(Original Article)

A Proof-of-Concept Study for an Intradermal Dose-Sparing Strategy: Non-Inferior Immunogenicity and Improved Systemic Tolerability of Fractional-Dose ChAdOx1-S SARS-CoV-2 Vaccine Compared to Intramuscular Administration

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(Received : 10 Nov. 25, Revised : 14 Dec. 25, Accepted : 19 Dec. 25)

Abstract

Background: The global shortage of SARS-CoV-2 vaccines and the necessity for repeated booster doses highlight an urgent need for dose-sparing strategies. The intradermal (ID) route, leveraging the skin's rich immune network, is highly attractive due to the high density of antigen-presenting cells (APCs) in the dermis, offering the potential to induce a robust immune response with a reduced antigen dose. This study was conducted to establish a Proof-of-Concept (PoC) for the feasibility of an ID fractional dose strategy.

Objective: This study aimed to establish a proof-of-concept for an ID dose-sparing strategy. Primary objectives were to evaluate the safety, tolerability, and immunogenicity of a fractional ID dose (0.1 mL, 1 x 1010 viral particles) compared to the standard IM dose (0.5 mL, 5 x1010 viral particles) as a booster in healthy adults.

Methods: This was a comparative, prospective, open-label, volunteer-controlled trial. Healthy adults (18-60 years old) who had previously completed a two-dose regimen of inactivated SARS-CoV-2 vaccine (Sinovac) were assigned to receive the ChAdOx1-S vaccine as a 3rd dose booster. The IM full dose group received 0.5 mL, 5 x 1010 vp, while the ID fractional dose group received 0.1 mL, 1 x 1010 vp. Safety, tolerability, and immunogenicity (Anti-S RBD antibody titers, converted to BAU/mL) were evaluated at Day 0 and Day 14 post-vaccination.

Results: A total of 60 volunteers were included. The Geometric Mean Titer (GMT) of Anti-S RBD antibodies was 10,203 BAU/mL (95%CI: 7,698 to 13,524) in the ID group and 10,337 BAU/mL (95%CI: 8,078 to 13,225) in the IM group. The GMT ratio (ID/IM) was 0.99 (95%CI: 0.77 to 1.27), which exceeded the pre-specified non-inferiority margin (0.67), demonstrating statistical non-inferiority of the fractional ID dose. The ID group demonstrated a favorable safety profile with significantly lower incidence of systemic adverse events (AEs) (e.g., Myalgia 10 % vs. 40 %, Headache 5 % vs. 30 %) compared to the IM group. Local reactions like

erythema (91 % vs. 11.1 %) and swelling (72 % vs. 11.1 %) were more common with ID administration. No serious adverse events were reported.

Conclusion: The ID administration of a one-fifth fractional dose of the ChAdOx1-S vaccine produces a robust, non-inferior immune response with a superior systemic safety profile. This study establishes a vital PoC for a Dose-Sparing strategy using viral vector vaccines. Beyond vaccine scarcity, the demonstrated efficiency of the ID route holds vast potential for future application in anti-cancer immunotherapy, specifically with personalized cancer vaccines.

Keywords: SARS-CoV-2 vaccine, intradermal; dose-sparing; immunogenicity

Royal Thai Air Force Medical Gazette, Vol. 71 No. 2 July - December 2025

(นิพนธ์ต้นฉบับ)

การศึกษาหลักฐานแนวคิดการให้วัคซีนขนาดต่ำ: ChAdOx1-S SARS-CoV-2 Vaccine ขนาด 1/5 ส่วนทางผิวหนังให้ภูมิคุ้มกันเทียบเท่าการฉีดกล้ามเนื้อ โดยมีอาการข้างเคียงน้อยกว่า

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บทคัดย่อ

ความเป็นมาและความสำคัญของปัญหา: การขาดแคลนวัคซีน SARS-COV-2 ทั่วโลกและความจำเป็นในการกระตุ้นซ้ำ ได้เน้นย้ำถึงความต้องการเร่งด่วนสำหรับกลยุทธ์การประหยัดโดส (Dose-Sparing Strategies) การให้ทางผิวหนัง (ID) เป็นทางเลือกที่น่าสนใจเนื่องจากมีความหนาแน่นของเซลล์นำเสนอแอนติเจน (APCS) สูงในชั้นหนังแท้ ซึ่งมีศักยภาพในการกระตุ้นภูมิคุ้มกันที่แข็งแกร่งด้วยปริมาณแอนติเจนที่ลดลง การศึกษานี้จัดทำขึ้นเพื่อสร้างหลักฐานแนวคิด (Proof-of-Concept: PoC) สำหรับความเป็นไปได้ของกลยุทธ์การให้วัคซีน ขนาดเศษส่วนทางผิวหนัง

วัตถุประสงค์: การศึกษานี้มีเป้าหมายเพื่อพิสูจน์แนวคิดของกลยุทธ์การประหยัดวัคซีนทางผิวหนังโดยใช้วัคซีน ChAdOx1-S วัตถุประสงค์หลักคือเพื่อประเมินความปลอดภัย ความทนทาน และการกระตุ้นภูมิคุ้มกันของวัคซีนขนาดเศษส่วนๆ ที่ฉีดผิวหนัง (0.1 มล., 1 X 1010 VP) เปรียบเทียบกับขนาดมาตรฐานที่ฉีดเข้ากล้ามเนื้อ (0.5 มล., 5 X 1010 VP) ในฐานะวัคซีนเข็มบูสเตอร์ในผู้ใหญ่สุขภาพดี

ระเบียบวิธีวิจัย: การศึกษานี้เป็นการศึกษาแบบเปรียบเทียบไปข้างหน้าและมีกลุ่มควบคุมกลุ่มตัวอย่างเป็นผู้ใหญ่สุขภาพดี (อายุ 18-60 ปี) ที่เคยได้รับวัคซีนซิโนแวคครบ 2 เข็ม จำนวนรวม 60 คน ได้รับการจัดให้ รับวัคซีน ChAdOx1-S เป็นโดสกระตุ้นครั้งที่ 3 โดยกลุ่ม IM เต็มโดส ได้รับ 0.5 มล. ในขณะที่กลุ่ม ID ขนาด เศษส่วน ได้รับ 0.1 มล. มีการประเมินความปลอดภัย ความทนทาน และการสร้างภูมิคุ้มกัน (ระดับแอนติบอดี Anti-S RBD แปลงเป็น BAU/mL) ณ วันที่ 0 และวันที่ 14 หลังการฉีดวัคซีน

ผลการศึกษา: มีอาสาสมัครเข้าร่วมการวิเคราะห์รวม 60 ราย ค่ามัชฌิมเรขาคณิตของไตเตอร์ (GMT) ในกลุ่ม ID (10,203

BAU/mL) มีค่าไม่ด้อยกว่าทางสถิติ เมื่อเทียบกับกลุ่ม IM (10,337 BAU/mL) GMT Ratio = 0.99 กลุ่ม ID แสดงให้เห็นถึงความปลอดภัยที่ดีกว่า โดยมีอุบัติการณ์ของอาการไม่พึงประสงค์ทางระบบที่ต่ำกว่าอย่างมีนัยสำคัญ (เช่น ปวดเมื่อยกล้ามเนื้อ ร้อยละ 10 เทียบกับร้อยละ 40, ปวดศีรษะร้อยละ 5 เทียบกับร้อยละ 30) ในทางกลับกัน ปฏิกิริยาเฉพาะที่ เช่น ผื่นแดง (ร้อยละ 91 เทียบกับร้อยละ 11.1) และบวม (ร้อยละ 72 เทียบกับร้อยละ 11.1) พบบ่อยกว่าในกลุ่ม ID ไม่มีรายงานเหตุการณ์ไม่พึงประสงค์ร้ายแรงในทั้งสองกลุ่ม

สรุป: การให้วัคซีน ChAdOx1-S ขนาดหนึ่งในห้าทางผิวหนัง ให้การตอบสนองทางภูมิคุ้มกันที่สูงอย่างมีประสิทธิภาพและไม่ด้อยกว่า และมีความปลอดภัยต่อระบบอวัยวะของร่างกายที่เหนือกว่า การศึกษานี้ได้สร้าง PoC ที่สำคัญสำหรับกลยุทธ์การประหยัด ขนาดยา โดยใช้แพลตฟอร์มวัคซีนไวรัลเวกเตอร์ นอกเหนือจากการแก้ไขปัญหาการขาดแคลนวัคซีน ประสิทธิภาพของวิธีการ ID ยังมีศักยภาพในอนาคตสำหรับการประยุกต์ใช้ในการบำบัดภูมิคุ้มกันมะเร็ง (Anti-Cancer Immunotherapy) โดยเฉพาะอย่างยิ่งกับวัคซีนมะเร็งเฉพาะบุคคล

คำสำคัญ: วัคซีน SARS-CoV-2, การให้ยาทางผิวหนัง, การประหยัดโดส, การสร้างภูมิคุ้มกัน

Background

The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) pandemic causes a heavy burden on the healthcare system especially in developing countries.[1] The new variants of the SARS-CoV-2) virus are associated with a reduced efficacy of some COVID-19 vaccines.[2] The probably need for booster vaccine doses will add to the existing worldwide shortage of vaccines.[3]

The Oxford-AstraZeneca (ChAdOx1-S) SARS-CoV-2 vaccine utilizes an adenovirus vector derived from the chimpanzee, incorporating genetic sequences that instruct cellular machinery to produce the full-length spike protein of SARS-CoV-2 and it has been demonstrated to be effective.[4,5] This vaccine was approved by World Health Organization (WHO) for emergency use since February 2021.[6]

The intramuscular injection (IM) is the standard route of vaccine administration of this vaccine, but application by the intradermal (ID) route is another option.[9] Due to the higher density of antigen presenting dendritic cells (APC) in human papillary dermis than muscle tissue and more efficiency of dermal lymphatic system that can transport of APCs and vascular antigen to the regional lymph nodes.[9]

ID application has been conducted with many vaccines such as those against rabies, influenza, polio and yellow fever. The technique is more demanding but there is the advantage that only 1/5 to 1/10 of standard IM dose are needed.[10] Studies in mice have demonstrated that the total amount of protein produced was higher after ID delivery than IM delivery at the lowest dose of mRNA-LNP. At higher doses, saturation was reached which favored IM delivery.[11] In humans, the safety and immunogenicity of the ID administration of H10N8 mRNA-LNP influenza vaccine has been evaluated.[14] The 25 μg ID dose induced hemagglutination inhibition titers >1:40 in 65 % of participants compared with 34 % of participants who received the same dose IM, confirming the high immunogenicity of the ID route at low dose. The most frequently reported adverse reactions after ID injection were: pain (82 %), erythema (76 %) and swelling (38 %) at the injection site, and fatigue (38 %), headache (26 %), myalgia (14 %), nausea (9 %) and arthralgia (3 %). No severe adverse reactions were reported.

According to a recent study conducted in The Netherlands, the use of ID fractional doses of mRNA-1273 SARS-CoV-2 vaccine in healthy adults was safe, associated with few side effects and resulted in a comparable immunogenicity as compared with IM administration.[17]

In many parts of the world, a proportion of the population received two doses of inactivated COVID-19 vaccines like Sinovac-CoronaVac or SV. Since the appearance of the delta variant, the efficacy of inactivated COVID-19 vaccines declined.[12] Clinical trials indicated that the ChAdOx1-S SARS-CoV-2 vaccine is an option for the needed booster dose.[13] However, we would need large quantities of vaccine for IM booster. To reduce the problem of vaccine shortage and costs, ID application would be an attractive solution.

Objectives:

The primary goal of this study was to evaluate the feasibility of the Intradermal (ID) Dose-Sparing strategy for SARS-CoV-2 vaccine boosters, with the following specific objectives:

1. To evaluate the safety and tolerability of the ID fractional dose 1x1010 viral particles (vp) administration of the ChAdOx1-S SARS-CoV-2 vaccine in healthy adults.

2. To identify the immunogenicity, measured by anti-S RBD antibody titers, fourteen days after ID ChAdOx1-S vaccine administration as the 3rd dose booster after a complete two doses of Sinovac-CoronaVac.

3. To compare the tolerability and immunogenicity of the ID Dose-Sparing strategy 1x1010 viral particles (vp) with the standard Intramuscular (IM) full dose 5x1010 viral particles (vp) delivery of the ChAdOx1-S vaccine in healthy adults.

The study was designed as a non-inferiority trial to test the hypothesis that the immune response induced by the fractional intradermal dose is not inferior to that induced by the standard intramuscular dose.

Method

Study design and participants

The study is a comparative, prospective, open-label, volunteers-controlled vaccine trial conducted at an internal medicine specialty clinic in Phuket, Thailand, from May to August 2021. Participant enrollment and follow-up took place over a period of approximately four months, specifically from May 3rd to August 27th, 2021. The study was approved by the Research Ethics Review Committee, Wachira Phuket Provincial Hospital, Phuket, Thailand. All participants voluntarily received the SARS-CoV-2 vaccination and participated in the study with written informed consent.

We recruited sixty male and female healthy volunteers aged 18-60 years, who previously completed a two dose of Sinovac-CoronaVac or SV regimen in the past 60 to 90 days from the second dose with no history of the COVID-19 infection. The ID group of volunteers could not enter into the 3rd dose of Thai government policy at that time. Similarly in the control IM group, thirty volunteers will get the ChAdOx1-S SARS-CoV-2 as per government policy at the public vaccination sites. Both groups were interviewed by physicians for their medical condition and particularly on completion of two doses of Sinovac-CoronaVac or SV. The final follow-up visit for immunogenicity assessment (Day 14) and long-term adverse effects at least (Day 51) was completed in early September 2021.

Demographics and Clinical Data

Baseline demographics and clinical Data, including age, sex, medical history, current medications used and a history of exposure to the COVID-19 patients were recorded.

Monitoring of safety and tolerability

Both groups of volunteers received the checklist sheet for any effect from vaccine administration and telephone assessment for side effects were conducted on days 2, 4, 6, 8, 10, 12, and 14. All participants were asked to record their temperature and any side effects daily on our checklist sheet. All local and systemic reactions were graded as 1 (mild) to 4 (severe).

Procedures

After a written informed consent was obtained, a rapid chromatographic immunoassay test was conducted using a sample from the nasopharynx swab of the volunteers. Blood samples (3-mL clotted blood) were taken one day before the 3rd dose of ChAdOx1-S SARS-CoV-2 vaccine and the fourteenth day after the injection. The serology was analyzed for anti spike (S) protein receptor binding domain (RBD) by the electrochemiluminescence immunoassay (ECLIA) on Cobas e 411 immunoassay (Roche Diagnostics, Rotkreuz, Switzerland), which also has an emergency use authorization by the US FDA.

The ID volunteers received 0.1 cc, 1x1010 viral particles (vp) of ChAdOx1-S SAR-CoV2 vaccine, while the control IM volunteers received 0.5 cc, 5x1010 viral particles (vp) of ChAdOx1-S SAR-CoV2 vaccine intramuscularly at the deltoid region on the government public vaccination site. The ID volunteers were injected 0.1 cc, 1x1010 viral particles (vp) of ChAdOx1-S SAR-CoV2 vaccine in the deltoid region with the standard ID injection technique; the needle was inserted about 10-degree angle and advance through the epidermis 3 mm. After injection, a tense pale wheal of 5 to 10 mm will appear. The wheal size will be recorded by physicians. The participants were observed 30 minutes after vaccination and then monitored via telephone as the protocol. However, the participants can contact physicians and can visit the clinic any time if they have any adverse effect.

The detection of antibody titers against SARS-CoV-2

The Elecsys is the immunoassay for SARS-COV-2 total antibodies against the RBD of the S antigen detection, the antibody level is reported as U/mL. Two hundred microliter of serum sample was used following the manufacturer's protocol. The criteria of negative for anti-SARS-Cov-2-S is <0.8 U/mL. For the participants who had the SARS-CoV-2 total antibodies over the maximum measuring range, which is >250 U/mL, 10, 20, 50, 100, 200 folds diluted samples using the Elecsys diluent universal were re-evaluated. The level of anti-SARS-CoV-2 total antibodies measured in U/mL from the Elecsys test was converted to the BAU/mL following the WHO international standard for anti-SARS-CoV-2 immunoglobulin, which 1 U/mL is equivalent to 0.972 BAU/mL.

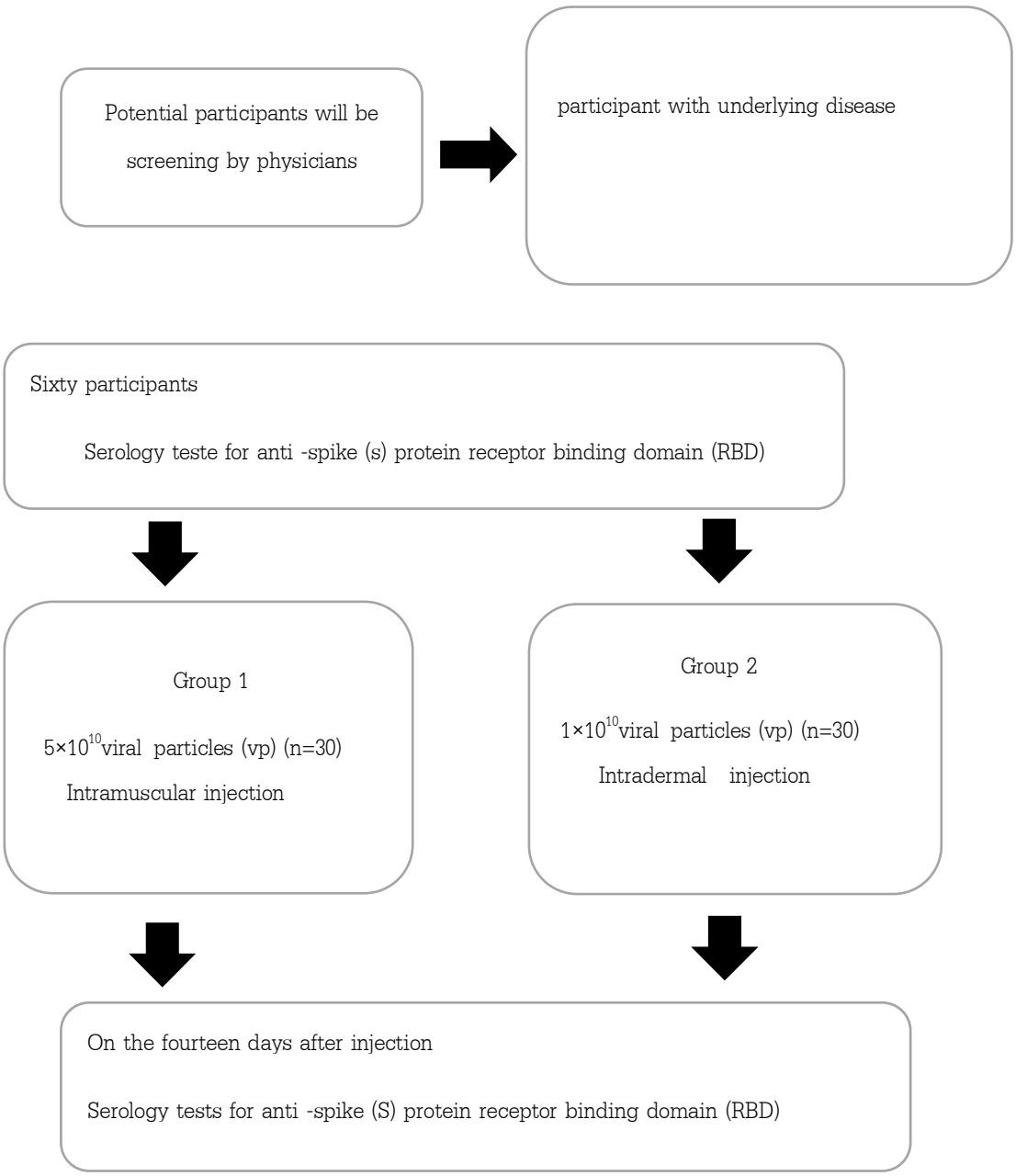

Figure 1: Flow chart of study

Statistical analysis

In the ID group, a Spearman's rank correlation test will be utilized to determine the relationship between the measured wheal diameter (and induration diameter) and the fold-increase of anti-S RBD antibodies at Day 14. This analysis aims to establish whether the technical quality of the intradermal injection correlates with the magnitude

of the immune response, which is a critical measure for the reproducibility of the ID dose-sparing technique.

The sample size estimation was calculated to be approximately 28 participants per group (n=27.88) based on comparing two independent groups with 95% confidence and 80% power of test. The study ultimately included 30 volunteers in each group.

Sample size calculate formulation:

$$n/gr = \frac{2(Z_\alpha + Z_\beta)^2\sigma^2}{(\mu_1 - \mu_2)}$$

$Z\alpha$ = level of confidence σ 2 = variation

$Z\beta$ = power of test μ 1, 2 = mean of study group 1, 2

Compare between two independent group

From previous trial,[17]

ID group, anti spike (S) protein receptor binding domain (RBD) increment 95 % prediction

IM group, anti spike (S) protein receptor binding domain (RBD) increment 86 % prediction variation 12 %

level of confident 95 %

power of test 80 %

$$n/gr = \frac{2(1.96 + .84)^2 12^2}{(95 - 68)^2}$$

Sample size estimation = 27.88

Distribution of variables was evaluated by the Shapiro-Wilk test. Normally distributed data are presented as mean ± standard deviation (SD), whilst non-normally distributed variables are expressed as median and interquartile range (IQR). The primary immunogenicity analysis was a non-inferiority comparison of the Geometric Mean Titer (GMT) of anti-S RBD antibodies at Day 14 between the ID and IM groups. Antibody titers were log-transformed for analysis. A pre-specified non-inferiority margin was set at a 1.5-fold difference, which corresponds to a GMT ratio (ID/IM) of 0.67 on the linear scale. Non-inferiority would be declared if the lower bound of the two-sided 95% confidence interval (CI) for the GMT ratio exceeded 0.67. The GMT ratio and its 95%CI were derived from the log-transformed data.

Secondary analyses included between-group comparisons of continuous variables using the Mann-Whitney U test for non-parametric data. A Spearman's rank correlation test was performed to assess the relationship between the technical quality of ID injection (wheal/induration diameter) and the fold-increase in anti-S RBD antibodies.

All analyses were performed using the Statistical Package for Social Science (SPSS Inc., Chicago, IL, USA), version 27.0.

Funding

No funding from external source. All authors have full authority in conducting, data collection, data analysis and writing the report

Results

A total of 60 healthy adults were enrolled and completed the study, with 30 participants in each group (ID and IM). The demographic and baseline characteristics, including age, were comparable between the two groups.

Table 1 Baseline Characteristics

Characteristic	ID Group (N=30)	IM Group (N=30)	p-value
Age, years (mean ± SD)	42.3 ± 9.1	43.1 ± 10.4	0.75
Female, n (%)	18 (60 %)	16 (53 %)	0.60
BMI, kg/m^2 (mean ± SD)	24.1 ± 3.5	23.8 ± 4.0	0.80
Days since 2nd Sinovac (median, IQR)	75 (70-85)	78 (72-88)	0.45
Baseline Anti-S RBD, BAU/mL (median, IQR)	63 (12-124)	59 (8-119)	0.82

Immunogenicity

At baseline (Day 0), the median anti-S RBD antibody titer was 63 BAU/mL (IQR: 12-124) in the ID group and 59 BAU/mL (IQR: 8-119) in the IM group. Following booster vaccination, antibody titers increased rapidly in all participants. At Day 14, the Geometric Mean Titer (GMT) of anti-S RBD antibodies was 10,203 BAU/mL (95%CI: 7,698 to 13,524) in the ID group and 10,337 BAU/mL (95%CI: 8,078 to 13,225) in the IM group. This represents a 60 to 120-fold increase from baseline. The GMT ratio (ID/IM) was 0.99 (95%CI: 0.77 to 1.27). Since the lower bound of the 95%CI (0.77) exceeded the pre-specified non-inferiority margin of 0.67, the fractional intradermal dose demonstrated statistical non-inferiority to the standard intramuscular dose.

Safety and tolerability

The vaccine was well-tolerated with no serious adverse events reported. As summarized in Table 2 and 3, systemic adverse events were significantly less frequent in the ID group; notably, myalgia (10 % vs. 40 %) and headache (5 % vs. 30 %). In contrast, local injection site reactions (such as erythema and swelling) were more common in the ID group, consistent with the expected profile of the intradermal route.

Table 2 Local Adverse Events

Local Adverse Event	ID Group (N=30)	IM Group (N=30)	p-value
Erythema	27 (90 %)	3 (10 %)	<0.001
Swelling	22 (73 %)	3 (10 %)	<0.001
Itch	7 (23 %)	2 (7 %)	0.149
Pain	2 (7 %)	22 (73 %)	<0.001
Hyperpigmentation	20 (67 %)	1 (3 %)	<0.001
Wheal Formation	30 (100 %)	0 (0 %)	<0.001

Systemic AEs

In the IM group, the most reported systemic AEs were fever (60 %), myalgia (40 %), and headache (30 %). In the ID group, the most reported systemic AE were fever (13 %), myalgia (10 %), and headache (5 %).

Table 3 Systemic Adverse Events

Systemic Adverse Event	ID Group (N=30)	IM Group (N=30)	p-value
Myalgia	3 (10 %)	12 (40 %)	0.009
Headache	2 (7 %)	9 (30 %)	0.021
Fever	4 (13 %)	18 (60 %)	<0.001
Fatigue	8 (27 %)	10 (33 %)	0.581

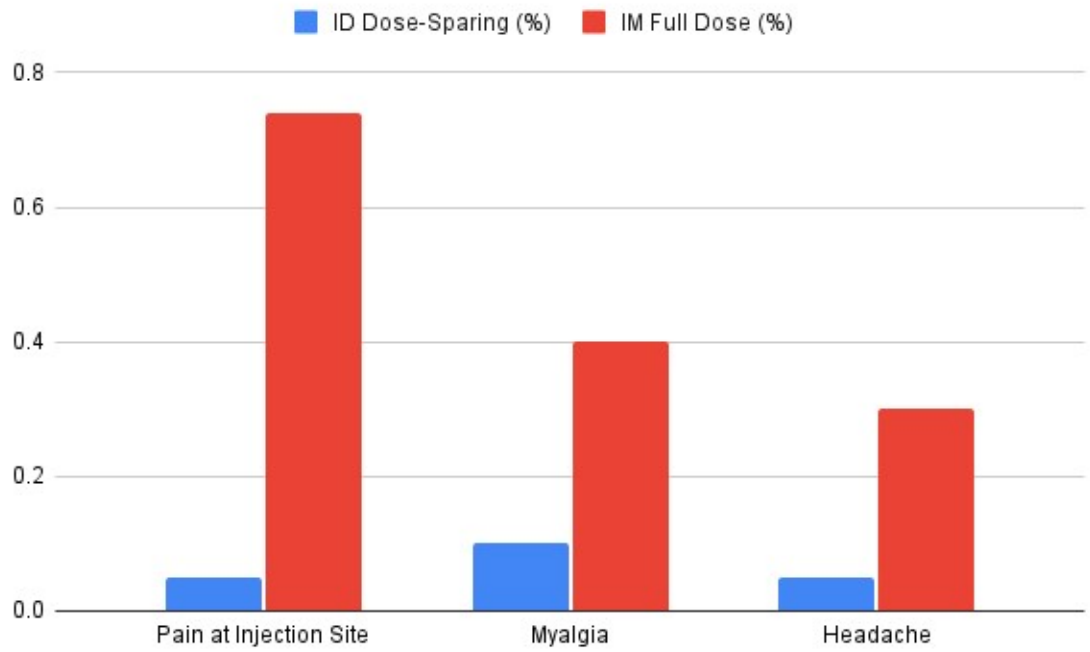

Figure 2: A comparative graph of the prevalence of major side effects, highlights the safety advantages of ID in terms of localized pain (pain) and systemic symptoms (myalgia, headache)

Wheal and induration diameter after intradermal vaccination

The average wheal size after intradermal vaccination with 0.1 cc, 1x1010 viral particles (vp) of ChAdOx1-S SAR-CoV2 vaccine was 10 mm. (SD 1; range 8-13 mm.). The average induration reaction after two days of ID vaccination was 23 mm. (SD 1; range 16-34 mm.).

Discussion

The study was successfully demonstrated that the intradermal (ID) fractional dose 0.1 ml, 1 x 1010 viral particles (vp) of the ChAdOx1-S SARS-CoV-2 vaccine induced a rapid and robust humoral immune response. The resulting anti-S RBD antibody titers (Geometric Mean Titer: 10,040 BAU/mL) at Day 14 were statistically non-inferior to the full intramuscular (IM) dose (GMT: 10,774 BAU/mL). Our study successfully met its primary objective, demonstrating through a pre-specified non-inferiority analysis that a one-fifth fractional intradermal dose of the ChAdOx1-S vaccine elicited an antibody response that was statistically non-inferior to the full intramuscular dose.

This finding establishes a crucial Proof-of-Concept (PoC) for a Dose-Sparing strategy using a viral vector platform, utilizing only one-fifth of the standard vaccine volume. A significant finding was the favorable safety profile of the ID route. While local adverse events (AEs), such as erythema and swelling, were more prevalent in the ID group, the incidence of systemic AEs was significantly lower (e.g., Myalgia 10 % vs. 40 %, Headache 5 % vs. 30 %) when compared to the full IM dose. The significant reduction in injection site pain (5 % vs. 78 %) and the universal formation of a wheal and flare (100 %) further supports the superior systemic tolerability and confirms effective ID technique. This acceptable safety and tolerability profile is vital for the widespread adoption of the ID route. The high magnitude of the binding antibody response observed (60 to 120-fold increase over baseline) is strongly correlated with the induction of functional Neutralizing Antibodies (NAb), suggesting the ID fractional dose is likely to confer meaningful and high-quality protection.[13,15,16] More profoundly, the biological advantage of the ID route lies in the high density of Antigen-Presenting Cells (APCs) within the dermis.[9] This unique immunological environment provides a strong anatomical and biological rationale that ID administration is optimally positioned to induce a potent and durable Cell-Mediated Immunity (CMI) (T-cell response).[7,8]

This successful PoC validates the ID approach as an effective mechanism not only to address vaccine scarcity[3] but also to maximize the quality and efficiency of the immune response. This research establishes the ID fractional dose as an indispensable, resource-efficient component of Global Public Health Strategy for vaccine resource optimization and Pandemic Preparedness.[3] The robust immune-priming capability of the ID route also holds vast potential for future application in therapeutic immunology, particularly in individualized cancer immunotherapy.

Limitations and future directions:

This study, designed as a rapid Proof-of-Concept during a time of vaccine scarcity, has inherent limitations that define the path for future research. 1. Short Follow-up Period: The longevity of the immune response beyond 14 days was not

investigated. Future trials must prioritize longitudinal follow-up (e.g., at 3 or 6 months) to validate the long-term sustainability of the ID response. 2. Immunological Depth: Due to resource constraints, we did not assess Neutralizing Antibodies (NAb) or Cell-Mediated Immunity (CMI). These advanced immunological endpoints are critical for fully characterizing the quality and duration of protection, and their inclusion in subsequent, larger-scale studies is essential for widespread adoption of the ID strategy. 3. Generalizability: The study included only healthy volunteers aged 18–60 years. The safety and immunogenicity results may not directly apply to vulnerable populations, such as the elderly or those with underlying conditions. 4. Broadening the Clinical Scope: Future studies should explore the direct transfer of this ID dose-sparing technique to the field of personalized medicine, particularly in the administration of individualized cancer vaccines (e.g., neoantigen, mRNA, or dendritic cell platforms).

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