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Research Article

N-protein vaccine Convacell® is effective against COVID-19: phase 3, randomized, double-blind, placebo-controlled clinical Trial

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Abstract

We have developed a Convacell®, a COVID-19 vaccine based on the conservative viral nucleocapsid (N) protein. The N protein is evolutionary conservative and is abundantly expressed on the surface of infected cells, allowing anti-N immune response generated by Convacell® to rapidly clear infected cells and provide long-lasting protection against COVID-19. Convacell® has been demonstrated to be safe and highly immunogenic, creating immune responses lasting over a year, in phase I/II and IIb clinical trials. Phase IIb clinical trial has also demonstrated that a single dose vaccination regimen with Convacell® is sufficient to provide an immune response.

Here we report the finding of the phase III clinical trial of Convacell®. Two groups of volunteers from Russia have been either vaccinated with a single dose of Convacell® or injected with placebo, and then monitored for incidence of COVID-19 and adverse effects. Anti-N antibody titers at admission were also analyzed, to take into account for potential effects of previous virus encounters. Disease incidence over 6 months results indicate an overall vaccine efficacy of 85.2% (95% confidence interval: 67.4-93.3%). Additionally, Convacell® has shown a good safety profile.

Keywords: COVID-19; vaccination; anti-N immune; anti-N antibody; anti-N IgG antibodies

1.Introduction

Anti-SARS-CoV-2 vaccines are hailed as the best and most cost-effective treatment for COVID-19 [1], crucial for protecting the vulnerable groups from the disease and lowering its economic and social impact [2–4]. We have developed a COVID-19 vaccine Convacell® based on the full-length nucleocapsid (N) protein of SARS-CoV-2, produced in an Escherichia coli recombinant protein platform [5]. COVID-19 vaccines based on the N

protein have already been described as promising in multiple papers [6–12]. Previously, Convacell® has been shown to be effective in protecting from severe disease in preclinical trials [5] and highly immunogenic and safe in phase I, II and IIb clinical trials [13]. Convacell® can be used for vaccination of the general populace. Additionally, Convacell® contains a different target antigen – protein N – and protects via a different dominant immune mechanism, compared to most currently available COVID-19 vaccines, which are based on the spike (S) protein of SARS-CoV-2. As such, we theorize it will be especially useful for people who are unable to generate an anti-S immune response (14–19). Unlike most common vaccines, Convacell® requires only a single dose to achieve protective effectiveness [13] and does not require booster doses to generate an immune response lasting up to a year [13]. In this study, we describe the results of the phase III of Convacell®'s clinical trials, which include assessments of Convacell®'s vaccine efficacy and safety.

Methods

Study design

The phase III study (NCT05726084) was planned to be prospective, multicenter, randomized, double-blind and placebo controlled. Study involve comparison between vaccine and placebo groups of volunteers to assess adverse effects (AE) and COVID-19 infection incidence and adherence to protocol. On-site monitoring by research centers ensured that Good Clinical Practices were followed throughout the course of the study. The protocol followed the Helsinki Declaration Guidelines and was firstly evaluated and approved by ethical committee at Ministry of Health of Russian Federation and then by independent onsite ethical committees. The study design included the possibility of preterm conclusion of the study after recruitment of either 33% or 66% of the target number of participants, if the gathered data allowed for conclusive assessment of vaccine efficacy in the vaccinated group greatly exceeding that in the placebo group.

The study recruited volunteers meeting all of the following criteria. Inclusion criteria:

1. Age >18.
2. Willing to sign an informed consent statement to participate in a clinical trial.
3. $18{,}5 \leq BMI \leq 30$ kg/m2, with body mass between 55 and 100 kg for men and between 45 and 100 kg for women.
4. Verified healthy status: no deviation from reference intervals in the results of standard clinical and laboratory tests.
5. Negative for human immunodeficiency virus (HIV), rapid plasma reagin (RPR), hepatitis B surface antigen (HBsAg), hepatitis C virus RNA (HCVRNA).
6. Haemodynamic and vital parameters within following reference intervals: heart rate 60–90 bpm, respiratory rate under 22 breaths per minute, systolic arterial pressure 100–139 mmHg, diastolic arterial pressure 60–89 mmHg.
7. Willing to keep a self-observation diary and attend control visits.
8. Willing to abstain from alcohol for 14 days before the beginning of the study and until its completion.
9. Willing to abstain from smoking for 48 hours before the beginning of the study and on the admission day.
10. For fertile women: negative pregnancy test and willing to use adequate contraception methods until the completion of the study and for at least two months after vaccination.
11. For fertile men: willing to use adequate contraception methods until the completion of the study or past vasectomy with confirmed azoospermia, partner willing to use at least 90% effective contraception methods or past tubal ligation or menopausal for at least 2 years.

Procedures

The volunteers in the study received one dose of a recombinant subunit COVID-19 vaccine based on the nucleocapsid protein of SARS-CoV-2, Convacell®, in the form of emulsion for intramuscular injection. The vaccine used in this study was produced according to the GMP by the Saint Petersburg Scientific Research Institute of Vaccines and Serums (SPbSRIVS). The one-dose vaccination regimen was chosen following the results of the earlier IIb study [13].

Each 0.5 ml dose of Convacell® contains 50 μg of recombinant SARS-CoV-2 nucleocapsid protein as the main active ingredient. Supplementary ingredients are 5 mg of (±)-α-tocopherol, 15 mg of squalane and 5 mg of polysorbate 80 in form of nanoemulsion.

The placebo formulation used in the study was identical to the vaccine formulation, with the exception of containing no SARS-CoV-2 nucleocapsid protein. Participants' sera were collected on screening and during unplanned visits. To obtain volunteer sera, volunteers' blood was collected into 6 ml vacuum tubes containing K2 EDTA as anticoagulant. The blood was processed to sera using centrifugation. The quantities of specific anti-N IgG antibodies in volunteer sera were assessed via the AdviseDx SARS-CoV-2 IgG II chemiluminescent microparticle immunoassay (Abbott Laboratories, Chicago, United States) and Anti-N IgG ELISA kit (St. Petersburg Research Institute of Epidemiology and microbiology, Russia). The standard manufactuter's protocol was followed.

Randomization and masking

Both the placebo and the vaccine doses appeared identical on external examination: as opaque milky-white suspension. Placebo and vaccine doses were supplied in visually identical vials containing no markings as to the nature of the suspension, except a numerical id-tag that was deliberately meaningless to participating volunteers and their physicians. Common practices included supplying both placebo and vaccine dose vials intermixed within same shipping containers. Staff that were unaware of assignments of subjects carried out data management and statistical analyses.

Outcomes

The main endpoint of this phase III study was the frequency of PCR-confirmed COVID- 19 infections among the participants, including asymptomatic infections, after 15 days since vaccination and until 6 months after vaccination. The secondary endpoints were: 1) the frequency of COVID-19, here meaning a SARS-CoV-2 infection coupled with at least one common COVID- 19 symptom (fever/chills, cough, shortness of breath or difficulty breathing, fatigue, muscle or body pain, headache, loss of taste or smell, sore throat, congestion or runny nose, nausea or vomiting, diarrhea) after 15 days since vaccination and until 6 months after vaccination; 2) the titers of anti-N IgG present at volunteer admission into the study and 3) the frequency of local and systemic AEs observed during the first 28 days since vaccination. AE were determined as disturbances in the tested vital parameters; or disorders that arose during the course of the study and were detected as the result of assessment by a professional physician. All AE that was observed during the course of the safety study were recorded, regardless of their putative association with the administration of the studied vaccine formulation. Severe AE (SAE) were defined as any AE that led to hospitalization of the volunteer and/or required immediate medical intervention, and/or led to the volunteer's death. Volunteers were instructed to visit the hospital if they considered themselves to be having COVID-19 symptoms, during this visit, volunteers were examined by professional physicians and all potential COVID-19 symptoms as well as the infection itself were recorded as AEs.

Statistical analysis

Formal sample size calculations were carried out via a validated copy of PASS 2021 software, version 21.0.5 (NCSS Statistical Software, United States), itself based on the works of O'Hagan, Stevens and Campbell in sample size statistics (20).

The mode used was "superiority by a margin test for vaccine efficacy using the ratio of two proportions." Vaccine efficacy (VE) for the placebo group was set to $\leq 30\%$, VE for the vaccinated group was set to 70%, infection incidence over a 6 months period was set to 0.6%, confidence interval was set to 95%, dropout rate during the study were set to 10%. Ratio between the placebo group and the vaccinated group was set to be 1:2.

The results indicated that 9505 participants were required to be included into the vaccinated group and 4753 into the vaccinated group, or 14258 participants were required in total. The total number of screened individuals to reach the needed number of participants was 17600. Due to the unknown future incidence of infection in the target population over the course of the study, during the planning stage, it was decided in the protocol to recruit participants in successive stages. Initially, 33% of the participants were enrolled, treated and observed. Due to higher incidence of COVID-19 in the sample population compared to the one assumed during the sample size calculation, the next stages of enrollment were judged to not be necessary to meet the planned endpoints and the clinical trial was concluded.

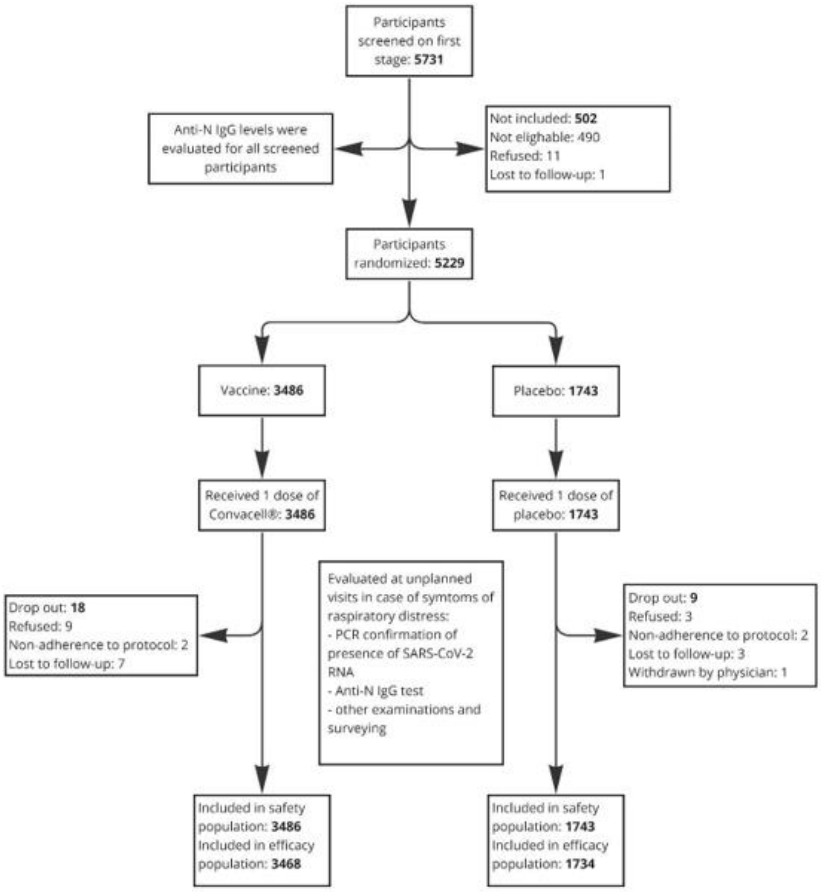

Figure 1: Schematic description of the clinical trial.

Results

Enrollment of volunteers started on May 18, 2023 and ended on August 9, 2023. Screening, randomization and populations details are given in Fig. 1. The observation for the last enrolled volunteer ended on February 11, 2024. For the "intention to treatment" efficacy population 8 cases of PCR confirmed COVID-19 were detected in the vaccine group, while in placebo group there were 27 cases. Vaccine efficacy (Fig. 2) assessment results indicate a high vaccine efficacy of 85.2% (95% confidence interval: 67.4-93.3%), with the attack rate in the vaccinated group being 5.78 times lower than in the placebo group (0.23% vs 1.55%). The primary endpoint regarding vaccine efficacy has been conclusively reached by the available data.

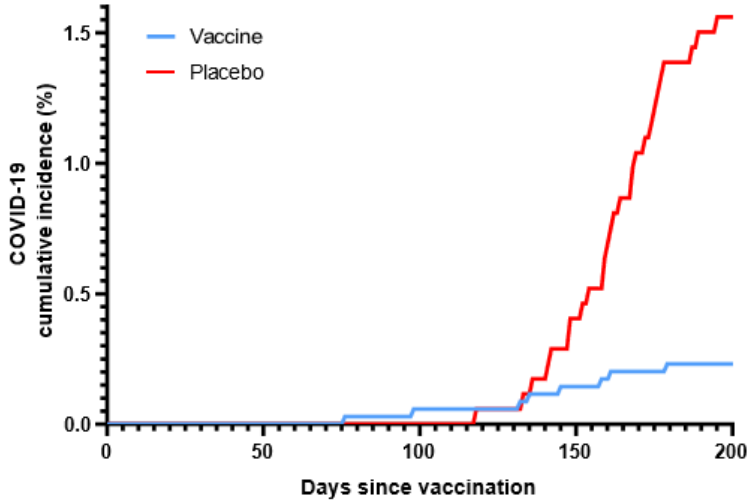

Figure 2: Vaccine efficacy graph showing cumulative incidence rate of COVID-19 among two groups of volunteers normalized for days after vaccination.

Analysis of anti-N antibody titers revealed that the fraction of participants with detectable anti-N antibody titers was 29.26% in the vaccinated group and 30.12% in the placebo group. As such, no major differences in rate of COVID-19 naivety between the two groups were discovered and the secondary endpoint regarding the titers of anti-N antibodies on randomization in participants has been reached.

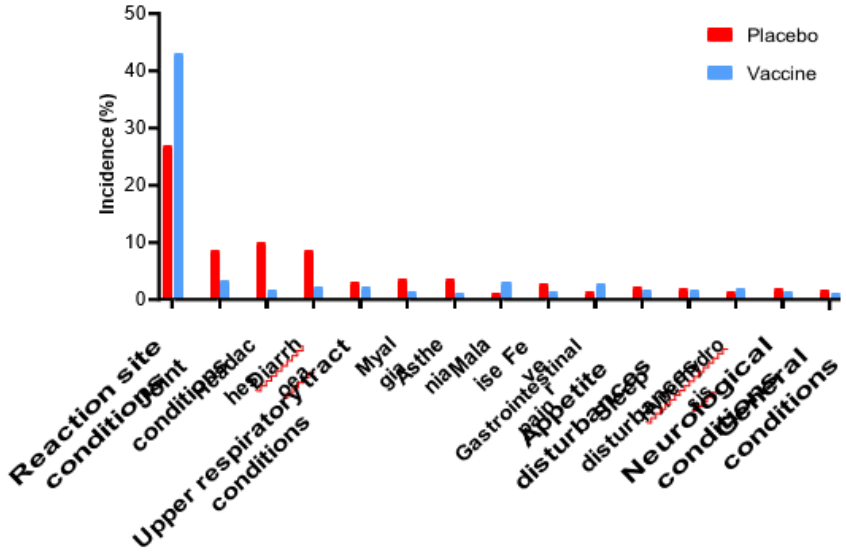

Figure 3: Incidence rates of adverse effects affecting more than 1% of participants, for both groups. Only adverse effects that has possible, probable, and definite causality are counted in figure.

Safety assessment of the vaccine demonstrated high safety throughout, with most commonly observed adverse effects (Fig. 3) being mild reaction site conditions or mild systemic disturbances. Notably, vaccinated group had higher incidence of adverse effects compared to the placebo group only with regards to reaction site conditions, malaise, gastrointestinal pain and hyperhidrosis

Discussion

All the planned endpoints have been successfully reached by the trial, which allows for the conclusion that, based on the obtained data, Convacell® has a high vaccine efficacy of 85.2%. Convacell®'s protection is evidently long lasting, given the lack of convergence between the placebo and vaccinated groups' COVID-19 incidence rates over the 180~195 days of observation in this study. Based on the immune response longevity data obtained in the previous phases of Convacell®'s study [13], we can reasonably assume that Convacell®'s protection should last at least a year, given that vaccinated individuals are positive for anti-N antibodies for at least a year after vaccination.

The usage of the internal N protein as the main antigen in Convacell® confers on it a number of advantages. The N protein is notably conservative [7,8,21–24], which confers onto Convacell®-generated immune responses both longevity [5] and broad cross-reactivity among SARS-CoV-2 variants [25]. The internal nature of the N protein in mature virions does not negatively affect Convacell®'s protectivity, as the N protein is highly expressed in infected cells [26,27] and exposed on their membranes [28,29], which allows such cells to be targeted and eliminated by cytotoxic T-cell [30–32] and natural killer (NK) cell action [33–35]. Currently, highly efficient infected cell clearance is theorized to be the main protective mechanism of Convacell®-generated immunity, based on the antibody-dependent NK cell activation data obtained in phase II of Convacell®'s clinical trials [13].

Convacell®'s recombinant E. coli protein platform confers onto it a very desirable safety profile. The most frequent adverse effects among vaccinated and placebo groups were local injection site reactions, with the rate of most frequent adverse effects – localized injection site reactions – observed in the vaccination group being considerably lower than that reported in the clinical trials of common COVID-19 vector vaccines. Overall, Convacell® demonstrated highly desirable qualities and good performance as a vaccine and can be considered as valuable COVID- 19 preventative measure

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