COVID-*e*Vax

STUDY TITLE
A PHASE I/II STUDY TO ASSESS THE SAFETY AND IMMUNOGENICITY OF COVID-*e*VAX, A CANDIDATE PLASMID DNA VACCINE FOR COVID-19, IN HEALTHY ADULT VOLUNTEERS

CLINICAL STUDY REPORT CODE: COV-1/2-01
EudraCT Number 2020-003734-20

PHASE OF DEVELOPMENT:	I/II
NAME OF TEST PRODUCT (AND REFERENCE THERAPY):	COVID-*e*Vax
DOSE(S):	0.5 mg, 1 mg, 2 mg Prime-Boost 2 mg Prime
DOSAGE FORM:	Solution for injection
MODE OF ADMINISTRATION:	Intramuscular
STUDY DESIGN:	Multicentre, open-label
INDICATION:	Protection from infection, morbidity and mortality caused by SARS-CoV-2
STUDY POPULATION:	Healthy male and female volunteers, aged 18 - 65
STUDY INITIATION DATE:	25Feb2021
STUDY TERMINATION DATE:	07Dec2021
Sponsor:	**Takis S.r.l.** Via Castel Romano 100 00128 Rome (RM), Italy
Partner:	**Rottapharm Biotech S.r.l.** Via Valosa di Sopra 9 20900 Monza (MB), Italy
Coordinating Investigators:	- Paolo Antonio Ascierto, MD Istituto Nazionale Tumori – Naples (Italy) - Paolo Bonfanti, MD University of Milano-Bicocca and San Gerardo Hospital – Monza (Italy)
Responsible Medical Officer:	Lucio Rovati, MD CEO/CSO/EMD Rottapharm Biotech

Document Revision History:
Version No. 01 - 29 November 2022

This study was conducted in accordance with the Declaration of Helsinki and in compliance with the International Conference on Harmonisation (ICH) Good Clinical Practices (GCP) regulations/guidelines. Essential documents will be retained in accordance with ICH GCP.

CONFIDENTIALITY STATEMENT
This confidential document is the property of Takis S.r.l. and Rottapharm Biotech S.r.l. The recipient agrees that no unpublished information contained herein may be disclosed without the prior written approval of Takis S.r.l. and Rottapharm Biotech S.r.l.

2. SYNOPSIS

Name of Sponsor/Company: *Takis S.r.l.*	*Individual Study Table Referring to Part of the Dossier* *Volume:* *Page:*	*(For National Authority Use only)*
Name of Finished Product: *COVID-eVax*		
Name of Active Ingredient: *DNA plasmid-based vaccine targeting the RBD portion of the SARS-CoV-2 Spike protein*		
Title of study: A PHASE I/II STUDY TO ASSESS THE SAFETY AND IMMUNOGENICITY OF COVID-*e*VAX, A CANDIDATE PLASMID DNA VACCINE FOR COVID-19, IN HEALTHY ADULT VOLUNTEERS Study Code Number: COV-1/2-01		
Study centres/Investigators: - Paolo Antonio Ascierto, MD Istituto Nazionale Tumori – Naples (Italy) - Paolo Bonfanti, MD University of Milano-Bicocca and San Gerardo Hospital – Monza (Italy) - Stefano Milleri, MD CRC Centro Ricerche Cliniche di Verona – Verona (Italy)		
Publication (reference): No publications		
Studied period (years): 2021 Date of first enrolment: 25Feb2021 Date of study end (last subject last visit): 07Dec2021		Phase of development: I/II
Objectives: Phase I (Dose Escalation) *Primary objective* • To assess the safety and reactogenicity of the candidate vaccine COVID-*e*Vax in healthy adult volunteers • To identify the dose(s)/schedule(s) to be used in the Phase II (Dose Expansion) *Secondary objectives* • To preliminarily assess the immunogenicity of the candidate vaccine COVID-*e*Vax in healthy adult volunteers		

Name of Sponsor/Company: *Takis S.r.l.*	*Individual Study Table Referring to Part of the Dossier* *Volume:* *Page:*	*(For National Authority Use only)*
Name of Finished Product: *COVID-eVax*		
Name of Active Ingredient: *DNA plasmid-based vaccine targeting the RBD portion of the SARS-CoV-2 Spike protein*		
Phase II (Dose Expansion) *Primary objective* • To assess the immunogenicity of the selected dose(s)/schedule(s) of the candidate vaccine COVID-*e*Vax in healthy adult volunteers *Secondary objective* • To assess the duration of the immune response of the selected dose(s)/schedule(s) of the candidate vaccine COVID-*e*Vax in healthy adult volunteers • To assess the (long-term post-administration) safety of the candidate vaccine COVID-*e*Vax in healthy adult volunteers		
Methodology: This was a multicentre, open-label Phase I/II study, with a first-in-human (FIH) dose escalation part (Phase I study) followed by an open-label single arm (or two-arm, randomized) dose expansion part (Phase II study) in males (M) and non-pregnant females (F), in a 1:1 M/F ratio within each cohort, aged 18 to 65 years, who are in good health and meet all eligibility criteria. The Phase I part of the study investigated the safety and preliminary immunogenicity of ascending doses (in terms of unit dose and dose schedule) of COVID-*e*Vax, to inform the decision to move to Phase II. Three escalating cohorts of 20 subjects each were planned sequentially, as follows: • Cohort 1: 0.5 mg prime and 0.5 mg boost (PB), 4 weeks apart - Total dose: 1 mg • Cohort 2: 1 mg prime and 1 mg boost (PB), 4 weeks apart - Total dose: 2 mg • Cohort 3: 2 mg prime and 2 mg boost (PB), 4 weeks apart - Total dose: 4 mg A further group was planned, (starting after completion of sentinel subjects of cohort 3), to test the prime schedule: • Cohort 4: 2 mg prime (P) - Total dose: 2 mg COVID-*e*Vax was administered both as prime-boost and prime only schedules (by IM route, and followed by electroporation)		

Name of Sponsor/Company: *Takis S.r.l.*	*Individual Study Table Referring to Part of the Dossier* *Volume:* *Page:*	*(For National Authority Use only)*
Name of Finished Product: *COVID-eVax*		
Name of Active Ingredient: *DNA plasmid-based vaccine targeting the RBD portion of the SARS-CoV-2 Spike protein*		
The Phase II part of the study was planned to expand 1 or 2 dose(s)/schedule(s) (the most promising), to primarily confirm immunogenicity and expand safety data of COVID-*e*Vax in a larger healthy subject population but was actually not conducted.		
Number of subjects planned: Phase I 80 subjects; Phase II 80/160 subjects Number of subjects analysed: Phase I 68 subjects; Phase II not applicable		
Diagnosis and main criteria for eligibility: Healthy male or female subjects aged 18-65 years, with BMI >18.5 and ≤30 kg/m2 and normal vital signs/ECG/lab values at inclusion. No significant medical history should be documented. Subjects should also have negative serological test for SARS-CoV-2 antibodies and nasopharingeal swab, as well as no signs of respiratory infection before first vaccination. Negative SARS-CoV-2 nasopharingeal swab, as well as no signs of respiratory infection should also be confirmed before the second vaccine administration.		

Test product, dose and mode of administration, batch number:
COVID-*e*Vax was to be administered by intramuscular injection of 0.5 mL (for all doses) into the deltoid muscle of the right arm, followed immediately by electroporation (EP).
The following EP parameters were used:

- Number of pulses per treatment = 4
- Voltage amplitude= 40V (corresponding to an electric field strength of 100 V/cm)
- EP pulse duration = 5 milliseconds/pulse
- Interval separating pulses = 5 milliseconds
- IM injection depth = 16 mm
- EP injection depth = 21 mm

The following IMP Batches were used:

Test drug	**IMP Batch number**	**Batch/ Packaging number**	**Expiry date**	**Storage requirements**
COVID-*e*Vax	TKSB01-P0120	TKSB01-P0120	06/2021	-20 ±5 °C
COVID-*e*Vax	TKSB01-P0120	TKSB01-P0120	10/2021*	-20 ±5 °C

Name of Sponsor/Company: *Takis S.r.l.*	*Individual Study Table Referring to Part of the Dossier* *Volume:* *Page:*	*(For National Authority Use only)*
Name of Finished Product: *COVID-eVax*		
Name of Active Ingredient: *DNA plasmid-based vaccine targeting the RBD portion of the SARS-CoV-2 Spike protein*		
Duration of treatment: The treatment consisted of a single (Prime group) or double (Prime-Boost group) COVID-*e*Vax intramuscular administration		
Reference therapy, dose and mode of administration, batch number: No control group was foreseen.		
Evaluation criteria: **Safety:** - Solicited local and systemic Adverse Events (AE) - Unsolicited AE - Laboratory examinations - Physical examination - Vital signs **Immunogenicity:** - Quantitative binding antibodies anti-S and anti-N SARS-CoV-2 proteins - SARS-CoV-2 neutralizing antibody titer - Antigen-specific cellular immune response		

Statistical Methods:
 	
Sample size
No formal sample size calculation was performed. In Phase I, 20 subjects for each dose were considered sufficient to assess safety and reactogenicity, while preliminarily assessing immunogenicity.
 	
Primary endpoint
Safety:
- Reactogenicity:
  - Incidence of solicited local AEs at the injection site
  - Incidence of solicited systemic AEs
- Incidence of unsolicited AEs
- Changes in safety laboratory parameters		

Name of Sponsor/Company: *Takis S.r.l.*	*Individual Study Table Referring to Part of the Dossier* *Volume:* *Page:*	*(For National Authority Use only)*
Name of Finished Product: *COVID-eVax*		
Name of Active Ingredient: *DNA plasmid-based vaccine targeting the RBD portion of the SARS-CoV-2 Spike protein*		

Secondary endpoints

Immunogenicity:

- Quantitative antibody titers, binding to the specific SARS-CoV-2 antigen, analysed as Geometric Mean Response (GMR) and Geometric Mean Fold Rise (GMFR) from baseline
- SARS-CoV-2 neutralizing antibody titer, analysed as Geometric Mean Titer (GMT) and GMFR from baseline
- Change from baseline in antigen-specific cellular immune responses to SARS-CoV-2 as determined by IFN-γ ELISpot
- Percentage of participants who seroconverted
- Duration of the immune response
- Incidence of unsolicited AEs through study completion

Safety analysis

Reactogenicity was based on the incidence of solicited local and systemic AEs reported in the subject diary during the 7-day period after each vaccination. The number and proportion of participants reporting each symptom after the first, after the second or after any vaccination were provided overall and by severity. Unsolicited AEs were summarized by MedDRA SOC and PT, considering AEs reported within the first 28 days after each vaccination and AEs reported from the first vaccination through the end of study. Laboratory parameters were summarized as absolute value and change from baseline at each timepoint.

Immunogenicity analysis

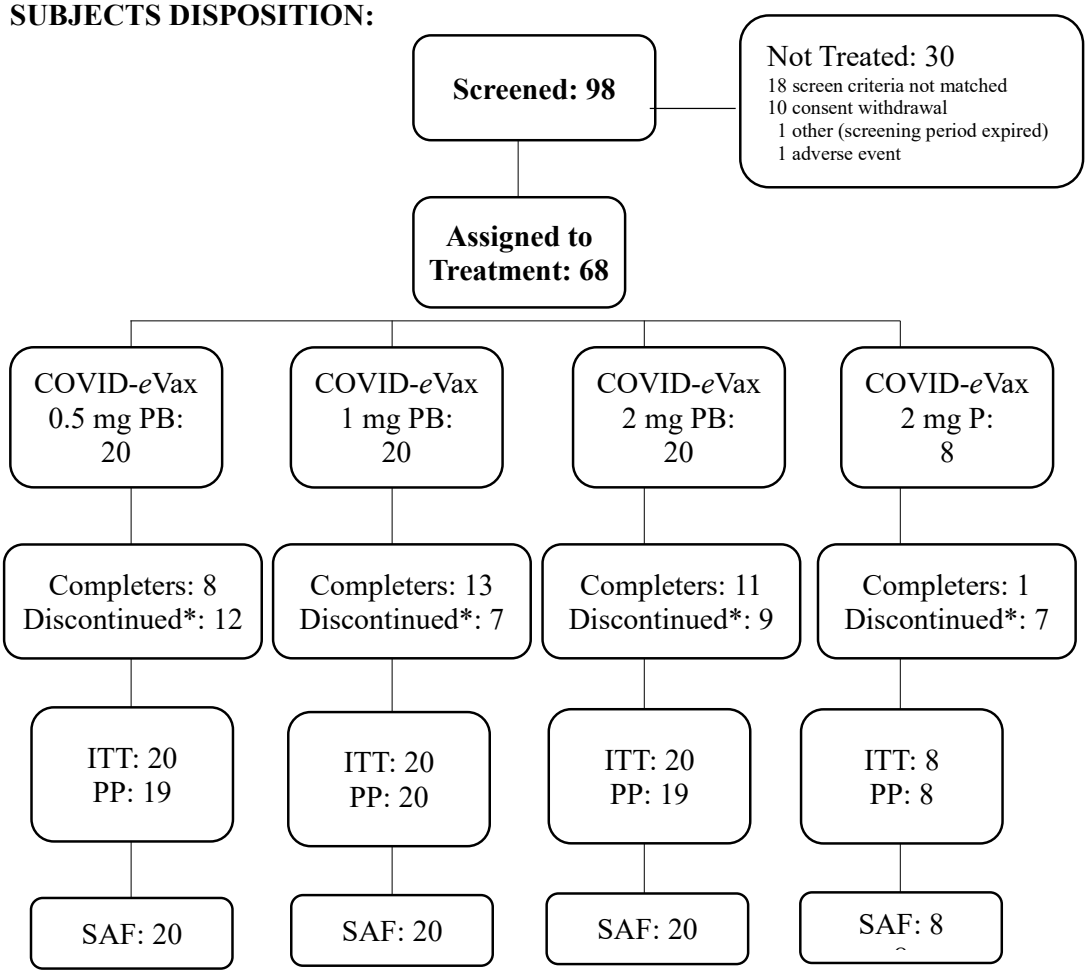
GMR (for S-binding antibodies), GMT (for neutralizing antibodies), and GMFR from baseline were summarized by timepoint. The proportion of participants who seroconverted and the proportion of participants with positive values were provided by timepoint. Seroconversion was defined as having an antibody level above the lower limit of quantification (LLOQ) post vaccination if the baseline level was below the LLOQ, or a 4-fold increase over baseline post vaccination if the baseline level was above the LLOQ. Positive values were defined as value ≥0.80 U/mL for binding antibodies and >5 titers for neutralizing antibodies. The antigen-specific cellular immune response to SARS-CoV-2, assessed by the IFN-γ ELISpot, was summarized at each timepoint as Geometric Mean (GM) and GMFR from baseline. Results were expressed as Spot Forming Cells (SFC) per 10^6 cells. Comparisons between post-

Name of Sponsor/Company: *Takis S.r.l.*	*Individual Study Table Referring to Part of the Dossier* *Volume:* *Page:*	*(For National Authority Use only)*
Name of Finished Product: *COVID-eVax*		
Name of Active Ingredient: *DNA plasmid-based vaccine targeting the RBD portion of the SARS-CoV-2 Spike protein*		

baseline timepoints and baseline were performed by means of Wilcoxon signed-rank test. Comparisons of proportion were performed by means of Chi-Square test.

SUMMARY - CONCLUSIONS:

SUBJECTS DISPOSITION:



** all discontinuations were due to subject decision to adhere to national vaccination campaign*

Name of Sponsor/Company: *Takis S.r.l.*	*Individual Study Table Referring to Part of the Dossier* *Volume:* *Page:*	*(For National Authority Use only)*
Name of Finished Product: *COVID-eVax*		
Name of Active Ingredient: *DNA plasmid-based vaccine targeting the RBD portion of the SARS-CoV-2 Spike protein*		

98 subjects were screened, 30 were not treated, so 68 subjects were assigned to study treatment and correctly received the planned treatment, 20 subjects in each of the PB groups and 8 subjects in the P group.

SAFETY RESULTS:
A total of 68 subjects were treated with COVID-*e*Vax; 60 subjects were treated twice, at a 4-week interval, while 8 subjects received a single treatment.

No deaths or SAEs were reported in the study, no subject left the study prematurely because of an AE, and no safety issues were identified during the safety data review conducted by the IDSMC during the dose escalation steps. The vaccine administration, including both the COVID-*e*Vax injection and the subsequent electroporation procedure, was well tolerated and successfully completed in all study participants.

Overall, solicited local AEs were mild or moderate. The most frequently reported solicited local AEs were tenderness (34 participants, 50%), pain at injection site (28 participants, 41%), and bruising (22 participants, 32%). No participants had redness or erythema. The proportion of participants with solicited local adverse events was quite similar between cohorts. Most of the solicited local AEs were observed after the first vaccination.

Overall, 38 (55.88%) participants reported at least one solicited systemic AE: 30 (44.12%) reported headache; 25 (36.76%) malaise/fatigue; 10 (14.71%) myalgia; 6 (8.82%) arthralgia; 6 (8.82%) nausea and 1 (1.47%) fever (maximum temperature 37.5°C for 2 days). The proportion of participants with solicited systemic AEs was similar between cohorts. Most of the solicited systemic AEs were reported after the first vaccination. Four (4) subjects experienced a severe solicited systemic AE: 1 headache after the second vaccination in the 0.5 mg PB cohort, 1 nausea after the first vaccination in the 1 mg PB cohort, and 1 malaise/fatigue and 1 headache after the first vaccination in the 2 mg PB cohort. A total of 11 participants (16%) reported in the diary to have used pain medications at least once in the 7 days after vaccination, mainly for headache.

Participants experiencing at least one unsolicited AE through 4 weeks after any vaccination were 8 (40.0%) in the 0.5 mg PB cohort, 7 (35.0%) each in the 1 and 2 mg PB cohorts and 1 (12.5%) in the 2 mg P cohort, with no relevant difference between occurrence after the first and the second vaccination or between cohorts. The majority of unsolicited AEs were sporadic mild to moderate episodes. The most frequently reported unsolicited AEs were contusion,

Name of Sponsor/Company: *Takis S.r.l.*	*Individual Study Table Referring to Part of the Dossier* *Volume:* *Page:*	*(For National Authority Use only)*
Name of Finished Product: *COVID-eVax*		
Name of Active Ingredient: *DNA plasmid-based vaccine targeting the RBD portion of the SARS-CoV-2 Spike protein*		

headache and blood creatine phosphokinase (CPK) increased. Three episodes of CPK increased were observed 1 week after the first or the second vaccination in 3 participants belonging to the 1 mg PB cohort; one episode was severe (CPK value 1909 U/L against a laboratory upper limit of 190 U/L), one was moderate (CPK value 651 U/L, upper limit 170 U/L) and one was mild (CPK value 376 U/L, upper limit 190 U/L), and the first two were considered by the Investigator as related to the electroporation procedure (to note, according to the Investigator, the severe episode was also probably due to a recent intense physical activity). Only one additional participant experienced a severe AE (hypertriglyceridemia, considered not related to the vaccine by the Investigator), while there were no grade 4 (life-threatening) AEs. A mild transient ischaemic attack with onset 17 days after the second vaccination was reported for a male smoker subject in the 0.5 mg PB cohort; the event was considered not related to the vaccine by the Investigator. The analysis of solicited and unsolicited AEs reported through study completion (i.e. up to 6 months) did not reveal any safety concerns.

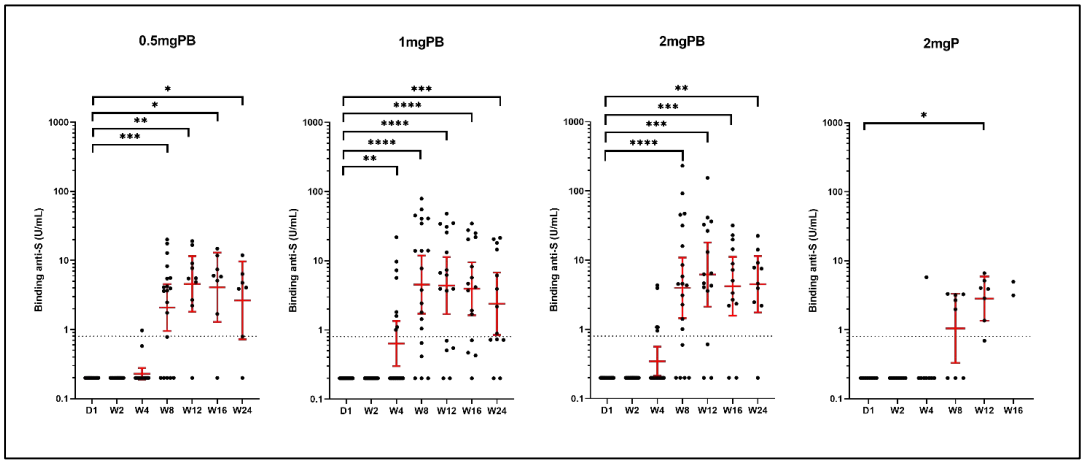
There were no other abnormal laboratory values that were deemed clinically significant, except for a mild blood bilirubin increase reported at week 1 for a participant in the 1 mg PB cohort considered not related to the vaccine by the Investigator, and a moderate neutropenia reported at day 3 for a participant in the 2 mg P cohort, considered by the Investigator a possible temporaneous effect of the vaccine.

No clinically significant findings were noted at ECG and vital signs remained stable immediately after vaccination and at subsequent follow-up visits.

Name of Sponsor/Company: *Takis S.r.l.*	*Individual Study Table Referring to Part of the Dossier* *Volume:* *Page:*	*(For National Authority Use only)*
Name of Finished Product: *COVID-eVax*		
Name of Active Ingredient: *DNA plasmid-based vaccine targeting the RBD portion of the SARS-CoV-2 Spike protein*		

IMMUNOGENICITY RESULTS:

COVID-*e*Vax vaccine induced a humoral response at all tested doses.

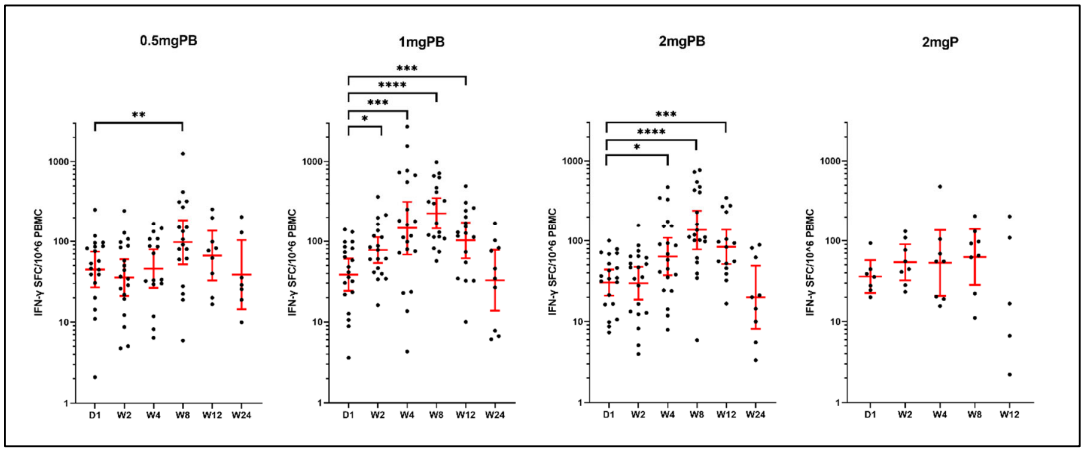


The binding anti-S antibodies specific for RBD became detectable at week 4 and peaked between week 8 and week 12 at all doses.
All PB cohorts had an at least 12-fold increase at week 12, peaking at 17 folds in the 2 mg PB cohort that still showed a 12-fold GMFR at week 24.
The vaccine-induced antibody response was lower in the 2 mg P cohort.
Comparison between post-baseline timepoints and day 1, performed by Wilcoxon signed-rank test, was statistically significant at all timepoints for the PB groups.
The proportion of evaluable participants with a positive binding antibody value was in the 80–90% range with 2 mg PB from week 8 onward and only slightly lower but less sustained in the other PB.

Neutralising antibody titer was absent in all subjects but 2, being one of them the subject with the highest concentration of binding anti-S antibodies.

Name of Sponsor/Company: *Takis S.r.l.*	*Individual Study Table Referring to Part of the Dossier* *Volume:* *Page:*	*(For National Authority Use only)*
Name of Finished Product: *COVID-eVax*		
Name of Active Ingredient: *DNA plasmid-based vaccine targeting the RBD portion of the SARS-CoV-2 Spike protein*		

In addition, a sustained cellular response was also observed.



In fact, the vaccine-induced T cells response was pronounced, with an approximately 5-fold increase in IFN-γ spot forming cells (SFC) per million peripheral blood mononuclear cells (PBMCs) in the 1 mg PB and 2 mg PB cohorts at week 8, and also at the lower dose 0.5 mg PB a 2-fold increase was observed.

Overall, the percentages of responders with positive binding anti-S antibodies and/or at least 2-fold increase in IFN-γ SFC at week 8 were 89%, 80% and 90% in the 0.5, 1 and 2 mg PB cohorts, respectively, while they were 62.5% in the 2 mg P cohort.

In the 2 mg PB cohort there was also a significant correlation between humoral and cellular response at week 8, not observed in the 1 mg PB cohort.

CONCLUSION:

This phase I/II clinical trial was aimed at investigating the safety and immunogenicity of COVID-*e*Vax, a candidate plasmid DNA vaccine for COVID-19.

The phase I dose-escalation part of the trial evaluated three cohorts (0.5, 1 and 2 mg prime-boos) receiving two doses 28 days apart, and an additional cohort (2 mg prime) receiving one dose only. The good progress of the national vaccination campaign against COVID-19 made impossible to complete the study as planned. Thus, enrolment in the last cohort of the phase I

Name of Sponsor/Company: *Takis S.r.l.*	*Individual Study Table Referring to Part of the Dossier* *Volume:* *Page:*	*(For National Authority Use only)*
Name of Finished Product: *COVID-eVax*		
Name of Active Ingredient: *DNA plasmid-based vaccine targeting the RBD portion of the SARS-CoV-2 Spike protein*		

study (prime cohort) was halted after 8 subjects and the dose expansion part of the trial (phase II) was not conducted.

COVID-*e*Vax was safe and promoted a distinct immunogenicity response in humans.

Reactogenicity was mainly mild and transient, generally lower after the second dose, and included bruising, an expected consequence of electroporation, similarly to transient blood CPK increase due to muscle stress, that was however observed only in few participants. Unsolicited AEs were sporadic and mild to moderate too.

COVID-*e*Vax induced an immune response at all tested prime-boost doses.

The best response was obtained with the highest 1 and 2 mg doses, inducing an immune response in up to 90% of the volunteers; particularly relevant was the cell-mediated response.

The cellular response mediated by COVID-*e*Vax appeared more efficient than the humoral effectiveness response, as supported by the correlations between the IFN-γ ELISpot data and the levels of binding antibodies.

In conclusion, this phase I clinical trial showed a tolerable safety profile and robust immunogenicity response especially in terms of cellular immunity of COVID-*e*Vax, a SARS-CoV-2 DNA-based vaccine candidate.

Additional phase II and III investigations are needed to understand if COVID-*e*Vax is able to induce a protective immunity. Moreover, it could be of interest to explore COVID-*e*Vax as a booster of pre-existing immunity induced by other technology platform vaccines.

Date of the report: 29 November 2022