Investigator Initiated Trial Protocol Template

Information on the CareDx Investigator Initiated Trial Protocol Template – please read before starting

The template is available for use by all investigators who are carrying out clinical research studies funded by CareDx. One of the requirements for IIT awards is the use of this template, where alternative GCP-compliant protocols will not be accepted.

Note that some of the sections of this template may not apply to your study and may be deleted, and while new sections not covered by this template may need to be added.

All advisory text and quotations from GCP are highlighted in yellow. These should all be deleted before finalising the document. All sample text is in 'basic text' style. This text of course will be altered or deleted as required while you produce the draft.

If not relevant, sections may be deleted entirely. There may also be instances where rearrangement of the subsections within section 8 is appropriate, in order to match with the order of study processes. Advisory text for deletion/rearrangement is highlighted in blue.

Repetition of information throughout the protocol is not necessary; it may be useful to cross-reference other sections of the protocol to avoid repetition.

Should you require any assistance, contact either Quynh Tran, Clinical Trials Manager, or Dr Sham Dholakia, Medical Director, as early as possible in the planning stage.

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Study Title: **A**ssessment of **D**onor Derived Cell Free DNA and **U**tility in **L**ung **T**ransplantation

Internal Reference Number / Short title: ADULT

IRB Ref: Will be submitted through University of Pittsburgh's IRB

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Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, FDA, host organisation, and members of the Research Ethics Committee, unless authorised to do so.

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1. SYNOPSIS

Study Title	The external validation and clinical utility assessment of AlloSure in Lung Transplantation	
Internal ref. no. / short title	ADULT	
Study Design	Prospective Cohort Study	
Study Participants	50	
Planned Sample Size	• Sample at baseline (prior to txp), then 24hours, 48hours, 72 hours post txp • Once a week for 3 months • Once monthly for months 6-12 • With any "For Cause" and surveillance biopsy	
Planned Study Period	12 months (option to add up to 24 additional months after review of data)	
	Objectives	**Outcome Measures**
Primary	Correlation between AlloSure dd-cfDNA and primary graft dysfunction, allograft rejection (ACR or AMR).	Histological assessment of tissue biopsy and pulmonary function test with paired AlloSure dd-cfDNA result – performed both 'For Cause' and 'Surveillance'
Secondary	Assessment of AlloSure in the predictive and prognostic value of diagnosing CLAD, allograft failure and premature death	DSA results, clinical tests, clinical details and events from patient records will be examined to assess the association with AlloSure.

2. ABBREVIATIONS

Define all unusual or 'technical' terms related to the project.

CRF	Case Report Form
dd-cfDNA	Donor Derived Cell Free DNA
GCP	Good Clinical Practice
GEP	Gene Expression Profile
GP	General Practitioner
ICF	Informed Consent Form
IRB	Institutional Review Board
PI	Principal Investigator
PIL	Participant/ Patient Information Leaflet
R&D	Research & Development Department
REB	Research Ethics Board/Committee
SOP	Standard Operating Procedure

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3. BACKGROUND AND RATIONALE

Lung transplantation (LTx) is the accepted treatment for advanced lung disease. The United Network for Organ Sharing (UNOS) reported 2057 lung transplants in the US in 2015 (www.unos.org/about/annual-report). Chambers et al published the 35th ISHLT lung transplant report in 2018 and despite recent advances in medical management of LTx, long-term survival after lung transplantation (LT) is worse than any other solid organ transplantation having a median survival: lung 6.5 years, heart 12.9 years, liver 8.5 years. Importantly the greatest cause of death is graft failure and infection in first year after which bronchiolitis obliterans syndrome (BOS) remains an issue which contributes to chronic lung allograft dysfunction (CLAD).

CLAD remains a major obstacle against long-term survival after LT, with the development of chronic lung allograft dysfunction, primary graft dysfunction and acute rejection (AR) having been identified as strong independent risk factors for the development of chronic lung allograft dysfunction. Acute rejection occurs in up to 90% of recipients and is responsible for approximately 4% of deaths in the first 30 days following transplantation. Chronic rejection occurs in up to 50% of recipients. Moreover, primary graft dysfunction could cause AR through the linkage between innate and adaptive immune responses after LT.

The clinical course of CLAD is therefore progressive with irreversible allograft injury that ultimately leads to allograft failure. Perhaps interventions at earlier stages before allograft injury becomes irreversible may delay or even prevent the development of CLAD and improve lung transplant outcomes.

Two clinical observations support this hypothesis. First, early post-transplant complications like primary graft dysfunction show a strong relationship with CLAD suggesting that allograft injury early after transplantation is a precursor of CLAD. Second, lung transplant patients undergo rigorous monitoring with bronchoscopies along with trans-bronchial biopsy, spirometry and other testing to detect and treat acute complications with the goal of preventing CLAD. Yet, CLAD still occurs an alarmingly high rate leading us to suspect the existence of allograft injury that is undetectable clinically and by monitoring tools.

Currently, fiberoptic bronchoscopy and transbronchial lung biopsy is the gold standard for detection of allograft dysfunction post LTx. However, this surveillance procedure is expensive and invasive. Scott et al. studied the sensitivity and specificity of transbronchial biopsy (TBB) in heart-lung and single LTx recipients and found eighteen samples per procedure were needed to reach 95% confidence of rejection detection.

In light of these limitations on the use of biopsy, new methods that aim to detect early rejection are needed. However, few studies have definitively demonstrated routine clinical utility at early clinical stages of presentation, since most methods rely on relatively non-specific and/ or insensitive biomarkers.

Recently, measurement of the plasma level of donor-derived cell-free DNA (dd-cf-DNA) was shown to be useful as a non-invasive diagnostic test for AR after cadaveric LT. Cell-free DNA (cf-DNA) consists mainly of 100-200 base-pair double stranded DNA fragments resulting from apoptosis, necrosis or release of nuclear DNA into the circulation. In the circulation, these fragments have a short half-life of 30minutes to 1.5 hours, because of rapid hepatic and renal clearance. Thus, dd-cf-DNA offers real-time monitoring of graft tissue damage.

Preliminary studies have employed the use of dd-cfDNA in the surveillance of lung allograft rejection and injection. The level of dd-cfDNA is diverse across the literature, and more research is needed to accurately determine thresholds for clinical utilization (Tanaka et al 2018; Khush et al abstract 2017; Agbor-Enoh et al 2017; Zou et al 2017; De Vlaminck et al 2015).

AlloSure takes advantage of the wide genomic difference between transplant donors and recipients, as well as the sensitivity of genome sequencing to identify and quantify circulating donor-derived cell-free DNA– dd-cfDNA. The test is broadly applicable across transplantation and has been used to detect acute rejection in kidney and heart

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transplantation. The objective of this is to assess the utility of AlloSure to quantitate allograft injury (both clinically-detected and clinically-silent) in the early post-transplant period and determine its relationship to allograft failure (CLAD or death).

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4. OBJECTIVES AND OUTCOME MEASURES

Objectives	Outcome Measures	Timepoint(s) of evaluation of this outcome measure (if applicable)
Primary Objective Correlation between dd-cfDNA and allograft rejection	Histological assessment of tissue biopsy with paired AlloSure dd-cfDNA result – performed both 'For Cause' and 'Surveillance'	AlloSure tests will be taken at regular intervals, timed with biopsy schedules (24 samples in 12 months) • Sample at baseline (prior to txp), then 24hours, 48hours, 72 hours post txp • Once a week for 3 months • Once monthly for months 6-12 • With any "For Cause" and surveillance biopsy
Secondary Objective Assessment of AlloSure in the predictive and prognostic value of diagnosing CLAD, allograft failure and premature death	Clinical details and events from patient records will be examined to assess the association with AlloSure.	Examination of patient EMR to correlate clinical data, other tests and outcomes with AlloSure results.

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5. STUDY DESIGN

Single Center Prospective Cohort Study on De-Novo Lung Transplant recipients. All new patients who are having lung transplants will be offered to participate and will be consented as part of their recruitment into the study. AlloSure will be drawn as part of routine blood draws and sent to CareDx for analysis of dd-cfDNA. No additional visits are anticipated outside normal routine care, with all data normally collected and stored in the hospital EMR, this will be examined with the AlloSure results to correlate outcomes.

6. PARTICIPANT IDENTIFICATION

6.1. Study Participants

- Participants with de-novo lung transplants

6.2. Inclusion Criteria

- Participant is willing and able to give informed consent for participation in the study.
- Male or Female, aged 18 years or above.
- Denovo lung transplant recipient
- Ability to understand written and spoken English

6.3. Exclusion Criteria

- Previous transplant or multi-organ transplant
- Unable to have blood draw for medical reason

7. STUDY PROCEDURES

An additional blood test will be taken (6mls) and with timed schedule to follow other routine blood tests. The anticipated testing schedule will involve 24 samples in the first 12 months.

- Sample at baseline (prior to txp), then 24hours, 48hours, 72 hours post txp
- Once a week for 3 months
- Once monthly for months 4-12
- With any “For Cause” biopsy -- Blood tests will also be paired with bronchial biopsies where appropriate.

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7.1. Recruitment

- Patients who are admitted for transplantation, will be identified and offered to participate in this study.

7.1. Screening and Eligibility Assessment

- 50 will be screened and recruited into the study to participate. This is achievable because on average our program transplants 90-100 patients per year. No additional screening from the standard assessments used for eligibility to proceed with transplant will be used. Demographics, medical history, concomitant medication, physical examination, ECG, laboratory tests, biopsies and samples, scans, recorded in the patient EMR record will be examined as part of this study.

7.2. Informed Consent

The participant must personally sign and date the latest approved version of the Informed Consent form before any study specific procedures are performed.

Written and verbal versions of the Participant Information and Informed Consent will be presented to the participants detailing the exact nature of the blood test and study; what it will involve for the participant in terms of having this additional blood test performed; the implications of the result and how it may impact clinical care. There are no known side effects and any risks involved in taking part. It will be clearly stated that the participant is free to withdraw from the study at any time for any reason without prejudice to future care, without affecting their legal rights, and with no obligation to give the reason for withdrawal.

The participant will be allowed as much time as wished to consider the information, and the opportunity to question the Investigator, their doctor or other independent parties to decide whether they will participate in the study. Written Informed Consent will then be obtained by means of participant dated signature and dated signature of the person who presented and obtained the Informed Consent. The person who obtained the consent must be suitably qualified and experienced, and have been authorised to do so by the Chief/Principal Investigator. A copy of the signed Informed Consent will be given to the participant. The original signed form will be retained at the study site.

7.3. Baseline Assessments

No additional baseline assessments outside of routine transplant care are necessary for this study all baseline assessments.

7.4. Subsequent Visits

All care will follow the standard clinical protocols of the hospital and transplant team, which includes follow up and what assessments will be conducted. There are no additional clinic visits, telephone assessments, or home visits by the study staff. Laboratory tests performed at these visits will be used to allow the additional draw of AlloSure.

7.5. Sample Handling

Blood samples and dd-cfDNA measurements

Venous blood was collected in Streck Cell-Free DNA BCT tubes prior to performance of bronchial biopsies and will be shipped to the central Clinical Laboratories Improvements Act (CLIA)-certified laboratory at CareDx, Inc. (Brisbane, CA) using FedEx. Details of the standardized specimen processing and analytical methods to determine the percentage of dd-cfDNA (AlloSure®) have been published. The targeted next generation sequencing assay employs highly polymorphic single nucleotide polymorphisms (SNPs) to quantify dd-cfDNA without need for separate genotyping of the recipient or the donor. All measurements were performed by laboratory technicians unaware of the clinical identity of the samples.

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7.6. Description of procedure

Venepuncture is the procedure of entering a vein with a needle, performed by a clinical professional trained in phlebotomy. The drawing of blood is part of standard routine care. Sometimes pain, redness and discomfort may occur around the needle site and so patients will be monitored by clinical staff. No additional venepunctures will be performed as part of this study

7.7. Discontinuation/Withdrawal of Participants from Study

Each participant has the right to withdraw from the study at any time. In addition, the Investigator may discontinue a participant from the study at any time if the Investigator considers it necessary for any reason including:

- Pregnancy
- Ineligibility (either arising during the study or retrospectively having been overlooked at screening)
- Significant protocol deviation
- Significant non-compliance with treatment regimen or study requirements
- Withdrawal of Consent
- Loss to follow up

Withdrawal from the study will result in exclusion of the data for that participant from analysis, with withdrawn participants being replaced.

7.8. Definition of End of Study

The end of study is the date of the last clinic visit and last AlloSure blood draw of the last participant.

8. STATISTICS AND ANALYSIS

8.1. Description of Statistical Methods

Data will be assessed for normality with the appropriate non-parametric tests performed. Data maybe pooled with other centers to increase the number of patients to reach a sample sufficient for a powered analysis.

8.2. The Number of Participants

This pilot studywill enrol 50 patients. It is un-powered and single centered. Acute rejection occurs in 90% of recipients within the first year, the primary outcome is likely to be achieved with this sample size.

9. DATA MANAGEMENT

9.1. Access to Data

Direct access will be granted to authorised representatives from the Sponsor and host institution for monitoring and/or audit of the study to ensure compliance with regulations.

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9.2. Data Recording and Record Keeping

All clinical data will be collected and stored as per routine clinical practice, normal procedures will be followed for entry and management. AlloSure results will be provided to clinical teams to enter into the EMR record, which is a encrypted, password protected closed system.

10. QUALITY ASSURANCE PROCEDURES

The study does not require monitoring or auditing, with the clinical team following the study design in accordance with the current approved protocol, GCP, relevant regulations and standard operating procedures.

11. ETHICAL AND REGULATORY CONSIDERATIONS

11.1. Declaration of Helsinki

The Investigator will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki.

11.2. Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in accordance with relevant regulations and with Good Clinical Practice.

11.3. Approvals

The protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to an appropriate Institutional Review Board (IRB), for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

11.4. Reporting

The PI shall submit once a year throughout the study, or on request, an Annual Progress report to the CareDx, IRB (where required), host organisation or coordinating center and Sponsor. In addition, an End of Study notification and final report will be submitted to the same parties.

11.5. Participant Confidentiality

The study staff will ensure that the participants' anonymity is maintained. The participants will be identified only by a participant ID number on all study documents and any electronic database, with the exception of the CRF, where participant initials may be added. All documents will be stored securely and only accessible by study staff and authorised personnel. The study will comply with the data protection, which requires data to be anonymised as soon as it is practical to do so.

11.6. Expenses and Benefits

No additional expenses or benefits will be made available to the patient, other than normal standard of care.

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12. FINANCE AND INSURANCE

12.1. Budget

A total budget $80,000 for the first year, with an option for more for each additional year after data collection and review.

Year 1 funding will support:

-enrolling patients

-collecting 24 blood samples from each enrolled patients

- following clinical outcomes regarding acute rejection

-reviewing PFTs and pathology from transbronchial biopsies

-data base build up and data analysis

13. PUBLICATION POLICY

The Investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Authors will acknowledge that AlloSure was provided by CareDx. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged.

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