Donor derived cell-free DNA and rejection of kidney allografts

MINIMAL RISKS AND CONSTRAINTS HUMAN RESEARCH STUDY

Version N°1.1 of 01/03/2020
Protocol code number:
ddcfDNA_study001

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PROTOCOL SIGNATURE PAGE

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The research is carried out in accordance with the current version of the protocol, with GCP and with all statutory and regulatory requirements.

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1 SUMMARY

Full title	Donor derived cell-free DNA and rejection of kidney allografts
Coordinating Investigator	Pr Alexandre Loupy
Scientific Director (if applicable)	Dr Olivier Aubert
Sponsor	Organ X Foundation
Scientific justification	Allograft rejection is a major problem worldwide and results in the loss of thousands of transplants every year, with devastating consequences for patients in terms of mortality, morbidity and quality of life. With a prevalence of approximately 20% in the first-year after kidney transplantation, allograft rejection also represents a considerable economic burden for health care systems with the associated downstream cost related to graft failure and return to dialysis. The standard strategy to monitor rejection is based on surveillance of nonspecific functional markers such as serum creatinine level, estimated glomerular filtration rate, proteinuria and circulating donor-specific anti-HLA antibodies (DSA), which lack sensitivity and specificity. The current gold standard for diagnosing allograft rejection is the biopsy which is a potentially harmful, costly and often unnecessary procedure. Recently, one promising non-invasive biomarkers has emerged in various fields such as oncology or gynecology for the monitoring of the patients: the cell-free DNA. In transplantation, donor-derived cell-free DNA (dd-cfDNA) detected in the blood of transplant recipients has been proposed as a noninvasive biomarker for the detection of rejection. However, dd-cfDNA has not been assessed in an unselected and deeply phenotyped population, with the comparison to the conventional parameters including functional, clinical or immunological factors to monitor patients and detect rejection.
Main objective and primary endpoint	**Main objectives** To assess the association of dd-cfDNA with the presence, activity and severity of allograft rejection, and determine whether dd-cfDNA adds value to standard of care monitoring parameters in detecting kidney allograft rejection

	Primary endpoint Kidney allograft rejection (all causes) Time Frame: Up to 10 years after kidney transplantation
Design of the study	Type of study: Multicenter international study Time Perspective: prospective
Population of study participants	Derivation cohort: Prospective cohort of kidney transplant recipients recruited in 2 French centers Validation cohort: kidney transplant recipients in 11 North-American centers and 1 Belgian center with clinical, biological, histological and immunological data, and dd-cfDNA assessment.
Inclusion criteria	• Recipients transplanted from a deceased or living donor, who undergone a kidney allograft biopsy with clinical, biological, histological and immunological data. Written informed consent at the time of transplantation for the center database.
Exclusion criteria	• Combined organ transplantation • Pregnant women • Bone marrow transplant
Interventions or product under investigation	This study involves minimal risk and constraints. The intervention consists in the evaluation of the risk of allograft rejection based on parameters measured as part of the standard of care and dd-cfDNA. No medicinal product is under investigation in this protocol.
Other interventions added by the study	Gene expression measurement in allograft biopsies
Expected benefits for the participants and for society	There will be no immediate individual benefit for the patient to participate in the study. This study will be the first that evaluates the independent association of dd-cfDNA with rejection and its added value in addition to conventional features to predict kidney allograft rejection. This first integrative system will improve the patient monitoring and will help physicians in decision-making process.

Minimal risks and constraints added by the study	The intervention added by the study represents minimal risk and constraints for the patient as it will be embedded within the standard of care. No additional puncture is performed; only one additional blood tube is taken for the measurement of dd-cfDNA.
Scope of the study	Kidney transplantation; non-invasive diagnosis of rejection for improving patient monitoring
Anticipated number of participants	Number of participants with post-transplant risk evaluation: Derivation prospective cohort: 1000 patients External validation cohort: 1500 patients
Number of sites	Multicenter international study involving 14 sites. **Derivation cohort**: • Saint-Louis Hospital, Paris, France • Necker Hospital, Paris, France **Validation cohort**: • Leuven Hospital, Leuven, Belgium • Cedars-Sinai, Los Angeles, USA • UCLA, Los Angeles, USA • Emory University, Atlanta, USA • Intermountain Medical Center Transplant Services, Murray, USA • Weill-Cornell Medical School, New York, USA • New York Hospital Medical Center, New York, USA • Virginia Commonwealth University, Richmond, USA • Washington University School of Medicine in St. Louis, St. Louis, pediatric department • Washington University School of Medicine in St. Louis, St. Louis, adult department • Transplant Institute, Tampa General Hospital, Tampa, USA • University of Wisconsin, Madison, USA
Schedule for the study	Inclusion period 2011- 2022; Participation period (follow-up): Derivation and validation cohorts: up to August 2022

Expected number of enrolments per site	Derivation cohort (n=1000) External validation cohorts (n=1500)
Statistical analysis	Continuous variables will be described using means and standard deviations (SDs) or median and the interquartile range. We will compare means and proportions between groups using Student's t-test, analysis of variance (ANOVA) (Mann-Whitney test for donor specific antibodies (DSA) mean fluorescent intensity) or the chi-square test (or Fisher's exact test if appropriate). We will evaluate the association between the dd-cfDNA levels and each allograft diagnosis (ABMR, TCMR, Mixed rejection, glomerulitis without rejection, polyomavirus nephritis, fibrosis, borderline changes, no specific lesions). Then, to investigate the association of dd-cfDNA with rejection severity, we will compare the mean dd-cfDNA level according to each active and chronic Banff lesion score (ranked from 0 to 3). ***Evaluation of the association between dd-cfDNA and rejection*** The associations between allograft rejection and clinical, functional, and immunologic factors will be first computed using logistic regression analyses. The factors identified in these analyses will be thereafter included in a final multivariable analysis if the p-value of the Wald test is < 0.1. Multivariable logistic regression analysis will be performed using a stepwise backward elimination until each predictor is associated with allograft rejection with a p-value below 0.05. The internal validity of the final model will be confirmed using a bootstrap procedure, which involves generating 1,000 datasets derived from resampling the original dataset and permitting the estimation of the biased corrected 95% CI and the accelerated bootstrap OR. ***Assessment of the added value of dd-cfDNA*** To evaluate the added value of dd-cfDNA, we will assess the performances of the reference model (including independent standard of care predictors of the final multivariable model) with and without dd-cfDNA to evaluate to risk of rejection. The performances of the models to predict rejection will be evaluated with the use of the accuracy of the model, which will be assessed based on its discrimination ability and calibration performance. For each model, the discrimination ability (i.e., the ability to separate patients with different prognoses) of the final model will be evaluated using Area under the curve (AUC). Calibration (the ability to provide unbiased rejection predictions in groups of similar patients) will be assessed based on a visual examination of the calibration plots.

	We will follow the TRIPOD (Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis) statement for reporting multivariable prediction model development and validation. ***Sensitivity analyses:*** We will investigate the independent association of dd-cfDNA with rejection and its added value in predicting rejection in different clinical scenarios (biopsies performed during the first year or after one-year post-transplantation, according to the subtypes of rejection, protocol biopsies or biopsies performed for clinical indication) and according to the patient ethnicity. We will use R (version 4.1.2, R Foundation for Statistical Computing) and STATA (v17) for the descriptive and prediction analyses. Values of $p<0.05$ will be considered significant, and all tests will be 2-tailed.
Sources of monetary support	The study is funded by the OrganX Foundation