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Diagnosis, prevention and treatment of acute respiratory infections

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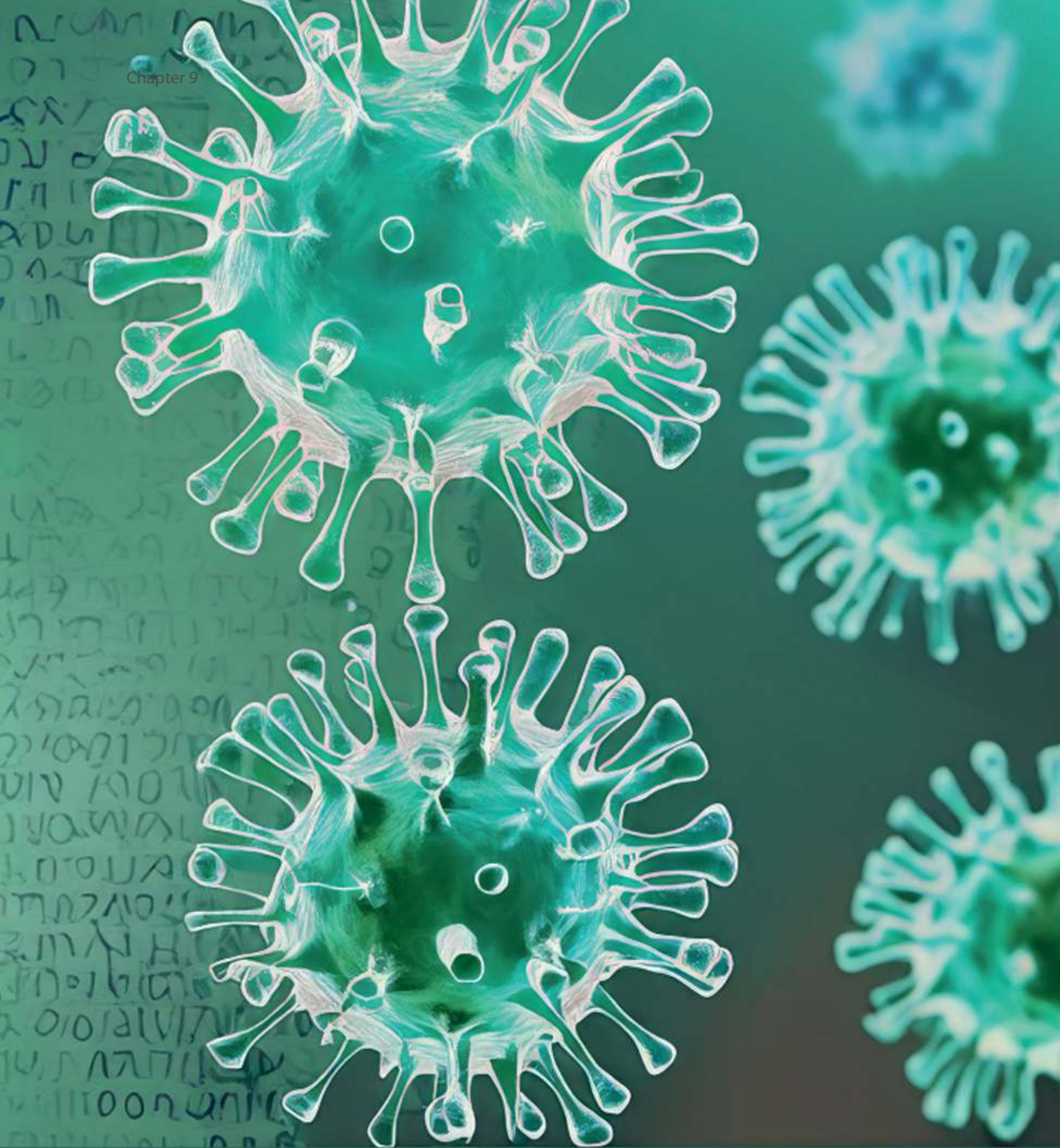
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Chapter 10

Summary and general discussion

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Despite being recently overshadowed by the effects of the COVID-19 pandemic, influenza (epidemics) continues to be a significant contributor to global infectious disease burden. At multiple times, both influenza and COVID-19 have resulted in overtaxing the (Dutch) healthcare system, with crowded emergency departments and overwhelmed hospitals, especially during the winter months (the respiratory season). To prevent an overwhelmed healthcare system, it is important to have effective prevention and treatment options. The general aim of this thesis is to address gaps in the scientific knowledge regarding SARS-CoV-2 and influenza, two major respiratory viruses, and how this can contribute to future (acute) care.

For centuries, humans have been affected by various types of respiratory viruses. Some of which have been known for longer, are more pathogenic, or are more prevalent than others. Respiratory viruses can cause a spectrum of illness, from the common cold to more severe pneumonia. Most studies examining the impact of respiratory viral infections (RVIs) or the effect of vaccines initially focused on healthy (young adult) populations. However, this population generally experiences less severe illness from a RVI compared to more vulnerable populations that are more likely to contract a virus or experience a more severe course of viral infections. The groups at increased risk for complications are determined by patient-related factors, such as the immune system and age, as well as underlying diseases like diabetes and respiratory conditions. Those having a known increased risk of severe course include older adults (65 years and older), pregnant women, young children, people with chronic conditions or immunocompromised patients.¹ Among immunocompromised patients, patients with a solid organ transplant (SOT) represent a very relevant group due to their severe immunocompromised state. While there has been considerable attention in the literature on RVIs in lung transplant recipients, a knowledge gap remains about patients who have undergone other SOTs. This distinction is important because, after lung transplantation, both the transplanted organ itself and the systemic immunosuppressed state contribute to the risk of more severe infections. In contrast, recipients of a SOT other than lungs are mainly affected by the immunosuppressed state alone, as the organ they received is not typically involved in the infection. Therefore, we conducted a retrospective, multicentre study in eight hospitals in the Netherlands to examine the clinical epidemiology, characteristics, and outcomes of RVIs in patients who received a liver, kidney, or pancreas transplant or a combination at the Leiden University Medical Centre (LUMC) over a period of ten years (chapter 2). SOT patients with a PCR positive for a respiratory virus between January 1, 2013 and July 1, 2024, were included. During the study period, 643 viruses were identified in 603 positive PCR test results among 460 patients. Co-infections were found in 38 patients, consisting of 36 cases of double infections and two cases of triple infections. SARS-CoV-2 and influenza were the most frequently identified viruses, followed by human rhinovirus (HRV) and respiratory syncytial virus (RSV). All RVIs were associated with high hospitalization rate (68%). Remarkably, influenza caused the least severe illness compared to the other viral pathogens, with the shortest hospital stay, lowest rates of ICU admission, and 30-day mortality, although it remains a significant cause

of hospitalization and severe acute RVI on its own. In contrast, an infection with SARS-CoV-2 was associated with a higher likelihood of a worse outcome. In addition, the presence of pulmonary consolidations on chest X-ray upon admission and a higher CURB-65 score were also associated with intensive care admission or 30-day mortality.

As asymptomatic patients or patients with mild symptoms of a RVI may not present themselves (at the hospital), the study may suffer from bias, which may lead to overestimation of disease severity. The severity of illness may vary between different viruses, leading to one RVI being overestimated more in severity compared to another. It should be noted, however, that these transplant patients have been clearly instructed to contact their treating hospital physician if they experience symptoms of a (respiratory) infection. However, we cannot say with certainty whether patients actually follow these instructions, due to the retrospective character of this study. To confirm our results, a prospective study would need to be conducted in the future. Conducting serial testing on patients during the winter season, for example through weekly nasal or throat swabs, could ensure that all infected individuals are identified, regardless of symptoms. However, this is not only time-consuming and expensive; it also required a large sample size, and it will be likely that fewer people are willing to participate in such a study.

Overall, chapter 2 demonstrated that the (clinical) consequences of RVIs in patients with a SOT were substantial. Averting (severe) disease through vaccination could be an important preventive measure. At the moment, there is a lot of attention on the RSV vaccine, which is available in the near future for children under one year and for older adults above 60 years.²⁻⁴ However, in chapter 2, it appears that the disease burden caused by RSV in immunocompromised individuals is comparable to the burden caused by other respiratory viruses, which receive less or no attention. Besides for RSV, vaccines are also available for influenza and SARS-CoV-2. While the SARS-CoV-2 vaccine has understandably only been developed in the past few years, the influenza vaccine has been available for much longer. The first influenza vaccine approved for wider dates from 1945.⁵ Extensive vaccine research has already been conducted, but these studies initially focused on healthy young adult populations. However, immunocompromised patients, such as SOT recipients, have an increased risk of influenza-related complications compared to healthy, immunocompetent individuals, as shown in chapter 2.^{6,7} In addition, SOT recipients require lifelong use of immunosuppressive medication, which affects the functionality of B- and T-cells. Consequently, the immunogenicity of the influenza vaccine in SOT recipients is reduced compared to immunocompetent persons. Studies have shown that the compromised immune system in SOT recipients has led to a reduced antibody response to influenza vaccines.⁸⁻¹¹ The benefits of influenza vaccination of SOT recipients have been reported in studies examining outcomes related to the survival of the organ transplant, severity of illness, or length of hospital stay. However, there are few studies assessing the real-world effectiveness (VE) of the influenza vaccine in this population. In chapter 3 we evaluated the VE in preventing (severe) symptomatic influenza infection in immunocompromised adults with a SOT. We retrospectively reviewed all patients who underwent organ transplantation at the LUMC and were tested for influenza using PCR between January 1, 2013, and July 1, 2024. We included patients who presented

with fever and/or respiratory symptoms at the LUMC or at one of the seven affiliated hospitals during the respiratory season. A total of 776 patients were included, of whom 174 tested positive for influenza (cases) and 602 were test-negative (controls). Among the controls, 183 had a positive PCR result for another viral pathogen, while 419 patients had a negative result for any virus. We assessed whether patients had received their influenza vaccination in the year prior to their PCR test. After adjusting for confounding variables, the average VE was found to be low (6.9%) and showed significant variability from year to year. Influenza-related outcomes such as length of hospital stay, need for ICU-admission or 30-day mortality did not differ between the vaccinated and unvaccinated groups. A significant limitation of this study is its retrospective nature. Patients with mild symptoms may have stayed at home, which would have excluded them from the study. This may have led to an underestimation of the VE, especially if these were individuals who had been vaccinated. Additionally, patients who have undergone SOT are advised to contact the hospital as soon as they have fever or (mild) respiratory symptoms, which leads us to believe that the impact on VE is minimal. Ideally, we would determine clinical VE in a prospective study, where everyone is instructed to report any (mild) respiratory symptoms or where everyone is periodically tested for influenza, allowing for the inclusion of asymptomatic individuals. In practice, however, such a study would require a significant amount of time and financial resources, as well as a large sample size to draw reliable conclusions. Moreover, it may be more challenging to find participants for a similar influenza study with a periodic testing policy, in contrast to previous COVID-19 studies where periodic testing was conducted.^{12,13} It would require much effort and time from the participants and it would not offer any direct consequences or benefits, unlike SARS-CoV-2, where test results had an impact on their vaccination passport, travel, social activities or work. In addition, it would be even more ideal to conduct a randomized controlled trial in which half of the patients receives the vaccine and the other half does not. If the VE is truly as low as our study suggests, this approach would not be unethical.

Despite the limitations due to the retrospective design, the calculated VE in this population is so low that it may actually lead to doubts about the usefulness of influenza vaccination among immunocompromised individuals and doctors. The vaccination degree in the Netherlands already indicates that not everyone gets vaccinated, and that vaccine hesitancy exists, as only 56.8% and 55.2% of the Dutch people who received an invitation for the influenza vaccination actually got vaccinated in 2022 and 2023, respectively.¹⁴ In our study, the vaccination degree was also low: 47%.

Thus, the low VE may raise the question whether the benefits outweigh the efforts and costs involved. However, our study focused on preventing (severe) influenza, while other studies have shown that the severity of illness in vaccinated SOT recipients is lower¹⁵⁻¹⁸, which is a strong argument in favour of annual vaccination. However, we found no difference in influenza-related outcomes between the vaccinated and unvaccinated group (chapter 2). It is possible that with our study we still do not accurately know whether the vaccine has prevented severe influenza, and if so, to what extent, as patients with mild symptoms may not have sought medical attention, despite our recommendation to do so. Nevertheless, the findings of our study call for a larger prospective method to determine the VE, and if the low effectiveness is

confirmed, a change in the current vaccination strategy is warranted. This could involve vaccinating later in the season (to reduce waning), applying a higher dose or administering repeated doses. The low VE could also be an argument for vaccinating health care personnel working in transplant departments and household contacts of the patients to reduce the burden of influenza infections.¹⁹

In the future, it would be valuable to have more data on such regional VE estimates. Ideally, it is worthwhile to prospectively monitor the effects of vaccinations, for example by using a platform approach^{20,21}, which could, among others, simultaneously measure antiviral activity and VE and which would also allow for the inclusion of other respiratory viruses.

Treatment of influenza

We have experienced several influenza pandemics over the past century (Spanish Flu, Mexican Flu) and more are likely to follow in future. During these pandemics, everyone is susceptible, but immunocompromised individuals are at greater risk for more severe outcomes. Therefore, appropriate treatment is crucial and is also a strategy to limit or prevent the severity of disease, besides vaccination. For many RVIs, no treatment is available. For influenza, neuraminidase inhibitors such as oseltamivir are the principal antiviral drug for early treatment of influenza, available since 1999. This therapy is used to reduce the severity and duration of the illness. While the use of oseltamivir is recommended, the evidence regarding the clinical benefits is inconsistent, in particular in SOT recipients. In clinical practice, treatment of influenza with oseltamivir in SOT recipients may be suboptimal as physicians may decide not administer antivirals when SOT recipients present with symptoms with a duration exceeding two days. However, the majority of patients come to a hospital after >48 hours of illness. On the other hand, prolonged shedding is common in SOT recipients.¹⁵ In a retrospective observational study of 173 lung transplant recipients, 22 of whom were infected with influenza, prolonged viral shedding (defined by RT-PCR on a swab) for ≥ 7 days was detected in 15 cases (68%), even though oseltamivir treatment was initiated as soon as influenza was suspected. Early treatment (<48h) was not associated with extended duration of shedding. The median duration of shedding was 21 days for influenza A and 9 days for influenza B. A high viral load at diagnosis was identified as a predictor of prolonged shedding.²² Additionally, a study involving 86 patients with influenza demonstrated a significantly longer shedding of 19 days among immunocompromised patients (primarily due to a malignancy or condition being treated with either a recent hematologic stem cell transplantation or immunosuppressive therapy), compared to a mean of 6.4 days among non-immunocompromised patients.²³ Starting therapy regardless of duration could be justified by prolonged viral shedding, as benefits are biologically plausible. In a retrospective study involving 390 patients with an influenza infection (of which 46% were immunocompromised), 30-day mortality and the composite endpoint (30-day mortality or ICU admission >48h after admission) were reduced by 9% and 11%, respectively, in the group treated with oseltamivir within 48 hours of admission, compared to those who did not receive oseltamivir.²⁴

In our study (chapter 2), 66% of the influenza positive SOT recipients was treated with oseltamivir. Unfortunately, the sample size of our study was too small to evaluate the effect of adequate treatment of oseltamivir (i.e. in the appropriate

dosage, initiated within 48 hours of hospital admission) on reducing influenza related complications. It would be valuable to address this question with future studies, by determining the effect of adequate treatment of oseltamivir on reducing influenza related complications, such as 30-day mortality, among adult influenza SOT recipients who require hospital admission.

COVID-19 pandemic

At the start of the pandemic, the focus of research was primarily on finding therapeutical approaches and vaccine development, as vaccines are by far the most effective public health strategy to control the pandemic. COVID-19 yields more than 400K hits in just five years on PubMed (in contrast to influenza, which only has 160K hits), indicating that a substantial amount of research on COVID-19 has been conducted in a relatively short timeframe. This was caused by several factors. The social pressure resulting from the COVID-19 pandemic was considerable, and the health effects of the pandemic were significant. Furthermore, the COVID-19 pandemic has caused the largest economic crisis in a century.²⁵ The closure of schools and workplaces resulted in significant unemployment and the permanent closure of many businesses.

Given that the pandemic posed an immediate threat to public health and economy, there was a rapid mobilization of resources and funding. Researchers, institutions and governments around the world collaborated extensively, and the high public interest drove researchers to share their findings quickly with both public and scientific community through pre-print publications, bypassing peer reviews. This accelerated the spread of knowledge and had a significant impact on global treatment guidelines. After identifying SARS-CoV-2 in China, the virus genome sequence was immediately made publicly, helping scientists quickly identify vaccines and treatment targets for COVID-19. In addition, previous research on coronaviruses (like SARS-CoV-1 and MERS) provided valuable insights into structure and behaviour of the virus.^{26,27} Research progress was further accelerated by substantial financial investments from governments, organizations and private sector. The Coalition for Epidemic Preparedness Innovations (CEPI) spent millions to fund vaccine development efforts, including our ID vaccination trial, enabling multiple vaccine candidates to enter clinical trials simultaneously.²⁸ Traditional vaccine approaches, such as inactivated, live-attenuated and protein-based vaccines had been the primary methods until then. However, mRNA-based vaccines, made possible by previous advances in genetic engineering²⁹, became a new approach to prevent severe infection.

Generally, developing a new vaccine often takes 10-15 years, following the established processes for vaccine development, clinical trials and regulatory approval.³⁰ However, shortly after the onset of the pandemic, in March 2020, the first phase I clinical trial of the mRNA-based vaccine (mRNA-1273, the Moderna vaccine) was started in the United States.²⁹ The phase I clinical trial for the mRNA-based vaccine from Pfizer-BioNTech started in April 2020, as well as the phase I/II study for the vector vaccine from AstraZeneca and for the inactivated virus vaccines from Sinovac and Sinopharm. Later that year, the phase I studies from Johnson & Johnson began. First results of these phase I trials were released in July 2020 and large-scale phase II/phase III vaccine trials began. The phase III trials have reported a high vaccine efficacy for several vaccines against symptomatic SARS-CoV-2, with the highest efficacy (>90%)

for the mRNA-based vaccines.²⁷ Two mRNA vaccines (mRNA-1273 from Moderna and BNT162b2 from Pfizer) received initial approval by the FDA (Food and Drug Administration) in the US and EMA (European Medicines Agency) in Europe at the end of 2020 and early 2021.²⁷ Several other vaccines have been approved in different parts of the world, such as CoronaVac, CoviVac, Oxford-AstraZeneca vaccine (ChAdOx1 nCoV-19), Sputnik V, and Johnson & Johnson vaccine.

Route of vaccination

All phase I/II/III trials for the new developed SARS-CoV-2 vaccines were conducted using IM vaccination, which is the standard route for vaccination. Other vaccination methods, such as ID vaccination, were not initially explored, while a significant global vaccine shortage was present from the start of the pandemic. ID vaccination is a dose-sparing injection technique. The vaccine is administered into the dermal papillary layer of the skin, which is rich in antigen-presenting cells (APCs), which are essential for initiating both the humoral and cellular immune response. A fractional ID dose of one-fifth or one-tenth of the standard IM dose can induce immune responses comparable to that generated by the standard dose given IM. This was already established for other infectious diseases.³¹ Based on previous research, our hypothesis was that a dose reduction of 80-90% could be an effective dose sparing strategy in mass emergency vaccination during the COVID-19 pandemic, and that two fractional doses of SARS-CoV-2 would be sufficient to elicit a protective antibody response. We tested this hypothesis in a proof-of-concept, dose-escalation, randomized study (chapter 4) involving healthy volunteers aged 18-30 who had not previously contracted COVID-19 and had not been vaccinated. The study followed a stepped dose-escalation strategy to determine the most appropriate vaccine dosage with a relatively small number of participants, paving the way for a larger follow-up study in the future. In the first part of this study, we reduced the dosage of the mRNA-1273 vaccine to 10% of the standard IM dose (10 µg), and ten participants were vaccinated ID. In the second part, 30 participants were 1:1 randomized between the 20 µg IM group and the 20 µg ID group, allowing us to compare the differences between the two administration routes in more detail. All patients receiving fractional doses of 10 and 20 µg showed a good and robust increase in (neutralizing) antibodies by day 43, comparable between the three groups of the first two parts of the study. The highest antibody response was observed in the 20 µg ID group. After 7 months, antibodies remained detectable in all three groups. The antibody concentrations observed after fractional dose vaccination fell within the range associated with high levels of protection in the phase III trial of mRNA-1273.³² Additionally, participants maintained a diary for the first 14 days to track any side effects following vaccination. None of the participants experienced severe adverse events. A limitation of the study was the inclusion of only healthy, young adults, meaning that the findings of safety and immunogenicity may not be applicable to the older population. A strength of our study, however, is that we compared the immunogenicity of the same antigen amount (20 µg) via different routes (IM and ID). A common criticism is that drawing firm conclusions about the dose-sparing potential of ID delivery from clinical trial data is often challenging due to limitations in the design of most studies. Most studies examining ID delivery have compared a reduced dose with the full dose administered via the standard IM or SC route. This, however, still

leaves open the possibility that similar dose-sparing benefits could be achieved with IM/ SC delivery as well.³¹ In our study, the highest antibody response was observed in the 20 µg ID group, suggesting a better immune response following ID delivery, although significance could not be determined due to the sample size.

The safety and immunogenicity findings from the first parts of the trial were promising and provided strong support for further investigation of ID vaccination using the mRNA-1273 vaccine. In chapter 5, we examined in a larger, randomized, non-inferiority study involving 150 participants whether the antibody response elicited by two fractional ID doses of the mRNA-1273 vaccine, was non-inferior to the antibody response of a control group that received standard dose IM vaccination. Additionally, this chapter explored the use of an easy-to-use, short ID microneedle, the Bella-mu® 1.4mm. Next, we also examined the T- and B-cell responses in a subgroup of participants. The non-inferiority margin was set at 15%. The primary outcome measure of our study was non-inferiority regarding the percentage of participants achieving seroconversion, based on 50% virus neutralization, measured on day 43 post-vaccination. We had to adjust the definition of seroconversion during the study because the original definition was based on a study that used a different neutralization assay (plaque reduction test).³³ instead of the microneutralization assay we employed. Furthermore, at the start of the study, there was no established cut-off value for neutralizing antibody concentrations that correlated with protection against COVID-19. Therefore, we chose to define seroconversion as a post-vaccination increase in neutralizing antibody concentration of at least 465 IU/mL, which was the lowest concentration measured in the IM group. The seroconversion rate on day 43 in the IM group was (as expected) 100%, while it was 86% (95% confidence interval (CI) -24.31 to -3.6) and 87% (95% CI -22.78 to -3.31) in the ID- standard needle (ID-SN) and ID-Bella-Mu® (ID-BM) groups, respectively. The lower limit of the 95% CI for the absolute difference in the proportion of seroconversion between the fractional and standard dose groups exceeded the pre-defined non-inferiority margin of 15%. On day 43, the neutralizing antibody concentrations showed a significant increase in all three groups, with the highest concentrations observed in the IM group, which 95% CI interval overlapped with the 95% CI of both the ID-SN and ID-BM groups. After 7 months, antibody levels remained elevated in all groups, with the highest concentrations in the IM group. While the SARS-CoV-2 B-cell responses were lower in the ID group, T-cell responses were comparable across the groups. The reported side effects were generally mild to moderate and of a temporary nature. The ID groups experienced more local side effects, such as pain, redness, or itching at the injection site. Systemic side effects, including fatigue, malaise, headache, and chills, were more frequently reported in the IM group, particularly after the second vaccination.

Although ID vaccination with a 1/5th dose of the mRNA-1273 vaccine did not meet the pre-specified non-inferiority criteria, the neutralizing antibody concentrations were significantly higher than the proposed proxy for protection against severe disease as found in other studies. Gilbert et al. estimated that a concentration of 300 BAU/mL at day 57 was linked to 90% protection against symptomatic COVID-19 (D614G variant) following vaccination with the mRNA-1273 vaccine.³² In our study, conducted during the wave of the Delta variant, all participants achieved sufficient SARS-CoV-2 spike S1

binding antibody levels exceeding 300 BAU/mL. Furthermore, all but one participant in the ID-SN group demonstrated a neutralizing antibody concentration above 100 IU/mL at day 43, indicating a high level of protection across all groups, despite not achieving the predefined non-inferiority criteria.

We conducted a unique study and to our knowledge, we are the first and only randomized-controlled study investigating the immunogenicity, reactogenicity and in-dept T- and B-cell responses after a primary series of ID mRNA-1273 vaccination. One other Thai study assessed various homo- and heterologous IM and ID vaccinations regimens as primary series, however the mRNA-1273 vaccine was not included. They reported comparable levels of SARS-CoV-2 anti-RBD IgG antibodies in the ID group compared to the IM group, except for BNT162b2.³⁴ Currently, research in a COVID-naïve population can no longer be conducted, as virtually there will be no one left who has not developed immunity against SARS-CoV-2, either through a COVID-19 infections or through vaccination.

In our view, the ID administration of a fractional dose of the mRNA-1273 vaccine (or other vaccines), with fewer side effects and limited loss of efficacy, could provide significant advantages from a public health and the economic point of view. This is particularly crucial during a pandemic, as it ultimately leads to lower rates of infection and mortality compared to a scenario where more individuals remain unvaccinated for extended periods because of vaccine shortage. Even with reduced effectiveness against symptomatic infection, fractional dose vaccination could still confer a high level of protection against severe disease at the population level, due to increased vaccine availability and a higher vaccination rates, which would facilitate quicker herd immunity against severe disease.³⁵ At this moment, there is no longer a shortage of COVID-19 vaccines, but in future pandemics, we recommend to evaluate dose-sparing fractional ID regimens early in the vaccine development phase compared to full-dose protocols. In addition, we recommend to employ this dose sparing method for general use in emergency outbreak situations.

Side effects SARS-CoV-2 vaccines

Shortly after the roll-out of SARS-CoV-2 vaccination, several potentially severe side effects were reported, including venous thrombosis. In chapter 6, we investigated whether fractional ID dosing would lead to a lower risk of thrombosis compared to the full dose administered intramuscularly. We specifically examined changes in indicators of a prothrombotic state, such as levels of coagulation factor VIII, fibrinogen, and D-dimer, as well as thrombin generation parameters post-vaccination. We also examined the association between these changes and the inflammatory response measured by high-sensitive C-reactive protein (hs-CRP).

Thrombin is an enzyme crucial for blood coagulation, activating various clotting factors. The five thrombin generation parameters are “lag time” (arbitrarily defined as the time until 10 nM thrombin is formed), “time to peak” (time to maximum thrombin generation), “peak height” (peak thrombin generation, “ETP or area under the curve” (endogenous thrombin potential), and “time to tail” (time to the end of thrombin generation). The ETP reflects the amount of thrombin the body can produce in response to bleeding and is considered the net result of two opposing factors that are active in the plasma: procoagulants and anticoagulants that promote thrombin

breakdown.

Vaccination with a full IM dose or a fractional ID dose of mRNA-1273 both resulted in a temporary prothrombotic state, as evidenced by changes in ETP, fibrinogen levels, and D-dimer (difference from day 1 to day 36). This change was most pronounced in the IM group. This increase in coagulability coincided with the inflammatory response to the vaccine. However, it should be noted that fibrinogen and factor VIII are both acute phase proteins, which explains their association with inflammation. The correlation between changes in ETP and inflammation appeared to be primarily due to a prolonged time required to fully inhibit thrombin generation, as there was a relatively stronger association of the time to tail with inflammation compared to lag time, time to peak, or peak height. Thus, ID vaccination with a fractional dose had a reduced effect on coagulation and inflammation compared to IM vaccination, providing an additional benefit that may further support the implementation of ID administered vaccines. Although the actual risk on VTE cannot be determined based on our study due to the small sample size, it is important to include this endpoint in future studies.

Booster vaccination

The rapid development and widespread implementation of SARS-CoV-2 vaccination have proven effective to control the COVID-19 pandemic. Lockdown measures could be lifted, allowing for a gradual return to normal life. However, with waning immunity following previous vaccinations and the threat of new highly contagious virus variants and possible resurgence of COVID-19, the decision was made to initiate a new round of vaccinations in the Netherlands. Starting in September 2022, persons of 12 years and older became eligible for a booster vaccination.

Having previously demonstrated that administering two fractional doses of 20 µg mRNA-1273 as a primary vaccination series was safe and immunogenic, we aimed to investigate whether this strategy would also be suitable as a booster method (chapter 7). The study described in chapter 5 was extended, and six months after the primary ID or IM vaccinations, a booster dose of the mRNA-1273 vaccine was administered to willing participants. Four groups of different ID and IM primary and booster combinations were compared. Vaccination regimens that included one or two fractional ID vaccine doses exhibited lower immunogenicity during this follow-up study. On day 29, anti-S1 antibody levels were significantly higher in the IM-IM group compared to the ID-IM and IM-ID groups, although the geometric mean fold ratio (GMFR) did not differ among the groups. Conversely, neutralization titers on day 29 were highest in the ID-IM group and lowest in the IM-ID group, with a significant difference between these two groups. Six months after the booster vaccination, all 27 remaining participants still had detectable antibodies. Anti-S1 antibody levels were significantly higher in the ID-ID group compared to the IM-ID group. Neutralization titers at this time point were also lower in the IM-ID group compared to the ID-IM and ID-ID groups. SARS-CoV-2 specific B-cells increased similarly across the IM-IM, ID-IM, and ID-ID groups. T-cell responses were comparable between the ID-ID group and the IM-IM and ID-IM groups. The reported side effects were mostly mild or moderate and temporary in nature. However, in the IM groups, more systemic side effects were reported.

Thus, booster vaccination with a fractional ID dose of 20 µg was found to be safe. The fact that the geometric mean fold ratio (GMFR) did not differ between the groups, despite variations in antibody levels, suggests a robust memory response, where the anamnestic response of memory B-cells rapidly facilitates the production of antibodies. This is an important finding for future pandemics: utilizing ID vaccinations with the mRNA vaccine as a dose-sparing strategy during the early phase of an outbreak would not compromise the ability to boost vaccinated individuals later in the outbreak with a regular dose when vaccine supplies increase. This makes fractional ID administration a viable option for low-risk groups during periods of limited vaccine availability or if costs are a limiting factor. In the acute stages of an epidemic, quickly immunizing a larger number of individuals can yield significantly more benefits than inducing higher antibody concentrations in a smaller group. The finding that individuals who were primarily vaccinated with a fractional ID dose responded excellently to boosters underscores the need to incorporate fractional dosing regimens in the early development phase of future mRNA vaccines.

Limitations of ID vaccination

Despite the various advantages of ID vaccination, there are also several limitations. First, the applicability and benefits of ID administration can vary by vaccine. As such, the degree of dose-sparing must be individually assessed for each vaccine in non-inferiority studies, as we did in our study. Additionally, it is important to consider the suitability of the vaccine type and formulation, particularly regarding the potential local adverse events induced by the chemical adjuvant, which is added to enhance the vaccination.³⁶ In addition, ID delivery of fractional doses of an existing vaccine formulation intended for IM injection must meet stringent regulatory requirements and approval processes, which can be time-consuming and resource-intensive.³⁷

Another common criticism is that the ID administration of a vaccine requires specific techniques and training, particularly when employing the Mantoux technique. Healthcare providers need to be trained in administering vaccines in this manner, which can require additional time and resources. To address this issue, new technologies are being developed to facilitate the administrations of ID vaccinations, including ID microneedles, needle-free injection devices (jet injectors), ID adapters, and microneedle arrays (microneedle patches). Chapter 5 examines the use of an easy-to-use microneedle- which allowed for perpendicular injection instead of using the Mantoux technique, with similar results with regard to immunogenicity and safety compared to the conventional Mantoux technique. Personally, I found that ID injection technique with the Bella-mu® needle was easier to master.

Innovative delivery methods such as needle-free injection systems are prioritized by CEPI because they can improve accessibility and efficiency in vaccine delivery. These technologies simplify administration, enhance the safety by reducing the risk of needle-stick injuries, and minimize waste by decreasing the need for sharps disposal, thus simplifying mass immunizations campaigns. We investigated the needle-free delivery method through microneedle patches in chapter 8.

Microneedle patches

Microneedle patches consist of needle-like structures arranged on a small patch,

typically less than a millimetre in length. These microneedles ideally perforate the stratum corneum without reaching the nociceptors, which are the pain receptors located in the skin. The vaccine is locally delivered by microneedle patches through diffusion to APCs in the dermis and epidermis. Various types of microneedles are being developed, including solid, drug-coated, dissolvable, and hollow microneedles, each with its own (dis)advantages.³⁸

In chapter 8, we investigated the safety and immunogenicity of needle-free ID administration of a fractional booster dose of 20 µg of the mRNA-1273 vaccine using a ceramic nanoporous microneedle patch (npMNA patch) developed by MyLife Technologies. This innovative microneedle technology features an interconnected nanofluidic network in both the microneedles and the reservoir of the backing layer, providing storage capacity for pharmaceutical formulations such as vaccines. The vaccine is pre-loaded onto the npMNA patch and is expected to diffuse into the interstitial fluid in the skin through the microneedle coating or from the nanopores.

A total of 20 participants were randomized into two groups, with one group (10 participants) receiving 20 µg (40% of the standard booster dose) of mRNA-1273 via the npMNA patch (ID-patch group). The control group (10 participants) received the same dose intramuscularly (IM group). At baseline, all participants had detectable antibodies. In the IM group, anti-S antibody concentrations showed a rapid increase on day 15, followed by a slight decline on day 29. However, none of the participants in the ID-patch group exhibited a response on day 15 or 29 after vaccination. Analysis of the remaining content showed that the majority of the vaccine was not present on the patch anymore. Therefore, we hypothesized that the mRNA vaccine was released by the patch, but was not delivered into the dermis. Evaluation of the loading procedure (by applying droplets of mRNA-1273 to the needle side of the npMNA, rather than solely on the needles themselves) revealed that only 10% of the load was present on the surface of the needle tips, while 90% was located on the backing plate (the surface of the npMNA between the needles). The lipid nanoparticles of mRNA-1273 are likely too large (approximately 180 nm) to diffuse from the backing plate through the cavities created by the needles into the skin.

Despite these disappointing findings, microneedle patches remain a promising alternative to traditional needle-based vaccinations for several reasons. First, there is a reduced risk of contamination compared to traditional needles, less training is required for personnel, and it may even allow for self-administration. Second, they offer a painless vaccination method, as they do not reach skin nerves. This is important, as fear against injection is one of the reasons for vaccine hesitancy, especially reported after the US FDA approval of SARS-CoV-2 vaccines.³⁹ Individuals with blood-injection-injury phobia were more likely to report COVID-19 vaccine hesitancy compared to individual without.⁴⁰ Addressing such fears will likely enhance the effectiveness of vaccinations programmes. In our study, we observed that particularly individuals who were afraid of needles were drawn to this option, hoping for a pain-free vaccination. In hindsight, they expressed high satisfaction with the microneedle arrays. In a post-hoc analysis of a n=1 study, I have personally experienced that administering a liquid intradermally using the microneedle patch is painless.

Future research should focus on this area and explore which needle-free options are most suitable for this purpose. New developed systems could encourage

both medical practitioners and patients to choose for ID vaccination. Perhaps innovative research⁴¹ in needle design, featuring more precise injection methods and easier-to-use devices, will spark renewed interest in this strategy of vaccination, potentially leading to the standardization of ID vaccination.

Vaccine hesitancy

Despite extensive evidence supporting the effectiveness and safety of vaccinations, an increasing number of individuals are hesitant to receive recommended vaccines or refuse them outright. Vaccine hesitancy issues are seen throughout the history.⁴² In the Netherlands, this was more evident during the last years, as the proportion of children vaccinated under the National Immunization Program has declined.⁴³ This seems to have particularly emerged since the COVID-19 pandemic, during which a significant amount of research was conducted on vaccine hesitancy.

COVID-19 vaccine hesitancy has been reported among various populations and there is heterogeneity among vaccine hesitant individuals.⁴⁴ By the end of 2022, 80.4% of those aged 12 years and older completed their primary SARS-CoV-2 vaccination series in the Netherlands. 62.1% of those aged over 18 years received a booster vaccination.⁴⁵ Reasons for vaccine hesitancy are diverse. Structural barriers to vaccination is one of the reported reasons: challenges in scheduling or attending appointments to vaccinate, difficulties in taking time off from work or school, distance to vaccination sites and language barriers. Other common reasons for vaccine hesitancy include uncertainty regarding VE, a desire to wait and see if vaccines are safe and concerns about potential side effects.^{46,47}

Our research shows that ID vaccination was safe and had resulted in fewer systemic side effects following SARS-CoV-2 vaccination, potentially resulting in less absenteeism from work or school (chapter 4, 5 and 7). This lower number of systemic side effects was regarded as an advantage by the participants. This could also contribute to reduced vaccine hesitancy and, therefore, higher vaccination degree. Using needle-free methods for vaccination (chapter 8) could further reduce vaccine hesitancy.

Treatment of COVID-19

Alongside preventing of (severe) infectious diseases with vaccinations, administering the right treatment is also crucial for maintaining manageable healthcare during pandemics and epidemics. At the onset of the COVID-19 pandemic, it was natural that there was limited knowledge regarding the treatment options for SARS-CoV-2. Consequently, numerous clinical trials have been conducted in a short timeframe to evaluate therapeutics against SARS-CoV-2. Many of these therapeutics are repurposed drugs (medications that were previously used for other indications) or registered agents with antiviral activity against coronaviruses in vitro.^{48,49} The TICO platform (Therapeutics for Inpatients with COVID-19) was established to rapidly evaluate the safety and efficacy of potential COVID-19 therapeutics, targeting either the host immune response to SARS-CoV-2 infection or viral control.

DARPin®, Designated Ankyrin Repeat Proteins, were a new class of engineered protein therapeutics, derived from naturally occurring ankyrin repeats and designed to bind with high affinity and specificity to other proteins.⁵⁰ Ensovibep is a DARPin,

designed to neutralize SARS-CoV-2 and it was the first DARPin anti-infective to enter human clinical trials (chapter 9). Ensovibep consists of three DARPins that bind to the RBD, which can simultaneously target the receptor-binding ridge on each RBD of the spike trimer, thereby locking the spike in an open confirmation and blocking the ACE2 binding site.⁵¹ Ensovibep was administered intravenously in two different doses (225 mg and 600 mg) to a small group of outpatients adults with mild to moderate COVID-19 in the early stages of their illness (median time from onset of symptoms was 5 days [range 2-8 days]), aiming to inhibit viral replication and prevent severe disease. This study demonstrated that both doses had a mean half-life of approximately two weeks. No apparent difference was observed in pharmacodynamic outcomes between high and low doses. Viral load in nasopharyngeal swab decreased rapidly in both dose groups during the first two weeks (5.1 and 5.3 log₁₀ copies/mL for the 225 mg and 600 mg dose, respectively). No serious side effects were observed, and the treatment was well tolerated; no antibody-dependent enhancement of infection was observed. All participants showed a reduction in their COVID-19 symptom score, particularly in the first week following ensovibep administration. However, the interpretation of pharmacodynamic parameters is limited due to the small patient group and the absence of a control group.

Following this study, the drug was further investigated in a larger phase 2a placebo controlled study (EMPATHY study), in non-hospitalized, symptomatic patients (enrolled within seven days of symptom onset), which confirmed antiviral activity with a significant reduction in viral load from baseline to day eight and demonstrated clinical benefits of ensovibep compared to placebo.⁵² In addition, fewer COVID-19 related hospitalizations and/or emergency departments visits and all-cause mortality were observed with ensovibep at all doses up to day 29 compared to placebo. Another double-blind, randomized-controlled clinical trial investigated the administration of 600 mg ensovibep in adults hospitalized with COVID-19 (median time since symptom onset was 8 days). They showed that ensovibep administration did not lead to improvement in hospitalized patients with COVID-19 compared to placebo.⁵⁰ Unfortunately, ensovibep did not pass the protocol-defined futility assessment, leading to a halt in further participant recruitment. The futility assessment was based on the analysis of two intermediate seven-category ordinal efficacy outcomes assessed at day 5 (pulmonary and pulmonary-plus ordinal scale). Based on an odds-ratio of 0.93 for the pulmonary outcome on day 5, the DSMB recommended that enrollment be stopped.

The results of these studies suggest that the antiviral ensovibep may be effective in preventing disease progression in non-hospitalized early symptomatic COVID-19 patients, rather than treating severe disease in hospitalized patients. Four other antiviral agents within the TICO platform showed similar results.⁵⁰ This can be explained by the pathogenesis of COVID-19. Antivirals are a class of medications specifically designed to treat viral infections, by inhibiting the development and replication of the virus. Therefore, they seem to be more suitable in the early stages of the disease. This has also been demonstrated for other SARS-CoV-2 antivirals, as they speed up viral clearance, expedite symptom resolution, reduce transmission and the likelihood of progression to severe disease.⁵³⁻⁵⁶ Hospitalized patients are particularly at a later stage of the disease, when the initial viral replication phase has already passed. The cause of hospitalization is likely more due to a dysregulated host immune response.

Therefore, other treatments beyond antiviral agents, such as immunomodulatory agents appear to be more effective⁵⁷, which are aimed at dampening the inflammatory response (like corticosteroids⁵⁸ and tocilizumab^{59,60}).

Measuring antibody responses to biotechnology-derived products (biologics), such as ensovibep, is important to ensure their safety and efficacy, as they have the potential to generate immune responses or immunogenicity through anti-drug antibodies (ADA). These ADAs can affect pharmacokinetics, pharmacodynamics and/or biological activity. In addition, they can pose significant safety risks, particularly when they involve functionally neutralizing antibodies.⁶¹ During the phase 2a clinical study (chapter 9), blood samples were collected before and throughout the study to detect circulating antibodies and assess immune responses. Pre-exposure samples revealed that one patient in the 225 mg group had pre-existing antibodies. Treatment-induced ADAs were detected in 5/6 (83%) patients in each dose group, with the onset of ADAs occurring between 14 and 91 days. For most patients (83%) who developed ADAs, the mono-exponential elimination of ensovibep remained unaffected. However, two patients exhibited increased elimination, both of them had a relatively early peak of ADA titers at day 29 compared to other patients. Most importantly, immunogenicity did not appear to impact ensovibep concentration within the first two weeks after dosing, a period which antiviral efficacy is expected to be most significant. The results of our study align with those of the phase 1 first-in-human trial of ensovibep. In this trial, a single IV administration of 3, 9 or 20 mg/kg bodyweight was given to 23 healthy subjects. All but two subjects who received ensovibep (n=17) developed ADAs at various time points during the study.⁶² The earliest detection of treatment-induced ADAs occurred on day 29. In seven subjects, development of ADAs was associated with a deviation from mono-exponential elimination. However, similar to our phase 2 study, ensovibep concentrations remained at expected levels for at least two week in all subjects, and for at least four weeks in 14/17 (83%) subjects. In this trial, one subject in the highest dose group (20mg/kg ensovibep) reported a hypersensitivity vasculitis. This subject also experienced a decline in drug exposure, first observed on day 22, and was confirmed to be ADA positive on day 50. Transient immune complex formation was considered the most likely cause. No correlation was found between maximum ADA levels and ensovibep dose, nor between initial ADA levels and ensovibep serum concentration at the time of the first positive ADA result. In the EMPATHY trial, ADAs were detected at the first assessment (day 15) after ensovibep administration in 45-50% of patients.⁵² The highest incidence was observed on day 61 (69%), with a slight decline by day 91 (64%). Despite this, significant viral load reduction was seen across all ensovibep doses through day 8, suggesting that the presence of ADAs, if any, did not affect efficacy outcomes. Moreover, the incidence of adverse events did not appear to differ between patients with and without ADAs. Taken together, the data from all studies suggest that the presence of ADAs did not impact the safety and/or efficacy profile of ensovibep. This is important for the development of future DARPins for new infectious diseases.

Today, SARS-CoV-2 is primarily a mild infection in vaccinated individuals and does not require specific antiviral treatment. However, in patients with underlying conditions, a compromised immune system (such as SOT patients) or older patients, COVID-19 can still be potentially dangerous, as is discussed in chapter 2. For these

patients, treatment with an antiviral should be considered to prevent disease progression. At the LUMC, the ritonavir-boosted nirmatrelvir (paxlovid) is the first-line treatment for this purpose. Ensovibep has not yet been officially approved for widespread use, as there is not yet enough data to fully approve the drug. To get approval, it must be reviewed by regulatory agencies such as the US FDA and the EMA. A comparison is needed with other approved antiviral treatments such as paxlovid to determine whether it offers a significant advantage in terms of efficacy or safety. Ensovibep does have several advantages compared to other antiviral therapies such as paxlovid. It has demonstrated activity against multiple variants of the virus, with the ability to neutralize a wide range of SARS-CoV-2 variants, including those that may partially evade the immune response.⁵¹ Additionally, it does not have significant interactions with liver enzymes, reducing the risk of adverse drug interactions. Moreover, it is administered as a single intravenous infusion, while other antivirals are often require multiple doses per day. The primary drawback, however, is the need of a hospital setting for administration.

The DARPIn platform itself presents several advantages for future infectious diseases. It is designed to quickly generate a wide range of multifunctional drug candidates that can bind to multiple targets simultaneously. With high production yields, it can be efficiently and rapid produced through *Eschericia coli* fermentation.⁵⁰ In addition, its adaptability allows for potential application to treat other viral infections beyond COVID-19. Consequently, ensovibep could serve as a foundation for therapies targeting various viral pathogens, offering flexibility for future antiviral strategies. Overall, DARPins continue to be a promising approach for the rapid development of antiviral treatments, particularly for early-stage therapy. In the future, DARPins could be further studied by treating mildly symptomatic patients early in their disease course. Acceleration in the rate of viral clearance could be determined as a surrogate for clinical benefit, as was done in the PLATCOV trial.⁶³

Current situation & future pandemics

The course of SARS-CoV-2 infection has changed significantly.⁶⁴ SARS-CoV-2 oropharyngeal clearance rates in uncomplicated SARS-CoV-2 infections substantially improved over the course of the pandemic, with the natural viral clearance in October 2023 being twice as fast as it was in September 2021.⁶³ The combination of natural immunity, public health measures, effective vaccines, improved treatments and evolving viral characteristics have brought the COVID-19 pandemic to a point where it is no longer considered an acute global emergency. Currently, the pressure on the (acute) healthcare caused by RVI's has been manageable in the Netherlands, although influenza virus leads to seasonal epidemic almost every year. However, it is inevitable that a new pandemic will occur in the future. The question, however, is when it will happen and which pathogen will be responsible. No-one can predict. At some point, a pathogen spill over from animals into people and begin to spread in a disease outbreak. The risk associated with this is expected to increase due to environmental change such as climate change, rapid urbanization, and deforestation. This changed ecological landscape creates new risks, both for the emergence of new pathogens and for the re-emergence of known pathogens.⁶⁵ Although predicting the next pandemic pathogen threat is challenging, preparing for it remains extremely important. Making

informed assumptions today and assessing previous efforts to plan and respond to disease outbreaks may facilitate a more proactive and effective response in the future. The COVID-19 pandemic has taught us a great deal. Our research on ID vaccinations as a primary vaccinations series has provided insights in how to respond in future pandemics, which may again involve vaccine inequity. In the next pandemic, it will be important to explore the ID route of administration early in the vaccine development process, alongside the currently standard IM route. If studies demonstrate that the ID route is (nearly) as effective as the IM route, it can gain approval from regulatory agencies, allowing it to be marketed and incorporated into guidelines. In addition, this method could allow for the vaccination of five times more individuals with the same dosage, maximizing potential lives saved. A broader reach at the expense of slightly reduced effectiveness can still lead to significant health benefits. In addition, the DARPIn platform represents a promising platform for future pandemics. The development of a DARPIn based treatment could be a good strategy to develop an antiviral for early symptomatic patients who are at high risk for progression to severe COVID-19 in a relatively short timeframe. Developing an oral variant is important in this context, as this is currently the main disadvantage for this non-hospitalized population.

Everyone, including the biopharmaceutical industry, has a role in pandemic preparedness. Only then we will be able to manage the next pandemic and improve (acute) care.

References

1. RIVM. Influenza richtlijn 2024 [Available from: <https://lci.rivm.nl/richtlijnen/influenza>.
2. GGD. RSV-vaccinatie vanaf najaar 2025 2024 [Available from: <https://www.ggdgv.nl/nieuws/rsv-vaccinatie-nog-niet-in-het-rijksvaccinatieprogramma/>.
3. RIVM. RSV-vaccinatie/immunisatie 2024 [Available from: <https://lci.rivm.nl/rsv-immunisatie>.
4. EMA. First vaccine to protect older adults from respiratory syncytial virus (RSV) infection 2023 [Available from: <https://www.ema.europa.eu/en/news/first-vaccine-protect-older-adults-respiratory-syncytial-virus-rsv-infection>.
5. WHO. History of the influenza vaccine [Available from: <https://www.who.int/news-room/spotlight/history-of-vaccination/history-of-influenza-vaccination>.
6. Ison MG. Influenza prevention and treatment in transplant recipients and immunocompromised hosts. *Influenza Other Respir Viruses*. 2013;7 Suppl 3(Suppl 3):60-6.
7. Vilchez RA, Fung J, Kusne S. The pathogenesis and management of influenza virus infection in organ transplant recipients. *Transpl Infect Dis*. 2002;4(4):177-82.
8. Hayney MS, Welter DL, Francois M, Reynolds AM, Love RB. Influenza vaccine antibody responses in lung transplant recipients. *Prog Transplant*. 2004;14(4):346-51.
9. Sakuraba A, Luna A, Micic D. A Systematic Review and Meta-Analysis of Serologic Response following Coronavirus Disease 2019 (COVID-19) Vaccination in Solid Organ Transplant Recipients. *Viruses*. 2022;14(8).
10. Mazzone PJ, Mossad SB, Mawhorter SD, Mehta AC, Schilz RJ, Maurer JR. The humoral immune response to influenza vaccination in lung transplant patients. *Eur Respir J*. 2001;18(6):971-6.
11. Mazzone PJ, Mossad SB, Mawhorter SD, Mehta AC, Mauer JR. Cell-mediated immune response to influenza vaccination in lung transplant recipients. *J Heart Lung Transplant*. 2004;23(10):1175-81.
12. Kojima N, Roshani A, Brobeck M, Baca A, Klausner JD. Incidence of SARS-CoV-2 infection among previously infected or vaccinated employees. *Int J Infect Dis*. 2022;118:21-3.
13. Lumley SF, O'Donnell D, Stoesser NE, Matthews PC, Howarth A, Hatch SB, et al. Antibody Status and Incidence of SARS-CoV-2 Infection in Health Care Workers. *N Engl J Med*. 2021;384(6):533-40.
14. NIVEL. Monitor vaccinatiegraad nationaal programma grieppreventie (NPG) 2023. 2024.
15. Kumar D, Ferreira VH, Blumberg E, Silveira F, Cordero E, Perez-Romero P, et al. A 5-Year Prospective Multicenter Evaluation of Influenza Infection in Transplant Recipients. *Clin Infect Dis*. 2018;67(9):1322-9.
16. Harboe ZB, Modin D, Gustafsson F, Perch M, Gislason G, Sørensen SS, et al. Effect of influenza vaccination in solid organ transplant recipients: A nationwide population-based cohort study. *Am J Transplant*. 2022;22(10):2409-17.
17. Perez-Romero P, Aydillo TA, Perez-Ordoñez A, Muñoz P, Moreno A, López-Medrano F, et al. Reduced incidence of pneumonia in influenza-vaccinated solid organ transplant recipients with influenza disease. *Clin Microbiol Infect*. 2012;18(12):E533-40.
18. Schuurmans MM, Tini GM, Dalar L, Fretz G, Benden C, Boehler A. Pandemic 2009 H1N1 influenza virus vaccination in lung transplant recipients: coverage, safety and clinical effectiveness in the Zurich cohort. *J Heart Lung Transplant*. 2011;30(6):685-90.
19. Sydnor E, Perl TM. Healthcare providers as sources of vaccine-preventable diseases. *Vaccine*. 2014;32(38):4814-22.
20. Doijen J, Heo I, Temmerman K, Vermeulen P, Diels A, Jaensch S, et al. A flexible,

- image-based, high-throughput platform encompassing in-depth cell profiling to identify broad-spectrum coronavirus antivirals with limited off-target effects. *Antiviral Res.* 2024;222:105789.
21. VirusBank Platform; Tools for pandemic preparedness 2025 [Available from: <https://virusbankplatform.be/>].
 22. Schuurmans MM, Isenring BD, Jungo C, Boeni J, Mueller NJ, Kohler M, et al. Clinical features and outcomes of influenza infections in lung transplant recipients: a single-season cohort study. *Transpl Infect Dis.* 2014;16(3):430-9.
 23. Memoli MJ, Athota R, Reed S, Czajkowski L, Bristol T, Proudfoot K, et al. The natural history of influenza infection in the severely immunocompromised vs nonimmunocompromised hosts. *Clin Infect Dis.* 2014;58(2):214-24.
 24. Groeneveld GH, Marbus SD, Ismail N, de Vries JJC, Schneeberger P, Oosterheert JJ, et al. Effectiveness of oseltamivir in reduction of complications and 30-day mortality in severe seasonal influenza infection. *Int J Antimicrob Agents.* 2020;56(5):106155.
 25. World development report: finance for an equitable recovery. Washington: World Bank Group; 2022.
 26. Pollet J, Chen WH, Strych U. Recombinant protein vaccines, a proven approach against coronavirus pandemics. *Adv Drug Deliv Rev.* 2021;170:71-82.
 27. Chakraborty C, Bhattacharya M, Dhama K. SARS-CoV-2 Vaccines, Vaccine Development Technologies, and Significant Efforts in Vaccine Development during the Pandemic: The Lessons Learned Might Help to Fight against the Next Pandemic. *Vaccines (Basel).* 2023;11(3).
 28. Kelland K. The race to future-proof coronavirus vaccines 2022 [Available from: <https://cepi.net/race-future-proof-coronavirus-vaccines-0>].
 29. Wang F, Kream RM, Stefano GB. An Evidence Based Perspective on mRNA-SARS-CoV-2 Vaccine Development. *Med Sci Monit.* 2020;26:e924700.
 30. Verch T, Trausch JJ, Shank-Retzlaff M. Principles of vaccine potency assays. *Bioanalysis.* 2018;10(3):163-80.
 31. WHO. Intradermal delivery of vaccines. A review of the literature and the potential for development for use in low- and middle- income countries. . 2009 August 27.
 32. Gilbert PB, Montefiori DC, McDermott AB, Fong Y, Benkeser D, Deng W, et al. Immune correlates analysis of the mRNA-1273 COVID-19 vaccine efficacy clinical trial. *Science.* 2022;375(6576):43-50.
 33. Jackson LA, Anderson EJ, Roupheal NG, Roberts PC, Makhene M, Coler RN, et al. An mRNA Vaccine against SARS-CoV-2 - Preliminary Report. *N Engl J Med.* 2020;383(20):1920-31.
 34. Chatsiricharoenkul S, Niyomnaitham S, Posen HJ, Toh ZQ, Licciardi PV, Wongprompitak P, et al. Safety and immunogenicity of intradermal administration of fractional dose CoronaVac®, ChAdOx1 nCoV-19 and BNT162b2 as primary series vaccination. *Front Immunol.* 2022;13:1010835.
 35. Więcek W, Ahuja A, Chaudhuri E, Kremer M, Simoes Gomes A, Snyder CM, et al. Testing fractional doses of COVID-19 vaccines. *Proc Natl Acad Sci U S A.* 2022;119(8).
 36. Chen X. Emerging adjuvants for intradermal vaccination. *Int J Pharm.* 2023;632:122559.
 37. Hickling JK, Jones KR, Friede M, Zehrung D, Chen D, Kristensen D. Intradermal delivery of vaccines: potential benefits and current challenges. *Bull World Health Organ.* 2011;89(3):221-6.
 38. Kim YC, Jarrahian C, Zehrung D, Mitragotri S, Prausnitz MR. Delivery systems for intradermal vaccination. *Curr Top Microbiol Immunol.* 2012;351:77-112.
 39. Adu P, Poopola T, Medvedev ON, Collings S, Mbinta J, Aspin C, et al. Implications for COVID-19 vaccine uptake: A systematic review. *J Infect Public Health.* 2023;16(3):441-66.

40. Freeman D, Lambe S, Yu LM, Freeman J, Chadwick A, Vaccari C, et al. Injection fears and COVID-19 vaccine hesitancy. *Psychol Med*. 2023;53(4):1185-95.
41. Nguyen HX, Banga AK. Advanced transdermal drug delivery system: A comprehensive review of microneedle technologies, novel designs, diverse applications, and critical challenges. *Int J Pharm*. 2024;670:125118.
42. Ryan J, Malinga T. Interventions for vaccine hesitancy. *Curr Opin Immunol*. 2021;71:89-91.
43. RIVM. Zorgen om dalende vaccinatiegraag Rijksvaccinatieprogramma 2023 [Available from: <https://www.rivm.nl/nieuws/zorgen-om-dalende-vaccinatiegraad-rijksvaccinatieprogramma>].
44. Cordina M, Lauri MA, Lauri J. Attitudes towards COVID-19 vaccination, vaccine hesitancy and intention to take the vaccine. *Pharm Pract (Granada)*. 2021;19(1):2317.
45. RIVM. Vaccinatiegraad COVID-19 Nederland, 2022. 2024.
46. Storey D. COVID-19 Vaccine Hesitancy. *Glob Health Sci Pract*. 2022;10(1).
47. Robertson E, Reeve KS, Niedzwiedz CL, Moore J, Blake M, Green M, et al. Predictors of COVID-19 vaccine hesitancy in the UK household longitudinal study. *Brain Behav Immun*. 2021;94:41-50.
48. Tao K, Tzou PL, Nouhin J, Bonilla H, Jagannathan P, Shafer RW. SARS-CoV-2 Antiviral Therapy. *Clin Microbiol Rev*. 2021;34(4):e0010921.
49. Edwards AM, Baric RS, Saphire EO, Ulmer JB. Stopping pandemics before they start: Lessons learned from SARS-CoV-2. *Science*. 2022;375(6585):1133-9.
50. Barkauskas C, Mylonakis E, Poulakou G, Young BE, Vock DM, Siegel L, et al. Efficacy and Safety of Ensovibep for Adults Hospitalized With COVID-19 : A Randomized Controlled Trial. *Ann Intern Med*. 2022;175(9):1266-74.
51. Rothenberger S, Hurdiss DL, Walser M, Malvezzi F, Mayor J, Ryter S, et al. The trispesific DARPIn ensovibep inhibits diverse SARS-CoV-2 variants. *Nat Biotechnol*. 2022;40(12):1845-54.
52. Kingsley J, Kumarasamy N, Abrishamian L, Bonten M, Igbinadolor A, Mekebebe-Reuter M, et al. The Designed Ankyrin Repeat Protein Antiviral Ensovibep for Nonhospitalized Patients With Coronavirus Disease 2019: Results From EMPATHY, a Randomized, Placebo-Controlled Phase 2 Study. *Open Forum Infect Dis*. 2024;11(6):ofae233.
53. Weinreich DM, Sivapalasingam S, Norton T, Ali S, Gao H, Bhore R, et al. REGEN-COV Antibody Combination and Outcomes in Outpatients with Covid-19. *N Engl J Med*. 2021;385(23):e81.
54. Gottlieb RL, Vaca CE, Paredes R, Mera J, Webb BJ, Perez G, et al. Early Remdesivir to Prevent Progression to Severe Covid-19 in Outpatients. *N Engl J Med*. 2022;386(4):305-15.
55. Hammond J, Leister-Tebbe H, Gardner A, Abreu P, Bao W, Wisemandle W, et al. Oral Nirmatrelvir for High-Risk, Nonhospitalized Adults with Covid-19. *N Engl J Med*. 2022;386(15):1397-408.
56. Jayk Bernal A, Gomes da Silva MM, Musungaie DB, Kovalchuk E, Gonzalez A, Delos Reyes V, et al. Molnupiravir for Oral Treatment of Covid-19 in Nonhospitalized Patients. *N Engl J Med*. 2022;386(6):509-20.
57. Ochani R, Asad A, Yasmin F, Shaikh S, Khalid H, Batra S, et al. COVID-19 pandemic: from origins to outcomes. A comprehensive review of viral pathogenesis, clinical manifestations, diagnostic evaluation, and management. *Infez Med*. 2021;29(1):20-36.
58. Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, Linsell L, et al. Dexamethasone in Hospitalized Patients with Covid-19. *N Engl J Med*. 2021;384(8):693-704.
59. Tocilizumab in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. *Lancet*. 2021;397(10285):1637-45.

60. Salama C, Han J, Yau L, Reiss WG, Kramer B, Neidhart JD, et al. Tocilizumab in Patients Hospitalized with Covid-19 Pneumonia. *N Engl J Med*. 2021;384(1):20-30.
61. Shankar G, Shores E, Wagner C, Mire-Sluis A. Scientific and regulatory considerations on the immunogenicity of biologics. *Trends Biotechnol*. 2006;24(6):274-80.
62. Stojcheva N, Gladman S, Soergel M, Zitt C, Drake R, Lockett T, et al. Ensovibep, a SARS-CoV-2 antiviral designed ankyrin repeat protein, is safe and well tolerated in healthy volunteers: Results of a first-in-human, ascending single-dose Phase 1 study. *Br J Clin Pharmacol*. 2023;89(7):2295-303.
63. Wongnak P, Schilling WHK, Jittamala P, Boyd S, Luvira V, Siripoon T, et al. Temporal changes in SARS-CoV-2 clearance kinetics and the optimal design of antiviral pharmacodynamic studies: an individual patient data meta-analysis of a randomised, controlled, adaptive platform study (PLATCOV). *Lancet Infect Dis*. 2024;24(9):953-63.
64. WHO. Statement on the fifteenth meeting of the IHR (2005) Emergency Committee on the COVID-19 pandemic 2023 [Available from: [https://www.who.int/news/item/05-05-2023-statement-on-the-fifteenth-meeting-of-the-international-health-regulations-\(2005\)-emergency-committee-regarding-the-coronavirus-disease-\(covid-19\)-pandemic](https://www.who.int/news/item/05-05-2023-statement-on-the-fifteenth-meeting-of-the-international-health-regulations-(2005)-emergency-committee-regarding-the-coronavirus-disease-(covid-19)-pandemic)].
65. CEPI. Disease X- what it is, and what it is not 2024 [Available from: <https://cepi.net/disease-x-what-it-and-what-it-not>].