**SAFE 21 STUDY**
Cell-free DNA testing versus invasive prenatal diagnosis in women at high-risk for trisomy 21: a multicenter open-label randomized trial

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# Statistical Analysis Plan

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English Version v1.1 (03.07.17)

## SIGNATURE PAGE OF THE STATISTICAL ANALYSIS PLAN

Research Biomedical Code No: P130601

ClinicalTrials.gov No: NCT02127515

> **STUDY TITLE:** Cell-free DNA testing versus invasive prenatal diagnosis in women at high-risk for trisomy 21: a multicentre open-label randomized trial

Version 1.1 (03.07.17)

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SAFE 21 – Statistical Analysis Plan

This document focusses and discusses the initial part of the statistical analysis plan (see section "analysis of the main endpoint and the diagnostic performance of the non-invasive test" in the protocol). As the health economic component and the analysis of the attitude of pregnant women and their decisions are managed by other teams, they will not be discussed in this document.

## 1. Statistical Analysis Team

The statistical analysis will be carried out at the Clinical Research Unit / ClC Paris Descartes Necker by Amandine Baptiste under the direction of Dr. Caroline Elie using the R software statistical package, version 3.3.1 (http: //cran.r-proiect. org).

The main statistical analysis will be performed following the completion of data collection. The accuracy and consistency of the data entered will be monitored from recruitment to delivery of the patients. All tests will use a level of significance set at 5%.

## 2. Sample size calculation

The number of patients required is based on the assumption of a significant reduction in the rate of miscarriages in the group undergoing cell free fetal DNA testing. In order to demonstrate a reduction in the miscarriage rate (80% power and $\alpha=0.05$) from 1.5% to 0.5% between those in traditional prenatal screening group and those in the cfDNA group, 1250 patients per group are required.

## 3. Analysis plan

### 3.1 Population test group

The test population will include all randomized patients, who have given written informed consent for participating in the study. Patients who have not given consent will be excluded from the database. For participants who have been randomized multiple times (either following a randomization error or because the patient was recruited and randomized in two different participating centers), only their first randomization will be used in the analysis.

### 3.2 Description of patient characteristics at inclusion

A descriptive analysis of the clinical features will be performed and detailed according to their randomization arm. Quantitative data will be expressed as means and standard deviations (or medians with interquartile ranges for non-normally distributed data), and as frequencies and percentages for qualitative data.

### 3.3 Primary outcome analysis

*Main analysis*

The main analysis will be performed using a modified intention to treat approach, *ie* all randomized patients will be analyzed in their randomization arm, regardless of screening method employed. Patients lost to follow up with an unknown pregnancy outcome will be

excluded. As there is a very small number of miscarriages expected in each of the two groups (ranging from 0.5% to 1.5%, n = 20 approximately), the only possible imputation method would be to consider them as failures, a multiple imputation model not being possible. However, considering these patients as failures could lead to an "artificial" difference between the two groups (attributable to different rates of missing pregnancy outcomes per group).

Patients who have undergone a termination of pregnancy for medical or personal reasons before 24 weeks' gestation will be considered as having not had a miscarriage.

The proportion of miscarriages occurring before 24 weeks' gestation in the cfDNA group will be compared to that of the standard prenatal screening group using a one-way Chi-squared test or Fisher's exact one-sided test if the conditions for a Chi-squared test are not met. Due to the high number of participating centers and the small number of randomized patients in many of these centers, the center, will not be taken into account.

*Sensitivity analyses*

A sensitivity analysis on the Per-Protocol population will also be conducted for the primary outcome. In this analysis, the data from randomized patients with the known screening method and a documented outcome of pregnancy, will be analyzed according to their allocated first-line screening mode group. Patients for whom the data for both exams are missing will be excluded.

### 3.4 Secondary analysis

The analysis of the secondary outcomes will be essentially descriptive, but comparisons between the groups may be conducted, using bilateral tests with a significance level of 5%. Comparisons of two means will be made by Student's test or if necessary by a non-parametric Wilcoxon test. Percentage comparisons will be made by the Pearson Chi-squared test or using Fisher's exact test if required.

*Standard prenatal screening method*

The percentage of invasive samples (amniocentesis, chorionic villus sampling or fetal cordocentesis) performed in each group will be described, as well as the percentage of abnormalities discovered as a result of these tests. In particular, we will also describe the rate of invasive samples taken following a cfDNA test and the reason for this (*ie* medical indication or maternal reassurance).

The rate of miscarriages and fetal deaths in utero following this method of screening will be calculated, regardless of the term of onset and in the month following screening.

*Cell free DNA method*

The time taken to obtain a result and the rate of uninterpretable results will be described. In this case, the subsequent examinations (Repeat cfDNA tests and /or standard prenatal screening methods) will be described.

The evaluation of the diagnostic performance of the cfDNA approach will be performed by the calculating the rates of false positives and false negatives as well as the sensitivity, the specificity and the positive and negative predictive values. The associated 95% confidence intervals will be calculated (binomial distribution). The reference test will be the karyotype (obtained by CVS or amniocentesis or postnatal karyotype), or the phenotype at birth.

Maternal characteristics (weight, height, medical and obstetric history, serum markers, etc.) of patients with a false positive or false negative result will be described.