Statistical analysis plan (SAP)

Statistical analysis plan presented below has been revised before data base lock.
We do not expect modifications of the initial analysis strategy. However, in case of occurrence of such validated modifications after the SAP, a modified SAP would be issued. The original SAP as well as the modified SAP will be kept in the study files, with the justification for any modification.

1 ANALYSIS PLAN

1.1 Description of planned statistical methods

Analysis will be performed by a statistician from URC-Est using SAS® software version 9.4 (or updated version) (SAS Institute Inc.) and Stata version 16 (Stata Corp). Partial data base lock will be performed for data from randomization to D28 to assess primary and secondary endpoints at D28. Final data base lock will be done at the end of the trial to assess secondary safety endpoints at 3 and 6 months.

A flow chart will be drawn according to consort statement. The proportion of eligible participants who refused to participate in the research or who did not come to the scheduled consultation will be described.

Baseline characteristics of patients will be described overall and per group.
Continuous variables will be summarized using descriptive statistics, i.e. number of subjects, mean, standard deviation (SD), median, inter quartile range, minimum and maximum depending on the variable distribution. Qualitative variables will be summarized by frequency and percentage.

The proportion of patients having been in contact with the virus before D0 on the one hand and after D0 on the other hand will be calculated (contact with the virus defined as positive if anti-nucleocapsid Ig G assay measured by ELISA on D0 and D28 positive or doubtful).

Primary endpoint assessment

The primary endpoint will be analyzed under non-inferiority hypothesis of vaccination with two doses of different vaccines (combined vaccination) compared to vaccination with two doses of the same vaccine (standard vaccination) (ie Pfizer Moderna versus Pfizer Pfizer and Moderna Pfizer versus Moderna Moderna) separately.

Therefore, the main analysis will be performed on the per protocol population with an additional sensitivity analysis on the intention-to-treat population. The non-inferiority of vaccination with two doses of different vaccines compared to vaccination with two doses of the same vaccine will be demonstrated if the two analyses are consistent.

The geometric means of the anti-SARS-CoV-2 IgG antibody titers (GMT) directed against the S1 domain of the Spike protein measured 28 days after the 2nd dose will be calculated in each group and transformed (log10).

For each mRNA vaccine, GMT will be compared between combined vaccination and standard vaccination under the hypothesis:
H0: GMT combined / GMT standard ≤ 0.61 or $log10$ GMT combined - $log10$ GMT standard ≤ -0.215;
H1: GMT combined / GMT standard > 0.61 or $log10$ GMT combined - $log10$ GMT standard > -0.215.
The geometric mean ratio (GMR) will be calculated as the antilogarithm of the difference between the mean of the log10 transformed anti-SARS-CoV-2 IgG antibody titer in the combined arm and that in the standard arm (as the reference).

Non-inferiority will be claimed if the lower limit of the 95% two-sided CI of the GMR is above 0.61 or lower limit of the 95% two-sided confidence interval (CI) of the geometric means difference is above -0.215.

As sensitivity analysis, we will test the above hypothesis using a linear mixed regression model on $log10$ GMT considering study site as random effect. The adjusted mean difference of $log10$ GMT will be presented with the two-sided 95% CI.

For each group, the anti-SARS-CoV-2 IgG antibody titers directed against the S1 domain of the Spike protein measured at D0 and D28 will be described as geometric means with 95% confidence intervals (by logarithmic transformation of the data, calculation of the arithmetic mean and its confidence interval and anti-log transformation of these mean and limits of the confidence interval). Plots will also be performed. The D28/D0 ratio will also be calculated and described as mentioned above.

Complementary analyses

The anti-SARS-CoV-2 IgG antibody titers directed against the S1 domain of the Spike protein measured at D0 will be compared between the 2 groups based on the first dose of vaccine received (i.e Pfizer or Moderna). A generalized linear mixed regression model, with a distribution adapted to the data and considering study site as random effect, will be used.

The anti-SARS-CoV-2 IgG antibody titers directed against the S1 domain of the Spike protein measured at D28 will be compared between the 4 groups. A generalized linear mixed regression model, with a distribution adapted to the data and considering study site as random effect, will be used. Comparisons between groups in pairs could be performed. A Bonferroni correction will then be applied depending on the number of tests performed.

For each group, the anti-SARS-CoV-2 neutralizing antibodies against original SARS Cov2 measured at D0 and D28 will be described as geometric means with 95% confidence intervals. Plots will also be performed. The D28/D0 ratio will also be calculated and described as mentioned above.

The anti-SARS-CoV-2 neutralizing antibodies against original SARS-CoV-2 measured at D0 will be compared between the 2 groups based on the first dose of vaccine received (i.e Pfizer or Moderna). A generalized linear mixed regression model, with a distribution adapted to the data and considering study site as random effect, will be used.

The anti-SARS-CoV-2 neutralizing antibodies against original SARS-CoV-2 measured at D28 will be compared between the 4 groups. A generalized linear mixed regression model, with a distribution adapted to the data and considering study site as random effect, will be used. Comparisons between groups in pairs could be performed. A Bonferroni correction will then be applied depending on the number of tests performed.

For 30 patients of each group, the anti-SARS-CoV-2 variants (alpha, delta…) neutralizing antibodies will be measured at D28. They will be described as geometric means with 95% confidence intervals. Plots will also be performed. Comparison between combined vaccination group and standard vaccination group will be performed using a generalized linear mixed regression model, with a distribution adapted to the data, considering study site as random effect.

Subgroup analysis will be conducted stratified by age (classes defined according to quartile values), sex (male and female), time between the 2 injections (<= 35 days and > 35 days) and antibody titers value at D0 (classes defined according to quartile values). GMT will be described by group of vaccination (pfizer-pfizer vs. pfizer-moderna and moderna-moderna vs. moderna-pfizer) and in each strata.

Safety assessment

Safety assessment will be analysed among safety population.
All adverse events (AE) will be coded and grouped into frequency tables. They will be presented by group, globally and by observation interval of the study (between D0 and D28, between D28 and M3, between M3 and M6). Proportions of 1) local AE and 2) systemic AE will be described globally and by randomisation group. Difference and 95%CI will also be performed using exact Clopper-Pearson

method. The overall frequencies and percentages of participants reporting these events, based on their severity and link to the vaccine, will be described.

As complementary analysis, proportion of COVID infections will be described globally and by randomisation group. Difference and 95%CI will also be performed using exact Clopper-Pearson method.

1.2 Hypotheses for calculating the required number of subjects

Preliminary data from our department suggest a geometric mean of anti-S1 antibody level measured by Elisa of 253 BAU / mL with a standard deviation of 0.54, 28 days after the second dose.

As the aim of the trial is not to register a new vaccine, the lower limit of the 95% CI for the combined vaccination / standard vaccination ratio of 0.61 is retained. This is encouraged by the lack of clinical impact observed even when the neutralizing activity of vaccinated sera was reduced by 45 to 50%.

The sample size calculation is based on the following assumptions:
- A non-inferiority margin corresponding to a geometric mean ratio (GMR) of 0.61 between vaccination with a second dose of a different vaccine and a second dose of the same vaccine (ie -0.215 in absolute difference on a logarithmic scale (base 10)).
- A standard deviation of the geometric mean of 0.54 on a logarithmic scale (base 10) based on our preliminary data.

The recruitment and randomization of 100 subjects per group, or 400 in total, will make it possible to achieve a power of 80% considering a one-sided alpha of 2.5%.

1.3 Anticipated level of statistical significance

For the primary clinical outcome analysis, the decision rule will be based on the lower bound of the 95% two-sided confidence interval.
No adjustment will be planned for multiplicity except for comparisons between groups in pairs (cf. complementary analysis).

1.4 Statistical criteria for termination of the study

Not applicable.

1.5 Method for taking into account missing, unused or invalid data

Missing data for the primary endpoint will be replaced by the geometric mean value of antibody levels observed in the group of the concerned subject.
Censored data reported as below the lower limit of detection/quantification will be imputed with a value equal to half of the threshold before transformation.
Other missing data will not be replaced.

1.6 Selection of populations

Intent to treat population (ITT): all randomized patients, regardless of the strategy received by the patient, except patient with positive or doubtful or missing NP antibodies at D0 and/or J28.

The per protocol (PP) population is defined as all patients randomized, treated without major protocol violations/deviations. Pre-defined major protocol violations/deviations are:

- Non-respect of eligibility criteria
- Non-respect of the randomized treatment allocation and/or duration (wrong vaccine received, second dose not received, wrong delay between the two doses)
- Missing data for the primary efficacy endpoints
- Patient with positive or doubtful NP antibodies at D0 and/or J28.

Major protocol deviation will be classified during a blinded data review before final data base lock.

Safety population is defined as all randomized patients who have received the second dose of vaccine.

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