Clinical study protocol

Immunological response after vaccination with the mRNA vaccine against COVID-19, Comirnaty, in immunosuppressed and immunocompetent individuals – an open-label, non-randomised, multicentre Phase IV study

EudraCT No.	2021-000175-37
Medicine	Comirnaty, mRNA-1273
Development phase	Phase IV
Indication	Active immunization to prevent COVID-19 caused by the SARS-CoV-2 virus
Design	Open-label, non-randomized study

Signature page

Sponsor: Karolinska University Hospital

I am responsible for ensuring that this protocol contains all the essential elements for conducting the study. I will share the protocol and all other important study-related information with the responsible investigators so that they can conduct the study correctly. I am aware of my responsibility to keep the staff working on the study informed and trained.

Sponsor representative Date

Name

Soo Aleman, Senior Physician, Associate Professor. Medical Unit Infectious Diseases, Karolinska University Hospital

Coordinating investigator

I have read this protocol and assessed that it contains all the essential elements for conducting the study. By signing this document, I agree to conduct the study in accordance with this protocol, the Declaration of Helsinki, ICH GCP (Good Clinical Practice), and the applicable national and international regulations governing this clinical study.
I will share the protocol and all other important study-related information with the staff and investigators participating in the study so that they can conduct the study correctly. I am aware of my responsibility to keep staff and investigators working on the study informed and trained on an ongoing basis.
I am aware that quality control will be carried out on the study in the form of monitoring, auditing, and possible inspection.

Coordinating investigator Date

Name

Soo Aleman, Senior Physician, Associate Professor. Medical Unit Infectious Diseases, Karolinska University Hospital

Principal investigator

I have read this protocol and assessed that it contains all the essential elements for conducting the study. By signing this document, I approve the conduct of the study in accordance with this protocol, the Declaration of Helsinki, ICH GCP (Good Clinical Practice), and the applicable national and international regulations governing this clinical study.
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Principal investigator's signature Date

Name

Synopsis

Protocol title	Immunological response after vaccination with the mRNA vaccine against COVID-19, Comirnaty, in immunosuppressed and immunocompetent individuals – an open-label, non-randomised, multicentre Phase IV study
English abbreviation of title	COVID-19 vaccination of immunodeficient persons (COVAXID)
EudraCT No	2021-000175-37
Medicinal	Comirnaty
Development phase	Phase IV
Indication	Active immunization to prevent COVID-19 caused by the SARS-CoV-2 virus
Design	Phase 4, open-label, non-randomized cohort study. The Comirnaty vaccine will be administered in two doses. Blood and saliva analyses will be performed to assess humoral and cellular responses. The occurrence of local or systemic reactogenicity, as well as AE/SAE/SUSAR, will be evaluated.
Background	
Objective	Comirnaty is an mRNA-based vaccine approved since December 2020, indicated for active immunization to prevent COVID-19 caused by the SARS-CoV-2 virus. The vaccine has been shown to be highly effective in clinical trials, but people with immunosuppressive diseases or immunosuppressive treatment were excluded from these trials. However, these individuals are at risk of serious complications, including increased mortality from COVID-19. There is currently a knowledge gap regarding how immunosuppressed patients, whether primary or secondary, respond immunologically to mRNA vaccines, including the current vaccine, and whether they extended protection against severe COVID-19. It is also unclear at present whether they react differently than healthy individuals in terms of side effects. **Primary objective**: to investigate the immunological effect of Comirnaty by measuring the incidence of seroconversion (development of antibodies against SARS-CoV-2 upon vaccination in seronegative individuals) after two vaccine doses.

Secondary objectives:

- To evaluate the safety and tolerability of the vaccine administered to the research subjects.
- To measure the incidence of SARS-CoV-2 infection documented by a positive PCR test during the study period.

Exploratory objectives:

- Proportion (95% CI) seroconverting to a positive response to SARS-CoV-2 IgG serology after two doses of vaccine, at 9 months, 12 months, 18 months, 24 months, 30 months, and 36 months after the second vaccine dose. This may mean that patients in routine healthcare have received one or more additional vaccine doses depending on the recommendations of the Public Health Agency of Sweden.
- To investigate the humoral response in more detail and analyze the cellular immune response after the vaccine has been administered.
- To examine the virus genome in breakthrough infections of SARS-CoV-2.
- To evaluate the significance of the oral and intestinal microbiome for vaccine efficacy in HIV-infected individuals.

Primary endpoint:

- Proportion (95% CI) seroconverting to a positive response to SARS-CoV-2 IgG serology test after two doses of vaccine, measured 2 weeks after the second vaccine dose.

Secondary endpoints:

- Proportion (95% CI) seroconverting to a positive response to SARS-CoV-2 IgG serology after two doses of vaccine, at 3 months and 6 months after the second vaccine dose.
- Proportion (95% CI) experiencing adverse events, including AE/SAE/SUSAR.
- Number and proportion (95% CI) who experience local reactions and systemic reactions.
- Proportion (95% CI) who receive a new diagnosis of SARS-CoV-2 infection through a positive PCR test.

Exploratory endpoints:

- Proportion (95% CI) seroconverting to a positive response to SARS-CoV-2 IgG serology after two doses of vaccine, at 9 months, 12 months, 18 months, 24 months, 30 months, and 36 months after the second vaccine dose.
- More detailed description of humoral and cellular immune responses at baseline and after vaccinations in blood and saliva at time points immediately before the first vaccine dose (day 0), day 10 after the first dose, immediately before dose 2 on day 21, and 2 weeks, 3 months, 6 months, 9 months, 12 months, 18 months, 24 months, 30 months, and 36 months after the second vaccine dose.
- Prevalence of escape mutations in SARS-CoV-2 sequencing in breakthrough infections.
- Prevalence of development/progression of graft versus host disease in allogeneic stem cell transplant recipients.
- Occurrence of biopsy-verified rejection in solid organ transplant recipients.
- Occurrence of viremia (defined as HIV RNA >50 copies/mL) after vaccination in individuals with HIV infection.
- Change in HIV DNA from study start to 3 and 6 months, 9 months, 12 months, 18 months, 24 months, 30 months, and 36 months after the second vaccine dose in individuals with HIV infection.
- Association between oral and gut microbiome and vaccine response in HIV-infected individuals and controls.

Investigational medicinal product Comirnaty	
Administration	Intramuscular injection
Study population	A total of 540 individuals who wish to be vaccinated against COVID-19 are planned to be included in the study. Within the primary study population of immunosuppressed individuals, 450 people are planned to be included. The goal is to include 90 people per patient category: - Patients with primary immunodeficiency - Patients with HIV infection - Patients who have undergone allogeneic stem cell transplantation/CAR T cell therapy - Patients who have undergone solid organ transplantation - Patients with chronic lymphocytic leukemia - A control group of 90 people without immunosuppressive disease is planned to be recruited in the following age groups: 18-39 years (n=30), 40-59 years (n=30), and ≥60 years (n=30).

Inclusion criteria

1. Individuals who are ≥18 years old

2a. Individuals with immunosuppressive disease who meet one of the following criteria, as determined by the investigator:

- Primary immunodeficiency
- HIV infection
- Previous allogeneic stem cell transplantation/CAR T cell therapy
- Undergone solid organ transplantation
- Chronic lymphocytic leukemia

or

2b. Individuals without immunosuppressive disease or treatment who, in the investigator's opinion, have no significant co-morbidity.

3. Signed written consent to participate in the study.

Exclusion criteria

1. Previous or ongoing COVID-19.
2. Coagulation disorders, other conditions associated with prolonged bleeding time, or anticoagulant treatments, which, according to the investigator, contraindicate intramuscular injection. Conditions that can be corrected with measures such as treatment with platelet concentrates, coagulation factors, or other measures for people on anticoagulants are not exclusion criteria.
3. Planned to receive another vaccine within 14 days prior to the first dose of the study vaccine, or during the period from the first dose of the study vaccine until 14 days after the second dose of the study vaccine, and vaccination with another vaccine that, in the investigator's opinion, cannot be planned outside these time periods.
4. Pregnancy or breastfeeding.
5. Hypersensitivity to the active substance or to any of the excipients included in the vaccine.
6. Individuals who are unable to understand the research subject information.
7. Individuals who, for other reasons, are deemed by the investigator to be unsuitable for inclusion.

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Statistics

The primary evaluation variable is the effect measured as the proportion of individuals who seroconvert in patients versus controls. For this, odds ratios are calculated and

analyzed using the Chi^2 test where α=0.05. With a patient group size of N=540, the power is >80% even with a conservatively estimated effect size of 0.15.

Time

First research subject included Q1 2021
Last research subject included Q2 2022 Last research subject completed Q2 2024

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2. BACKGROUND INFORMATION AND RATIONALE

2.1. Introduction

December 2019 marked the third re-emergence in the 21st century of a new coronavirus (SARS-CoV-2), which can cause respiratory infections and acute respiratory disease. The disease is now known as "coronavirus disease 2019," COVID-19 (Zhu et al, 2019). The outbreak of COVID-19 was first identified in Wuhan, China, presumably from a live animal market, and has caused a rapid increase in the number of infections and deaths across Chinese borders, and has also become a serious threat to the general public globally, including in Sweden. As of January 2021,
>10,000 COVID-19-related deaths have been reported in Sweden, according to the Public Health Agency of Sweden's report.

To prevent the spread of the virus or reduce the incidence of disease and control the pandemic, several COVID-19 vaccines are now in development, using different platforms and technologies. The development of these vaccines has progressed at a rapid pace, with the recent approval of the first vaccines, Comirnaty and mRNA-1273, by the European Medicines Agency (EMA). Both of these vaccines use the messenger RNA (mRNA) platform, where non-replicating RNA is formulated with lipid nanoparticles. This is to deliver genetic material that codes for the SARS-CoV-2 spike protein (found on the surface of the virus). An mRNA vaccine can stimulate both neutralizing antibodies and a cellular immune response to the spike antigen (Sahin U et al, 2020).

In a phase 2/3 study evaluating the efficacy of Comirnaty in 43,548 participants (21,720 of whom received the active substance), a 95.0% efficacy in reducing the risk of symptomatic COVID-19 was observed (Polack FP et al, 2020). In another phase 3 study evaluating the efficacy of mRNA-1273 in approximately 30,000 people, 94.1% efficacy was observed (Baden LR et al, 2020). However, people with immunosuppression were generally excluded from the clinical trials. Efficacy, safety, tolerability, and immunogenicity have not been evaluated in immunosuppressed individuals.

There is therefore a lack of knowledge about the immunological response in immunosuppressed individuals after vaccination with mRNA-based vaccines against COVID-19. A phase 1/2 study analyzing antibody responses after Comirnaty showed that IgG antibody titers against SARS-CoV-2 peaked on day 28 after the first vaccine dose, while neutralizing antibodies did not reach high levels until after the second vaccine dose, with the highest titer on day 35 after the first dose (no time points other than day 35 were analyzed in this study) (Mulligan MJ et al). Another study showed that IgG titers peaked on day 29 after the first dose, with a gradual decline on days 43 and 50 to day 85 after the first dose, with similar kinetics for neutralizing antibodies (Sahlin U et al, 2020).

IgG antibodies against SARS-CoV-2 in saliva have been shown to correlate well with antibodies in the blood in a study (manuscript) conducted through a collaboration between Karolinska Institutet and the Royal Institute of Technology (Margaret Sällberg Chen and Peter Nilsson's research groups). It would be advantageous if it were possible to measure antibodies in saliva, instead of invasive blood sampling, in the general population and in immunosuppressed individuals. In addition, the presence of antibodies in saliva may indicate some mucosal immunity, with further knowledge development. There is therefore a need to investigate antibody responses in saliva samples as well.

Studies have been published indicating that the gut microbiome may be important for the immunological response after vaccination (de Jong SE et al. 2020). Certain immunosuppressed patient groups receive frequent antibiotic treatments that can affect the microbiome (Hagan T et al. 2019), which can make it difficult to study any association between the microbiome and vaccines. However, people infected with HIV (human immunodeficiency virus) do not generally use antibiotics more frequently

antibiotic use than the general population, which is why this can be studied. Other patient groups, such as those with primary immunodeficiency or those on immunosuppressive drugs, etc., may have more frequent antibiotic treatment. The composition of the gut flora is strongly linked to ethnicity, diet, and body mass index (Deschasaux M et al, 2018).

In-depth analysis of cellular immunity is not a routine method in healthcare and requires advanced methods with specific expertise and very resource-intensive laboratory work, which is why the number of samples cannot be as high as for antibody analyses. For this type of immunological study, up to 20 individuals per group is a well-established number in the literature (Miller JD et al, 2008). These analyses also require handling within a certain time frame with manual cell extraction before freezing the samples, which is why samples cannot be submitted to regular sampling units in healthcare and usually require a physical visit to the study unit.

We have chosen to only investigate the immunological effect after vaccination with one of the available vaccines in order to make the study group more homogeneous. Comirnaty has been chosen due to potentially higher availability of the vaccine, based on the predicted volumes of vaccine that are and will be available in the Stockholm Region in the early phase of vaccine rollout.

Addition in version 5.0: Knowledge about the duration of the immune response after vaccination is currently limited, so the study will be extended with sampling for up to 24 months, see section 5.2 and Table 2. Our results after day 35 in this clinical trial, i.e. two weeks after the second vaccine dose, have shown that the chance of seroconversion was significantly lower (60 times) in immunosuppressed patients compared to healthy controls (Bergman Petal, 2021). The seroconversion rate was 72% in all immunosuppressed patients, with large variations in seroconversion rate and antibody titers among different immunosuppressed patient groups, with the lowest seroconversion rate of 43% in organ transplant recipients and 63% in patients with chronic lymphocytic leukemia. Immunosuppressive treatments with mycophenolate mofetil (MMF) and ibrutinib had a significant negative impact on seroconversion. Other reports have also shown lower seroconversion rates in immunosuppressed patients (Diefenbach C et al, 2021; Firket L et al, 2021; Hagin D et al, 2021; Herzog Tzarfati K et al, 2021; Rabinowich L et al, 2021). Emerging virus variants such as B.1.617.2 (delta) may further impair protection against infection and severe COVID-19 after mRNA vaccination (Bernal JL et al, 2021). New informed consent for the extended study will be obtained. Sampling intervals will be every three to six months. The interval may change and vary between different patient groups, depending on the current state of knowledge. Information on additional vaccinations with Comirnaty, within the framework of routine healthcare, will be obtained from the Vaccinera registry.

Addition in version 7.0: The study will be extended with two additional visits (month 30 and month 36). Our results with data up to 12 months after the second vaccine dose in this clinical trial have shown that the third vaccine dose significantly increased spike IgG responses against all tested SARS-CoV-2 variants (Wu-Hu.1, B.1.1.7 (Alpha), B.1.351 (Beta), B.1.617/AY.4 (Delta), B.1.640.2 (IHU), and the following 5 omicron variants B1.1.529/BA.1, BA.1+L452R, BA.1.1, BA.2, and BA.3 (submitted manuscript). Neutralization capacity against all variants also increased except for omicron. However, omicron-specific neutralization increased after the 4th dose or after the 3rd dose and SARS-CoV-2 infection in many of the patient subgroups. We plan to further follow up on vaccination responses, as it is expected that additional booster doses and/or new infections with potential new variants may arise during the continued follow-up period.

2.2. Expected results

We expect that some immunosuppressed patients will have a lower degree of immune activation and thereby potentially poorer immunity, compared with

immunocompetent individuals. Individuals who have an absence or partial deficiency in their ability to form antibodies can be expected to develop T cell immunity in certain cases. The degree of immunological response is expected to decrease over time, but it is difficult to predict the duration of a strong immunological response, as we currently lack long-term data. Reactogenicity may be milder in immunosuppressed individuals compared to immunocompetent individuals.

We expect the antibody response to decrease over time, but booster doses and/or new SARS-CoV-2 infections may increase the antibody response, while in some individuals a low/negative antibody response may continue. With the development of new variants, neutralisation capacity may change.

3. BENEFIT-RISK ASSESSMENT

3.1. Side effects

According to the product summary, the most commonly reported side effects after vaccination with Comirnaty were pain at the injection site, fatigue, headache, muscle pain, chills, joint pain, and fever. Side effects usually began within two days after vaccination and disappeared after 1-2 days. These side effects were more frequent after the second dose than after the first dose.

Table 1: Side effects of Comirnaty in clinical trials according to the summary of product characteristics

Organ system	**Very common (≥ 1/10)**	**Common (≥ 1/100,< 1/10)**	**Uncommon (≥ 1/1,000, < 1/100)**	**Rare (≥ 1/10,000, < 1/1,000**	**Frequency not known (cannot be estimated from the available data)**
Blood and lymphatic system			Lymphadenopathy		
Immune system					Anaphylaxis, hypersensitivity
Psychiatric disorders			Insomnia		
Central and peripheral nervous system	Headache			Acute peripheral facialis-pares†	
Gastrointestinal tract		Nausea			
Musculoskeletal system and Connective tissue	Arthralgia, myalgia		Pain in the extremities		
General symptoms and/or symptoms at the administration site	Pain at the injection site, fatigue, chills, fever*, swelling at the injection site site	Redness at the injection site	Feeling unwell, itching at the injection site		

*A higher frequency of fever was observed after the second dose.

3.2. Benefit-risk assessment

Despite side effects, the benefits of vaccines greatly outweigh the risks, as complications from COVID-19 can be very serious, especially in high-risk groups. For example, one study showed that one in three patients with chronic lymphocytic leukemia (CLL) who were hospitalized due to COVID-19 infection died (Mato A et al. 2020). A similar mortality risk has also been reported after stem cell transplantation (Ljungman P et al. 2020). Even healthy individuals without risk factors can experience severe complications from COVID-19. All individuals in Sweden, including those with immunosuppressive conditions, are recommended to be vaccinated against COVID-19 by the Public Health Agency of Sweden. Both the primary population with immunosuppression and control subjects would therefore have been offered vaccination within routine care, regardless of participation in the study.

For a large number of generally immunocompetent individuals, Comirnaty has not been shown to cause serious side effects (>30,000), except for anaphylactic reactions in a small number of individuals. We do not expect a higher safety risk in the primary population with immunosuppression in the study. Rather, the opposite may be true, that reactogenic reactions may be milder in the primary population.

A routine venous blood sample, where a small volume of blood (<50 ml) is taken, is standard practice in healthcare and does not involve any significant risks. The discomfort of the venipuncture is negligible. In routine healthcare and at each site, protective measures are in place to prevent research subjects from becoming infected with SARS-CoV-2 during their visits. In addition, clear information is provided to all visitors not to come to the hospital if they have COVID-19 symptoms. Face masks and visors will be used during patient contact.

Study participants will receive information about the development of antibodies against SARS-CoV-2, with an IgG test used in clinical routine in the Stockholm Region. This test cannot distinguish between antibody formation after vaccination or SARS-CoV-2 infection, but since we perform SARS-CoV-2 antibody serology and PCR tests at baseline, as well as SARS-CoV-2 PCR tests for COVID-19 symptoms during the study period, it is highly likely that those who seroconvert without a diagnosis of COVID-19 do so because of vaccination. Other than that, there's no direct benefit for participants to take part in the immunological evaluation in the study, but knowledge that can be generated at the group level can indirectly benefit participants, as it can give a deeper indication of immunity in the group with the current diagnosis. This is because patients with immunosuppression are considered to be at risk for severe COVID-19 and may potentially have a reduced response to the COVID-19 vaccine compared to people without immunodeficiency. As these individuals have generally not been included (except for a few HIV-infected individuals) in previous clinical trials of Comirnaty or other mRNA-based vaccines, there is a knowledge gap. The study will give us a better understanding of the immunological response after vaccination with mRNA-based COVID-19 vaccines, both on the humoral and cellular sides. This may lead to better mechanistic knowledge of whether specific parameters correlate with a poorer adaptive immune response or protection after vaccination. For society, vaccines not only protect the individuals who receive them, but can also potentially reduce the risk of developing mutations that can occur in people with compromised immune systems after SARS-CoV-2 infection and prolonged replication of the virus.

The extended study (version 5.0), which only involves blood sampling, is not expected to pose any significant risk to the research subjects, as sampling in the most severely ill groups is performed frequently in routine healthcare without any significant risks, except for possible risks of local bleeding, which usually stops spontaneously.

The study will be extended by two additional visits, at month 30 and month 36 (version 7.0), and will involve research subjects providing blood and saliva samples on these occasions and stool samples (for the HIV group and healthy controls) at month 36 after renewed consent.

Overall, the benefits are considered to outweigh the risks in the study because the risks are expected to be minimal in both immunosuppressed and immunocompetent individuals, while there is a great need to analyze and understand different aspects of the immune system's response for the reasons mentioned above.

4. OBJECTIVE AND ENDPOINTS

4.1. Objective

The primary objective is to investigate the immunological effect of Comirnaty by measuring the incidence of seroconversion (development of antibodies against SARS-CoV-2 upon vaccination in seronegative individuals) after 2 vaccine doses.

Secondary objectives:

- To evaluate safety and tolerability of the given vaccine in the research subjects.
- To measure the incidence of SARS-CoV-2 infection documented by a positive PCR test during the study period.

Exploratory objectives:

- Proportion (95% CI) seroconverting to a positive response to SARS-CoV-2 IgG serology after two doses of vaccine, at 9 months, 12 months, 18 months, 24 months, 30 months, and 36 months after the second vaccine dose. This may mean that patients in routine healthcare have received one or more additional vaccine doses depending on the recommendations of the Public Health Agency of Sweden.
- To investigate the humoral response in more detail and analyze the cellular immune response after the vaccine has been administered.
- To examine the virus genome in breakthrough infections of SARS-CoV-2.
- To evaluate the importance of the oral and intestinal microbiome for vaccine efficacy in HIV-infected individuals.

4.2. Endpoints

Primary endpoint:

- Proportion (95% CI) seroconverting to a positive response to SARS-CoV-2 IgG Serology test after two doses of vaccine, measured 2 weeks after the second vaccine dose.

Secondary endpoints:

- Proportion (95% CI) seroconverting to a positive response to SARS-CoV-2 IgG serology after two doses of vaccine, at 3 months and 6 months after the second vaccine dose.
- Proportion (95% CI) experiencing adverse events, with AE/SAE/SUSAR.
- Number and proportion (95% CI) who experience local reactions and systemic reactions.
- Proportion (95% CI) who receive a new diagnosis of SARS-CoV-2 infection through a positive PCR test.

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Exploratory endpoints:

- Proportion (95% CI) seroconverting to a positive response to SARS-CoV-2 IgG serology after two doses of vaccine, at 9 months, 12 months, 18 months, 24 months, 30 months, and 36 months after the second vaccine dose. This may mean that patients in routine healthcare have received one or more additional vaccine doses depending on the recommendations of the Public Health Agency of Sweden.
- A more detailed description of humoral and cellular immune responses at baseline and after vaccinations in blood and saliva at time points immediately before the first vaccine dose (day 0), day 10 after the first dose, immediately before dose 2 on day 21, and 2 weeks, 3 months, 6 months, 9 months, 12 months, 18 months, 24 months, 30 months, and 36 months after the second vaccine dose.
- Prevalence of escape mutations in SARS-CoV-2 sequencing in breakthrough infections.
- Occurrence of development/progression of graft versus host disease in allogeneic stem cell transplant recipients.
- Occurrence of biopsy-verified rejection in solid organ transplant recipients.
- Occurrence of viremia (defined as HIV RNA >50 copies/mL) after vaccination in individuals with HIV infection.
- Change in HIV DNA from study start to 3 and 6 months, 9 months, 12 months, 18 months, 24 months, 30 months, and 36 months after the second vaccine dose in individuals with HIV infection.
- Association between oral and gut microbiome and vaccine response in HIV-infected individuals and controls.

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5. STUDY DESIGN

5.1. Assessments and study procedures

5.1.1. Study participants

Immunosuppressed individuals (primary study population) and immunocompetent individuals (control population) are planned to receive two doses of the Comirnaty vaccine in an open-label cohort study without randomization or blinding. Study participants would have been offered vaccination within the Stockholm Region, regardless of whether they participated in the study or not.

A total of 540 individuals who wish to be vaccinated against COVID-19 are planned to be included in the study.

Within the primary study population of immunosuppressed individuals, 450 individuals are planned to be included. The goal is to include 90 individuals per patient category:

- Patients with primary
- immunodeficiency Patients with HIV
- infection
  Patients who have undergone allogeneic stem cell transplantation/CAR T cell therapy
- Patients who have undergone solid organ transplantation Patients with
- chronic lymphocytic leukemia

We plan to include patient groups with both primary immunodeficiency and secondary immunodeficiency (HIV infection, allogeneic stem cell transplantation/CAR (chimeric antigen receptor) T cell therapy, solid organ transplantation, and chronic lymphocytic leukemia) among a large and heterogeneous group of all immunosuppressed patients in specialized care at the hospital. This is because these patient groups may be interesting to study from an immunological point of view, as different parts of the immune system are suppressed, and we have well-characterized patient groups at participating study units that are suitable for study. The patients attend regular check-ups at the outpatient clinic at Karolinska University Hospital and may form the basis for inclusion.

The control group (90 people in total) is planned to be recruited in the following age groups: 18-39 years (n=30), 40-59 years (n=30), and >60 years (n=30).

5.1.2. Test subject population

Recruitment of the primary population will take place at Karolinska University Hospital. For the patient group with primary immunodeficiency, an application for data extraction from the PIDcare quality registry (National Quality Registry for Primary Immunodeficiency Diseases) will be made to identify patients who can be included in the study. The data extraction will cover diagnosis and lab values (latest IgG, IgA, IgM, and lymphocyte profile).
The control group will be recruited, for example, from among staff at Karolinska University Hospital and, if necessary, from other healthcare units or facilities, such as primary care, via the Alltid öppet app, recruitment flyers, or advertising. Healthcare personnel are recruited as a control group because of easier access to sampling and because this is a priority group for vaccination among healthy individuals. Depending on when the study can start versus the vaccine rollout for healthcare personnel, others such as relatives of healthcare personnel or study participants may also be eligible for recruitment. The age category ≥60 years may be difficult to recruit among healthcare personnel, which is why external recruitment outside the hospital is necessary.

5.1.3. Inclusion criteria

1. Individuals who are ≥18 years old.

2a. Individuals with immunosuppressive disease who meet one of the following criteria:

- Primary immunodeficiency

- HIV infection
- Review of allogeneic stem cell transplantation/CAR T cell therapy
- Review of solid organ transplantation
- Chronic lymphocytic leukemia

or

2b. Individuals without immunosuppressive disease or treatment who, in the investigator's opinion, have no significant co-morbidity.

3. Signed written consent to participate in the study.

5.1.4. Exclusion criteria

1. Previous or ongoing COVID-19.
2. Has coagulation disorders, other conditions associated with prolonged bleeding time, or anticoagulant treatments, which, according to the investigator, contraindicate intramuscular injection. Conditions that can be corrected with measures such as treatment with platelet concentrates, coagulation factors, or other measures for people on anticoagulants are not exclusion criteria.
3. Planned to receive another vaccine within 14 days prior to the first dose of the study vaccine, or during the period from the first dose of the study vaccine until 14 days after the second dose of the study vaccine, and vaccination with another vaccine that, in the investigator's opinion, cannot be planned outside these time periods.
4. Pregnancy or breastfeeding.
5. Hypersensitivity to the active substance or to any of the excipients contained in the vaccine.
6. Individuals who are unable to understand the research subject information. Individuals who, for other reasons, are deemed by the investigator to be unsuitable for inclusion.

5.2 Study procedures

5.2.1. Screening visit

Screening is done via video visit or as a physical visit. During the COVID-19 pandemic, the majority of routine visits for outpatients have been converted from physical visits to video visits/telephone contacts within routine healthcare, in order to reduce the spread and infection of SARS-CoV-2, especially for risk groups. Screening visits will therefore be conducted via video visits using the Alltid öppet app (used at Karolinska University Hospital for routine video visits), as far as possible. Mobile Bank ID is required to access Alltid öppet, and some IT knowledge is needed, so not all study participants will be able to use this.
Informed consent will be obtained during the screening visit. Electronic signing will take place via another app during video visits, and during physical visits, consent will be obtained via signing on paper.

The following procedures will be performed:

- Written/electronic informed consent.

- Demographic data will be collected, including baseline characteristics that may be relevant to underlying disease, co-morbidity, ongoing medication, and recently completed immunosuppressive treatments (within 4 weeks) (Appendix 1). Patient group-specific treatments prior to screening will be collected as described in 5.5.2. This includes a review of planned vaccinations with other vaccines within 14 days prior to the first dose of the study vaccine, or if another vaccine is planned to be administered during the period from the first dose of the study vaccine until 14 days after the second dose of the study vaccine. The investigator will assess whether this can be postponed so that no other vaccine is administered during the specified period.
- If the visit is a physical visit, a pregnancy test is performed using a urine sample for women of childbearing age at this time, otherwise it is performed on day 0.
- If the visit is a physical visit, clinical laboratory samples are taken (see Table 2 for specifications).
- If the visit is a physical visit, samples for HIV RNA and HIV DNA are taken, only for HIV-infected individuals.
- HIV-infected individuals and control subjects are offered participation in a study component investigating the significance of the microbiome for vaccine efficacy. Individuals who agree to participate in this component will be sent fecal sample collection kits to their homes after the video visit, or the kits will be given to them directly during the physical visit. The person can then send the fecal sample by mail to the study unit during the screening period, or drop it off on site on day 0, before or after the injection of the first vaccine dose. Saliva samples submitted in the study can be used for both antibody response and oral microbiome analyses.

5.2.2. Day 0

If study participants meet all inclusion criteria and no exclusion criteria, they can proceed to day 0, either on the same day or on another day within 28 days after screening. Day 0 visits take place at the study unit.

On day 0, the first vaccine dose is given according to the product summary, and the vaccination is registered in an internet-based reporting system for vaccinations according to healthcare routine (Vaccinera). The study unit where vaccinations are planned to be given will have the necessary equipment and medicines (adrenaline, cortisone, and antihistamine) on hand for a potential anaphylactic reaction. A doctor will be available.

Individuals with an increased risk of bleeding, such as known coagulopathy or anticoagulant treatments, will be managed according to section 5.3.1.

Day 0 samples will be taken before injection of the first dose of vaccine. Sampling according to the schedule in Table
2. For blood sample volume at each visit and total in the study for each subgroup of study participants, please see Appendix 2). Saliva will be collected by having study participants spit saliva into a clean jar for 5 minutes. It is also possible to provide a saliva sample after the vaccine dose.

Two groups of sampling are planned:
A. Group with sampling without cellular immunity.

B. Group with cellular immunity sampling (at least 40 study participants per patient group)

These two sampling groups are divided to give sampling group A participants the opportunity to also provide blood samples at sampling units at Karolinska University Laboratory (in addition to the study unit) during certain visits in the study (day 10 after the first dose, and 2 weeks, 3 months, and 6 months after vaccine dose 2). This is because it may be easier for these individuals to travel to these units for sampling. As all analyses of cellular immunity require sampling at the study unit, this may mean several visits to the unit for sampling group B patients (4 extra visits). The number of sampling occasions is the same for both groups, but the total blood volume throughout the study period is approximately 80 ml higher in sampling group B. Subgroups to these two groups (A and B) are whether the person is HIV-infected or not. This is because HIV RNA and DNA samples are taken from HIV-infected individuals at specific times. The amount of blood taken is up to 10 ml higher per sampling occasion for HIV-infected individuals than for other groups.

The diary that the study participant must fill in to monitor adverse events can either be completed in eDagbok via the Alltid öppet app or on paper, depending on what is possible for the study participant. This is due to the need for access to mobile Bank ID and certain IT skills required to use an app (e.g., difficulty for older people).

<u>Sampling group A:</u>

- Review of health declaration questions in Vaccinate, according to care routine before vaccination.
- Vital parameters: Blood pressure, pulse, temperature, and respiratory rate are measured. If the temperature is ≥ 38 degrees, the study participant is rescheduled for the vaccine dose injection on another day, when the person has been fever-free for at least 48 hours.
- Review of new medications since the screening visit (except if the screening visit is on the same day as day 1). If the study participant has received any other vaccine within 14 days
- For women of childbearing age, a pregnancy test is performed using a urine sample if this has not been done during the screening visit.
- Sampling of clinical laboratory samples including blood count (hemoglobin, white blood cells, and platelets) and differential count (neutrophils, lymphocytes, monocytes, basophils, and eosinophils), liver function (ALT, AST, bilirubin, ALP), and creatinine, as well as IgG analysis, if not taken at the screening visit.
- Sampling for SARS-CoV-2 serology and more detailed analyses of humoral immunity (blood).
- Sampling for humoral immunity (saliva).
- Sampling for SARS-CoV-2 PCR test (nasopharynx, NPH).
- Fecal sampling for HIV-infected individuals and controls, for those who have agreed to this part and the sample has not been submitted after the screening visit.
- Sampling for HIV-RNA and HIV-DNA, only for HIV-infected individuals, if this has not been taken during the screening visit.

- Vaccine dose 1 is administered (unless contraindications have been identified following health declaration questions).
- Study participants remain at the healthcare facility for at least 15 minutes (30 minutes for those with bleeding disorders) after vaccination.
- A diary is given to the study participant/information about the eDiary.

Sampling group B:

- Review of health declaration questions in Vaccinate, according to care routine.
- Vital parameters: Blood pressure, pulse, temperature, and respiratory rate are measured. If the temperature is ≥ 38 degrees, the study participant is rescheduled for the vaccine dose on another day, when the person has been fever-free for at least 48 hours.
- Review of new medications since the screening visit (unless the screening visit is on the same day as day 1).
- For women of childbearing age, a pregnancy test is performed using a urine sample if this has not been done during the screening visit.
- Clinical laboratory tests including blood count (hemoglobin, white blood cells, and platelets) and differential count (neutrophils, lymphocytes, monocytes, basophils, and eosinophils), liver function (ALT, AST, bilirubin, ALP), creatinine, and IgG analysis, if not taken at the screening visit.
- Testing for SARS-CoV-2 serology and more detailed analyses of humoral immunity (blood).
- Sampling for humoral immunity (saliva).
- Sampling for analysis of cellular immunity (blood).
- Sampling for SARS-CoV-2 PCR test (nasopharynx, NPH).
- Sampling of faeces for HIV patients and controls, for those who have agreed to this part and samples have not been submitted after the screening visit.
- Sampling for HIV-RNA and HIV-DNA, only for HIV patients if this has not been done during the screening visit.
- Vaccine dose 1 is given (unless contraindications have emerged after health declaration questions).
- Study participants remain at the healthcare facility for at least 15 minutes (30 minutes for haemophiliacs) after vaccination.
- A diary is given to the study participant/information about the eDiary.

5.2.3. 10-day follow-up (10-day FU) after the first vaccine dose (+/-3 days)

Sampling group A
All contact between study participants and study staff (research nurse or investigator) can be done remotely by phone or chat, if the Always Open function is available. Can also be done on site, if it coincides with the study participant's regular visit to the study unit or if the study participant prefers to be sampled at the study unit.

Blood samples can be taken at any of the Karolinska University Laboratory's sampling units in Stockholm, and saliva samples can be sent in a stamped envelope to the Karolinska Institute.

- Review of the diary (local and systemic reactogenicity).
- Review of adverse events (AE/SAE/SUSAR).
- Review of concomitant medication (all new drugs, including immunosuppressive/immunoglobulin treatments).
- Testing for SARS-CoV-2 serology and more detailed analyses of humoral immunity (blood).
- Sampling for humoral immunity (saliva).

Sampling group B

All procedures are performed at the study unit, as sampling for peripheral blood mononuclear cell (PBMC) samples is required for cellular immunity at the study unit:

- Review of the diary (local and systemic reactogenicity).
- Review of adverse events (AE/SAE/SUSAR).
- Review of concomitant medication (all new drugs, including immunosuppressive/immunoglobulin treatments).
- Sampling for SARS-CoV-2 serology and more detailed analyses of humoral immunity (blood).
- Sampling for humoral immunity (saliva).
- Sampling for analysis of cellular immunity (blood).

5.2.4. Day 21 after first vaccine dose (+/-3 days, vaccine dose 2 given)

The following procedures will be performed at the study unit with study participants present. Sampling will be performed prior to vaccination.

Sampling group A

- Review of health declaration questions in Vaccinate, according to care routine before vaccination.
- Vital parameters: Blood pressure, pulse, temperature, and respiratory rate are measured. If the temperature is ≥ 38 degrees, the study participant is rescheduled for vaccination on another day, when the person has been fever-free for at least 48 hours.
- Review of adverse events (AE/SAE/SUSAR).
- Review of concomitant medication (all new drugs, including immunosuppressive/immunoglobulin treatments).
- Sampling for SARS-CoV-2 IgG serology and more detailed analyses of humoral immunity (blood).
- Sampling for humoral immunity (saliva).
- Sampling of clinical laboratory tests including blood count (hemoglobin, white blood cells, and platelets) and differential count (neutrophils, lymphocytes, monocytes, basophils, and eosinophils), liver function (ALAT, ASAT, bilirubin, ALP), and creatinine.

- Sampling of HIV-RNA for HIV-infected individuals.
- Vaccine dose 2 is given (in case of anaphylaxis or other significant side effect likely to be related to Comirnaty, this dose will not be given).
- Study participants remain at the healthcare facility for at least 15 minutes (30 minutes for those with bleeding disorders) after vaccination.
- A diary is given to the study participant/information about the eDiary.

Sampling group B
All procedures are performed at the study unit:

- Review of health declaration questions in Vaccinate, according to care routine before vaccination.
- Vital parameters: Blood pressure, pulse, temperature, and respiratory rate are measured. If the temperature is ≥ 38 degrees, the study participant is rescheduled for the vaccine dose injection on another day, when the person has been fever-free for at least 48 hours.
- Review of adverse events (AE/SAE/SUSAR).
- Review of concomitant medication (all new drugs, including immunosuppressive/immunoglobulin treatments).
- Sampling for SARS-CoV-2 IgG serology and more detailed analyses of humoral immunity (blood).
- Sampling for humoral immunity (saliva).
- Sampling of clinical laboratory tests including blood count (hemoglobin, white blood cells, and platelets) and differential count (neutrophils, lymphocytes, monocytes, basophils, and eosinophils), liver function (ALAT, ASAT, bilirubin, ALP), and creatinine.
- Sampling of HIV-RNA for HIV-infected individuals.
- Sampling for analysis of cellular immunity (blood).
- Vaccine dose 2 is administered (in the event of anaphylaxis or other significant side effects likely to be related to Comirnaty, this dose will not be administered).
- Study participants remain at the healthcare facility for at least 15 minutes (30 minutes for people with bleeding disorders) after vaccination.
- A diary is given to the study participant/information about the eDiary.

5.2.5. 2-week follow-up (2-week FU) after the second vaccine dose (+/-3 days)

Sampling group A
All contact between study participants and study staff (research nurse or investigator) can be done remotely by phone (or chat, if the Always Open function is available). Can also be done on site, if it coincides with the study participant's regular visit to the study unit, or if the study participant prefers to be sampled at the study unit). Blood samples can be taken at any of the Karolinska University Laboratory's

sampling units in Stockholm, and saliva samples can be sent in a stamped envelope to Karolinska Institutet:

- Review of the diary (local and systemic reactogenicity).
- Review of adverse events (AE/SAE/SUSAR).
- Review of concomitant medication (all drugs from, including immunosuppressive/immunoglobulin treatments).
- Sampling for SARS-CoV-2 IgG serology and more detailed analyses of humoral immunity (blood).
- Sampling for humoral immunity (saliva).
- Sampling of clinical laboratory samples including blood count (hemoglobin, white blood cells, and platelets) and differential count (neutrophils, lymphocytes, monocytes, basophils, and eosinophils), liver function (ALAT, ASAT, bilirubin, ALP), and creatinine.
- Sampling of HIV-RNA for HIV-infected individuals.

Sampling group B

All procedures are performed at the study unit:

- Review of the diary (local and systemic reactogenicity).
- Review of adverse events (AE/SAE/SUSAR).
- Review of concomitant medication (all drugs from, including immunosuppressive/immunoglobulin treatments).
- Testing for SARS-CoV-2 IgG serology and more detailed analyses of humoral immunity (blood).
- Sampling for humoral immunity (saliva).
- Sampling of clinical laboratory tests including blood count (hemoglobin, white blood cells, and platelets) and differential count (neutrophils, lymphocytes, monocytes, basophils, and eosinophils), liver function (ALAT, ASAT, bilirubin, ALP), and creatinine.
- Sampling of HIV-RNA for HIV-infected individuals.
- Sampling for analysis of cellular immunity (blood), increased blood volume for all patient groups.

5.2.6. 6-week follow-up (6-week FU) after second vaccine dose (+/-3 days; remote contact)

Only remote contact via telephone (or chat, if the Always Open function is available) with the study participant.

- Review of adverse events (SAE/SUSAR, last date of reporting period for these).
- Review of concomitant medication (only immunosuppressive/immunoglobulin treatments).

5.2.7. 3-month follow-up (3 m FU) after second vaccine dose (+/- 2 weeks)

Sampling group A

All contact between study participants and study staff (research nurse or investigator) can be done remotely by phone (or chat, if the Always Open function is available. Can also be done on site, if it coincides with the study participant's regular visit to the study unit, or if the study participant prefers to be sampled at the study unit). Blood samples can be taken at any of the Karolinska University Laboratory's sampling units in Stockholm, and saliva samples can be sent in a stamped envelope to the Karolinska Institute.

- Review of concomitant medication (immunosuppressive/immunoglobulin treatments only).
- Sampling for SARS-CoV-2 IgG serology and more detailed analyses of humoral immunity (blood).
- Sampling for humoral immunity (saliva).
- Sampling for HIV-RNA and HIV-DNA for HIV-infected individuals.

Sampling group B
All procedures are performed at the study unit.

- Review of concomitant medication (immunosuppressive/immunoglobulin treatments only).
- Sampling for SARS-CoV-2 IgG serology and more detailed analyses of humoral immunity (blood).
- Sampling for humoral immunity (saliva).
- Sampling for HIV-RNA and HIV-DNA for HIV-infected individuals.
- Sampling for analysis of cellular immunity (blood).

5.2.8. 6-month follow-up (6 m FU) after second vaccine dose (+/- 2 weeks)

Sampling group A
All contact between study participants and study staff (research nurse or investigator) can be done remotely by telephone (or chat, if the Always Open function is available. Can also be done on site, if it coincides with the study participant's regular visit to the study unit, or if the study participant prefers to be sampled at the study unit). Blood samples can be taken at any of the Karolinska University Laboratory's sampling units in Stockholm, and saliva samples can be sent in a prepaid envelope to the Karolinska Institute.

- Review of concomitant medication (immunosuppressive/immunoglobulin treatments only).
- Sampling for SARS-CoV-2 IgG serology and more detailed analyses of humoral immunity (blood).
- Sampling for humoral immunity (saliva).
- Sampling for HIV-RNA and HIV-DNA for HIV-infected individuals.

Sampling group B
All procedures are performed at the study unit:

- Review of concomitant medication (immunosuppressive/immunoglobulin treatments only).
- Sampling for SARS-CoV-2 IgG serology and more detailed analyses of humoral immunity (blood).
- Sampling for humoral immunity (saliva).
- Sampling of HIV RNA and HIV DNA for HIV-infected individuals.

Sampling for analysis of cellular immunity (blood).

5.2.9. 9-month follow-up (9 m FU) after second vaccine dose (+/- 3 weeks)

New informed consent obtained before study procedure. Sampling group A

All contact between study participants and study staff (research nurse or investigator) can be done remotely by telephone (or chat, if the Always Open function is available. Can also be done on site, if coincides with the study participant's regular visit to the study unit, or if the study participant prefers to be sampled at the study unit). Blood samples can be taken at any of the Karolinska University Laboratory's sampling units in Stockholm, and saliva samples can be sent in a stamped envelope to the Karolinska Institute.

- Written informed consent regarding extended study
- Review of concomitant medication (only newly initiated immunosuppressive/immunoglobulin treatments).
- Note any additional vaccination with Comirnaty or other COVID-19 vaccination in the eCRF (concurrent treatment).
- Ask the study participant about COVID-19
- Sampling for SARS-CoV-2 IgG serology and more detailed analyses of humoral immunity (blood).
- Testing for humoral immunity (saliva).
- Sampling for HIV-RNA and HIV-DNA for HIV-infected individuals.

Sampling group B

All procedures are performed at the study unit:

- Written informed consent regarding extended study
- Review of concomitant medication (only newly initiated immunosuppressive/immunoglobulin treatments).
- Note any additional vaccination with Comirnaty or other COVID-19 vaccination in the eCRF (concurrent treatment)
- Ask the study participant about COVID-19
- Sampling for SARS-CoV-2 IgG serology and more detailed analyses of humoral immunity (blood).
- Sampling for humoral immunity (saliva).
- Sampling for HIV-RNA and HIV-DNA for HIV-infected individuals.
- Sampling for analysis of cellular immunity (blood).

5.2.10. 12-month follow-up (12 m FU) after second vaccine dose (+/- 3 weeks)

Sampling group A

All contact between study participants and study staff (research nurse or investigator) can be done remotely by telephone (or chat, if the Always Open function is available. Can also be done on site, if it coincides with the study participant's regular visit to

the study unit, or if the study participant prefers to be sampled at the study unit). Blood samples can be taken at any of the Karolinska University Laboratory's sampling units in Stockholm, and saliva samples can be sent in a prepaid envelope to the Karolinska Institute.

- Review of concomitant medication (only newly initiated immunosuppressive/immunoglobulin treatments).
- Note any additional vaccination with Comirnaty or other COVID-19 vaccination in the eCRF (concurrent treatment).
- Ask the study participant about COVID-19.
- Sampling for SARS-CoV-2 IgG serology and more detailed analyses of humoral immunity (blood).
- Testing for humoral immunity (saliva).
- Sampling for HIV-RNA and HIV-DNA for HIV-infected individuals.

Sampling group B
All procedures are performed at the study unit:

- Review of concomitant medication (only newly initiated immunosuppressive/immunoglobulin treatments).
- Note any additional Comirnaty or other COVID-19 vaccinations in the eCRF (concurrent treatment).
- Ask the study participant about COVID-19
- Testing for SARS-CoV-2 IgG serology and more detailed analyses of humoral immunity (blood).
- Sampling for humoral immunity (saliva).
- Sampling for HIV-RNA and HIV-DNA for HIV-infected individuals.
- Sampling for analysis of cellular immunity (blood).

5.2.11. 18-month follow-up (18 m FU) after second vaccine dose (+/- 3 weeks)

Sampling group A
All contact between study participants and study staff (research nurse or investigator) can be done remotely by telephone (or chat, if the Always Open function is available. Can also be done on site, if it coincides with the study participant's regular visit to the study unit, or if the study participant prefers to be sampled at the study unit). Blood samples can be taken at any of the Karolinska University Laboratory's sampling units in Stockholm, and saliva samples can be sent in a prepaid envelope to the Karolinska Institute.

- Review of concomitant medication (only newly initiated immunosuppressive/immunoglobulin treatments).
- Note any additional Comirnaty or other COVID-19 vaccinations in the eCRF (concomitant treatment).
- Ask the study participant about COVID-19.
- Testing for SARS-CoV-2 IgG serology and more detailed analyses of humoral immunity (blood).
- Sampling for humoral immunity (saliva).
- Sampling for HIV-RNA and HIV-DNA for HIV-infected individuals.

Sampling group B
All procedures are performed at the study unit:

- Review of concomitant medication (only newly initiated immunosuppressive/immunoglobulin treatments).
- Note any additional vaccination with Comirnaty or other COVID-19 vaccination in the eCRF (concomitant treatment).
- Ask the study participant about COVID-19.
- Testing for SARS-CoV-2 IgG serology and more detailed analyses of humoral immunity (blood).
- Testing for humoral immunity (saliva).
- Sampling for HIV-RNA and HIV-DNA for HIV-infected individuals.
- Sampling for analysis of cellular immunity (blood).

5.2.12. 24-month follow-up (24 m FU) after second vaccine dose (+/- 3 weeks)

Sampling group A
All contact between study participants and study staff (research nurse or investigator) can be done remotely by telephone (or chat, if the Always Open function is available. Can also be done on site, if it coincides with the study participant's regular visit to the study unit, or if the study participant prefers to be sampled at the study unit). Blood samples can be taken at any of the Karolinska University Laboratory's sampling units in Stockholm, and saliva samples can be sent in a stamped envelope to the Karolinska Institute.

- Review of concomitant medication (only newly initiated immunosuppressive/immunoglobulin treatments).
- Note any additional vaccination with Comirnaty or other COVID-19 vaccination in the eCRF (concurrent treatment).
- Ask the study participant about COVID-19.
- Sampling for SARS-CoV-2 IgG serology and more detailed analyses of humoral immunity (blood).
- Testing for humoral immunity (saliva).
- Sampling of HIV-RNA and HIV-DNA for HIV-infected individuals.
- Fecal sampling for HIV patients and controls, for those individuals who have agreed to this part.

Sampling group B
All procedures are performed at the study unit:

- Review of concomitant medication (only newly initiated immunosuppressive/immunoglobulin treatments).
- Note any additional vaccination with Comirnaty or other COVID-19 vaccination in the eCRF (concomitant treatment).
- Ask the study participant about COVID-19
- Sampling for SARS-CoV-2 IgG serology and more detailed analyses of humoral immunity (blood).
- Sampling for humoral immunity (saliva).
- Sampling for HIV-RNA and HIV-DNA for HIV-infected individuals.
- Sampling for analysis of cellular immunity (blood).
- Fecal sampling for HIV patients and controls, for those who have agreed to this part.

5.2.13. 30-month follow-up (30 m FU) after second vaccine dose (+/- 3 weeks)

New informed consent obtained before study procedure. Sampling groups A

and B

All contact between study participants and study staff (research nurse or investigator) can be done remotely by telephone (or chat, if the Always Open function is available. Can also be done on site, if it coincides with the study participant's regular visit to the study unit, or if the study participant prefers to be sampled at the study unit). Blood samples can be taken at any of the Karolinska University Laboratory's sampling units in Stockholm, and saliva samples can be sent in a stamped envelope to the Karolinska Institute.

- Review of concomitant medication (only newly initiated immunosuppressive/immunoglobulin treatments).
- Note any additional vaccination with Comirnaty or other COVID-19 vaccination in the eCRF (concurrent treatment).
- Ask the study participant about COVID-19.
- Sampling for SARS-CoV-2 IgG serology and more detailed analyses of humoral immunity (blood).
- Sampling for humoral immunity (saliva).
- Sampling of HIV-RNA and HIV-DNA for HIV-infected individuals.

Study participants will receive a SARS-CoV-2 antigen test kit for saliva sampling in case of COVID symptoms. Study participants will only contact the research staff if they test positive or need more test kits.

5.2.14. 36-month follow-up (36 m FU) after second vaccine dose (+/- 3 weeks)

Sampling group A

All contact between study participants and study staff (research nurse or investigator) can be done remotely by phone (or chat, if the Always Open function is available. Can also be done on site, if it coincides with the study participant's regular visit to the study unit, or if the study participant prefers to be sampled at the study unit). Blood samples can be taken at any of the Karolinska University Laboratory's sampling units in Stockholm, and saliva samples can be sent in a stamped envelope to the Karolinska Institute.

- Review of concomitant medication (only newly initiated immunosuppressive/immunoglobulin treatments).
- Note any additional vaccination with Comirnaty or other COVID-19 vaccination in the eCRF (concurrent treatment).
- Ask the study participant about COVID-19.
- Sampling for SARS-CoV-2 IgG serology and more detailed analyses of humoral immunity (blood).
- Sampling for humoral immunity (saliva).
- Sampling of HIV RNA and HIV DNA for HIV-infected individuals.
- Fecal sampling for HIV patients and controls, for those individuals who have agreed to this part.

Sampling group B
All procedures are performed at the study unit:

- Review of concomitant medication (only newly initiated immunosuppressive/immunoglobulin treatments).
- Note any additional vaccination with Comirnaty or other COVID-19 vaccination in the eCRF (concurrent treatment).
- Ask the study participant about COVID-19
- Sampling for SARS-CoV-2 IgG serology and more detailed analyses of humoral immunity (blood).
- Sampling for humoral immunity (saliva).
- Sampling for HIV-RNA and HIV-DNA for HIV-infected individuals.
- Fecal sampling for HIV patients and controls, for those who have agreed to this part
- Sampling for analysis of cellular immunity (blood).

If there are too few study participants in group B at this visit, some study participants from group A may be asked to provide group B samples.

Study participants will be given a SARS-CoV-2 antigen test kit to take home/be sent home for saliva sampling in case of COVID symptoms. Study participants will only contact the research staff if they test positive or need more test kits.

5.3. Procedures for the entire study period

5.3.1. Measures to reduce the risk of bleeding after injection in people with coagulopathy or anticoagulants

To minimize the risk and complications of bleeding, the following measures will be taken for people with coagulopathy or anticoagulant treatments as follows (www.janusinfo.se).

- Anyone with coagulopathy, anticoagulants, or otherwise at increased risk of bleeding will be observed for at least 30 minutes after vaccination.
- All individuals with bleeding disorders should be vaccinated after contacting the coagulation clinic at Karolinska University Hospital and follow their recommendations regarding treatment with possible factor therapy beforehand.
- Study participants with thrombocytopenia <50x10 g/L are given an injection, only after measures have been taken to raise the platelet count, e.g. with platelet concentrate.
- For individuals treated with Waran/warfarin: vaccination can be given at PK INR <2.8, based on a PK test taken within seven days.
- For study participants treated with Waran in combination with ASA or clopidogrel (dual therapy) or a combination of all three drugs (triple therapy), we recommend that vaccination can be given at PK INR 1.8–2.0. For these patients, there should be a current PK INR.
- For study participants receiving NOAC treatment:
- The injection should be given as close to the next dose as possible. The plasma concentration of the drug is then at its lowest and

the blood-thinning effect is at its lowest. This minimises the risk of bleeding during the injection.

- After vaccination, at least two hours should elapse before the patient takes the next dose of NOAC.
- For study participants receiving low molecular weight heparin: The daily injection should be given as long as possible before vaccination, i.e., the vaccine should be given shortly before the injection of low molecular weight heparin (LMWH).
- Compression is applied for approximately ten minutes after injection for all individuals receiving anticoagulant therapy.

5.3.2. Physical examinations

During the COVID-19 pandemic, physical outpatient visits for routine care at the hospital have been replaced with video visits or telephone contacts as much as possible to reduce the risk of exposure to SARS-CoV-2, especially for immunosuppressed patients who are also considered to be at higher risk of severe complications from COVID-19. This is therefore an established way of monitoring these patients in routine care. To reduce the risk of exposure to SARS-CoV-2, even when screening research subjects and during the study period, we plan to screen research subjects via video visits. During such a visit, electronic consent is planned to be obtained from the research subject and study doctor via an app, using an established and certified method, i.e. without a physical examination. However, screening via remote contact requires access to mobile Bank ID and a certain level of technical knowledge on the part of the research subject, which is why individuals (especially older individuals) who are unable or unwilling to have screening visits via remote contact in this manner will be offered a physical visit for screening. At both vaccination occasions, doctors will be on site who can perform a targeted and symptom-driven physical examination if necessary.

The rationale behind planning only targeted and symptom-driven physical examinations as needed during the study period is that we do not expect any specific side effects in the primary population related to Comirnaty, which is already an approved drug. In terms of side effects, it may rather be the opposite due to immunosuppression, with a lower frequency and severity of reactogenicity or autoimmune reactions as side effects of Comirnaty. Vaccination efficacy may prove to be suboptimal in this population, and patients therefore need to be protected from SARS-CoV-2 infection as much as possible, even in connection with measures taken in this study. The patients are well known to the clinics, where the usual follow-ups will take place within routine care during the study period. The most severely ill patients will continue to be closely monitored in routine care for their underlying disease, with physical examinations as needed in routine care.

The control group will also not undergo any physical examinations, except for targeted and symptom-driven physical examinations as needed during the study period. Comirnaty has been studied in >30,000 healthy individuals without any signs of serious side effects except anaphylaxis, which research subjects are still observed for, and no physical examinations would have been offered to them if they had received vaccination in routine healthcare.

5.3.3. Features via the "Always Open" app and consent application

1.1.1.1 Always open

In collaboration with the Stockholm County Council (SLSO), we plan to build process support for this study within "Always open", an application (app) developed by the Stockholm County Council (SLSO) that offers various healthcare contacts such as video meetings, chat with healthcare personnel, etc. All logins to the app are done with mobile Bank ID. The app is used routinely in routine healthcare, especially in outpatient care. All health information that appears via the app will be recorded in the patient's medical records, which constitute source data.

We plan to use this app to:

- Have video visits with research subjects during screening and, if necessary, during the study period.
- As a complement, use the app to send pictures of the injection site when necessary. Any pictures will be printed and scanned into the patient's medical record.
- Record local and systemic reactogenicity using eDagbok, where study participants self-record their reactions daily for 7 days after the vaccine dose. Information from Alltid öppet will be recorded in the patient's medical records, which constitute source data. A paper version of the diary can be filled in if eDagbok cannot be used.
- Chat with study participants at the predefined contact times. Chat content will be recorded in the patient's medical records, in the same way as if communication had taken place via a telephone call. Test results from SARS-CoV-2 serology can be communicated via chat at the end of the study.
- Give study participants the opportunity to ask questions to study staff during the study period.

1.1.1.2 Application for electronic consent

Where possible, we will use an app that has already been used in clinical trials. Through this app, we will obtain consent from the patient electronically and remotely, while the study doctor signs the consent form during a video visit at screening.

5.3.4. SARS-CoV-2 serology results for study participants

SARS-CoV-2 serology results will be communicated to study participants (these are analyzed at an accredited and validated laboratory, Karolinska University Laboratory), either by letter or telephone response, or chat via the "Alltid öppet" app before the follow-up visit 6 months after the second vaccine dose. In the two extension parts of the study, antibody analyses will be performed on an ongoing basis, which is why SARS-CoV-2 IgG serology results will be provided to study participants no later than 3 months after sampling.

5.3.5. PCR test

If any of these symptoms reappear during the study period, study participants are asked to contact study staff for information and to have their symptoms noted. SARS-CoV-2 PCR tests in the nasopharynx will be taken to check for SARS-CoV-2 infection: cough, fever ≥ 38 degrees, breathing difficulties, runny nose, nasal congestion, sore throat, headache, nausea, muscle and joint pain, impaired sense of smell and taste, or diarrhea, which is routine in healthcare. Study participants report symptoms via Alltid Öppet (Always Open) with a referral for testing within routine healthcare.

Data on the occurrence of positive SARS-CoV-2 PCR tests will be collected, as well as the occurrence of diagnoses of pneumonia or thromboembolic complications in connection with COVID-19, and treatments given for COVID-19 or complications. Sequencing will be performed on the PCR sample.

The severity of COVID-19 will be graded using the highest degree reached on the scale during the period of illness (no later than 4 weeks after symptom onset), according to the following ordinal scale (Beigel JH et al., 2020):

1. Not hospitalized, with no restrictions on activities.
2. Not hospitalized, but with limited activities and/or need for oxygen therapy at home.

3. Hospitalized, but does not require extra oxygen and no longer needs ongoing medical care.
4. Hospitalized and does not require oxygen therapy, but requires ongoing medical care.
5. Hospitalized and requires oxygen therapy.
6. Hospitalized with a need for noninvasive ventilation (NIV) or use of high-flow oxygen.
7. Hospitalized with mechanical ventilation or ECMO (extracorporeal membrane oxygenation) treatment.
8. Death.

5.4. Description of analyses of humoral and cellular immune responses

In this exploratory part, we intend to investigate various aspects of the humoral and cellular immune response. Certain analyses are planned, as described below, but changes may occur. This is due to the rapid development of knowledge in the field of COVID-19 vaccines, and also depending on what emerges from the interim analysis in the study, where certain analyses may need to be added. However, the main purpose of the analyses will remain the same in this part, i.e. to investigate what happens immunologically after vaccination. Some samples will be analysed immediately, while others will be frozen pending analysis.

5.4.1. IgG antibodies against SARS-CoV-2

Antibodies against the spike protein in SARS-CoV-2 will be analyzed at an accredited laboratory, Clinical Microbiology, Karolinska University Hospital.

5.4.2. Detailed analysis of antibody response

i) Antibody levels. Analyzed using serological analysis that includes two different versions of the spike protein (a soluble trimer form of the spike glycoprotein stabilized in the pre-fusion conformation and the receptor-binding domain) and a C-terminal domain (representation of the nucleocapsid protein) at the SciLife laboratory. The method has been validated in a large cohort, showing a sensitivity of 99.7% and a specificity of 100%. Variants of the spike glycoprotein such as "the British variant B.1.1.7" and the "South African variant 501.V2" will be able to be implemented for these analyses.

ii) Neutralization: The ability of antibodies to inhibit the interaction between the spike protein and the ACE2 receptor will be evaluated by incubating serum with fluorescently labeled spike protein to detect the amount of spike protein that can still bind to microbeads; a pseudoneutralization assay. This method has been evaluated against a micro-neutralization assay and has been shown to correlate well. By using these two methods, we will be able to compare the total amount of spike-recognizing antibodies with the amount that can inhibit receptor binding.

5.4.3. Antibody analyses in saliva

Antibody analyses are performed in the same way as blood analyses, i.e., using the multiplex platform at the SciLife laboratory, with optimized dilution. Other protein analyses are Luminex/Elisa-based analyses. Virus-inactivated samples are used in all analyses. Saliva samples are handled at the ANA Futura Laboratory, Department of Odontology, Karolinska Institutet.

5.4.4. T and B cell immunity

In the different groups, we will study the reactions of immune cells, including the levels and functions of molecules produced by these cells (e.g., antibodies, cytokines, and chemokines). Of particular importance are studies of the development of protective antibodies and virus-specific T cells against the viruses targeted by the vaccines. Our hypothesis is based on the fact that individuals with

vaccinated individuals with immunodeficiency will have lower levels of protective antibodies and virus-specific T cells than individuals in the control groups.

T cells:
i) Cytokine stimulation assays will be used to identify T cell responses and assess their phenotype and functional properties before and after vaccination. ELISPOT analysis will be performed on patients to determine whether the vaccine groups have an IFNg-positive T cell response after spike peptide stimulation. More in-depth analysis with flow cytometry will be performed for some patients in each group to identify the function, phenotype, and helper function of SARS-CoV-2-specific CD4+ T cells, including B helper analyses. SARS-CoV-2-specific CD8+ T cell analysis will focus on cytotoxic capacity, homeostatic potential, and tissue display markers. Cells will be stimulated with overlapping spike peptides for 10 hours and analyzed using targeted and unbiased strategies to identify different characteristics of the responses. Multimer staining may also be performed, and HLA typing will therefore be performed on patients.
ii) DNA and RNA sequencing: Some patients with poorer or more robust antibody responses will be selected for DNA and RNA sequencing analysis of SARS-CoV-2-specific CD4+ or CD8+ T cells. These are performed to gain a deeper understanding of what type of T cells are correlated with an effective antibody response. For CD8+ T cells, we will use SARS-CoV-2-specific peptide-MHC-I multimers, and for CD4+ T cells, we will stimulate with relevant SARS-CoV-2 peptides and sort based on activation-induced markers to identify cells of interest for further sequencing analysis.

B cells:
To evaluate spike glycoprotein-specific memory B cell responses, we will compare a subset of individuals longitudinally in each group. ELISPOT analysis will be used as a first approach to detect patients with memory B cells against the spike protein before and after vaccination. Using fluorescently labeled spike protein together with relevant B cell markers to identify different subsets and isotype usage, we will further define and enumerate spike-specific B cells by flow cytometry. These studies will evaluate the fluctuations in spike-specific B cell memory after vaccination. With this method, there is also the prospect of isolating and sequencing B cells as described above for T cells.

The techniques described above are well established at the Center for Infectious Medicine, Karolinska Institutet, Karolinska University Hospital, Huddinge, and the researchers responsible have extensive experience in functional and phenotypic analysis of immune cells using the above-mentioned methods.

5.4.5. Microbiome analysis

There is growing awareness that the gut microbiome can influence the outcome of vaccinations (T Hagen et al, Cell 2019, 178(6)). For this reason, faeces and saliva samples will be collected from patients with HIV infection and controls at baseline. Bacterial DNA will be extracted from faeces and saliva. The 16SRNA gene and metagenome (in selected patients) will then be sequenced and analysed using bioinformatic techniques. All analyses are established at the ANA Futura Laboratory/Institution for Laboratory Medicine, Karolinska Institutet.

5.4.6. Biological sampling procedures

Handling, storage, and destruction of biological samples

Appendix 2 specifies the volume of blood per research subject and visit. Depending on which sampling group the person belongs to and whether or not the person belongs to the HIV-infected group, the volume varies from 7 ml to 104 ml per visit and research subject. The total volume of blood per research subject throughout the study varies between 132.5 ml and 572.5 ml over a period of approximately 3 years. By way of comparison, blood donors usually give 450 ml of blood per visit, with men able to give

a maximum of 4 times per year and women a maximum of 3 times per year. However, some patients in different risk groups cannot tolerate blood draws of the same volume as healthy blood donors. The responsible investigator is therefore responsible for assessing whether the sample volume is acceptable for the patient from a medical point of view.

Even after the clinical trial has ended, we will continue with the analyses for another ten years. We also want to store the remaining samples because we expect that the results from this first study will lead to new hypotheses and studies. For these, we will then seek new ethical approvals where necessary. The samples will be destroyed after 10 years if no new approval is sought.

5.4.7. Biobank

Research samples will be collected and stored in accordance with the Biobank Act (2002:297).

The samples will be stored in coded form, which means that they cannot be directly traced back to any individual person. Each sample has a unique code to prevent mix-ups. The samples and the associated identification list (code key) will be stored separately from each other and protected from access by unauthorized persons.

Samples may be sent to another laboratory for analysis within Sweden, the EEA, or the EU. In such cases, an MTA will be drawn up.

5.4.8. Study participants with a new diagnosis of COVID-19 during the study period

If the patient receives a virologically confirmed COVID-19 diagnosis in healthcare outside the study, this information, including the date of a positive test, will be collected from the study participant's medical records. The presence of a diagnosis of pneumonia or thromboembolic complications in connection with COVID-19 will be noted, as well as any treatment given for COVID-19 or complications. Sequencing will be performed on the PCR sample.

The severity of COVID-19 will be graded using the highest degree reached on the scale during the period of illness (no later than 4 weeks after symptom onset), according to the following ordinal scale (Beigel JH et al., 2020):

1. Not hospitalized, with no restrictions on activities.
2. Not hospitalized, but with restrictions on activities and/or need for oxygen therapy at home.
3. Admitted to hospital, but does not require extra oxygen and no longer needs ongoing medical care.
4. Hospitalized and does not require oxygen therapy, but requires ongoing medical care.
5. Hospitalized and requires oxygen therapy.
6. Hospitalized with a need for noninvasive ventilation (NIV) or high-flow oxygen therapy.
7. Hospitalized with mechanical ventilation or ECMO (extracorporeal membrane oxygenation) treatment.
8. Death.

Table 2: Overview of study design for study participants receiving Comirnaty

Visit No.	Screening	1	2	3	4	5	6	7	8	9	10	11	12	13
Administration Comirnaty		dose 1		dose 2										
Study contacts		d 0	10 d FU (vacc 1)	d 21	2 weeks FU (vacc 2)	6 weeks FU (vacc 2)	3 months FU (vacc 2)	6 months FU (vacc 2)	9 months FU (vacc 2)	12 months (vacc 2)	18 months (vacc 2)	24 months (vacc 2)	30 months (vacc 2)	36 months (vacc 2)
Time span	0-28 days before visit 1	Day 0	+/-3 days	+/-3 days	+/-3d	+/-3d	+/- 2 w	+/- 3v	+/- 3v	+/- 3v	+/- 3v	+/- 3 weeks	+/- 3v	+/- 3v
Contacts and sampling[1] for sampling group A without cellular analyses														
Study visits to the study unit		X		X										
Remote contact (telephone/chat) by nurse/tester			X		X	X								
Review of health declaration questions in Vaccinate and/or registration in Vaccinate		X		X										
Sampling of clinical laboratory samples	X[1]	(X)[1,2]		X[1,2]	X[1]									
SARS-CoV-2 serology and extended humoral immunity sampling immunity (blood and saliva)		X[2]	X[3]	X[2]	X[3]		X[3]	X[3]	X[3]	X[3]	X[3]	X[3]	X[3]	X[3]
Contacts and sampling[1] for sampling group B with cellular analyses														
Study visits		X	X	X	X		X	X	X	X	X	X	X	X
Review of health declaration questions in Vaccinate and/or registration in Vaccinate		X		X				X	X	X	X	X	X	X
Sampling of clinical laboratory samples	X[1]	(X)[1,2]		X[1,2]	X[1]									
SARS-CoV-2 sampling serology and humoral immunity (blood and saliva)		X[2]	X	X[2]	X		X[3]	X	X	X	X	X	X	X
Sampling for cellular immunity analyses (blood)		X[2]	X	X[2]	X		X	X	X	X	X	X		X[14]

Visit no.	Screening	1	2	3	4	5	6	7	8	9	10	11	12	13
Study contacts		d 0	10 d FU (vacc 1)	d 21	2 v FU (vacc 2)	6v FU (vacc 2)	3 months FU (vacc 2)	6 months FU (vacc 2)	9 months FU (vacc 2)	12 months (vacc 2)	18 months (vacc 2)	24 months (vacc 2)	30 months (vacc 2)	36 months (vacc 2)
Time span	0-28 days before visit 1	Day 0	+/-3 days	+/-3 days	+/-3d	+/-3d	+/- 2 w	+/- 3v	+/- 3v	+/- 3v	+/- 3v	+/- 3v	+/- 3v	+/- 3v
Other procedures for both sampling groups A and B														
Informed consent	X								X				X	
Inclusion/exclusion criteria	X	X												
Demographics	X													
Concomitant medication[4]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Symptom-driven physical examination [5]														
Vital signs [6]		X		X										
15/30 min post-vaccination observation period		X		X										
Handing out of diary		X		X										
Review of diary for local and systemic reactogenicity[7]			X		X									
Collection AE[8]			X	X	X									
Collection SAE/SUSAR [9]			X	X	X	X								
Sampling SARS-CoV-2 PCR test		X[2.10]												
Pregnancy test for women of childbearing age (urine)	X[11]	(X)[11]												
Microbiome samples for HIV-infected individuals and control subjects, if they have agreed to this part (fecal sample)	X[12]											X[12]		X[12]
For HIV-infected individuals, HIV DNA sample	X[13]	(X)[13]					X	X	X	X	X	X	X	X
For HIV-infected individuals, HIV RNA test	X[13]	(X)[13]		X	X		X	X	X	X	X	X	X	X
Note any additional vaccination with Comirnaty								X	X	X	X	X	X	X
Question about Covid 19								X	X	X	X	X	X[15]	X[15]

Abbreviations: d= day; w= week; m= month; vac= vaccination, FU= follow-up (follow-up time is counted as time after the last vaccination); AE = adverse events, i.e., undesirable medical events; SAE = severe adverse events, i.e., serious undesirable medical events; SUSAR = suspected unexpected severe adverse reaction, i.e., suspected, unforeseen, serious undesirable reactions (SUSAR). Footnote.

[1] Includes blood count (hemoglobin, white blood cells, and platelets) and differential count (neutrophils, lymphocytes, monocytes, basophils, and eosinophils), liver function (ALT, AST, bilirubin, ALP), and creatinine. IgG analysis in blood only at screening. If the screening visit is conducted via video contact, these samples will be taken on day 0, before the injection of the first vaccine dose.

[2] Samples will be taken before vaccination.

[3] Blood samples can be submitted at Karolinska University Laboratory's sampling units in Stockholm, and study participants do not need to be at the study unit. Saliva samples can be sent by mail.

[4] During screening visits, previous medications/treatments are noted in accordance with 5.5.2. All medications are noted during screening visits. During the period from the day of the vaccine dose until 14 days after, all medications are recorded after each vaccine dose. During other periods during the study, only new immunosuppressive/immunoglobulin treatments are noted, in accordance with 5.5.2.

[5] Targeted examinations for symptoms during the study period, if necessary.

[6] Blood pressure, pulse, temperature, and respiratory rate are measured.

[7] Diary is issued on day 0/study participants receive an e-diary.

[8] AE is monitored from the first and second vaccine doses until 14 days after each vaccine dose.

[9] SAE/SUSAR is monitored within the period from the first vaccination day to 6 weeks after the second vaccine dose.

[10] Nasopharynx sample on day 0 and, if necessary, in case of COVID-19 symptoms. COVID-19 symptoms are: cough, fever≥ e 38 degrees, breathing difficulties, runny nose, nasal congestion, sore throat, headache, nausea, muscle and joint pain, impaired sense of smell and taste, or diarrhea.

[11] If the screening visit takes place via video contact, a pregnancy test will be performed on day 0, before the first vaccine dose is administered.

[12] HIV-infected individuals and control subjects are asked to participate in a study section on the significance of the microbiome for vaccine efficacy. For individuals who agree to participate in this section, sample material for fecal sampling is sent to the individual's home after the video visit during screening, at 24 and 36 months, or the material will be given to the person directly during a physical visit. The person can then send the fecal sample by mail to the study unit during the screening period, or leave it on site on day 1, before the injection of the first vaccine dose.

[13] HIV RNA and DNA are only taken from HIV-infected individuals. If visiting in person, these samples are planned to be taken during the screening period, or alternatively on day 0 before injection of vaccine dose 1.

[14] Applies only to sampling group B.

[15]. Study participants will take home/be sent a SARS-CoV-2 antigen test kit for saliva sampling in case of COVID symptoms. Study participants will only contact the research staff if the test is positive or if more test kits are needed.

5.5. Evaluations and data collection in CRF

5.5.1. Demographics

Demographic data and other baseline characteristics will be obtained and recorded at screening.

5.5.2. Medical history and medications

Medical history, only ongoing drug treatments for the underlying disease, and recently completed immunosuppressive treatment (i.e., <4 weeks from inclusion) are recorded at the screening visit. Ongoing immunosuppressive treatments are divided into steroid and non-steroid treatments.

Specific treatments for individual patient groups are recorded at the screening visit as follows:

Patient group	Parameter
Patients with HIV infection	Antibiotic treatment during the last 3 months.
Patients who have undergone solid organ transplantation	Rejection treatment during the last 6 months. Thymoglobulin treatment during the last 12 months. Rituximab treatment during the last 12 months. Chemotherapy during the past 12 months.
Patients with CLL	Fludarabine (/cyclophosphamide (FC) in combination with rituximab (FCR) during the last 6-30 months. Bedamustine in combination with rituximab (BR) within the last 6-30 months. Rituximab-containing treatment during the last 6 months.

All new medications during the period from the vaccination date to 14 days after each vaccine dose are recorded (i.e., both after vaccine dose 1 and vaccine dose 2). The rationale for this time limit is that if certain adverse events occur during the AE observation period, an assessment must be made as to whether they are related to the study drug or another medication. Although no interaction studies have been conducted in previous clinical trials, we do not expect any drug interactions between the study drug and other drugs. We have already excluded individuals who are scheduled to receive any other vaccination in the exclusion criteria. However, any newly initiated immunosuppressive or immunoglobulin treatments after day 0 (screening) will be recorded, as this may affect the efficacy of the vaccine.

5.5.3. Reactogenicity (Diary)

Each study participant will be provided with a diary. Study participants will record and rate the severity of symptoms in a diary according to 5.8.3. The evaluation will take place from the day of vaccination until 7 days after the dose, after each vaccine dose.

A reactogenicity event recorded in the diary will not be recorded as an AE unless it meets the criteria for an SAE.

5.5.4. Adverse events

Please refer to sections 5.7 and 5.9.

5.5.5. Targeted and symptom-driven physical examination

Examination of various organs/systems indicated by signs or symptoms reported by the study participant and clinically indicated will be performed according to schedule or between/after study visits during the study period.

Any clinically significant change in the findings of the physical examination will be recorded as an AE, unless the event is due to an existing medical condition or is due to treatment given for the existing medical condition.

5.5.6. Vital parameters

At the times specified in the schedule (Table 2), systolic and diastolic blood pressure, pulse, temperature, and respiratory rate will be measured and recorded. Any clinically significant abnormal vital sign value will be recorded as an AE, unless it is deemed not to be due to an existing medical condition or treatment given for the existing medical condition.

5.5.7. Clinical laboratory tests

The predefined safety samples according to 5.2 will be taken and recorded. Any abnormal sample value that is considered clinically significant by the investigator should be recorded as an AE, unless it is due to an existing medical condition or is due to treatment given for the existing medical condition.

5.5.8. Pregnancy test

A urine test will be performed on all women of childbearing age and the results will be recorded.

5.5.9. Serum SARS-CoV-2 serology

Test results, whether positive or negative, will be recorded for samples taken according to the schedule in Table 2, and the results will be communicated to study participants verbally or by letter. However, this test cannot distinguish between the presence of antibodies after vaccination or SARS-CoV-2 infection. Samples are planned to be analyzed at Clinical Microbiology, Karolinska University Hospital (an accredited and validated laboratory) according to standard healthcare procedures. If a method for quantitative measurement of antibodies has been established at this laboratory, antibody titers will also be noted in addition to positive or negative results.

5.5.10. Diagnosis of SARS-CoV-2 positivity/COVID-19

Study participants who have been diagnosed with positive SARS-CoV-2 or COVID-19 within healthcare are registered with the date and symptoms. COVID-19 severity is graded according to section 5.3.5.

5.6. Investigational medicinal products

5.6.1. Description of investigational medicinal products

Comirnaty: Concentrate for solution for injection, dispersion (sterile concentrate). The vaccine is a white to off-white frozen dispersion (pH: 6.9-7.9).
Comirnaty is administered intramuscularly after dilution with sodium chloride as a vaccination series consisting of 2 doses (0.3 ml each) at least 21 days apart. Sodium chloride will be ordered from the hospital pharmacy according to clinical routine.

5.6.2. Labeling and handling of investigational medicinal products

The vaccine is distributed via Region Stockholm. The vaccine will be handled and administered at the respective clinics. Therefore, no study-specific labeling of the investigational medicinal product will be done.
The investigational medicinal product will be stored until vaccination in accordance with the instructions in the summary of product characteristics.

5.6.3. Method of administration

Comirnaty will be prepared by healthcare professionals using aseptic technique to ensure that the prepared dispersion is sterile. Thawing and dilution will be performed according to the summary of product characteristics. Intramuscular administration of Comirnaty is planned to take place at

study unit. Close observation for at least 30 minutes after vaccination for people with bleeding disorders. Other study participants will be observed for at least 15 minutes after vaccination. Those who have experienced an anaphylactic reaction after the first dose will not receive a second dose of the vaccine.

5.6.4. Traceability and compliance with treatment

Administration of the investigational medicinal product will be documented at each unit with information such as who administered the product, the research subject, date, time, and batch number. Any remaining investigational medicinal product will be destroyed at each clinic.

5.6.5. Concomitant use of other drugs

All simultaneous use of other drugs will be documented in the CRF.

5.6.6. Treatment after the end of the study

After the end of the study, patients will receive treatment according to clinical routine.

5.7. Definitions of adverse events

5.7.1. Incident/Adverse event (AE)

Any incident (AE), or worsening of an existing medical condition in a research subject who has received an investigational drug, regardless of whether it is causally related to the treatment or not, i.e., it may be an adverse and undesirable sign (including an abnormal laboratory finding), symptom, or disease that is temporally associated with the use of an investigational medicinal product, regardless of whether it is related to the investigational medicinal product or not.

However, events that are an expected consequence of the underlying disease, such as worsening of the underlying disease, will not be reported as an AE. This is because we do not expect Comirnaty to affect the progression of the underlying disease.

5.7.2. Serious incident/Serious adverse event (SAE)

Any incident that:

- results in death
- is life-threatening
- requires hospitalization or prolongs hospitalization
- causes permanent or significant disability or impairment
- results in a congenital injury/malformation
- is serious for other reasons as described below

Medical and scientific judgment should be used to determine whether an event is "serious" and whether it would be reportable in other situations, such as important medical events that may not be directly life-threatening or result in death or hospitalization, but may jeopardise the research subject or may require intervention to prevent one of the other outcomes listed in the definition above. These should also be considered SAEs.

However, events that are an expected consequence of the underlying disease, such as worsening of the underlying disease, will not be reported as SAEs. This is because we do not expect Comirnaty to affect the progression of the underlying disease.

Hospitalization due to day surgery, elective surgery, or admission that was planned before the start of the study for a pre-existing medical condition that has not worsened

is not considered an SAE.

The investigator is responsible for assessing the severity (serious or non-serious). If the incident is considered serious, it should be reported as a serious adverse event (SAE) by the investigator to the sponsor.

5.7.3. Suspected unexpected serious adverse reactions (SUSAR)

An adverse reaction/event that is serious, unexpected, and suspected to be caused by the treatment, i.e., adverse reactions that are not previously described (in the summary of product characteristics).

5.8. Assessment of adverse reactions

5.8.1. Assessment of severity

Each incident should be classified by the investigator as mild, moderate, or severe.

- Mild: The event is relatively mild and transient in nature but does not affect the normal life of the research subject.
- Moderate: The event causes functional impairment but does not affect health. The event may be sufficiently unpleasant and interfere with normal activities but does not completely prevent them.
- Pronounced: The event causes impairment of function or working capacity or poses a health risk to the research subject.

5.8.2. Assessment of causality

The investigator is responsible for determining whether there is a causal relationship between the AE/SAE and the use of an investigational medicinal product. AEs suspected of being related to the investigational medicinal product will be followed up until the research subject has recovered or is well cared for and on the road to recovery. All AEs should be categorized as either probably related, possibly related, or probably unrelated according to the definition below:

- Probable: Clinical event, including abnormal laboratory tests, occurring within a reasonable time after administration of the intervention/study product. Unlikely to be attributable to underlying disease or other drugs.
- Possible: Clinical event, including laboratory tests, occurring within a reasonable time after administration of the intervention/study product. The event can be explained by the investigational medicinal product and its occurrence is reasonable in connection with the use of the investigational medicinal product, but there is insufficient information to establish the connection. The event can be explained by underlying disease or other medicinal products.
- Unlikely: Clinical event, including abnormal laboratory test results, but not reasonable in relation to the use of the intervention/investigational product. The event is unlikely to be related to the intervention/investigational product and can be explained by other drugs or underlying disease.
- Not classifiable: The event cannot be classified due to lack of information or because the event is not verified.

5.8.3. Assessment of local and systemic reactogenicity

1.1.1.3 Assessment of local reaction at the injection site

The occurrence of pain, redness, and swelling at the injection site is reported in a diary completed by the patient for the period from the vaccine dose to 7 days after the dose for both the first and second vaccine doses. Local bleeding is observed by staff after injection during the observation period. For individuals who have difficulty filling in a diary, study staff will contact the patient on days 2 and 7 after the vaccine dose to collect data by telephone.

The rationale for the 7-day period after the vaccine dose is that local reactions have been studied in 8,183 study participants within 7 days after injection, and these reactions usually began within two days after vaccination and disappeared after 1-2 days. Only 1% reported severe pain, and a lower frequency was reported for redness or swelling. These reactions were less common in people over 55 than in younger people. We do not expect a higher frequency or more severe degree of these local reactions, possibly the opposite in immunosuppressed individuals. An exception may be local bleeding at the injection site, due to existing medical conditions with coagulopathy or anticoagulant treatments.

Table 1. Local Reaction Grading Scale

	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Pain at the injection site	Does not interfere with activity	Interferes with activity	Prevents daily activity	Emergency room visit or hospitalization for severe pain
Redness	>2.0 cm to 5.0 cm (5 to 10 measuring device units)	>5.0 cm to 10.0 cm (11 to 20 measuring device units)	>10 cm (≥21 measuring device units)	Necrosis or exfoliative dermatitis
Swelling	>2.0 cm to 5.0 cm (5 to 10 measuring device units)	>5.0 cm to 10.0 cm (11 to 20 measuring device units)	>10 cm (≥21 measuring device units)	Necrosis

1.1.1.4 Assessment of systemic reactogenicity

The occurrence of fever (yes/no, if yes, temperature), fatigue, headache, chills, vomiting, diarrhea, new or worsening muscle pain, new or worsening joint pain is reported by the study subject in a diary for 7 days after dose 1 and dose 2. For individuals who have difficulty completing a diary, study staff will contact the patient on days 2 and 7 after the vaccine dose to collect data by telephone.

Table 2. Systemic Event Grading Scale

	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Vomiting	1-2 times in 24 hours	>2 times in 24 hours	Requires IV hydration	Emergency room visit or hospitalization for hypotensive shock
Diarrhea	2 to 3 loose stools in 24 hours	4 to 5 loose stools in 24 hours	6 or more loose stools in 24 hours	Emergency room visit or hospitalization for severe diarrhea
Headache	Does not interfere with activity	Some interference with activity	Prevents daily routine activity	Emergency room visit or hospitalization for severe headache
Fatigue/ tiredness	Does not interfere with activity	Some interference with activity	Prevents daily routine activity	Emergency room visit or hospitalization for severe fatigue

5.8.4. eDiary

We are looking into the possibility of setting up an eDiary via Alltid öppet, where login with mobile Bank ID is required to access the diary and where study participants can register their local or systemic reactogenicity via the Alltid öppet mobile app. Study staff will have access to this data. This will be offered to study participants who are technically capable and have access to a mobile Bank ID. Alltid öppet is used in routine healthcare to send patient data, which is then entered into the patient's medical records. Similarly, we will manually record data from the eDiary in patients' medical records.

5.9. Reporting of adverse events

5.9.1. Reporting of adverse events (AE)

- All adverse events should be recorded in a special AE form in the CRF for the period from the day of the first and second vaccine doses until 14 days after each vaccine dose. Exceptions are adverse events that are an expected consequence of the underlying disease, such as worsening of the underlying disease.
- Rationale for the above point: In Phase I and Phase II/III studies of Comirnaty, AEs were collected for the period from informed consent to one month after the second dose. As this has already been studied in approximately 21,000 people with a favorable safety profile, and we do not expect multiple AEs or autoimmune reactions in immunosuppressed individuals, the observation periods are limited to those above. As we expect frequent medical events of a non-serious nature in the majority of the included primary population due to the existing medical condition, such events are excluded from data collection.

5.9.2. Reporting of serious adverse events (SAEs)

- All serious adverse medical events (SAEs) must be noted in the CRF for the period from the first vaccine dose to 6 weeks after the second dose. Exceptions are medical events that are an expected consequence of the underlying disease, such as worsening of the underlying disease.

- SAEs must be reported to the sponsor on a special SAE form within 24 hours of the investigator becoming aware of the SAE. Follow-up information describing the outcome and management of the SAE must be reported as soon as that information is available. The original must be entered in the CRF.
- Rationale for the above points: In Phase I and Phase II/III studies of Comirnaty, SAEs were collected for the period from informed consent to six months after the second dose. As this has already been studied in approximately 21,000 people with a favorable safety profile regarding SAE, and we do not expect multiple SAE or autoimmune reactions in immunosuppressed individuals, the observation periods are limited to those above. As we can expect serious medical events in the majority of the included primary population due to the existing medical condition.

5.9.3. Reporting of suspected, unexpected, serious adverse reactions (SUSAR)

- All suspected, unexpected, serious adverse reactions (SUSARs) should be noted in the CRF for the period from the first vaccine dose to 6 weeks after the second dose. Exceptions are medical events that are an expected consequence of the underlying disease, such as worsening of the underlying disease.
- SUSARs that are fatal or life-threatening shall be reported to the Swedish Medical Products Agency within 7 days of the sponsor becoming aware of the event. Relevant follow-up information shall then be submitted within a further 8 days.
- Other SUSARs shall be reported as soon as possible and no later than 15 days after they have come to the sponsor's attention. The sponsor is responsible for reporting all relevant information about suspected, unforeseen, serious adverse reactions to the Swedish Medical Products Agency.

5.9.4. Procedure in case of emergency or pregnancy

If a serious or urgent situation arises, the sponsor and investigator are obliged to immediately take the urgent safety measures necessary to protect the research subjects. Examples of such measures may include discontinuing the clinical trial, temporarily or introducing additional monitoring measures. The sponsor shall notify the Swedish Medical Products Agency and the Ethical Review Authority as soon as possible of the urgent safety measures taken by the investigator or sponsor.

Any pregnancies among research subjects will be monitored until delivery. If the fetus/child has any form of congenital malformation, this will be reported as a serious adverse event (SAE).

There is limited experience with the use of Comirnaty in pregnant women. However, animal studies have not shown any direct or indirect harmful effects on embryo/fetal development, birth, or postnatal development, according to the summary of product characteristics.

5.9.5. Annual safety report (Development Safety Update Report, DSUR)

During the study, an annual safety report (DSUR) will be sent to the Swedish Medical Products Agency. The report contains a summary of the SAEs and SUSARs that have occurred, a summary assessment of the safety of the research subjects included in the study, and information on whether the benefit-risk assessment has changed since the study was approved.

5.9.6. Reference safety information

The reference safety information is described in the summary of product characteristics (SPC) for Comirnaty, sections 4.3, 4.4, and 4.8.

5.9.7. Discontinuation criteria

Participants may choose to withdraw from the study at any time without giving a reason. Specific

grounds for **discontinuation of study treatment**

- The participant withdraws their consent
- Participants who do not meet the compliance requirements
- If SUSAR, SAE, severe AE, or other medical condition arises, where continued study participation could pose a risk to the participant, in the investigator's judgment, the study drug treatment will be discontinued. The participant will then be offered medical follow-up within the framework of the study.
- Participants who become pregnant during the study
- Severe/systemic allergic reaction to the vaccine.
- The patient has not completed follow-up (lost to follow-up).
- The investigator has the right to discontinue participation in the study if there are reasons that may affect the individual's safety.
- The relevant authority may terminate the study

If an individual withdraws, information collected during the time they were participating in the study will be used to perform the analyses described. A follow-up visit will be offered to individuals who have discontinued their participation.

No Data and Safety Monitoring Committee (DSMC) is planned to be established in this open-label, non-randomized study. This is because Comirnaty has already been studied in approximately 21,000 individuals regarding its safety profile. This is an open-label study, and we do not expect an increased number of AE/SAE/SUSAR in primary populations.

6. STATISTICS

6.1. Determination of the number of subjects

The primary purpose of this study is to study the immune response after the mRNA vaccine Comirnaty in a small study population (Phase IV study) in a descriptive or exploratory manner. The number of research subjects in the patient groups is based on the number of available patients who could be included at the respective clinics and the feasibility at a time when vaccination has already begun in Sweden. Several of these diseases/conditions are rare, with Karolinska University Hospital having the largest cohorts in Sweden, but with a limited number of patients available to participate in clinical trials.

The primary evaluation variable is the effect measured as the proportion of individuals who seroconvert in patients versus controls. For this, odds ratios and

analyzed using the Chi2 test where α=0.05. With a patient group size of N=540, the power is >80% even with a conservatively estimated effect size of 0.15.

6.2. Analysis populations

Population	Description of population
Full analysis set population	All study participants who have received at least one dose of vaccine
Population with two doses of vaccine	All study participants who received two doses of vaccine and had a test result at 2 weeks after the second vaccine dose.
Population with evaluable reactogenicity after dose 1	All study participants who have received at least one dose of vaccine and have at least one measurement of reactogenicity after the first dose.
Population with Evaluable reactogenicity after dose 2	All study participants who have received two doses of vaccine, and had at least one measurement of reactogenicity after the second dose.

The number and percentage of study participants who are enrolled in the study, complete the study, and discontinue the study will be presented. Reasons for discontinuation will also be presented.

6.3. Planned analyses

6.3.1. Descriptive analyses

- The number and percentage (95% CI) of study participants with co-morbidity and other baseline characteristics.
- Previous and ongoing treatments will be summarized.
- Description of abnormal findings on complete or targeted physical examinations, vital parameters, clinical laboratory tests, and pregnancy tests.

6.3.2. Analyses of primary endpoint:

- Proportion (95% CI) receiving a positive SARS-CoV-2 IgG serology test result after two vaccine doses, three weeks after the second vaccine dose in the "population with two doses of vaccine," and having a negative SARS-CoV-2 serology test result at baseline, i.e., seroconverting.
- Chi^2 test is used for comparisons with the control group.

6.3.3. Analyses of secondary endpoints

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- Proportion (95% CI) seroconverting to a positive response to SARS-CoV-2 IgG serology after two doses of vaccine, at 3 months and 6 months after the second vaccine dose, measured in the "two-dose vaccine population" compared with the control group.
- Proportion (95% CI) experiencing adverse events, with AE/SAE/SUSAR. Number and proportion (95% CI) experiencing local reactions and systemic reactions are measured in the "Full analysis set population".
- Proportion (95% CI) receiving a new diagnosis of SARS-CoV-2 infection through a positive PCR test is measured in the "Full analysis set population."

- Comparisons are made with the control group.

6.3.4. Analyses of exploratory endpoints

Descriptive analyses of the¨ Full analysis set population¨ in subgroups:

- Proportion (95% CI) seroconverting to a positive response to SARS-CoV-2 IgG serology after two doses of vaccine, at 9 months, 12 months, 18 months, 24 months, 30 months, and 36 months after the second vaccine dose, measured in the "Two doses of vaccine population" compared with the control group.
- Humoral and cellular immune response at baseline and after vaccinations in blood and saliva
- Prevalence of escape mutations in SARS-CoV-2 sequencing in breakthrough infections.
- Prevalence of development/progression of graft-versus-host disease in allogeneic stem cell transplant recipients.
- Occurrence of biopsy-verified rejection in solid organ transplant recipients.
- Occurrence of viremia (defined as HIV RNA >50 copies/ml) after vaccination in individuals with HIV infection.
- Change in HIV DNA from study start to 3 and 6 months, 9 months, 12 months, 18 months, 24 months, 30 months, and 36 months after the second vaccine dose in people with HIV infection.
- Oral/intestinal microbiome and vaccine response in HIV-infected individuals and controls.

6.4. Statistical methods

Continuous variables will be presented with mean and standard deviation [SD] for normally distributed values, and median and interquartile range (IQR) for non-normally distributed values. Categorical variables will be presented as frequency distributions.

Comparisons between patient groups and the control group will be made using the Chi-square test. P-values will be adjusted for multiple comparisons where necessary.

7 QUALITY CONTROL AND QUALITY ASSURANCE

7.1 Quality assurance and sponsor monitoring

It is the sponsor's responsibility to oversee the quality of the study. This is done, among other things, by performing a risk analysis of the study as a whole.

The sponsor is responsible for the study's monitoring plan, which should be based on the identified risks. The sponsor is also responsible for following up on the risks during the course of the study.

It is the sponsor's responsibility to ensure that staff are trained in the study.

7.2 Monitoring

To ensure that the study is conducted according to the protocol, that data is collected, documented, and reported in accordance with ICH-GCP (Good Clinical Practice) and applicable ethical and regulatory requirements, the study will be monitored before, during, and after the study. Monitoring is performed according to the study's monitoring plan and aims to ensure that the rights, safety, and well- being of the research subjects are safeguarded and that the data in the CRF is complete, accurate, and consistent with the source data.

Monitoring for this study will be carried out with the help of KTA (Karolinska Trial Alliance) support. The appointed monitor has documented GCP knowledge. The monitor will continuously check that the study is being conducted in accordance with the approved study protocol and complies with ICH-GCP. The monitor will also check that data is collected in such a way that it can be verified with source data. The respective operations manager will grant the monitor access to the CRF, patient records, and other source data.

Deviations from protocols or regulations will be documented and handled in accordance with the monitoring plan.

7.3 Source data

Records will be kept in accordance with current practice and the Patient Data Act (SFS 2008:355). Patient participation in the study will be noted in the medical records. Patient data will be collected according to the protocol and recorded continuously in study-specific patient forms (CRF) in electronic format. The collected data originates from source documents at the clinic, such as lab reports.

7.4 Deviations or serious violations

Violations and deviations from trial protocols, GCP, and other regulations that significantly and directly affect, or are highly likely to affect, research subjects in Sweden or the scientific value of the trial must be reported immediately within 7 days (from knowledge) to LV. It is the sponsor's responsibility to assess the consequences of any deviations that have occurred and, accordingly, to decide whether LV should be informed.

Minor deviations that do not affect the integrity or safety of research subjects, or significantly affect the scientific value of the trial, shall be documented in the trial documentation by the principal investigator and the sponsor.

7.5 Inspection

Authorized representatives of the sponsor and competent authorities may conduct inspections at the trial site, including source data verification. The investigator shall ensure that all source documents are available for inspection. The purpose of an inspection is to systematically and independently review all study-related activities and documents to determine whether these activities were performed,
recorded, analyzed, and reported correctly in accordance with the protocol, Good Clinical Practice (GCP), and applicable regulations.

8 ETHICS

8.1 Compliance with protocols, GCP, and regulations

The study will be conducted in accordance with the protocol, the Declaration of Helsinki, ICH-GCP (Good Clinical Practice), and the applicable national and international regulations relating to this clinical study. This is to ensure the safety and integrity of the research subjects as well as the quality of the data collected.

8.2 Ethical review of the study

The final study protocol for drug trials must, as part of the application for a clinical drug trial permit, be approved by both the Ethical Review Authority and the Swedish Medical Products Agency before the trial can be conducted. The final version of the informed consent form and other information provided to the research subjects must be approved or given a written positive opinion by the Ethical Review Authority. The Ethical Review Authority and the Swedish Medical Products Agency must be informed of any changes to the protocol in accordance with applicable requirements.

8.3 Procedure for obtaining informed consent

The principal investigator at each trial site shall ensure that the research subject is given full and adequate verbal and written information about the study, its purpose, any risks and benefits, and inclusion/exclusion criteria. Research subjects must also be informed that they are free to withdraw from the study at any time without having to give a reason. The research subject shall be given the opportunity to ask questions and be allowed time to consider the information provided. If the person chooses to participate, the research subject and the investigator shall sign the consent form. A copy of the research subject information and the signed consent form shall be given to the research subject. The research subject's signed and dated informed consent must be obtained before any study-specific activity is performed in the study. Each research subject participating in the study will be identified by a research subject number on a research subject identification list. The research subject consents to monitors and inspectors having access to their medical records and other source data. If new information becomes available during the study, the research subject has the right to reconsider whether he/she wishes to continue participating.

In this study, informed consent may be signed on paper forms or electronically with Bank ID. The study population also includes English-speaking research subjects. These subjects will be informed in English and will sign an English version of the informed consent form. Signed informed consent for participation in the two extension parts of the study will be obtained before sampling begins.

8.4 Data protection

If any part of the data processing is carried out by another organisation, within or outside the EU, appropriate agreements and/or other documentation will be drawn up to ensure that the processing is carried out in accordance with the provisions of the Data Protection Regulation and other relevant legislation before any data transfer takes place.
The content of the informed consent form complies with relevant privacy and data protection legislation. The research subject information and informed consent form will provide research subjects with complete information about how their study data will be collected, used, and disclosed. The research subject information and informed consent form will explain how study data will be stored to maintain confidentiality in accordance with national data legislation.
All data processed by the sponsor will be pseudonymized and identified only by study code.

The informed consent form will also explain that for data verification purposes, authorized representatives of the sponsor, as well as the relevant authority, may require access to parts of hospital or study records relevant to the study, including the research subject's medical history.

8.5 Insurance

Research subjects are insured through patient injury insurance and drug insurance.

8.6 Significant changes

Significant changes to the signed protocol are only possible through approved protocol amendments and by agreement of all responsible persons. Information about non-significant changes must be clearly noted in the amended protocol.

In the event that significant changes to the protocol (e.g., change of primary objective, primary or secondary variables, method of measuring primary variable, change of investigational medicinal product or dosage) are to be made during the course of the study, approval must be obtained from the Ethical Review Authority and the Swedish Medical Products Agency before the changes are implemented. A change involving a new trial site, new investigator, and/or new research subject information shall only be approved by the Ethical Review Authority.

Non-substantial changes must be recorded and included in the documentation when it is subsequently submitted, for example in any subsequent notification of a substantial change or in connection with the reporting of the end of the trial.

8.7 Collection, handling, and archiving of data

Research subjects participating in the study are coded with specific research subject numbers. All research subjects are registered on a subject enrolment and identification list that links the research subject's name and personal identification number with a research subject number.

All data shall be recorded, handled, and stored in a manner that enables accurate reporting, interpretation, and verification. The complete investigator's brochure and source documents will be archived for at least 10 years after the study is completed. Source data in patient record systems will be stored and archived in accordance with the regulations of the respective hospital region.

8.7.1 Case Report Form (Researcher Form)

Data collected in the study will be recorded in a Case Report Form (CRF) where research subjects are identified only by a study-specific code number. An electronic CRF (eCRF) will be used in the study. The investigator shall ensure that the data is recorded and that any corrections to the CRF are made in accordance with the study protocol and the instructions. The investigator shall ensure that the recorded data is accurate, complete, and that reporting is carried out according to the predetermined timelines. The investigator shall sign the completed CRF. A copy of the completed CRF will be archived at the trial site.

The code key that links the study-specific research participant number to the person's name and personal identification number is stored securely and separately from other medical records at the respective study unit. No unauthorized person will have access to the code key. The code key is stored with the investigator. All data presentations are anonymized.

9. STUDY COMPLETION, REPORTING, AND PUBLICATION

The study is concluded when the last trial subject has completed the final follow-up.
Decisions regarding early termination of the study in one or more patient groups, or the entire study, are made by the sponsor in consultation with the coordinating investigator and the study steering committee. The steering committee consists of coordinating investigators, principal investigators at each study unit, and co-investigator Per Ljungman, who is an expert in the field of vaccines. The steering committee will hold regular meetings to monitor the progress of the study, both in parts and as a whole, and will serve in an advisory capacity in the study, for example, regarding ethical issues or complex problems that

arise during the course of the study. Furthermore, the Steering Committee is tasked with providing advice on whether the entire study or parts of it need to be changed or stopped due to safety or efficacy concerns.

Any changes will be made after approval of the amendment application by the Swedish Medical Products Agency and the Ethical Review Authority.

No later than 90 days after the end of the study, an EU-wide document (Declaration of End of Trial Notification) must be sent to the Swedish Medical Products Agency

The study results will be summarized and submitted to the Swedish Medical Products Agency no later than one year after the trial has ended. When the study is completed, the Swedish Ethical Review Authority will be notified. The results will be summarized in a manuscript with the aim of publication in scientific journals, and the EudraCT number of the trial will be stated at the end of the introductory abstract in order to comply with the requirements of the ICMJE (International Committee for Medical Journal Editors) for publication in medical scientific journals. Interim analyses may be published.

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11. APPENDIX

11.1 Appendix 1 - List of parameters for screening visits and during the study period for eCRF

A base module is created for all patients, followed by specific modules for each patient group. The "healthy controls" group has its own base module in eCRF.

Base module: All patients (primary population)

Gender:
M/F Age:
Diagnosis for inclusion: (Primary immunodeficiency/HIV/allogeneic stem cell transplantation or CAR T cell therapy/Solid organ transplantation/CLL)
Immunosuppressive treatment in the last 4 weeks prior to inclusion: yes/no Ongoing medication at inclusion:

- Non-immunosuppressive drugs: yes/no, if yes, which ones
- Ongoing immunosuppressive treatment, steroid: yes/no, if yes, for how long
- Ongoing immunosuppressive treatment, non-steroid: yes/no, if yes, which

Patient group 1. Patients with primary immunodeficiency

Primary diagnosis:
Confirmed genetic mutation: yes/no, if yes, which gene?
Other comorbidities relevant to vaccine response: yes/no (if yes, describe)
Malignancy: yes/no, if yes, describe which?
Lung disease: yes/no, if yes, describe which?
Autoimmune disease: yes/no, if yes, describe which? Immunoglobulin treatment: yes/no
Rituximab Yes/No. If yes: When was the last dose?

Patient group 2. Patients with HIV infection

Ethnicity: Caucasian, Black, Oriental, Latin, Other.
HIV diagnosis date:
Latest CD4: date, number, percentage Nadir CD4: date, number, percentage, Latest HIV RNA:
Opportunistic infection: yes/no, if yes, which infection and date Number of years of antiviral treatment:
Antiviral treatment: (which)
BMI:
Diet – normal diet/vegetarian/other: [if other, describe]
Antibiotic treatment during the last 3 months: yes/no, if yes, describe which antibiotic and duration.

Patient group 3. Patients who have undergone allogeneic stem cell transplantation/CAR T cell therapy

Primary diagnosis:
Stage of disease at transplantation Type of donor
Stem cell source Conditioning regimens GVHD prophylaxis
Acute GVHD Yes/No and severity Chronic GVHD Yes/No and severity

Ongoing immunosuppression (non-steroidal) Yes/No and type
Ongoing corticosteroid therapy Yes/No, severity, and type of medication Any significant opportunistic infection prior to vaccination (CMV, EBV, adenovirus, invasive fungal disease)
Previous rituximab Yes/No. If yes:
When? Obliterative bronchiolitis Yes/No

Patient group 4. Patients who have undergone solid organ transplantation/CAR T cell therapy

Primary diagnosis: Blood group

Number of months after transplantation (most recent):

Induction treatment (No/yes - thymoglobulin/yes - dacluzimab)

Basic immunosuppression: (Cyclosporine A/Tacrolimus/Everolimus/Sirolimus)

Antimetabolite treatment: (Azathioprine/MMF)

Steroids: (Yes/No)

Rejection treatment <6 months (yes/no)

Rejection treatment <3 months (yes/no)

Thymoglobulin treatment in the past year (yes/no)

Rituximab treatment in the past year (yes/no)

Chemotherapy in the past year (no/yes, and if yes, which type?)

CMV/EBV/BK viremia in the last 3 months (yes/no)

Patient group 5. Patients with chronic lymphocytic leukemia

Rai stage (at start of vaccination)

- 0
- I
- II
- III
- IV
- UNK

Cytogenetics FISH (last performed, specify date):

- del(17p)/TP53 mutation (>10%)
- del(11q)
- trisomy 12
- del(13q)
- normal
- Not done

IgHV mutation status:

- Mutated
- Unmutated
- Not done

Previous treatment:
Yes/No Number of treatment lines:
Subgroups:

1. Indolent and untreated: Yes/No

2. Ongoing ibrutinib: Yes/No
   Start date
   Dose at inclusion in vaccine study: 140/280/420 mg/d

3. -FCR within the last 6-30 months: Yes/No
   Start date
   End date
   -BR within the last 6-30 months: Yes/No Start date
   End date
   -Rituximab-containing treatment ongoing or within the last 6 months: Yes/No Date of last dose:

Own base module: Healthy controls

Gender:
M/F Age:
Diagnosis/es:
Medications in the last 4 weeks:
Current medications:
Ethnicity - Caucasian, Black, Oriental, Latino, other
BMI
Diet - normal diet/vegetarian/other
Antibiotic treatment in the last 3 months - yes/no, if yes, describe which antibiotic/duration

11.2 Appendix 2 - Amount of blood samples at each study visit

Visit	Screening/1	2	3	4	5	6	7
Visits	**Screening/d 0**	**10 FU**	**d 21**	**2 weeks FU (vacc. 2)**	**6w FU (vacc 2)**	**3 months FU (vacc. 2)**	**6 months FU (vaccine 2)**
Sampling group A (not HIV infected)	17.5 ml	7 ml	14 ml	14 ml	-	7 ml	7 ml
Sampling group A (HIV infected)	21.5 ml	7 ml	18 ml	18 ml	-	11 ml	11 ml
Sampling group B (not HIV infected)	53.5 ml	43 ml	50	50 ml	-	43 ml	43 ml
Sampling group B (HIV infected)	57.5 ml	43 ml	54 ml	54 ml	-	47 ml	47 ml

Visit	8	9	10	11	12	13	
	9 months FU (vacc 2)	**12 months FU (vacc 2)**	**18 months FU (vacc 2)**	**24 months FU (vacc 2)**	**30 months FU (vacc 2)**	**36 months FU (vacc 2)**	**Total**
Sampling group A (not HIV infected)	11 ml	11 ml	11 ml	11 ml	11 ml	11 ml	132.5 ml
Sampling group A (HIV infected)	21	21 ml	21	21	21	21 ml	212.5 ml
Sampling group B (not HIV infected)	11	47 ml	47	47 ml	11 ml	47 ml	492.5 ml
Sampling group B (HIV infected)	21	57 ml	57 ml	57	21	57 ml	572.5 ml