatment of BOS in double lung transplant recipients.

8.2 Purpose of the Trial

Currently, there is no approved medicinal product for the prevention or treatment of BOS after lung transplantation. All attempts for therapeutic intervention to alter the incidence or the progression of BOS are empirical and investigational and are usually based on center-specific evidence. This circumstance explains the diversity and disharmony of therapeutic concepts in the lung transplant community. There are SoC practices established to manage BOS that develops after lung transplantation, but no therapeutic regimens have conclusively demonstrated evidence of significant benefit in the prevention or treatment of BOS. Adequately designed and executed, randomized, controlled trials to assess efficacy and safety are needed to identify optimal therapies for established BOS.

The hypothesis that local delivery of CsA by aerosol inhalation will achieve higher intrapulmonary concentration than by systemic administration alone, and will result in decreased allograft rejection with limited toxicity compared to similar systemic doses due to minimal absorption of drug into the blood circulation, has been substantiated by the studies described in Section 7.1.4.

In particular, the investigator-initiated trial AI001 provided evidence of L-CsA's potential to delay progression of BOS when administered as add-on inhalation therapy as compared to SoC for systemic BOS therapy. In combination with the acceptable safety profile of L-CsA, observed in clinical trial 12011.201 when patients were treated for up to two years, L-CsA has the potential to provide superior efficacy over current best practice BOS therapy. The purpose of this trial is to confirm the efficacy and safety of L-CsA in a distinct lung transplant population, i.e., in double lung transplant recipients.

9 TRIAL DESIGN

9.1 Endpoints

9.1.1 Primary Endpoint

Mean change in FEV_1 (mL) from baseline to Week 48.

CCI [redacted]

[redacted]

CCI [redacted]

[redacted]

Safety:

- Adverse events (including infections and malignancies)
- Acute tolerability of IMP
- Clinical laboratory parameters
- Renal Function
- Vital signs
- Physical examinations

9.2 Trial Design

This is a Phase III, prospective, multi-center, randomized, controlled clinical trial in the treatment of BOS in adult recipients of a bilateral pulmonary allograft. Approximately 160-170 patients are planned for enrollment. The clinical trial will be conducted in approximately 43 centers worldwide. Patients will be randomly allocated to receive either L-CsA plus SoC or SoC alone for the treatment of BOS. Patients will be monitored every 4-8 weeks over 48 weeks for efficacy parameters and for all safety evaluations.

The rationale for not using a placebo control is driven by concerns that a sucrose-containing formulation could increase a potential risk of pulmonary infection without the benefit of L-CsA. Sucrose is a lyoprotectant in the manufacturing process. After carefully weighing alternative options for lyoprotection, no sugar-free alternatives that qualify as real placebo (i.e., undistinguishable by means of appearance, taste or smell) could be identified. Therefore, a randomized controlled trial versus SoC is the only suitable alternative.

An open-label clinical trial generally bears the potential regarding biased patient treatment and care, and thus trial outcome. However, for this trial the risk of bias is considered low because of the following reasons:

- Currently, the general strategy to treat BOS is augmentation of immunosuppressive therapy to the highest tolerable level unless limited by systemic toxicity. Since application of L-CsA via inhalation has not shown to lead to an additional systemic drug burden, dose reductions or adaptations of other components of the immunosuppressive cocktail will not be required and are not desired.
- Furthermore, the selected primary endpoint FEV_1 is an objective parameter and its measurement will be performed according to recommended guidelines of ATS/ERS which have to be respected by each participating center. Following this methodology, a subjective and intentional manipulation of outcome is highly unlikely even if health care professional and patient are aware of the treatment allocation.
- The pulmonary function technicians, respiratory therapists, or physiotherapists who perform spirometry at each site will be blinded to each study patient's study treatment assignment.
- Finally, the correctness and validity of individual FEV_1 curves and their resulting value has to be approved in a central and blinded reading by a pulmonary expert who is independent and not involved in patients' care or treatment.

The implementation of these measures shall guarantee that the reported data of the primary outcome will be free of bias induced by the open-label character of the clinical trial.

A similar Phase III clinical trial, BT – L-CsA – 301 – SLT (BOSTON-1) will be conducted in patients who have undergone single-lung transplantations. Patients who complete either BOSTON-1 or BOSTON-2, will be eligible to participate in a follow-on study, BT – L-CsA – 303 – FU (BOSTON-3).

9.3 Stratification and Randomization

At the baseline visit (see Section 14.2.2), after all inclusion and exclusion criteria have been fully evaluated, eligible patients will be assigned to the two treatment groups at a ratio of 1:1 by means of block randomization. Randomization will be performed by a statistician otherwise not involved with the trial, using validated random number generator software. The randomization code of each patient will be provided to the Investigator after compliance with all eligibility criteria has been confirmed, through the electronic Case Report Form (eCRF) into which the generated random code list will be uploaded. Each patient will be assigned a number as part of the randomization process. The number assigned to a patient will become that patient's unique treatment number throughout the clinical trial and cannot be re-assigned to another patient.

The randomization will be stratified by Screening FEV_1 of ≥ 81% of personal best FEV_1 value post-transplant versus Screening FEV_1 between 80-51% of personal best FEV_1 value post-transplant, by age (< 55 versus ≥ 55 years), and by region (North America versus all other countries together). The block size will be withheld from the investigators in order to reduce the predictability of the treatment assignment and is therefore not mentioned in this study protocol.

9.3.1 Blinding

The pulmonary function technicians, respiratory therapists, or physiotherapists who conduct spirometry on site will be blinded to treatment assignment. Patients and other unblinded personnel will be asked to not share treatment assignment information with the pulmonary function technicians, respiratory therapists, and physiotherapists. The statistician who performed the interim sample size re-assessment was blinded to study treatment assignment. For details of the blinding of the Data Monitoring Committee members, see Section 13.2. For details of the blinding of the Adjudication Committee members, see Section 13.3. Clinical trial monitors, treating physicians, study nurses, study coordinators, and enrolled patients will not be blinded to study treatment assignment.

9.4 Investigational Medicinal Product: Treatment, Dosage and Administration

9.4.1 Formulation of L-CsA

The composition of the L-CsA formulation is shown in Table 5. It is a lyophilisate intended for reconstitution with 0.25% saline. The formulation is developed for inhalation use and adjusted to physiological tolerable values of [redacted]

Table 5. Qualitative Composition of the IMP

Ingredient	Function	Quality
Cyclosporine A	Active ingredient	Ph Eur[1]
CCI		

1: European Pharmacopeia
2: United States Pharmacopeia

The particle size of the liposomes is in the range of 40-100 nm with a polydispersity index of not more than 0.50, which indicates the width of the size distribution. Values of polydispersity index above 0.5 indicate a broad distribution. For colloidal disperse systems a PI of 0.20 to 0.50 is appropriate.

The IMP L-CsA and 0.25% saline for reconstitution will be supplied by:

Zambon SpA
Via Lillo del Duca, 10
Bresso (MI) 20091
Italy

9.4.2 Treatment and Dosage

Basic Immunosuppression:

Regardless of treatment allocation, all participants will receive Standard of Care (SoC). Eligible patients should be on a maintenance regimen of immunosuppressive agents including tacrolimus, a second agent such as but not limited to MMF or azathioprine, and a systemic corticosteroid such as prednisone as third agent. The regimen must be stable for within 4 weeks prior to randomization with respect to the therapeutic agents. Patients receiving azithromycin for prophylaxis or treatment of BOS, must be on a stable regimen for at least 4-weeks prior to randomization and will continue to receive azithromycin during the trial as deemed appropriate by the study investigator.

After screening, patients complying with all inclusion and exclusion criteria (see Sections 10.1 and 10.2) will be randomized to one of the following treatments:

Group A (aerosolized L-CsA treatment plus Standard of Care BOS Therapy):

- L-CsA 10 mg/2.5 mL twice daily for 48 weeks

  Plus

- Standard of Care (as directed by treating physician)

Group B (Standard of Care alone):

- Standard of Care (as directed by treating physician)

9.4.3 Administration of L-CsA

Patients randomized to L-CsA treatment will receive L-CsA inhalation therapy in addition to SoC as outlined below.

Prior use in clinical trial 12011.201 and clinical trial AI001 created no evidence suggesting L-CsA was intolerable to patients. However, patients with BOS typically have inflamed airways which could increase the likelihood of bronchospasm. Thus, to minimize risk to patients randomized to L-CsA, patients will receive training on the use of the device CCI [redacted]. In addition, during all subsequent scheduled visits the CCI [redacted].

Each patient will ideally receive two L-CsA administrations per day, CCI [redacted]. The inhalations are scheduled to be taken approximately CCI [redacted]. Instructions for administration should be followed as described in the Instructions For Use.

If patients develop dyspnea or signs of drug intolerance at any point during the course of the trial, the site investigator should take appropriate actions, immediately. If clinically indicated, L-CsA will be held and re-challenged and/or discontinued. Patients will be encouraged to complete all remaining study visits through the end of the trial.

Patients who demonstrate signs of acute infection or acute rejection should be evaluated by the treating physician and treated appropriately. L-CsA should be continued if tolerated during this period. If L-CsA is held, it should be tracked in the eCRFs. The reasons for discontinuation or dosage adjustments have to be documented accordingly with specification of the date and reason on the respective case report form.

For those patients on inhalation therapy, reasons for temporary interruption may include the following:

- Significant acute respiratory infection requiring antimicrobial therapy until a clinical response is documented

- Respiratory failure from any cause until the event has resolved
- Mechanical ventilation
- If a significant decline in FEV_1 CCI should occur associated with breathlessness or other respiratory symptoms.

Patients temporarily interrupting L-CsA inhalation will be asked to return for clinical trial visits according to the visit schedule defined in the protocol but shall not receive IMP. If there is full clinical resolution of the reasons for temporary interruption, the Investigator may attempt to re-introduce the IMP at a later time. If the patient agrees, she/he will resume all mandatory evaluations according to the study protocol until end of treatment (Week 48).

CCI

CCI

9.5 Investigational Medicinal Product Packaging, Labeling and Storage

9.5.1 Packaging and Labeling of IMP

Packaging

The investigational medicinal product will be provided as 10 mg L-CsA lyophilisate CCI [redacted]

Labeling: CCI [redacted] (U.S. Version. Labelling will meet applicable regulatory requirements.)

- Protocol No.: BT – L-CsA – 302 – DLT
- 10 mg L-CsA, powder for nebulization solution.
- For inhalation use.
- Batch No.:
- Expiry Date: mm/yyyy
- Zambon SpA.

Labeling: Saline 0.25% Solution (U.S. Version. Labelling will meet applicable regulatory requirements.)

- Protocol No.: BT – L-CsA – 302 – DLT
- NaCl 0.25%, solvent for L-CsA
- LOT No.:
- EXP: mm/yyyy
  Zambon SpA

Labeling: CCI [redacted] (U.S. Version. Labelling will meet applicable regulatory requirements.)

- Protocol No.: BT – L-CsA – 302 – DLT
- FOR DOUBLE LUNG TRANSPLANT PATIENTS ONLY
- CCI [redacted]
- For inhalation use.
- LOT No.:
- Box No.:
- Expiry Date: mm/yyyy
- Inhale CCI [redacted], according to the instructions from your research team.
- Do not store above 77 °F (25 °C), do not freeze. Protect from light.

- Keep out of reach of children.
- FOR CLINICAL TRIAL USE ONLY
- Return all unused medication to the investigator.
- Sponsor: Zambon S.p.A., Via Lillo del Duca 10, 20091 Bresso (MI), Italy,
- Phone: +39 02 665241
- Investigator name:
- Site No.:
- Patient No.:
- Visit No.:

9.5.2 Storage

The unopened IMP must be kept in a secure location carefully stored not above 77 °F (25 °C) protected from light.

CCI [redacted]

9.6 Stopping Rules and Discontinuation Criteria

9.6.1 Patient Level

Regular termination of clinical trial participation:

The investigational treatment will be terminated after 48 weeks (End of Treatment [EoT]). The treatment period will be followed by a follow-up of 4 week duration (End of Study [EOS]). Patients who enroll in Clinical Trial BT – L-CsA – 303 – FU (BOSTON-3) will forego the 4-week follow-up period of BOSTON-2 and will enroll directly into BOSTON-3 at the Week 48 Visit. For these patients, the End of Treatment (EoT) visit will be the EOS visit.

Any patients with ongoing SAEs will be followed-up until recovery or stabilization of the SAE. If a patient has enrolled in BOSTON-3, pharmacovigilance will be continuously assessed in the scope of the follow-up clinical trial.

Premature termination of clinical trial participation:

- Patients may discontinue participation in the clinical trial at any time by revoking their informed consent.
- The Investigator may withdraw a patient at any time if it is in the best interest of the patient based upon clinical assessments.
- Patient is lost to follow-up.
- Re-transplantation. Patients who are eligible for and/or listed for re-transplantation will continue to participate in the trial until they receive re-transplantation surgery.
- Pregnancy (the patient will be followed via a pregnancy registry).
- Zambon SpA may choose to terminate the study at a site or as a whole for reasons such as, but not limited to, medical futility, safety concerns, or poor study site performance.

Patients stopping to take study treatment but not revoking their consent will be encouraged to continue the visits planned until the scheduled end and perform all the important safety and efficacy assessment for the full duration of the study.

If the patient fails to return for clinical follow-up, the patient will be contacted by telephone or a letter will be sent requesting a mandatory clinic visit and the reason for non-compliance will be documented.

9.6.2 Center Level

Regular termination of clinical trial centers:

All centers will be closed after the pre-defined number of patients have been randomized (i.e., approximately 220) in the BOSTON 1 and the present BOSTON 2 study combined and the patients enrolled have completed clinical trial participation.

Premature termination of a clinical trial center:

The sponsor may close a center for the following reasons:

- In case of repeated and continuous violations of GCP regulations
- Inaccurate or incomplete data collection
- Falsification of records
- Failure to adhere to the protocol
- Organizational or structural changes in the center which challenge the requirements or qualification for clinical trial participation (e.g., change of investigator, shift of center activities etc.).

In case a center is closed for any of the reasons above, efforts will be made to ensure the safety and data reliability of ongoing patients until clinical trial completion.

9.6.3 Study Level

Regular termination of the clinical trial:

The clinical trial will be considered complete after the pre-defined number of patients (i.e., approximately 220) have been randomized in the BOSTON 1 and the present study combined, and the patients have completed clinical trial participation (last patient last visit).

Premature termination of the clinical trial:

- Serious Safety concerns that indicate a potential health hazard caused by treatment with the IMP or SoC (termination due to safety issues).

Zambon SpA may choose to terminate the study as a whole for reasons such as, but not limited to, medical futility or safety concerns. In this case, adverse event data will be reported to FDA/Competent Authorities according to applicable regulatory requirements. The Investigator must notify his IRB/EC of clinical trial closure.

9.7 Investigational Medicinal Product: Supply and Accountability

Each Investigator is responsible for ensuring that deliveries of IMP CCI and other clinical trial materials from Zambon SpA are correctly received and recorded, that these are handled and stored safely and properly, and that they are used in accordance with this protocol. CCI

Unused and used L-CsA CCI must be returned to Zambon SpA or destroyed on-site after drug accountability is performed or at least at termination of the clinical trial when overall drug accountability has been completed.A list of L-CsA, CCI , and other materials received, used, returned, or destroyed must be prepared, completed and signed by the Investigator; any discrepancies must be accounted for.

COVID-19 specific consideration: in case the planned on-site visit can not be performed, the Principal Investigator (PI) or the Sub-Investigator and/or an appropriately qualified site study team member can arrange for the delivery of the IMP directly to patient home, if allowed by local regulation and the site IRBs/ECs.

10 SELECTION AND WITHDRAWAL OF PATIENTS

10.1 Patient Inclusion Criteria

1. Adult patients of ≥ 18 years who received a double lung transplant at least 12 months prior to Screening.
2. Patients with BOS diagnosis defined as CLAD-BOS phenotype with:
   a) Screening FEV_1 between 85-51% of personal best FEV_1 value post-transplant.

   OR

   b) Screening FEV_1 >85% of personal best FEV_1 associated with EITHER a ≥ 200 mL decrease in FEV_1 in the previous 12 months OR according to medical history showing BOS progression.
3. Diagnosis of CLAD-BOS must be made at least 12 months after lung transplantation and
   a) within 12 months prior to the screening visit.

   OR

   b) more than 12 months from screening and patient must have shown a decline in FEV1 ≥ 200ml in the previous 12 months before screening, which is not due to acute infection or acute organ rejection.
4. Patients in whom the diagnosis of BOS has been confirmed by the elimination of other possible causes of obstructive or restrictive lung disease (CLAD – RAS phenotype, see Protocol Specific Definitions).
5. Patients should be on a drug maintenance regimen of immunosuppressive agents including tacrolimus, a second agent such as but not limited to MMF or azathioprine, and a systemic corticosteroid such as prednisone as third agent. The regimen must be stable within 4 weeks prior to randomization with respect to the therapeutic agents. In case a patient is also receiving concomitant azithromycin for prophylaxis or treatment of BOS, in addition to the previously described immunosuppressive regimen, azithromycin must be on a stable regimen for at least 4-weeks prior to randomization.
6. Patients capable of understanding the purposes and risks of the clinical trial, who have given written informed consent and agree to comply with the clinical trial requirements/visit schedules, and who are capable of aerosol inhalation. Patients must consent to retrieve prespecified data from the historic medical record (e.g., information related to the transplant surgery; spirometry data; medication use).
7. Women of childbearing potential must have a negative serum or urine pregnancy test within 7 days prior to randomization and must agree to use one of the methods of contraception listed in Appendix II through their End of Study Visit.
8. Patients have no concomitant diagnoses that are considered fatal within one year (12 months) of Screening.

10.2 Patient Exclusion Criteria

1. Patients with confirmed other causes for loss of lung function, such as acute infection, acute rejection, restrictive allograft syndrome (CLAD – RAS phenotype, see Protocol Specific Definitions), etc.
2. Cystic Fibrosis patients with multi-drug resistant infections not responding to available anti-microbial therapies.
3. Patients with acute antibody-mediated rejection at Screening. In this context, clinically stable patients (as judged by the Investigator) with detectable levels of donor specific antibodies (DSA) at the Screening Visit are eligible for the study.
4. Active bacterial, viral, or fungal infection not successfully resolved at least 4 weeks prior to the Screening Visit. Patients with chronic infection or colonization who are clinically stable as per judgement of the Investigator are eligible for the study
5. Mechanical ventilation (including CPAP) within 12 weeks prior to Randomization.
6. Patients with uncontrolled hypertension.
7. Patient has baseline resting oxygen saturation of < 89% on room air or use of supplemental oxygen at rest.
8. Evidence of functional airway stenosis (e.g., bronchomalacia/tracheomalacia, airway stents, or airways requiring balloon dilatations to maintain patency) with onset after the initial diagnosis of BOS and ongoing at Screening and/or Randomization Visit.
9. Known hypersensitivity to L-CsA or cyclosporine A.
10. Patients with chronic renal failure defined as serum creatinine > 2.5 mg/dL at screening or requiring chronic dialysis.
11. Patients with liver disease and serum bilirubin > 3-fold upper limit of normal range or transaminases > 2.5 upper limit of normal range.
12. Patients with active malignancy within the previous 2 years, including post-transplant lymphoproliferative disorder, with the exception of treated, localized basal and squamous cell carcinomas.
13. Pregnant women or women who are unwilling to use appropriate birth control to avoid pregnancy through their End of Study Visit.
14. Women who are currently breastfeeding.
15. Receipt of an investigational drug as part of a clinical trial within 4 weeks prior to the Screening Visit. This is defined as any treatment that is implemented under an Investigational New Drug (IND) or compassionate use.
16. Patients who have received extracorporeal photophoresis (ECP) for treatment of BOS within 1 month prior to Randomization.
17. Patients who are currently participating in an interventional clinical trial.
18. Psychiatric disorders or altered mental status precluding understanding of the informed consent process and/or completion of the necessary procedures.

19. Any co-existing medical condition that in the Investigator's judgment will substantially increase the risk associated with the patient's participation in the clinical trial.

10.3 Patient Withdrawal Criteria

In the event of patient withdrawal, the Investigator must complete the appropriate end of study form in the eCRF as soon as possible after withdrawal, stating the reason and date of withdrawal. An automated e-mail alert will be generated by the eCRF system and will be sent to the Clinical Research Organization (CRO).

10.3.1 Procedures for patients Withdrawal and Type and Timing of Data Collection

The patient is free to withdraw from the clinical trial for any reason and at any time without giving a reason for doing so and without penalty or prejudice. The Investigator is also free to terminate a patient's involvement in the clinical trial at any time if the patient's clinical condition warrants it and if it is in the best interest of the patient based upon clinical assessments.

If the subject decides to withdraw the consent or the Investigator decide to withdrawn a patient from the study, he/she should perform:

- End of Treatment Visit (Visit 9) PRIOR to discontinuation, whenever possible (the reason for premature withdrawal should always be documented)
- A final clinical trial visit (Visit 10) at least 1 month after Visit 9, if possible

If subjects permanently discontinue study treatment and maintain consent, or the Investigator decide to withdrawn a patient from study treatment, patients should be followed for additional outcome information. Subjects should be encouraged to continue the participation in the clinical trial until the scheduled end, regardless of the treatment arm. Subjects should perform all study visits required by Study Protocol performing all important safety and efficacy assessments for the full duration of the Study.

It is also possible that the Competent Authority(ies), IRBs/ECs or the sponsor request termination of the clinical trial, if there are concerns about conduct or safety.

Reasons for withdrawal are outlined in detail in Section 9.6.1

The reasons for withdrawal must be recorded in the eCRF.

10.3.1.1 Additional information for the Investigator in case of decision to withdraw a patient from IMP:

To support the Investigator in his/her decision to withdraw a patient from IMP, it is recommended to use the maximum intensity of any IMP-related adverse events (see Section 13.1.1.3) as the basis

for evaluation. If the patient reports any severe adverse events that are determined to be related to L-CsA, the Investigator should consider withdrawing the patient from IMP.

Spirometry using the CCI spirometer will be performed CCI after inhalation of L-CsA during the supervised treatments on Day 1. Patients may be evaluated in the clinic on Day 2 for further observation with administration of L-CsA if warranted in the Investigator's opinion based upon Day-1 L-CsA administration. A reduction in the FEV_1 of CCI associated with clinical symptoms of breathlessness or other respiratory symptoms will be considered intolerability of the IMP and grounds for discontinuation of inhalation.

10.3.2 Replacement of Withdrawn Patients

Patients withdrawn before randomization (e. g., screening failures) will be replaced until the pre-defined number of randomized patients has been reached. If a subject fails the screening process due to not meeting inclusion/exclusion criteria, a re-screening is allowed. Randomized patients will not be replaced. The eligibility of prematurely withdrawn patients for the different analysis populations will be determined in a blind data review meeting with consideration of the definition of these analysis populations provided in Section 15.1.

10.3.3 Follow-up of Withdrawn Patients

Patients stopping to take study treatment but not revoking their consent will be encouraged to continue the visits planned until the scheduled end and perform all the important safety and efficacy assessment for the full duration of the study.

All patients withdrawn from the clinical trial prematurely will be followed for AEs and SAEs for further 4 weeks. Any patient with ongoing SAEs will be followed until recovery or stabilization of the SAE.

The 4-week safety follow-up will be obsolete if a patient completes participation in the clinical trial and consents to participate in Study BT – L-CsA – 303 – FU (BOSTON-3). In this case, safety will be continuously assessed in the scope of the follow-up clinical trial.

10.3.4 Completion of Clinical Trial and Loss to Follow-up

Patients will be considered to have completed the clinical trial if they were followed-up through Study Visit 9 (Study Week 48).

A patient will be considered lost-to-follow-up only if no contact has been established by the time the clinical trial is completed such that there is insufficient information to determine the patient's status at Visit 9 (Week 48). The Investigator should document attempts to re-establish contact with any missing patient throughout the clinical trial period. If contact with a missing patient is re-established, IMP inhalation and follow-up should resume according to the protocol.

11 TREATMENT OF PATIENTS

11.1 Standard of Care Alone

Patients randomized to the control arm will receive only the Standard of Care regimen as prescribed by the treating physician and as described in Section 9.4.2.

Any changes in Standard of Care treatment will be documented in the eCRF.

11.2 L-CsA plus Standard of Care

Patients randomized to the L-CsA arm will receive L-CsA 10 mg twice daily in addition to SoC as prescribed by the treating physician and as described in Section 9.4.2. L-CsA will be administered as 10 mg/2.5 mL inhalation via the CCI for 48 weeks.

Any changes in Standard of Care treatment will be documented in the eCRF.

11.3 Concurrent Treatment or Medication

All participants randomized, whether enrolled in the L-CsA plus SoC arm or the Standard of Care arm of this protocol, will receive standard of care therapies for the maintenance of their allograft. SoC includes maintenance immunosuppressive medication, prophylaxis against common opportunistic infections, and all other necessary medications and therapies for the optimal care of the patient. This also include vaccination against CoV-SARS 2. Changes to immunosuppression and use of azithromycin are also considered SoC. All changes in the concurrent treatment or medication will be administered according to site's standard of care.

11.3.1 Immunosuppressive Medication

Maintenance Immunosuppressive Therapy

Maintenance immunosuppressive therapy including tacrolimus, a second agent such as but not limited to MMF or azathioprine, and a systemic corticosteroid such as prednisone as third agent will be administered according to institutional standards. Dosages, dates of start and stop or change in dosage will be recorded on the respective eCRF.

Systemic tacrolimus doses should not need to be adjusted based on prior experience using aerosolized cyclosporine A. However, systemic tacrolimus doses should be adjusted based upon renal function. Should elevations of creatinine occur during the course of the trial by 30% from baseline at randomization, tacrolimus levels given systemically should be reduced by approximately 30% from baseline and levels should be followed and creatinine monitored according to standard protocols at the discretion of the Investigator.

In the event of renal insufficiency (creatinine > 2.5 mg/dL), the dose of oral tacrolimus may be reduced by 30% and the creatinine should be followed by subsequent monthly blood analyses. Alternatively, a switch from Calcineurin Inhibitor (CNI) to mTOR inhibitors should be considered according to investigators judgement.

Augmentation or Change of Immunosuppression

Augmentation or change of immunosuppression/immunomodulation following randomization of the patient into the clinical trial must be recorded on the concomitant medication in the eCRFs.

All efforts should be made to maintain the type of immunosuppressive drugs at inclusion throughout the study. Changes in doses should be driven by medical considerations. Should a fourth immunosuppressive drug be needed, an mTOR inhibitor can be considered during the study if regarded only as strictly necessary, based on the Investigator's judgement.

11.3.2 Prophylaxis and Therapy of Infections

All patients will receive standard infectious disease prophylaxis and therapy consisting of appropriately targeted antibiotics, antivirals and/or antifungals according to institutional standards. Dates of start and stop or change in dosage will be recorded on the respective eCRF.

11.3.3 Azithromycin

Patients receiving azithromycin prior to randomization (for prophylaxis or treatment of BOS), must be on a stable regimen for at least 4 weeks prior to randomization. These patients can continue to receive azithromycin during the clinical trial as deemed appropriate by the investigator.

11.3.4 Other Medication

All concurrent treatment or medications given within 30 days prior to Randomization and until Visit 10/EoS, will be recorded in the CRF.

The use of any inhaled formulation of Cyclosporine A other than the IMP is strictly prohibited during the complete clinical trial period. Topical formulations (e.g., eye drops) containing CsA are allowed. Patients should not receive systemic Cyclosporine A at any time during Screening and Randomization. If medically indicated after Randomization, the treating physician can exchange Tacrolimus for systemic Cyclosporine A.

All inhaled medications, except investigational inhaled medications, are permissible during the trial and should be administered in the following order: inhaled bronchodilators, inhaled corticosteroids, inhaled antifungals/antibiotics, and then inhaled L-CsA. Spirometry must be performed without the use of bronchodilators (see Section 12.1.1.1) and preferably before but at least 2 hours after the last administration of inhaled antifungals/antibiotics.

To standardize spirometry across the two treatment arms, patients should not use short-acting bronchodilators (e.g., albuterol) 2 hours prior to spirometry. Patients on scheduled Long-Acting Beta Agonists (LABAs) or Long-Acting Muscarinic Antagonists (LAMAs) should ideally have spirometry done at the same time of day following their scheduled dose. Patients have to note their use and timing of bronchodilators carefully.

11.3.5 CCI

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The reason for non-compliance shall be documented accordingly on the respective CRF.

12 ASSESSMENT OF EFFICACY

12.1 Methods and Timing of Efficacy Assessments and Analyses

12.1.1 Spirometry

12.1.1.1 [redacted]

For on-site spirometry each clinical trial site will be provided with a [redacted]), which will stay on site. Clinical trial personnel at each site will be trained and certified on the use of protocol specific software installed on the [redacted] Spirometry will be measured according to American Thoracic Society (ATS)/European Respiratory Society (ERS) spirometry guidelines at each visit [Miller 2005a; Miller 2005b; Wanger 2005; Pellegrino 2005]. Three acceptable maneuvers will be obtained and the largest value for FVC and FEV_1 will be recorded. The [redacted] from the maneuver with the largest sum of FEV_1 + FVC will be recorded. FEV_1, FVC, and [redacted] will be recorded in absolute terms. Centralized overread will be applied to all maneuvers obtained.

It is well known that spirometry relies on the cooperation between the patient and examiner and that the results obtained will depend on technical as well as personal factors. Therefore, all efforts will be undertaken to have a particular patient assessed by the same examiner. To reduce the variability of the results, the method should be standardized as far as possible.

Within-maneuver acceptability criteria:

Individual spirograms are acceptable if:

- They are free from artefacts
  - Cough during the first second of exhalation
  - Glottis closure that influences the measurement
  - Early termination or cut-off
  - Effort that is not maximal throughout
  - Leak
  - Obstructed mouthpiece
- They have good starts
  - Extrapolated volume < 5% of FVC or 0.15 L whichever is greater
- They show satisfactory exhalation
  - Duration of ≥ 6 seconds or a plateau in the volume-time curve or if the patient cannot or should not continue to exhale.

Between-maneuver repeatability criteria:

After three acceptable spirograms have been obtained, apply the following test:

- The two largest values of FVC must be within 0.150 L of each other

- The two largest values of FEV_1 must be within 0.150 L of each other.

If both of these criteria are met, the test session may be concluded.

If both of these criteria are not met, continue testing until:

- Both of the criteria are met with analysis of additional acceptable spirograms
  or
- A total of eight tests have been performed (optional)
  or
- The patient cannot or should not continue.

Save, as a minimum, three acceptable and repeatable maneuvers.

12.1.1.2 CCI

Each site will receive portable spirometers (CCI) for newly enrolled patients to be used at all on-site and remote visits. Clinical trial personnel will be trained in the use of the CCI , patients will be trained by the clinical trial personnel in the use of the device at screening. Refresher training for patients will be provided at all subsequent clinic visits (and during remote visits, if required). The patient will receive written instructions on how to use the CCI to perform spirometry correctly.

Spirometry on the CCI should be performed using the same within and between maneuver criteria that would be applied during on-site spirometry testing as described above. The largest value for FVC and FEV_1 will be recorded. The CCI from the maneuver with the largest sum of FEV_1 + FVC will be recorded. FEV_1, FVC, and CCI will be recorded in absolute terms. As a minimum, three acceptable and repeatable manoeuvres will be recorded.

To assist the clinical trial personnel and patient to achieve the spirometry within (acceptability) and between (repeatability) manoeuvre criteria described above [Miller 2005a; Miller 2005b; Wanger 2005; Pellegrino 2005], the CCI will be programmed to give feedback. For example, if an individual spirogram did not meet the start of test criteria, the device will indicate to the patient there was a slow start of test.

Spirometry data stored on the CCI device will be transmitted to a central database, via a GSM cradle from the patients home and be subject to centralized overread.

12.1.1.3 General Considerations

For the determination of eligibility at Screening, only spirometry data obtained with the CCI spirometer will be used.

To standardize spirometry across the two treatment arms, patients should not use a short-acting bronchodilator (e.g., albuterol) 2 hours prior to spirometry. If patients are on scheduled LABAs or LAMAs then the time from last dose to spirometry should be consistent throughout the study. Spirometry should ideally be performed before, or a minimum of 2 hours following the last inhalation of inhaled antifungals/antibiotics.

All inhaled medications, except investigational inhaled medications, are permissible during the trial and should be administered in the following order: inhaled bronchodilators, inhaled corticosteroids, inhaled antifungals/antibiotics, and then inhaled L-CsA.

Spirometry must always be performed before L-CsA inhalation.

12.1.1.4 Onsite Visits

On-site spirometry should be performed by blinded personnel.

At every visit, spirometry will be measured using the CCI spirometers. Instructions for Use (including additional information on accuracy checks, cleaning of the device and further support) for both devices are available in the Study Manual. At least three acceptable and repeatable maneuvers on each device should be obtained at each visit.

At Visit 1, the spirometry at CCI post IMP will only be performed using the CCI

Sufficient rest between both spirometry sessions should be granted to the subject at the Investigator's discretion. For the CCI spirometry session, procedures identical to those to be used at home will be followed.

12.1.1.5 Remote Visits

If patients cannot attend clinic visits due to COVID-19, remote visits using guided home spirometry may be performed (FEV_1, CCI $_5$, and FVC) on the CCI device. For remote visits, the clinical trial personnel will telephone the patient and guide them through performing spirometry on the CCI device (for further assessments during remote visits, see Section 14.2.8).

Home spirometry should be scheduled around the same time as the usual clinic visits, whenever possible, to minimize the effects of circadian variation of lung function measurements.

In case measurements are not satisfactory after central overread, home spirometry may be repeated as close to the initially scheduled remote visit as possible.

12.1.2 Progression of BOS

Due to the patchy distribution of affected areas in the pulmonary allograft, chronic rejection and BOS are not uniformly detectable by biopsy. This method has turned out to be too unspecific and harmful for routine diagnostic use. Therefore, non-invasive spirometry has been routinely

established using standardized airflow measurements which nowadays is used to determine FEV_1 as a surrogate marker for the diagnosis of BOS, its classification and its control of progression.

Progression of BOS during the treatment period is defined as a further decline of FEV_1 of ≥ 10% or ≥ 200 mL from Baseline FEV_1 and absolute decrease in [redacted] of > 5% that is confirmed by measurements that are taken with [redacted] spirometer at least 2 weeks apart, OR worsening in BOS grade OR Re-transplantation, OR Death from respiratory failure.
If a patient has an event which meets one of the criteria for progression of BOS, the Investigator should report the event via the eCRF.

12.1.3 Augmentation or Change of Immunosuppression

Augmentation or change of immunosuppression/immunomodulation must be recorded in the eCRFs.

12.1.4 Re-transplantation

Re-transplantation is defined as the date that a patient undergoes lung re-transplantation surgery. The reason for re-transplantation will be recorded. At the time of re-transplantation, the patient will be withdrawn from the study.

12.1.5 Death

If a patient dies during the clinical trial, the medical monitor should be contacted as soon as is feasible. The cause of death and a clinical summary will be requested.

12.1.6 Acute Rejection and its Severity

A patient with symptoms of a concomitant medical condition should be evaluated as clinically indicated. If a patient is diagnosed with acute rejection, the diagnosis should be recorded according to the following classification scheme. Any medications used to treat acute rejection should be recorded on the concomitant medication eCRF. The grade of acute rejection, airway inflammation, chronic airway rejection and chronic vascular rejection will be evaluated by the Investigator according to the revised working formulation for classification and grading of pulmonary allograft rejection [Stewart 2007] (Table 6):

Table 6. Revised Working Formulation for Classification and Grading of Pulmonary Allograft Rejection

A: Acute rejection	
Grade 0	None
Grade 1	Minimal
Grade 2	Mild
Grade 3	Moderate
Grade 4	Severe
B: Airway inflammation	
Grade 0	None
Grade 1R	Low grade
Grade 2R	High grade
Grade X	Ungradable
C: Chronic airway rejection – obliterative bronchiolitis	
0	Absent
1	Present
D: Chronic vascular rejection – accelerated graft vascular sclerosis	

"R" denotes revised grade to avoid confusion with 1996 scheme.

12.1.7 Antibody-Mediated Rejection

While less common in patients more than one-year post-transplant, if the treating physician makes the diagnosis of antibody-mediated rejection (AMR), this should be recorded in the eCRF. The diagnostic criteria and a working consensus definition established by the ISHLT with the aim of determining criteria for pulmonary AMR and establishing a definition should be used to support the diagnosis [Levine 2016]. See Appendix III for further information on Antibody-Mediated Rejection.

12.1.8 Quality of Life Questionnaire (EQ-5D-5L)

The EQ-5D-5L is a standardized measure of health status developed by the EuroQol Group in order to provide a simple, generic measure of health for clinical appraisal. Patients will be asked to manually complete the EQ-5D-5L at Visits 1, 4, 6, and 9/EoT and results will be entered into the eCRFs by site staff.

12.1.9 Hospitalizations

Hospitalizations for any reason other than a scheduled procedure should be considered as serious adverse event and should be recorded in the eCRFs with admission date, discharge date, reason for admission, and additional diagnoses. If a patient requires mechanical ventilation for any reason other than a planned surgical procedure, dates of mechanical ventilation, and reason for mechanical ventilation should be recorded separately. A Hospitalization Discharge Summary may be requested by the Medical Monitor and shared with the DMC and/or AC.

13 ASSESSMENT OF SAFETY

13.1 Safety Parameters

Safety Assessments at every visit include physical examination, vital signs, adverse event reporting, and clinical laboratory parameters (performed at the local labs in each study site).

Acute tolerability of IMP during initial dosing will be assessed by spirometry before and after inhalation.

COVID-19 specific consideration: In case the planned on-site visit cannot be performed, the PI or the Sub-Investigator and/or an appropriately qualified site study team member must call the patient to assess any potential adverse events (AEs) and to confirm the patient's status and wellbeing (see also Section 14.2.8).

13.1.1 Adverse Events (AEs)

13.1.1.1 Definitions

Adverse Event (AE)

An AE is any untoward medical occurrence in a patient after administration of a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH Guidelines for Good Clinical Practice, E6(R2), 9 November 2016).

This includes worsening of a pre-existing condition or increase in frequency of a pre-existing condition. An adverse event is considered serious if it meets any of the serious criteria listed below. To ensure no confusion or misunderstanding of the difference between the terms "serious" and "severe", which are not synonymous, the following clarification is provided:

The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious", which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning. Seriousness (not severity) serves a guide for defining regulatory reporting obligations.

The official definition also extends to AEs occurring during the off-treatment follow-up period.

Each patient will be closely observed and questioned for AEs during the clinical trial period with non-leading questions (e.g., how do you feel?). The patients will be instructed to report to the clinical trial staff immediately any symptoms and/or signs which occur between the scheduled observation times.

All AEs reported by the patient or observed by the Investigator or hospital personnel will be documented in the respective eCRF. The following information regarding each adverse event will be obtained: date and time of onset and resolution (duration), serious or non-serious (as defined below), severity, treatment required, outcome, relationship to certain medication, and if the AE caused withdrawal from the clinical trial.

In addition to the Investigator's own description of the AE, each AE event will be coded by data management according to the Medical Dictionary for Regulatory Activities (MedDRA) code list. The verbatim term will be recorded in the eCRF.

Abnormal laboratory test results will be reported as AEs if they are indicative of a clinical condition that meets the criteria for an AE.

Adverse Drug Reaction (ADR)

Adverse drug reactions (ADRs) are all noxious and unintended responses to a medicinal product related to any dose that a causal relationship between the medicinal product and an adverse event is at least a reasonable possibility, i.e., the relationship cannot be ruled out. An unexpected ADR is any adverse reaction not identified in nature, frequency, or intensity in the current Investigator's Brochure.

Serious Adverse Event (SAE)

Each adverse event is to be classified by the Investigator as SERIOUS or NON-SERIOUS.

The International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use defines a serious adverse event as any adverse event occurring at any dose that results in any of the following outcomes:

- Death
- A life-threatening event (immediate risk of death at the time of the event)
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly/birth defect
- Other important medical event (Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious.)

13.1.1.2 Relationship to Clinical Trial Treatment Assignment

All adverse events will be evaluated by the Investigator for potential relationship to the clinical trial treatment assignment (L-CsA plus SoC or SoC alone) in the following categories:

- **Unrelated**: An AE for which there is no evidence of any causal relationship with the clinical trial treatment assignment
- **Possible**: An AE that might be due to the use of the clinical trial treatment assignment. An alternative explanation, e.g., concomitant drug(s), concomitant disease(s), is inconclusive. The relationship in time is reasonable; therefore, the causal relationship cannot be excluded.
- **Definite**: An adverse event that follows a reasonable temporal sequence from administration of the clinical trial treatment assignment; that follows a known or expected response pattern to the suspected clinical trial treatment assignment.

13.1.1.3 Maximum Intensity

All adverse events will be graded according to the following:

- **Mild**: Event requiring no special treatment and generally does not interfere with usual activities.
- **Moderate**: Event that impairs usual activities but may be ameliorated by simple therapeutic maneuvers.
- **Severe***: Event which impairs usual activities and requires intervention.

* There is a distinction between a severe adverse event and a serious adverse event; a severe reaction is not a serious adverse event unless it meets one of the criteria for serious events (see Section 13.1.1.1).

13.1.1.4 Serious Adverse Event (SAE) Reporting

Any serious adverse events occurring

- between the first clinical trial procedure after obtaining informed consent and within 4 weeks after the completion of the EoT visit, whether or not considered related to the clinical trial treatment assignment
  or
- at any time after completion of the last follow-up and coming to the attention of the Investigator, if it is judged as related to the patient's participation in the clinical trial

must be reported to CCI IMMEDIATELY, but within 24 hours upon knowledge, as follows:

- Document the event in the eCRF within 24 hours of discovery
- Submit the SAE report by FAX or e-mail it to a list of predefined recipients.
- Follow-up the SAE until the outcome is determined, providing periodic updates in the eCRF
- Provide any additional information if requested

Contacts of CCI are as follows:

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13.1.1.4.1 Timelines for Suspected Unexpected Serious Adverse Reaction (SUSAR) reporting to Competent Authorities and IRBs/ECs

All suspected unexpected serious adverse reactions (SUSARs) will be reported by the Sponsor to the Competent Authorities and to the IRBs/ECs concerned as soon as possible, but within a maximum of 15 days (fatal or life-threatening SUSARs within a maximum of 7 days) of first knowledge.

Relevant follow-up information of fatal or life-threatening SUSARs will be communicated subsequently within an additional eight days.

13.1.1.4.2 Other Events to be Treated as Serious Adverse Events

Exposure to drug during pregnancy/lactation:

In principle, pregnancy and the lactation period are exclusion criteria. In the event of a pregnancy occurring during the course of the clinical trial, the patient must be withdrawn from all IMP treatment immediately and permanently. The Sponsor must be notified without delay and the patient followed during the entire course of the pregnancy and postpartum period. Prenatal and neonatal outcomes must be recorded even if they are completely normal and without AEs. The SAE reporting procedure should be followed, even though pregnancy is not considered an SAE. No "serious criterion" should be checked. The SAE report form is solely used to ensure expedited reporting.

If any clinical trial patient becomes pregnant during the study period, the Investigator must contact Zambon SpA as the Sponsor of the clinical trial or its designee to discuss the management of the patient.

13.1.1.4.3 Investigational Medicinal Product (IMP) Overdosing

An overdose is a deliberate or inadvertent administration of a treatment at a dose higher than specified in the protocol. For the purposes of this clinical trial, any dose of aerosolized IMP administered to a patient that exceeds the dose foreseen in the protocol by more than 50% over 4 weeks should be reported as an overdose.

The Investigator/Sponsor responsibilities in regard to reporting SAEs are in accordance with the US Food and Drug Administration and other Competent Authorities concerned.

13.1.1.4.4 Events not Regarded as Adverse Events/Serious Adverse Events

The following events will be not regarded as AEs/SAEs:

- Pre-scheduled (before any trial-related activities commenced) hospitalizations/surgeries/ interventions
- Trial endpoint-related worsening; this will be assessed in the endpoint analysis.

13.1.1.4.5 Duration of Follow-up after Adverse Events

All adverse events should be followed until they are resolved or until a stable clinical endpoint is reached. The occurrence of AEs and SAEs will be monitored until 4 weeks after the EoT visit. Any patient with ongoing SAEs will be followed until recovery or stabilization of the SAE.

COVID-19 specific considerations: All confirmed cases of COVID-19, occurring after the patient has provided informed consent and until 4 weeks after the EoT visit, must be reported as SAE, and reported to CCI **within 24 hours <u>upon knowledge</u>**.

The seriousness criterion for COVID-19 hospitalized patients should be "hospitalization"; if the patient was not admitted to a hospital or the event is not resulting in prolonged hospitalization, the seriousness citerion "medically significant" should be used.
In all cases, details of the patient's symptoms, treatment and any clinically relevant information, should be entered in the SAE forms, as appropriate.

13.1.2 Acute Tolerability of L-CsA

Acute tolerability of L-CsA during initial dosing will be determined by measuring spirometry CCI [redacted]. FEV_1 will be measured at prior to dosing with L-CsA. A decline of CCI associated with symptoms could warrant IMP discontinuation.

13.1.3 Infections

Acute infections will be evaluated according to the following criteria:

- <u>Bacterial infection:</u> bacterial infections will be diagnosed by conventional laboratory culture methods in combination with clinical symptoms [Bonvillain 2007]. Pneumonia requires presence of new worsening infiltrates by chest radiography and growth of organisms in Bronchoalveolar Lavage (BAL) or sputum samples with anti-microbial treatment.
- <u>Fungal infection:</u> the revised consensus definitions of the European Organization for Research and Treatment of Cancer / Mycosis Study Group will be used for the diagnosis of fungal infections [De Pauw 2008]. Fungal infections will be classified as proven, probable or possible according to host, clinical and microbiological criteria.
- <u>Viral infection:</u> the diagnosis of viral infection requires a 4-fold increase in serial serum samples and/or one of the following: a newly positive IgM antibody titer associated with clinical signs/symptoms requiring antiviral therapy; sero-conversion; virus isolation; or

histopathologic evidence of viral infection [Bonvillain 2007]. The diagnosis of viral pneumonia requires impairment of either pulmonary mechanisms or gas exchange, or reduction in gas transfer in the appropriate clinical context.

Occurrence of acute infections will be monitored permanently throughout the complete clinical trial period. Sputum and BAL samples will be collected, if indicated, and monitored for the presence of bacterial, viral and fungal pathogens.

An infection is defined as any acute infection requiring antimicrobial therapy. All acute infections will be reported as AEs. Information captured on the AE CRF will include: date of onset, pathogen identified, resolution, and action taken.

In the case of an acute infection episode fulfilling the criteria of a serious adverse event, the reporting timelines as laid down in Section 13.1.1.4 have to be adhered to.

The cumulative incidence of acute infections as well as the number of acute infections per patient will be summarized by treatment group.

The samples should be evaluated by the local certified laboratory of each center. When needed due to unexpected circumstances and upon clinical judgment (e.g. patient travelling, acute event happening in a location distant to clinical center etc.), samples could also be analyzed by a different laboratory as per patient convenience and the related results will be provided to the Investigator.

13.1.4 Malignancies

All malignancies must be confirmed by histopathology. Malignancies will be documented on the AE CRF.

13.1.5 Clinical Laboratory

Laboratory assessments will be performed following the routine protocols at each study visit by the local laboratory at each site.

The following clinical laboratory parameters have to be documented:

- White Blood Cell (WBC) count with differential
- Red Blood Cell (RBC) count
- Platelet count
- Alkaline phosphatase (AP)
- Alanine Aminotransferase (ALT)
- Aspartate Aminotransferase (AST)
- Gamma Glutamyl Transferase (γ-GT)
- Blood Urea Nitrogen (BUN)
- Serum bilirubin
- Serum creatinine

Laboratory assessments listed above will be performed at a certified local facility.

13.1.5.1 Pregnancy Test

Serum samples from women of childbearing potential will be collected for pregnancy tests at screening and with all scheduled blood draws. Positive results will be recorded in the eCRF and appropriate actions will be taken. At Visit 1, a serum or urine pregnancy test must be obtained within 7 days prior to randomization. A urine pregnancy test is allowed at this visit so that the result can be obtained rapidly in order to not delay randomization.

13.1.5.2 Cyclosporine A and Tacrolimus Whole Blood Trough Levels

A whole blood sample for the determination of cyclosporine A and/or tacrolimus trough level will be collected at each visit. In patients allocated to L-CsA inhalation, the sample should be collected within 60 minutes before inhalation. The mean cyclosporine A and/or tacrolimus trough level will be compared across treatment groups. Blood samples will be evaluated by the local laboratories.

13.1.5.3 Donor Specific Antibody Test

Serum samples for donor specific antibody testing will be collected at Screening, and at Visits 4, 6, and 9. If a DSA test performed within 4 weeks before screening is available, a further DSA test at screening visit will not be mandatory and the previous result will be reported in eCRF.

13.1.5.4 Renal Function

As lung transplant patients frequently present with symptoms of renal impairment special emphasis is paid for the key parameter of renal function, i.e., serum creatinine. The classification of renal dysfunction shall follow a unique terminology to describe the disorder (Table 7):

Table 7. Terminology of renal disorders

Proposed Term	Medical Condition	Preferred Term
Blood creatinine abnormal or Enhanced creatinine level or Increase of creatinine level	Temporarily increased creatinine values due to medical or nutrition condition	Blood creatinine increased
Renal impairment	Continuously increased creatinine values due to medical condition No treatment required	Renal impairment
	Continuously increased creatinine values due to medical condition Treatment required and laboratory values clinically significant	
Acute renal dysfunction	Creatinine value temporarily over 2.5 mg/dL Possible actions: Fluid administration; adjustment of TAC/CsA or co-medication	Renal impairment
Severe renal dysfunction	Creatinine value stable over 2.5 mg/dL Treatment required, but adjustment of TAC/CsA or co-medication not effective anymore; CNI toxicity	Renal impairment
Chronic severe renal dysfunction or Renal failure	Creatinine value stable over 2.5 mg/dL Dialysis or renal transplantation required	Renal impairment Renal Failure

Terminology of renal disorders table is prepared in accordance with Dictionary MedDRA - 23.1

13.1.6 Vital Signs

Vital signs will be assessed at each visit.

The following vital signs will be recorded:

Systolic and diastolic blood pressure (sitting, after 5 minutes of rest), pulse rate (sitting, after 5 minutes of rest), body temperature (the same method should be used for each patient and throughout the clinical trial), and respiratory rate.

Abnormal values will be summarized by Common Toxicity Criteria (CTC) AE grade (version 5.0) for each patient.

13.1.7 Physical Examinations

A physical examination will be carried out at the Screening Visit and at every subsequent visit. The following body systems will be examined: body weight, cardiovascular, respiratory, nervous, gastrointestinal, hepatic, renal, dermatological, musculoskeletal, extremities, eyes, ears, nose, throat, and lymphatic. Patient height will be measured at Screening Visit only.

Abnormal findings will be summarized for each patient.

13.2 Data Monitoring Committee (DMC)

An independent Data Monitoring Committee will be established to monitor the safety of IMP throughout the studies BT – L-CsA – 301 – SLT and BT – L-CsA – 302 – DLT. The DMC will monitor safety by evaluating the safety analysis generated by the unblinded independent statistician during the course of the clinical trial. Details of the safety analyses will be included in the DMC's Charter. The DMC will evaluate treatment groups for possible trends in adverse events, determine whether the basic clinical trial assumptions remain valid, evaluate whether the overall integrity, scientific merit and conduct of the clinical trial remain acceptable, and make recommendations to the Sponsor.

The DMC will peform a comparison between the rate of observed COVID-19 cases, rate of patient's withdrawal, and rate of missing data among trial participants in both treatment arms.

Members of the DMC will be unblinded to the treatment group label, i.e., the DMC will know which group is L-CsA + Standard of Care and which group is Standard of Care alone. The DMC will evaluate all safety and acute tolerability parameters, to be detailed in the DMC charter.

Safety and acute tolerability parameters include adverse events, acute tolerability of L-CsA, infections, malignancies, clinical laboratory data (white blood cell count with differential, red blood cell count, platelet count, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase, blood urea nitrogen, serum bilirubin, serum creatinine), vital signs, physical examinations and FEV_1. The statistics provided will be merely descriptive.

13.3 Adjudication Committee (AC)

The AC – consisting of three independent physicians, with documented expertise in the clinical management of lung transplantation and diagnosis and treatment of BOS - will maintain the role of reviewing the BOS progression data in blind condition once each participating subject completes the study period, with the aim to ensure consistent and complete recording of data.
If necessary, the AC may query the sites to ask for clarifications.
In case there are discrepancies between the assessment of the AC and that of the investigator re: BOS progression events, the CRO will notify the investigator. In all cases, the final decision as to whether or not specific event qualifies as BOS progression stands with the Investigator.
Details of the AC activities will be included in the AC's Charter.

14 VISIT SCHEDULE

All clinical trial visits, investigations and activities are listed in the Clinical Trial Schedule of Activities below. Further details on the methodology of these investigations and activities are described in detail in Section 12 and 13. The investigator will adhere to the visit and procedure schedule as closely as possible.

All patients assigned to a randomization number will be followed according to the protocol unless consent for follow-up is withdrawn. The Sponsor must be notified of all deviations from the protocol visit schedule or evaluations, and these visits/evaluations, if applicable, must be rescheduled or performed at the closest possible time to the original schedule.

For all patients randomized to L-CsA, proper patient understanding and competency of the CCI [redacted] use will be ensured in an individual training by the responsible clinic personnel. The patient will be administering the drug independently under supervision to verify patient competency with the equipment and drug tolerability.

After randomization, patients will visit the clinic every 4-8 weeks throughout the 48-week duration of the treatment period.

It is recommended that the Investigators contact the patient approximately 1 to 2 weeks prior to the next visit to remind the patient about the next scheduled visit. Patients will be instructed to call clinical trial personnel to report any abnormalities, including any hospitalizations or doctor visits during the interval between visits and to come to the clinical trial site if medical evaluation is needed and urgency of the situation permits. For emergency and other unscheduled visits to a medical facility other than the clinical trial site, medical records of the visit should be obtained by the Investigator.

If a patient prematurely discontinues clinical trial participation for any reason apart death, re-transplantation or withdrawal of consent, he/she will be encouraged to complete the follow-up

visits planned in the protocol. All patients who prematurely discontinue the study, including the ones who undergo re-transplantation or withdraw their consent for further participation in the clinical trial, should complete at least the evaluation listed under “End of Treatment Visit” (Visit 9) prior to discontinuation whenever possible (the reason for premature withdrawal should always be documented).

Schedule of Activities

Clinical Trial Period	**Screening**	**Baseline**	**Treatment**				
Visit Number	**-**	**V 1**	**V 2**	**V 3**	**V 4**	**V 5**	**V 6**
Week & Window	**W -4 to W 0**	**W 0 Day 1**	**W 4 ± 7 Days**	**W 8 ± 7 Days**	**W 12 ± 7 Days**	**W 16 ± 7 Days**	**W 24 ± 14 Days**
Historical Spirometry (from patient's medical file)	X						
Informed Consent[1]	X						
Demographics/Medical History	X	X					
Donor-specific Antibody Test	X[9]				X[8]		X[8]
Inclusion/Exclusion	X	X (confirmation of screening)					
Serum/Urine Pregnancy Test[2]	X	X[2]	X[8]	X[8]	X[8]	X[8]	X[8]
Physical Examination[3]	X	X	X	X	X	X	X
Vital signs[4]	X	X	X	X	X	X	X
Clinical Laboratory Tests	X	X[10]	X[8]	X[8]	X[8]	X[8]	X[8]
Spirometry[5]	X	X	X	X	X	X	X
Randomization		X					
L-CsA Administration[6]		Twice daily administration of 10 mg L-CsA					
Adherence via CCI		Permanently throughout the complete clinical trial period (for Israel: after EoT)					
Blood Sampling (CNI, mTor inhibitors)[7]	X	X	X[8]	X[8]	X[8]	X[8]	X[8]
Concomitant Medication	X	X	X	X	X	X	X
Adverse Events	Permanent assessment throughout the complete clinical trial period						
Acute L-CsA Tolerability[6]		X					
Drug Accountability[6]			X	X	X	X	X
EQ-5D-5L		X			X		X

Clinical Trial Period	**Treatment**				**Follow-Up**
Visit Number	**V7**	**V8**	**V9 / EoT**	**Remote Visit** V 2 – V8	**V10 / EoS**
Week/Month & Window	**W 32 ± 14 Days**	**W 40 ± 14 Days**	**W 48 ± 14 Days**		**W 52** CC [redacted]
Donor-specific Antibody Test			X[8]		
Serum/Urine Pregnancy Test[2]	X[8]	X[8]	X[8]		X[8]
Physical Examination[3]	X	X	X		X
Vital Signs[4]	X	X	X		X
Clinical Laboratory Tests	X[8]	X[8]	X[8]		X[8]
Spirometry[5]	X	X	X	X	X
L-CsA Administration[6]	Twice daily administration of 10 mg L-CsA				
Adherence via CCI [redacted]	Permanently throughout the complete clinical trial period (for Israel: after EoT)				
Blood Sampling (CNI, mTor inhibitors)[7]	X[8]	X[8]	X[8]		X[8]
Concomitant Medication	X	X	X	X	X
Adverse Events	Permanent assessment throughout the complete clinical trial period				
Acute L-CsA Tolerability[6]					
Drug Accountability[6]	X	X	X		
EQ-5D-5L			X		

NOTE: The Screening phase (time between screening and randomization visit) last from one day to up to 4 weeks.

[1] Conduct prior to any screening activities.

[2] Not more than 7 days prior to Randomization. Urine pregnancy test is allowed at Visit 1 only. Austria only: Urine pregnancy tests will be performed every month according to local law (Visits 5 through 9).

[3] Including body weight, height (Screening Visit only), cardiovascular, respiratory, nervous, gastrointestinal, hepatic, renal, dermatological, musculoskeletal, extremities, eyes, ears, nose, throat, and lymphatic.

[4] Including systolic and diastolic blood pressure (sitting, after 5 minutes of rest), pulse rate (sitting, after 5 minutes of rest), body temperature (the same method should be used for each patient and throughout the clinical trial), and respiratory rate.

[5] At all visits, spirometry will be performed on the CCI [redacted] and on the CCI [redacted] as per Section 12.1.1.1. At the Baseline Visit (V1), serial spirometry is to be performed prior to Randomization (CCI [redacted] and CCI [redacted] CCI [redacted] after completion of L-CsA dose (or after Randomization to SoC). Remote visits will be performed CCI [redacted]. Visit 10 spirometry will be performed in those patients not rolling over to Boston 3 on the CCI [redacted] only.

[6] Only in patients randomized to receive L-CsA treatment.

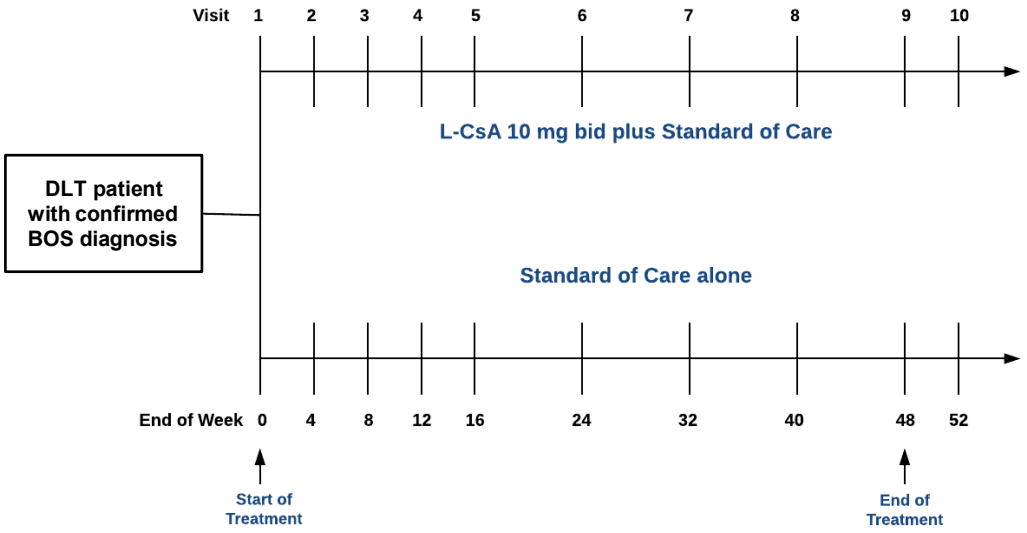
[7] within 60 min. prior to next CNI (cyclosporine A and/or tacrolimus) administration. Blood levels of mTor inhibitors will be taken only if applicable, i.e., if patient is treated with mTor inhibitors.

[8] ±3 days of the Visit date.

[9] If a DSA test performed within 4 weeks before screening is available, a further DSA test at screening visit will not be mandatory.

[10] Laboratory tests at V1 are not mandatory in case laboratory tests at screening visit have been performed within 2 weeks from V1.

14.1 Clinical Trial Flow Chart



14.2 Study Visits

14.2.1 Screening

Eligible patients who express interest in participating in the trial must sign an IRB/EC approved informed consent form (ICF) prior to initiation of any clinical trial activities. Patients who fulfill all of the inclusion criteria and none of the exclusion criteria may be enrolled into the clinical trial.

Documentation of the patient's consent and eligibility must be completed by the Investigator or his/her designee. All patients who fail the screening activities will be documented in the screening failure log.

The following screening activities will be performed during the screening period (up to 4 weeks prior to Visit 1):

- **Informed Consent:** Obtain informed consent prior to performing any clinical trial activities.
- **Demographics:** Year of birth, gender, ethnic group, date of body measurements, height and weight.
- **Medical History:** Details related to each patient's lung transplant will be recorded and will include: underlying condition (cause of lung failure), date, type of transplantation, donor demographics, donor cause of death, donor/recipient Human Leukocyte Antigen (HLA)-status, induction therapy, antiviral/antifungal/antibacterial therapy, cold ischemia time, donor/recipient CMV-status, donor/recipient Epstein Barr Virus (EBV)-status, acute lung injury. All other significant medical conditions (including GERD) and procedures (including past surgeries) occurring before the ICF is signed will be recorded as medical history. Females will be queried regarding their reproductive histories.
  - **Confirmation of screening FEV_1 above 51% of personal best FEV_1 value post-transplant.**
  - **Proof of diagnosis** will be recorded.

- **Donor-specific antibody assay:** Collect a blood sample for this assay at the Screening Visit. If DSA test performed within 4 weeks before screening is available, a further DSA test at screening visit will not be mandatory
- **Inclusion/Exclusion Criteria:** Evaluate eligibility for the clinical per the inclusion/exclusion criteria.
- **Serum Pregnancy Test:** Will be obtained in women of childbearing potential (see Appendix II). A serum or urine pregnancy test must be obtained within 7 days prior to randomization.
- **Physical Examination:** Body weight and height, cardiovascular, respiratory, nervous, gastrointestinal, hepatic, renal, dermatological, musculoskeletal, extremities, eyes, ears, nose, and throat, and lymphatic.
- **Vital Signs:** Blood pressure, heart rate, body temperature, respiratory rate.

- **Clinical Laboratory Tests:** All parameters listed in Section 13.1.5 of the protocol, to be performed in the local laboratory at the study site.
- CCI (CCI Patients will be trained by site personnel on the use of this portable spirometer.

- CCI **(on** CCI **):** FEV_1, CCI, FVC, CCI ratio
  A spirometry assessment will be performed on CCI as per Section 12.1.1.

- **Document historical spirometry data** from personal best post-transplant prior to Screening, plus
  - Date of personal best FEV_1 post-transplant and all lung function values available (FVC [absolute], FEV_1 [absolute], CCI [absolute])
  - Dates of meeting BOS 0-p or BOS 1 criteria and all lung function values available (FVC, FEV_1, CCI)
- **Blood Sampling:** For determination of serum levels of calcineurin inhibitors and mTor inhibitors
- **Dosages of maintenance immunosuppression:** record on the Concomitant Medication eCRF
- **Concomitant Medications/Therapies:** Name of drug, dosage, duration
- **Adverse Events**

14.2.2 Visit 1 / Baseline Visit

Baseline Prior to Start of Treatment

- Verify Informed Consent was obtained
- Verify Demographics/Medical History
- Verify Inclusion/Exclusion Criteria were met at the Screening Visit
- Repeat Serum (or Urine) Pregnancy Test, if result was obtained more than 7 days prior to the visit
- Physical examination
- Vital signs
- Clinical laboratory tests. Not mandatory in case laboratory tests at screening visit have been performed within 2 weeks from Visit 1
- CCI CCI); if needed, refresher training for CCI Patients will be provided with the portable spirometer at the Visit 1 / Baseline Visit only if the patient will be randomized in the study
- Randomization
- Blood sampling for serum levels of calcineurin inhibitors and mTor inhibitors

- Concomitant medications/therapies
- Adverse events
- EQ-5D-5L Questionnaire

Start of Treatment

Following activities will be performed (in patients randomized to L-CsA):

- Train patient on CCI use, drug storage and inhalation technique
- L-CsA Administration: only in patients randomized to receive L-CsA treatment. Supervise first inhalation.
- Activate CCI function in the clinical trial center
- Emphasize importance of adherence to IMP
- Emphasize importance of adherence to usual medications as taken prior to participating in the trial
- CCI (CCI) at CCI after completion of IMP inhalation
- Queries about general health status
- Verify patient is familiar with drug administration and use of nebulizer device

Prior to release from clinic, adequate drug and the nebulizer system for home use will be dispensed to each patient.

Following activities will be performed (in patients randomized to Standard of Care alone):

- Queries about general health status
- Emphasize importance of adherence to usual medications as taken prior to participating in the trial
- CCI (CCI only) at CCI after randomization

14.2.3 Visit 2 through Visit 9 / EoT

- Physical examination
- Vital signs
- Clinical laboratory tests
- Serum pregnancy test (for Austria only: additional monthly urine pregnancy tests between the trial Visits 5 through 9)
- CCI (CCI); if needed, refresher training for CCI
- L-CsA Administration
- Blood sampling serum levels of calcineurin inhibitors and mTor inhibitors
- Concomitant medications/therapies
- Adverse events
- Hospitalization

- Adherence via CCI
- Drug Accountability

14.2.4 Visits 1, 4, 6, and 9 / EoT

- Euro Quality of Life Questionnaire (EQ-5D- 5L)

14.2.5 Visit 10 / End of Study (EoS)

Only performed:

In patients not rolling over to Boston 3.

In patients who decided to withdraw the consent during the study (if possible) [see section 10.3.1]

- Physical examination
- Vital signs
- Clinical laboratory tests
- Serum pregnancy test
- CCI
- Blood sampling for serum levels of calcineurin inhibitors and mTor inhibitors
- Concomitant medications/therapies
- Adverse events
- Hospitalization

14.2.6 Permanently throughout the complete clinical trial period

- Adverse events

14.2.7 Unscheduled Visits

Whenever medically warranted, the Investigator may ask the patient to appear or the patient may elect to appear at the clinic for an unscheduled visit. All medical measures performed as appropriate shall be documented in the Case Report Form for unscheduled visits.

14.2.8 Remote Visits (V2 - V8)

Mandatory on site visits are Screening Visit, Visit 1 and Visit 9. Every effort will be made to have also all planned V2 to V8 and unscheduled visits at the study site. However, if one of these visits cannot be performed at site due to COVID-19, the site will schedule a remote visit with the patient according to the visit windows as defined in the Schedule of Activities. The scheduled time and the sequence of procedures (i.e. spirometry, IMP inhalation) should be the same as during on-site visits. The PI or Sub-Investigator and/or an appropriately qualified site study team member must call the patient to

- capture any potential adverse events (AEs) and to confirm the patient's status and wellbeing.
- capture any changes in concomitant medication
- check IMP supply with patient (if required re-supply needs to be arranged, see Section 9.7)

- provide refresher training for CCI if needed (for patients enrolled under protocol version 3.0 or later)
- guide the patient through a spirometry session using the CCI device (see Section 12.1.1.). Home spirometry should be scheduled around the same time as the usual clinic visits, whenever possible, to minimize the effects of circadian variation of lung function measurements (for patients enrolled under protocol version 3.0 or later).
- remind the patient to bring their CCI with them to their next clinic visit so that it can be checked and returned to them for continued use.
- remind the patient of IMP inhalation (to be performed after home spirometry assessment has been completed, for further details see also Section 12.1.1.4)

The PI or Sub-Investigator must confirm if, in their opinion, the patient is stable and suitable for prolonged home-treatment, and that patient is in agreement to continuing home-treatment until their next on-site study visit can occur.

All medical measures performed as appropriate shall be documented in the eCRF.

15 STATISTICS

This section presents an overview of the planned analyses. Final analyses are not limited to the summaries described herein. The Statistical Analysis Plan (SAP) will provide a detailed description of the planned statistical analyses and data summaries. If circumstances arise during the clinical trial that make these analyses inappropriate or if improved methods become available, the SAP may be revised. If discrepancies exist between the text of the statistical analysis as planned in the protocol and the final SAP, the final SAP will define the planned analysis of record. Reasons for such discrepancies will be described in the final Clinical Study Report (CSR).

This is a Phase III, prospective, multicenter, randomized, controlled trial with one blinded, adaptive interim analysis for sample size re-estimation and final identification of the model to be used for the primary analysis.

The primary outcome measure of the trial is the difference in mean FEV_1 (mL) change from baseline to Week 48 between the L-CsA plus SoC and SoC alone groups[redacted]

For the purpose of this study, "baseline FEV_1" is the mean of the best FEV_1 obtained at the Screening Visit and the best FEV_1 obtained at the Baseline (randomization) Visit.

For all spirometer-based measurements see Section 12.1.1.

Data will be listed by patient and treatment group.

15.1 Analysis Data Sets

The following analysis data sets will be analyzed:

Safety analysis set (SAFSet)

The SAF is defined as all randomized patients in the SoC group and all randomized patients receiving at least one dose of IMP in the L-CsA plus SoC group. All data collected after baseline to the end of clinical trial participation will be included in the safety summaries. Patients will be analysed according to the treatment they actually received.

Full analysis set (FAS)

The FAS is defined as all randomized patients. Patients who receive augmentation of SoC therapy, are re-transplanted, or die before the first post-baseline assessment of the primary outcome measure will be retained in the FAS. Patients will be analysed according to the treatment group to which they were randomized.

A Data Review Meeting (DRM) will be used to determine if patients should be excluded from the FAS in cases of significant, major protocol deviations that would interfere with the assessment of treatment efficacy (e.g., patients not suffering from the condition under investigation).

Per protocol Set (PPS)

The PPS is defined as all patients included in the FAS:

- who complete randomized treatment as scheduled or who are withdrawn prematurely due to lack of efficacy or lack of tolerability of the clinical trial treatment, and
- for whom no major protocol deviations interfering with the assessment of treatment efficacy are observed.

Eligibility for the analysis data sets will be determined in DRMs held after closing the database before the interim analysis and before the final analysis. Protocol deviations will be classified as 'major' when a significant influence on the assessment of the primary outcome measure for treatment efficacy cannot be excluded. Comprehensive justification for the classification of a protocol deviation as 'major' will be given in the DRM minutes as well as in the integrated CSR. Any exclusion of patients from the FAS will require individual justification.

The primary analysis of treatment efficacy will be performed in the FAS. An additional efficacy analysis in the PPS will be provided as a sensitivity analysis. Any data pertaining to safety and tolerability will be analyzed in the SAF.

15.2 Statistical and Analytical Methods

15.2.1 Descriptive Statistics

The data recorded in the case report forms will be summarized as appropriate, per treatment group and for the total group, using the following descriptive measures:

- All continuous study assessments will be summarized using descriptive statistics (i.e., n, mean, standard deviation, median, Q1, Q3, minimum, and maximum).
- All categorical study assessments will be summarized by time point, as applicable, using frequency counts and percentages.
- Discrete ordinal or higher-level data will be summarized as continuous data, but a tabulation of categories may be included additionally depending on the number of categories.

For all measures analyzed descriptively (i.e., measures to characterize the treatment groups and their baseline status, efficacy, and safety outcomes), tabulations comparing the treatment groups will be prepared. For outcomes measured more than once, the analyses will include tabulations of the measured values by visit as well as an analysis of change from baseline. Where provided, all confidence intervals will be two-sided 95% confidence intervals.

15.2.2 Characterization and Baseline Comparability of Treatment Groups

The number and percentage of patients who complete and discontinue the study treatment and the number and percentage of patients who complete and discontinue the clinical trial as well as reasons for early discontinuation (of both study treatments) will be presented.

Descriptive statistics will be presented for demographic, anthropometric, medical history, and antibody-response data.

15.2.3 Analysis of Efficacy

15.2.3.1 Primary endpoint

Analysis population and estimand

FEV_1 data collected from the on site CCI spirometer will be considered primary, while the data collected with the CCI home spirometer will be used for supportive analyses.

The primary efficacy parameter is the difference in mean FEV_1 change from baseline to Week 48 between L-CsA plus SoC and SoC alone in the FAS population. Intercurrent events will be ignored, in line with the "treatment policy strategy" (ICH E9 addendum on estimands and sensitivity analysis in clinical trials to the guideline on statistical principles for clinical trials). After intercurrent events such as stopping treatment with L-CsA, augmentation of SoC therapy etc., FEV_1 measurements will still be collected and used in the analysis. Intercurrent events preventing the use of FEV_1 measurements after the event are death, re-transplantation, and withdrawal of the patient's consent. It is anticipated that these events will be rare in this patient population. The estimand will be estimated as if these events had not happened, i.e., the unobserved FEV_1 data of such patients will be modeled until the scheduled end of treatment at Week 48.

Working hypothesis

The alterative hypothesis (H_1) for this trial is that L-CsA plus SoC is superior to SoC alone in preserving FEV_1, i. e., under H_1 it is expected that patients treated with L-CsA plus SoC will exhibit a smaller mean FEV_1 decline between baseline and the end of Week 48 than those who receive SoC alone.

The analysis model

The primary efficacy analysis will be carried out with a linear mixed model (LMM) for repeated measurements, using all available FEV_1 measurements. The dependent variables are the absolute change from baseline of FEV_1 measurements at visits 2 (i. e., the first post-baseline visit) to 9 (Week 48).

A flexible and robust model will be chosen for the random part of the LMM according to a pre-specified stepwise procedure as described in detail in the SAP. The fixed part of the LMM is specified as follows. Time will be represented in a flexible and robust way using cubic splines, as described in detail in the SAP. The model will contain the following covariates (apart from the

intercept): time splines, treatment, the interactions of time splines by treatment, baseline FEV_1, the interactions of time splines with the baseline FEV_1, region (North America versus all other countries together), age (< 55 versus ≥ 55 years), and use of azithromycin at randomization. The primary efficacy estimand will be estimated from the model as the adjusted difference between groups in mean FEV_1 change from baseline after 48 weeks. The one-sided p-value and the two sided 95% confidence interval will be provided. Superiority of L-CsA will be demonstrated by a statistically significant result (defined as $p<0.025$, one-sided) in favor of the L-CsA plus SoC treatment.

In case few data are collected at intermediate timepoints between baseline and Week 48 (see section 15.3 on interim analysis), the LMM model will be replaced by an ANCOVA model on the changes in FEV_1 from baseline to Week 48, using the same stratification factors and the same covariates described for the LMM

Sensitivity analyses

Intercurrent missing values and values missing due to death, re-transplantation and withdrawal of consent are assumed to be Missing At Random (MAR). Sensitivity analyses to the MAR assumption will be performed using different approaches, including selection modelling, simultaneous modelling of repeated FEV_1 outcome and time to drop out, and controlled missing values imputation, and an analysis where re-transplantation and death are included in the definition of outcome variable and estimand as worst outcomes.

In addition to use of LMM to analyze the primary endpoint, simple t-test comparing the difference in mean FEV1 change from baseline to Week 48 between the treatment group in the FAS population including a 95% confidence interval for the treatment difference of the mean FEV1 change between the two treatment groups will be performed.

Further sensitivity analyses will be described in the SAP.

CCI

CCI

[redacted]

CCI [redacted]

15.2.3.3 Type I error level control

The over-all type I error rate of the clinical trial will be $\alpha = 0.025$ one-sided, corresponding to $\alpha = 0.05$ two-sided.

Strong control of the clinical trial-wise type I error level will be achieved by a-priori ordering of hypotheses:

1. The primary endpoint (mean change in FEV_1 from baseline to Week 48) will be tested first.
2. CCI [redacted]

All tests will be performed using a local type I error level of $\alpha = 0.025$ (one-sided).

If testing stops due to failure to reject a null hypothesis on a particular level of the hierarchy, all lower level tests will still be performed but will be considered descriptive.

CCI [redacted]

15.2.4 Cumulative Maintenance Immunosuppression

The total immunosuppression administered from the day of randomization will be evaluated. At each trial visit, the doses of calcineurin inhibitors (e.g., tacrolimus), antimetabolic agents (e.g., mycophenolate mofetil), and corticosteroids will be recorded by the Investigator on the concomitant medications eCRF. The cumulative dose of maintenance immunosuppressive treatment will be summarized for each group and compared descriptively.

15.2.5 Analysis of Safety

Safety outcomes are listed in Section 13.1. All safety measures will be compared descriptively between the treatment groups in accordance with the concept of descriptive data analysis in the SAS population. Treatment group comparisons of safety outcomes will be based on statistical tests and confidence intervals (if applicable).

15.2.5.1 Extent of Exposure

Exposure to IMP will be confirmed based on the report of IMP vials returned and dosing recorded in the clinical database.

15.2.5.2 Adverse Events

Adverse events (AEs) will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Events will be identified as treatment-emergent (TEAE) and non-treatment-emergent (Non-TEAE) on the basis of the date of onset relative to the date of randomization.

Summaries (number and percentage of events as well as of patients with any events of a particular type) of treatment-emergent AEs (by system organ class and preferred term) will be provided. Number (%) of patients experiencing TEAEs by severity, relationship to IMP, seriousness, and action taken with regard to IMP will be summarized. Number (%) of patients with TEAEs leading to discontinuation of clinical trial participation will also be summarized.

15.2.5.3 Laboratory and Other Safety Evaluations

For clinical safety laboratory test results and vital signs, the numeric values and corresponding changes from baseline will be summarized using descriptive statistics by parameter and time point. In addition to the tables showing the distribution parameters, laboratory measures will be presented in 'shift tables' that show the position of all patients relative to the applicable reference range at clinical trial entry, broken down by the position at clinical trial exit. Common Toxicity Criteria (CTC version 5.0) will be applied to abnormal laboratory test results and abnormal vital signs.

Parameters reflecting acute tolerability of IMP (spirometry, cough, or dyspnea) will be summarized.

15.3 Interim Analysis

Approximately one-two months before the last patient of the initial sample size is projected to be randomized in the clinical trial, an interim analysis was to be performed with the following objectives:

- Re-estimation of the standard deviation of the primary endpoint.
- Evaluation of the actual amount of FEV_1 data collected at all timepoints using the CCI spirometer.
- Evaluation of the number of drop-outs and the number and pattern of the missing data.
- Appropriateness of the analysis based on the anticipated longitudinal model (LMM) vs. the analysis based on the ANCOVA model in the light of the collected data.

All analyses were to be performed on blinded data available at that time.

For the re-estimation of the standard deviation, using the methodology described in Kieser and Friede (2003), no unblinding is required and no adjustment of the significance level α is necessary.

If the sample size calculated on the basis of the new value for the standard deviation is higher than the initial estimate, the sample size of the trial might be increased accordingly in order to preserve the pre-specified power. If the re-calculated sample size is lower than the initial estimate, the clinical trial was to be continued with the initial sample size (110 patients).

Full details of the procedure for sample size re-estimation and for performing the other analyses were to be provided in the SAP.

The interim analysis has been performed by an independent, blinded statistician on 01/Dec/2021.

The conclusions were as follows:

- The LMM is applicable and therefore this model will be used for the primary analysis, while ANCOVA will be used as an additional sensitivity analysis
- The sample size is approximately adequate for this model but considered small for ANCOVA.

The statistician communicated these conclusions to the Sponsor. Confidentiality of results was ensured and no interim analysis results were provided to the Sponsor or the Investigators.

15.4 Missing Data

See Sections 15.2.3.1 and 15.2.3.2.

15.5 Sample Size Considerations

The initial sample size was based on the following consideration. Assuming a treatment group difference for mean FEV_1 change from baseline of 200 mL and a standard deviation of 350 mL at the end of treatment Week 48, an independent samples t-test with a one-sided type I error level of α=0.025 will have at least 80% power to reject the null hypothesis when the sample size in each treatment group is at least 50 patients. To compensate for a loss of power due to premature withdrawals, a total of 110 patients will initially be randomized.

Results of the interim analysis have been presented in Section 15.3.

Following FDA recommendation to obtain an adequately sized safety database for L-CsA inhalation and reach the originally planned sample size for BOSTON 1 and 2 studies combined, randomization will be stopped upon achievement of a total of approximately 220 patients in the present BOSTON-2 and BOSTON-1 studies.

Since the BOSTON-1 study is experiencing serious enrolment delay, it is anticipated that approximately 160-170 patients will be randomized in the present study and 50- 60 patients in BOSTON 1. This sample size will provide a more reasonable power for the planned sensitivity analyses, including ANCOVA, for the present study (BOSTON-2).

16 DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents and data records include but are not limited to: hospital records, clinical and office charts, laboratory notes, memoranda, patients' diaries or questionnaires or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilms or magnetic media, X-rays, patient files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

Case report forms, all copies of test results, and clinical trial-related regulatory documents [e.g., Informed Consents, Institutional Review Board (IRB)/Ethics Committee (EC) approvals/correspondence, etc.] must be available always for regulatory agency inspection and review by the Sponsor or its designee. During the periodic site monitoring visits, the source documents will be verified against data entered onto the eCRF to assure that all data is accurately and completely reflected on the patient's eCRF.

17 QUALITY CONTROL AND QUALITY ASSURANCE

This clinical trial will be monitored by the Sponsor or its designee according to ICH GCP (E6R2) guidelines. A site visit will be held prior to initiation of patient enrollment. The protocol, CRFs, IMP supplies, and relevant procedures will be explained in detail at the Site Initiation Visit. Subsequent to patient enrollment, a study site monitor from the Sponsor or its designee will review the eCRFs and source documents to ensure that the study is conducted according to the protocol and ICH GCP guidelines.

To ensure compliance with ICH GCP guidelines and all applicable regulatory requirements, the Sponsor or its designee may conduct a quality assurance audit. Regulatory agencies may also conduct a regulatory inspection of this clinical trial. Such audits or inspections can occur at any time during or after completion of the clinical trial. If audits or inspections occur, the Investigator and the institution agree to allow the auditor/inspector direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the auditor/inspector to discuss findings and any relevant issues.

18 INSTITUTIONAL REVIEW BOARD (IRB)/ETHICS COMMITTEE (EC)

The protocol and informed consent form(s) for this clinical trial must be approved by an appropriately constituted Institutional Review Board (IRB)/Ethics committee (EC) as defined by local requirements. A list of the IRB/EC voting members, their titles or occupation, and their institutional affiliations (if available and allowable under EU law) and/or the IRB/EC general assurance number, if applicable, must be submitted to the Sponsor or its designee to be archived in the Trial Master File.

Any documents that the IRB/EC may need to fulfill its responsibilities, such as protocol amendments, and information concerning patient recruitment, payment or compensation procedures, or information from the Sponsor or its designee will be submitted to the IRB/EC. The IRB's/EC's written approval/favorable opinion of the protocol and the informed consent form/patient information leaflet will be in the possession of the Investigator before the clinical trial is initiated. The IRB's/EC's unconditional approval statement/favorable opinion will be transmitted by the Investigator to the Zambon SpAor its designee prior to shipment of IMP supplies to the site. This approval/favorable opinion must refer to the clinical trial by exact protocol title and number, and should identify the documents reviewed and the date of review.

Protocol modifications or changes may not be initiated without prior written IRB/EC approval/favorable opinion except when necessary to eliminate immediate hazards to the patients or when the change(s) involves only logistical or administrative aspects of the clinical trial. Such modifications will be submitted to the IRB/EC and written verification that the modification was submitted should be obtained.

The IRB/EC must be informed by the Investigator of informed consent changes or revisions of other documents originally submitted for review; serious and/or unexpected adverse events occurring during the clinical trial; new information that may affect adversely affect the safety of the patients or the conduct of the clinical trial; an annual update and/or request for re-approval; and when the clinical trial has been completed.

The Investigator will ensure that the conduct of the clinical trial conforms to the Declaration of Helsinki (current revision) and with applicable national laws and regulations for clinical research. Before starting this clinical trial, the protocol will be submitted to each center's Institutional Review Board/Ethics Committee for evaluation at each site. As required, the clinical trial will not start before the IRB/EC has given approval/favorable opinion.

Periodic status reports and adverse events must be submitted to the IRB/EC according to the IRB/EC reporting requirements. The IRB/EC must also be notified of completion of the clinical trial and a final report must be submitted to the IRB/EC in accordance with the IRB/EC reporting requirements. The Investigator must maintain an accurate and complete record of all communication, reports and submissions to the IRB/EC.

19 PATIENT INFORMED CONSENT

Patients will be required to sign a statement of informed consent that meets the requirements of the Code of Federal Regulation (US FDA Title 21 CFR 50), local regulations, ICH GCP guidelines, and the IRB/EC of the center. The medical record will include a statement that written informed consent was obtained before the patient was enrolled in the clinical trial and the date written consent was obtained.

Members of the treating team will review the nature of the clinical trial, its purpose, the procedures involved, the expected duration, the potential risks and benefits and alternative therapies including best supportive care. Patients must be informed that participation in the clinical trial is voluntary, he/she may withdraw from the clinical trial at any time and withdrawal from the trial will not affect his/her subsequent medical treatment or relationship with the treating physician. Financial costs that will or may be incurred as a result of participation in the trial, as well as the efforts to maintain patient confidentiality will also be discussed.

This consent must be dated and retained by the Investigator/Designee as part of the clinical trial records. A copy of the informed consent form must be given to the patient. If the patient signed the informed consent more than 30 days prior to the date of randomization, it should be documented that consent has not been rescinded. Alternatively, the site should follow the IRB/EC's requirement for re-consenting a patient.

If an Experimental Patient's Bill of Rights is applicable at the Investigator's site, that form must also be prepared and signed by each patient and retained as a part of the required clinical trial records. A copy of the Bill of Rights must be given to the patient or the patient's legally authorized representative.

A copy of the IRB/EC approved consent form must be submitted to Zambon SpA or its designee prior to shipment of IMP supplies to the Investigator. Each patient's signed informed consent must be kept on file by the Investigator for regulatory authority and Sponsor inspection at any time.

The HIPAA Privacy rule authorization or GDRP language must be included in the Informed Consent/authorization form (or a separate authorization document) and approved by the IRB/EC (or Privacy board) as applicable.

The Declaration of Helsinki, as amended (version of Fortaleza, 2013), recommendations guiding doctors in clinical research must be signed by the Investigator and returned to the Sponsor or its designee. A copy must also be kept on file by the Investigator.

20 NOTIFICATION TO AUTHORITIES

The protocol and any applicable documentation (e.g., patient information, informed consent form) will be notified to the regulatory authorities in compliance with applicable US, Israeli and European regulations.

21 DATA HANDLING AND RECORD KEEPING

21.1 Confidentiality

To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records will be identified by a coded number and initials only. All clinical trial records will be kept at each center in a secure location. Clinical information will not be released without written permission of the patient, except as necessary for monitoring or inspections by the authorities or a designee of the Sponsor. The Investigator must also comply with all applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996).

The Investigator must assure that the privacy of the patients, including their personal identity and all personal medical information, will be maintained at all times. In CRFs and other documents patients will not be identified by their names, but by an identification code (e.g., by an arbitrary, unique patient number).

The Investigator agrees that all information received from Zambon SpA including but not limited to this protocol, eCRF, and any other clinical trial information, remain the sole and exclusive property of Zambon SpA during the conduct of the clinical trial and thereafter. This information is not to be disclosed to any third party (except employees or agents directly involved in the conduct of the trial or as required by law) without prior written consent from Zambon SpA. The Investigator further agrees to take all reasonable precautions to prevent the disclosure by any employee or agent of the investigative site to any third party or otherwise into the public domain.

21.2 Completion of Electronic Case Report Forms

An electronic case report form (eCRF) will be used in this clinical trial. Staff authorized to enter or modify data on the eCRF will be assigned an appropriate user role:

- study coordinators or nurses may enter, modify, or delete data and may enter, modify, or delete responses to queries;
- investigators may perform the same activities as study coordinators. Moreover, they may authorize data by entering an electronic signature.

Any data entries, modifications, and deletions will be recorded in an automated audit trail together with a time stamp and the identification code of the person performing the entry / modification / deletion.

To access the eCRF authorized users will receive a unique user name and password that will also be required for data authorization (investigators only). These credentials are strictly personal and confidential and must not be shared with anyone.

Investigators will be provided with detailed eCRF Completion Guidelines that will identify the required data points to be collected, how to document them and when these data should be documented. Appropriate training in electronic data capture and support will be provided.

It is the responsibility of the Investigator to maintain adequate and accurate eCRFs to record (according to the eCRF Completion Guidelines) all observations and other data pertinent to the clinical trial obtained during scheduled, remote or unscheduled visits. All eCRFs should be fully completed to ensure accurate data interpretation**.**

All eCRF entries should be made within a maximum of 5 business days of a study visit by a designated and trained member of the site staff. The Investigator must ensure the accuracy, completeness legibility and timeliness of data reported in the eCRF and all required reports. Any change or correction to an eCRF must be documented as instructed and explained (if necessary) in the clinic notes, this applies to both written and electronic changes. The patient's medical source documents must support any changes made.

Data reported in the eCRF that are derived from source documents should be consistent with the source documents or the discrepancies should be explained.

The investigator must review and electronically sign the completed eCRF casebook to verify its accuracy.

The main objective is to obtain those data required by the protocol in a complete, accurate, legible, and timely fashion. These data in the eCRF should be consistent with the relevant source documents.

The eCRFs and the corresponding electronic database shall be processed, evaluated, and stored in anonymous form in accordance with the Personal data-protection regulations.

These data recorded in the course of this clinical trial may periodically be remotely reviewed and compared to the electronic database. The computerized handling of these data after receipt of the eCRFs may generate additional requests via electronic queries to which the Investigator is obliged to respond by confirming or modifying these data questioned. These requests with their responses will be available in the eCRFs held by the Investigator and Sponsor.

The eCRFs are regulatory documents and must be suitable for electronic submission to authorities.

The Investigator must keep a separate patient identification list showing code numbers, names, and dates of birth to allow unambiguous identification of each patient included in the clinical trial. A note will be made in the hospital medical records that the patient is participating in a clinical trial. The eCRF will be completed appropriately.

21.3 Source Data and Patient Files

The Investigator has to keep a paper or electronic patient file for every patient participating in the clinical trial. In this patient file, the available demographic and medical information of a patient has to be documented, in particular the following: name, date of birth, sex, height, weight, patient history, concomitant diseases and concomitant medication (including changes during the clinical trial), statement of entry into the clinical trial, trial identification, patient number, the date of

informed consent, all study visit dates, pre-defined performed examinations and clinical findings, observed AEs (if applicable) and reason for withdrawal from clinical trial treatment or from the trial (if applicable). It should be possible to verify the inclusion and exclusion criteria for the clinical trial from the available data in this file. It must be possible to identify each patient by using this patient file.

Additionally, any other documents with source data, especially original printouts of data that were generated by technical equipment have to be filed. This may include laboratory value listings, ECG recordings, X-rays, CT scans, etc. (if applicable). All these documents have to bear at least the patient identification and the printing date printed by the recording device to indicate to which patient and to which procedure the document belongs. The medical evaluation of such records should be documented as necessary and signed/dated by the Investigator.

Printouts of computerized patient files must be signed and dated by the Investigator, and kept as a source document.

21.4 Investigator File and Archiving

The Sponsor will provide, and the investigator will maintain an Investigator Site File (ISF) containing all essential documents for the clinical trial. The ISF will contain each patient's eCRF pdf after the database has been locked. The investigator should maintain a record of the location(s) of their respective essential documents including source documents. The storage system used during the trial and for archiving (irrespective of the type of media used) should provide for document identification, version history, search and retrieval.

Essential documents are subject to audit and inspection at all times and will be archived according to applicable regulatory requirements.

21.5 Monitoring, Quality Assurance, and Inspection by Authorities

This clinical trial is to be conducted in accordance with ICH GCP E6 (R2) and applicable US, Israeli and European regulations. Monitoring and auditing of the trial will be the responsibility of the Sponsor or a designee authorized by the Sponsor.

To ensure compliance with ICH GCP guidelines and all applicable regulatory requirements, the Sponsor or its designee may conduct quality assurance audits. Regulatory agencies may also conduct a regulatory inspection of this clinical trial. Such audits or inspections can occur at any time during or after completion of the clinical trial. If audits or inspections occur, the Investigator and the institution agree to allow the auditor/inspector direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the auditor/inspector to discuss findings and any relevant issues and to implement corrective and preventive actions for such findings.

21.6 Data Management and Data Control

The Sponsor will be responsible for the processing and quality control of the data. Data management and filing will be carried out as described in the contracted CRO's SOPs for clinical trials.

Prior and concurrent medications within 30 days of randomization will be listed. Prior/concurrent medications will be coded using the World Health Organization (WHO-DD) classification system and grouped by drug class and preferred term. AEs and medical history terms will be coded using MedDRA.

The eCRF data and patient data listings will be archived by the Sponsor for the lifetime of the product. No clinical trial document or image should be destroyed without prior written agreement between the Sponsor and the Investigator. Should the Investigator wish to assign the clinical trial records to another party or move them to another location, advance written notice should be given to the Sponsor.

22 FINANCING AND INSURANCE

A separate financial agreement (Clinical Investigators' Agreement) will be made between each Investigator and the Sponsor before the IMP is delivered. The Sponsor will ensure financing of the complete clinical trial.

The clinical trial is covered under a Sponsor group combined liability insurance policy. The certificate of insurance and an information leaflet containing essential information about the insurance coverage will be provided upon request.

23 PUBLICATIONS

After conclusion of the clinical trial, an integrated clinical and statistical study report will be prepared by the Sponsor or designee. The first publication of the clinical trial will comprise of data from the entire clinical trial population enrolled at all sites. Any publications of the results, either in part or in total (abstracts in journals or newspapers, oral presentations, etc.) by Investigators or their representatives will require pre-submission review by the Sponsor. The Sponsor is entitled to delay publication in order to obtain patent protection. For more details regarding publications, refer to the clinical trial agreement/Investigator trial agreement.

The results of the clinical trial will be published and/or presented in scientific meetings in a timely manner. Any formal publication of clinical trial results will be a collaborative effort between the Sponsor and the Investigator(s). All manuscripts and abstracts will be reviewed and approved in writing by the Sponsor prior to submission.

Policies regarding the publication of the clinical trial results are defined in the Clinical Investigator's Agreement.

24 REFERENCES

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25 APPENDIX I – CORE INFORMED CONSENT FORM

The informed consent form must be adapted to local requirements but must contain at least the following points:

- That the trial involves research.
- The purpose of the trial.
- The trial treatment(s) and the probability for random assignment to each treatment.
- The trial procedures to be followed, including all invasive procedures.
- The patient's responsibilities.
- Those aspects of the trial that are experimental.
- The reasonably foreseeable risks or inconveniences to the patient and, when applicable, to an embryo, foetus, or nursing infant.
- The reasonably expected benefits. When there is no intended clinical benefit to the patient, the patient should be made aware of this.
- The alternative procedure(s) or course(s) of treatment that may be available to the patient, and their important potential benefits and risks.
- The compensation and/or treatment available to the patient in the event of trial related patient injury.
- The anticipated prorated payment, if any, to the patient for participating in the trial.
- The anticipated expenses, if any, to the patient for participating in the trial.
- That the patient‘s participation in the trial is voluntary and that the patient may refuse to participate or withdraw from the trial, at any time, without penalty or loss of benefits to which the patient is otherwise entitled.
- That the monitor(s), the auditor(s), the IRB/IEC, and the regulatory authority(ies) will be granted direct access to the patient‘s original medical records for verification of clinical trial procedures and/or data, without violating the confidentiality of the patient, to the extent permitted by the applicable laws and regulations and that, by signing a written informed consent form, the patient is authorizing such access.
- That records identifying the patient will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available. If the results of the trial are published, the patient's identity will remain confidential.
- That the patient will be informed in a timely manner if information becomes available that may be relevant to the patient‘s willingness to continue participation in the trial.

- The person(s) to contact for further information regarding the trial and the rights of trial patients, and whom to contact in the event of trial-related injury.
- The foreseeable circumstances and/or reasons under which the patient‘s participation in the trial may be terminated.
- The expected duration of the patient‘s participation in the trial.
- The approximate number of patients involved in the trial.
- Information regarding the trial can be found on clinicaltrials.gov.

26 APPENDIX II – ACCEPTABLE METHODS OF CONTRACEPTION

A woman is considered of childbearing potential, i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile. The status of post-menopausal is defined as no menses for 12 months without an alternative medical cause and/or the age of 60 years.

According to the Clinical Trial Facilitation Group (CTFG) dated 15/04/2014, the following methods are allowed for contraception during the clinical trial with a failure rate of less than 1%:

- combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation[1]:

  o oral
  o intravaginal o transdermal

- progestogen-only hormonal contraception associated with inhibition of ovulation[1]:

  o oral
  o injectable
  o implantable[2]

- intrauterine device (IUD)[2]
- intrauterine hormone-releasing system (IUS)[2]
- bilateral tubal occlusion[2]
- vasectomised partner[2,3]
- sexual abstinence[4]

  From results of previous clinical trials with aerosolized L-CsA potential interactions between the IMP and hormonal contraceptives have not been investigated. It is recognized that hormonal contraceptives can increase serum levels of systemically dosed cyclosporine, but cyclosporine does not alter the efficacy of hormonal contraceptives. Subjects in the trial will be monitored for systemic cyclosporine levels.

[1] Hormonal contraception may be susceptible to interaction with the IMP, which may reduce the efficacy of the contraception method

[2] Contraception methods that in the context of this guidance are considered to have low user dependency.

[3] Vasectomised partner is a highly effective birth control method provided that partner is the sole sexual partner of the WOCBP trial participant and that the vasectomised partner has received medical assessment of the surgical success.

[4] In the context of this guidance sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject.

27 APPENDIX III – ANTIBODY-MEDIATED REJECTION

Key diagnostic criteria include the presence of antibodies directed toward donor human leukocyte antigens and characteristic lung histology with or without evidence of complement 4d within the graft. Exclusion of other causes of allograft dysfunction increases confidence in the diagnosis but is not essential. Pulmonary AMR may be clinical (allograft dysfunction which can be asymptomatic) or sub-clinical (normal allograft function). Both clinical and sub-clinical AMR were further sub-categorized into 3 mutually exclusive possibilities (definite, probable and possible). These categories were based on the degree of certainty related to the presence or absence of a number of pathologic, serologic, clinical and immunologic criteria (Table 8 and Table 9).

Table 8. Definition and diagnostic certainty of clinical pulmonary AMR

	Allograft dysfunction	Other causes excluded	Lung histology	Lung biopsy C4d	DSA
Definite	+	+	+	+	+
Probable	+	+	+	-	+
Probable	+	+	+	+	-
Probable	+	+	-	+	+
Probable	+	-	+	+	+
Possible	+	+	+	-	-
Possible	+	+	-	-	+
Possible	+	+	-	+	-
Possible	+	-	+	+	-
Possible	+	-	+	-	+
Possible	+	-	-	+	+

DSA, donor-specific antibodies; +, item present; - item absent or missing

Table 9. Definition and diagnostic certainty of sub-clinical pulmonary AMR

	Lung histology	Lung biopsy C4d	DSA
Definite	+	+	+
Probable	+	-	+
Probable	-	+	+
Probable	+	+	-
Possible	+	-	-
Possible	-	+	-
Possible	-	-	+

DSA, donor-specific antibodies; +, item present; - item absent or missing

28 APPENDIX IV – STUDY PROTOCOL VERSIONS HISTORY OF CHANGES

Study Protocol Version 6.0 dated 7-April-2023
The Study Protocol version 6.0 (dated 7-April-2023) has been issued CCI [redacted] for amendment to Study Protocol BOSTON-2 version 5.0 submitted on December 8, 2022, IND 078854 L-Cyclosporine A. This amendment is considered to be substantial according to the guidance on the request to the competent authorities for authorisation of substantial amendments (2010/C 82/01). CCI [redacted] Full descriptions of substantial and other minor changes or clarifications are provided in the Summary of Changes Document.

Study Protocol Version 5.0 dated 28-October-2022
The Study Protocol version 5.0 (Amendment 4) has been issued to CCI [redacted] ensure the adequacy of the safety database and to implement the efforts to minimize missing data in both studies. This amendment is considered to be substantial based on the criteria set forth in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the European Union. CCI [redacted] Full description of substantial and other minor changes/clarifications are provided in the Summary of Changes Document.

Study Protocol Version 4.0 dated 19-January-2021
CCI [redacted] CCI [redacted] Only discussions (type C meeting) and the EMA guidelines on clinical trial conducted during the COVID-19 contingency was added. This amendment had considered to be substantial based on the criteria set forth in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the European Union.

Full description of substantial and other minor changes are provided in the Summary of Changes Document.

Study Protocol Version 3.0 dated_9-June-2020
The Study Protocol version 3.0 (Amendment 2) has been issued to add the COVID-19 related measures in order to ensure patient safety and efficacy data collection in case a given on-site visits cannot take place due to COVID-19 outbreak, including the possibility to perform remote visits, to carry out spirometry examination at patient home and the IMP re-supply at patient home. Furthermore the Eligibility Criteria have been reviewed. The amendment had considered to be substantial based on the criteria set forth in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the European Union. Full description of substantial and other minor changes/clarifications are provided in the Summary of Changes Document.

Study Protocol Version 2.0 dated 30-April-2019
The Study Protocol version 2.0 (Amendment 1) has been issued to review the Eligibility Criteria, Treatment of Patients, Assessment of Efficacy and Safety, Visits Schedule and Statistical considerations. This amendment had considered to be substantial based on the criteria set forth in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the European Union. Full description of substantial and other minor changes/clarifications are provided in the Summary of Changes Document.