The sharpest tool in the shed: question-based clinical development of vaccines to address global health priorities

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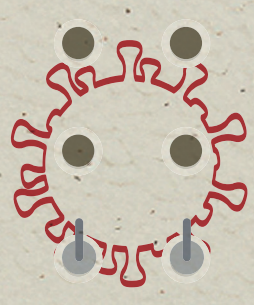
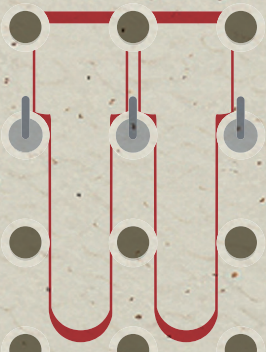
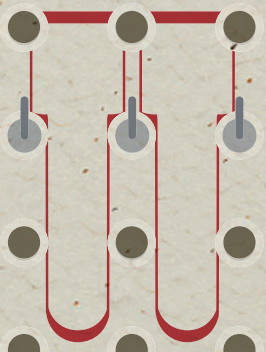

Geert Roozen

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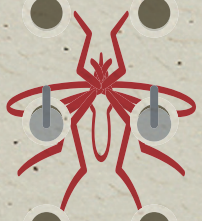
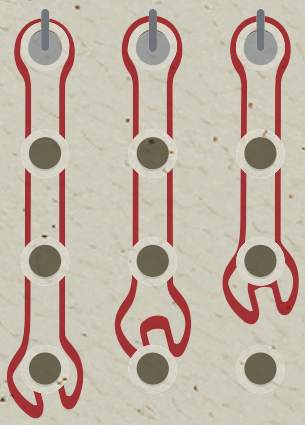
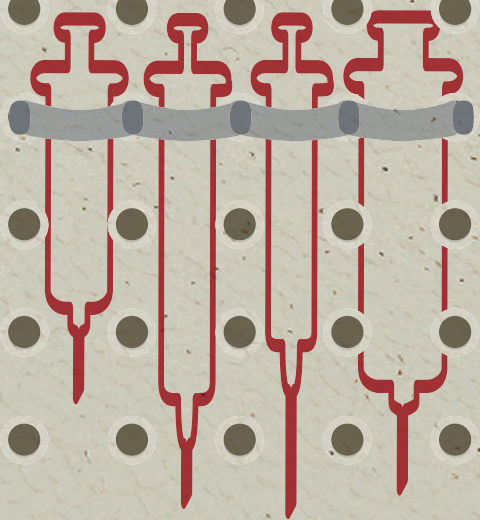


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Geert Roozen

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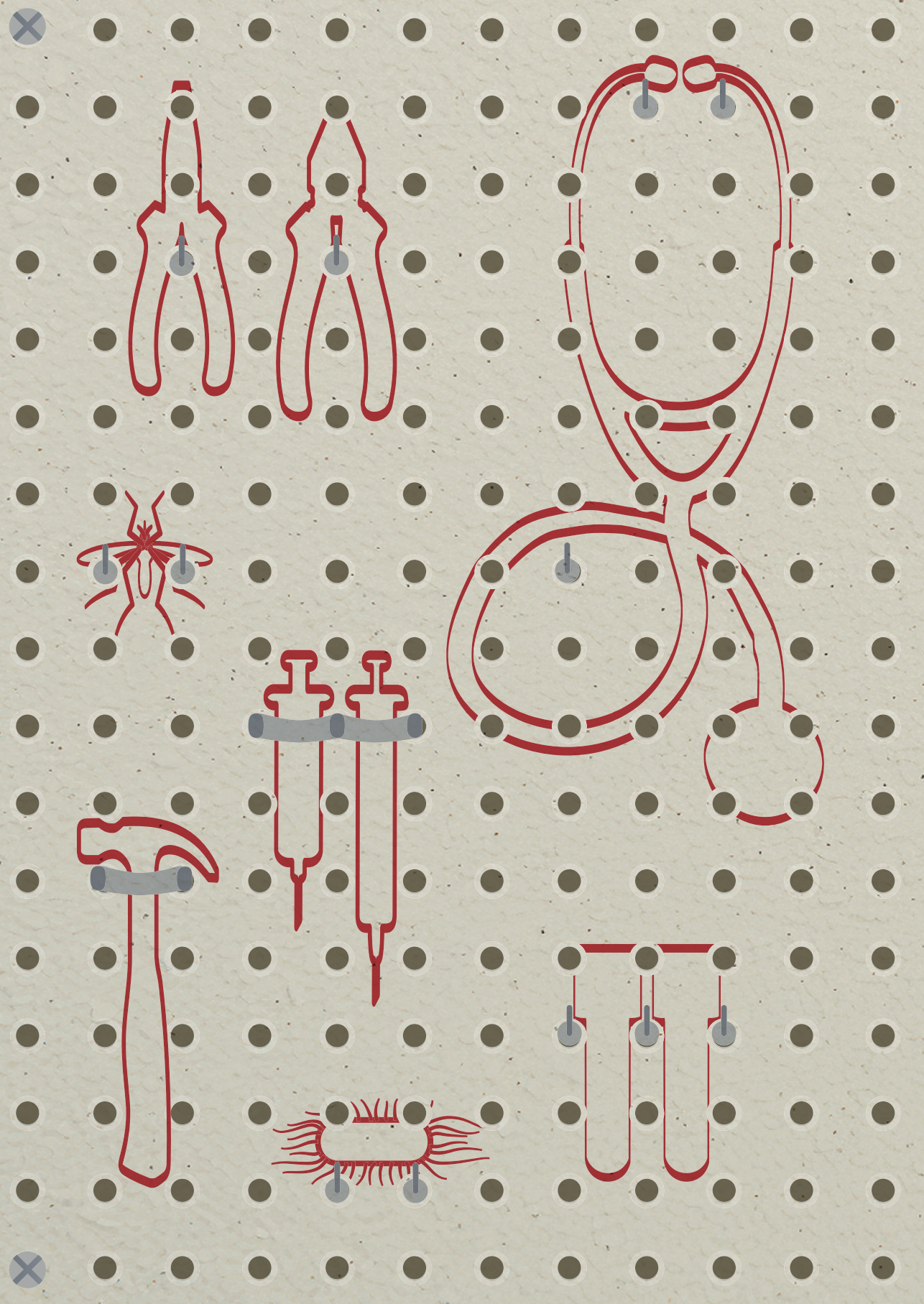
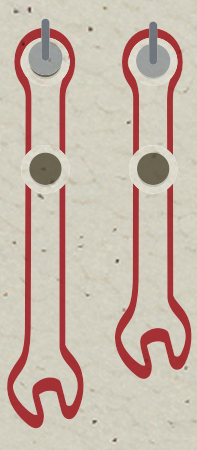
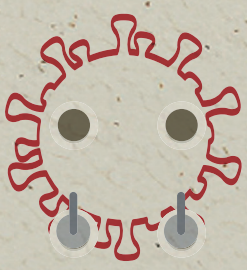




1

General Introduction



Infectious diseases have been the predominant cause of death throughout human history.[1] Epidemics have decimated ancient civilizations[2], and in many wars, more people died of infections than by bullets or bombs.[3] In 2021, more than twice as many people died from an infectious disease like malaria (1.1% of the global population) as all deaths from conflicts (0.14%), natural disasters (0.0014%), and malnutrition (0.32%) combined.[4]

Vaccines have played a significant role in the global fight against infectious diseases in recent decades. In 1974, the World Health Organization (WHO) introduced the Expanded Program on Immunization (EPI) to make life-saving vaccines available to the entire world population. Since the introduction of EPI, vaccination has averted an estimated 154 million deaths, including 146 million among children below the age of five.[5] Since vaccines have an immense potential to positively impact global health, developing new vaccines is a top scientific priority.

The most critical vaccines to develop or improve are those with the greatest potential to prevent mortality and safe life years on a global scale, as they offer the highest impact on global health (Fig. 1).[6] Two important scenarios where high-impact vaccine are especially needed include:

1. A poorly contained infectious disease with high mortality in a low-resource setting: developing a vaccine for a disease that currently lacks preventive measures or effective containment, can have a substantial positive impact. This scenario is more likely to occur in low- and middle-income countries (LMICs) where healthcare systems may struggle to provide adequate treatment, elevating mortality rates of a disease which increases the importance of preventive vaccines;
2. An outbreak of a novel pathogen: in case of an epi- or pandemic in a population with limited or no pre-existing immunity, introducing a new and effective vaccine can dramatically reduce the disease's impact.

Both scenarios present distinct challenges regarding vaccine development and implementation. Commercial vaccine developers typically allocate more resources to developing vaccines with higher revenue potential, resulting in limited funding for vaccines targeting diseases that predominantly burden low-resource settings. Additionally, in the case of a pandemic vaccine, there will always be a scarcity of vaccine doses at its moment of introduction, complicating the availability and distribution of the novel vaccine.

Challenges for the development and implementation of new high-impact vaccines are multifaceted and complex, encompassing not only scientific but also societal, economic, and logistical dimensions. Addressing them requires collaboration and insights from policymakers and industry stakeholders. Nonetheless, scientists in non-commercial institutions can also play an import role in developing these new vaccines. Certain biomedical and scientific aspects can be addressed by designing and conducting optimized clinical trials initiated by academia or other not-for-profit research organizations. Such trials can focus on facilitating the

advancement of vaccine development and implementation, even when resources are limited. In this thesis, some of the most pressing challenges associated with vaccine development will be addressed and an evaluation will be made on how publicly initiated clinical trials can contribute to overcoming them.

The next section will begin with a brief description on how pharmaceutical companies generally manage clinical vaccine research, followed by an exploration of a new approach to clinical development. This approach will then be assessed for its potential to support publicly funded researchers in advancing vaccine development that holds significant global health relevance. In subsequent sections, reasons for the typical underfunding of this research by commercial vaccine producers will be discussed, along with ways in which academia and other non-commercial organizations can help to fill this gap.

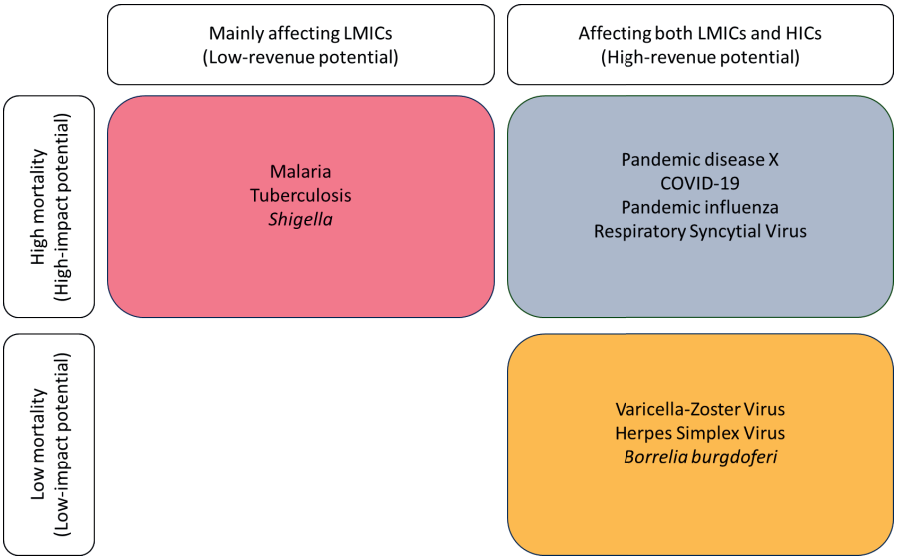


Figure 1. High-impact potential versus high-revenue potential

Vaccines targeting diseases with a high disease burden, do not necessarily have a high-revenue potential. Non-commercial organizations should strive to address vaccine development for these diseases, as they may be of less interest to the pharmaceutical industry.

Clinical vaccine development

Conventionally, new potential drugs, including vaccines, identified during preclinical development undergo three phases of clinical trials. In Phase I, approximately 10 to 100 participants receive the new vaccine, with several doses tested based on preclinical animal studies. The primary objective of Phase I is to assess safety and dosage. In Phase II, about 50 to 500 individuals are vaccinated to evaluate immunogenicity and tolerability. This phase may involve testing two or three dosing regimens and may include specific target groups, such as

older adults or minors. Phase III trials aim to gather the majority of safety and efficacy data, typically involving 1000 to 10 000 participants, depending on the disease's incidence and transmission rates. Generally, only one dose, dosing regimen, or route of administration is assessed in Phase III.

Clinical trials are expensive, particularly Phase III trials. As a candidate vaccine progresses through clinical development, the number of participants increases, leading to longer processing times and requiring a larger staff to conduct the trials, which further escalates costs. Although trial expenses can vary substantially depending on the setting, the disease and the product being tested, Phase I trials cost around $3 million, while Phase III trials can reach $20 to $75 million.[7] Consequently, only major pharmaceutical companies generally have the resources to conduct these large clinical trials necessary for market approval of a candidate vaccines.

A major downside of the three-phases paradigm is that that it does not take the specific properties of a candidate drug and the target disease into account. This increases the risk of advancing candidate drugs with low potential into late-phase development. As an alternative, the Question-Based Clinical Development (QBCD) method has been proposed.[8] Rather than rigidly adhering to the three standard phases of clinical development, QBCD proposes the identification of so-called key questions that are essential for the specific drug being developed, drawing from, but not limited to, the five general QBCD questions:

1. Does the biologically active compound reach the site of action?
2. Does the compound produce its intended pharmacological effect?
3. Does the compound have beneficial effects on the disease or its pathophysiology?
4. What is the therapeutic window of the new drug?
5. How do the sources of variability in drug response within the target population affect product development?

Taking these scientific key questions into account, the QBCD method evaluates them together with development risks and financial considerations to determine the optimal development path for the new drug.[8] In QBCD, clinical trials are not designed to follow the three phases, but to answer key questions in the most effective order.

QBCD does not bypass the need for a clinical trial evaluating efficacy and safety in a large population. However, it does help answer the questions resulting from the specific biomedical properties of the vaccine, the target disease, and the target population. Having these insights early in the research process, accelerates vaccine development and reduces vaccine development costs. When a well-designed clinical trial already reveals significant flaws in a candidate vaccine early in the development process, costly and unsuccessful trials later on can be prevented. This so-called fail-fast principle ensures efficient funding allocation to candidate vaccines with highest potential for success.

While the three-phase paradigm primarily focuses on advancing a candidate vaccine from early-phase clinical trials to market approval, QBCD better allows for the identification of key questions that are relevant for global health and equitable vaccine access. Therefore, QBCD is particularly well-suited for academia or other non-commercial organizations aiming to contribute to the development or improvement of high-impact vaccines. Even with limited funding, these institutions can use QBCD to initiate non-commercial research that expedites the development of high-impact vaccines and enhances vaccine equity and accessibility by identifying and answering key questions.

The next two sections aim to identify relevant key questions for publicly initiated development of high-impact vaccines. First, challenges for vaccine development for diseases predominantly prevalent in LMICs will be evaluated. This includes outlining financial and immunological challenges and pinpointing knowledge gaps that can be addressed by non-commercial research. Second, challenges for vaccine dose-finding and optimization during a pandemic will be discussed. This section will explain how publicly initiated research can address these key questions, especially those that are not addressed by vaccine produces, even after a vaccine has received market approval.

Vaccine development for low- and middle-income countries

Limited vaccine research is conducted in LMICs, and much of it is led by external stakeholders. In 1990 the term "90/10 gap" was introduced to highlight that less than 10% of global research funding was allocated to health issues prevalent in LMICs, even though these countries bear over 90% of the global burden of preventable mortality.[9] Although the nature of the 90/10 gap has changed, a large inequality in research funding persists to this day (Fig. 2). Diseases with low morbidity but a high revenue potential in high-income countries (HICs) receive disproportionately more funding, while research into diseases that impose a high global burden remains severely underfunded.[9]

Historically, most vaccines were developed for a dual market, where profits would be maximized by selling at high prices in HICs, enabling pharmaceutical companies to offer the same vaccines for a lower price in LMICs.[10] However, this model fails for vaccines targeting diseases predominantly affecting LMICs. Pharmaceutical companies in HICs are often reluctant to invest in these vaccines due to their limited revenue potential. As a result, manufacturers from LMICs started to enter the market in the 1980s.[10] These manufacturers, united in the Developing Countries Vaccine Manufacturers Network (DCVMN), generate their income through the high-volume sale of low-cost vaccines in LMICs, rather than through high-margin sales in HICs.[11] Although DCVMN members now represent 18% of the vaccine market by volume, their share in revenue remains only 5% (and even 49% and 6%, respectively, when excluding COVID-19 vaccines).[12] This high-volume, low-revenue business model substantially limits DCMVN members to invest in product innovation, resulting in less vaccine research aimed at LMIC populations. Of the 94 vaccines and biologicals that got market approval between 2000 and 2011, only eight were directed at diseases affecting populations in low-income countries, and

only 1% of the clinical trials in 2011 were researching these diseases.[13] In the entire period from 2012 to 2018, only one vaccine for a disease mainly affecting low-resource settings got market approval.[14]

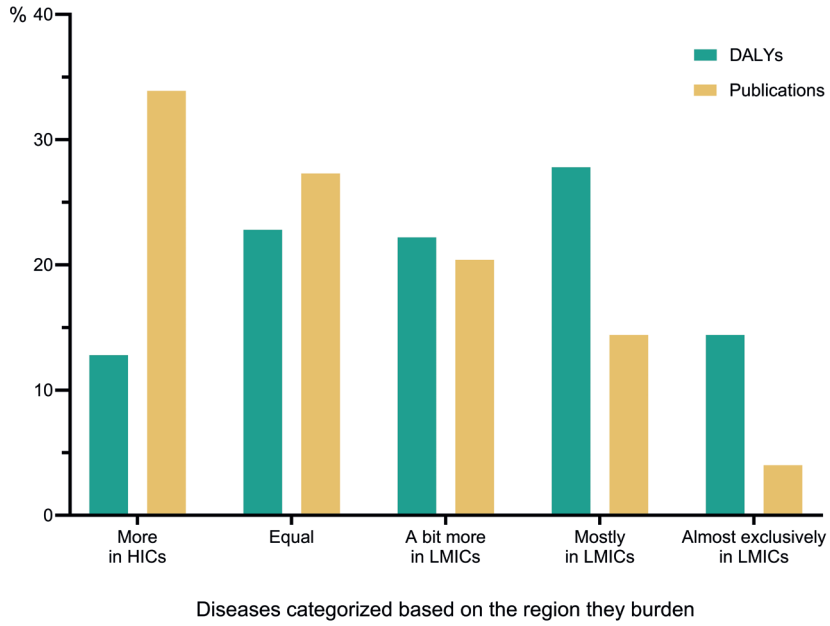


Figure 2. Global disease burden (DALYs) in relation to global research output (scientific publications)

The nature of the 90/10 gap has changed but a large inequality still exists. Globally, 34% of research addresses diseases that cause more burden in HICs, while causing only 13% of the global burden. Vice versa, only 4% of research focusses on diseases that exclusively burden LMICs, but attribute to 14% of the global burden.

DALY = disability-adjusted life year, HIC = high-income country, LMICs = low-and-middle-income countries.

Figure based on data from: Yegros-Yegros A, van de Klippe W, Abad-Garcia MF, et al. Exploring why global health needs are unmet by research efforts: the potential influences of geography, industry and publication incentives. Health Research Policy and Systems. 2020;18(1):47.

Given that the vaccine market incentivizes the development for HICs, there exists a gap in vaccine development for low-resource settings. Until LMICs establish the capacity to develop their own vaccines, (semi-)public institutions funded by HICs (and upper-middle income countries) should strive to compensate this unbalance. To use their funding optimally, these institutions must focus on identifying the right key questions that will help design trials that enable fast failure of vaccine candidates with low potential and accelerate the development of those with high potential.

An additional difficulty resulting from the fact that most vaccines are developed in and for HICs is vaccine hyporesponsiveness: lower performance of vaccines in specific populations. For

example, the BCG vaccine and vaccines targeting yellow fever virus, Ebola virus, and rotavirus are known to induce lesser immune responses in populations from LMICs than in populations in HICs.[15] Although genetic differences play a role here, about 70% of the hyporesponsiveness is thought to be caused by environmental factors such as differences in food intake, microbiome and exposure to different micro-organisms and parasites.[15]

Key question 5 of QBCD ("How do the sources of variability in drug response within the target population affect product development?") encourages vaccine developers to address the issue of hyporesponsiveness. Evaluating varying doses of different antigen and adjuvant combinations to determine the most immunogenic options is typically conducted early in clinical development. If a vaccine targets a disease in LMICs, but these initial developmental decisions are solely based on trials in HIC populations, it risks suboptimal performance in its intended population. For this reason, tailoring vaccines to LMIC populations early on is crucial. While regulatory or risk considerations may sometimes necessitate initial trials in HICs (especially if the vaccine has been developed there), moving vaccine development to LMICs as early as possible will lead to better vaccine development.

Beyond addressing hyporesponsiveness, numerous other key questions can be formulated regarding vaccine properties specifically relevant for LMICs. Examples are improved storage stability, and reduced production costs, and easier administration methods. To further tailor new vaccines to LMICs needs, expanding local scientific capacity and strengthened local regulatory and ethical oversight in LMICs are essential. This would support the conduct of high-quality clinical trials and allow for context-specific research by local scientists. Despite considerable progress, continued support from international academic and public initiatives, aided by global health organizations, can further advance infrastructure and training for local scientists and regulators.[16] Increased scientific capacity would empower local researchers to initiate non-commercial research that aligns vaccine development with local needs.

Part I of this thesis will discuss two trials that aim to contribute to addressing key questions on vaccine development for LMICs.

Dose optimization during a pandemic

With the majority of the world population living in cities, the ever-increasing number of international flights, and the proximity in which high numbers of people and livestock live together, it is inevitable that new epidemics, and potentially pandemics, will arise, albeit hard to predict when and where they will strike. The COVID-19 pandemic has shown that we now have more tools than ever to respond to emerging infectious diseases quickly: the new mRNA and viral vector vaccines (together with established technologies like protein vaccines) have proven very effective for the rapid development of vaccines that have saved an estimated 14 million lives in the first year after their introduction alone.[17] The pandemic showed that when Phase I, II, and III studies run overlapping, regulatory authorities conduct their reviews as soon as new evidence comes in ("rolling review"), and large-scale production of new vaccines starts

before approval (with the financial risks covered by governments), pharmaceutical companies can develop, market and mass produce new vaccines in about two years, instead of the usual 10-plus year process.[18-21]

When developing a vaccine, it is challenging to determine the most fit dose. Generally, developers have information on toxicity and immunogenicity in animal models and extrapolate this, with a safety margin, to a first-in-human dose for the Phase I trial. Then, the dose is escalated until the maximum tolerated dose has been identified. Based on these results, two or three candidate doses are selected for Phase II. Based on Phase II results, the most immunogenic dose that is still tolerable is chosen for Phase III.

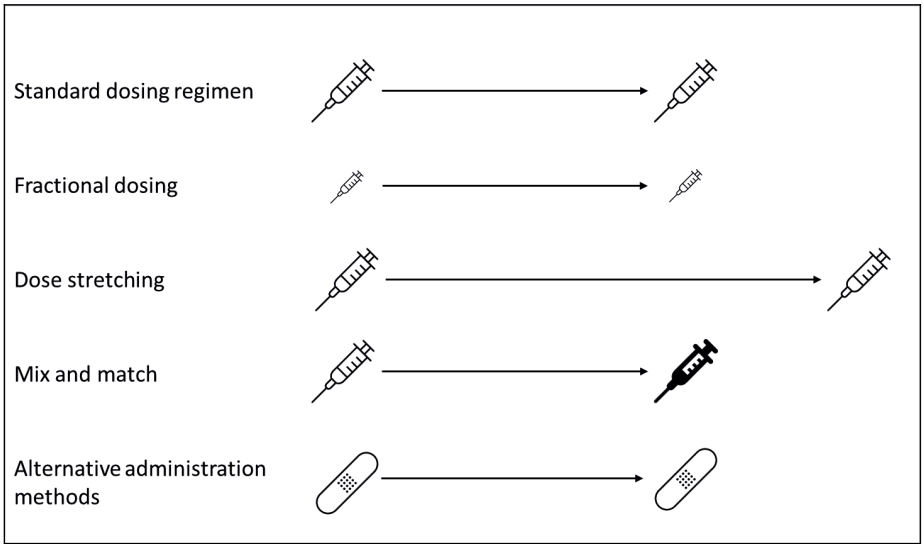


Figure 3. Different strategies for dose optimization

Although this is the fastest method to identify a tolerable and effective dose, it often results in a dose that exceeds the minimally required amount to elicit sufficient immune levels to prevent most morbidity and mortality on a population level. For optimal future vaccine implementation, it is essential to gather additional information on strategies that enhance vaccine availability ("dose sparing"), such as the reducing vaccine doses ("fractional dosing"), extending intervals between doses ("dose stretching"), employing alternative administration methods (e.g. intradermal delivery), and adopting immunization regimens that combine different types of vaccines ("mix and match") (Fig. 3). Addressing these additional key questions on dose optimization is usually not a priority for vaccine manufacturers, as it requires additional trials that are not strictly necessary for market approval. Since clinical development costs typically contribute more to vaccine production expenses than the costs of raw materials[22], there is often little commercial incentive to incorporate elaborate dose optimization in the standard development process. However, such efforts can be very valuable

for society as a whole. Determining the minimal dose required to provide sufficient protection against a novel pathogen is particularly relevant in the context of a pandemic, when vaccine shortages will inevitably occur. From a global health perspective, rapidly increasing herd immunity with lower doses in a larger population is preferable to inducing higher immunity in a smaller group of people.

Part II of this thesis will discuss publicly initiated clinical trials that addressed these issues on vaccine dose-optimization during the COVID-19 pandemic. Other examples of key questions regarding pandemic vaccines that are important for society as a whole, but are not profitable and therefore not addressed by industry, will also be discussed.

Outline

Part I (**Chapter 2** and **Chapter 3**) will discuss trials for developing vaccines for diseases with a (primary) target population of children in LMICs, particularly malaria and shigellosis. **Chapter 2** reports on a controlled human malaria study conducted at LUMC that assessed the protective efficacy of a single immunization with a genetically attenuated malaria parasite. **Chapter 3** describes a study protocol for a trial to evaluate the safety, tolerability, and immunogenicity of a novel *Shigella* vaccine and adjuvant combination in Dutch and Zambian adults.

Part II (**Chapters 4-8**) will focus on how publicly funded clinical trials can contribute to research into dose-sparing strategies and innovation, particularly for COVID-19 mRNA vaccines. This part will discuss a Viewpoint article and four post-licensure studies into dose optimization conducted during the pandemic. In the Viewpoint article in **Chapter 4**, we describe which locally initiated publicly funded initiatives were researching COVID-19 vaccine dose-optimization. We propose more centralized coordination and stimulation of this research to fully harness its potential. **Chapter 5** describes a proof-of-concept trial for fractional intradermal administration of the mRNA-1273 COVID-19 vaccine (Moderna Spikevax®). In **Chapter 6,** this concept is tested in a larger non-inferiority study design, and the trial reported in **Chapter 7** evaluates its potential as a booster dose. In **Chapter 8**, fractional intradermal dosing of mRNA-1273 is assessed as a pragmatic approach to vaccinating patients with a suspected allergic reaction to their first mRNA COVID-19 vaccine.

Chapter 9 summarizes the findings and aims to situate them within a broader context. This chapter assesses whether and how the studies presented in **Part I** and **Part II** contributed to the formulation and answering of key questions that were unlikely to have been addressed by commercial developers.

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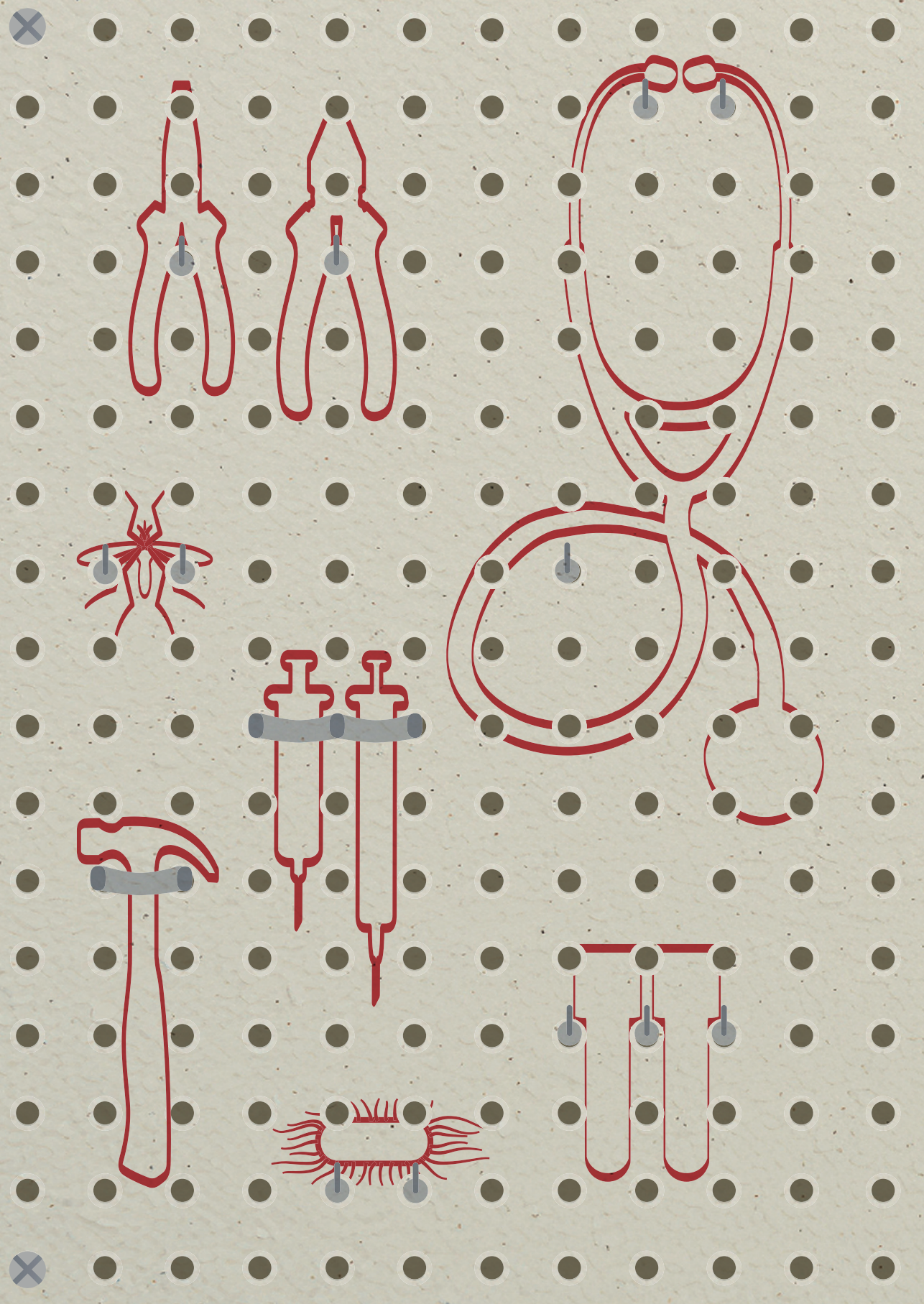
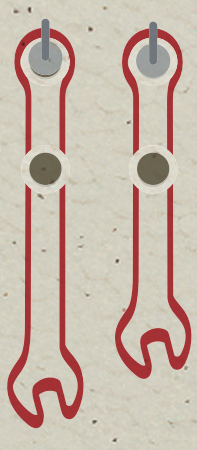
Part I

Vaccine Development for Low- and Middle-Income Countries





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Single Immunization with Genetically Attenuated *PfΔmei2* (GA2) Parasites by Mosquito Bite in Controlled Human Malaria Infection: a Placebo-Controlled Randomized Trial

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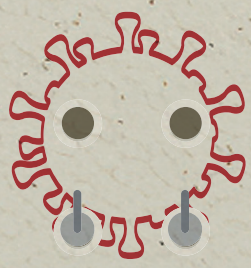

Abstract

Malaria vaccines consisting of metabolically active *Plasmodium falciparum* (*Pf*) sporozoites can offer improved protection compared to currently deployed subunit vaccines. In a previous study, we demonstrated the superior protective efficacy of a three-dose regimen of late-arresting genetically attenuated parasites administered by mosquito bite (GA2-MB) as compared to early-arresting counterparts (GA1-MB) against a homologous controlled human malaria infection (CHMI). Encouraged by these results, we explored the potency of a single GA2-MB immunization in a placebo-controlled randomized trial. Primary outcomes were safety and tolerability, time-to-parasitemia and protective efficacy. Humoral and cellular immunological results were considered secondary outcomes. Here, we report safe administration of GA2-MB with no breakthrough malaria and sterile protection in 9/10 participants at 6 weeks after a single immunization with 50 GA2-infected mosquitoes, compared with 0/5 mock-immunized participants, against a homologous CHMI. Immunization increased circulating *Pf*-specific polyfunctional effector memory $CD4^+$ T-cells co-expressing tumor necrosis factor-α and interleukin-2. This unprecedented 90% protective efficacy after a single low dose immunization holds great promise for the potency of GA2 immunization. Future studies should demonstrate if GA2 is similarly efficacious in pre-exposed populations and if the favorable safety profile reported here holds up in larger groups. ClinicalTrials.gov identifier: NCT05468606.

Main

Each year more than 600 000 people die from malaria, mainly children under the age of five[1], making it the fifth leading cause of child mortality worldwide.[2] Although the widespread deployment of both RTS,S and R21 subunit vaccines in regions with moderate-to-high transmission marks tremendous progress in reducing malaria-related morbidity and mortality[3,4], their limited efficacy and need for boosters to sustain protection call for ongoing effort into development of improved vaccines with increased potency to achieve high level durable protection that can ultimately break transmission.[5] Immunization strategies based on the use of whole *Plasmodium falciparum* (*Pf*) sporozoites have the potential to provide this much sought after high level protection, particularly late liver stage arresting parasites that are attenuated genetically through the knock-out of genes that are crucial for the development of blood-stage disease.[6-9]

Previously, we demonstrated that genetically attenuated *Pf* sporozoites can be safely administered to humans by injection and mosquito bite.[10,11] Through targeted gene deletion, we created two different parasite lines based on the *Pf*NF54 strain: early-arresting *PfΔb9/Δslarp* (GA1)[12] and late-arresting *PfΔmei2* (GA2).[8] We demonstrated with three immunizations through mosquito bites that parasites arresting development late in the liver at day 6 post-infection are much more potent in inducing protection as compared to early-arresting counterparts (89% vs. 13% protection in a controlled human malaria infection [CHMI]).[11] The high level protection was accompanied by potent circulating cellular memory responses, potentially against late-liver stage antigens.[11]

In previous studies involving immunization with sporozoites under chemoprophylaxis, detailed parasite detection by *Pf*qPCR during immunization regimens indicated that 10-90% of previously malaria-naïve participants become parasitemic after the second immunization [13-18], suggesting that single immunization with parasites that reach the late liver stage can provide varying levels of immunity. We hypothesized that GA2 might also have that potential and we decided to assess the efficacy of a single GA2-immunisation regimen against a homologous CHMI.

Results

We enrolled 15 participants in a randomized double-blind placebo-controlled trial. Details on recruitment and participant characteristics can be found in Fig. 1 and Table 1. Participants were exposed to the bites of 50 (±5) GA2-infected or uninfected *Anopheles stephensi* mosquitoes (GA2-MB or placebo, respectively) on April 12th 2023 (Fig. 2A). For one participant in the placebo group, the target dose of 45-55 blood fed mosquitoes was not reached at immunization (Fig. 2B). Six weeks later, all participants underwent CHMI through the bites of five mosquitoes infected with unattenuated homologous wild-type *Pf* parasites. After CHMI, blood feedings

were confirmed in either five infected mosquitoes (6/10 GA2-MB participants and 4/5 in the placebo group) or four infected mosquitoes (4/10 GA2-MB participants and 1/5 in the placebo group) (Extended Data Fig. 1A).

Study visits were held on the day before immunization, on day 6, 9 and 14 post-immunization, on the day before CHMI, daily from day 6 to 21 post-CHMI, and on day 28, 31 and 35 post-CHMI. During these visits, adverse events were collected, safety was assessed and a highly sensitive quantitative polymerase chain reaction (qPCR) analysis for *Pf* in whole blood was performed. The lowest limit of detection for the *Pf*qPCR was 50 parasites/mL blood. Escape treatment (three-day regimen of atovaquone/proguanil) was provided at a concentration of >100 parasites/mL or at day 28 after CHMI.

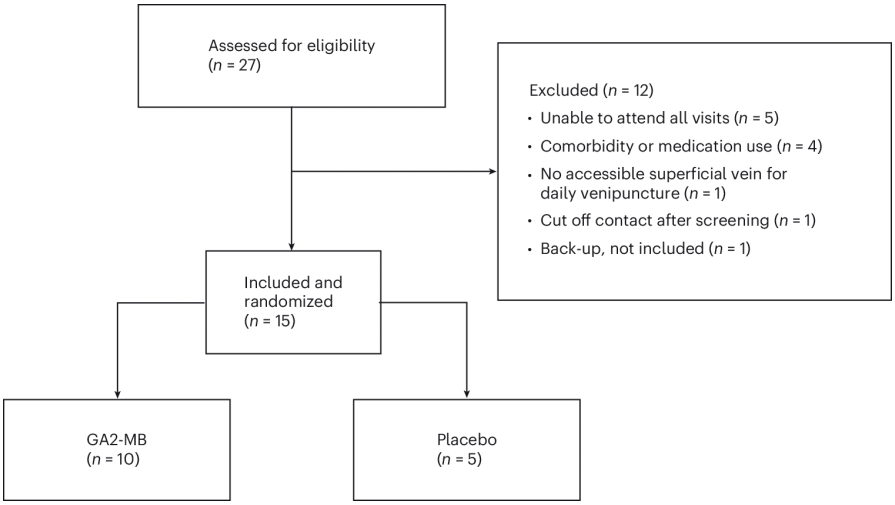

Figure 1. CONSORT diagram on recruitment and inclusions

Between 13 February 2023 and 17 March 2023, 27 persons were screened of whom 15 were included, randomized, (mock-)immunized and infected with wild-type malaria parasites in a controlled human malaria infection.

Study visits were held on the day before immunization, on day 6, 9 and 14 post-immunization, on the day before CHMI, daily from day 6 to 21 post-CHMI, and on day 28, 31 and 35 post-CHMI. During these visits, adverse events were collected, safety was assessed and a highly sensitive quantitative polymerase chain reaction (qPCR) analysis for *Pf* in whole blood was performed. The lowest limit of detection for the *Pf*qPCR was 50 parasites/mL blood. Escape treatment (three-day regimen of atovaquone/proguanil) was provided at a concentration of >100 parasites/mL or at day 28 after CHMI.

Table 1. Baseline characteristics of participants at screening

	GA2-MB (n=10)	Placebo (n=5)	Total (n=15)
Age			
Mean (SD)	24 (3)	24 (5)	24 (4)
Median (range)	23 (21-30)	25 (19-31)	23 (19-31)
Sex			
Male (%)	4 (40.0)	3 (60.0)	7 (46.7)
Female (%)	6 (60.0)	2 (40.0)	8 (53.3)
BMI			
Mean (SD)	23.9 (3.3)	22.7 (2.1)	23.5 (2.9)
Median (range)	23.8 (19.4-28.6)	23.7 (20.3-25.0)	23.7 (19.4-28.6)

BMI = body mass index; SD = standard deviation.

Primary outcomes

The single immunization with GA2-MB was safe and well tolerated with no study-related serious adverse events or breakthrough malaria. Neither were there any parasite concentrations ≥50 parasites/mL detected in any blood sample after immunization. The mosquito bites led to severe itch in one participant and moderate swelling and mild blistering in another participant, for which topical corticosteroids were prescribed. All other adverse events after immunization were mild to moderate. Further details on safety and tolerability can be found in Extended Data Table 1.

We found that 9/10 (90%) participants in the GA2-MB group were fully protected against *Pf* malaria and remained *Pf*qPCR-negative until day 28 post-CHMI (Fig. 2C). In contrast, all participants in the placebo group became parasitemic (log-rank test: $p<0.0001$). While the median time-to-parasitemia in the placebo group was 9 days (range: 7-11 days), detection of parasitemia in the one unprotected GA2-MB participant was considerably delayed to day 13 (Extended Data Fig. 1B). When assessing protection in solely the participants that received a dose of five infected blood fed mosquitoes at CHMI (not a pre-specified analysis in our protocol), we found a protective efficacy of 83% (5/6 GA2-MB participants protected vs. 0/4 placebo participants).

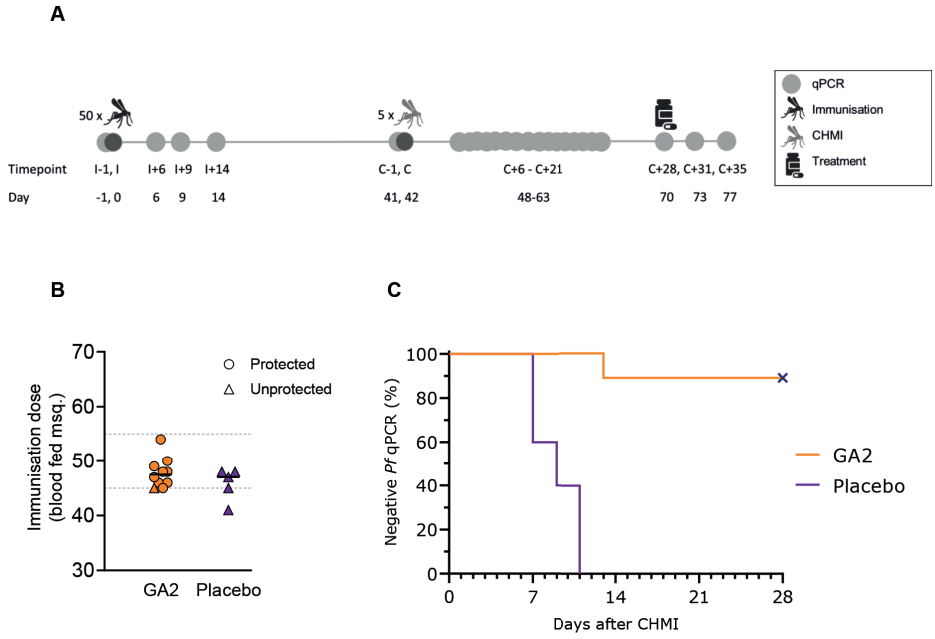

Figure 2. Study design, immunization dose and protection against controlled human malaria infection

A. Schematic overview of study design. **B.** Number of blood fed mosquitoes per participant at immunization. Orange represents GA2-MB, purple represents placebo. Circles represent protected participants and triangles unprotected. Black horizontal lines represent the median. Dotted grey line represents target dose (45 – 55). **C**. Kaplan-Meier curve of percentage of participants that had a negative *Pf*qPCR (<100 parasites/mL) in peripheral blood after CHMI. X = censored; Log-rank test = p<0.0001. CHMI = controlled human malaria infection; *Pf* = *Plasmodium falciparum;* qPCR = quantitative polymerase chain reaction.

Secondary outcomes

We assessed antibody responses one day before CHMI (C-1) and detected significantly higher levels of antibodies targeting *Pf* circumsporozoite protein (*Pf*CSP) but not key late-liver and blood-stage antigens *Pf* apical membrane antigen-1 (*Pf*AMA-1) and *Pf* merozoite surface protein-1 (*Pf*MSP-1) in GA2-MB participants compared to placebo (Fig. 3A). *Pf*-specific cellular immunity in GA2-MB participants assessed by stimulation of peripheral blood mononuclear cells (PBMCs) with *Pf*-infected red blood cells (*Pf*RBCs), surrogate for late liver stage antigens, and uninfected RBCs (unRBCs) showed a strong type-1 pro-inflammatory (interferon-γ [IFN-γ], tumor necrosis factor-α [TNF-α], and interleukin-2 [IL-2]), and a moderate type-2 anti-inflammatory (IL-4, IL-5 and IL-13) and regulatory (IL-10) profile in $CD4^+$ and $V\delta2^+$ γδ T cells but not in $CD8^+$, $V\delta2^-$ γδ and natural killer T cells (Fig. 3B, Extended Data Fig. 2-4). GA2-MB elicited higher frequencies of poly-functional $CD4^+$ and $V\delta2^+$ γδ T cells expressing more than one type-1 cytokine, in comparison to placebo (Fig. 3C). While $CD4^+$ T cells preferentially co-expressed TNF-α and IL-2 with or without IFN-γ, $V\delta2^+$ γδ T cells co-expressed high levels of IFN-γ and TNF-α

with or without IL-2. We observed a relatively minor proportion of type-1 poly-functional CD4⁺ and Vδ2⁺ γδ T cells co-expressing type-2 cytokines (Extended Data Fig. 5). Poly-functional CD4⁺ T cells, but not Vδ2⁺ γδ T cells, were enriched among memory T cells (CD3⁺CD45RA⁻), indicating the capacity of single GA2-MB immunization to form *Pf*-specific cellular memory (Fig. 3D, Extended Data Fig. 6A-F). These GA2-induced memory T cells preferentially acquired effector memory phenotype as early as 2 weeks post-immunization and remained high during the post-CHMI time point, while central memory T cells were induced at much lower frequency (Fig. 3E, Extended Data Fig. 6G and H).

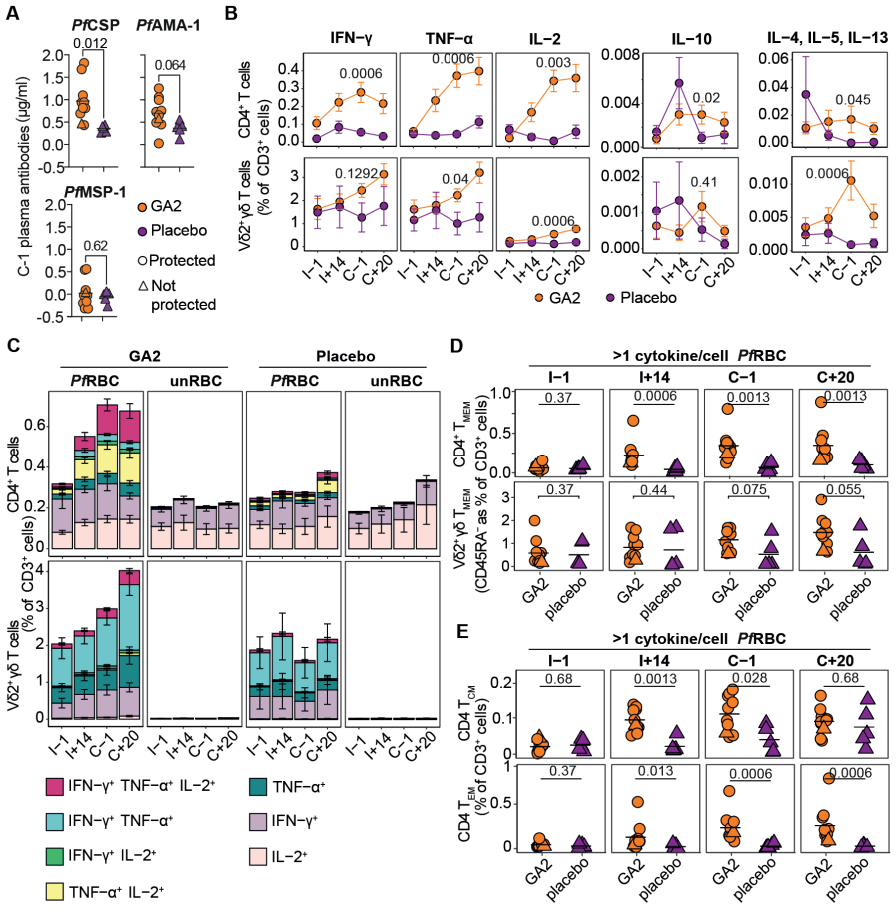

Figure 3. Prominent polyfunctional CD4⁺ memory T cell response in GA2-single-immunised participants

A. Plasma antibody levels against the indicated *Plasmodium falciparum* (*Pf*) antigens on the day before CHMI (C-1). Values are $\log_{10}$ transformed. **B**. Frequency of CD4⁺ and Vδ2⁺ γδ T cells expressing the indicated cytokines upon stimulation with *Pf* infected red blood cells (*Pf*RBC) corrected for uninfected RBC (unRBC) stimulation. **C**. Frequency of CD4⁺ and Vδ2⁺ γδ T cells expressing single or more than one of the

indicated cytokines per cell upon *Pf*RBC and unRBC stimulation. **D**. Frequency of $CD45RA^{-}$ $CD4^{+}$ (top) and $V\delta2^{+}$ γδ (bottom) memory T cells (T_{MEM}) among polyfunctional cells at the indicated time points upon *Pf*RBC stimulation. **D**. Frequency of central (T_{CM}; top) and effector (T_{EM}; bottom) memory cells among polyfunctional $CD4^{+}$ T cells.
Data corresponding to the GA2-MB and the placebo group are indicated in orange and purple, respectively (A, B, D, and E). Filled circles and triangles indicate the data from individual participants and the horizontal black line indicates arithmetic mean (A,D,E). Filled circles and error bars indicate arithmetic mean and standard error of mean, respectively (B). Bar charts represent arithmetic means and error bars represent standard error of mean (C). Groups were compared using a two tailed Mann-Whitney U test (A, B [at C-1], D, and E).

Discussion

In this study, we demonstrated the capacity of a single immunization with 50 GA2-infected mosquitoes to protect 90% of malaria-naïve individuals against a homologous CHMI. So far, malaria vaccines have always been tested in regimens of two or more immunizations, but single immunization has important potential advantages over multiple immunizations with regards to implementations in endemic settings as well as for travelers' vaccinations. Although high level protective efficacy after immunization with sporozoites has been observed before,[11, 13-19] never has protective efficacy been demonstrated in a CHMI after one immunization. Surprisingly, this high level of protective efficacy seems to be similar to three immunizations with GA2-infected mosquito bites,[11] suggesting that the boosting effect of additional immunizations is limited. Further research into the dynamics of immune components in a larger cohort of participants is needed to understand changes on an individual basis and to evaluate how these changes relate to protection. Additionally, the longevity of the immune response and protective efficacy after both single and triple GA2-MB immunization needs to be further evaluated and compared.

Blood-stage breakthrough infections have been observed after immunizing mice with *Plasmodium yoelli Δmei2*.[20] When taking the current and our previous GA2-MB study[11] together, a total of 50 participants have now been exposed to GA2-MB: 15 participants to 15 GA2-MB and 35 participants to 50 GA-MB (nine of whom underwent three exposures). None of these participants have developed breakthrough malaria. This is in line with results in mice with humanized livers that were exposed to *PfΔmei2* and did not develop breakthrough blood infections either.[8] Future studies should demonstrate if this favorable safety profile of GA2 holds up in larger populations or if genetically attenuated parasites with more knockouts beside *mei2* are warranted to eliminate the risk of breakthrough infections after immunizations.[21]

Due to the lack of knowledge on immunogenic late liver stage antigens and the technical limitations to generate large numbers of infected hepatocytes suitable for *in vitro* stimulation, *Pf*RBCs were used as a surrogate antigenic source in this study, similar to previously published clinical studies.[10, 17, 19] Furthermore, preferential liver resident capacity of *Pf*-specific $CD8^{+}$ T

cells may have thwarted our attempts to detect them in peripheral blood samples. In previous sporozoite immunization studies, polyfunctional $CD4^+$ and $V\delta2^+$ γδ T cells expressing pro-inflammatory cytokines, particularly IFN-γ, were associated with protection.[11, 13, 17, 19] However, after single GA2-MB immunization we find the production of pro-inflammatory cytokines other than IFN-γ (notably TNF-α and IL-2) by $CD4^+$ T cells to be more pronounced, in addition to an increase in effector rather than central memory phenotypes which differentiate early after immunization and persist throughout the CHMI follow-up period.

A limitation of our study is the small sample size of healthy malaria-naïve participants that do not adequately represent the target population for malaria vaccines in endemic areas. Additionally, administration of GA2 through mosquito bites is not a feasible method for large scale immunization campaigns. To translate the high-level protective efficacy of GA2-MB to an amenable way of vaccine administration through parenteral immunization, future studies need to assess whether aseptically purified, vialed and cryopreserved sporozoites with the *mei2* deletion are as safe and as efficacious as GA2-MB in this study and similarly potent in malaria endemic areas.

Nonetheless, our finding that a single immunization with GA2-MB can induce high-level protection against a homologous CHMI provides strong support for the further clinical development of potentially highly potent next generation single-immunization malaria vaccines based on late-arresting genetically attenuated sporozoites.

Methods

Study design and recruitment

A randomized, double-blind, placebo-controlled trial with a CHMI was conducted from February to November 2023 at the Leiden University Medical Center, in Leiden, the Netherlands. Fifteen malaria-naïve participants aged 15 to 30 were included after a health assessment including medical history, physical examination, a general laboratory evaluation including hematology and biochemistry assessment, a drugs test to exclude cocaine and amphetamine use, and an electrocardiography. Female participants were counselled to use adequate contraception throughout the study and were tested for pregnancy with a serum beta-HCG test on both the day before immunization (I-1) and the day before CHMI (C-1). All participants provided written informed consent.

Ten participants were immunized with the bites of 45 to 55 GA2-infected mosquitoes, and five participants received a mock-immunization with uninfected mosquitoes as a placebo. Six weeks after immunization, all 15 participants underwent a homologous CHMI with the bites of five wild-type *Pf*3D7-infected mosquitoes. From day 6 to day 21 after CHMI (C+6 to C+21), participants were closely followed with daily ambulatory visits for the collection of adverse events (AEs), safety assessment, blood sampling, and highly sensitive quantitative polymerase

chain reaction (qPCR) analysis for *Pf* on whole blood as previously described (lowest limit of detection was 50 parasites/mL).[22] Participants were treated with a three-day regimen of atovaquone/proguanil when they exhibited parasitemia (*Pf*qPCR>100 parasites/mL) or at day 28 after CHMI (C+28). AEs were recorded by participants in a diary. AEs were graded in four categories (mild, moderate, severe and serious) that were prespecified per protocol. Both participants and investigators were blinded to intervention. Mosquito cages were prepared by technicians independent from the clinical investigators. Randomization was carried out by an independent member of the study team. Safety, time-to-parasitemia and protective efficacy were the primary outcomes. Secondary study outcomes were humoral and cellular immunology results. Data capture was done using an electronic case report form (Castor CDMS version 2023.1.x.x).

The trial protocol was approved by the Dutch Central Committee for Research involving Human Subjects (CCMO, file number NL82130.000.22) and registered at ClinicalTrials.gov (NCT05468606), and EudraCT (2022-002646-40).

Parasite culturing, mosquito rearing and exposures

The characterization of GA2 and its generation from *Pf*NF54, its genetic backbone, has been described previously.[8] The wild-type parasite used for the CHMI (*Pf*3D7) is a clone of the *Pf*NF54 parasite strain. Parasites were cultured in standard conditions using semi-automated shaker culture systems[23] and subsequently fed to female *Anopheles stephensi* mosquitoes by standard membrane feeding.[24] Mosquitoes were reared and infected following standard procedures at the insectary of the LUMC following established methods.[24] Production of parasites and mosquitoes underwent strict quality control before release by a qualified person. Fourteen days after feeding the parasites to the mosquitoes, a sample of twenty mosquitoes was taken from every mosquito batch (consisting of 200 to 500 mosquitoes) to assess sporozoite yield in the mosquito salivary glands. Only batches that had an average yield of at least 1000 sporozoites per mosquito were used for exposure to participants. For the immunization, the average yield of the batches was 34 000 and 67 000 sporozoites per mosquito and for the challenge the yield of the batches ranged from 11 300 to 35 300 sporozoites per mosquito.

Exposure of mosquitoes to participants was done using small cages with mesh-covered openings that were applied for 15 minutes to the deltoid region (immunization) or inner lower arm (CHMI). After exposure, mosquitoes were dissected to confirm feeding. Additionally, after CHMI exposures, salivary glands were dissected and microscopically assessed for the presence of sporozoites. At immunization, exposures were repeated until the target dose of 45 to 55 mosquitoes was reached or up to a maximum of three times. For CHMI, the procedure was repeated until five infected mosquitoes had taken a blood meal or up to a maximum of four exposures.

Pf antigen binding antibody measurements in ELISA

ELISAs were performed as described before.[11] In brief, half area 96-well high binding plates were coated overnight at 4 °C with 1 µg/mL of antigen at 25 µl/well in 0.1 M sodium carbonate buffer (pH 9.6). Upon blocking with 5% skim milk in phosphate-buffered saline for two hours, serially diluted plasma samples (starting dilution of 1:500 serially diluted eight steps each by 1:2.5) were incubated for two hours. Bound antibodies were detected with 450 nm absorbance using goat-anti-human IgG conjugated with horseradish peroxidase and 3,3',5,5'-Tetramethylbenzidine substrate development stopped with 10% sulfuric acid. Standard curve developed using polyclonal IgG with known concentration was used for normalization. Measures from at least two independent experiments with a coefficient of variance below 30% were considered for analysis.

T cell response measurement using flow cytometry

Cellular response using *Pf* infected red blood cells (RBC) stimulation was performed as described before(5). In brief, PBMCs stimulated with RBCs from a healthy blood donor either as uninfected (unRBC) or infected with *Pf* (*Pf*RBC) for 24 h during which 10 µg/mL Brefeldin A (Sigma) was added at 4 h post stimulation. Cells were stained with a panel of antibodies (Extended Data Table 2) to identify T cell subsets (CD56, $\gamma\delta^{-}V\delta2$ T cell receptor, CCR7, CD3, CD4, CD8, CD25 and CD11c), cytokine expression (IFN-γ, TNF-α, IL-10, IL-2, IL-4, IL-5, IL-13) and phenotype (CD45RA and CCR7). For fixation and intracellular staining Intracellular Fixation & Permeabilization Buffer Set (Invitrogen) was used. To stain dead cells Aqua Live/Dead dye (Invitrogen) was used. Cells were acquired on the 3-laser spectral analyzer Aurora (configuration 16V-14B-8R) and analyzed using FlowJo 10.8.2 as described in Extended Data Fig. 2 and 3. Frequency of *Pf*-specific cytokine positive cells in *Pf*RBC stimulated samples after subtraction of the same gate on the same sample stimulated with unRBC are reported for analysis. Frequencies of cytokine positive $CD4^{+}$ and $\gamma\delta^{+}V\delta2$ cells were calculated as a percentage of $CD3^{+}$ cells by using the frequencies of total $CD4^{+}$ and $\gamma\delta^{+}V\delta2$ cells, respectively.

Statistical analysis

Baseline characteristics of participants are reported as both means with standard deviations and medians with range for continuous variables and as frequencies with percentages for categorical variables. The incidence of AEs is reported as frequencies with percentages (risk). Time-to-parasitemia is reported as a Kaplan-Meier graph and the difference between groups is evaluated using a log-rank test. Antibody concentrations are reported in µg per mL and frequency of responding cells as a percentage of the indicated population, both as arithmetic means with standard error of means. Antibody concentrations and cell populations are compared between groups with a two tailed Mann-Whitney U test.

Figures were produced in GraphPad Prism (version 9.3.1) and RStudio (version 4.2.1).

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Extended Data

Extended Data is available online: https://www.nature.com/articles/s41591-024-03347-2

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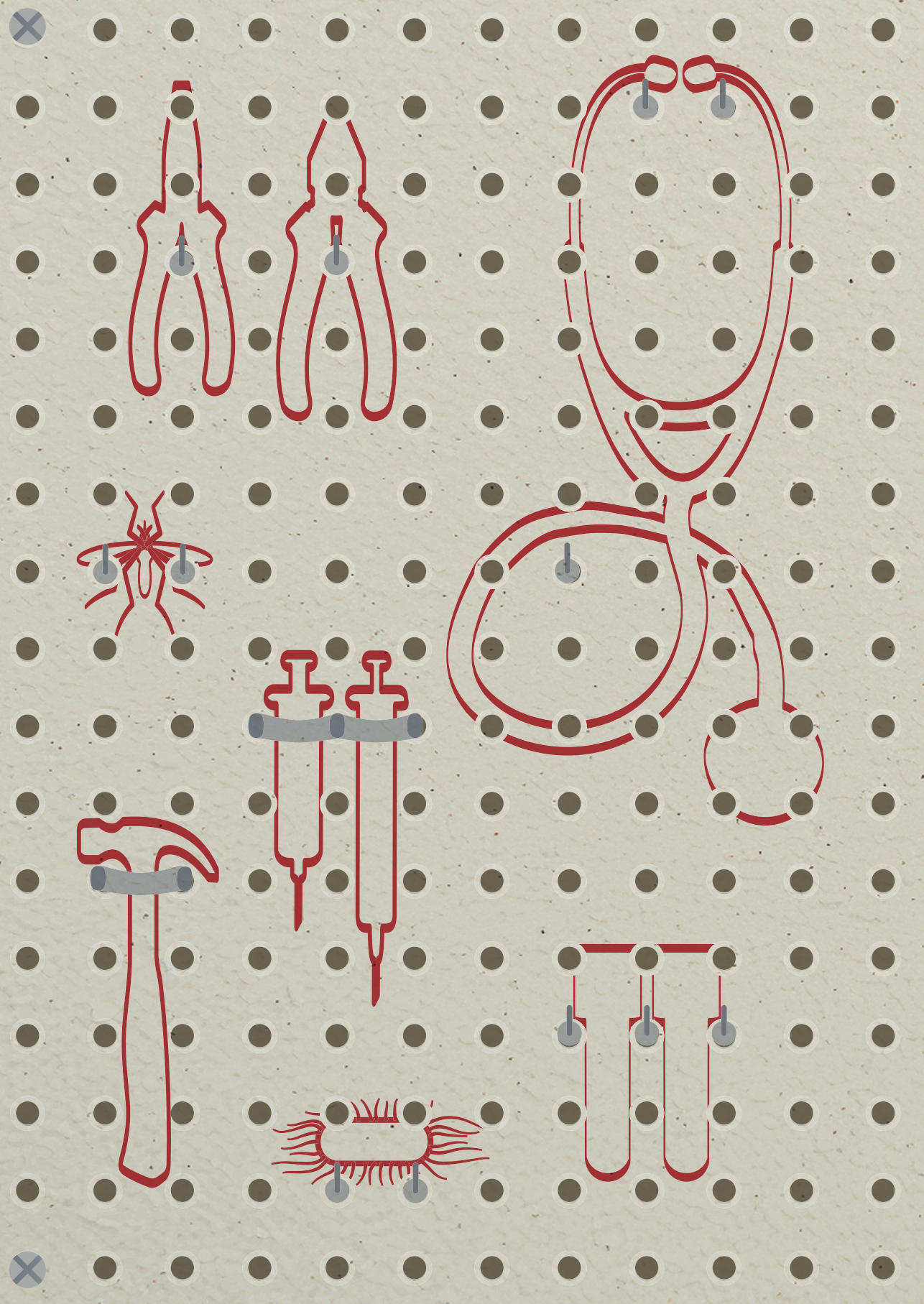

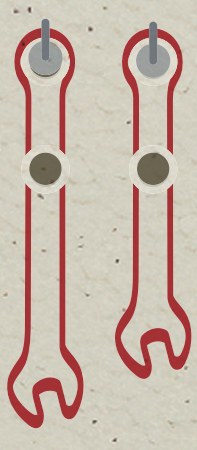

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Safety, Tolerability, and Immunogenicity of the Invaplex$_{AR\text{-}Detox}$ *Shigella* Vaccine Co-Administered with the dmLT Adjuvant in Dutch and Zambian Adults: Study Protocol for a Multi-Center, Randomized, Double-Blind, Placebo-Controlled, Dose-Escalation Phase Ia/b Clinical Trial

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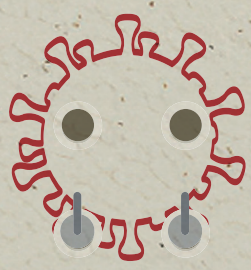
Vaccines (2025)



Abstract

Background

Shigella infections remain endemic in places with poor sanitation and are a leading cause of diarrheal mortality globally, as well as a major contributor to gut enteropathy and stunting. There are currently no licensed vaccines for shigellosis but it has been estimated that an effective vaccine could avert 590 000 deaths over a 20-year period. A challenge to effective *Shigella* vaccine development has been the low immunogenicity and protective efficacy of candidate *Shigella* vaccines in infants and young children. Additionally, a new vaccine might be less immunogenic in a highly endemic setting compared to a low endemic setting ("vaccine hyporesponsiveness"). The use of a potent adjuvant enhancing both mucosal and systemic immunity might overcome these problems. Invaplex$_{AR\text{-}Detox}$ is an injectable *Shigella* vaccine that uses a novel combination of conserved invasion plasmid antigen proteins and a serotype-specific bacterial lipopolysaccharide attenuated for safe intramuscular administration. The adjuvant dmLT has been shown to enhance *Shigella* immune responses in mice and has safely been administered intramuscularly and was shown to enhance immune responses in healthy volunteers when given in combination with other antigens in Phase I trials. This article describes the protocol of a study that will be the first to assess the safety, tolerability and immunogenicity of Invaplex$_{AR\text{-}Detox}$ co-administered with dmLT in healthy adults in low-endemic and high-endemic settings.

Methods

In a multi-center, randomized, double-blind and placebo-controlled dose-escalation Phase Ia/b trial, the safety, tolerability and immunogenicity of three intramuscular vaccinations administered 4 weeks apart with 2.5 µg or 10 µg of Invaplex$_{AR\text{-}Detox}$ vaccine, alone or in combination with 0.1 µg of the dmLT adjuvant, will first be assessed in a total of 50 healthy Dutch adults (Phase Ia) and subsequently in 35 healthy Zambian adults (Phase Ib) aged 18 to 50 years. The primary outcome is safety and secondary outcomes are humoral and cellular immune responses to the adjuvanted or non-adjuvanted vaccine.

Discussion

This trial is part of the ShigaPlexIM project that aims to advance the early clinical development of an injectable *Shigella* vaccine and to make the vaccine available for late-stage clinical development. This trial addresses the issue of hyporesponsiveness in an early stage of clinical development by testing the vaccine and adjuvant in an endemic setting (Zambia), after the first-in-human administration and the dose-escalation has proven safe and tolerable in a low-endemic setting (Netherlands). Besides strengthening the vaccine pipeline against a major diarrheal disease, another goal of the ShigaPlexIM project is to stimulate capacity building and to strengthen global North-South relations in clinical research.

Trial registration

EU CT number: 2023-506394-35-02
ClinicalTrials.gov identifier: NCT05961059

Introduction

Shigellosis is an acute invasive enteric infection caused by the gram-negative, non-motile *Shigella* bacillus. *S. flexneri* is the main serogroup found in low-income countries (~60% of isolates), followed by *S. sonnei* (~15-25%).[1, 2] In middle- and high-income countries, *S. sonnei* is most common (~60%), followed by *S. flexneri* (~15-20%).[3, 4]

Shigella is transmitted through the fecal-oral route, and its infective dose is very low. This gives rise to outbreaks in situations with poor sanitation, enabling the disease to disseminate rapidly by person-to-person contact.[3] Shigellosis clinically manifests after an incubation period of 1 to 4 days with symptoms of fever, malaise, anorexia and sometimes vomiting. *Shigella* infection can cause bloody diarrhea but in mild infections watery diarrhea can be the only clinical manifestation.[3] Additionally, local invasion and intestinal inflammation induced by *Shigella* infection are risk factors for the subsequent development of gut enteropathy, malnutrition and stunting in children.[5, 6] Shigellosis is a leading cause of diarrheal deaths globally[3] and is the fourth most common cause of moderate to severe diarrhea in young children living in low- and middle-income countries (LMICs).[7] In 2016, shigellosis caused over 200 000 deaths of which more than 60 000 were children under 5 years old.[8] A recent systematic review found *Shigella* to be the second leading cause of diarrhea associated mortality in LMICs.[9]

There is currently no licensed vaccine available for shigellosis.[10] Following a rise in antibiotic resistance combined with the high burden of infection, particularly in LMICs, the World Health Organization (WHO) has identified *Shigella* as a priority bacterial pathogen and has urged the development of a *Shigella* vaccine.[(11-13)] It has been estimated that *Shigella* vaccination could avert 43 million cases of stunting and 590 000 deaths over a 20-year period and would be highly cost-efficient.[14, 15]

Invaplex$_{AR\text{-}Detox}$ is a subunit *Shigella* vaccine that contains conserved invasion plasmid antigen (Ipa) proteins (IpaB and IpaC) together with an *S. flexneri* 2a serotype-specific bacterial lipopolysaccharide (LPS). In addition to *S. flexneri* 2a, the recombinantly-produced conserved proteins IpaB and IpaC could also offer protection against *S. flexneri* serotypes 3 and 6, and *S. sonnei*.[16] The LPS is generated from a genetically attenuated msbB *Shigella* mutant that expresses underacylated and therefore detoxified lipid A, thus enabling safe intramuscular (IM) vaccine administration.[17] Previously, a Phase I trial (NCT03869333) was conducted in 58 adults in the United States, assessing three different dose levels (2.5, 10, and 25 µg) of Invaplex$_{AR\text{-}Detox}$ given IM in a three-dose course. All three dose levels of Invaplex$_{AR\text{-}Detox}$ were well tolerated and highly immunogenic, inducing strong systemic antibody responses to all three *Shigella* antigens contained within the vaccine. The magnitude of the LPS-specific serum IgG responses was comparable to or exceeded antibody levels observed after infection with *S. flexneri* 2a.[18, 19] Antibody responses to the IpaB and IpaC antigens induced by the vaccine also approached levels that have been associated with protection against shigellosis.[20] Although these results are promising in the adult population, historically, reduced immunogenicity and protective

efficacy of *Shigella* vaccines has been seen in infants and thus provides a rationale for adding an adjuvant to enhance immunogenicity.[12, 21]

A promising adjuvant candidate is the double mutant (LT R192G/L211A) enterotoxigenic *E. coli* heat labile toxin (dmLT)[22] that has previously been shown to enhance both systemic and mucosal immune responses to *Shigella* antigens in mice.[23] Furthermore, dmLT (up to 0.5 µg) has been administered IM in a Phase I trial of a prototype enterotoxigenic *E. coli* vaccine antigen and was found to be safe and significantly improved antibody responses to this vaccine antigen.[24]

This current study will be the first clinical trial assessing the safety, tolerability and immunogenicity of the Invaplex$_{AR\text{-}Detox}$ vaccine adjuvanted with dmLT. The first-in-human and dose-escalation part of the trial will be conducted in the Netherlands, in a setting with low *Shigella* endemicity, and therefore with immunologically naïve participants. The second part of the trial will take place in an endemic setting in Zambia.

Materials and Methods

Design

This will be a multi-center, randomized, double-blind, placebo-controlled dose-escalation Phase Ia/b clinical trial assessing the safety, tolerability and immunogenicity of three IM vaccinations given 28 (±5) days apart. The vaccine will consist of either a 2.5 or 10 µg dose of the Invaplex$_{AR\text{-}Detox}$ vaccine administered alone or in combination with a 0.1 µg dose of the dmLT adjuvant and will be compared to placebo (saline solution). A total of 85 healthy participants aged 18 to 50 years will be recruited: 50 in the Netherlands and 35 in Zambia. Inclusion- and exclusion criteria are reported in Table 1.

Table 1. Inclusion- and exclusion criteria

Inclusion criteria	-	Healthy adult, male or female, aged 18 to 50 years (inclusive) at the time of inclusion (= first vaccination).
	-	Provide written informed consent before initiation of any study procedure.
	-	Available to complete all study visits and procedures.
	-	Negative stool PCR test for *Shigella.*
	-	Women of childbearing potential: negative pregnancy test at screening and before each study vaccine administration. Women of childbearing potential must agree to use continuous highly effective contraception to avoid pregnancy during the study, starting at least 4 weeks before the first vaccine dose, until 3 months following the last vaccine dose.

Table 1. Inclusion- and exclusion criteria (continued)

Exclusion criteria	-	Any history or evidence of clinically relevant chronic medical conditions (such as: psychiatric conditions, diabetes mellitus, hypertension [treated by medication], autoimmune disorders, immunodeficiencies, cardiovascular disease, renal disease, or inflammatory bowel disease). Trial physicians (in consultation with the principal investigator) will use clinical judgment on a case-by-case basis to assess safety risks under this criterion.
	-	Current use of immunosuppressive medications (except for antihistamines and topical or inhalation corticosteroids).
	-	Women who are a) currently nursing or b) who are pregnant or planning to become pregnant during the study period plus 3 months beyond the last vaccine dose.
	-	Participation in research involving another investigational product (defined as receipt of an investigational product or exposure to an invasive investigational device) 30 days before the first vaccination or anytime through the last in-clinic study safety visit.
	-	Positive blood test for HBsAg, HCV, or HIV.
	-	Clinically significant abnormalities on basic laboratory screening tests.
	-	Systemic antimicrobial treatment (i.e., topical treatments are not an exclusion criterion) within 1 week before the first vaccine dose (temporary exclusion).
	-	Known hypersensitivity to compounds in the vaccine or adjuvant or other known drug allergies that may increase the risk of adverse events.
	-	Regular use (weekly or more often) of antidiarrheal, anti-constipation, or antacid therapy.
	-	Abnormal stool pattern (fewer than 3 stools per week or more than 3 stools per day) on a regular basis; loose or liquid stools on other than an occasional basis.
	-	Personal or family history of inflammatory arthritis.
	-	Proven allergy to any substance in the Invaplex$_{AR\text{-}Detox}$ vaccine or dmLT or history of anaphylactic reaction to any other vaccine.
	-	Exclusionary skin disease history/findings that would confound assessment or prevent appropriate monitoring of local adverse events, or possibly increase the risk of local adverse events.
	-	Recent (<3 months) history of gastroenteritis.
	-	Received previous licensed or experimental *Shigella* vaccine, dmLT or live *Shigella* challenge.
	-	Any severe medical condition that might place the participant at increased risk of adverse events according to the clinical judgment of the study clinicians in consultation with the PI.
	-	Any planned vaccination within 14 days before the first vaccine dose until the last in-clinic visit, with the exception of SARS-CoV-2 vaccines or influenza vaccines.

HBsAg = hepatitis B surface antigen; HCV = hepatitis C virus; HIV = human immunodeficiency virus; PCR = polymerase chain reaction; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

In the first part of the trial (Phase Ia), which will include the dose-escalation evaluation, the participants will be enrolled in two cohorts (A and B) at the Leiden University Medical Center (LUMC) in Leiden, the Netherlands (Fig. 1). In the second part of the trial (Phase Ib), the participants will be enrolled in a single cohort (C) at the Centre for Infectious Disease Research in Zambia (CIDRZ) in Lusaka, Zambia.

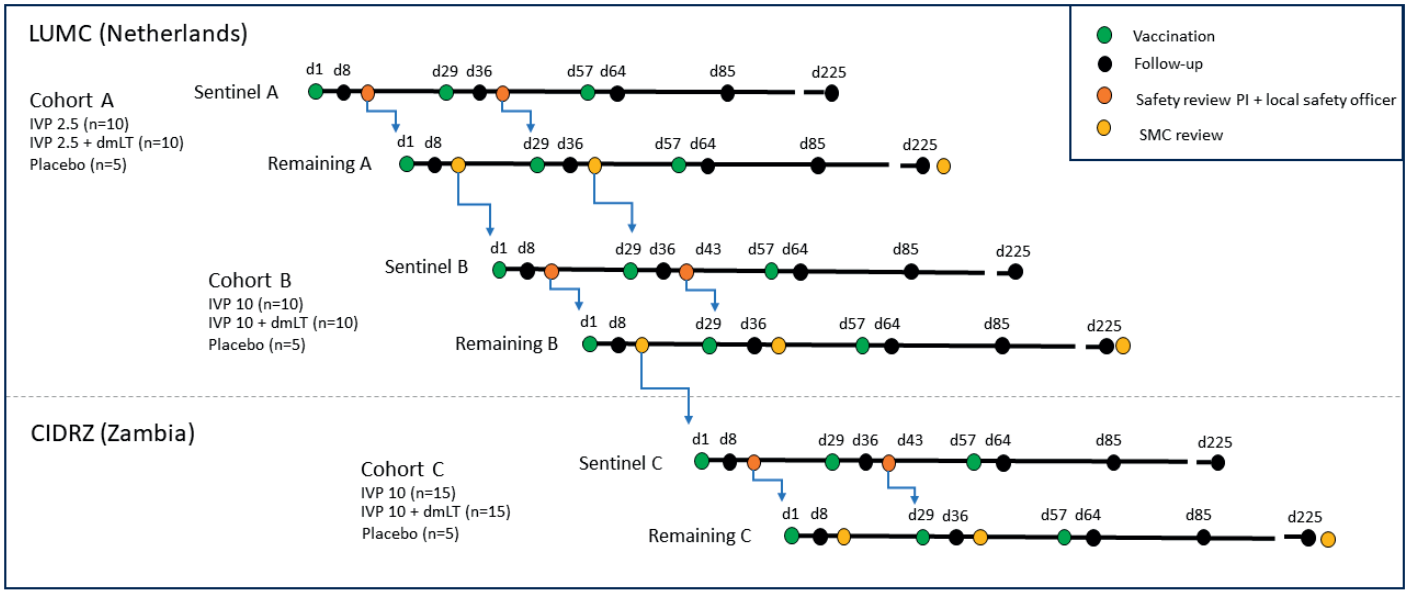


Figure 1. Study design

Participants are vaccinated on days 1, 29, and 57. In each sentinel cohort, a safety review by the PI and a local safety officer will take place after the first vaccination (between days 8 and 29) and the second vaccination (between days 36 and 57). In the remaining cohorts, an SMC review will take place on these same time points. Only after these reviews have found the vaccine and adjuvant to be safe and well-tolerable, can further vaccination of the subsequent cohort take place (indicated by blue arrows).

CIDRZ = Center for Infectious Disease Research in Zambia; IVP 2.5 = 2.5 µg Invaplex$_{AR\text{-}Detox}$; IVP 10 = 10 µg Invaplex$_{AR\text{-}Detox}$; LUMC = Leiden University Medical Center; PI = principal investigator; SMC = safety monitoring committee.

In cohort A, a total of 25 participants will receive 2.5 µg Invaplex$_{AR\text{-}Detox}$ with or without 0.1 µg dmLT, or a placebo in a 2:2:1 ratio, respectively (Table 2). In cohort B, a total of 25 participants will receive 10 µg Invaplex$_{AR\text{-}Detox}$ with or without 0.1 µg dmLT, or a placebo in a 2:2:1 ratio, respectively. In cohort C, a total of 35 participants will receive 10 µg Invaplex$_{AR\text{-}Detox}$ with or without 0.1 µg dmLT, or a placebo in a 3:3:1 ratio, respectively. As an additional safety measure, all three cohorts will be divided into a sentinel cohort and a remaining cohort. A sentinel cohort consists of seven participants (three receiving the vaccine alone, three receiving the adjuvanted vaccine and one participant receiving a placebo). The 7 participants in a sentinel cohort will be vaccinated on the same day with a 30-minute interval. Only if the vaccine and adjuvant are safe and well tolerated in the sentinel cohort in the seven days following administration will the remaining participants of the cohort receive their dose. At both sites, an independent local safety officer will monitor trial progress and safety and will decide together with the local principal investigator (PI) if the remainder of the cohort will be vaccinated.

Table 2. Trial sites, cohorts and groups with corresponding vaccine and adjuvant doses

Site	Cohort	Group	Invaplex$_{AR\text{-}Detox}$ (µg)	dmLT (µg)	N
LUMC	A	A1	2.5	---	10
		A2	2.5	0.1	10
		A3	Placebo	---	5
		- Sentinel cohort (A1: n=3, A2: n=3, A3: n=1) to be dosed first, followed by the remaining cohort 1 week later. - Advancement to cohort B based on safety data through 7 days post first dose.			
	B	B1	10	---	10
		B2	10	0.1	10
		B3	Placebo	---	5
		- Sentinel cohort (B1: n=3, B2: n=3, B3: n=1) to be dosed first, followed by the remaining cohort 1 week later. - Advancement to cohort C based on safety data through 7 days post first dose.			
CIDRZ	C	C1	10*	---	15
		C2	10*	0.1	15
		C3	Placebo	---	5
		- Sentinel cohort (C1: n=3, C2: n=3, C3: n=1) to be dosed first, followed by the remaining cohort 1 week later. - Advancement to age-descending study (Phase IIa at CIDRZ) based on safety data from cohorts A and B (Phase Ia at LUMC) through 6 months and safety data from cohort C through 7 days post third dose.			

* In case the 10 µg dose is not well tolerated or deemed unsafe by the safety monitoring committee, the committee can decide to proceed to cohort C with the 2.5 µg dose.
CIDRZ = Center for Infectious Drug Research in Zambia; LUMC = Leiden University Medical Center.

Participants will register adverse events (AEs) and body temperature on a daily basis in a paper diary. They will return for a study visit 7 days post-vaccination for the collection of samples and a safety assessment. In Zambia, investigators will perform home visits in the week after

each vaccination to ensure adequate recording of AEs and body temperature. After the third vaccination, there will be a visit at 7 and 28 days after vaccination. Twenty-four weeks after the third vaccination, the last visit will take place. The decision to proceed from one cohort to the next will be based on the recommendation of the safety monitoring committee (SMC). The SMC will consist of two local safety officers with two additional clinicians: a pediatrician and an expert in vaccines for gastrointestinal infectious diseases. The local safety officer may be an in-house expert at a study site, but not a member of the study team. The two additional SMC members will have no affiliation to the study sites. One of the independent SMC members will be appointed as chair and has a casting vote in case of a tie. If the 10 µg Invaplex$_{AR\text{-}Detox}$ dosage is not well tolerated in cohort B or deemed unsafe by the SMC, the committee may recommend proceeding to cohort C with the 2.5 µg Invaplex$_{AR\text{-}Detox}$ dosage.

Participants

At the LUMC in the Netherlands, recruitment of healthy volunteers from the general population will be performed through advertisements, social media and institutional websites. Advertisement material will be subject to ethical review before the start of recruitment. At CIDRZ in Zambia, volunteers will be recruited from Matero General Hospital, CIDRZ clinical research site and the surrounding community. Individuals should be able to understand and comply with planned study visits and procedures. After a detailed explanation of the trial, participants will have to provide written informed consent prior to any of the trial procedures. Pregnant or breastfeeding women, individuals who recently had gastro-enteritis, and individuals with an abnormal stool pattern or regular users of antidiarrheal, anti-constipation, or antiacid therapy are excluded. Women of childbearing potential must agree to use continuous highly effective contraception to avoid pregnancy during the trial, for at least 4 weeks before the first vaccination and for 3 months following the last vaccine dose.

Screening

During a screening visit, information on demographics, self-reported medical and medication history, and alcohol, drugs and cigarette use will be recorded. A general physical examination including vital sign measurements will be performed and samples will be collected for laboratory tests including hematology (e.g. hemoglobin, red blood cell count, platelet count), serum chemistry (e.g. creatinine, sodium, potassium, blood urea nitrogen, transaminases), serology (human immunodeficiency virus, hepatitis B and C) and a diagnostic polymerase chain reaction (PCR) for *Shigella* on a stool sample. Women of childbearing potential will also be subjected to a urine or serum pregnancy test, both at screening and before each vaccination. Individuals with a clinically significant abnormal test result or a positive *Shigella* PCR will be excluded from the trial.

Randomization and blinding

Before the first vaccination in a sentinel or remaining cohort takes place, a randomization list will be prepared by at least two members of the pharmacy team not otherwise involved in the trial. The allocation ratios will be 3:3:1 for all sentinel cohorts, 7:7:4 for remaining

cohorts A and B, and 3:3:1 for remaining cohort C (Table 2). The randomization list will link each randomization number to the corresponding intervention (vaccine alone, adjuvanted vaccine or placebo). The pharmacy team will prepare the investigational products and will assign the corresponding randomization number to each product. Investigators will receive the investigational product labeled with a randomization number. The investigator will administer the investigational product to the participant and will record the corresponding randomization number for the said participant in the electronic case report form (eCRF). This participant-randomization number combination will be communicated to the pharmacy team to allocate the correct investigational product to the participant during the subsequent administrations. Both investigators and participants will be blinded to the intervention. Each participant will remain blinded until the end of the trial. After data cleaning, the database will be locked, followed by unblinding of the cohorts.

Vaccination

Vaccination will be done through IM injection. The first vaccine dose will be administered in the left deltoid, the second in the right deltoid, and the third in the left deltoid, to ensure that local side effects can be correctly attributed to the corresponding administration. In sentinel cohorts, there will be at least 30 minutes between the vaccination of subsequent participants. In the remaining cohorts, this delay will be at least 15 minutes. After vaccination, participants will be observed for at least 30 minutes to ensure neither hypersensitivity nor anaphylactic reaction occurs.

Outcomes

The primary outcome is safety. The safety evaluation will include registration of all solicited and unsolicited AEs during the whole trial period (Table 3). All reported AEs will be classified according to the International Classification of Diseases version 11 (ICD-11) and causality and severity will be assessed by investigators (Table 3). Primary outcome measures will consist of all solicited AEs considered to be possibly, probably, or definitely related to vaccination occurring within 7 days following each vaccination and all unsolicited AEs occurring within 28 days following each vaccination. There are three pre-specified safety stopping rules (Table 4). If a stopping rule is met, the study is put on a temporary halt and no further vaccines will be administered until the temporary halt is suspended. AE collection and blood collection will still continue. If not done already, the hospital pharmacist will break the study blind for the affected volunteers and the SMC will be informed about the group allocation. The trial participants and all investigators will remain blinded. The SMC will review the safety data and recommend the study sponsor on study (dis)continuation.

Table 3. Adverse events classification

Solicited*	
Local	Pain/tenderness, erythema, induration/swelling, pruritus, ipsilateral axillary lymphadenopathy, and depigmentation.
Systemic	Fever, chills, headache, fatigue, malaise, nausea/vomiting, painful/swollen joints, myalgia, diarrhea, abdominal pain, and neuralgia.
Severity	
Mild	Awareness of symptoms that are easily tolerated and do not interfere with usual daily activity.
Moderate	Discomfort that interferes with or limits usual daily activity but does not require professional medical attention.
Severe	Disabling, with subsequent inability to perform the usual daily activity, resulting in absence from work or school, requiring bed rest during the majority of the day and may need professional medical attention.
Serious	Requires emergency room visit or hospitalization or results in persistent or significant disability or death.
Relatedness	
Not related	A relationship to the administration of the investigational product cannot be reasonably established or another etiology is known to have caused the adverse event or is highly likely to have caused it.
Unlikely	A relationship to the administration of the investigational product is unlikely; however, it cannot be ruled out.
Possibly	There is a potential association between the event and administration of the investigational product; however, there is an alternative etiology that is more likely.
Probably	Administration of the investigational product is the most likely cause; however, there are alternative reasonable explanations, even though less likely.
Definitely	Administration of the investigational product is the only reasonable cause; another etiology causing the adverse event is not known.

*Only symptoms with an onset after a vaccination until the 7th subsequent day after that vaccination will be considered solicited.

The secondary outcome is immunogenicity. To assess serum anti-LPS, anti-IpaB, and anti-IpaC IgG and IgA antibody responses and to perform serum bactericidal assays (SBA) to *S. flexneri* 2a (strain 2457T), serum samples will be collected at days 1 (baseline), 29, 57, 64, 85, and 225 and kept frozen at -20 °C until tested. Peripheral blood mononuclear cells will be collected and cryopreserved at days 1, 8, 64, and 225 to assess B and T cell responses to the LPS, IpaB and IpaC antigens. Further exploratory humoral and cellular immune assays using the cryopreserved serum and mononuclear cell samples may be performed as resources allow.

Table 4. Pre-specified stopping rules

1. A serious adverse event occurs that is possibly, probably, or definitely related to Invaplex$_{AR\text{-}Detox}$ and/or dmLT.
2. In case of a blinded assessment: if more than 25% of participants in one cohort (i.e., sentinel cohort and remaining cohort combined) experience one or more severe adverse events that are possibly, probably, or definitely related to vaccination and last more than 48 hours.
3. In case of an unblinded assessment: if more than 50% of participants in one dosing group experience one or more severe adverse events that are possibly, probably, or definitely related to vaccination and last more than 48 hours.

Data management and monitoring

An eCRF will be used at both sites to facilitate direct data entry by the investigators for visit reports and AE registration. Other documentation (e.g. paper diaries, laboratory reports or supplementary medical records) may form part of the source documentation for a study participant. These source documents will be filed and stored at the site where they were produced. To create the eCRF, a validated system with audit, data and edit trail will be used to comply with Good Clinical Practice (GCP) standards for electronic data entry and export. Both sites will be monitored by an independent monitor to ensure the study will be conducted in accordance with the study protocol and GCP standards.

Statistical analysis and sample size

Safety will be assessed in the intention-to-treat (ITT) population. The ITT population will be defined as all participants included in the trial (i.e., all participants who received at least one vaccine dose). Safety data will be grouped by study cohort A, B and C, except for control participants who received the placebo and will be grouped together. AEs will be reported as both frequencies and percentages. Rates will be compared by Pearson's Chi-square test (or Fisher's exact test if assumptions are not met for Pearson's Chi-square).

Immunological analyses will be performed on the per protocol (PP) population. The PP population will be adapted to each analysis time point and parameter and will include all participants who received at least two vaccinations and have baseline and postvaccination data for the immunogenicity variable of interest. Participants with missing baseline data or major protocol deviations prior to the analysis time point that are likely to affect immunology results will be excluded. Analyses of immunological data will be grouped by cohorts A, B, and C, except for control participants who received the placebo and will be grouped together. Additionally, an analysis per dose level (2.5 and 10 µg) may be performed.

Reciprocal endpoint titers less than the starting dilution (the lowest limit of quantification) of the assay will be assigned a value of half the starting dilution for computational purposes. In general, descriptive statistics (mean and SD of $\log_{10}$ titers, geometric mean titer [GMT] and 95% CI, median, range) will be tabulated by cohort and time point. The two-sided 95% CI will be obtained using a t-distribution. Additionally, the geometric mean fold-rise from baseline (GMFR) will be computed (based on the difference in log titer of post-baseline measurement

minus baseline) and summarized in the same manner. For GMTs and GMFRs, between-group comparisons will be examined with ANOVA. The normality of the log-transformed continuous outcomes will be assessed using goodness-of-fit tests based on the empirical distribution function and by inspection of the normal probability plot. If normality assumptions are not satisfied, then the Kruskal-Wallis test will be used.

The number and proportion of responders (participants who seroconvert with a ≥ 4-fold increase in endpoint titer between baseline and post-vaccination samples), together with exact Clopper-Pearson 95% CIs will be tabulated by cohort and time point. If appropriate, between-group comparisons will be examined with Fisher's exact test unless assumptions are fulfilled for the χ^2 test.

A sample size of 10 participants per intervention group will allow for a reasonable initial assessment of the AE profiles of the first-in-human administration of the adjuvanted vaccine (cohort A) and the subsequent dose escalation (cohort B). A group size of 15 participants in cohort C will provide 80% power to detect AEs with an assumed true event rate of 10% in each intervention group separately.

Discussion

This trial is part of the ShigaPlexIM project (www.shigaplexim.eu), a collaborative project funded by the European & Developing Countries Trials Partnership 2 (EDCTP2). ShigaPlexIM aims to advance the early clinical development of an adjuvanted injectable *Shigella* vaccine and to make the vaccine available for late-stage clinical development. Prior to the clinical trial, data was collected in a surveillance study on the incidence of shigellosis in Burkina Faso and Zambia among children under 5 presenting with moderate to severe diarrhea at primary healthcare facilities to provide an epidemiologic basis for further clinical development and late-stage clinical trial design (manuscript in preparation).

In a previous trial (NCT03869333), a three-dose regimen of $\text{Invaplex}_{\text{AR-Detox}}$ was safe and immunogenic in US adults, inducing high anti-LPS, anti-IpaB and anti-IpaC antibody titers. Previous vaccines have shown to be less immunogenic in endemic settings due to genetic differences and differences in environmental factors such as food intake, microbiome and exposure to different micro-organisms and parasites; a phenomenon called vaccine hyporesponsiveness.[25] The trial described here aims to address the issue of hyporesponsiveness in an early stage of clinical development by testing the $\text{Invaplex}_{\text{AR-Detox}}$ vaccine in combination with an adjuvant in a highly endemic setting after the first-in-human administration and the dose-escalation has proven safe and tolerable in a low endemic setting.

Even though the protective immune mechanisms against shigellosis are not fully understood, antibodies against the cell wall LPS antigen are believed to play a primary role, with additional

protective effects from an immune response against virulence proteins IpaA, B, C and D and VirG.[20, 26, 27] The current formulation of Invaplex$_{AR\text{-}Detox}$ should induce immune responses to *S. flexneri* serotypes 2a, 3a, and 6. LPS from *S. sonnei* will be added at a later stage of product development to optimize serotype coverage: *S. flexneri* 2a, 3a, and 6, and *S. sonnei* together cover about 80% of the strains causing shigellosis.[2]

As defined by the WHO, the primary target population for a new *Shigella* vaccine consists of infants and young children.[12, 13] After the current trial in adults, the intention is to conduct a Phase IIa age de-escalation study in Zambia and/or Burkina Faso to test the vaccine and adjuvant sequentially in adolescents, young children and infants. The data on the safety, tolerability, and immunogenicity of the current trial in adults will enable an informed decision on proceeding to a Phase IIa study and will provide the foundation for the design of such a trial. An age de-escalation study in an African setting will answer the question if Invaplex$_{AR\text{-}Detox}$ with or without dmLT will be able to overcome problems of low immunogenicity in the target population that have hampered the clinical development and implementation of *Shigella* vaccines in the past.[12, 21]

Besides strengthening the vaccine pipeline against a major diarrheal disease, another goal of the ShigaPlexIM project is to stimulate capacity building and to provide support for formal education and training of study staff. The close collaboration will develop capacity in clinical trial conduct and clinical immunology and will strengthen both global South-South and global North-South networks. To further strengthen these connections, the creation of new PhD positions and dedicated capacity building events are separate deliverables of the ShigaPlexIM project.

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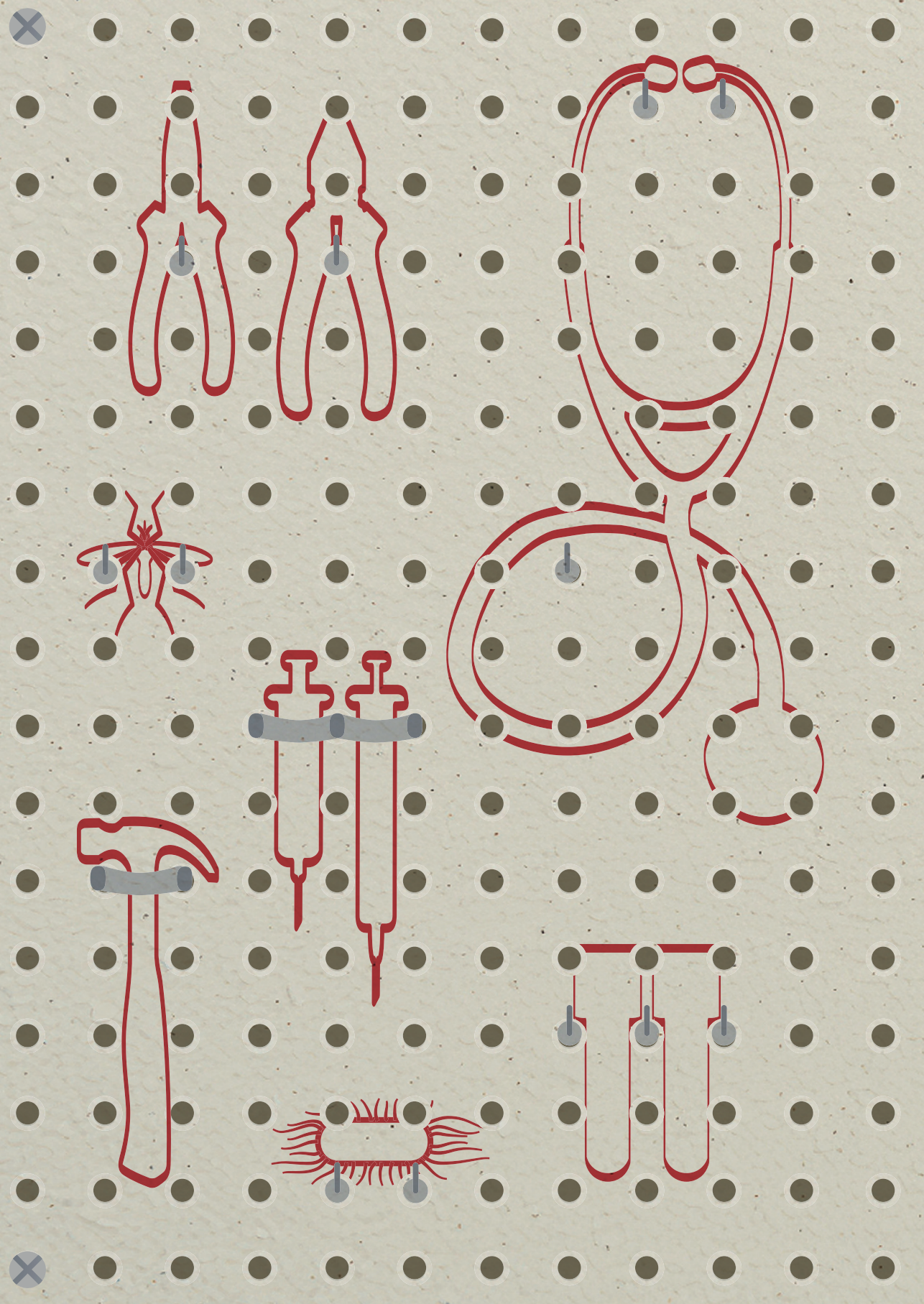
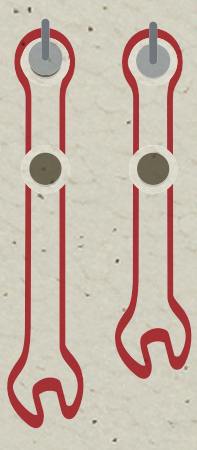
Part II

Dose-Optimization Strategies During a Pandemic



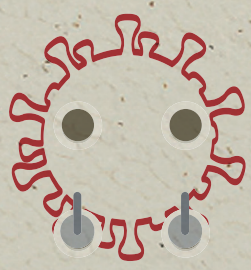


4

COVID-19 Vaccine Dose Sparing: Strategies to Improve Vaccine Equity and Pandemic Preparedness

Geert V.T. Roozen, Anna H.E. Roukens, Meta Roestenberg

Lancet Global Health (2022)



Abstract

Despite tremendous efforts, worldwide COVID-19 vaccination coverage is lagging. Dose-sparing strategies for COVID-19 vaccines can increase vaccine availability to address the global crisis. Several clinical trials evaluating dose sparing are currently under way. However, to rapidly provide solid scientific justification for different dose-sparing strategies, joint coordinated action involving both public and private parties is needed. In this Viewpoint, we provide examples of approaches to vaccine dose-sparing that have previously been evaluated in clinical trials to improve vaccine availability and reflect on the origin of their funding. With a focus on the current COVID-19 pandemic, we stress the need for expedited testing of vaccine dose-sparing strategies in endemic or epidemic infectious diseases. However, we argue that the establishment of a mechanism through which dose-sparing opportunities are systematically identified, scientifically tested, and ultimately implemented will prove to be valuable beyond the current pandemic for infectious diseases product development and pandemic preparedness in the future.

Introduction

The emergence of the SARS-CoV-2 coronavirus has burdened health systems worldwide. COVID-19 still poses a threat to global health, with nearly 67 000 new fatalities a week, as of February.[1] Additionally, the measures to contain COVID-19 take an enormous social and economic toll.

Vaccination is a highly cost-effective tool to curtail cases in epidemic and pandemic infectious diseases. At a remarkable pace, new COVID-19 vaccines have been developed, tested and registered. Currently, there are nine vaccines that are used widely, effectively reducing infection, severe disease and death worldwide.[2] Real world data from Israel, the United Kingdom (UK), Sweden and the United States, showed that full vaccination with the BNT162b2 (Pfizer-BioNTech Comirnaty®) or mRNA-1273 (Moderna Spikevax®) vaccine protected adults for 61-92% against infection, 80-87% against hospitalization and 85% against death for the viral variants that were prevalent at the time these studies were conducted.[3]

Although highly effective vaccines are available and have a proven effect on pandemic control, less than 15% of people in low-income countries have been (partially) vaccinated so far.[4] This is in stark contrast to high-income countries (HICs), where more than 180 vaccinations per 100 citizens have been given.[4] This leaves a staggering 2.7 billion people still to be vaccinated globally. COVID-19 Vaccines Global Access (COVAX), aimed to provide enough vaccines to vaccinate 40% of the adult population of 92 lower income economies participating in the COVAX Advance Market Commitment by the end of 2021, but reached only 20% by the end of the year.[5] The delay in vaccination leads to enormous preventable morbidity and mortality and puts more strain on health care systems that were already heavily burdened before the pandemic.[6]

Increasing access to vaccines in low- and middle income countries (LMICs) is a complex challenge: limited supplies in vaccines, HIC vaccine nationalism,[7] vaccine hesitancy, and complications in distribution and registration[6, 8] all play a part. Although these problems require societal, political, logistical and infrastructural solutions, scientific justification for alternative dose-sparing strategies are needed to facilitate resolution of shortages.

New approaches to dose sparing and vaccination regimens

Fractional dosing

Fractional dosing, administering only a part of a registered dose, has been an important strategy to provide more vaccine doses in epidemic circumstances in the past. In 2016, a yellow fever epidemic plagued Angola and the Democratic Republic of Congo (DRC) with an estimated 7 000 cases.[9] Faced with a substantial global shortage, the World Health Organization (WHO)

reviewed the available evidence, and advised on fractional dosing to combat the epidemic.[10] Together with the WHO, the DRC government launched a vaccination campaign with a one fifth fractional dose. In one week, more than seven million people were vaccinated, preventing the spread of the disease in the capital Kinshasa.[11]

In most dose-finding studies for COVID-19 vaccines, several doses (based on results from animal testing) were evaluated for tolerability and immunogenicity. During that initial period of vaccine development in early 2021, it was unclear whether antibody concentrations and T cell responses would correlate to protective efficacy, so the most certain strategy was to continue with the highest tolerated dose from the Phase 2 trial into the Phase 3 trial. For the mRNA-1273,[12] ChAdOx1 nCoV-19 (AstraZeneca Vaxzevria®)[13] and BNT162b2[14] vaccines, this meant that the highest dose from the dose-finding trial was used in the larger efficacy trial. This makes it likely that some of these vaccines are actually over-dosing and that lower doses would probably lead to comparable, or overall acceptable protective efficacy.[15]

Whereas fractional doses have been investigated to booster fully vaccinated populations, trials comparing fractional versus full dose priming regimens are scarce. One such a study has been conducted by La Jolla Centre for Immunology funded by the United States National Health Institute, which evaluated immunogenicity in participants 6 months after receiving a 1/4th fractional primary regimen of the mRNA-1273 vaccine. Despite the fact that neutralizing antibody responses in the low-dose vaccine group about half of those vaccinated with the registered dose,[16] we can now estimate that even the low-dose would yield a more than 80% efficacy base on the model created by Khoury et al.[17] A second example is the fractional dosing scheme unintentionally introduced in a sub-group of the Phase 3 study of the ChadOx1 nCoV-19 vaccine. Participants in this group were primed with a half dose, followed by a regular dose booster. This led to a protective efficacy of 90% (95% confidence interval: 67% to 97%).[18]

The results of these trials underline that there is no absolute linear correlation between dose and efficacy, e.g. a one fifth fractional dose does not reduce efficacy to one fifth. As a consequence fractional dosing will yield higher levels of cumulative immunity with the same amount of vaccine.[15] In times of an outbreak, fractionation can thus provide an immediate solution which should be considered when dealing with vaccine shortages.[15]

Intradermal vaccination

Intradermal (ID) vaccination provides opportunities to further increase vaccine efficacy of fractioned doses by administering the vaccine into the dermis, which is rich in antigen-presenting cells. Consequently, ID vaccination requires a lower dose than intramuscular (IM) vaccination, making it a valid strategy for dose sparing. ID vaccination is already in use for influenza and rabies vaccination where non-inferiority for immunogenicity has been demonstrated when administering a 20% to 60% fractional dose.[19] For the tuberculosis vaccine (Bacillus Calmette-Guérin [BCG]), ID administration is already the standard of care

and the WHO approves ID vaccination as a way of dose sparing for rabies and inactivated polio vaccine.[20, 21]

We have previously assessed the safety and immunogenicity of both a one tenth and a 1/5th fractional ID vaccination with the mRNA-1273 vaccine. Funded through crowdfunding and philanthropic organizations, we found this strategy to be safe and well tolerated. Both low-dose regimens elicited higher anti-spike (anti-S) and anti-regional binding domain (anti-RBD) IgG concentrations than in a comparative convalescent serum group and comparable to a group that received the full IM dose.[22] A larger study to compare levels of neutralizing antibodies head-to-head with the registered IM dose is on its way (EudraCT: 2021-000454-26).

Concerns about the ID vaccination technique have been posited as potential drawbacks for large scale implementation as a dose-sparing method. Although ID vaccination is technically more challenging than IM vaccination, the technique can be acquired after some training and is already used extensively for BCG vaccination worldwide. After ID vaccination, the appearance of a wheal provides immediate feedback on correct administration of the vaccine, facilitating training and quality control. Additionally, novel application devices such as intradermal applicators or needle-free injection devices can further facilitate mass vaccination campaigns.[23]

Heterologous vaccine regimens

To increase flexibility of vaccination programs in times of vaccine shortage, knowledge about "mix and match" strategies is crucial when different vaccines are available. Various publicly funded studies have shown that combinations of ChAdOx1 nCov-19, Ad26.CoV.S (Janssen Jcovden®), mRNA-1273 and the BNT162b2 vaccines are safe, well tolerated and immunogenic, sometimes even more immunogenic than homologous regimens.[24-28] A study from the United States Institute of Allergy and Infectious Diseases found that homologous regimens increased neutralizing antibody titers 4.2-20 fold, whereas heterologous regimens increased titers 6.2-76fold.[24] In all trials, regimens that contained at least one mRNA vaccine induced higher neutralizing antibody titers than regimens that only contained viral vector vaccines.[24, 27, 28]

Early knowledge on heterologous regimens can assure continuation of vaccination programs when supplies of certain vaccines are delayed and others are still available. The use of heterologous regimens can also aid campaigns where one vaccine is temporarily not given due to safety concerns.

Dose stretching

In December 2020, the UK government decided to prioritize giving the first COVID-19 vaccine to as many people in at-risk groups as possible, rather than providing second vaccinations. A study funded by the National Institute for Health Research, AstraZeneca and others evaluated the dose stretching approach. The study found that a longer interval between two doses of the ChAdOx1 nCov-19 vaccine led to higher antibody levels than shorter intervals. Antibody levels

were 923 ELISA Units with an 8–12 week interval; 1860 ELISA Units with a 15–25 week interval, and 3738 ELISA Units with a 44–45 week interval.[29] Concerns were raised that expanding the fraction of the population with partial immunity could increase selection for vaccine-escape variants. However, others argued that the corresponding reduction in prevalence and incidence reduced the rate at which new variants are generated and the speed of adaptation.[30] The dose stretching approach enabled the UK to provide at least one vaccine to almost half of its population in the first 3 months of its vaccination campaign.[4] This example illustrates how central coordination and rapidly launched trials can aid in making policies that improve vaccine access.

Pandemic preparedness

During the COVID-19 pandemic, new vaccines were developed at an unprecedented pace. The development process was accelerated in multiple ways: running the different clinical testing phases in parallel, rolling reviews by the regulatory authorities, and starting large-scale production before regulatory approval (Figure 1).[31] However, upscaling of production capacity takes time and currently there are still not nearly enough vaccines to meet global needs. As of December 2021, COVAX has distributed 1.2 billion doses to LMICs.[5] If these doses had been administered with a 1/5th fractionation, the entire eligible population of countries receiving COVAX vaccines could have already been fully vaccinated with these vaccines.[32]

In future pandemics, it is inevitable that we will be confronted with vaccine shortages once again when new vaccines become available. That is why dose-sparing mechanisms should be identified and tested as soon as new vaccines have demonstrated to be safe (Figure 1). Ideally, such dose-sparing approaches are immediately evaluated in parallel with the pre-licensure Phase 2 and 3 trials. However, in this stage of development it is still unclear whether a vaccine will be licensed at all and it therefore stands to reason to evaluate dose sparing only after licensure. In post-licensure Phase 2 trials, promising dose-sparing strategies could be quickly evaluated, followed by larger post-licensure Phase 3 trials to assess efficacy of these strategies. With the identification of immunological correlates of protection, these Phase 3 trials would not necessarily have to be as large-scale as the initial Phase 3 trials.[17]

By the time pharmaceutical companies have registered and marketed a new vaccine, there is little financial incentive to evaluate dose-sparing mechanisms. As the aforementioned trials illustrate, dose-sparing trials are typically initiated in the public scientific domain.

In the current COVID-19 crisis, dose-sparing trials eventually came to be as governments rolled out their national vaccination campaigns, which provided access to vaccines for public institutions to conduct trials with. In most places, this happened 3 to 4 months after the first vaccines got licensed. This is a considerable delay given the only very short timelines of clinical development to licensure (around 10 months). Ideally, dose-sparing strategies are

tested immediately after licensure as part of coordinated effort between industry and public parties to improve global access. Research funding bodies that use public money to fund the development of vaccines should use these financial investments as leverage to demand trial designs that assess dose-sparing regimens, not only in Phase 1 but also in the later stages of clinical development.

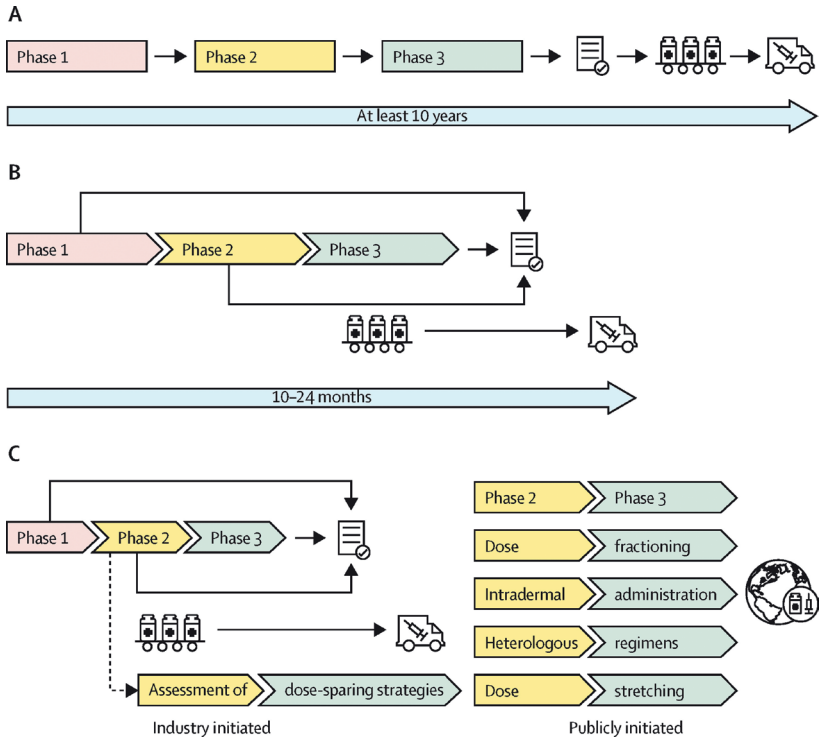


Figure 1. Vaccine development: conventional, COVID-19 and future pandemics

A. Conventional vaccine development: sequential clinical trial phases followed by regulatory review, production and distribution. **B.** Vaccine development during COVID-19 pandemic: clinical trial phases overlap; regulatory authorities apply rolling review procedures and pharmaceutical companies start production before approval (financial risk partly covered by governments). **C.** Optimal future pandemic preparedness: pre-approval phases as in B, after which international public body stimulates and coordinates new trials to evaluate strategies to improve global vaccine access. Promising strategies are evaluated in Phase 2/3 trials. Ideally, this evaluation already starts as soon as industry-initiated Phase 2 is completed.

Adapted with permission, from: Krammer F. SARS-CoV-2 vaccines in development. Nature. 2020;586(7830):516-27.

Currently, there is no infrastructure in place to systematically coordinate and fund post-licensure trials. CEPI has made a first attempt by launching a funding opportunity for trials assessing fractional dosing, but again this application of dose-sparing vaccination is intended as a way of boosting immunity in fully vaccinated populations.[33] Albeit important, insights

gained by this initiative will only benefit countries whose populations have for the most part been fully vaccinated, and not those countries that are still at the beginning of their vaccination campaigns. We thus argue that joint, coordinated efforts are needed to provide the infrastructure for rapid testing of dose sparing.

Improving worldwide immunity against COVID-19 is a multifaceted challenge involving limited vaccine supplies, vaccine hesitancy and logistical problems. Overcoming these difficulties requires coordination, collaboration and a globalist view on health. Creative scientific innovations can provide a solid foundation to a comprehensive approach that includes societal, political, logistical and infrastructural solutions to improve the availability of vaccines. At the same time, these innovations require robust scientific evidence to avoid providing substandard vaccines to LMICs.

We believe that in times of shortage, the scientific community and the pharmaceutical industry have a moral obligation to rapidly identify and test dose-sparing strategies and unleash the full potential of available vaccine doses to save lives. Creating the infrastructure to collaboratively conduct post-licensure trials will not only help address one of the biggest global health challenges so far, but also contribute to our preparedness for new pandemics that will undoubtedly follow.

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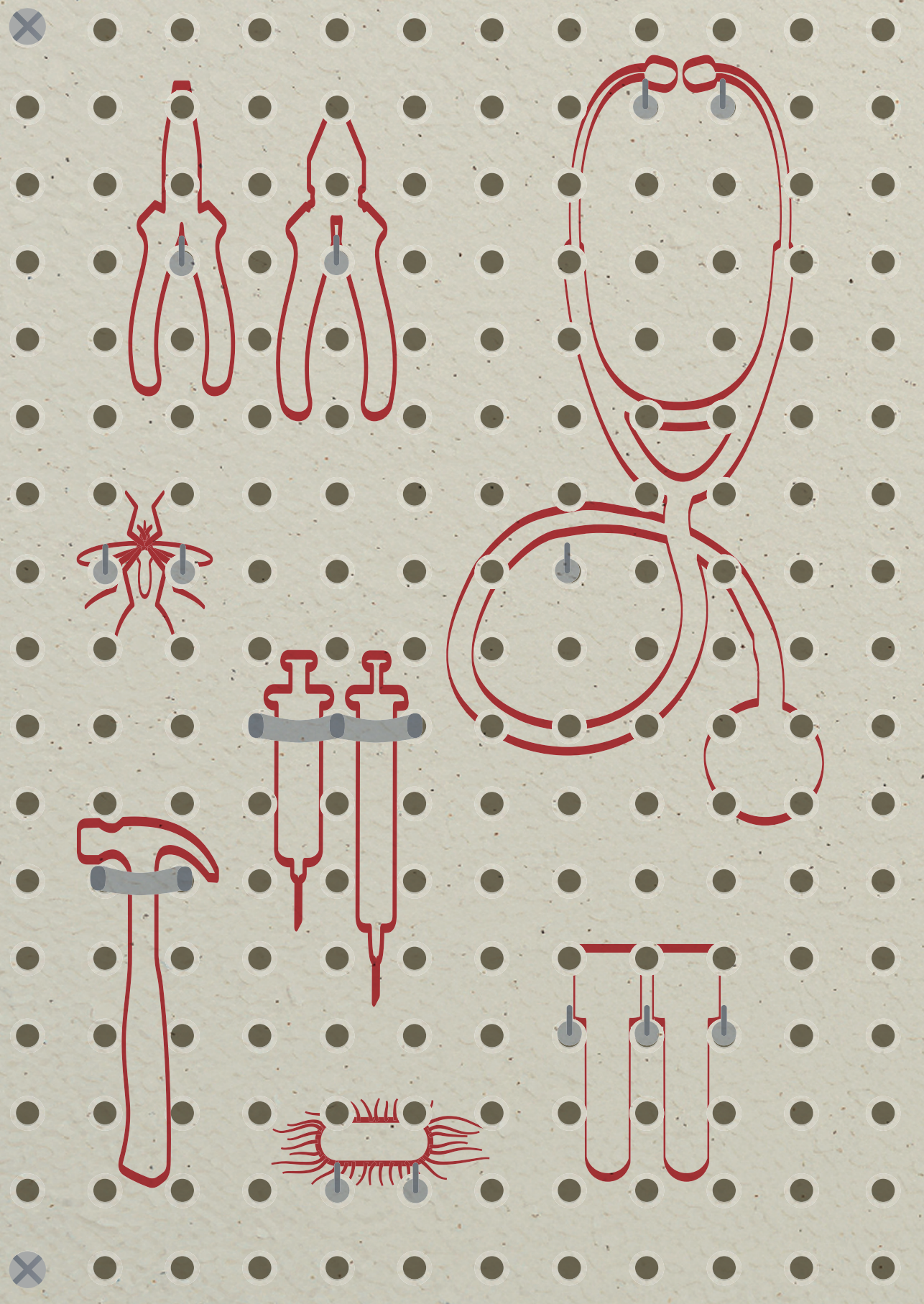
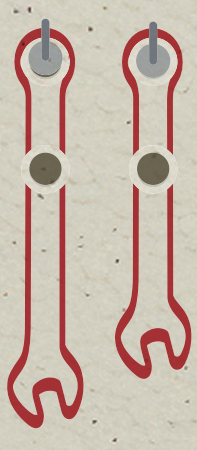


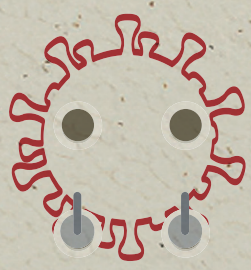


5

Safety and Immunogenicity of Intradermal Fractional Dose Administration of the mRNA-1273 Vaccine: A Proof-of-Concept Study

Geert V.T. Roozen, Margaretha L.M. Prins, Rob van Binnendijk, Gerco den Hartog, Vincent P. Kuiper, Corine Prins, Jacqueline J. Janse, Annelieke C. Kruithof, Mariet C.W. Feltkamp, Marjan Kuijer, Frits R. Rosendaal, M.D., Meta Roestenberg, Leo G. Visser, Anna H.E. Roukens

Annals of Internal Medicine (2022)



Abstract

Background

There is an urgent need for fair and equitable access to safe and effective COVID-19 vaccines. Intradermal (ID) delivery is a dose-sparing technique that can be used to immunize more people with the same limited vaccine stockpile.[1] The papillary dermis contains a higher density of antigen-presenting cells than muscle tissue; therefore, ID delivery of a fractional vaccine dose into this skin layer can be as effective as intramuscular (IM) administration of the standard dose.[2]

Objective

To assess the safety, tolerability, and immunogenicity of ID fractional dose administration of the mRNA-1273 (Moderna Spikevax®) vaccine as a potential dose-sparing strategy.

Methods and findings

We conducted a proof-of-concept, dose-escalation, open-label, randomized controlled trial in a tertiary medical center in Leiden, the Netherlands. Participants were recruited in April and May 2021 from a database of people who had previously shown interest in participating in upcoming COVID-19 vaccine trials. Eligible participants were healthy adults aged 18 to 30 years with no history of COVID-19. At every visit, participants were screened for past SARS-CoV-2 infection via serologic testing and polymerase chain reaction and were excluded from further participation if results were positive.

In part one, 10 participants received 10 μg of mRNA-1273 vaccine (0.05 mL [1/10th of the standard dose]) intradermally at days 1 and 29. In part two, 30 participants were randomly assigned in a 1:1 ratio to receive a 20-μg dose of mRNA-1273 (0.01 mL [1/5th of the standard dose]) either intradermally or intramuscularly at days 1 and 29. All ID vaccinations were administered using a Becton Dickinson U-100 Micro-Fine insulin syringe with an integrated 29G needle.

Diaries were used to collect self-reported local and systemic adverse events for 14 days after every vaccination (Supplement Sections A and B). Concentrations of IgG- and IgA-binding antibodies against SARS-CoV-2 spike S1 and receptor-binding domain (RBD) and virus neutralization titers were measured at day 36, day 43, and month 7 (Fig. 1).

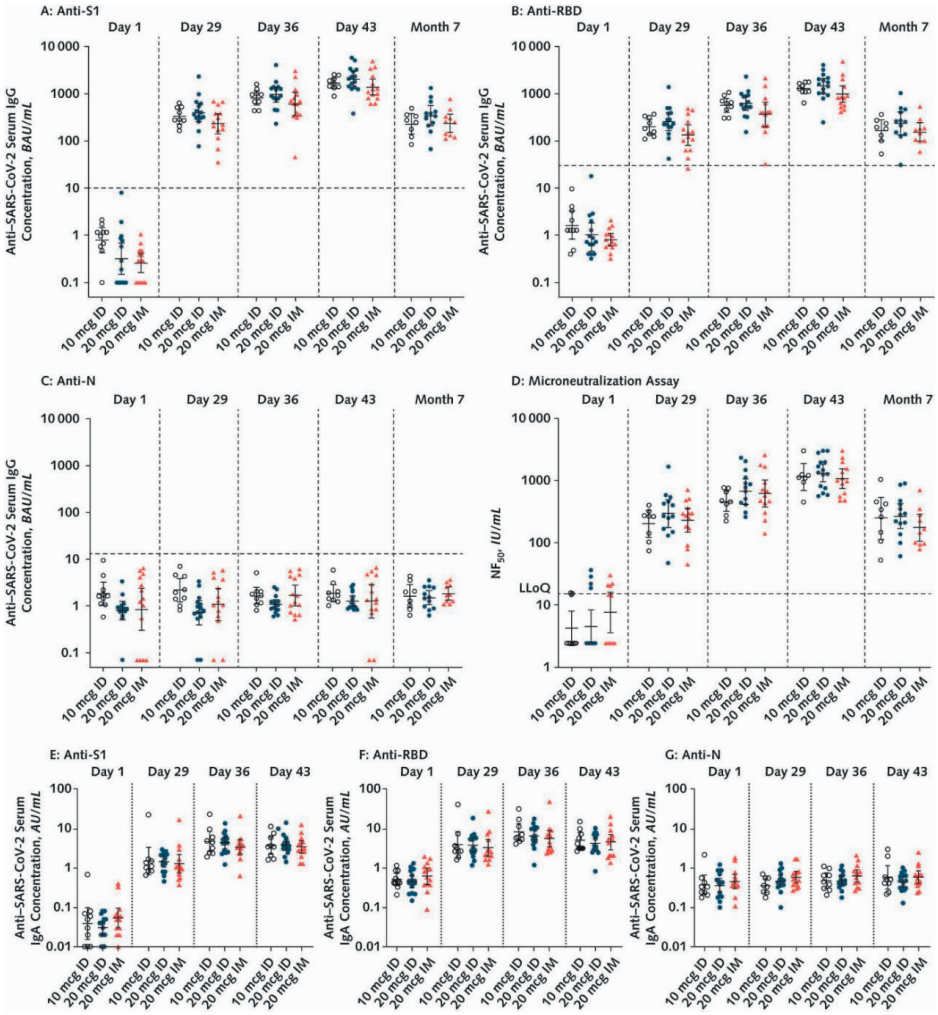


Figure 1. Serum anti–SARS-CoV-2 antibody concentrations

A, B, and C. Anti–SARS-CoV-2 serum IgG concentrations for anti-S1 (A), anti-RBD (B), and anti-N (C), assessed by bead-based immunoassay and reported in BAUs per milliliter. Data at day 1 (before receipt of the first vaccination), day 29 (before receipt of the second vaccination), day 36 (1 week after receipt of the second vaccination), day 43 (2 weeks after receipt of the second vaccination), and month 7 (half a year after receipt of the second vaccination) are reported for the 10- µg ID dose, the 20- µg ID dose, and the 20-µg IM dose. Horizontal dashed lines represent the cutoff for seropositivity (10 BAU/mL for anti-S1, 30 BAU/mL for anti-RBD, and 14 BAU/mL for anti-N). **D.** Wild-type SARS-CoV-2 (Wuhan strain) microneutralization assay. NF_{50} is reported in international units per milliliter. The conversion rate from titer to international units was 1 / 4.064. The horizontal dashed line represents the LLoQ at 15 IU/mL (titer of 62). Values with an NF_{50} below the LLoQ were set to 2 IU/mL (titer of 10). Values above the upper limit of quantitation (n=2 in the 20-µg ID group and 1 in the 20-µg IM group at day 43) were set to 2953 IU/mL (titer of 12 000). **E, F, and G.** Anti–SARS-CoV-2 serum IgA concentrations for anti-S1 (E), anti-RBD (F), and anti-N (G), assessed by bead-based immunoassay and reported in AUs per milliliter. Data at days 1, 29,

36, and 43 are reported for the 10-µg ID dose, the 20-µg ID dose, and the 20-µg IM dose.
Each symbol represents a sample from an individual participant at a certain time point. Error bars represent geometric mean concentrations with 95% confidence intervals.
Anti-N = anti-nucleocapsid; anti-RBD = anti–receptor-binding domain; anti-S1 = anti–spike S1; AU = arbitrary unit; BAU = binding antibody unit; ID = intradermal; IM = intramuscular; LLoQ = lower limit of quantitation; NF_{50} = neutralizing factor at 50% normalized against an international reference serum.

Thirty-eight of 40 participants remained in the study through day 43, and 31 remained through month 7 (Supplement Section C). The main reasons for premature study termination were COVID-19 or receipt of an additional vaccination elsewhere (Supplement Section C).

The average wheal sizes after the first and second 10-µg ID vaccinations were 8 mm (SD: 1) and 7 mm (SD: 1), respectively. For the 20-µg dose, the average wheal sizes were 8 mm (SD: 1) and 10 mm (SD: 1), respectively, for the first and second vaccinations.

No serious adverse events occurred. The most commonly reported adverse events were short-lasting and consisted of mild pain, itching, erythema, and swelling at the injection site (Table 1, Supplement Sections E and F). One participant in the 20-µg ID group reported severe erythema of more than 10 cm in diameter and moderate swelling. This lasted 6 days and was self-limiting and well tolerated (Supplement Section G).

Table 1. Local and systemic adverse events related to vaccination

Event	Vaccination 1			Vaccination 2		
	10 µg ID (n=10)	20 µg ID (n=15)	20 µg IM (n=15)	10 µg ID (n=9)	20 µg ID (n=15)	20 µg IM (n=14)
Local adverse events						
Mild hyperpigmentation	1 (10.0)	2 (13.3)	0 (0.0)	2 (22.2)	1 (6.7)	0 (0.0)
Mild local muscle stiffness	4 (40.0)	4 (26.7)	12 (80.0)	0 (0.0)	6 (40.0)	9 (64.3)
Moderate local muscle stiffness	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (6.7)	0 (0.0)
Mild itching at injection site	2 (20.0)	8 (80.0)	0 (0.0)	3 (33.3)	10 (66.7)	0 (0.0)
Mild pain at injection site	7 (70.0)	11 (73.3)	12 (80.0)	6 (66.7)	11 (73.3)	9 (64.3)
Moderate pain at injection site	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (13.3)	0 (0.0)
Mild swelling	1 (10.0)	5 (33.3)	1 (6.7)	1 (11.1)	2 (13.3)	2 (14.3)
Moderate swelling	0 (0.0)	3 (20.0)	0 (0.0)	0 (0.0)	3 (20.0)	0 (0.0)
Mild erythema	3 (30.0)	10 (60.0)	0 (0.0)	5 (55.6)	3 (20.0)	2 (14.3)
Moderate erythema	0 (0.0)	2 (13.3)	1 (6.7)	0 (0.0)	10 (66.7)	0 (0.0)
Severe erythema	0 (0.0)	1 (6.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Table 1. Local and systemic adverse events related to vaccination (continued)

Event	Vaccination 1			Vaccination 2		
	10 µg ID (n=10)	20 µg ID (n=15)	20 µg IM (n=15)	10 µg ID (n=9)	20 µg ID (n=15)	20 µg IM (n=14)
Mild axillar lymphadenopathy	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (13.3)	0 (0.0)
Systemic adverse events						
Mild nausea and vomiting	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (6.7)	0 (0.0)
Mild myalgia	0 (0.0)	2 (13.3)	2 (13.3)	0 (0.0)	3 (20.0)	2 (14.3)
Moderate myalgia	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (6.7)	2 (14.3)
Mild headache	0 (0.0)	3 (20.0)	5 (33.3)	0 (0.0)	3 (20.0)	3 (21.4)
Moderate headache	1 (10.0)	1 (6.7)	0 (0.0)	0 (0.0)	2 (13.3)	2 (13.4)
Mild diarrhea	0 (0.0)	0 (0.0)	1 (6.7)	0 (0.0)	0 (0.0)	0 (0.0)
Mild chills	0 (0.0)	1 (6.7)	1 (6.7)	0 (0.0)	1 (6.7)	1 (7.1)
Moderate chills	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (7.1)
Mild fever	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Mild fatigue and malaise	0 (0.0)	5 (20.0)	5 (20.0)	0 (0.0)	5 (20.0)	1 (7.1)
Moderate fatigue and malaise	0 (0.0)	1 (6.7)	0 (0.0)	0 (0.0)	5 (20.0)	3 (21.4)
Mild arthralgia	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Moderate arthralgia	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (7.1)

Data are numbers (percentages) of participants who experienced the adverse event. All adverse events possibly, probably, or definitely related to the vaccine in the 28 days after administration are reported. Grade 4 (potentially life-threatening) adverse events did not occur.
ID = intradermal; IM = intramuscular.

All participants showed robust antibody responses at day 43 that were still detectable at month 7. The binding antibody responses for anti-S1 IgG, anti-RBD IgG, and neutralizing antibodies showed similar patterns (Fig. 1 A-D, Supplement Sections H and I). At day 43, geometric mean concentrations of neutralizing antibody were 1115 IU/mL (95% CI: 669 to 1858 IU/mL) for the 10-µg group, 1300 IU/mL (CI: 941 to 1796 IU/mL) for the 20-µg ID group, and 1052 IU/mL (CI: 733 to 1509 IU/mL) for the 20-µg IM group. The IgA responses were similar between groups, independent of dose or method of administration (Fig. 1E-G, Supplement Section J).

Discussion

Intradermal delivery of a two-dose regimen of mRNA-1273 vaccine at 10 or 20 µg was safe, was well tolerated, and induced durable antibody responses.

This study has two limitations. First, although the IgG, IgA, and neutralizing antibody concentrations were highest in the 20-µg ID group, the sample size did not allow demonstration of statistically significant superiority of ID over IM injection. Second, only healthy volunteers aged 18 to 30 years were included, so the findings on safety and immunogenicity may not apply to the general population.

Although true vaccine efficacy depends on several factors, antibody concentrations measured after fractional dose vaccination in our trial are within the ranges that correlated with high levels of protection in the mRNA-1273 Phase III trial.[3] This is especially true for the 20-µg ID group.

In conclusion, the safety and immunogenicity results from this trial strongly support advancement of the investigation of ID vaccination with the mRNA-1273 vaccine.

Acknowledgment

The authors thank all of the trial participants for their contributions and the members of the data safety monitoring board for their invaluable advice. They also thank all of the people who donated to the crowdfunding that supported the trial. The authors acknowledge the support of CEPI and the participation of Viroclinics for performance of the neutralization assay.

Supplemental material

Supplemental material is available online: https://www.acpjournals.org/doi/10.7326/M22-2089

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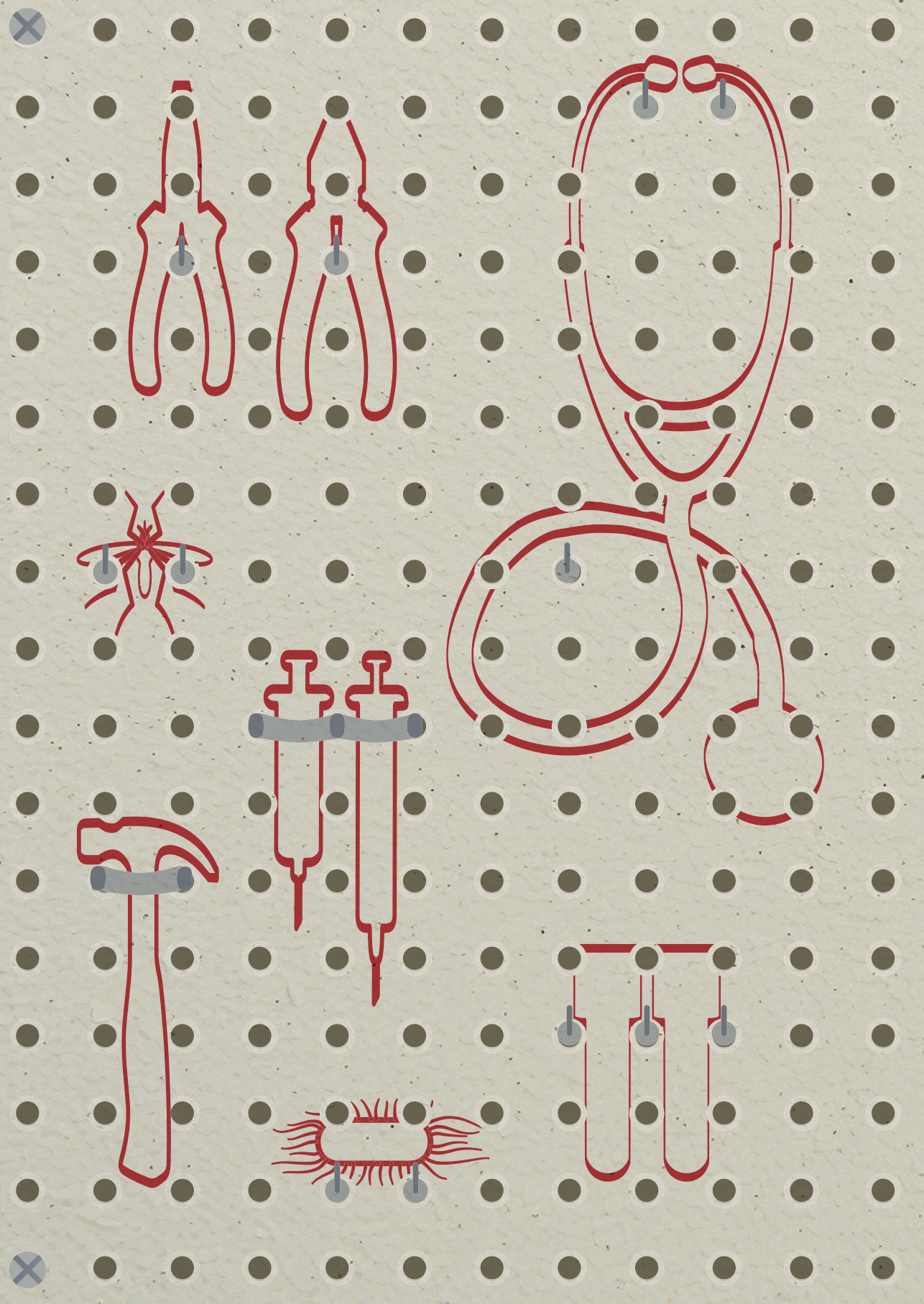

6

Immunogenicity and Reactogenicity of Intradermal mRNA-1273 SARS-CoV-2 Vaccination: a Non-Inferiority, Randomized-Controlled Trial

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NPJ Vaccines (2024)

Abstract

Fractional dosing can be a cost-effective vaccination strategy to accelerate individual and herd immunity in a pandemic. We assessed the immunogenicity and safety of primary intradermal (ID) vaccination, with a 1/5th dose compared with the standard intramuscular (IM) dose of mRNA-1273 (Moderna Spikevax®) in SARS-CoV-2 naïve persons. We conducted an open-label, non-inferiority, randomized controlled trial in the Netherlands between June and December 2021. One hundred and fifty healthy and SARS-CoV-2 naïve participants, aged 18–30 years, were randomized (1:1:1) to receive either two doses of 20 µg mRNA-1273 ID with a standard needle (SN) or the Bella-mu® needle (BM), or two doses of 100 µg IM, 28 days apart. The primary outcome was non-inferiority in seroconversion rates at day 43 (D43), defined as a neutralizing antibody concentration threshold of 465 IU/mL, the lowest response in the IM group. The non-inferiority margin was set at −15%. Neutralizing antibody concentrations at D43 were 1789 (95% CI: 1488–2150) in the IM and 1263 (951–1676) and 1295 (1020–1645) in the ID-SN and ID-BM groups, respectively. The absolute difference in seroconversion proportion between fractional and standard-dose groups was −13.95% (−24.31 to −3.60) for the IDSN and −13.04% (−22.78 to −3.31) for the ID-BM group and exceeded the predefined non-inferiority margin. Although ID vaccination with 1/5th dose of mRNA-1273 did not meet the predefined non-inferior criteria, the neutralizing antibody concentrations in these groups are far above the proposed proxy for protection against severe disease (100 IU/mL), justifying this strategy in times of vaccine scarcity to accelerate mass protection against severe disease.

Introduction

Safe and effective vaccines have proven to be the cornerstone of success in the battle against SARS-CoV-2 during the COVID-19 pandemic, but vaccine inequity remains a challenge across the globe.[1, 2] Vaccine dose-sparing techniques, such as intradermal (ID) administration, may offer an important advantage in (emergency) mass immunization campaigns as more people can be vaccinated with the same stockpile, with the potential additional advantage of fewer side effects.[3]Modeling has shown that, even if vaccine efficacy of fractional dose is lower than that of full dose vaccination, fractional dosing of SARS-CoV-2 vaccines could be a very cost-effective vaccination strategy and reduce a large number of deaths in lower- and middle-income countries.[4]

In ID administration, the vaccine is introduced directly into the papillary dermis, where antigen-presenting cells are abundantly present. A 1/10th or 1/5th fractional vaccine dose can induce protective immune responses equivalent to the standard dose delivered intramuscularly (IM), as has been shown for many vaccines such as rabies, yellow fever, poliomyelitis, and seasonal influenza vaccine.[5] Since ID delivery is considered technically more difficult than IM vaccination, novel ID devices are being developed.[6] We chose the mRNA-1273 vaccine (Moderna Spikevax®) for ID delivery because at the beginning of the COVID-19 pandemic, only mRNA vaccines (mRNA-1273 and BNT162b2 vaccine [Pfizer-BioNTech Comirnaty®]) were available in the Netherlands. In addition, ID delivery had never been studied with an mRNA vaccine and if this was safe and effective, it could have major implications for the future of mRNA vaccines.

Recently, we demonstrated the safety and immunogenicity of two doses of 10 µg or 20 µg mRNA-1273 at 28-days-interval through the ID route in a proof-of-concept study.[77] The SARS-CoV2-spike-S1 and -RBD IgG-binding antibodies generated by 10 µg or 20 µg mRNA-1273 vaccine ID were similar in magnitude to the levels seen in subjects from an age-matched cohort vaccinated with 100 µg IM. These results justified a larger randomized-controlled, non-inferiority study. We investigated whether virus neutralizing antibody and binding antibody concentration elicited by two 1/5th doses of mRNA-1273 vaccine given at a 28-day interval by ID vaccination were non-inferior to those of a control group receiving two standard doses of mRNA-1273 vaccine. Additionally, we measured SARS-CoV-2-specific memory B- and T cell responses. Finally, we evaluated the performance of an easy-to-use ID microneedle to facilitate ID delivery on a wider scale

Results

Trial population

Between June 14th and July 8th of 2021, 165 participants were assessed for eligibility (Fig. 1). One-hundred and fifty eligible participants were enrolled and randomized to receive either 20 µg mRNA-1273 ID-SN (n=50), 20 µg mRNA-1273 ID-BM (n=50) or 100 µg mRNA-1273 IM (n=50). The participants' characteristics are shown in Supplementary Table 8. The median age was 22 years, and 63/150 (42%) of participants were female. All 150 participants received at least one vaccine dose. One hundred and forty-one participants (94%) received a second dose and completed all scheduled safety visits.

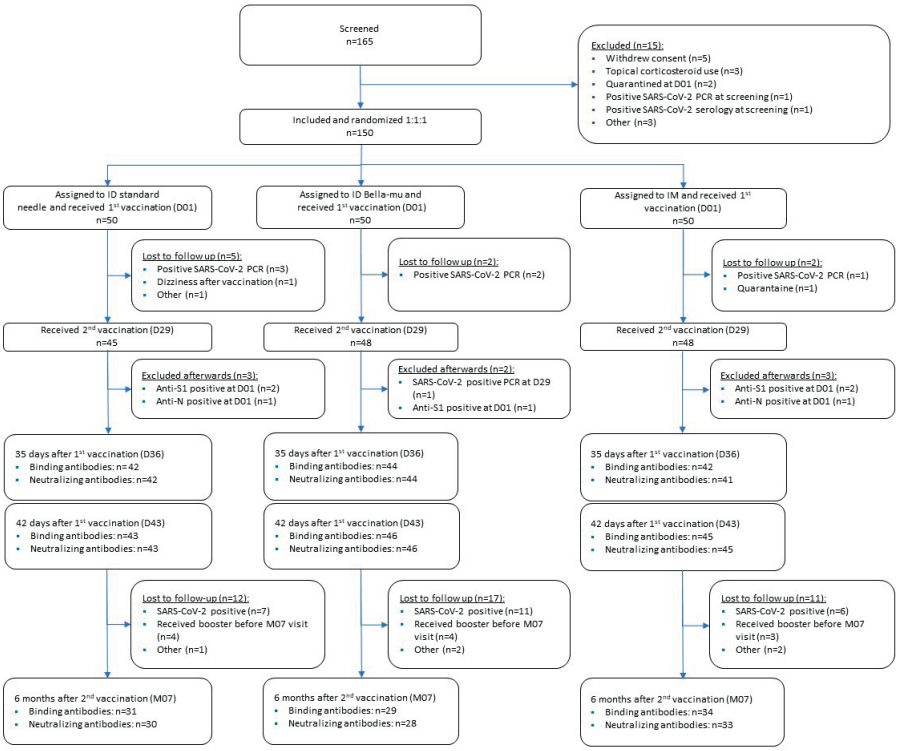

Figure 1. Flow-chart of inclusions

Among the 141 participants receiving a second vaccination, seven participants were excluded from the immunogenicity analysis afterwards due to seropositivity for IgG anti-S1 or anti-N at baseline, indicating an earlier unrecognized SARS-CoV-2 infection. One of them was one of the two participants who ended the study prematurely due to dizziness.
D = day; ID = intradermal; IM = intramuscular; M = months.

Several participants were excluded from the analysis for various reasons. This included participants with a positive SARS-CoV-2 PCR before D29 (n=6), participants who showed signs of past SARSCoV-2 infection based on baseline seropositivity for anti-S1 and/or anti-N IgG antibodies (n=7), and two seronegative participants from the IM group who displayed activated SARS-CoV-2-spikespecific B cells prior to vaccination, indicating recent infection. These two participants were excluded in the PP in-depth B cell analysis but not from the immunogenicity analysis to avoid bias, as in-depth B cell analysis was not performed in all participants. The ITT population for immunogenicity at D43 included 134 participants. Forty participants were excluded between D43 and M07, mainly due to intercurrent SARS-CoV-2 infection (n=24) or booster vaccination through the national vaccination campaign (n=11). The immunogenicity analysis at M07 included 94 participants who were tested for IgG-binding antibodies, 91 of which were also analyzed for virus neutralization.

All injections were considered successful, however, ID vaccination with the Bella-mu® needle elicited slightly smaller wheals (8 mm; IQR: 7–9; 95% CI: 8–9) than standard technique ID vaccination (9 mm; IQR: 9–10; 95% CI: 9–9).

Neutralization and binding antibody responses

The seroconversion rate at D43 was 100% in the IM group, whereas in the ID-SN and ID-BM groups, it was 86% (95% CI: 73.2–94.1) and 87% (74.8–94.5) (Table 1). The lower limit of the 95% CI for the difference in response compared with the IM group exceeded the predefined non-inferiority margin for both ID groups.

Table 1. Seroconversion and neutralization at day 43

	Total (n)	Seroconversion			Neutralization concentration, IU/mL (95% CI)
		n	% (95% CI)	Difference in response (%)	
20 µg ID-SN	43	37	86% (73.2-94.1)	-13.95% (24.31 to -3.60)	1263 (951-1676)
20 µg ID-BM	46	40	87% (74.8-94.5)	-13.04% (-22.78 to -3.31)	1295 (1020-1645)
100 µg IM	45	45	100 (93.6-100.0)	Ref	1789 (1488-2150)

BM = Bella-mu® needle; CI = confidence intervals; ID = intradermal; IM = intramuscular; IU = international units.

GMCs of neutralizing antibodies at D43 were highest in the standard dose IM group (Fig. 2A, Supplementary Table 9), with mean concentrations of 1789 (1488–2150) in the standard dose IM group and 1263 (95% CI 951–1676) and 1295 (1020–1645) in the ID-SN and ID-BM groups, respectively, with overlapping 95% CIs. At D43, GMCs of IgG-binding antibodies against SARS-CoV-2- spike-S1 were lower in the fractional dose ID groups than in the standard dose IM group, but 95% CIs were also overlapping (Fig. 2C, Supplementary Table 9). Similar results were observed for antibodies against SARS-CoV-2-spike-RBD (Supplementary Fig. 4, Supplementary Table 9).

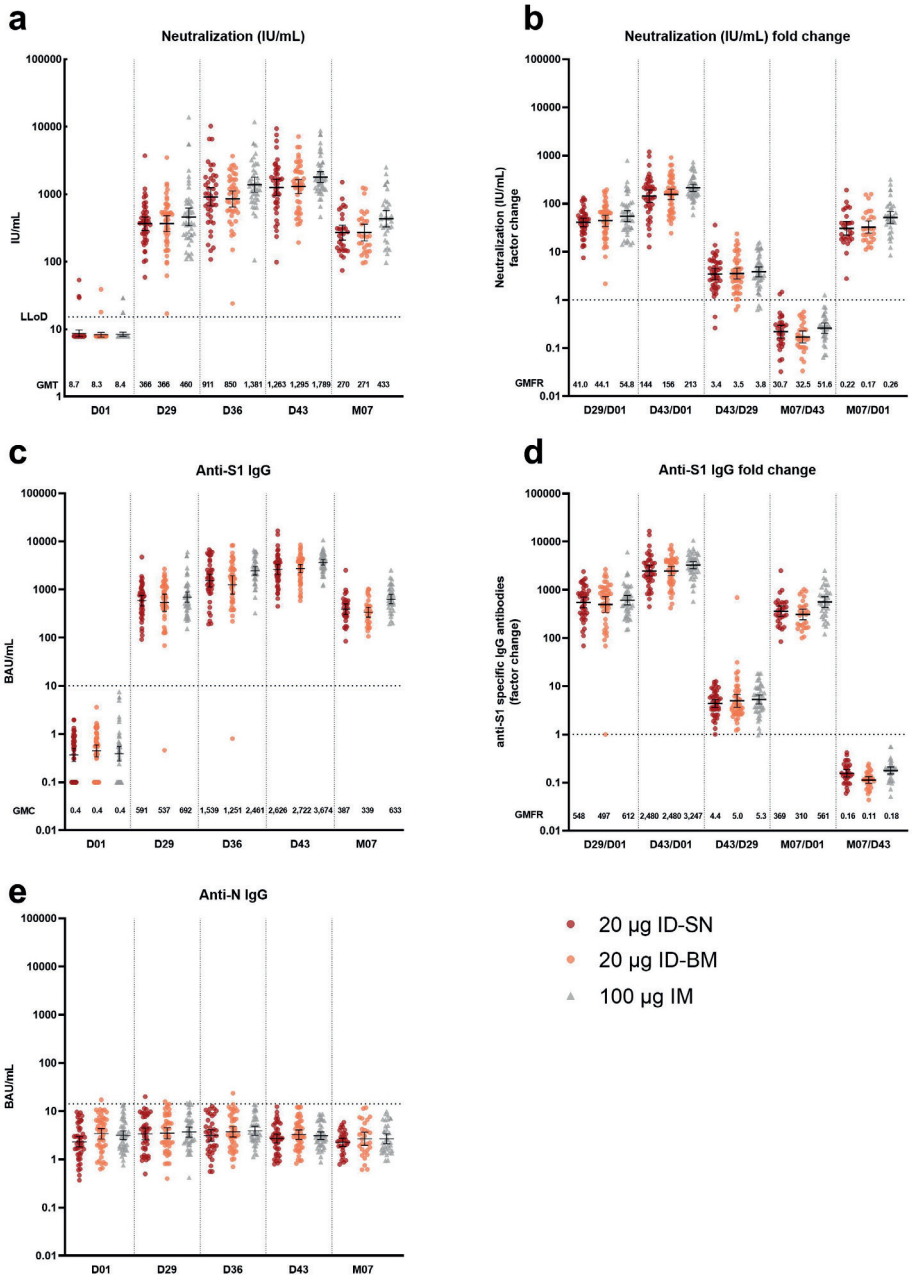

Figure 2. SARS-CoV-2 specific immune responses

A. Virus neutralization concentrations in international units per mL. Horizontal dotted lines represent the LLoD (=15.26 IU/mL). Results below the LLoD were arbitrarily set to half the LLoD. **B.** Neutralization concentration fold change. **C.** SARS-CoV-2 S1-specific IgG antibody concentrations by bead-based multiplex immunoassay (MIA) in binding antibody units per mL in the three groups at each timepoint.

Cut-off for seropositivity = 10.08 BAU/mL. **D.** Anti-S1-specific binding antibodies fold change. **E.** SARS-CoV-2 anti-N- specific IgG antibody concentrations by bead-based immunoassay (MIA) in binding antibody units per mL. Cut-off for seropositivity = 14.3 BAU/mL.
Each symbol represents a sample from an individual participant. Error bars represent the geometric mean with 95% confidence intervals. Horizontal dotted lines represents the cut-off for seropositivity (A and C) or a factor increase of 1, i.e., no increase or decrease (B and D). For the calculations of the fold change, values below 1 were set to 1 (B and D).
Anti-N = anti-nucleocapsid; anti-RBD = anti–receptor-binding domain; anti-S1 = anti–spike S1; BAU = binding antibody unit; BM = Bella-mu® needle; GMC = geometric mean concentration; GMFR = geometric mean fold rise; D = days; ID = intradermal; IM = intramuscular; IU/mL = international units per mL; LLoD = lower limit of detection; M = months; SN = standard needle.

At M07, GMCs remained elevated in all groups and were highest in the IM group (433; 95% CI 328–573) compared to both ID groups: 270 (209–349) in the ID-SN and 271 (205–359) in the IDBM group, with overlapping 95% CIs. Similar results were observed for the GMCs of the SARS-CoV-2 binding antibodies.

The change in GMCs between the different timepoints is shown in Fig. 2B and Supplementary Table 10.

B cell responses

Higher frequencies of SARS-CoV-2-spike-specific B cells were detected at D29, D43, and at M07 in participants receiving IM vaccination, compared to ID-SN vaccinated participants (Fig. 3A). The frequencies of SARS-CoV-2-spike-specific B cells increased further during the 7 months after first vaccination in both groups and the fold-change of percentages of SARS-CoV-2-spike-specific B cells at D43/29 and M07/D43 were similar between groups (Fig. 3B). Participants that received ID-SN vaccination had significantly more unswitched SARS-CoV-2 spike-specific B cells at D29 (IgMD) and D43 (IgD and IgMD), and significantly fewer IgG-switched B cells at D43, than IM vaccinated participants (Fig. 3C). No significant differences between isotypes were observed at M07, with almost all SARS-CoV-2 spike-specific B cells switched to IgG in both groups. Percentages of IgG-positive SARS-CoV-2-specific B cells correlated with the anti-S1-specific IgG antibody concentrations (Fig. 3D).

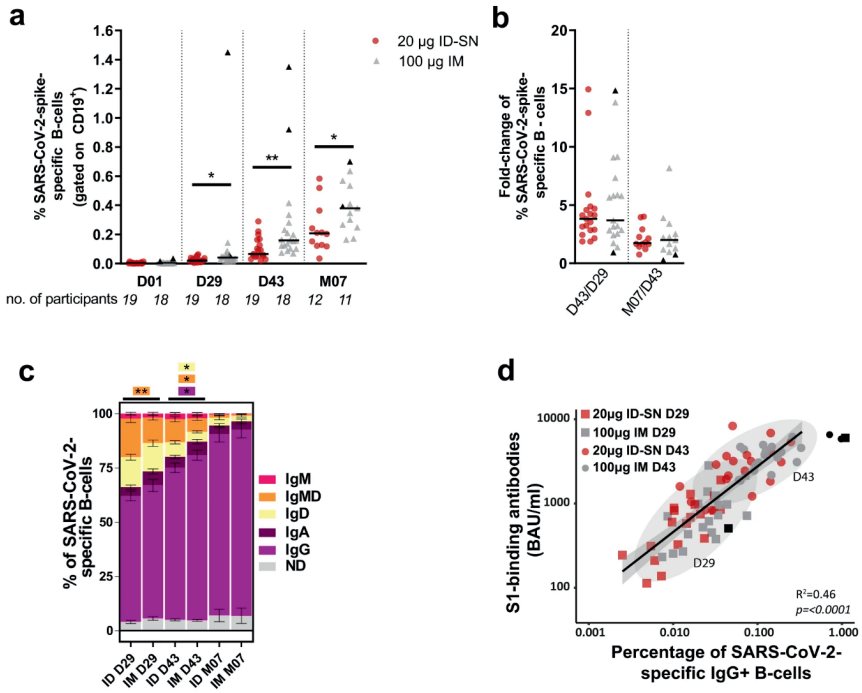

Figure 3. B cell compartment and the immunogenicity of intradermal and intramuscular delivery of mRNA SARS-CoV-2 vaccine, according to the per-protocol analysis

A. Percentages of B cells specific for SARS-CoV-2-spike-protein, shown as frequencies from total B cells per individual and vaccine delivery (IM, grey vs ID-SN, red). **B**. The fold-change of the frequencies of SARS-CoV-2-spike-specific B cells, 2 weeks after the second dose (D43/D29) and around 6 months after D43 (M07/D43). **C.** Isotype usage of SARS-CoV-2-spike-specific B cells as stacked bars at each timepoint for each vaccine delivery. **D.** Correlation plot between IgG$^+$ titers and IgG$^+$ SARS-CoV-2-spike-specific B cells. 95% CI are shown as ellipse for each timepoint. Pearson correlation analysis results are depicted and linear regression results shown as a black line with shaded 95% CI.
Individuals and median values are shown. The black data points (A, B, and D) represent the two individuals with the presence of SARS-CoV-2-specific B cells at D01 prior to vaccination, which were excluded in the PP analysis. Including these participants in the ITT analysis did not change the outcome. Individuals and median values are shown. Groups were compared using a Mann-Whitney U tests (* = p<0.05, ** = p<0.01). Anti-S1 = anti–spike S1; BAU = binding antibody unit; CI = confidence intervals; D = days; ID = intradermal; IM = intramuscular; ITT = intention-to-treat; M = months; PP = per-protocol; SN = standard needle.

T cell responses

Frequencies of spike-specific CD4$^+$ T cells increased with each dose in both groups until D43 and decreased slightly at M07 (Fig. 4A). At D43 and M07, all IM and ID-SN vaccinated individuals had a SARS-CoV-2-spike-specific CD4$^+$ T cell response above threshold (Fig. 4B).

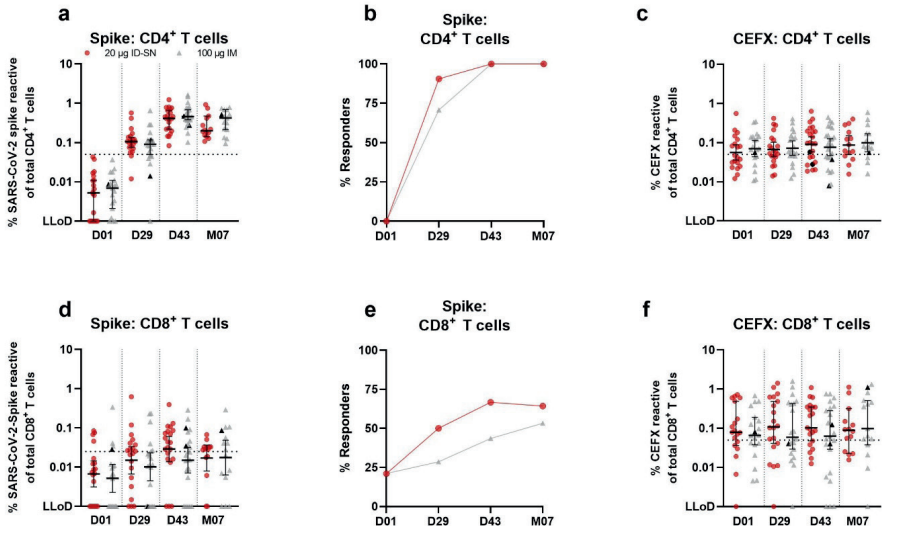

Figure 4. mRNA-1273 induced SARS-CoV-2-specific T cell responses

A. Frequency of spike-specific CD4$^+$ T cells in time. Spike-specific CD4$^+$ T cells were defined as the frequency of CD154$^+$ and/or CD137$^+$ cells of total CD4$^+$ T cells, corrected for background in DMSO control. Dotted line represents threshold for a response. **B.** Frequency of individuals with a spike-specific CD4$^+$ T cell response above threshold. **C**. Frequency of CEFX-specific CD4$^+$ T cells in time. **D.** Frequency of spike-specific CD8$^+$ T cells in time. Spike-specific CD8$^+$ T cells were defined as the frequency CD69$^+$ and/or CD137$^+$ cells of total CD8$^+$ T cells, corrected for background in DMSO control. Dotted line represents threshold for a response. **E.** Frequency of individuals with a spike-specific CD8$^+$ T cell response above threshold. **F.** Frequency of CEFX-specific CD8$^+$ T cells in time.
Each point represents a single subject. Error bars represent the median with 95% CI. The dotted line indicates limit of quantification. A pool of peptides derived from CEFX was used as a positive control and DMSO as a negative control. Black symbols in the IM group represent the two participants with suspected previous SARS-CoV-2 infection, based on SARS-CoV-2-spike-specific B-cells prior to vaccination (A, C, D, F). Groups were compared using a Mann-Whitney U tests (* = p<0.05, ** = p<0.01, *** = p<0.001).
CEFX = CMV, EBV, Flu and extra; CI = confidence intervals; D = days; DMSO = dimethyl sulfoxide; ID = intradermal; IM = intramuscular; LLoD = lower limit of detection; M = months; SN = standard needle.

In general, the SARS-CoV-2-specific CD8$^+$ T cell responses were lower and more variable compared to CD4$^+$ T cell responses (Fig. 4D, E and Supplementary Fig. 10C). For more details on the in-depth analysis of the B- and T cell response, see the Supplementary Appendix (Supplement I and J).

Vaccine safety

No serious AEs or severe COVID-19 cases were reported, and no pre-specified stopping rules were met. Solicited local and systemic AEs were mostly mild or moderate and transient in nature both after the first and second vaccination (Fig. 5 and Supplementary Table 13). Twenty-three of 150 participants (15.3%) had one or more severe (grade 3) AEs (Supplementary Tables 15 and 16), which were self-limiting and resolved within a few days.

Frequencies of AEs in the ID-SN and ID-BM groups were more or less the same (Supplementary Table 13). The three most commonly reported local AEs after ID injection were pain, erythema, and itch at the injection site (Fig. 5). Systemic AEs such as fatigue and malaise, headache, and chills, were more frequently reported in the IM group, especially after the second vaccination. The most common systemic solicited AEs after ID vaccination were fatigue and headache.

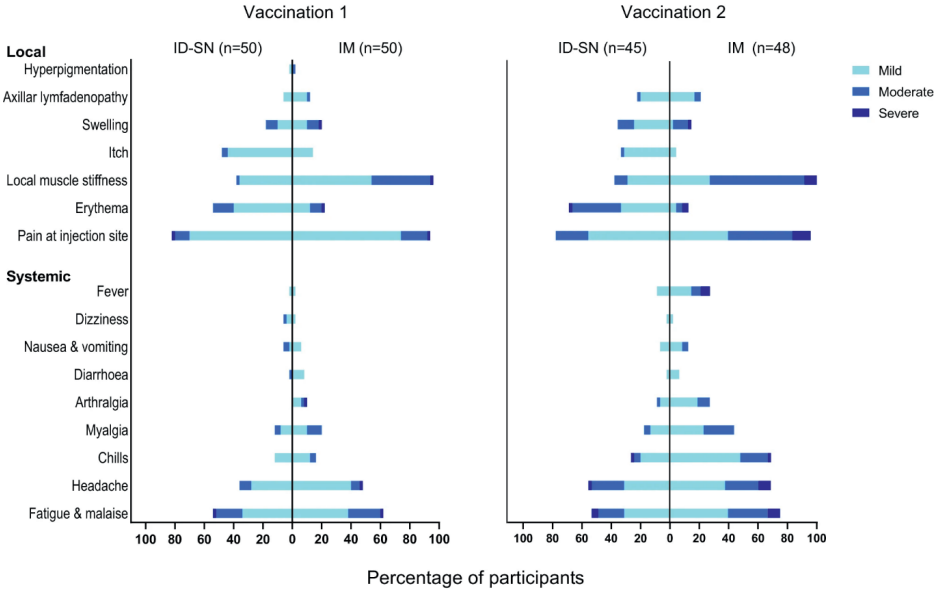

Figure 5. Adverse events related to vaccine administration in the ID-SN group and IM group

All adverse events possibly, probably or definitely related to the vaccination in the following 28 days after the first and second vaccine administration are reported. Adverse events are categorized as mild, moderate or severe. Grade 4 (potentially life threatening) adverse events did not occur. Hyperpigmentation, itch and dizziness are unsolicited adverse events. For adverse events in the ID-BM group, see Supplementary Table 12.
BM= Bella-mu® needle; ID = intradermal; IM = intramuscular; SN= standard needle.

Discussion

Intradermal delivery of two 1/5th fractional doses of the mRNA1273 vaccine given at a 28-day interval, either by standard needle or Bella-mu® 1.4 microneedle, elicited high levels of neutralizing antibody concentrations at D43 but did not meet non-inferior criteria compared with two standard doses of mRNA-1273 IM. In addition, SARS-CoV-2 B cells were also slightly lower in the ID groups, but T cell responses were comparable. Finally, ID vaccination elicited milder systemic AEs.

To our knowledge, this is the first randomized-controlled study in which the immunogenicity reactogenicity and in-depth T and B cell responses were evaluated after a primary ID vaccination

series with a fractional dose of mRNA-1273 vaccine. A study from Thailand evaluating different homo- and heterologous IM and ID regimens as primary series demonstrated that two ID doses generated similar SARS-CoV-2 anti-RBD IgG-antibodies as their respective standard IM-IM regimens, except for homologous BNT162b2 delivered ID.[8] However, the mRNA-1273 vaccine regimen was not evaluated.

Both binding and neutralizing antibodies have been proposed as a proxy for protection against symptomatic and severe COVID-19 disease.[9-13] However, all studies found that the level of protection evolved gradually with neutralization titer. Consequently, no specific cut-off level exists below which individuals lack protection or above which protection is guaranteed. In addition, establishing a universal threshold in international units poses challenges due to the absence of standardized assays across various studies.[14] At the start of this study, no cut-off level regarding neutralizing antibodies was known. Therefore, the definition of seroconversion for this study was based on a study from Jackson et al.[15] using the plaque reduction test, which is different from the MNA used in our study. Since the predefined seroconversion could not be used in our assessment of noninferiority, we chose the lowest neutralization concentration of the IM group (control group) as the cut-off for seroconversion, which was 465 IU/mL. Analysis of the Phase III study of mRNA-1273 suggested that protection against symptomatic COVID-19 disease was 91% and 96% with a day 57 neutralizing antibody concentration of 100 and 1000 IU/mL (50% virus neutralization), respectively.[11, 16] In addition, Gilbert et al. estimated that a level of 300 BAU/mL at day 57 was associated with 90% protection against symptomatic COVID-19 (D614G variant) by the mRNA-1273 vaccine16. In our study (during the wave with the Delta variant), all participants developed an adequate SARS-CoV-2-spike-S1 binding antibody concentration above 300 BAU/mL, and all except one participant (of the ID-SN group) showed a neutralizing antibody concentration above 100 IU/mL at D43, indicating a high level of protection in all groups, despite not meeting the predefined non-inferiority criteria.

Cellular immunity plays a key role in controlling disease severity. Thus, analyzing B- and T cell responses is necessary to provide further insight into the effectiveness and durability of the adaptive immune response.[17] Evidence also indicates that T cell responses are less likely to be affected by spike antigen mutations associated with variants of concern (VOC) compared to antibody response (17-19). We showed that priming with the first vaccine dose resulted in a lower frequency of SARS-CoV-2-specific B cells in both ID groups at all follow-up time points. However, the response was equally effective and the immunization kinetics were comparable, with similar phenotypical SARS-CoV-2-specific B cells to standard dose IM delivery. Both the 20 µg and the 100 µg dose elicited a rapid $CD4^+$ response after the first and second vaccination, consistent with other studies.[12, 15, 18, 20-24]

Also consistent with other studies, we observed more local AEs with ID than IM vaccination; however, these were predominantly mild or moderate. More importantly, ID administration led to a lower incidence of systemic AEs than IM vaccination. This could have important

consequences, as fewer systemic side effects may lead to less absenteeism and higher vaccine acceptance in vaccine-hesitant individuals.[25, 26]

The Bella-Mu® microneedle showed comparable results regarding immunogenicity and safety when comparing it with the standard needle, making it a good alternative for ID vaccination.

Our study has several limitations. Firstly, we failed to meet the sample size to establish non-inferiority in the proportion of participants with seroconversion due to the exclusion of participants with COVID-19. In addition, we had to adapt the definition of seroconversion rate to the MNA we used in our study, resulting in a different, very strict cut-off. Thirdly, our cohort consisted of young, healthy individuals, limiting generalizability to older individuals. Fourthly, participants were not blinded to allocation, which could have introduced bias in AE reporting. Lastly, we analyzed cellular results in a subgroup of 50 participants, two of whom were unknowingly exposed to SARS-CoV-2 without detectable SARS-CoV-2 anti-S or anti-N IgG at inclusion. There is also a possibility that other participants not included in the subgroup were also pre-exposed. We believe that randomization balanced the distribution of pre-exposed participants across the study groups.

In conclusion, our data support reducing the dose to 1/5th of the mRNA-1273 vaccine, administered intradermally, in terms of immunogenicity and safety, despite somewhat lower neutralizing antibody concentrations. Sero-epidemiological studies suggest that even with reduced efficacy against symptomatic infection, fractional dose vaccination could still provide high levels of protection against severe disease on the population level through increased availability (and speed) of vaccination. This would ultimately reduce total infections and death, compared to a scenario where more people remain unvaccinated for a longer period.[26] As such, fractional dose mRNA-1273 vaccine delivered intradermally could have important public health and economic benefits, with fewer side effects and minor loss of efficacy, making it a preferable option for achieving herd immunity quickly. Currently, with high vaccination rates and fewer severe cases due to the decreased severity of the Omicron variant in combination with pre-existent immunity, vaccine coverage is less urgent. However, in case of the emergence of a new, more virulent VOC, boosting with a new vaccine does become more urgent as there will be high and fast vaccine coverage. Therefore, in future pandemics, it would be advisable to evaluate dose-sparing fractional ID doses versus full-dose priming regimens early on during drug development.

Methods

Study design

We performed an open-label, randomized controlled trial at the vaccination clinic of the Leiden University Medical Center (LUMC), a tertiary referral hospital in the Netherlands, in collaboration with the Center for Human Drug Research (CHDR), Leiden, The Netherlands. The

trial was approved by the Medical Ethical Committee Leiden-Den Haag-Delft and registered in the International Clinical Trials Registry Platform (EUCTR2021-000454-26-NL). The study was done in accordance with Good Clinical Practice Guidelines and monitored by an independent Data and Safety Monitoring Board.

Participants

Healthy adults between 18 and 30 years and without a history of laboratory-confirmed or self-reported SARS-CoV-2 infection were eligible. Other main exclusion criteria were prior SARS-CoV-2 vaccination, immunodeficiency or autoimmune disease, use of corticosteroids, and pregnancy (see the protocol for a full list). All participants provided written informed consent before enrollment.

Randomization and blinding

Participants were randomized by block randomization in a 1:1:1 ratio to receive either a fractional dose of 20 µg mRNA-1273 ID through a standard needle (ID-SN) or through the Bella-mu® 1.4 mm microneedle (ID-BM) or standard dose of 100 µg mRNA1273 vaccine IM. Participants and investigators were aware of allocation, given the different routes of administration. Laboratory personnel assessing outcomes were blinded to allocation.

Procedures

The vaccine was prepared according to the manufacturer's instructions. At day 1 (D01), a 1/5th ID dose of 0.1 mL was injected in the deltoid region with a standard needle and syringe (Becton Dickinson U-100 Micro-Fine insulin syringes with integrated 29 G needle) or with a Bella-mu® 1.4 mm microneedle. The standard needle was inserted at a 5-to-15-degree angle and advanced approximately 3 mm through the epidermis to ensure that the entire bevel was covered by the skin using the Mantoux technique.[27] The Bella-mu® 1.4 mm microneedle was placed perpendicularly onto the skin until the hub loosely touched the surface of the skin, and then the vaccine was injected at a controlled depth of about 1 mm. After each ID injection, a wheal appeared on the skin, which was quantified in mm as a quality indicator of the vaccination technique, with a cut-off diameter of 6 mm or more.[28] Participants in the IM group received the standard dose of 0.5 mL in the deltoid muscle. The second dose was administered on the contralateral side.

Participants were followed up by telephone calls on days 2, 4, 8, and 15 after each vaccination and by on-site visits on day 29 (D29), day 36, day 43, and month 7 (M07). Participants recorded the nature and severity of any (un)solicited local and systemic AE and the use of medication in a diary up to 14 days following each vaccination (Supplement D). All AEs were assessed according to a standardized grading scale (Supplementary Tables 1–3) and to the International Classification of Disease-10 (ICD-10) terms. Stopping rules were applied in case any grade 4 AE occurred or a grade 3 AE was reported more than once (Supplement B).

We collected blood samples at D01 and at each scheduled onsite follow-up visit. Serum samples were separated, aliquoted, and stored at −80 °C until analysis.

Immunogenicity

SARS-CoV-2-spike-S1 and -RBD IgG-binding antibodies in serum were measured by a bead-based multiplex immunoassay (MIA) based on Luminex technology.[29, 30] Antibody concentrations were interpolated using a 5-parameter fit of a serum pool calibrated against the WHO international reference (NIBSC, no 20/136) and reported in binding antibody units per mL (BAU/mL).[29] Seropositivity was defined as a SARS-CoV-2-spike-S1 and -RBD antibody concentration of more than 10 and more than 30 BAU/mL, respectively.[19]

We measured neutralizing antibody concentrations against SARS-CoV-2 D614G by micro-neutralization assay (MNA), as previously described.[31] In short, heat-inactivated serum samples were diluted two-fold in a 96-well plate, and 75 µl/well of diluted wild-type SARS-CoV-2 virus was added. After 1 hour incubation at 37 °C, the virus-antibody mixture was added to Vero E6 cells (ECACC, cat. No. 85020206). After overnight incubation at 37 °C, cells were fixed with formaldehyde. Virus-infected foci were visualized by SARS-CoV-2 immunostaining (ImmunoSpot S6 UltraV analyzer with BioSpot counting module [Cellular Technologies Europe]), and foci were counted with SoftMax Pro (Molecular Devices, cat. no. SMP7X GXP SINGLE COMP or SMP7X GXP SERVER). Neutralization titer was expressed as ND_{50}, i.e., the serum dilution at which infection of Vero E6 cells was reduced by 50%, compared to the positive control. Neutralizing titers of the serum samples were also calibrated against an international reference serum (1st WHO International Standard for anti-SARSCoV2 antibody [20/136])[32] and are reported in IU/mL. The lower limit of detection was 15.25 IU/mL.

In a subgroup of participants from the ID-SN (n=26) and IM group (n=24), we collected additional blood samples and isolated peripheral blood mononuclear cells (PBMC) to perform an in-depth analysis of T- and B cell responses to SARS-CoV-2 antigens. The analysis of these immune responses is described in the Supplementary Appendix (Supplements E and F). Briefly, immunophenotyping of SARS-CoV-2-spike protein-specific B cells was performed by flow cytometry. Spike-specific T cells were detected by flow cytometry using peptide stimulation followed by intracellular (cytokine) staining and, in parallel, peptide-HLA tetramer technology.

Intercurrent COVID-19 infection

Before enrollment and at every study visit, participants were screened for SARS-CoV-2 infection by serology (SARS-CoV-2 anti-nucleocapsid [anti-N] IgG antibodies [Alinity m SARS-CoV-2 assay, Abbot Molecular, IL, USA] and MIA) and SARS-CoV-2 PCR of a mid-turbinate/ throat swab. Participants who tested positive were withdrawn from the study.

Outcomes

The primary outcome was non-inferiority in the proportion of participants with seroconversion, as determined by 50% virus neutralization, measured on D43 after vaccination for fractional

dose ID (SN or BM) compared with standard dose IM. Seroconversion was defined as a post-vaccination rise in neutralizing antibody concentration of at least 465 IU/mL, which was the lowest concentration measured in the IM group. Safety was also a primary outcome and included the nature and severity of local and systemic related AE up to 14 days after each vaccination. Secondary outcomes included geometric mean concentrations (GMC) of binding and neutralizing antibodies at D01, D29, D36, and M07 and geometric mean fold rise (GMFR) between consecutive time points.

Statistical analysis

For the primary endpoint analysis, a non-inferiority margin of 15% was set for the difference in response between the fractional ID doses and the standard IM dose. We based the sample size on the Phase-I dose-escalation study of Jackson et al.[15] We assumed >90% seroconversion after the standard IM dose and considered that reduction to 75% seroconversion with fractional ID dose would still provide sufficient protection against severe disease on a population scale.[10] Based on these assumptions, we defined seroconversion as an antibody titer of ≥128, measured by an 80% plaque reduction test (PRNT80). A sample size of 55 participants per study group was required to detect a non-inferiority margin of 15%, with 80% power, 5% significance level for a one-sided test, and accounting for 10% loss to follow-up. In total, 165 participants were to be recruited.

We compared the ID fractional dose (ID-SN and ID-BM) groups pairwise with the standard IM dose in an intention-to-treat (ITT) population, which included all eligible randomized participants who were seronegative at baseline and who remained negative for SARS-CoV-2 anti-N IgG-binding antibodies during the study, with at least one valid antibody test result.

Neutralizing antibodies were expressed as GMC, geometric mean titers (GMT, Supplements), and GMFR with corresponding 95% geometric confidence interval (CI). Any ND_{50} concentration reported as seronegative (limit of quantification [LOQ] < 15.3) was converted to LOQ/2. Non-inferiority was demonstrated if the lower bound of the two-sided 95% CI for the seroconversion rate difference between the ID and IM groups was smaller than 15%. GMFR was calculated as the mean of the difference of logarithmically transformed test results (later time point minus earlier time point) and transformed back to the original scale. Levels of IgG-binding antibodies against SARS-CoV-2-spike-S1 and -RBD were expressed as GMC with a 2-sided 95% geometric CI. To enable ratio calculation for the GMFR for D29/D01, D43/D01, and M07/D01, any SARS-CoV-2-spike-S1 and -RBD antibody concentration at D01 reported below 1 was set to 1.

mRNA-1273-induced T cell responses were analyzed in the ITT subgroup population. B cell responses to SARS-CoV-2 antigens were assessed in the per-protocol (PP) and ITT populations. The ITT population included all participants in the subgroup from the ID-SN (n=26) and IM group (n=24), whereas the PP population excluded participants in the subgroup who had SARS-CoV-2 specific B cells at baseline.

Safety outcomes were assessed in the ITT population, including all randomized participants who received at least one dose of mRNA-1273 vaccine, including those with COVID-19 illness. The safety endpoints, except wheal diameter, are presented as counts and percentages. Wheal diameter was reported as median with interquartile range.

Statistical analyses were done using IBM SPSS Statistics for Windows, version 25.0. Armonk, New York: IBM Corp. Graphs were made using Graphpad version 9.3.1 for Windows, GraphPad Software, San Diego. California.

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Supplementary information

The supplements are available online: https://www.nature.com/articles/s41541-023-00785-w

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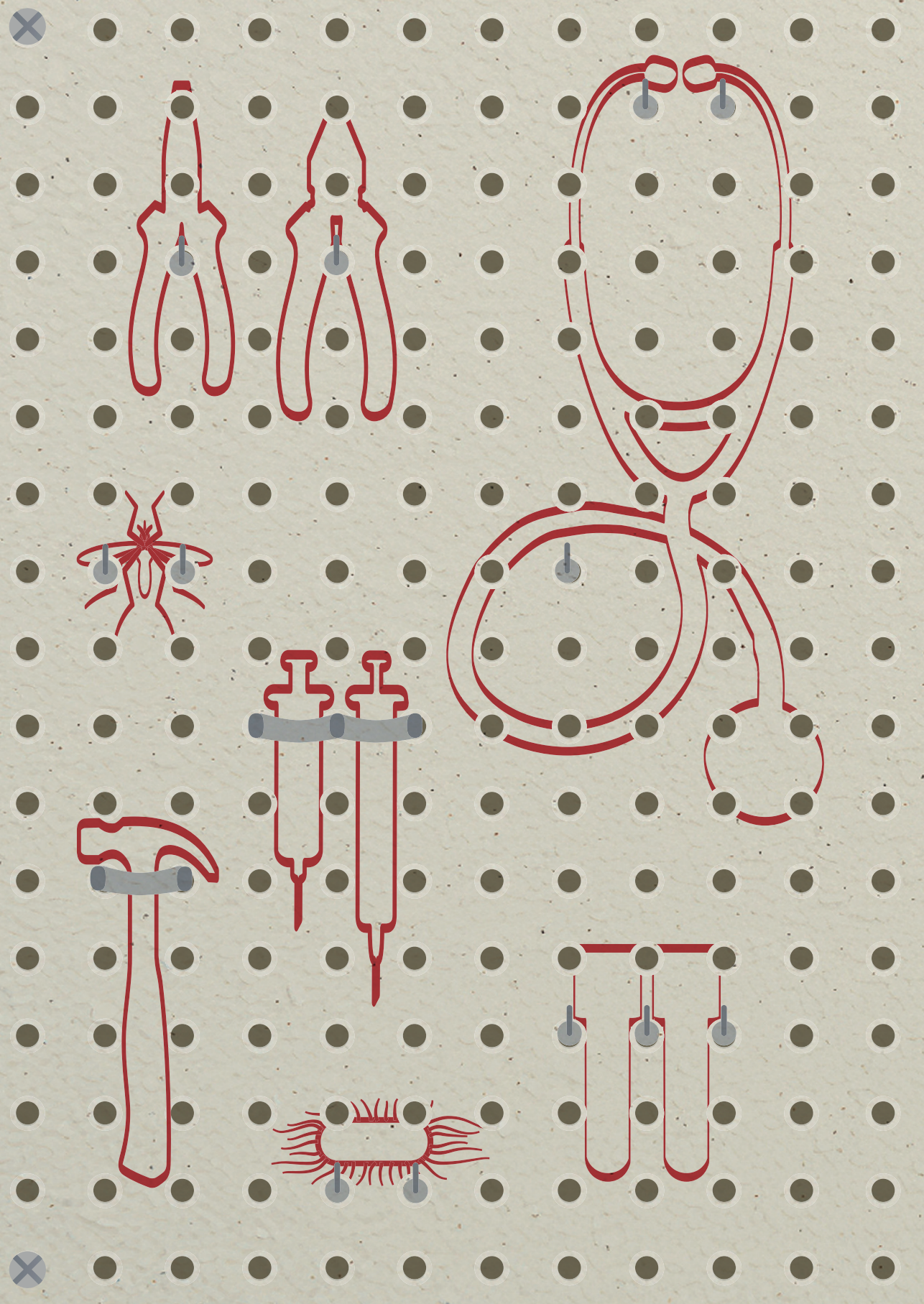

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Intradermal Delivery of the Third Dose of the mRNA-1273 SARS-CoV-2 Vaccine: Safety and Immunogenicity of a Fractional Booster Dose

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Clinical Microbiology and Infection (2024)

Abstract

Objectives

The aim of this study was to assess safety and immunogenicity of a dose-sparing fractional intradermal (ID) booster strategy with the mRNA-1273 COVID-19 vaccine.

Methods

COVID-19 naïve adults aged 18 to 30 years were recruited from a previous study on primary vaccination regimens that compared 20 µg ID vaccinations with 100 µg IM vaccinations with mRNA-1273 (Moderna Spikevax®) as the primary vaccination series. Participants previously immunized with ID regimens were randomly assigned (1:1) to receive a fractional ID booster dose (20 µg) or the standard-of-care intramuscular (IM) booster dose (50 µg) of the mRNA-1273 vaccine, 6 months after completing their primary series (ID-ID and ID-IM group, respectively). Participants that had received a full dose IM regimen as the primary series, received the IM standard-of-care booster dose (IM-IM group). Additionally, COVID-19 naïve individuals aged 18 to 40 years that had received an IM mRNA vaccine as the primary series were recruited from the general population to receive a fractional ID booster dose (IM-ID group). Immunogenicity was assessed using IgG anti-spike antibody responses and neutralizing capacity against SARS-CoV-2. Cellular immune responses were measured in a sub-group. Safety and tolerability were monitored.

Results

In January 2022, 129 participants were included. Fractional ID boosting was safe and well-tolerated, with fewer systemic adverse events compared to IM boosting. At day 28 post-booster, anti-spike S1 IgG geometric mean concentrations were 9,106 (95% confidence interval: 7150-11 597) binding antibody units (BAU)/mL in the IM-IM group and 4357 (3003-6322) BAU/mL, 6629 (4913-8946) BAU/mL, and 5264 (4032-6873) BAU/mL in the ID-IM, ID-ID, and IM-ID groups, respectively.

Conclusions

ID boosting provides robust immune responses and is a viable dose-sparing strategy for mRNA COVID-19 vaccines. The favorable side-effect profile supports its potential in reducing vaccine hesitancy. Fractional dosing strategies should be considered early in the clinical development of future mRNA vaccines to enhance vaccine availability and pandemic preparedness.

Introduction

The swift development and widespread distribution of SARS-CoV-2 vaccines have proven highly effective in mitigating the severe consequences of COVID-19.[1] The mRNA-1273 vaccine (Moderna Spikevax®) demonstrated a protective efficacy of 93% after two intramuscular (IM) doses of 100 µg as a primary series, after a median follow-up of 5.3 months.[2] A booster dose of mRNA-1273 with 50 µg IM led to 1.7-fold (95% confidence interval (CI) 1.5 - 1.9) higher neutralizing antibody titers than those at 28 days after the second injection of the primary series.[3]

While many countries have already conducted multiple booster rounds, a major disparity in booster administration between high-income and low- and middle-income countries remains. One of the causes are the high vaccine costs.[4] Fractional dosing strategies could be a cost effective method to increase vaccine coverage,[5] and may contribute to vaccine uptake in populations with fear of side effects as a reason for vaccine hesitancy.[6, 7]

Given the higher density of antigen presenting cells in the dermis as compared to muscle tissue, the skin is an obvious site for fractional dose delivery. Previously, we have shown that two fractional intradermal (ID) doses of 20 µg mRNA-1273 as a primary series were safe and highly immunogenic.[8, 9] To evaluate whether this strategy is also suitable for the mRNA-1273 booster, we extended the trial and administered a booster dose 6 months after the primary ID or IM series. We measured the immune responses elicited by a fractional (20 µg) ID booster or a full dose (50 µg) IM booster after either a fractional ID primary series or a regular (100 µg) IM primary series.

Methods

Study design and population

This open-label, partly randomized controlled clinical trial was performed at the Leiden University Medical Center, a tertiary health care facility in the Netherlands. This is a follow-up study of a previous trial comparing safety and immunogenicity in healthy SARS-CoV-2-naïve adults who were randomly assigned to primary vaccination with either two fractional doses of 20 µg of mRNA-1273 through the ID route or the standard regimen with two doses of 100 µg of mRNA-1273 vaccine through the IM route (EU Clinical Trials Register: EUCTR2021-000454-26-NL). The vaccine was manufactured in Switzerland.

Participants of the original trial (aged 18 to 30) received a booster (third) dose 6 months after completing the primary vaccination series. In the original trial, two methods of ID administration were assessed: ID delivery according to the Mantoux technique and perpendicular ID administration using an ultra-short Bella-mu® 1.4 micro needle (U-Needle BV, Enschede, The Netherlands). Since both methods yielded similar antibody responses,

participants were grouped together in the current trial and randomly assigned (1:1) to receive either a standard IM booster dose of 50 µg mRNA-1273 (ID-IM group) or a fractional ID booster dose of 20 µg mRNA-1273 (ID-ID group). Participants who had initially received two IM doses of 100 µg mRNA-1273 were assigned to the standard IM booster dose of 50 µg mRNA-1273 (IM-IM group).

For comparison, a fourth group of healthy adults (ages 18 to 40 years) was included, having received a primary series with two IM doses of either 100 µg mRNA-1273 or 30 µg of BNT16b2 (Pfizer-BioNTech Comirnaty®) through the Dutch Municipal Health Service 4 to 8 months earlier. This fourth group received a fractional ID booster dose of 20 µg mRNA-1273 (IM-ID group).

Participants with a history of COVID-19 or immunodeficiency, were excluded from the study. All participants were screened for recent or current SARS-CoV-2 infection before enrolment and at every on-site visit by anti-SARS-CoV-2-nucleocapsid (anti-N) antibodies and SARS-CoV-2 PCR of a mid-turbinate/throat swab and they were asked for positive COVID-19 antigen self-tests of PCRs at the municipal health care center. Participants were excluded when tested positive.

Written informed consent was obtained from all participants. The trial was done in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice. The study was reviewed and approved by the Medical Ethics Committee of Leiden, The Hague, Delft (NL 76702.058.21).

Randomization and blinding

Randomization of ID-primed participants (block sizes of 4) was done using sealed envelopes. Clinical investigators and participants were unblinded to the administration route. Laboratory personnel remained blinded for the study groups.

Vaccination procedure

Vaccine was prepared according to the manufacturer's instructions. A single dose of 50 µg mRNA-1273 vaccine was administered to participants in the IM-IM and ID-IM group as a 0.25 mL IM injection in the deltoid muscle. The participants in the ID-ID group and the IM-ID group received a single dose of 20 µg mRNA-1273 vaccine as a 0.1 mL ID injection in the skin of the deltoid region. Participants in the IM-ID group were vaccinated intradermally with a Becton Dickinson U-100 Micro-Fine™ insulin syringe with integrated 29G needle, using the Mantoux technique. Participants in the ID-ID group were vaccinated using the Bella-mu® 1.4 mm micro-needle. A clearly visible wheal was indicative of a successful ID injection, with a minimally required diameter of 6 mm.

Monitoring of tolerability and safety

Participants were asked to record solicited and unsolicited adverse events (AEs) for 14 days following vaccination (Supplements 1). The severity of AEs was graded according to a standard grading scale (Supplements 1). Solicited AEs included local and systemic reactions.

Immunogenicity analysis

Blood samples for immunological analyses were taken before booster vaccine administration, 28 days after vaccination and 6 months (25 to 27 weeks) after vaccination.

Binding IgG antibody responses against the receptor binding domain (RBD) and to the S1 subunit of the spike protein and the nucleocapsid (N) of SARS-CoV-2 were measured in serum as previously described.[8] Concentrations were expressed as international binding antibody units per mL (BAU/mL). Anti-N IgG concentrations >14.3 BAU/ml were considered proof of a convalescent SARS-CoV-2 infection.

The presence of antibodies with neutralizing capacity against SARS-CoV-2 was measured using a microneutralization assay (MNA) as previously described.[10] Serum dilutions ranging from 1:10 to 1:10240 were tested, and the first dilution that resulted in zero plaque formation was reported as $PRNT_{100}$.

In the original trial, a subset of 25 participants of each group was selected to assess cellular immune responses. From those who remained in the booster study (13 in the IM-IM group, 4 in the ID-IM group and 6 in the ID-ID group), peripheral blood mononuclear cells were isolated. SARS-CoV-2 spike-specific B and T cell responses were measured before and 28 days after vaccination, as described previously[9] and in Supplements 4 and 5.

Statistical analysis and sample size

No formal power calculation was performed as all participants of the original trial were eligible to participate in the current study. The sample size of the IM-ID group of 40 was based on the expected group size of the other groups.

All participants that received a booster vaccination were included in the intention-to-treat (ITT) population. The per protocol (PP) population comprised all participants who received a booster vaccination with a negative SARS-CoV-2 PCR at the day of vaccination (PCR results became available on the day after vaccine administration). Safety was assessed in the ITT population and immunogenicity in the PP population.

Primary outcome was defined as the S1 and RBD binding antibody concentrations at day 28 post-vaccination. Neutralizing capacity was a secondary outcome measure. The ID-IM, ID-ID and IM-ID groups were compared pairwise to the IM-IM control group. No adjustments for antibody concentrations before booster were made since the objective was to assess the entire vaccination regimen (primary series + booster). Binding antibody concentrations were reported in geometric mean concentrations (GMCs) with two-sided 95% CI and neutralizing antibodies were reported in geometric mean titers (GMTs) with two-sided 95% CI. Geometric mean fold rises (GMFRs) with two-sided 95% CI were used to report the changes in antibody concentrations and titers during follow-up. At every time point, the four groups were compared with each other using a non-parametric test that adjusts for multiple comparison (Dunn's multiple comparison test).

The study protocol specified a non-inferiority analysis as primary outcome for the original trial. Since the sample size was not powered for the booster part of the trial and there were no non-inferiority criteria pre-defined for this part of the study, no non-inferiority analysis was performed.

All statistical analyses were performed using SPSS Statistics version 25.0 (IBM Corp, Armonk, New York). A p-value below 0.05 was considered significant.

Results

Participants

Details on the recruitment and loss to follow-up are shown in Fig. 1. In January 2022, a total of 129 participants received a booster: 31 in the IM-IM group, 27 in the ID-IM group, 28 in the ID-ID group and 43 in the IM-ID group. Two participants in the ID-IM group and three participants in the IM-ID group were excluded from the PP population because of a positive PCR for SARS-CoV-2 on the day of the booster. At the primary endpoint 28 days after the booster, 94/124 (75.8%) of the participants in the PP population were still in the trial. COVID-19 was the main reason for exclusion during the trial, which was balanced among the groups (Fig. 1).

Baseline characteristics of the participants are reported in Table 1. The IM-ID group contained relatively more females and was slightly older compared to the other groups. In the IM-ID group 88.4% of the participants had received a regimen with the BNT126b2 vaccine in the primary immunization series, whereas the other groups had only received the mRNA-1273 vaccine.

Table 1. Characteristics of participants at inclusion (intention-to-treat population)

	Primary series 100 µg IM Booster 50 µg IM IM-IM	Primary series 20 µg ID Booster 50 µg IM ID-IM	Primary series 20 µg ID Booster 20 µg ID Bella-mu® ID-ID	Primary series 100 µg IM* Booster 20 µg ID standard needle IM-ID
n	31	27	28	43
Female, n (%)	14 (45.2)	11 (40.7)	11 (39.3)	25 (58.1)
Age in years, mean (SD)	24.1 (3.5)	23.1 (3.4)	23.0 (3.2)	26.8 (5.7)
BMI in kg/m^2, mean (SD)	24.2 (4.1)	24.9 (4.3)	23.8 (5.0)	23.4 (3.2)
Primary series with BNT162b2, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	38 (88.4)
Time between primary series and booster in days, mean (SD)	176 (3)	176 (3)	177 (3)	176 (17)

* All primary series and booster vaccinations were with mRNA-1273, except in case of a primary series with BNT162b2, which was 30 µg.
BMI = body mass index; ID = intradermal; IM = intramuscular; SD = standard deviation.

Serological immunogenicity

Details on antibody concentrations and neutralizing capacity can be found in Fig. 2 and Table 2. At 28 days after booster administrations, the anti-S1 GMC of the IM-IM group (9,106 [95% confidence interval: 7150 – 11 597]) was significantly higher than that of the ID-IM (4,357 [3003 - 6322], p=0.01) and the IM-ID group (5264 [4032 - 6873], p=0.02). GMFRs (booster + 28 days/ pre-booster) for anti-S1 IgG did not differ between groups. Neutralization GMTs at 28 days after booster vaccination were 445 (331 - 598) in the IM-IM group, 598 (415 – 863) in the ID-IM group, 440 (287 – 674) in the ID-ID group and 234 (168 – 355) in the IM-ID group. Neutralization titers in the ID-IM group were significantly higher than in the IM-ID group at this time point (p=0.02).

Six months after booster vaccination, all 27 remaining participants had detectable antibody levels, and anti-S1 GMCs were significantly higher for the ID-ID group compared to the IM-ID group (p=0.045). Neutralization GMTs 6 months after booster were lower for IM-ID group compared to the ID-IM group (p=0.04) and the ID-ID group (p=0.02).

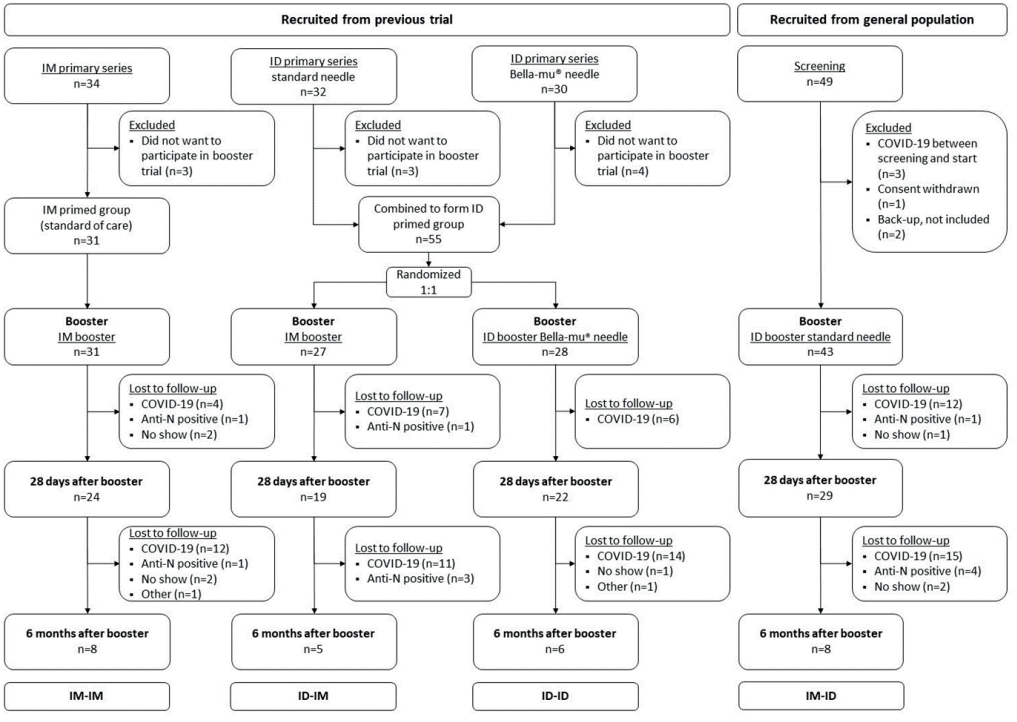


Figure 1. Flowchart of inclusions, exclusions and loss to follow-up of study participants

The current trial is a continuation of a previous trial that compared the safety and immunogenicity of two intradermally administered fractional doses of 20 µg of mRNA-1273 to the standard immunization regimen with two intramuscular doses of 100 µg of mRNA-1273 vaccine (EU Clinical Trials Register: EUCTR2021-000454-26-NL). Participants who received IM vaccinations in the primary series in the previous trial, were recruited to receive a 50 µg IM booster (IM-IM group, standard of care). Participants who received a fractional ID vaccination in the primary series in the previous trial, were recruited to receive a 50 µg IM booster (ID-IM group) or a 20 µg ID booster (ID-ID group). A fourth group was recruited from the general population to receive a fractional ID booster dose of 20 µg mRNA-1273 (IM-ID group). Anti-N = anti-nucleocapsid; ID = intradermal; IM = intramuscular.

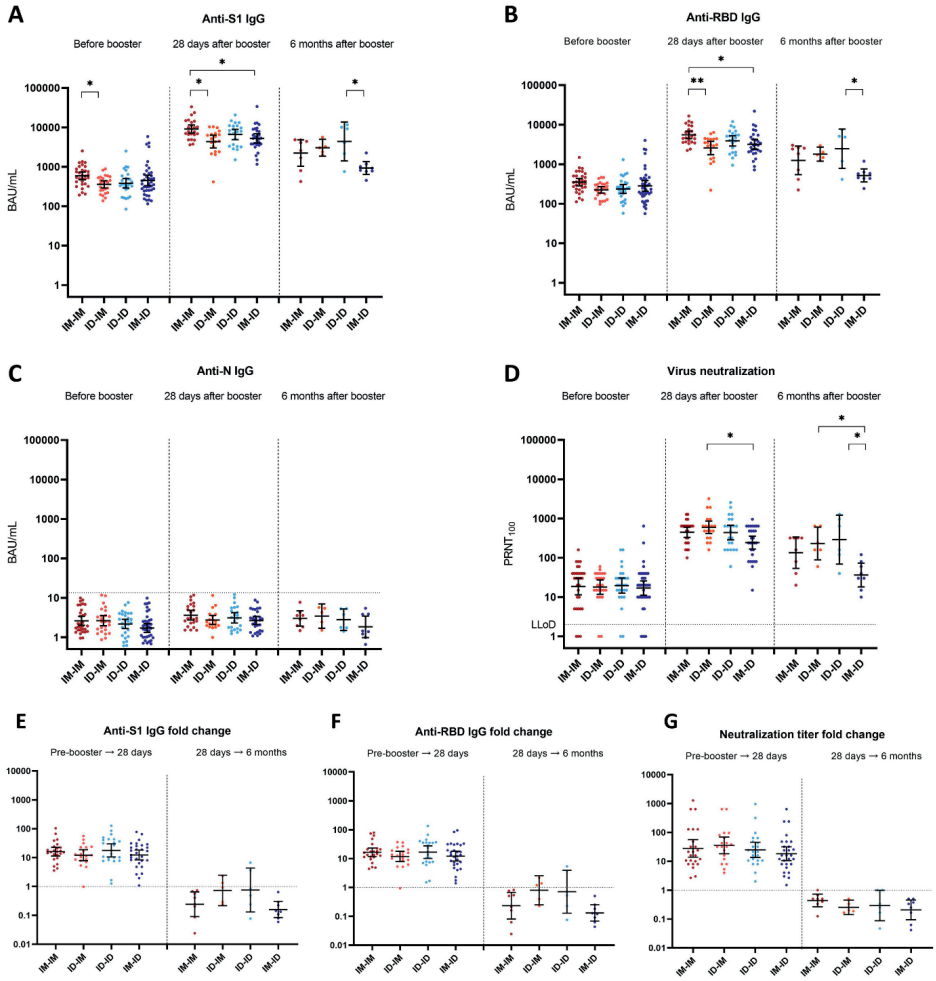


Figure 2. Antibody responses

A. Anti-S1 IgG antibody concentrations in BAU/mL. **B.** Anti-RBD IgG concentrations in BAU/mL. **C.** Anti-N IgG titers in BAU/mL. Horizontal dotted line represents the cut-off for seropositivity (14.3 BAU/mL). **D.** Virus neutralization titers. Horizontal dotted line represents the lower limit of detection. Results below the limit of detection were arbitrarily set to 1. **E.** Anti-S1 IgG fold change. Horizontal dotted line represents a fold change of 1 (no increase and no decrease). **F.** Anti-RBD IgG fold change. Horizontal dotted line represents a fold change of 1 (no increase and no decrease). **G.** Neutralization titer fold change. Horizontal dotted line represents a fold change of 1 (no increase and no decrease).

Every dot represents the result of a single participant at that time point. Error bars represent geometric means with a two-sided 95% confidence intervals. IM-IM stands for IM primary series and IM booster, ID-IM stands for ID primary series and IM booster, etc. Groups were compared using a Dunn's multiple comparison tests (* = $p<0.05$, ** = $p<0.01$).

Anti-N = anti-nucleocapsid; anti-RBD = anti-receptor-binding domain; anti-S1 = anti–spike S1; BAU = binding antibody units; ID = intradermal; IM = intramuscular; PRNT = plaque reduction neutralization test.

Table 2. Antibody concentrations reported in GMCs (IgG) and GMTs (neutralization) and fold change reported in GMFR (per protocol population)

	IM-IM	ID-IM	ID-ID	IM-ID
Pre-booster				
n	31	25	28	40
Anti-S1 IgG, BAU/mL (95% CI)	588 (471-743)	359 (291-442)	383 (291-505)	453 (334-613)
Anti-RBD IgG, BAU/mL (95% CI)	352 (283-437)	224 (186-272)	237 (183-306)	285 (209-387)
Neutralization, $PRNT_{100}$ (95% CI)	19 (11-30)	18 (12-27)	20 (13-30)	18 (12-26)
Booster + 28 days				
n	24	19	22	29
Anti-S1 IgG, BAU/mL (95% CI)	9106 (7150-11 597)	4357 (3003-6322)	6629 (4913-8946)	5264 (4032-6873)
Anti-S1 IgG, GMFR (95% CI)*	16.1 (11.5-22.7)	12.1 (7.8-18.7)	17.8 (10.5-30.0)	12.3 (8.4-18.2)
Anti-RBD IgG, BAU/mL (95% CI)	5535 (4430-6916)	2588 (1761-3804)	3900 (2903-5239)	3192 (2404-4239)
Anti-RBD IgG, GMFR (95% CI)*	16.6 (11.9-23.2)	11.9 (7.9-17.9)	16.7 (10.2-27.4)	12.7 (2.8-18.1)
Neutralization, $PRNT_{100}$ (95% CI)	445 (331-598)	598 (415-863)	440 (287-674)	234 (168-355)
Neutralization, GMFR (95% CI)*	26.8 (13.1-54.8)	34.1 (17.5-66.4)	23.3 (12.4-43.9)	17.0 (10.2-28.4)
Booster + 6 months				
n	8	5	6	8
Anti-S1 IgG, BAU/mL (95% CI)	2,242 (1037-4845)	3,055 (1873-4984)	4,398 (1418-13645)	929 (636-1358)
Anti-S1 IgG, GMFR (95% CI)**	0.2 (0.1-0.7)	0.7 (0.2-2.5)	0.7 (0.1-4.3)	0.2 (0.1-0.3)
Anti-RBD IgG, BAU/mL (95% CI)	1,248 (544-2862)	1,795 (1190-2707)	2,466 (787-7733)	520 (357-757)
Anti-RBD IgG, GMFR (95% CI)**	0.2 (0.1-0.7)	0.8 (0.3-2.6)	0.7 (0.1-4.0)	0.1 (0.1-0.3)
Neutralization, $PRNT_{100}$ (95% CI)	135 (54-336)	230 (89-601)	291 (69-1218)	36 (18-73)
Neutralization, GMFR (95% CI)**	0.4 (0.2-0.7)	0.3 (0.1-0.5)	0.3 (0.1-0.5)	0.2 (0.1-0.5)

* GMFR = geometric mean (booster + 28 days) / geometric mean (pre-booster).
** GMFR = geometric mean (booster + 6 months) / geometric mean (booster + 28 days).
Anti-RBD = anti-receptor-binding domain; anti-S1 = anti–spike S1; BAU = binding antibody units; CI = confidence interval; GMC = geometric mean concentration; GMFR = geometric mean fold rise; GMT = geometric mean titer; ID = intradermal; IM = intramuscular; PRNT = plaque reduction neutralization test.

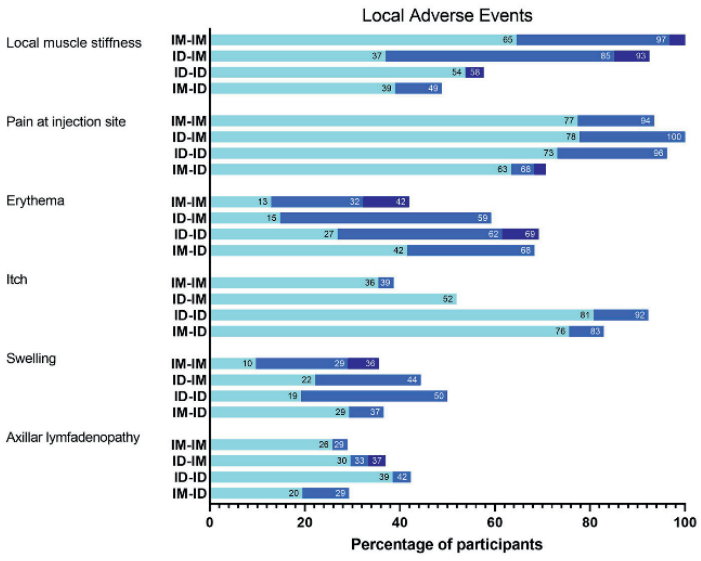
Cellular immunogenicity

The frequencies of spike-specific $CD4^+$ and $CD8^+$ T cell responses, and B cell responses before and after booster were comparable between the ID-ID, the IM-IM and the ID-IM groups (Supplements 4, Fig. S3 for T cells and Supplements 5, Fig. S4 for B cells). Percentages of IgG positive SARS-CoV-2 specific B cells correlated with the anti-S1 IgG antibody titers before booster, while no correlation was found 28 days after boosting (Fig. S4C).

Safety and tolerability

No acute or serious adverse reactions and no grade 4 AEs occurred after vaccine administration. Mild and moderate pain at the injection site was the most prevalent AE (Fig. 3). Local muscle stiffness was more severe and more prevalent in IM boosted groups whereas itch at the injection site did occur more in ID boosted groups. Systemic AEs were more prevalent in IM boosted groups. This was especially true for nausea and vomiting, headache, chills and fever.

One of the participants in the ID-IM group developed hyperpigmentation on her left hand and arm and face 2 days after the IM booster (Supplements 3, Fig. S2), which fully resolved within a week. Relatedness with vaccine administration was unclear.



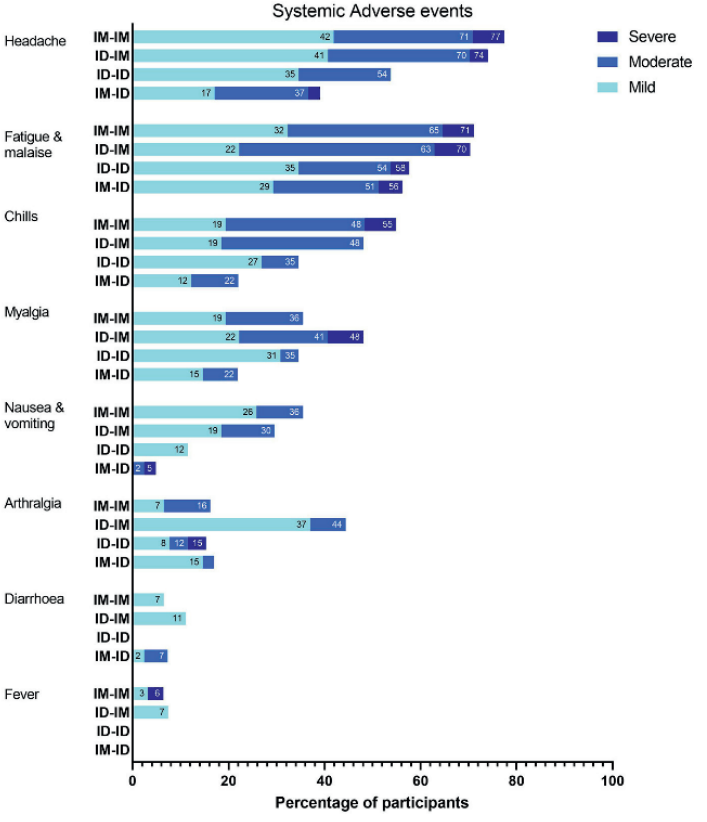


Figure 3. Local and systemic adverse events after booster vaccination

Solicited adverse events related to vaccination reported for 28 days following administration. IM-IM stands for IM primary series and IM booster, ID-IM stands for ID primary series and IM booster, etc.
ID = intradermal; IM = intramuscular.

Discussion

In this study, we compared the safety, tolerability, and immunogenicity of a fractional ID booster dose (20 µg mRNA-1273 COVID-19 vaccine) to the standard of care (50 µg IM dose), in both ID and IM primed, COVID-19 naïve healthy persons. Fractional ID boosting was safe and well tolerated, with fewer systemic AEs compared to IM boosting. While regimens that contained one or two fractional ID vaccine doses exhibit slightly lower immunogenicity, all participants had anti-S1 IgG concentrations >300 BAU/L, which has been previously associated with 90% (77 – 94%) protective efficacy against SARS-CoV-2 infection.[11]

Other studies with ID boosting of COVID-19 vaccines have been conducted (12-18), but to our knowledge, this is the first to assess an ID primary-booster regimen consisting solely of mRNA COVID-19 vaccines. Moreover, it is the first to evaluate the booster response in individuals who have received ID vaccinations as their primary series. We demonstrate that these ID-primed individuals reached high antibody concentrations and that their fold change in SARS-CoV-2 spike-specific B cells did not differ compared to the controls, which suggest a good memory response. This is an important finding for future pandemics as in the acute stages of an epidemic, rapidly immunizing a larger number of people can yield greater benefits in preventing mortality than inducing higher antibody concentrations in a smaller group.[19,20] The excellent boostability of individuals primed with a fractional ID dose, emphasizes the need to incorporate fractional dosing regimens early in the clinical development of future mRNA vaccines to improve vaccine availability and pandemic preparedness.[21]

Participants that received an ID booster reported less systemic AEs than IM boosted participants monitored in the first 2 weeks after the booster, which is in accordance with other studies assessing ID boosting with COVID-19 vaccines (12-14, 17, 18). When recruiting the participants for the IM-ID group, we noted increased interest among individuals hesitant to receive another full dose. Although there is a multitude of reasons why people are reluctant to getting a COVID-19 vaccination, an important concern is side effects (22-26). Given its favorable systemic AE profile compared to IM vaccination, ID vaccination may thus be a method to decrease vaccine hesitancy.

This study has some limitations. Firstly, the findings in a relatively young and healthy population may not apply to older populations or those with co-morbidities, necessitating further study in older persons. Additionally, the IM-ID group was recruited from the general population, with nearly 90% having received BNT162b2 as their primary series, which was the preferred vaccine for adults in the Netherlands. This disparity hampers direct comparison with the other groups and may also explain the observed lower binding and neutralizing antibody responses. Previous research shows that on average, a BNT162-mRNA-1273 prime-booster combination leads to circa 15% lower antibody concentrations and circa 30% lower neutralizing antibody titers compared to a prime-booster regimen solely comprising mRNA-1273.[27] Lastly, the high exclusion rate during the study due to COVID-19 has led to relatively small groups that may

not have sufficient power to identify subtle differences in immunogenicity or side effects. This factor also limits the ability to draw conclusions on long term immunogenicity since a rather small number of participants remained in the study up to 6 months post-booster. Exclusions due to COVID-19 may also have introduced a survival bias.

In conclusion, we have demonstrated that a fractional ID booster of the mRNA-1273 vaccine elicits a robust immune response, which supports ID administration as a dose-sparing strategy for mRNA vaccines in a future epidemic or when a new more virulent variant of concern arises.

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Supplementary materials

The supplements are available online: https://www.clinicalmicrobiologyandinfection.com/article/S1198-743X(24)00159-9/fulltext

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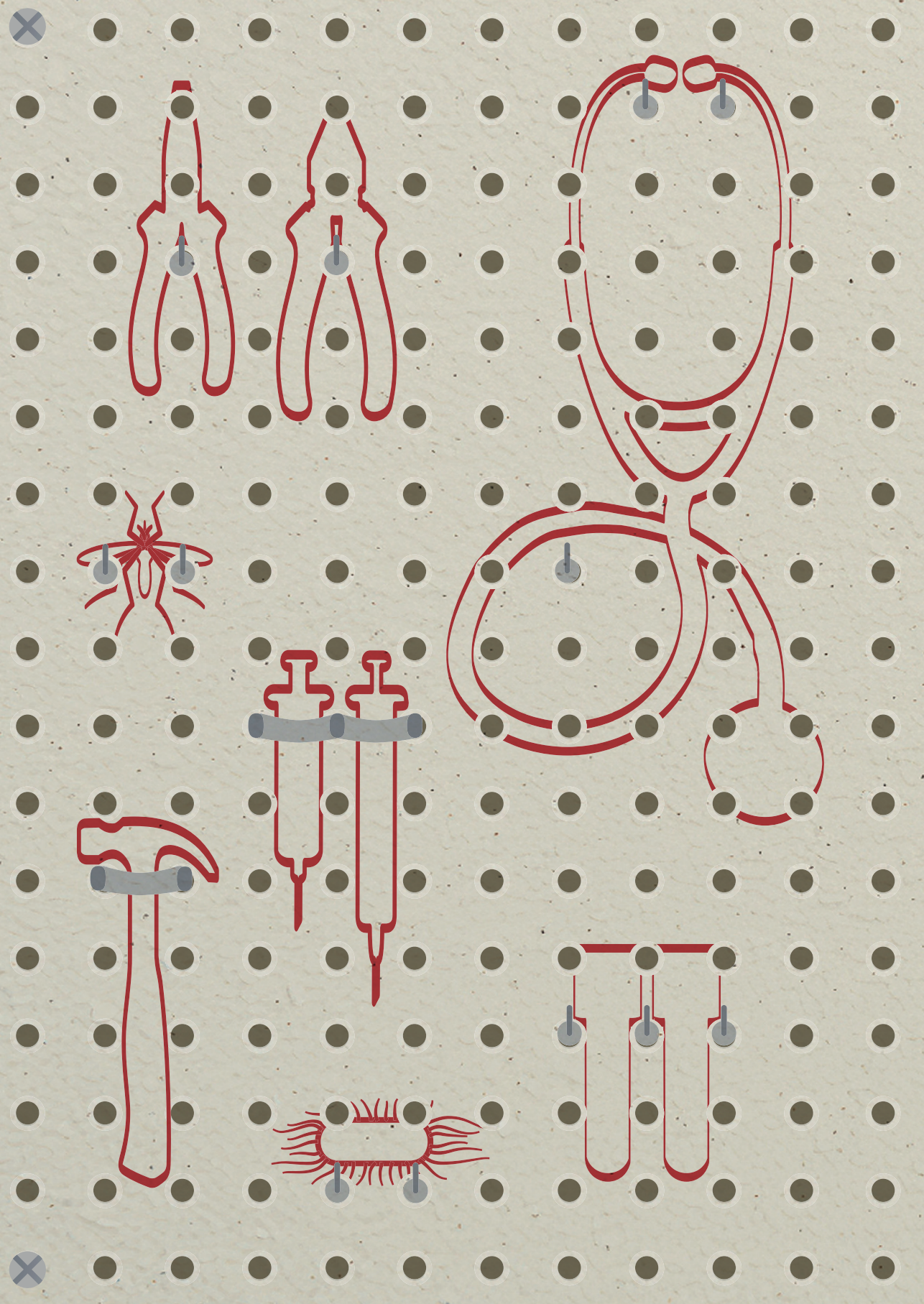
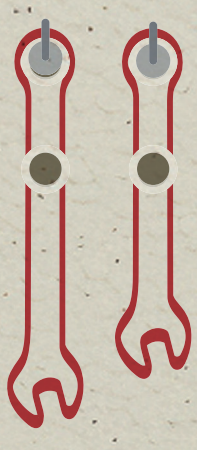


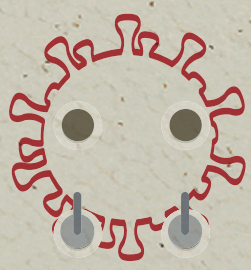


8

Intradermal Fractional Dose Vaccination as a Method to Vaccinate Individuals with Suspected Allergy to mRNA COVID-19 Vaccines

Geert V.T. Roozen, Alexandra Granger, Rob S. van Binnendijk, Gerco den Hartog, Meta Roestenberg, Leo G. Visser, Anna H.E. Roukens

Vaccine (2024)



Abstract

Suspected allergic reactions after mRNA COVID-19 vaccination withheld multiple individuals from getting fully vaccinated during the pandemic. We vaccinated adults who had experienced possible allergic symptoms after their first intramuscular dose of a COVID-19 mRNA vaccine with a 1/5th fractional intradermal test dose of the mRNA-COVID-19 vaccine 1273 (Moderna Spikevax®). No anaphylactic reactions were observed after intradermal vaccination (n=56). Serum anti-S1 IgG concentrations were measured using a bead-based multiplex assay four weeks after vaccinations. Antibody concentrations were compared with a previously collected nationwide cohort that had received two intramuscular doses of mRNA-1273. Antibody responses in all subjects tested (n=47) were comparable to standard of care intramuscular dosing. Fractional intradermal dosing of mRNA COVID-19 vaccines may provide a pragmatic solution that is safe, time efficient compared to skin prick testing, dose sparing and immunogenic in individuals with suspected vaccine allergy.

Introduction

Even though severe allergic reactions to mRNA COVID-19 vaccines are rare[1], the global immunization campaigns in the COVID-19 pandemic, with around 1.5 billion mRNA COVID-19 vaccines administered in the European Union and United States alone,[2, 3] inherently led to a large number of people with suspected allergic reactions to the first dose. This stressful experience made these persons anxious to receive any further vaccinations.[4] In some cases, physicians working at these vaccination centers were also hesitant to further vaccinate these individuals.[4]

Anaphylaxis is an acute mast-cell mediated severe hypersensitivity reaction with multi-organ involvement that can present as a life-threatening respiratory impairment or shock.[5] Anaphylaxis is very rare after vaccination with mRNA COVID-19; only occurring about 1.3 to 17 times per million doses.[1] Even in persons with an immediate allergic reaction of any severity to a first mRNA COVID-19 vaccine, the absolute risk of a second-dose severe reaction to the same mRNA COVID-19 vaccine is as low as 0.16% (95% confidence intervals: 0.01%-2.94%).[1]

The European Network of Drug Allergy and the European Academy of Allergy and Clinical Immunology recommended a skin prick test for persons with a generalized immediate reaction to any mRNA vaccine,[6] even though the sensitivity of this method (using the vaccine as the skin test reagent) is very low (0.2, 0.01–0.52).[7] For this group graded dosing has been proposed, i.e. splitting the vaccine dose in multiple (equal or increasing) fractional doses that are administered at intervals (usually around 30 to 60 minutes),[6, 8] but there is no data supporting whether this intervention will lead to fewer allergic reactions.

Since the added value of the skin prick test and graded dosing were unclear and since these methods were practically unfeasible in times of a pandemic due to their time consuming nature, we chose a more pragmatic approach of fractional dosing followed by an observation period. During the first rounds of COVID-19 vaccinations in the Netherlands, we have administered a fractional dose (20 µg, equal to 1/5th of the standard 100 µg dose) of the mRNA-1273 COVID-19 vaccine (Moderna Spikevax®) in individuals with suspected allergic responses after their first COVID-19 vaccination. We hypothesized that the intradermal (ID) route in patients would be an efficacious immunization method with a reduced potential allergen exposure and concomitant risk. Previously, we have shown that a fractional ID dose induced immune responses that are comparable to the responses induced by a full dose administered through the intramuscular (IM) route in healthy, non-allergic persons.[9-11] In terms of vaccine dose used, fractional ID administration holds the middle between a skin prick test and graded dosing. It can thus be used both as a method to assess hypersensitivity responses in people with alleged allergies and at the same time to encourage hesitant persons to get vaccinated. In the Netherlands, this method is standard practice for yellow fever vaccination of patients traveling to a highly endemic region, who are at risk of hypersensitivity to the vaccine due to a suspected chicken egg allergy.[12, 13]

To assess if the 1/5th fractional ID dose was also sufficient as a means of immunization in this population, we measured antibody responses four weeks after ID vaccination. Here, we report on observed symptoms and immunogenicity associated with an allergic reaction after fractional ID administration.

Methods

This was a retrospective chart review study that was approved by the Institutional Review Board of the Leiden University Medical Center (LUMC) in the Netherlands for observational COVID-19 studies. The information reported here is the result of a retrospective assessment of an off-label vaccination method that was applied ad-hoc to provide patients with a suspected allergy to COVID-19 vaccines with a possibility to get vaccinated rapidly during the pandemic.

From January 2021 to February 2022, adult patients in the region of Leiden, The Hague and Delft in the Netherlands who had a suspected allergic reaction to their first COVID-19 vaccination were referred to the outpatient vaccination clinic of the LUMC. Some patients were referred without previous vaccination because they were hesitant to undergo COVID-19 vaccination due to (suspected) allergic reactions to multiple allergens including PEG in the past. Referrals were made by general practitioners, allergists, and Municipal Health Services. The outpatient vaccination clinic serves as a specialized travel and vaccination clinic and is situated within the tertiary university hospital LUMC.

Referred patients were first contacted by phone by an Infectious Diseases consultant who registered co-morbidities, allergies, medication use, and self-reported symptoms that occurred after the first COVID-19 vaccination in the electronic patient file of the LUMC. If the risk of anaphylaxis was considered high (i.e. if their self-reported symptoms met the SPEAC case definition of anaphylaxis[14]), patients were planned to receive an ID vaccination at the LUMC day unit under close medical supervision with an intravenous drip installed before vaccination to treat anaphylaxis if needed. All other patients were scheduled at the outpatient vaccination clinic. Patients gave consent to use their data for future research. Patients who had typical type 1 allergic symptoms, e.g. urticarial rash or angio-oedema after the first vaccination were advised to take oral antihistamines before their second vaccination at the LUMC.

A 20 µg (0.1 ml) dose of the mRNA-1273 vaccine was administered ID in the deltoid region using a Becton Dickinson U-100 Micro-Fine insulin syringes with integrated 29 G needle as described previously.[9,10] Patients received 0.1 ml 0.9% saline solution in the deltoid region of the contralateral arm as a control. After vaccination, all patients were observed for at least 30 minutes by a nurse with a doctor on site. If, after the observation period, the cutaneous wheal of the ID vaccination was at least twice the size of the ID saline injection, the patient was considered to have a positive skin test.[13] Patients with a negative skin test were offered to receive the remainder of the vaccine dose IM to provide the opportunity to receive the

standard of care regimen. Patients were asked to return to the vaccination clinic four weeks after vaccination for the collection of a blood sample for immunogenicity analysis. SARS-CoV-2-spike-S1 (Wuhan-Hu-1) and nucleocapsid (N)-binding antibodies in serum were measured by a bead-based multiplex immunoassay (MIA) based on Luminex technology as described previously[10] and reported in geometric mean concentrations (GMC) of Binding Antibody Units per mL (BAU/mL). Anti-N IgG concentrations above 14.3 BAU/mL were considered indicative of a convalescent COVID-19 infection.[15] Patients were registered in the national vaccination database as fully vaccinated if they had received two vaccinations and their serum anti-S1 IgG concentration was 300 BAU/ml or higher, corresponding with a vaccine efficacy of about 90% in the mRNA-1273 phase III clinical trial.[16]

The retrospective chart review took place in February 2022, when the electronic patient files from the LUMC outpatient vaccination clinic were reviewed. Firstly, the goal of this study was to evaluate the occurrence and severity of allergic symptoms and/or anaphylaxis[14] after the ID administration of the second mRNA COVID-19 vaccine dose in patients with a suspected allergy based on symptoms they had experienced after receiving their first mRNA COVID-19 vaccination (hypersensitivity analysis). Secondly, this study aimed to determine whether a second fractional ID vaccination with mRNA-1273 induced sufficient humoral immune responses. Therefore, only patients who had received a first vaccination with an mRNA COVID-19 vaccine through the IM route and a fractional ID dose as a second vaccination were included; patients who had received a non-mRNA COVID-19 as their first vaccination, or patients who received their first vaccination through the ID route were excluded. All eligible patients who had their symptoms recorded after the ID vaccination were included in the hypersensitivity assessment. Symptoms experienced by patients after receiving the remainder of the dose IM were not included in the hypersensitivity assessment. All eligible patients from whom a serology sample was taken and who did not receive the remainder of their second dose IM, were included in the immunogenicity assessment. The information collected in the review comprised demographics, co-morbidities, self-reported symptoms after the first full-dose vaccination, symptoms and signs observed by a healthcare worker in the first half hour after the second fractional ID dose and antibody responses one month later.

For comparison of immunogenicity, antibody concentrations were compared with a previously collected cohort of the PIENTER-Corona study. PIENTER-Corona is a nationwide study conducted by the National Institute for Public Health and the Environment including all age groups of the Dutch general population, with serum samples collected at regular time intervals irrespective of the moment of infection or vaccination.[17] For the reference group of this study, all sera from PIENTER-Corona were selected that had been collected from adults vaccinated with two full IM doses of mRNA-1273 between two weeks to ten weeks after their second vaccination.

The PIENTER-Corona study was approved by the medical ethical committee MED-U, Nieuwegein, the Netherlands and registered in the Netherlands Trial Register under number NL8473 (https://onderzoekmetmensen.nl/nl/trial/21435).

Results

Eighty-five records were screened (Fig. 1). Of those, 56 were included in the hypersensitivity assessment and 47 in the immunogenicity assessment.

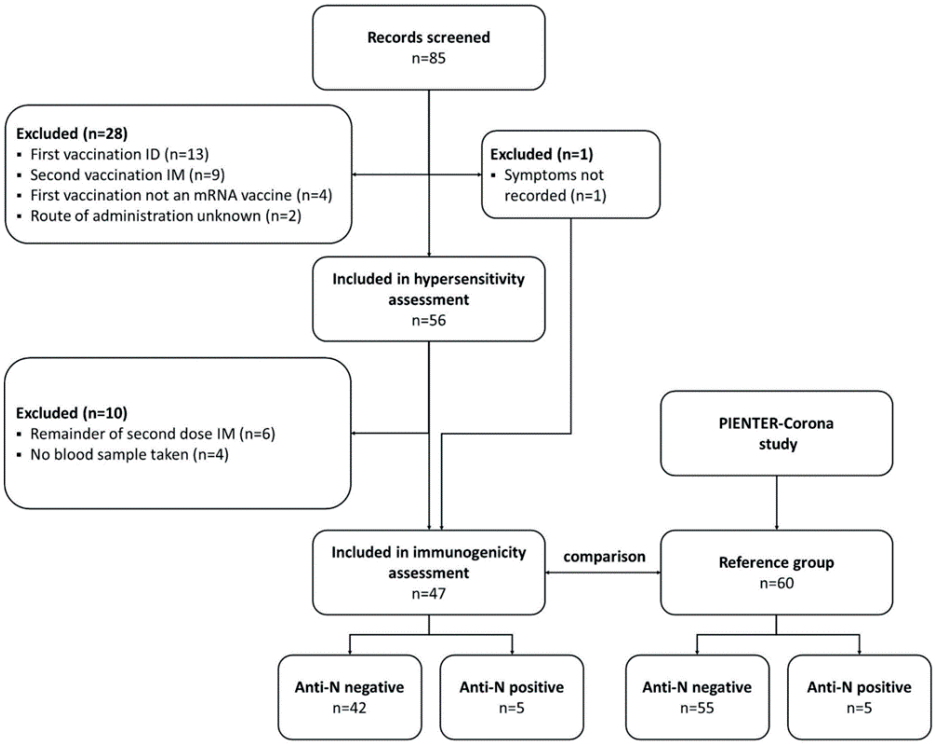


Figure 1. Flowchart of records screened and reasons for in- and exclusion in hypersensitivity assessment and immunogenicity assessment

Patients who received their first vaccination as a full IM dose with a mRNA COVID-19 vaccine and their second dose as fractional ID dose were included. All patients of whom symptoms were recorded after vaccinations were included in hypersensitivity assessment. All patients who had their blood sample taken and had not received the remainder of the second dose IM were included in the immunogenicity assessment. As a comparison, sixty sera were selected from adults in the PIENTER -Corona study that had been collected two to ten weeks after their second vaccination with full dose mRNA-1273 IM. The PIENTER study was a nationwide observational study including all age groups of the Dutch general population. Immunogenicity of patients who had a history of COVID-19 (based on anti-N IgG serology) is reported separately.
Anti-N = anti-nucleocapsid; ID = intradermal; IM = intramuscular.

Two out of 56 (3.6%) patients received their ID vaccination at the LUMC day unit because their self-reported symptoms after their first vaccination satisfied the criteria of an anaphylactic reaction according to the SPEAC case definition.[14] Although a wide variety of symptoms were self-reported after the first vaccination, only a fraction of these were observed after the second vaccination at the LUMC (Table 1). Angioedema, which was the most self-reported symptom after the first vaccination, was not observed after the second vaccination in this cohort. Thirty-four patients (60.7%) had used an oral antihistamine before ID administration of the vaccine. Dizziness or light headedness, localized itch and erythema were the most observed symptoms and signs after the ID vaccination. None of the patients, including the two high-risk patients vaccinated on the day unit, developed severe hypersensitivity reactions or anaphylaxis (according to the SPEAC case definition[14]). Although frequencies were not formally recorded, we noticed that many patients were very anxious before and during the observation period. This was also reflected by the fact that only six patients with a negative skin test accepted the offer of receiving the remaining dose IM. Most patients with a negative skin test rejected this offer because they still feared hypersensitivity reactions if they were to receive the full dose.

The characteristics of the patients included in the immunogenicity assessment were as a group very similar to the selected reference group (in terms of age, sex and time between the second vaccination and sampling) (Table 2). The main difference was that the majority of patients referred to the vaccination clinic had received the BNT162b2 COVID-19 vaccine (Pfizer-BioNTech Comirnaty®) as their first vaccination, together with the fact that the interval between the two vaccinations in the primary series was longer than for the reference group. Co-morbidities related to an atopic constitution such as asthma, hay fever, and drug and food allergies were higher in the patients with suspected vaccine allergies, compared to the reference group.

Anti-spike-S1 IgG concentrations are reported in Fig. 2. All patients vaccinated with a fractional ID dose at had detectable anti-spike-S1 IgG antibodies. On average, patients with a suspected allergy had higher antibody concentrations than the reference group from the general population, but confidence intervals were overlapping. This was also true for patients with a history of COVID-19. There were no significant differences in antibody responses between patients using antihistamines compared to patients not using antihistamines or for patients with positive skin tests compared to patients without positive skin tests (results not shown), but groups were too small to make sound comparisons.

Table 1. Hypersensitivity assessment: symptoms after first and second mRNA COVID-19 vaccination

N=56	First vaccination (standard dose IM) Self-reported	Second vaccination (fractional dose ID) Observed
First vaccination, n (%)		
mRNA-1273	7 (12.5)	56 (100)
BNT162b2	49 (87.5)	0 (0.0)
Symptoms, n (%)		
Loss of consciousness	2 (3.6)	0 (0.0)
Tingling of lips or mouth	2 (3.6)	0 (0.0)
Palpitations	2 (3.6)	1 (1.8)
Vomiting or nausea	3 (5.4)	0 (0.0)
Diarrhea	3 (5.4)	0 (0.0)
Shivering	4 (7.1)	0 (0.0)
Hypotension	4 (7.1)	0 (0.0)
Fever	4 (7.1)	0 (0.0)
Stridor	5 (8.9)	0 (0.0)
Headache	10 (17.9)	4 (7.1)
Dizziness or light headedness	11 (19.6)	9 (16.1)
Urticaria	14 (25.0)	2 (3.6)
Bronchospasm	19 (33.9)	3 (5.4)
Itch (other than vaccination site)	20 (35.7)	10 (17.9)
Erythema (other than vaccination site)	20 (35.7)	14 (25.0)
Angioedema	33 (58.9)	0 (0.0)
Positive skin test, n (%)		
Yes	N/A	31 (55.4)
No	N/A	18 (32.1)
Missing	N/A	7 (12.5)

Symptoms after first vaccination (full dose IM vaccination with mRNA-1273 or BNT162b2 at general practitioner or municipal public health service) are self-reported. Symptoms after second vaccination ($1/5^{th}$ fractional ID dose of mRNA-1273) are from observation by a health care worker. Patients received 0.1 ml 0.9% saline solution in the deltoid region of the contralateral arm as a control. The skin test was positive if the cutaneous wheal of the ID vaccination was at least twice the size of the ID saline injection after 30 minutes.
ID = intradermal; IM = intramuscular.

Table 2. Characteristics of patients in immunogenicity assessment

	LUMC 20 µg ID (n=47)	PIENTER-Corona 100 µg IM (n=60)
Mean age, years (SD)	46 (16)	46 (12)
Sex, n (%)		
Male	2 (4.3)	8 (13.3)
Female	45 (95.7)	52 (86.7)
First vaccination, n (%)		
mRNA-1273	7 (14.9)	60 (100)
BNT162b2	40 (85.1)	0 (0.0)
Vaccination interval between 1st and 2nd dose, days (SD)	143 (68)	33 (12)
Time since 2nd dose, days (SD)	28 (7)	38 (14)
Co-morbidities, n (%)		
Diabetes	1 (2.1)	1 (1.7)
Hypertension	5 (10.6)	8 (13.3)
Hay fever	13 (27.7)	5 (8.3)
Asthma	15 (31.9)	3 (5.0)
Food allergy	23 (48.9)	0 (0.0)
Drug allergy	31 (66.0)	2 (3.3)

Patients received a full IM dose of mRNA-1273 or BNT162b2 as a first vaccination and a second vaccination with a 20 µg ID dose with mRNA-1273 (LUMC cohort). The reference group received the standard regimen with two IM vaccinations with 100 µg mRNA-1273 (PIENTER-Corona cohort). Co-morbidities, including allergies, are self-reported.
ID = intradermal; IM = intramuscular; SD = standard deviation.

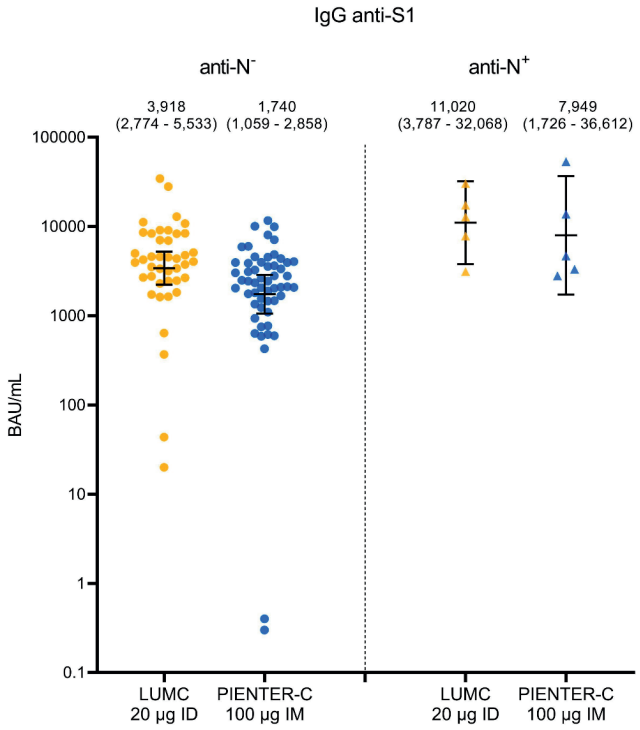


Figure 2. Immunogenicity assessment: anti-spike-S1 IgG concentrations

SARS-CoV-2-spike-S1 binding antibodies in serum were measured by a bead-based multiplex immunoassay (MIA) based on Luminex technology and are reported in geometric mean concentrations of binding antibody units per milliliter (BAU/mL). Every data point represents a separate patient. Patients with a history of COVID-19 (anti-N positive) are reported as separate groups. Error bars represent GMC with 95% confidence intervals.
Anti-S1 = anti-spike-1; anti-N = anti-nucleocapsid; BAU = binding antibody units; ID = intradermal; IM = intramuscular; GMC = geometric mean concentrations.

Discussion

This study provides evidence that a fractional dose of the mRNA-1273 vaccine administered through the ID route is an effective way to vaccinate individuals with a suspected allergic reaction after their first vaccination with an mRNA COVID-19 vaccine. This is in line with our previous study, which shows that an immunization regimen with fractional ID vaccine dose induces adequate immune responses.[10] A trend towards a higher antibody response in the ID vaccinated group was observed, but this was most probably caused by the longer interval between the two vaccinations in this group. Previously, it has been shown for mRNA COVID-19 vaccine BNT162b2 that an immunization regimen with a longer interval is more immunogenic.[18]

We observed no anaphylaxis in our patients, which is not surprising given the low incidence of anaphylaxis after mRNA COVID-19 vaccines, even in those with allergic reactions to previous administrations.[1] The markedly lower number of symptoms observed after the second vaccination compared to the self-reported symptoms after the first vaccination is noteworthy and can have multiple explanations. The reduction in dose may have caused fewer symptoms than the full IM dose. The fact that the majority of patients had taken oral antihistamines before the second vaccination may have also lowered the incidence of symptoms. Additionally, the fact that the ID vaccination was performed with the mRNA-1273 vaccine and the majority of patients had received a first dose with the BNT162b2 vaccine, that is composed of slightly different components may also have led to less symptoms. Finally, symptoms self-reported after the first vaccination may have been stress-related rather than hypersensitivity signals. Receiving the vaccine in a small outpatient clinic (contrary to a large municipal vaccination center) while being observed by an experienced medical professional, may be reassuring for patients and may have thus contributed to fewer experienced symptoms after the second vaccination.

A limitation of this study is the fact that the serology before the second vaccination is lacking, and therefore we could not calculate a fold increase of anti-SARS-CoV-2 antibody concentration. In addition, the majority of patients were COVID-naïve, whereas most people currently have had at least one episode of COVID-19. However, the results in the anti-N IgG positive groups do suggest that our results also apply to individuals with a history of COVID-19. Although the results reported here are from the primary vaccination series, we believe ID administration can also be used to give booster vaccinations to patients with suspected allergies, as we have previously shown that fractional ID dosing induces immune responses comparable to full IM booster dose administrations.[11]

Some have proposed, that due to the extremely low risk of anaphylaxis, and the lack of evidence for skin prick testing or graded dosing, individuals with a suspected allergy to mRNA COVID-19 vaccines can receive the regular full IM dose according to the standard of care as long as it is in a setting where there is experience with handling anaphylactic reactions.[1] This does not take into consideration that general practitioners and health care workers at mass vaccination clinics might be hesitant to administer further vaccinations to these individuals. Even if the risk of anaphylaxis is low from an evidence-based point of view, it also fails to consider that it can be quite stressful for patients to receive another full dose when they previously have experienced symptoms that they might have associated with an anaphylactic reaction. In these situations, fractional ID dosing may provide a pragmatic solution that is time efficient and sufficiently immunogenic.

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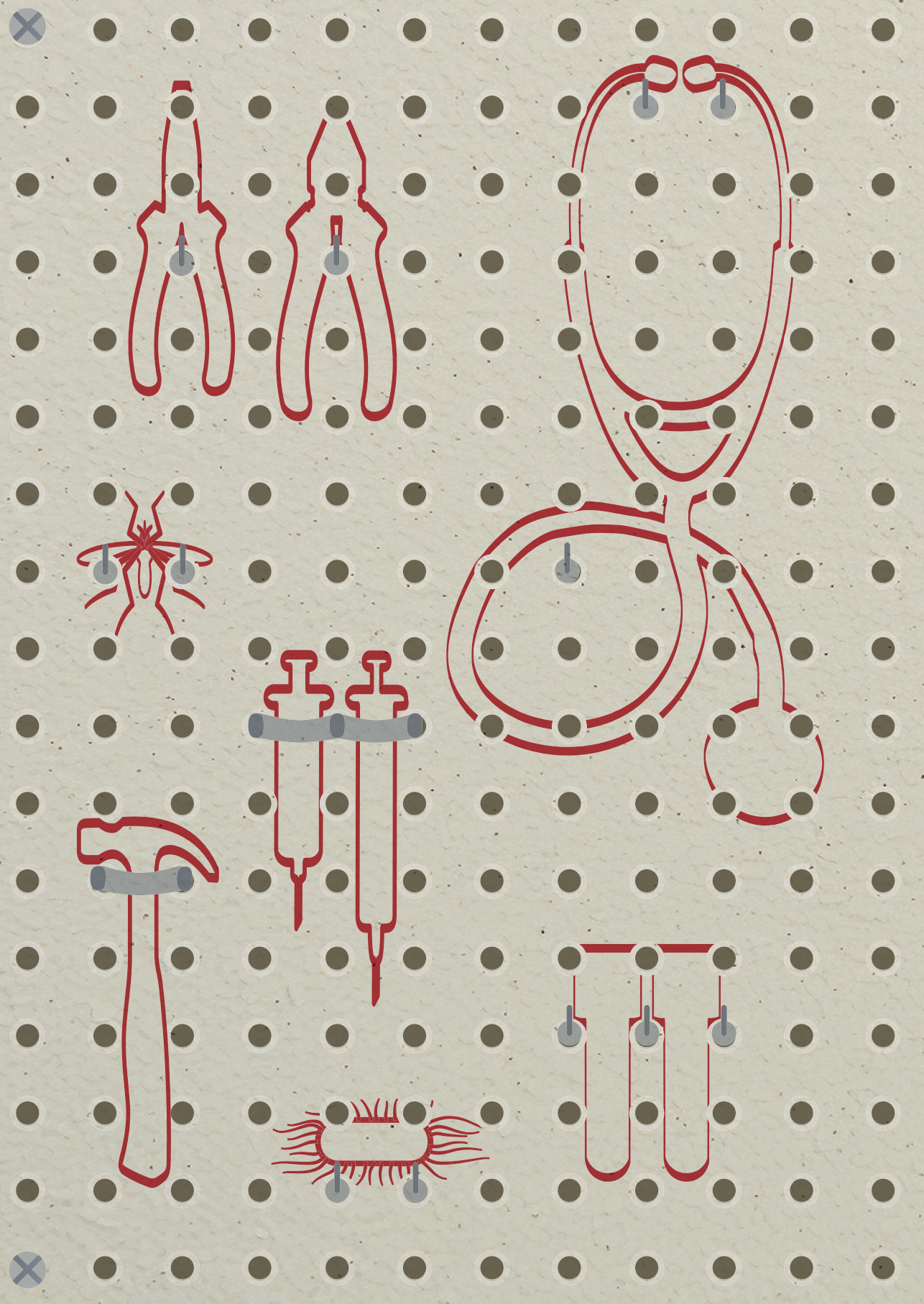

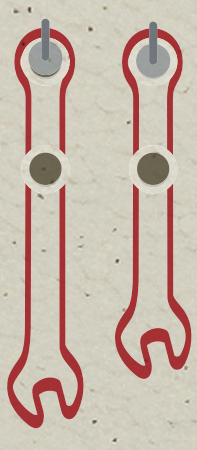

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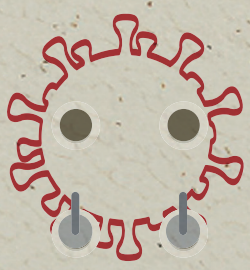
Discussion



This final chapter summarizes the findings presented in the previous chapters and situates them within the broader context of vaccine development for malaria (**Chapter 2**) and shigellosis (**Chapter 3**), as well as dose optimization of COVID-19 vaccines during the pandemic (**Chapter 4-8**). Furthermore, it evaluates the contributions of these publicly initiated trials to enhancing vaccine accessibility and development. Finally, this chapter examines how the principles of Question-Based Clinical Development (QBCD), introduced in **Chapter 1**, can drive the ongoing advancement of these vaccines.

Whole-sporozoite malaria vaccine development

Chapter 2 presents a study on dose optimization of a whole-sporozoite (WSp) immunization approach for malaria. Malaria is a disease caused by the *Plasmodium* parasite, which is transmitted between humans by mosquitoes. Each year, approximately 600 000 people die from malaria, mostly children below the age of five.[1] The *Plasmodium falciparum* (*Pf*) parasite is the leading cause of malaria-related mortality.[1] To date, *Pf* is the only parasite for which a vaccine has been developed and broadly implemented outside of clinical research settings.

RTS,S and R21 are the two malaria vaccines that currently have received prequalification by the World Health Organization (WHO). Both are subunit vaccines that consist of nanoparticles covered with the circumsporozoite protein (CSP) in combination with an adjuvant to increase immunogenicity.[2, 3] In regions with moderate to high seasonal transmission where seasonal malaria chemoprevention is provided, a three-dose regimen of either of the two vaccines prevents approximately 75% of clinical malaria episodes for about one year when administered to young children at the start of the malaria season.[2-4] However, protective efficacy is substantially lower when the vaccinations are given outside of this timing (50-70%), when given to infants (approximately 40%), in areas with high perennial transmission (20-35%), or in the second year after receiving a booster dose (40-60%).[2-4] As of February 2024, nearly 10 000 children in Cameroon and Burkina Faso have received the RTS,S vaccine, and RTS,S and R21 are planned for introduction in a total of 19 African countries.[5]

The development and implementations of these two vaccines mark important milestones in the fight against malaria. Current immunization regimens result in a decrease of morbidity and mortality among vaccinated individuals. However, achieving a substantial reduction in community-level transmission requires next-generation malaria vaccines capable of inducing robust and durable protection from blood-stage disease (e.g. 90% for more than a year), preferably with simpler dosing schemes.[6]

Since malaria predominantly affects LMICs, a new malaria vaccine holds limited revenue potential (**Chapter 1**). As a result, a substantial part of research into the development of next-generation malaria vaccines is not conducted by the pharmaceutical industry but by public and other non-commercial research institutions. Adhering to QBCD and the fail-fast principle ensures an efficient use of limited available funding.

A specific strategy to facilitate this process is the evaluation of experimental malaria vaccines through small-scale trials using a controlled human malaria infection (CHMI). In CHMI trials, participants are randomized to receive either the vaccine or a placebo and then exposed to wild-type malaria parasites via infected mosquito bites or intravenous inoculation with sporozoites. Participants are monitored daily and receive antimalarial treatment as soon as parasites are detected in their peripheral blood (parasitemia). The proportion of participants in the vaccinated group who are protected against infection as well as the time-to-parasitemia in those who are unprotected provides valuable insights into how the vaccine may perform in larger, late-stage field trials in malaria-endemic regions.[7] The use of CHMI allows for early down selection of ineffective vaccine candidates, thereby reducing the financial burden associated with unsuccessful late-stage development.[8]

A promising approach to develop the next generation of malaria vaccines is immunization with attenuated WSp, which may improve both the efficacy and durability of protection against malaria. WSp are metabolically active *Pf* parasites that expose the immune system to a broader range of antigens comparted to subunit vaccines.[9] Strong supportive evidence for this idea comes from immunization with chemoprophylaxis and sporozoites (CPS), in which infective sporozoites are administered under the cover of a prophylactic drug that is only effective against the erythrocytic stage of the disease.[9] When evaluated in a CHMI, CPS is found to elicit high levels of protective efficacy,[10-13] which is durable as well.[14]

Through genetic modification, the Leiden University Medical Center (LUMC) has developed a *Pf* parasite in which the *mei2* gene, vital for the parasite's intrahepatic development, has been removed. This genetically attenuated *PfΔmei2* parasite (GA2) demonstrates complete growth arrest in the liver after approximately 6 days,[15] thus mimicking the CPS method but without inducing symptoms associated with blood-stage disease. Three administrations of GA2 to human participants, delivered through the bites of 50 mosquitoes, were safe and did not cause breakthrough parasitemia. Three weeks later, eight out of nine participants were protected against a subsequent CHMI.[16] While these results supported the potential for further development of a three-dose regimen, the key question arose whether a single immunization could induce similar high-level protection. A next-generation vaccine with a single-immunization regimen would vastly simplify implementation in vaccination campaigns, compared to the current vaccines that require three vaccinations and a booster.

To address this, the trial described in **Chapter 2** was conducted, evaluating the efficacy of a single-dose regimen. The findings demonstrated that a single immunization with GA2 provided protection against CHMI. Six weeks post-immunization, 90% of participants (9/10) in the GA2 group were protected, compared to non in the control (0/5).

These results mark an important step forward in the development potent vaccines based on genetically attenuated parasites. In line with QBCD, several key questions can be identified that require an answer to effectively advance this concept into late-stage development:

1. What is the optimal dose? Can a lower dose also induce sufficient immunity?
2. How durable is the immunity?
3. What is the protective efficacy of a vialed genetically attenuated WSp vaccine that can be administered parenterally?[17]
4. Can a genetically attenuated WSp vaccine induce equivalent efficacy in pre-exposed populations in malaria-endemic regions?

The first question aligns with general question 5 of the QBCD framework: "What is the therapeutic window of the drug?" (**Chapter 1**). To address this, an ongoing trial at LUMC is investigating the protective efficacy of GA2 immunization at a lower dose (NCT05468606). Additionally, another trial will address protection durability by rechallenging participants at least one year post-immunization (NCT06293339).

It is important to note that the current administration method, relying on mosquito bites, is not feasible for large-scale implementation. Another genetically attenuated WSp vaccine has been developed that can be stored in liquid nitrogen and administered through intravenous injection.[18] This vaccine, termed LARC2, has the same *mei2* gene deletion as GA2 and an additional knockout of the *linup* gene.[18] A first-in-human trial with LARC2 is planned and its results will be pivotal for the future of genetically attenuated WSp malaria vaccine development.

The fourth key question formulated above pertains to the issue of vaccine hyporesponsiveness.[19] Previously tested WSp approaches that used radiation-attenuation instead of genetic attenuation reported lower efficacy and lower humoral and cellular immune responses in pre-exposed African populations compared to malaria-naive adults in the United States.[17] Despite this knowledge, radiation-attenuated WSp vaccines were subsequently tested in multiple large-scale clinical trials in malaria endemic regions.[17] This strategy arguably deviated from the principles of QBCD and the fail-fast approach, suggesting that research funds could have been allocated more optimally. While formulating key question is central to QBCD, the framework also emphasizes the importance of addressing these questions in a prioritized, optimal sequence; often the most challenging aspect of the strategy. According to QBCD, both financial and scientific factors must guide the selection of the optimal development path.[20] There are financial and environmental barriers that complicate the conduction of CHMI trials in malaria-endemic regions, but genetically attenuated WSp vaccines developed in the Global North should eventually be tested target populations. Assessing the efficacy and immune responses in pre-exposed African populations should be a priority early in the development process, to avoid the missteps made with previous WSp vaccine candidates.

If hyporesponsiveness in the target population is identified, a potential solution could be the identification of potent adjuvants to enhance the immunogenicity of WSp immunization. While substantial research has been conducted on adjuvants for subunit malaria vaccines, little is known about adjunvating WSp vaccines. Preliminary rodent studies have yielded

promising results,[21-23] but further research in humans is needed to determine the viability of this approach.

If the key questions discussed here could successfully be addressed, a genetically attenuated WSp malaria vaccine candidate could progress to large-scale clinical trials to evaluate safety and efficacy in populations in malaria-endemic regions. Achieving this goal would represent a major step toward the development of a highly effective next-generation malaria vaccine.

Development of a new Shigella vaccine

Shigellosis is an enteric infection caused by *Shigella* bacteria, which induces inflammation that can lead to gut enteropathy, malnutrition, and stunting in children. Symptoms typically include fever, malaise, anorexia, vomiting, and most prominently, (bloody) diarrhea. Shigellosis is estimated to be the second leading cause of diarrhea-associated mortality in LMICs. Currently, there is no licensed *Shigella* vaccine available, but modelling suggests that the introduction of an effective vaccine could avert 43 million cases of stunting and 590 000 deaths over a 20-year period.[24]

Chapter 3 outlines the protocol for an upcoming study to evaluate the safety and immunogenicity of a candidate *Shigella* vaccine and adjuvant. The vaccine candidate, Invaplex$_{AR\text{-}Detox}$, has previously been proven safe and immunogenic in adults in the United States (NCT03869333). According to the WHO Preferred Product Characteristics (PPC) for *Shigella* vaccines, the target population are infants and young children under five living in LMICs, where the disease burden is highest.[25] However, prior studies with *Shigella* vaccine candidates have shown low or absent immune responses in children under three.[26, 27] Vaccine hyporesponsiveness, due to different genetic and environmental factors, may further limit the immune response in LMIC populations. Addressing these challenges will require optimization of the vaccine to overcome variability in the target population, in line with general key question 5 of QBCD (**Chapter 1**)

The study protocol described in **Chapter 3** is specifically designed to address this question. The safety and immunogenicity of Invaplex$_{AR\text{-}Detox}$ will be assessed both with and without dmLT, a candidate adjuvant shown to significantly enhance immune responses to *Shigella* antigens in mice.[28, 29] To address hyporesponsiveness, the third study cohort will involve Zambian adults, following two dose-escalation cohorts in Dutch adults to confirm safety and tolerability.

This early evaluation in the target setting ensures rapid conformation of dmLT's adjuvating potential or, alternatively, facilitates the termination of this strategy if dmLT added benefits are minimal ("fail fast"). If Invaplex$_{AR\text{-}Detox}$ combined with dmLT proves to be safe and immunogenetic, a subsequent age-descending study is planned to evaluate the vaccine and adjuvant in adolescents, young children, and infants in a country with a high *Shigella* disease burden (either Burkina Faso or Zambia). This trial will ultimately answer the key question on immunogenicity in the target population.

Beyond advancing the vaccine pipeline for shigellosis, the study is part of a broader project that aims to strengthen the capacity of ethical reviewing and clinical research in the African countries participating in the consortium. While most countries in the WHO African region have national ethical committees installed, more than halve indicate a need for capacity strengthening on health research ethics, including clinical trials.[30] International collaborations for vaccine development (both between HICs and LMICs and among LMICs) can enhance this development. National regulatory authorities can be strengthened in clinical trial evaluation and authorization through an exchange of knowledge and best practices with experienced foreign regulators.[31]

The study protocol described in **Chapter 3** exemplifies how publicly funded initiatives can foster partnerships between scientific organizations in the Global North and the Global South, facilitating the exchange of expertise. A parallel submission process of the study protocol to the Dutch and Zambian review boards proved efficient and provided valuable insights to researchers in both countries on local regulatory priorities on study design, ethical principles and good manufacturing practices of investigational products. To further promote knowledge sharing, a two-day capacity building program was organized to bring together researchers from Burkina Faso, Zambia, and The Netherlands, as well regulators from Zambia, to exchange experiences on vaccine trials and discuss ethical and regulatory principles of clinical trials with Dutch regulators.

Intradermal administration and fractional dosing of mRNA COVID-19 vaccines

In early 2020, the world was hit by the COVID-19 pandemic, the largest since the 1918 influenza outbreak. By the end of 2024, more than 7 million confirmed COVID-19-related deaths have been reported[32], though the true death toll is likely substantially higher.

The pandemic demonstrated that when the need for a vaccine in high-income countries (HICs) is high, pharmaceutical companies are willing and capable of accelerating the development and production of vaccines, particularly when HIC governments cover most financial risks.[33-36] Clinical trials were quickly rolled out, addressing key questions (**Chapter 1**) required to acquire (emergency) licensure, like “What is the highest tolerated and safe dose?”, “Is the vaccine immunogenic?” and “Does the induced immune response protect against infection and/or severe disease?” However, other key questions were not addressed, particularly those relevant to advancing vaccine equity but less directly beneficial to the vaccine producer.

In **Chapter 4,** we described that questions like “Which reduced dose can induce sufficient immunity levels to vaccinate more people with the same amount of vaccine?” or “Can the interval between vaccinations be stretched in the initial stage of the vaccination campaign?” are typically not addressed by vaccine producers, but by publicly funded research institutes. Although these studies generated important insights, they were primarily local initiatives and relied on the enthusiasm and dedication of individual research groups rather than being coordinated in a centralized effort. To address this gap, we proposed establishing a pandemic

preparedness vaccine development pipeline, coordinated by an international public body, such as the World Health Organization (WHO) or the Coalition for Epidemic Preparedness Innovations (CEPI). Under this framework, post-licensure trials should systematically evaluate reduced doses, alternative dosing methods, and revised vaccination regimens as soon as pharmaceutical companies successfully completed a late-phase clinical trials for vaccines targeting pandemic pathogens. Furthermore, governments investing in vaccine candidates and guaranteeing their purchase should require producers to assess at least some dose optimization during large pre-licensure trials.

Chapters 5 - **7** present findings from post-licensure studies on the mRNA-1273 COVID-19 vaccine (Moderna Spikevax®). These trials evaluated the safety, tolerability, and immunogenicity of an intradermally administered dose as a dose-sparing strategy. The rationale for these trials is based on two principles.

First, it was assumed that the registered vaccine dose may be excessive and that a reduced dose could still elicit sufficient immune responses. Determining the optimal dose during vaccine development is challenging because the dose-response relationship is usually not linear but concave or S-curved (Fig. 1). This means that a major reduction of the dose often leads to a minor reduction in effectiveness.[37] Fractionating doses to vaccinate five or ten times more individuals, even with slightly lower immune responses, could greatly enhance herd immunity, reducing mortality and morbidity on a population level.[38] As discussed in **Chapter 1**, during clinical vaccine development, often the highest tolerated dose instead of the lowest necessary dose is selected. As a result, a lot of the vaccine is effectively wasted.

Second, the studies were based on the hypothesis that the dermis, which contains more immune cells than muscle tissue, might produce a stronger immune response to vaccine antigens (Fig. 2). Intradermal administration could therefore induce a stronger immune response than intramuscular administration.[39] A review comparing immune responses from intradermally and intramuscularly administered vaccines at equivalent doses found this hypothesis holds true for some vaccines.[40] For others, equivalent doses administered intradermally produced similar immune responses as intramuscularly administered doses.[40]

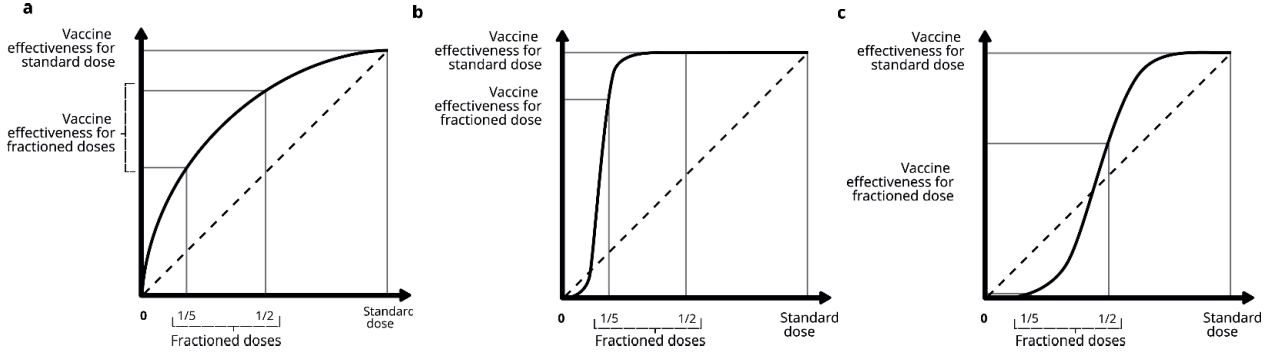


Figure 1. Relationship between vaccine dose and effectiveness is not linear

Three scenarios with different vaccine dose-effectiveness relationships. In all three examples, providing halved doses to a certain number of people could provide a greater level of population immunity than would providing standard doses to half as many people. For reference, the population immunity conferred by vaccination could be estimated via the vaccination coverage multiplied by vaccination effectiveness. Horizontal axis represents vaccine dose, and vertical axis represents vaccine efficacy. The dashed diagonal line is included for reference.

A. Solid line indicates a scenario in which there is a concave relationship between vaccine efficacy and dosage, whereby a half dose could provide considerably more than half the effectiveness of the standard dose. In this example a 1/5th fractioned dose leads to considerably less effectiveness in the individual but would still be beneficial on a population level if twice as many people receive a vaccination. **B.** A different scenario: in this example there is an S-curved relationship between dose and effectiveness. Here, a halved dose has the same effectiveness as the standard dose and a 1/5th fractioned dose still has an effectiveness of approximately 80%. **C.** Another scenario in which there is an S-curved relationship between dose and efficacy. Here, a 1/5th fractioned dose does not provide any protective effectiveness. A halved dose will still provide population benefits if it leads to twice as many people getting vaccinated but would come with a substantial reduction in effectiveness on the individual level.

Used with permission, adapted from: Cowling BJ, Lim WW, Cobey S. Fractionation of COVID-19 vaccine doses could extend limited supplies and reduce mortality. Nature Medicine. 2021;27(8):1321-3. Figure adaptation by Jori Jansen.

The trials described in **Part II** were conducted using the mRNA-1273 vaccine shortly after it received market approval in the European Union. This vaccine was selected because it was the first COVID-19 vaccine available for research purposes and among the first ever mRNA vaccines to receive market approval in the Netherlands.

Chapter 5 presents the first ever study to report on intradermal administration of a mRNA vaccine. Forty participants were assigned to receive either an intradermal administration of 10 µg (1/10th fractional dose) or 20 µg (1/5th fractional dose), or a 20 µg intramuscular dose as the control group. Although the small sample size prevented definitive conclusion about whether intradermal administration yields superior immune responses compared to intramuscular administration, the trial demonstrated that reduced doses elicited antibody levels correlating with high levels of protection as observed in the mRNA-1273 Phase III trial conducted by Moderna.[41]

Chapter 6 reports on a trial involving 150 participants comparing the 20 µg intradermal dose, delivered either with a conventional small-gauge needle or a novel microneedle that enables easy perpendicular administrations (Bella-mu® needle), to the standard-of-care 100 µg intramuscular dose. Both intradermal methods induced robust antibody responses that were slightly lower than those observed in the intramuscularly vaccinated control group. Analysis of cellular immune responses revealed comparable or slightly better T-cell responses in intradermally vaccinated participants (standard needle) compared to the control group, although B-cell responses were somewhat reduced.

Chapter 7 presents the findings from a trial of 129 participants, including 80 who had participated in the trial described in **Chapter 6**. This study demonstrated that robust antibody levels could also be induced by administering a fractional intradermal dose as a booster. Furthermore, participants who received their primary vaccination series intradermally showed similar booster responses to those in the control group.

The results from **Chapters 5 - 7** highlight the immunogenic potential of intradermally administered mRNA-1273 and represent the first step toward new administration methods for mRNA vaccines. Microneedles, such as the Bella-mu needle used in the trial in **Chapter 4**, could facilitate efficient intradermal mass vaccination campaigns. However, even greater progress may be achieved with needle-free innovations like jet injection or permeabilization techniques, which could reduce vaccine hesitancy associated with fear of needles.[42] Moreover, international global health organizations like UNICEF, PATH, CEPI, and GAVI are mobilizing efforts to develop dermal patches covered with microneedles, a so-called vaccine-containing microarray patch (VMAP) that can be stored outside the fridge for a few days.[43-46] VMAPs could directly contribute to increasing global immunization coverage, especially in low-resource settings where problems with maintaining the cold chain make it challenging to complete the last mile of vaccine distribution.[46] However, developing new VMAPs for different vaccines requires addressing specific key questions for each type of vaccine. In a recent study assessing

a VMAP loaded with the mRNA-1273 COVID-19 vaccine, the dermal patch failed to induce an immune response, possibly because the mRNA lipid nanoparticles were too large to diffuse from the ceramic VMAP.[47]

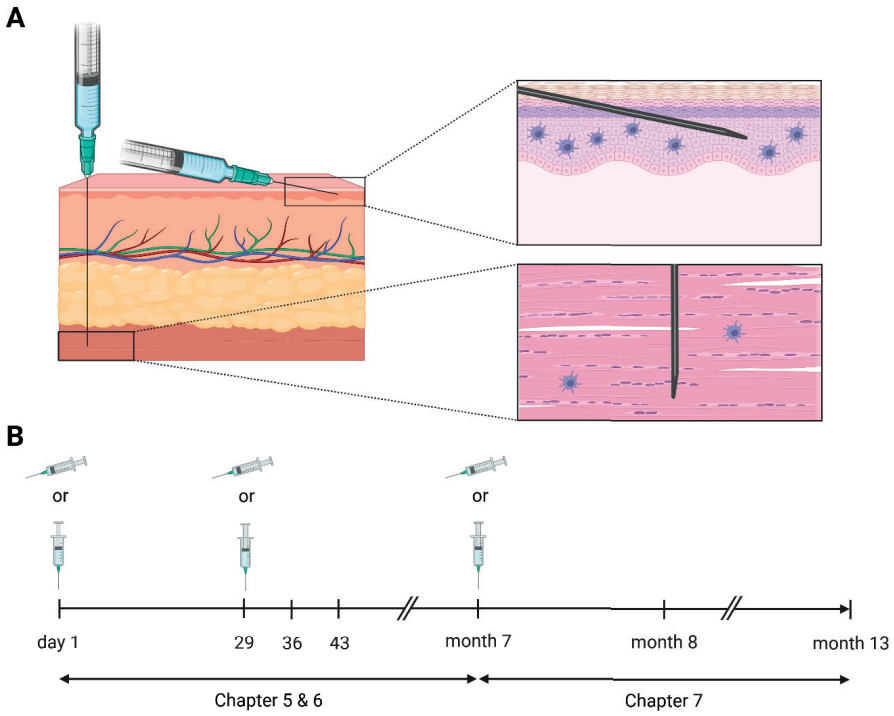


Figure 2. Rationale and design of the intradermal vaccination trials described in Part II

A. Intradermal vaccination requires administration of a fractional dose in the stratum spinosum of the dermis, which is rich in antigen-presenting cells. Intramuscular vaccination requires administration is in the muscle tissue which is low in antigen-presenting cells. **B.** In the primary series, two vaccinations were given at a one-month interval, with additional blood collections 1 week, 2 weeks and 6 months after the second dose. The booster dose was administered 6 months after the second dose, with additional blood collections 1 month and 6 months after the booster.
Figure created with BioRender.

Chapter 8 focuses on a post-licensure study evaluating intradermal administration of the mRNA-1273 in individuals with suspected allergies to mRNA COVID-19 vaccines. This study included 56 patients who were referred to the vaccination outpatient clinic of LUMC after experiencing suspected allergic reactions to their first mRNA COVID-19 vaccination. At the clinic, these patients received a 20 µg intradermal dose of mRNA-1273 to simultaneously assess their allergic response in a controlled setting and complete their primary immunization regimen. Antibody levels measured in 47 patients after intradermal vaccination were comparable to those in a historical reference group that received the standard intramuscular regimen. Importantly, none of the 56 patients experienced anaphylaxis or a severe allergic

reaction, suggesting that intradermal vaccination could offer a pragmatic solution for individuals with suspected allergies to mRNA COVID-19 vaccines.

The studies in **Part II** highlight the importance of post-licensure vaccine research to evaluate dose-optimization strategies and improve global vaccine access during a pandemic. Addressing these challenges asks for a question-based development approach focused on improving equity and accessibility, extending beyond the Phase I-III paradigm that prioritizes rapid market approval. Even after a vaccine receives (preliminary) market approval, key questions can be formulated that address gaps in vaccine equity and accessibility. While commercial incentives to tackle these issues are often lacking, the studies presented here exemplify how publicly initiated and socially driven research can bridge knowledge gaps that pharmaceutical companies do not address.

Conclusion

Most vaccines continue to be clinically developed through standardized steps: small early-phase clinical trials to establish first-in-human administration and identify the highest tolerated dose, followed by large, late-phase clinical trials to assess protective efficacy. Clinical development can be improved by the QBCD framework: formulating key questions critical for development, prioritizing these questions based on scientific and economic arguments, and then designing trials to optimally address these questions.[20] Strategies to answer these fundamental questions more efficiently include involving target populations in early-phase clinical trials and utilizing controlled human infection models to evaluate protective efficacy earlier in the development process.

In times of vaccine shortages, key questions regarding dose optimization, such as fractional dosing, dose stretching, or alternative administration methods become particularly relevant. However, there are currently no incentives for pharmaceutical companies to pursue dose optimization once a vaccine has received market approval. Such studies require additional financial investments and could reduce revenues if findings suggest that one dose is sufficient to immunize multiple people. Consequently, investigators from academia and not-for-profit organizations play a pivotal role in addressing these important questions.

The studies presented in **Part II** of this thesis are among numerous publicly funded initiatives that investigated optimal dosing strategies, demonstrating that a rapid scientific response is feasible. These studies highlight how publicly initiated trials can successfully formulate and answer vaccine-related key questions, ultimately contributing to pandemic response efforts and enhancing preparedness. Nevertheless, these initiatives were not centrally coordinated. Even when substantial evidence supporting fractional dosing strategies emerged, policymakers remained hesitant to implement these findings. This reluctancy likely stems from

concerns about deviating from registered doses and regimens unless more systematically structured and large-scale evidence supporting dose-sparing strategies is presented.

Such evidence can only be generated if governments or international public bodies take control for overseeing and coordinating the post-licensure research. This would allow for the identification of knowledge gaps and ensure that all dose-optimization questions are addressed. During the COVID-19 pandemic, governments covered the financial risks associated with vaccine development.[34-36] Governments should use this position of influence as leverage to demand the evaluation of fractional doses and alternative administration methods in large, late-phase trials. Furthermore, central coordination of post-licensure research would ensure oversight by identifying knowledge gaps, prioritization, distribution of research question across institutions, and consolidation of findings. These efforts would create a robust framework to rapidly deliver scientific sound evidence on dose optimization aiding policymakers in effectively rolling out vaccination campaigns during pandemics.

During periods of vaccines shortages, it is especially difficult for LMICs to acquire pandemic vaccines. Vaccine nationalism of HICs drives up the price during a pandemic, putting LMICs at the back of the queue for vaccine distribution. During the COVID-19 pandemic, initiatives like COVID-19 Vaccines Global Access (COVAX) aimed to counter these inequities to and promote fairer vaccine allocation, achieving considerable but limited success.[48] If similar disparities occur in future pandemics, dose-sparing strategies will hold even greater significance for LMICs than for HICs, since vaccine shortages will exacerbate due to their resource constraints.

For diseases primarily affecting LMICs, vaccine development faces challenges by market failure. Pharmaceutical companies prioritize diseases with a predominant burden in HICs due to their higher potential to generate profits.[49-51] To address this, the international scientific community should strive to establish a sustainable system in which researchers from the Global South develop, test and deploy vaccines tailored to diseases and the needs in their regions. This will reduce dependency on pharmaceutical companies in the Global North as it will empower LMICs to address their own public health priorities more effectively. Currently, this ideal is far from realized. Until then, academic institutions in HICs can support vaccine development for resource-limited settings by addressing key questions relevant for diseases with high prevalence in LMICs. Rather than conducting trials as external researcher from the Global North, collaborations with local researchers from the Global South should be forged to create equitable partnerships.[52] Such collaborations contribute to capacity building, which is essential for enabling researcher in the Global South to independently develop, test, and license future vaccines for disease that impose high burdens on their populations.

Every newly developed vaccine represents a valuable asset for humanity. However, vaccines targeting diseases with high pediatric mortality or those with significant pandemic potential have the greatest impact to transform global health outcomes.[53] Historically, vaccine development priorities haven been driven by economic incentives and opportunities for

profit.[49] Academia and other non-commercial research institutes can counterbalance these market forces. They can ensure optimal allocation of their limited resources by designing clinical trials that address the most pressing key questions. By doing so, they foster innovative vaccine development that prioritizes global health needs.

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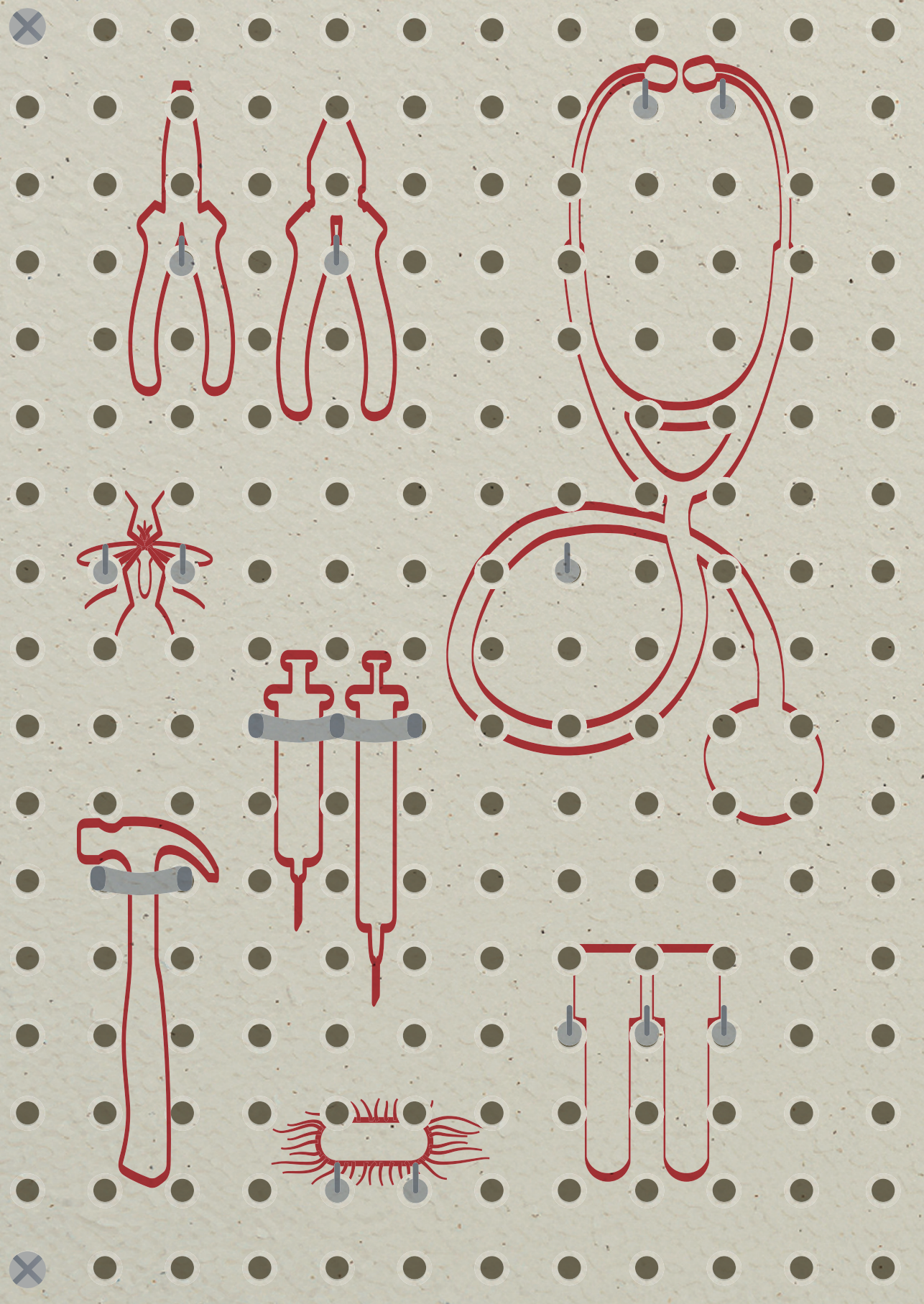

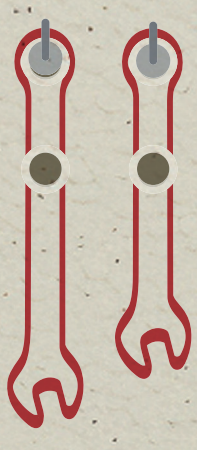

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Appendices

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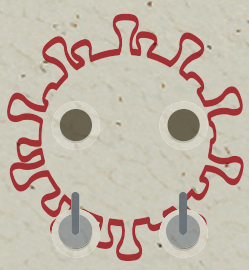

English summary

Vaccines are crucial to global health, preventing millions of deaths each year. Newly developed vaccines can have the greatest impact in preventing mortality and saving life years when targeting a deadly disease primarily affecting children or against an international outbreak of a novel pathogen (or variant). Given their importance, development of these vaccines together with strategies to ensure equitable vaccine access should be considered a top scientific priority. Because these priorities do not always align with commercial interests, pharmaceutical companies hardly ever address these issues. Therefore, vaccine development should not be left solely to the commercial sector, but public bodies and academia must actively participate, with governments closely monitoring and supporting these efforts, directly or indirectly.

In general, clinical vaccine development adheres to the three-phase paradigm. First, a Phase I study evaluates the first-in-human vaccine administration, followed by a Phase II study in which the highest tolerated dose is established. Then, a large Phase III study is conducted to assess the protective efficacy of that dose.

This thesis argues that stepping away from this paradigm can positively influence the development process through decreasing time and costs, and that public bodies and academia can play a pivotal role here. Instead of rigidly following the three-phase model, vaccine development should be guided by identifying key questions based on the biomedical properties of the vaccine and the target disease. Based on scientific and financial arguments, the most optimal order for answering these questions must be determined. Trials should then be designed to address these questions in this most efficient sequence, a concept termed Question-Based Clinical Development (QBCD).

QBCD does not eliminate the need for expensive late-stage clinical trials to evaluate safety and efficacy in a broad population. However, early identification and answering of key questions can minimize the risk of advancing unsuccessful candidate vaccines to late-stage development. Early termination of the development of candidates with low potential has been described as the “fail-fast approach.” This strategy can help save resources, which is particularly important for developing vaccines targeting diseases primarily affecting low- and middle-income countries (LMICs) with limited funding. In a pandemic setting, not only development speed should be prioritized, but also key questions that facilitate widespread vaccine availability after market approval.

This thesis is divided in two parts. In **Part I**, two studies are presented that are designed with the QBCD principles in mind and aim to advance the development of a vaccine against malaria and *Shigella*. **Part II** consists of four chapters that describe studies that aimed to increase the availability of COVID-19 vaccines during the pandemic by addressing key questions on dose optimization.

Part I

Chapter 2 presents findings of a controlled human malaria infection (CHMI) study. In a CHMI study, healthy study participants are deliberately infected with malaria parasites under controlled conditions; they are tested for malaria daily and receive antimalarial treatment immediately once they test positive. A CHMI study is an efficient way to test the protective efficacy of a vaccine in early-stage clinical development without the need for a large clinical trial in a malaria-endemic area. The trial in **Chapter 2** examined the protective efficacy of the GA2-parasite, a genetically attenuated malaria parasite that can infect the liver but does not cause malaria. Previous work found that three immunizations by mosquito bite protected 8 out of 9 participants from a CHMI. The current study revealed that after only one immunization with the GA2-parasite by mosquito bites, 9 out of 10 participants were protected against a controlled infection. This has never been shown before for malaria immunization and could substantially benefit vaccine implementation and traveler's vaccination.

Before this immunization method can be developed into a vaccine ready for late-stage development, it will be critical to investigate key questions on the longevity of the protection, performance in pre-exposed populations living in malaria-endemic areas, and whether similar efficacy can be achieved when using an injectable form of the GA2-parasite.

Chapter 3 reports on a study protocol for a trial that will be conducted by an international consortium with African, European, and North American partners. The trial will assess the safety and immunogenicity of a new *Shigella* vaccine ($Invaplex_{AR\text{-}Detox}$) and adjuvant (dmLT). The vaccine has previously proven safe and immunogenic in adults in the United States. However, previous *Shigella* vaccines were ineffective in the target population (infants and young children in LMICs). Therefore, the study will assess whether the vaccine's immunogenicity can be improved further by adding an adjuvant, an immunostimulant compound. Once the first-in-human and dose-escalation part of the trial has been completed in Dutch adults, the rest of the study will be conducted in Zambia to test the immunogenicity of the vaccine and adjuvant in a *Shigella*-endemic setting. The most important key question for this vaccine and adjuvant combination will be its immunogenicity in children and young adults. To address this, the study consortium aims to perform an age-de-escalation study if the trial described in **Chapter 3** generates promising results.

Part I

Chapter 4 reviews clinical COVID-19 vaccine trials from the early stage of the pandemic. These trials evaluated dose optimization strategies to increase the number of people who could be vaccinated with the same amount of vaccine. To improve pandemic preparedness, we argue that such publicly funded clinical trials should be initiated by international public bodies and governments. Moreover, these institutions should oversee and coordinate all post-licensure vaccine research to ensure effective research and implementation of dose optimization and other strategies that can improve equitable distribution of vaccines. Ideally, pharmaceutical companies are incentivized to integrate dose optimization into vaccine development as well.

Chapters 5-8 present the results of four studies with the mRNA-1273 COVID-19 vaccine (Moderna Spikevax®) conducted soon after the vaccine's market approval in the European Union. We showed that a 1/5th dose can safely be administered intradermally (**Chapter 5**) and that this method induces sufficient immunogenicity for both the primary vaccination series (**Chapter 6**) and the booster vaccination (**Chapter 7**). **Chapter 8** demonstrates that intradermal administration is a pragmatic solution for (suspected) allergy to mRNA COVID-19 vaccines.

Conclusion

Part I highlights the broader issue of vaccine development for diseases endemic to LMICs, where market failure often leads to underinvestment. The studies on malaria and *Shigella* vaccines exemplify how publicly initiated research can fill this gap by addressing key questions that pharmaceutical companies may not prioritize. The ultimate goal is for LMICs to independently develop, test, and license vaccines, reducing their reliance on developers in high-income countries. Until this ideal is achieved, partnerships between researchers from the Global North and the Global South can help build capacity and foster knowledge exchange.

Part II underlines the importance of dose optimization strategies for vaccine development and the role that publicly initiated research can play in this regard. Stronger public oversight and more research into dose optimization can improve equitable vaccine access, particularly during a pandemic.

In conclusion, this thesis demonstrates how academic institutions and publicly funded research can expedite vaccine innovation. By addressing research areas and knowledge gaps that are of little interest to the commercial sector they can play a critical role in advancing the development of vaccines for global health priorities.

10

Nederlandse samenvatting

Vaccinaties redden jaarlijks miljoenen levens en vormen daarmee een cruciaal onderdeel van de mondiale gezondheidszorg. De introductie van een vaccin tegen een ziekte die vooral jonge kinderen treft, kan enorm veel levens(jaren) redden. Evenzo kan de snelle ontwikkeling van een vaccin bij een grote uitbraak van een nieuwe ziekteverwekker (of variant) een enorme positieve impact hebben.

Gezien het belang van vaccins voor de wereldwijde volksgezondheid, moet het een wetenschappelijke topprioriteit zijn om nieuwe vaccins te ontwikkelen en vaccins breder beschikbaar te maken. Omdat sommige van deze belangrijke vaccins commercieel oninteressant zijn, kan dit proces niet uitsluitend aan farmaceutische bedrijven worden overgelaten. Publieke instellingen en universiteiten spelen hierin een essentiële rol en overheden zouden deze rol actief moeten ondersteunen en stimuleren, zowel direct als indirect.

De klinische ontwikkeling van vaccins verloopt doorgaans in drie fasen: in een fase-I-studie wordt het vaccin voor het eerst toegediend aan een klein aantal mensen. In fase II wordt in een iets grotere groep de hoogste dosis vastgesteld die goed te verdragen valt. Tenslotte wordt in fase III in een grote groep studiedeelnemers onderzocht in hoeverre het vaccin bescherming biedt tegen de beoogde infectie.

In dit proefschrift betoog ik dat er niet te star aan deze klassieke fasering vastgehouden moet worden. In plaats daarvan, is het beter om in kaart te brengen wat de cruciale vragen (*key questions*) zijn die van belang zijn voor de ontwikkeling van een specifiek vaccin, afhankelijk van de biomedische eigenschappen van het vaccin en de verwekker waartegen het vaccin gericht is. Op basis van biomedische en financiële argumenten moet vervolgens worden bepaald in welke volgorde deze vragen het best beantwoord kunnen worden. Klinische vaccinstudies moeten daarom zodanig worden ontworpen dat die de belangrijkste *key questions* zo vroeg mogelijk worden onderzocht. Deze aanpak wordt in de literatuur aangeduid als *Question-Based Clinical Development* (QBCD).

Publieke instellingen en universiteiten kunnen hierin een sleutelrol spelen. Zij kunnen onderzoek doen naar *key questions* waar de farmaceutische industrie minder interesse voor heeft, bijvoorbeeld omdat er geen commercieel belang mee gemoeid is. Dit geldt voor vaccins tegen ziekten die met name lage- en middeninkomenslanden (*low- and middle-income countries*, LMIC's) treffen, maar ook voor strategieën om bestaande vaccins breder toegankelijk te maken.

QBCD betekent niet dat er in een later stadium geen grote klinische studies meer nodig zijn om de veiligheid en effectiviteit van een vaccin in een grote onderzoeksgroep te testen. Wel kan het vroeg beantwoorden van de belangrijkste vragen helpen te voorkomen dat er grootschalige

(en dus dure) studies worden uitgevoerd met vaccins die uiteindelijk niet succesvol blijken te zijn. Dit principe staat bekend als de *fail-fast*-strategie: vaccins met een lage kans van slagen zo vroeg mogelijk in het ontwikkelingsproces uitsluiten. Dit bespaart tijd en geld, wat extra relevant is bij vaccins voor LMIC's, waar vaak beperkte financiering voor beschikbaar is.

Bij een pandemie moet het doel niet alleen zijn om zo snel mogelijk een werkzaam vaccin te ontwikkelen, maar ook om ervoor te zorgen dat het vaccin voor iedereen beschikbaar is zodra het op de markt komt.

De hoofdstukken in **Deel I** gaan over vaccinontwikkeling voor infectieziekten uit LMIC's, namelijk malaria en darminfecties met de *Shigella*-bacterie. Deze studies richten zich op *key questions* die essentieel zijn om de ontwikkeling van deze vaccins een stap verder te helpen.

De hoofdstukken in **Deel II** van dit proefschrift behandelen studies die tijdens de COVID-19-pandemie zijn verricht met als doel de nieuwe COVID-19-vaccins breder beschikbaar te maken met dezelfde hoeveelheid vaccin (dosisoptimalisatie). Nadat de eerste vaccins op de markt kwamen, richtten deze studies zich op vragen rondom dosisoptimalisatie die de producent nog niet beantwoord had.

Deel I - Question-based klinische vaccinontwikkeling voor malaria en Shigella

Hoofdstuk 2 bespreekt de resultaten van een gecontroleerde malaria-infectiestudie (*controlled human malaria infection,* CHMI), waarin gezonde studiedeelnemers op een veilige en gecontroleerde manier worden blootgesteld aan malariaparasieten. De deelnemers worden dagelijks getest op malaria en worden direct behandeld als de test positief is. Zo kan vroeg in het ontwikkelingsproces de beschermende werking van een vaccin worden onderzocht, zonder dat hiervoor een grote klinische studie in malariagebied nodig is. Deze studie onderzoekt de beschermende werking van de GA2-parasiet; een genetisch verzwakte malariaparasiet die wel de lever kan infecteren maar geen malaria kan veroorzaken. Een eerdere studie toonde aan dat na drie immunisaties waarbij GA2 door muggen werd toegediend, acht van de negen deelnemers beschermd waren tegen een gecontroleerde infectie. De huidige studie toont aan dat na slechts één enkele immunisatie met GA2 via muggenbeten negen van de tien deelnemers beschermd zijn tegen een CHMI. Dit is nog nooit eerder aangetoond bij een malariavaccin en kan grote voordelen hebben voor vaccinatiecampagnes en reizigersvaccinaties.

Voordat deze immunisatiemethode ontwikkeld kan worden tot een vaccin dat klaar is voor het late stadium van klinische ontwikkeling zullen er in toekomstige studies nog een aantal *key questions* beantwoord moeten worden. Vervolgonderzoek moet inzicht bieden in de duur van de bescherming, de werking in mensen die eerder zijn blootgesteld aan malaria en of dezelfde mate van bescherming kan worden opgewekt met een injecteerbaar vaccin.

Hoofdstuk 3 beschrijft een studieprotocol voor de evaluatie van nieuw *Shigella*-vaccin en een adjuvans (een hulpstof die het immuunsysteem stimuleert). Dit onderzoek zal worden

uitgevoerd door een consortium bestaande uit Afrikaanse, Europese en Noord-Amerikaanse onderzoekers. De belangrijkste studie-uitkomsten worden de veiligheid van vaccin en adjuvans en hun vermogen om een reactie van het immuunsysteem op te wekken (immunogeniciteit).

Een eerdere studie in volwassenen uit de Verenigde Staten toonde aan dat het vaccin veilig is en een goede immunogeniciteit heeft. Echter, eerder onderzochte *Shigella*-vaccins bleken ineffectief in de doelpopulatie (zuigelingen en jonge kinderen in LMIC's). Daarom zal de huidige studie onderzoeken of de immunogeniciteit van het vaccin nog verder verbeterd kan worden door toevoeging van het adjuvans. Eerst zal de veiligheid en dosering van vaccin en adjuvans worden onderzocht in Nederlandse volwassenen. Het tweede deel van de studie zal in Zambia worden verricht met als doel de immunogeniciteit van vaccin en adjuvans te testen in een omgeving waar *Shigella*-infecties veel voorkomen. De belangrijkste vervolgvraag is of deze vaccin-adjuvanscombinatie een goede immuunreactie kan opwekken bij zuigelingen en jonge kinderen. Daarom is het doel van het onderzoeksconsortium om een vervolgstudie in kinderen op te zetten indien de huidige studie veelbelovende resultaten oplevert.

Deel II – Dosisoptimalisatie tijdens een pandemie

Het artikel in **Hoofdstuk 4** biedt een overzicht van de klinische studies uit het beginstadium van de COVID-19-pandemie die strategieën voor dosisoptimalisatie onderzochten. Het doel van dosisoptimalisatie is om met dezelfde hoeveelheid vaccin meer mensen te kunnen vaccineren. Opvallend is dat al deze studies werden gefinancierd door publieke middelen en niet door farmaceutische bedrijven. Daarom pleit het artikel voor een beter gecoördineerde publieke aanpak, zodat *key questions* over dosisoptimalisatie sneller beantwoord worden en vaccins eerlijker verdeeld kunnen worden. Idealiter worden farmaceutische bedrijven ook gestimuleerd om dosisoptimalisatie te integreren in hun vaccinontwikkeling.

Hoofdstukken 5-8 beschrijven vier studies met het mRNA-1273 (Moderna Spikevax®) COVID-19 vaccin die zijn uitgevoerd kort nadat dit vaccin in de Europese Unie op de markt kwam. Hierin tonen we aan dat een 1/5^{e} dosis veilig in de huid kan worden toegediend (**Hoofdstuk 5**) en dat deze methode een toereikende immuunreactie opwekt voor zowel de primaire vaccinaties (**Hoofdstuk 6**) als de booster (**Hoofdstuk 7**). **Hoofdstuk 8** laat zien dat huidvaccinatie een pragmatische oplossing kan zijn voor mensen met een (veronderstelde) allergie voor mRNA-vaccins.

Conclusie

Deel I benadrukt het belang van vaccinontwikkeling voor ziekten die vooral LMIC's treffen. Marktfalen leidt ertoe dat hier slechts beperkt in wordt geïnvesteerd. Het vaccinonderzoek naar malaria en *Shigella* laat zien hoe publiek geïnitieerd onderzoek *key questions* kan beantwoorden die voor de farmaceutische industrie geen prioriteit hebben.

Het uiteindelijke doel is dat LMIC's zelf nieuwe vaccins zullen ontwikkelen, testen en op de markt zullen brengen waardoor deze landen minder afhankelijk worden van ontwikkelaars

in hoge-inkomstenlanden. Totdat deze ideale situatie realiteit is geworden, kunnen samenwerkingsverbanden tussen het Globale Noorden en het Globale Zuiden bijdragen aan capaciteitsversterking en kennisuitwisseling.

Deel II benadrukt het belang van dosisoptimalisatie en de rol die publiek geïnitieerd onderzoek hierin kan spelen. Betere publieke coördinatie en meer onderzoek naar dosisoptimalisatie kunnen een rechtvaardige toegang tot vaccins bevorderen, met name ten tijde van een pandemie.

Dit proefschrift laat zien dat academische instellingen en publiek gefinancierd onderzoek vaccininnovatie kunnen stimuleren. Door kennislacunes te belichten en onderzoek te verrichten naar onderwerpen die voor de commerciële sector minder aantrekkelijk zijn, kunnen deze instellingen een sleutelrol spelen in de ontwikkeling van vaccins die essentieel zijn voor het verbeteren van de mondiale volksgezondheid.

List of publications

In this thesis

Roozen GVT, Sukwa N, Chirwa M, White JA, Estrada M, et al. *Safety, tolerability, and immunogenicity of the Invaplex$_{AR\text{-}Detox}$ Shigella vaccine co-administered with the dmLT adjuvant in Dutch and Zambian adults: study protocol for a multi-center, randomized, double-blind, placebo-controlled, dose-escalation Phase Ia/b clinical trial.* Vaccines 2025;13(1):48.

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Curriculum Vitae

Geert Roozen was born on the 19th of June 1993 in Sanyati, Zimbabwe. He grew up in Leeuwarden where he attended Piter Jelles Stedelijk Gymnasium. In 2011, he went to study medicine at Utrecht University.

During his medical degree, he did a research project at Ndlovu Medical Center in Mpumalanga, South Africa, under supervision of dr. Alinda Vos and dr. Kerstin Klipstein-Grobusch of the Julius Center and dr. Karine Scheuermaier of the University of the Witwatersrand. The project focused on the determinants of cardiovascular disease in people living with HIV in urban and rural areas. Subsequently, he did a research project at Baragwanath Hospital in Johannesburg on echocardiographic abnormalities in people living with HIV, under supervision of dr. Ruchika Meel.

After his graduation in 2019, he worked for one year as an intern-physician (ANIOS) at the Department of Internal Medicine at Tergooi Hospitals in Hilversum and Blaricum. In 2020, he started his PhD research in early-stage clinical vaccine development at Leiden University Medical Center, under supervision of prof. dr. Meta Roestenberg and dr. Anna Roukens. During his PhD, he completed the course “Question-Based Clinical Development” at Paul Janssen Future Lab in Leiden and the course “Development and Implementation of Vaccines in Global Health” at the ISGlobal institute of the University of Barcelona. He presented the results of his research at the ESCMID Conference in Copenhagen and the ASTMH Annual Meeting in New Orleans.

After completing his PhD, he worked for six weeks at Medical Volunteers International in Athens, an organization that provides primary health care to refugees and asylum seekers. He is currently working as an intern-physician (ANIOS) at the Department of Internal Medicine at the Haga Hospital in The Hague.

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