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Immunogenicity and Safety of SpikoGen®, an Adjuvanted Recombinant SARS-CoV-2 Spike Protein as a Heterologous Third Booster Dose in Kidney Transplant Patients: A Single-Arm Clinical Trial

Mohsen Nafar , Narges Mostafaloo , Ahmad Firouzan , Fatemeh Poorrezagholi , Fariba Samadian , Nooshin Dalili , Saghar Barati , Nassim Anjidani , Hamidreza Kafi , Ramin Shahpari , Mahdiye Bayat , Soha Kianipour , Shiva Samavat

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Immunogenicity and Safety of SpikoGen®, an Adjuvanted Recombinant SARS-CoV-2 Spike Protein as a Heterologous Third Booster Dose in Kidney Transplant Patients: A Single-Arm Clinical Trial

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Author contributions

MN conceptualized the study. NM, AF, FP, FS, ND were involved in performing the research. SB wrote the original draft of the manuscript. NA, HK and SK were involved in organization, and conducting the study. RSH and MB performed the statistical analysis. SHS was involved in conceptualization, trial design and performing the study. All authors critically reviewed the manuscript and approved the final version. All authors had full access to all data in the studies and

had final responsibility for the decision to submit for publication.

Role of funder

This study was funded by CinnaGen Co. The funder had no role in the trial design, data collection, analysis or interpretation of the results or any other steps of preparing the manuscript for submission.

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Highlights:

- SpikoGen® vaccine induced considerable immune responses in kidney transplant patients.
- The antibody levels were above the protective levels 30 days after the third dose.
- Heterologous third dose of SpikoGen® can be considered in immunocompromised patients.

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Abstract

Purpose

Studies have shown that immunocompromised patients have suboptimal responses to COVID-19 vaccines leading to approval of a need for booster doses in this population. SpikoGen® is a subunit recombinant spike protein vaccine combined with Advax-CpG55.2™ adjuvant to protect against COVID-19. This vaccine was shown to be safe, immunogenic and efficacious to reduce the risk of COVID-19 including severe disease in previous clinical trials. However, the effects of this vaccine have not been assessed in immunocompromised patients. This study sought to assess the immunogenicity and safety of SpikoGen® vaccine as a third booster dose in kidney transplant patients taking immunosuppressive therapy who have received their primary vaccination based on an inactivated whole virus platform (Sinopharm).

Methods

This was a single-arm trial performed on 43 kidney transplant patients. The participants received a single booster dose of SpikoGen® vaccine 1 to 3 months after primary vaccination with 2 doses of Sinopharm vaccine. Immunogenicity assessments were performed at baseline and 30 days after the booster dose. The primary outcomes were seroconversion rates of anti-S_1 and surrogate virus neutralizing antibodies. Safety outcomes included the incidence of solicited and unsolicited adverse events in the seven days and one month after the booster dose, respectively.

Findings

SpikoGen® vaccine induced positive humoral and cellular responses 30 days after the booster dose in those seropositive or seronegative after two primary doses of Sinopharm vaccine. Thirty days post the SpikoGen® vaccine booster, seroconversion rates were 35.29% (95% CI; 19.75% to 53.51%) to anti-S_1 and 29.41% (95% CI; 13.27% to 46.57%) to surrogate neutralizing antibodies. The most common local and systemic reported solicited adverse events were injection site pain and fatigue, which were largely mild and transient. No serious adverse events were reported.

Implications

A single booster dose of SpikoGen® vaccine given 1-3 months after primary vaccination with 2 doses of Sinopharm vaccine induced positive humoral and cellular immune responses in immunosuppressed renal transplant patients thereby achieving spike antibody levels predictive of protection. It should be noted that this study was performed as just a single center study, and it will be important for future large multi-center studies to extend these results to other immunocompromised patient groups.

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Introduction

As of September 2022, SARS-CoV-2 is still infecting many people worldwide with vaccination playing an important role in helping to reduce the burden of disease. However, the problems of rapidly waning immunity after vaccination as well as immune escape caused by new variants, including B.1.617 or B.1.1.529, has led to calls for booster vaccinations to restore waning immunity and protection.

Low rates of seroconversion have been reported in solid organ transplant recipients receiving mRNA SARS-CoV-2 vaccines [1, 2]. In a study of BNT162b2 on renal transplant recipients, a third vaccine dose led to induction of neutralizing antibodies in populations with and without response to the primary vaccination [3]. In another study, third dose of BNT162b2 restored neutralizing titers of anti-receptor binding domain (RBD) antibodies in 40% of participants who had not responded to the previous vaccination course [4]. Based on all the positive findings, a meta-analysis has also recommended a third dose of mRNA vaccines in patients with solid organ transplantation considering its enhanced effects of immunogenicity and acceptable safety profile [5].

SpikoGen® is an adjuvanted recombinant S protein trimer vaccine that was shown to induce strong humoral and cellular responses in previous Phase 2 and a pivotal phase 3 trial demonstrating

positive safety, immunogenicity and efficacy, resulting in emergency use authorization from Iran's Food and Drug Administration in October 2021 [6].

As patients under immunosuppressive therapy were excluded from the previous SpikoGen® clinical trials, its immunogenicity and safety has not previously been assessed in this population. Hence, this study aimed to investigate the immunogenicity and safety of a SpikoGen® booster shot in kidney transplant patients on immunosuppressive therapy who had previously received primary vaccination with 2 doses of an inactivated whole virus platform (Sinopharm) vaccine.

Methods

Setting

This study was a single-arm open-label prospective clinical trial conducted on 43 patients during February and march 2022 at Shahid Labbafinezhad clinic; affiliated with Shahid Beheshti University of Medical Sciences, Tehran, Iran, which is considered a referral center for kidney transplantation in Iran.

Patients

Renal transplant patients 18 years of age and older were eligible to enter the study if they had received a primary course of vaccination with Sinopharm vaccine 1 to 3 months earlier. The exclusion criteria were as follows: pregnant or lactating women; patients with active infection or symptoms of COVID-19 at screening visit; history of COVID-19 between the primary vaccination and the third booster dose; active cytomegalovirus (CMV) infection; history of receiving rituximab

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or intravenous immunoglobulin (IVIg) during the past 6 months; patients with a history of severe allergic reactions (e.g. anaphylaxis) to the study vaccine or any components of the vaccine; subjects who had received any other investigational product within 30 days prior to the screening visit or intend to participate in other clinical studies during this trial; history of transplant rejection during the past 30 days; subjects with special circumstances which may increase the risk of participating in the study or interfering with the evaluation of the primary endpoints of the study according to the researcher's opinion.

Informed Consent

All participants signed the informed consent form before enrollment. The study was approved by the Ethics Committee of Urology and Nephrology Research Center; Shahid Beheshti university of medical Sciences (ethics code number: **IR.SBMU.UNRC.REC.1400.017**). The trial was registered at the Iranian Registry of Clinical Trials with the registration code **IRCT20150303021315N28** and ClinicaTrial.gov with the registration code **NCT05285384**.

Interventions

A total of 43 eligible patients were included in this study (Figure 1). Medical histories, and Immunogenicity assessments were done at baseline and 30 days after the booster dose. Eligible participants received a single booster dose of 25 µg SpikoGen® in their deltoid muscle.

Outcomes

The primary outcomes were the seroconversion rates of anti-S_1-binding IgG and neutralizing antibodies measured via a surrogate virus neutralizing test (sVNT) 1 month after the booster dose.

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Secondary outcomes included the geometric mean fold rise (GMFR) of anti-S_1 and neutralizing antibodies 1 month after the SpikoGen® booster dose. T cell responses were also assessed at baseline and 1 month after the booster dose using an interferon gamma-release assay after stimulation with spike protein peptides. Other secondary outcomes included immunogenicity assessments in subgroups of participants with and without previous humoral response to their primary vaccination course. Patients with primary response were defined as positive status of anti-S_1 **or** neutralizing antibodies at baseline. Whereas, patients without response to the primary vaccination were defined as being negative for anti-S_1 and neutralizing antibodies at baseline.

Seroconversion was defined as a change in the status of antibody levels from negative to positive based on the prespecified commercial ELISA kits threshold in the seronegative populations. In the baseline seropositive population, seroconversion was defined as at least a four-fold rise in the antibody levels on day 30. T cell responses were performed based on QuantiFERON SARS-CoV-2 RUO (Qiagen, Germany) toolset. Tube 1 contained CD4+ epitopes (AG1) and tube 2 contained CD4+ and CD8+ epitopes (AG2) from the spike protein. The levels of interferon-gamma in plasma samples were reported in international units per milliliter according to manufacturer's instructions.

Safety assessments were performed as secondary outcomes including the occurrence of local and systemic solicited adverse events for seven days after the booster dose. Unsolicited adverse events were assessed until 1 month after the booster dose. These outcomes were reported based on Medical Dictionary for Regulatory Activities (MedDRA) classification. The participant's severity score was assessed based on the FDA toxicity grading scale [7].

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Statistical Analysis

The initial recruitment target of 100 subjects was planned according to the available population of kidney transplant patients who had not received the booster dose. This estimation was not based on any power calculation, with 43 patients ultimately being enrolled. All patients who received a booster dose of the study treatment were included in the safety population. Safety was presented as incidences and percentages of subjects with solicited (local and systemic) and unsolicited adverse events. The immunogenicity outcomes were reported based on 34 available samples of patients who received a booster dose of the vaccine.

Missing data were not imputed. No multiplicity adjustments were made in this study. Continuous data were compared using a t-test and categorical data were assessed using chi-squared or Fisher exact test. Participants with seroconversion due to vaccination were provided with two-sided 95% CI using the Clopper-Pearson method. Hypothesis testing was two-sided and p-values of less than 0.05 were considered significant. The 95% CIs for Geometric mean concentration (GMC) and Geometric mean fold rise (GMFR) were calculated based on the t-distribution of the log-transformed values then back-transformed to the original scale in each time point for presentation. The sub-group analyses were performed based on baseline characteristics. Wilcoxon tests were used to compare the paired samples to evaluate the difference in the concentrations of IFN-γ and antibody levels. We used R (version 4.0.1) and STATA 14 for all Statistical analyses.

Results

In total, 43 patients were enrolled in the study. Screening process of the participants are provided in the CONSORT diagram in Figure 1. Table 1 shows the demographic data and baseline

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characteristics of the participants including drug histories, the reason for transplantation, seropositivity status at baseline, days passed from transplantation, history of rejection, number of transplantations, type of transplantation and history of new onset diabetes after transplantation.

Figure 1. Screening and randomization of the participants.

Table 1. Demographics and clinical characteristics of the patients at baseline

Immunogenicity outcomes

Post-booster day 30, seroconversion rate of anti- S_1 IgG was 35.29% (95% CI; 19.75% to 53.51%). The seroconversion rate of neutralizing antibodies was 29.41% (95% CI; 13.27% to 46.57%). The results of the GMFR and subgroup analysis based on the baseline characteristics are provided in Table 2.

Table 2.Seroconversion rate and GMFR of S_1-IgG (RU\mL) and sVNT (ug/mL)

Results of the T-cell response are provided in Table 3. As the Table shows, the interferon-γ response in blood samples taken one month after the booster dose was significantly higher after stimulation with antigen 2 (CD4+ and CD8+ epitopes) when compared to baseline levels. While the interferon-γ response to antigen 1 also trended higher at Day 30 when compared to Day 0. However, this did not reach statistical significance.

Table 3. Interferon-γ released assay after stimulation with Antigen1 & Antigen2 (IU/mL)

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Safety outcomes

The results of the solicited adverse events after the booster injection are provided in Table 4. As can be seen, injection site pain was the most common adverse event among the participants (44.19%). Among systemic complications, fatigue was the most reported adverse event, being detected in 10 (23.26%) patients. The results of the unsolicited adverse events are provided in Table 5.

Table 4. number and percentage of subjects experiencing solicited local and systemic adverse events by symptoms based on maximum toxicity grading scale.

Table 5. number and percentage of subjects experiencing unsolicited adverse events

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Post-hoc analysis

The GMC results for anti-S_1 binding IgG and neutralizing antibodies are provided in Table 6. As the Table shows, the anti-S_1 and neutralizing antibody levels were significantly greater at Day 30 over baseline values in the pooled population and those seropositive and seronegative after the primary vaccination course.

Table 6. GMC of S_1-IgG (RU\mL) and neutralizing IgG (ug/mL) in the participants

The use of WHO standards allowed us to convert the S_1 antibody results to WHO binding antibody units (BAU). The results are provided in Table 7.

A total of 25/34 (73.5%) of the participants receiving the SpikoGen® booster dose, achieved S_1 antibody levels of equal or greater than 154 BAU/ml 30 days post-booster.

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Table 7. GMC of S_1-IgG based on (BAU\mL)

Discussion

The results of this study show that the SpikoGen® vaccine is able to induce humoral and cellular responses in kidney transplant patients under treatment with immunosuppressors. The 3rd booster dose of SpikoGen® vaccine significantly elevated anti-S_1 and neutralizing antibody levels in patients who had previously received primary vaccination with an inactivated whole virus vaccine (Sinopharm). This boosting effect was robust in both baseline seropositive and seronegative patients.

Previous studies have shown weak immune response to two doses of other SARS-CoV-2 vaccines in patients with solid organ transplantation under immunosuppressive therapy [2]. According to our baseline results, after 1 to 3 months of a second dose of an inactivated whole virus platform ~ 35% of the patients had no detectable anti-S_1 binding or neutralizing antibodies. A booster dose of SpikoGen® vaccine successfully induced both humoral and cellular immune responses in these immunosuppressed populations.

A study reported a seroconversion rate of 44%, 30 days after a third dose of mRNA COVID-19 vaccine in solid-organ transplant recipients without response to the primary vaccination [8]. The seroconversion rate of ~ 35% in the pooled participants, ~ 32% and ~ 41% in participants with and without primary response to the inactivated whole virus platform in our study, appears comparable to that study. In individuals primed with inactivated vaccine, exposure to the much larger amount

of spike protein in SpikoGen® vaccine may better stimulate a recall memory B cell response, helping explain the antibody response induced by SpikoGen® vaccine despite the lack of apparent response to the primary vaccination course. The Advax-CpG adjuvant in the SpikoGen® may also have contributed to this response in non-responders to the primary vaccine course.

How might the spike antibody levels achieved with the SpikoGen® booster dose in solid-organ transplantation recipients in our study translate into actual SARS-CoV-2 protection? Goldblatt et al., suggested the ratio of trial participants who achieve a level of 154 BAU/ml, could be used to predict the level of protection against wild type virus and for studies with antibody distributions that enabled precise estimation of thresholds, this threshold was considered to be 60 BAU/ml [9]. Based on this protection algorithm, the percentage of subjects with spike IgG levels above 154 BAU/ml 30 days after the SpikoGen® booster dose, were 25/34 (73.5%). Notably, the mean concentrations of anti-S_1 antibodies after the booster dose even in those without a primary response to the Sinopharm vaccine was greater than the cut-off of 60 BAU/ml. These results would predict a SpikoGen® booster dose efficacy against symptomatic infection in these transplant patients of ~ 70% against the ancestral strains. This algorithm came from a ChAdOx1 viral vector vaccine study showing an anti-S_1 levels of 54 BAU/ml translated to a 60% efficacy against symptomatic infection with Alpha (B.1.1.7), 113 BAU/ml with a 70% efficacy and 264 BAU/ml with 80% vaccine efficacy [10]. Notably, this algorithm was derived on antibody responses obtained in immunocompetent subjects, whereas our prediction of 70% SpikoGen® efficacy against symptomatic infection was achieved in immunosuppressed transplant patients. The most important outcome of vaccination, however, is protection against severe disease. Notably, in its pivotal Phase 3 trial, SpikoGen® vaccine provided ~78% protection against severe disease caused by the delta variant [6]. This is consistent with trial results for other COVID-19 vaccines, which

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have all consistently shown higher levels of protection against severe disease caused by SARS-CoV-2 than against just symptomatic infection. This provides additional confidence that the booster dose of SpikoGen® vaccine in renal transplant patients should provide even more robust protection against severe disease as predicted by this algorithm for symptomatic infection.

Overall, our results confirm that a third dose of a spike-protein based vaccine may be beneficial at an interval of 1-3 months after an initial primary vaccine course in immunocompromised patients. This early third booster dose could be particularly important given the rise of new vaccine-resistant variants such as Omicron to which vaccine immunity wanes even faster than against the ancestral strains [11].

While humoral responses are clearly highly important, T cell responses may also play an important role in SARS-CoV-2 protection in immunocompromised patients [12]. SpikoGen® vaccine induced a considerable rise in interferon-gamma responses after stimulation with CD4+ and CD8+ (AG2) spike protein epitopes, consistent with previous trials of SpikoGen® showing its ability to induce T cell responses [13]. Notably, Advax-CpG adjuvant has been shown to induce potent anti-viral CD8+ T cell responses [14]. In this booster study, T-cell interferon gamma responses were increased by the SpikoGen® booster which is a valuable finding in immunocompromised patients where the activation of both arms of the immune response may be particularly important for protection.

Based on the safety results, the SpikoGen® booster shot was well tolerated and the solicited adverse events were consistent with those normally seen after vaccination. The vast majority of adverse reactions were mild and short-lived with full recovery, consistent with the clean safety profile seen in previous SpikoGen® trials [6, 15, 16].

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A recently published SpikoGen® booster trial in immune-competent participants who had received a primary vaccination course of inactivated whole virus or other vaccines 4-6 months earlier showed a seroconversion rate of 68% in anti-S_1 antibodies and 80% in neutralizing antibodies 14 days after the booster dose [15]. While the seroconversion rates were lower in the current trial, this previous booster trial was in immunocompetent healthy individuals whereas the current trial was in transplant recipients on immune-suppressive therapy, in whom seroconversion rates would be expected to be significantly lower.

This study had some limitations including the relatively low number of transplant patients and the lack of a suitable control group. At the time of study design, we planned to enroll 100 subjects. However, according to the national vaccination program, most of the available subjects already became vaccinated during the approval of the ethical committee, and we could only recruit 43 subjects. Among these subjects, only 34 samples were available for immunogenicity results. The values of other 9 samples could not be assessed because of the leaking and broken blood specimen containers. Another limitation of this study includes the lack of measurement for actual infection although an attempt was made to try and predict protection, by extrapolating from antibody levels. Furthermore, the results were based on just a single time point 30 days post the booster dose and it is not currently known how the spike antibody responses induced by the booster dose might decay over time, whether additional boosters of SpikoGen® may be required and, if so, what the optimal dose window for such additional boosters may be. Similarly, it is not currently known what levels of protection this might afford against evolving Omicron variants. A final limitation was that this study was performed as just a single center study, and it will be important for future large multi-center studies to extend these results to other immunocompromised patient groups.

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Conclusion

In renal transplant patients that had received a primary course of an inactivated whole virus vaccine 1-3 months previously, a single booster dose of heterologous SpikoGen® - a recombinant spike protein vaccine- induced positive humoral and cellular immune responses predictive of protection against SARS-CoV-2 infection.

Acknowledgements

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Data availability statement

Anonymous participant data will be available upon a reasonable request to the corresponding author.

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Declarations of Competing Interest

SB, NA, HK, RSH, MB, and SK are members of the Orchid Pharmed medical department which is in collaboration with CinnaGen company with respect to conducting clinical trials. The remaining authors have no other relevant affiliations.

References

1. Boyarsky, B.J., et al., *Immunogenicity of a Single Dose of SARS-CoV-2 Messenger RNA Vaccine in Solid Organ Transplant Recipients.* JAMA, 2021. **325**(17): p. 1784-1786.

2. Boyarsky, B.J., et al., *Antibody Response to 2-Dose SARS-CoV-2 mRNA Vaccine Series in Solid Organ Transplant Recipients.* JAMA, 2021. **325**(21): p. 2204-2206.
3. Hod, T., et al., *BNT162b2 Third Booster Dose Significantly Increases the Humoral Response Assessed by Both RBD IgG and Neutralizing Antibodies in Renal Transplant Recipients.* Transpl Int, 2022. **35**: p. 10239.
4. Charmetant, X., et al., *Infection or a third dose of mRNA vaccine elicits neutralizing antibody responses against SARS-CoV-2 in kidney transplant recipients.* Sci Transl Med, 2022. **14**(636): p. eabl6141.
5. Efros, O., et al., *Efficacy and Safety of Third Dose of the COVID-19 Vaccine among Solid Organ Transplant Recipients: A Systemic Review and Meta-Analysis.* Vaccines (Basel), 2022. **10**(1).
6. Tabarsi, P., et al., *Safety and immunogenicity of SpikoGen®, an advax-cpg55.2-adjuvanted sars-cov-2 spike protein vaccine: a phase 2 randomized placebo-controlled trial in both seropositive and seronegative populations.* Clin Microbiol Infect, 2022.
7. Popmihajlov, Z., et al., *A post hoc analysis utilizing the FDA toxicity grading scale to assess injection site adverse events following immunization with the live attenuated Zoster Vaccine (ZVL).* Hum Vaccin Immunother, 2018. **14**(12): p. 2916-2920.
8. Kamar, N., et al., *Three Doses of an mRNA Covid-19 Vaccine in Solid-Organ Transplant Recipients.* N Engl J Med, 2021. **385**(7): p. 661-662.
9. Goldblatt, D., et al., *Towards a population-based threshold of protection for COVID-19 vaccines.* Vaccine, 2022. **40**(2): p. 306-315.
10. Feng, S., et al., *Correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection.* Nat Med, 2021. **27**(11): p. 2032-2040.
11. Evans, J.P., et al., *Neutralizing antibody responses elicited by SARS-CoV-2 mRNA vaccination wane over time and are boosted by breakthrough infection.* Sci Transl Med, 2022. **14**(637): p. eabn8057.
12. Grifoni, A., et al., *SARS-CoV-2 human T cell epitopes: Adaptive immune response against COVID-19.* Cell Host Microbe, 2021. **29**(7): p. 1076-1092.
13. Tabarsi, P., et al., *Safety and immunogenicity of SpikoGen(R), an advax-cpg55.2-adjuvanted sars-cov-2 spike protein vaccine: a phase 2 randomized placebo-controlled trial in both seropositive and seronegative populations.* Clin Microbiol Infect, 2022.
14. Li, H., et al., *Novel adjuvants enhance immune responses elicited by a replication-defective human cytomegalovirus vaccine in nonhuman primates.* Vaccine, 2021. **39**(51): p. 7446-7456.
15. Tabarsi, P., et al., *Immunogenicity and safety of SpikoGen®, an adjuvanted recombinant SARS-CoV-2 spike protein vaccine as a homologous and heterologous booster vaccination: A randomized placebo-controlled trial.* Immunology, 2022.
16. Tabarsi, P., et al., *Evaluating the Efficacy and Safety of SpikoGen®, an Advax-CpG55.2-adjuvanted SARS-CoV-2 Spike Protein Vaccine: A Phase 3 Randomized Placebo-Controlled Trial.* Clin Microbiol Infect, 2022.

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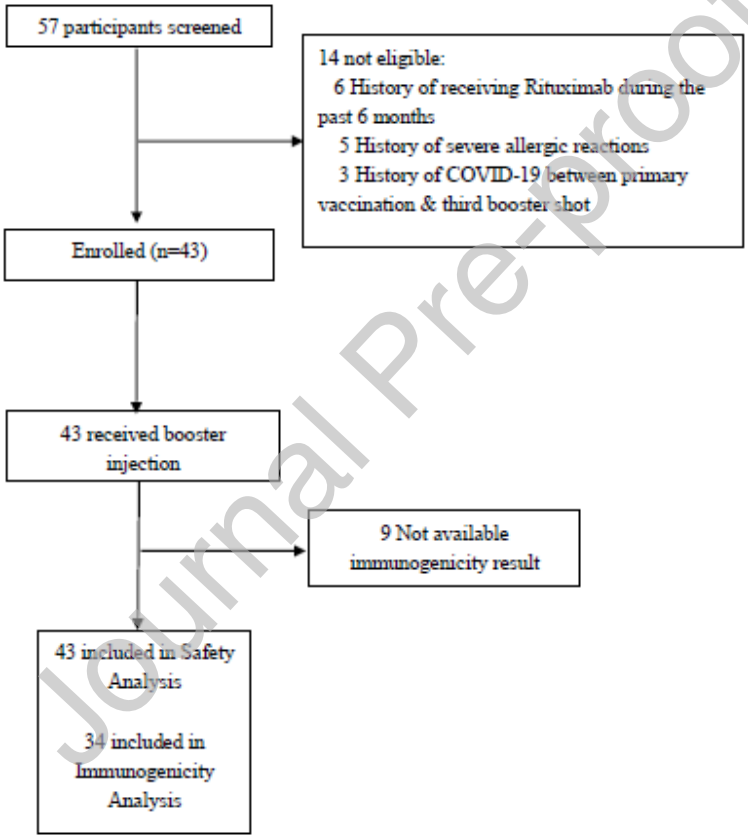

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Characteristics	SpikoGen® (N = 43)
Age(y)—Mean ±SD	41.99±11.83
Gender— n (%)	
Male	28 (65.12)
Female	15 (34.88)
Medication— n (%)	
Cyclosporine	18 (41.86)
Tacrolimus	24 (55.81)
Sirolimus	1 (2.33)
Everolimus	0 (0)
Prednisolone	43 (100)
Mycophenolate Mofetil	41 (95.35)
Azathioprine	1 (2.33)
Reason for transplantation— n (%)	
Autosomal dominant polycystic kidney disease (ADPKD)	3 (6.98)
Diabetes mellitus	6 (13.95)
Glomerulonephritis	9 (20.93)
Hypertension	15 (34.88)
Nephrolithiasis	1 (2.33)
Other	15 (34.88)
Patients without primary response to Sinopharm vaccine	12(35.29)
Days from last transplantation—Mean ±SD	2775.42 ±2110.78
History of rejection—n (%)	0 (0)
Transplantation Type—n (%)	
Living	23 (53.49)
Deceased	20 (46.51)
Transplantation count—n (%)	
0	1 (2.33)
1	36 (83.72)
2	6 (13.95)
New onset diabetes after transplantation—n (%)	2 (4.65)

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Population set		SCR (%) (95% CI)		GMFR (95% CI)	
		S_1-IgG	sVNT	S_1-IgG	sVNT
Pooled		12 (35.29) (19.75-53.51)	10 (29.41) (13.27-46.55)	5.01 (2.41-10.45)	3.51 (1.97-6.25)
	Without primary response to Sinopharm	5 (41.67) (15.17-72.33)	4 (33.33) (9.92-65.11)	15.58 (2.37-102.23)	4.77 (1.07-21.17)
	With primary response to Sinopharm	7 (31.82) (13.86-54.87)	6 (27.27) (10.73-50.22)	2.70 (1.65-4.41)	2.96 (1.77-4.97)
Gender					
	male	9 (40.91) (20.71-63.65)	8 (36.36) (17.20-59.34)	8.45 (2.95-24.23)	4.45 (1.99-10.00)
	Female	3 (25) (5.49-57.19)	2 (16.67) (2.09-48.41)	1.92 (1.03-3.59)	2.26 (1.02-5.01)
Medication					
	Cyclosporine	4 (26.67) (7.79-55.10)	3 (20.00) (4.33-48.09)	2.62 (1.38-4.98)	2.54 (1.36-4.75)
	Tacrolimus	8(42.11) (20.25-66.50)	7 (36.84) (16.29-61.64)	8.37 (2.46-28.45)	4.52 (1.75-11.71)
	Prednisolone	12 (35.29) (19.75-53.51)	10 (29.41) (15.10-47.48)	5.01 (2.41-10.45)	3.51 (1.97-6.25)
	Mycophenolate Mofetil	11 (33.33) (17.96-51.83)	9 (27.27) (13.30-45.52)	4.92 (2.31-10.48)	3.34 (1.85-6.00)
Medical History					
	Diabetes mellitus	2 (33.33) (4.33-77.72)	2 (33.33) (4.33-77.72)	15.99 (0.67-380.08)	6.95 (0.46-105.61)
	Glomerulonephritis	2 (40.00) (5.27-85.34)	1 (20.00) (0.51-71.64)	3.55 (0.45-27.88)	1.92 (0.40-9.21)
	Hypertension	5 (41.67) (15.17-72.33)	5 (41.67) (15.17-72.33)	6.00 (1.75-20.59)	5.12 (1.53-17.22)
	Other	3 (23.08) (5.04-53.81)	2 (15.38) (1.92-45.45)	2.81 (0.94-8.41)	2.23 (1.16-4.27)
Transplantation Type					
	Living	7 (36.84) (16.29-61.64)	6 (31.58) (12.58-56.55)	5.34 (1.75-16.38)	4.54 (1.84-11.16)
	Deceased	5 (33.33)	4 (26.67)	4.62	2.53

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	(11.82-61.62)	(7.79-55.10)	(1.64-13.00)	(1.20-5.32)

Note: Percentages for seroconversion rate (SCR) were calculated as a number of subjects who reported the event divided by the total number of subjects in each population set with non-missing data multiply 100. The 95% confidence interval (CI) for SCR was calculated using the exact Clopper-Pearson method. The 95% confidence interval (CI) for geometric mean fold rise (GMFR) was calculated based on the t-distribution of the log-transformed values, then back-transformed to the original scale for presentation.

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IFN-γ	Antigen1		Antigen2	
	Day 0	Day 30	Day 0	Day 30
Median	0.23	0.35	0.22	0.47
IQR	(0.15-0.50)	(0.22-0.62)	(0.15-0.41)	(0.29-0.93)
*P-Value	0.20		0.03	

*Based on Wilcoxon test

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Symptom	Grade	SpikoGen® N=43
Any solicited local AE	1	20 (46.51)
	2	2 (4.65)
Injection site erythema	1	1 (2.33)
Injection site pain	1	19 (44.19)
	2	2 (4.65)
Injection site swelling/induration	1	2 (4.65)
Any solicited systemic AE	1	14 (32.56)
	2	5 (11.63)
Chills	1	1 (2.33)
	2	1 (2.33)
Fatigue	1	10 (23.26)
	2	1 (2.33)
Arthralgia	1	2 (4.65)
Myalgia	1	5 (11.63)
	2	1 (2.33)
Headache	1	2 (4.65)
	2	3 (6.98)

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System Organ Class/Preferred Term	SpikoGen N=43
Any adverse event	9 (20.93)
Gastrointestinal disorders	1 (2.33)
Diarrhoea	1 (2.33)
General disorders and administration site conditions	1 (2.33)
Fatigue	1 (2.33)
Nervous system disorders	3 (6.98)
Dizziness	2 (4.65)
Parosmia/dysgeusia	1 (2.33)
Respiratory, thoracic and mediastinal disorders	6 (13.95)
Cough	2 (4.65)
Dyspnoea	2 (4.65)
Oropharyngeal pain	2 (4.65)
Rhinorrhoea	2 (4.65)
Skin and subcutaneous tissue disorders	1 (2.33)
Pruritus	1 (2.33)
Vascular disorders	1 (2.33)
Hypotension	1 (2.33)

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S_1-IgG	Pooled		Without primary response to Sinopharm vaccine		With primary response to Sinopharm vaccine	
	Day 0	**Day 30**	**Day 0**	**Day 30**	**Day 0**	**Day 30**
GMC	8.96	44.92	0.33	5.15	54.17	146.31
95% CI	(3.56-22.57)	(19.38-104.12)	(0.27-0.41)	(0.81-32.71)	(32.36-90.68)	(105.22-203.45)
*__P-Value__	<0.001		0.01		<0.001	
Neutralizing IgG						
GMC	4.16	14.60	0.62	2.97	11.74	34.79
95% CI	(2.14-8.09)	(7.05-30.21)	(0.45-0.86)	(0.72-12.33)	(5.92-23.26)	(18.70-64.73)
*__P-Value__	<0.001		0.04		<0.001	

*Based on paired t-test

Note: The 95% confidence interval (CI) for geometric mean was calculated based on the t-distribution of the log-transformed values, then back-transformed to the original scale for presentation

Journal Pre-proof

S_1-IgG	Pooled		Without primary response to Sinopharm		With primary response to Sinopharm	
	Day 0	**Day 30**	**Day 0**	**Day 30**	**Day 0**	**Day 30**
GMC	89.48	243.07	21.06	72.75	196.99	469.37
95% CI	(56.28-142.28)	(150.78-391-86)	(20.71-21.42)	(27.21-194.52)	(128.52-301.92)	(354.20-621.98)

Note: The 95% confidence interval (CI) for geometric mean was calculated based on the t-distribution of the log-transformed values, then back-transformed to the original scale for presentation

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