CLINICAL STUDY PROTOCOL

Title: Prospective study to evaluate the effects of Raloxifene therapy on SARS-CoV-2 immunity after COVID-19 vaccination (RALOXIVAXI)

Study Number:	RLX0121
EudraCT Number:	2021-002476-39
Investigational Product:	No drug will be administered. The trial will enrol subjects who are already receiving Raloxifene, Alendronate, Vit. D + Calcium, or no therapy as a prevention strategy or treatment for osteoporosis
Phase of the study:	IV
Protocol Version - Date:	Version No. 1.1 - Final 07.06.2021

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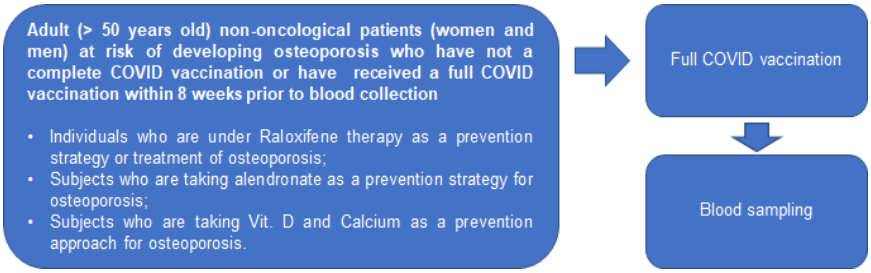
List of Abbreviations and Definitions of Terms

bAb	Binding Antibody
EC	Ethics Committee
e-CRF/CRF	Electronic/Case Report Form
EU	European Union
GCP	Good Clinical Practice
GMFRs	Geometric mean fold rises
GMTs	Geometric mean titers
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
mRNA	messenger ribonucleic acid
nAb	Neutralizing Antibody
Vit.	Vitamin D

1. SYNOPSIS

Study Number	RLX0121
Short Title / Project ID:	RALOXIVAXI
Title of the Study:	Prospective study to evaluate the effects of Raloxifene therapy on SARS-CoV-2 immunity after COVID-19 vaccination
EudraCT N°	2021-002476-39
Study Centers (Country)	This is a monocentric study. Clinical Trials Center: Unità Operativa Complessa Malattie Infettive e Tropicali, Azienda Ospedaliera di Padova, Via Giustiniani 2, 35128 Padova Italy
Development Phase	IV
Background and Rationale:	In December 2019, a novel coronavirus, designated SARS-CoV-2, caused an international outbreak of respiratory illness termed COVID-19. The vaccines so far approved use messenger ribonucleic acid (mRNA) containing instructions to synthesize the Spike protein of the virus, necessary to enter cells. Spike protein is an important antigenic determinant capable of activating the immune system thus initiating an immune response able to counteract SARS-CoV-2 entering in case of viral exposure. According to several studies and clinical observations, sex hormones appear to be involved in risk of infection and development of COVID-19 disease. A recent study on female oncological population indicates a reduced prevalence of SARS-CoV-2 infection in hormonal-driven cancer patients under SERM (Selective estrogen receptor modulators) therapy with respect to other antiestrogenic therapies. Among their functions, estrogens are implicated in regulation of inflammation and innate and adaptive immune response. Moreover, raloxifene is able to modulate the immune response by inhibiting not only viral infection but also modulating a series of spike-induced effects on immune and non-immune cells. Based on these data we put forward the hypothesis that estrogen modulator drugs may ameliorate the immune response to vaccines, thus protecting subjects from onset of severe disease. It is of high general interest to verify from a clinical standpoint this hypothesis by setting up a prospective study to evaluate the onset of immune response to vaccines, and the incidence of SARS-CoV-2 infection in subjects who are treated with SERMs, or other drugs commonly used to treat or prevent osteoporosis.
Objective	The overall aim of the study is to assess the impact of Raloxifene treatment SARS-CoV-2 immunity after vaccination. The study will be conducted in non-oncological female patients older than 50 years. Participants to the study will be classified into 4 cohorts according to their ongoing osteoporosis treatment and preventive treatment (no randomization is planned): GROUP 0: Subjects who are not receiving any osteoporosis preventive treatment or osteoporosis treatment. GROUP 1: Patients receiving Vitamin D and Calcium as supplements as a prevention strategy or treatment of osteoporosis.

	GROUP 2: Patients under treatment with alendronate. GROUP 3: Patients who receive Raloxifene as a prevention strategy or treatment for osteoporosis. **Study objectives** The **primary objective** is to verify if Raloxifene treatment is able to stimulate the immunization against Sars-Covid-2 compared(descriptively) to other osteoporosis treatments/prevention strategies vaccination. The **secondary objective** is to evaluate if Raloxifene is able to boost COVID-19 vaccine's efficacy by descriptively comparing the number of Sars-CoV-2 cases in the different groups after
Study Design and Methodology	This trial is a prospective, proof of concept, study. Raloxifene is a medication used to prevent and treat osteoporosis in postmenopausal women . 
Number of Participants:	Actual sample size within each group will depend on the prevalence of the treatments for osteoporosis in the population that is going to receive COVID-19 vaccine. For this reason and given the objective of the study, sample size calculation within each group is not based on hypothesis testing but on the precision of the estimation of primary endpoint.
Inclusion / Exclusion criteria:	Participants should fulfil the following criteria. **Inclusion criteria:** ● Females > 50 years old ● Patients who are under Raloxifene therapy as a prevention strategy or treatment of osteoporosis OR ● Subjects who are taking alendronate as a prevention strategy for osteoporosis OR ● Subjects who are taking Vit. D and Calcium as a prevention approach for osteoporosis OR ● Subjects who are not receiving any osteoporosis preventive treatment or treatment ● AND Who have received the first dose of a COVID vaccination, or who have received the full vaccination within 8 weeks prior to the first planned blood collection. All approved COVID vaccines are accepted. **Exclusion criteria:** ● Subjects who are SARS-CoV-2 positive ● Subjects who have received the complete COVID vaccination more than 8 weeks prior to the first planned blood collection ● Immunosuppressive or immunodeficient state, including human immunodeficiency virus (HIV) infection, asplenia, and recurrent severe infections ● Subjects who have received systemic immunosuppressants or

	immune-modifying drugs for >14 days in total within 6 months prior to the first planned blood collection (for corticosteroids ≥20 milligrams (mg)/day of prednisone equivalent) ● Subjects who have received systemic immunoglobulins or blood products within 3 months prior to the first planned blood collection ● Subjects who have received chemotherapy, radiation therapy or have undergone surgical intervention for cancer in the last 5 years
Endpoints:	**<u>Study endpoints</u>** **Primary endpoint:** quantification of SARS-CoV-2 Specific Neutralizing Antibody (nAb) and S protein-specific Binding Antibody (bAb) levels in patients' blood serum and measurement of GMTs (geometric mean titers) and GMFRs (geometric mean fold rises) in SARS-CoV-2 Specific Neutralizing Antibody (nAb) and S protein-specific Binding Antibody (bAb) at the different timepoints[3,4]. **Secondary endpoint:** proportion of participants tested positive for Sars-CoV-2 in the period from complete COVID vaccination to the end of the study (week 16 after complete vaccination) in the different groups[1,2].
Study Product / Intervention/ Control:	No drug will be administered. The trial will enrol subjects who are already receiving Raloxifene, Alendronate, Vit. D + Calcium, or no therapy as a prevention strategy or treatment for osteoporosis. Therefore, no randomization procedure is required. Masking: single (Health worker who collect and analyse the blood samples).

Project Duration, schedule:	The duration of the study per participant will be around 16 weeks (day of enrolment followed by completion of vaccination - if needed - and then monthly assessments until week 16). The study is expected to last approximately 6 months from first enrolment to completion of the last blood sample collection.
Project assessments, procedures:	Participants in the study will be classified into one of the 4 cohorts according to the type of treatment/prevention therapy for osteoporosis. <u>Investigations before trial enrolment</u>: - Physical examination and medical history - Nasopharyngeal swab (NPS) to exclude positivity to Sars-CoV-2. <u>Investigations during the trial:</u> For all four groups, the first blood sampling will be performed on the day of the dose completing the vaccine (for subjects enrolled before the completion of the vaccine) or 8 weeks after the completion of the vaccine (for subjects enrolled after the complete vaccination). In all subjects, 8 ml of the blood sample will be collected in an EDTA tube for analysis of SARS-CoV-2 Specific Neutralizing Antibody (nAb) and S protein-specific Binding Antibody (bAb) and their GMTs and GMFRs. Complete hematology tests will also be done as a routine investigation. A rapid-swab test will be taken at every blood sampling to assess the SARS-CoV-2 negativity in asymptomatic patients. (Symptomatic patients are subjected to current regulations). Patients will be sampled and according to Table 1.
Study Schedule:	Month Year of First-Participant-In (planned): June 2021 Month Year of Last-Participant-Out (planned): November 2021
Statistical Considerations:	Baseline characteristics will be described by means of standard summary statistical measures. Within each cohort the two-sided 95% confidence interval based on t-test distribution will be computed for the SARS-CoV-2 Specific Neutralizing Antibody (nAb) and S protein-specific Binding Antibody (bAb) levels. No formal comparisons between arms are planned, but the following analysis will be performed for descriptive purpose: • The difference of the mean variation of nAb and bAb from baseline between the four groups will be analysed by Student's *t* test or analogue non-parametric methods. ANOVA will be considered for providing estimates of the difference after adjustment for baseline factors. This approach will be also used for all other comparisons requiring analysis of continuous data. • The differences in proportion of participants tested positive for Sars-CoV-2 in the period from complete COVID vaccination to the end of the study will be analysed with Mantel-Haenszel chi-squared test and summarised with the odds ratio with appropriate confidence intervals (95% CI). This approach will be also used for all the other comparisons requiring analysis of categorical data. • The difference between time to event (time to COVID-19 positivity) will be summarised with hazard ratio, median and restricted mean statistics with appropriate 95% CI.

Other methodological considerations:	As this is a proof of concept study, no formal statistical hypothesis testing will be done.
Risk-Benefit statement:	Clinical risk is related to the procedures of taking the blood samples. Project participants will benefit from a monthly screening for SARS-CoV-2 infection and of a complete hematological exam. The inconveniences for the patients are represented by the repeated (max x4) blood samples (for a total of 24 ml of blood).

2. STUDY SCHEDULE

Table 1: Schedule of assessments

Please see Measurements and Procedures

Groups 0, 1 ,2 and 3

Patients enrolled before the completion of the vaccine

	Day of complete vaccination	Week 8	Week 12	Week 16
Physical examination and medical history	X			
Patients' information and consent	X			
Demographic	X			
Patient diary and symptoms assessment	X	X	X	X
Haematology	X	X	X	X
Blood sample	X	X	X	X
Rapid Antigen Test	X	X	X	X

Patient enrolled with a complete vaccination

	Week 8	Week 12	Week 16	
Physical examination and medical history	X			
Patients' information and consent	X			

Demographic	X			
Patient diary and symptoms assessment	X	X	X	
Haematology	X	X	X	
Blood sample	X	X	X	
Rapid Antigen Test	X	X	X	X

3. ETHICAL AND REGULATORY ASPECTS

3.1 Ethical Conduct of Study

The study will be conducted in full compliance with ICH guidelines for good clinical practice (GCP) and in accordance with the ethical principles that have their origins in the Declaration of Helsinki. Before the study will be conducted, the protocol, the proposed patient information and consent form as well as other study-specific documents shall be submitted to the competent Ethics Committee (EC). Any subsequent amendments must be submitted to this institution as well.

The decision of the EC concerning the conduct of the study will be made in writing to the Sponsor-Investigator before commencement of this study. The clinical study can only begin once approval from the required authority has been received. Any additional requirements imposed by the authority shall be implemented.

3.2 Ethics Committee (EC) and Competent Authorities (CA)

The Sponsor and the Investigator ensure that approval from the competent EC is sought for the clinical study.

No substantial changes will be made to the protocol without prior EC approval, except where necessary to eliminate apparent immediate hazards to study participants.

The Sponsor will obtain the necessary approval from the Competent Authorities, as needed, prior to initiation of the study. The study will not be started until written approval from the relevant Competent Authorities (or no objection within the timeframe set by the local regulation, as applicable) has been received by the Sponsor.

3.3 Participant Information and Informed Consent

The investigator will explain to each participant the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits and any discomfort it may entail. Each participant will be informed that the participation in the study is voluntary and that he may withdraw from the study at any time and that withdrawal of consent will not affect his subsequent medical assistance and treatment.

The participant must be informed that his medical records may be examined by authorized individuals other than their treating physician.

All participants in the study will be provided an information sheet and a consent form describing the study and providing sufficient information for the participant to make an informed decision about his participation in the study. The patient should not sign the informed consent on the same day when it is delivered to him to ensure adequate time for reflection and discussion with relatives or other trusted persons.

The patient information sheet and the consent form will be submitted to the EC to be reviewed and approved. The formal consent of a participant, using the approved consent form, must be obtained before the participant undergoes collection of blood samples required for the study.

The participant should read and consider the statement before signing and dating the informed consent form and should be given a copy of the signed document. The consent form must also be signed and dated by the investigator (or his designee) and it will be retained as part of the study records.

3.4 Participant privacy and safety

All information obtained during the conduct of the study will be regarded as confidential. An agreement for disclosure will be obtained in writing by the patient and will be included in the ICF. Patient's data collected during the study will be handled in accordance with applicable data

protection laws and regulations.

On the CRFs, patients will be identified ONLY by the assigned patient number. If patient names are included on copies of documents submitted to the Sponsor, the names will be obliterated or masked, and the assigned patient number added to the document.

The Investigator should keep a separate log (Patient Master List) of patient's codes, names and addresses.

The Project Leader affirms and upholds the principle of the participants' right to dignity, privacy and health and that the project team shall comply with applicable privacy laws.

Anonymity of the participants shall be guaranteed when presenting the data at scientific meetings or publishing them in scientific journals.

Participant confidentiality will be further ensured by utilising identification code numbers to correspond to medical information in the computer files.

For data verification purposes, authorised representatives of the Sponsor, a competent authority, or an ethics committee may require direct access to parts of the medical records relevant to the project, including participants' medical history.

3.5 Early termination of project

The Sponsor may terminate the study prematurely in case of new findings that make the continuation of this clinical study unwise.

3.6 Amendments, Changes

If necessary, the Sponsor may amend the protocol after its first approval by the EC.

As general rule, substantial amendments are only implemented after approval of the EC.

Under emergency circumstances, deviations from the protocol to protect the rights, safety and well-being of human subjects may proceed without prior approval of EC. Such deviations shall be documented and reported to the EC as soon as possible and in any case within 7 days.

4. INTRODUCTION

4.1 Background

In December 2019, a novel coronavirus, designated SARS-CoV-2, caused an international outbreak of respiratory illness termed COVID-19[5-7].
SARS-CoV-2 viruses infect people by using the surface protein Spike to enter cells, where they can reproduce. All vaccines have been developed to induce a response to block the Spike protein, thus preventing cell infection. The vaccines so far approved use messenger ribonucleic acid (mRNA) containing instructions for the cells to synthesize Spike proteins in the vaccinated subject. This protein is considered an important antigenic determinant capable of activating the immune system thus initiating a protective immune response[8]. Once produced, the proteins stimulate the immune system to produce specific antibodies that, in case of exposure to viral infection, block the Spike proteins preventing their entry into the cells.

According to clinical observation and several studies, men have higher rates of COVID-19 related symptoms and mortality when compared to women. These evidences suggest that sex hormones may be involved by affecting SARS-CoV-2 infection and subsequent development of disease[9]. Recent studies demonstrated prostate cancer patients receiving Androgen-deprivation Therapy (ADT) present a lower risk of infection by SARS-CoV-2 and to develop COVID-19 disease, when compared to untreated prostate cancer patients[10]. Moreover, in another study on female oncological population, a reduced prevalence of SARS-CoV-2 infection in hormonal driven patients under SERM (Selective estrogen receptor modulators) therapy but not upon treatment that inhibits estrogen synthesis, was observed[11]. This suggests the potential role of steroid hormones in COVID-19 and underlines the potential use of drugs acting on estrogen receptors as therapeutic strategy.

4.2 Rationale for the research project

Based on these data we put forward the hypothesis that estrogen modulator drugs may ameliorate the immune response to vaccines, thus protecting subjects from onset of severe disease. Estrogens, in addition to their primary functions in the regulation of bone homeostasis and secondary sexual characteristics, play also a key role in the regulation of inflammation and immune response to infection. This action may be particularly relevant in COVID-19 infection[12]. Estrogens are actively involved in the regulation of innate and adaptive immune responses, as well as in the control of the cytokine storm in inflammatory conditions[13,14]. Their activity is mediated by nuclear Estrogen Receptors (ERs) that act as “hormone-activated transcription factors”. The SARS-CoV-2 spike protein interferes with the signalling pathways of estrogen receptors, probably due to the presence in the SARS-CoV-2 spike protein of an aminoacidic (LxxLL) motif, homolog to the motif of the nuclear coactivator 1 (NCOA1).

Interestingly, recent observations on specific cell types prompted Dompé to investigate a new potential effect of raloxifene in inhibiting spike-induced cytotoxicity. Experimental data confirm the ability of raloxifene to modulate the immune response by inhibiting not only viral infection but also modulating a series of spike-induced effects on immune and non-immune cells that impact on the immune response to vaccines, and on vaccine tolerability. On this basis, Dompé believes that it is of high general interest to verify from a clinical standpoint this hypothesis by setting up a prospective study to evaluate the onset of immune response to vaccines, and the incidence of SARS-CoV-2 infection in subjects who are treated with SERMs, or other drugs commonly used to treat or prevent osteoporosis.

4.3 Risk-Benefit Assessment

Clinical risk is related to the procedures of taking the blood samples. There is no immediate benefit to the project participant, but future patients with the same clinical condition could benefit due to a

better understanding of the disease. The inconveniences for the patients are represented by the repeated (max x4) blood samples (for a total of 24 ml of blood).

5 OBJECTIVES AND ENDPOINTS

5.1 Primary and secondary objectives

The overall aim of the study is to assess the impact of Raloxifene treatment SARS-CoV-2 immunity after vaccination.

The study will be conducted in female non-oncological patients older than 50 years.

Participants to the study will be classified into 4 cohorts according to their ongoing osteoporosis treatment and preventive treatment (no randomization is planned):

GROUP 0: Subjects who are not receiving any osteoporosis preventive treatment or treatment.

GROUP 1: Patients receiving Vitamin D and Calcium as supplements as a prevention strategy or treatment of osteoporosis.

GROUP 2: Patients under treatment with alendronate.

GROUP 3: Patients who receive Raloxifene as a prevention strategy or treatment of osteoporosis.

The **primary objective** of the study is to evaluate if Raloxifene is able to boost COVID vaccine's efficacy by comparing the number of Sars-CoV-2 cases in the different groups after vaccination.

The **secondary objective** of the study is to verify if Raloxifene treatment is able to stimulate the immunization against Sars-CoV-2 compared to other osteoporosis treatments/prevention strategies.

5.2 Primary and secondary endpoints

The **primary endpoint** of the study is to quantificate SARS-CoV-2 Specific Neutralizing Antibody (nAb) and S protein-specific Binding Antibody (bAb) levels in patients' blood serum and measurement of GMTs (geometric mean titers) and GMFRs (geometric mean fold rises) in SARS-CoV-2 Specific Neutralizing Antibody (nAb) and S protein-specific Binding Antibody (bAb) at the different timepoints.

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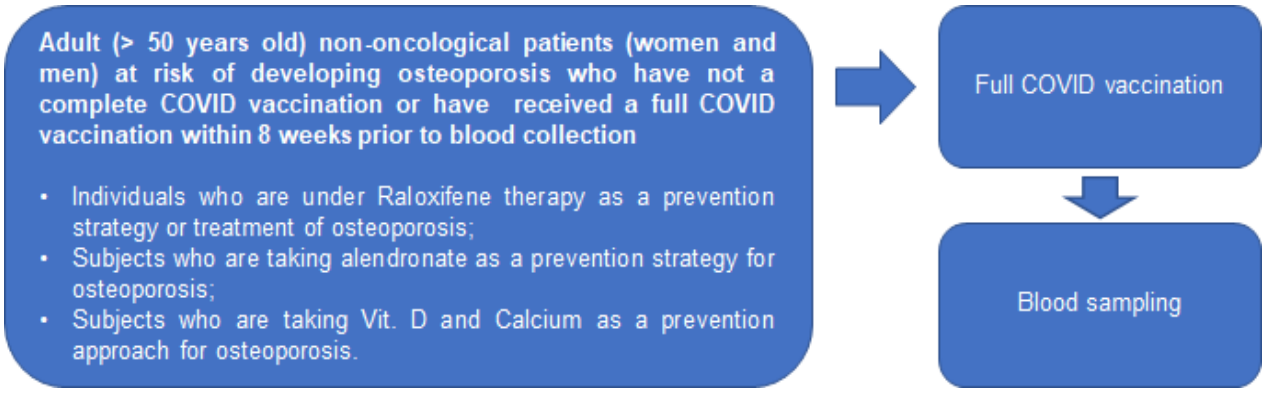
The **secondary endpoint** of the study is to evaluate the proportion of participants tested positive for Sars-CoV-2 in the period from complete COVID vaccination to the end of the study (week 16 after complete vaccination) in the different groups

6 PROJECT DESIGN

6.1 Type of research and general project design

This trial is a prospective, proof of concept study.



6.2 Procedures

Participants in the study will be assigned to the 4 cohorts according to the type of treatment/prevention therapy for osteoporosis.

Investigations before trial enrolment:

- Physical examination and medical history

- Nasopharyngeal swab (NPS) to exclude positivity to Sars-CoV-2.

Investigations during the trial:

For all four groups, the first blood sampling will be performed on the day of the dose completing the vaccine (for subjects enrolled before the completion of the vaccine) or 8 weeks after the completion of the vaccine (for subjects enrolled after the complete vaccination).

In all subjects, 8 ml of the blood sample will be collected in an EDTA tube for analysis of SARS-CoV-2 Specific Neutralizing Antibody (nAb) and S protein-specific Binding Antibody (bAb) and their GMTs and GMFRs. Complete hematology tests will also be done as a routine investigation. A rapid-swab test will be taken at every blood sampling to assess the SARS-CoV-2 negativity in asymptomatic patients. (Symptomatic patients are subjected to current regulations).

Patients will be sampled and according to Table 1.

6.3 Recruitment and Screening

Adult female non-oncological subjects who have received the first dose of a COVID-19 vaccination, or subjects who have received the full vaccination within 8 weeks prior to the first planned blood collection, that are also receiving Raloxifene, Alendronate, Vit. D + Calcium, or no therapy as a prevention strategy or treatment for osteoporosis, will be invited to participate and will receive written and oral information about the trial.

Patients signing the informed consent and fulfilling the inclusion/exclusion criteria will be enrolled in the trial and will undergo the following investigations:

Investigations before trial treatment:

- Physical examination and medical history
- Nasopharyngeal swab (NPS)

Investigations during the trial:

For all four groups, the first blood sampling will be performed on the day of the dose completing the vaccine (for subjects enrolled before the completion of the vaccine) or 8 weeks after the completion of the vaccine (for subjects enrolled after the complete vaccination).

In all subjects, 8 ml of the blood sample will be collected in an EDTA tube for analysis of SARS-CoV-2 Specific Neutralizing Antibody (nAb) and S protein-specific Binding Antibody (bAb) and their GMTs and GMFRs. Complete hematology tests will also be done as a routine investigation.

Patients will be sampled and according to Table 1.

6.4 Methods of minimising bias

All the samples will be processed by the day after the collection to avoid degradation and variability in the analyses.

7 PROJECT POPULATION

Actual sample size within each group will depend on the prevalence of the treatments for osteoporosis in the population that is going to receive its first dose of vaccine.

7.1 Inclusion criteria

- Females > 50 years old
- Patients who are under Raloxifene therapy as a prevention strategy or treatment of osteoporosis OR
- Subjects who are taking alendronate as a prevention strategy for osteoporosis OR
- Subjects who are taking Vit. D and Calcium as a prevention approach for osteoporosis OR
- Subjects who are not receiving any osteoporosis preventive treatment or treatment
  AND
- who have received the first dose of a COVID vaccination, or who have received the full vaccination within 8 weeks prior to the first planned blood collection. All approved COVID vaccines are accepted.

7.2 Exclusion criteria

- Subjects who are SARS-CoV-2 positive
- Subjects who have received the complete COVID vaccination more than 8 weeks prior to the first planned blood collection
- Immunosuppressive or immunodeficient state, including human immunodeficiency virus (HIV) infection, asplenia, and recurrent severe infections
- Subjects who have received systemic immunosuppressants or immune-modifying drugs for >14 days in total within 6 months prior to the first planned blood collection (for corticosteroids ≥20 milligrams (mg)/day of prednisone equivalent)
- Subjects who have received systemic immunoglobulins or blood products within 3 months prior to the first planned blood collection
- Subjects who have received chemotherapy, radiation therapy or have undergone surgical intervention for cancer in the last 5 years

7.3 Criteria for withdrawal / discontinuation of participants

Patients will be taken off study if they withdraw their consent to participate.

8 PROJECT ASSESSMENTS

8.1 Project flow chart

See Table 1.

8.2 Assessments of primary endpoint

The **primary endpoint** of the study is to evaluate the proportion of participants tested positive for Sars-CoV-2 in the period from complete COVID-19 vaccination to the end of the study (week 16 after complete vaccination) in the different groups. A participant is defined as a COVID-19 case if their first case of SARS-CoV-2 RT-PCR-positive symptomatic illness occurs.

9 STATISTICAL METHODOLOGY

9.1 Determination of Sample Size

Actual sample size within each group will depend on the prevalence of the treatments for osteoporosis in the population that is going to receive its first dose of vaccine. For this reason and given the objective of the study, sample size calculation within each group is not based on hypothesis testing but on the precision of the estimation of primary endpoint. Figure 1 represents the precision (expressed as half-width of the 95% confidence interval of the estimation in Standard Deviation units) that can be anticipated given a sample size and, for exemplification, Table 2 reports some values on which the Figure 1 is based.

Figure 1: Precision by sample size within each group

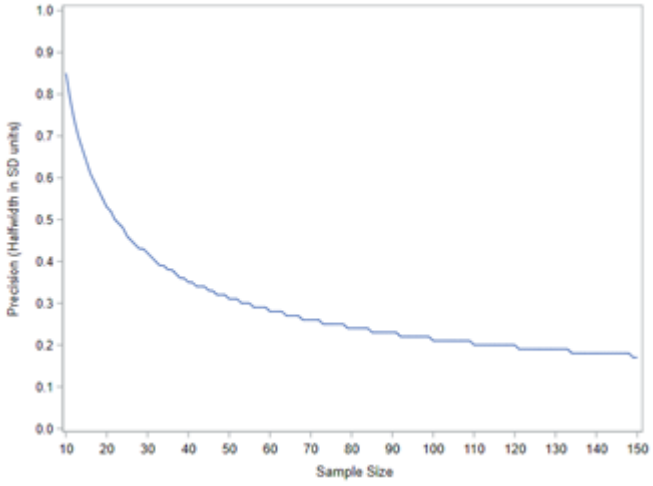


Table 2: Example of precisions by sample size within each group

Sample size within a cohort	Half-Width of 95% C.I. (units of SD)
10	0.85
25	0.46

50	0.31
75	0.25
100	0.21
125	0.19
150	0.17

9.2 Planned analysis

All patient data collected will be listed by patient.

Appropriate descriptive statistics will be produced, according to the variable. For continuous data n, mean, standard deviation, median and range (minimum and maximum) will be presented. For categorical data, frequency distributions and percentages will be presented. If appropriate, confidence intervals around the mean or the proportions will be presented.

Within each cohort the two-sided 95% confidence interval based on t-test distribution will be computed for the SARS-CoV-2 Specific Neutralizing Antibody (nAb) and S protein-specific Binding Antibody (bAb) levels. Similarly, the proportion of participants tested positive for Sars-CoV-2 in the period from complete COVID vaccination to the end of the study (week 16 after complete vaccination) in the different groups will be assessed by means of 95% confidence interval.

No formal comparisons between arms are planned, but the following analysis will be performed for descriptive purpose:

- The difference of the mean variation of nAb and bAb from baseline between the four groups will be analysed by Student's t test or analogue non-parametric methods. ANOVA will be considered for providing estimates of the difference after adjustment for baseline factors. This approach will be also used for all other comparisons requiring analysis of continuous data.
- The differences in proportion of participants tested positive for Sars-CoV-2 in the period from complete COVID vaccination to the end of the study will be analysed with Mantel-Haenszel chi-squared test and summarised with the odds ratio with appropriate confidence intervals (95% CI). This approach will be also used for all the other comparisons requiring analysis of categorical data.
- The difference between time to event (time to COVID-19 positivity) will be summarised with hazard ratio, median and restricted mean statistics with appropriate 95% CI.

10 DATA AND QUALITY MANAGEMENT

The study will be conducted according to the ICH-GCP and national regulation. Quality assurance and control will follow the Sponsor's Standard Operating Procedures (SOPs).

The Principal Investigator is responsible for proper training of all personnel delegated to perform study-related activities and for supervising the conduct of the study at the site.

10.1 Data handling and record keeping / archiving

All study-related documents (essential documents and source documents) are archived at the study site under the responsibility of the Investigator.

10.1.1 Case Report Form

Electronic Case Report Forms (eCRFs) will be used for data collection. Data can be entered and modified only by the Investigator or other staff delegated in writing by the Investigator. The access to the eCRFs will be restricted to authorized staff who will be identified by a unique username. Each person authorized to view and/or edit eCRF data will use a personal confidential password to enter the system and all actions will automatically be tracked.

The completed case report forms are an integral part of the study records.

Quality control will be set up to ensure completeness and inconsistency reconciliation.

10.2 Confidentiality, Data Protection

Data generation, transmission, storage and analysis of health-related personal data and the storage of biological samples within this project will follow strictly the current European legal requirements for data protection and will be performed according to the General Data Protection Regulation (EU) 2016/679.

Health related personal data captured during this project and biological samples from participants are strictly confidential and disclosure to third parties is prohibited; coding will safeguard participants' confidentiality.

Data protection: project data shall be handled with uttermost discretion and only be accessible to authorised personnel. Include a statement that direct access to source documents will be permitted for purposes of monitoring, audits or inspections and should declare who will have access to the project plan, dataset, statistical code, etc. during and after the research project (publication, dissemination).

10.3 Coding

The Investigator assigns a code to each patient entered in the study before any sample is taken. The code is given using a subsequent numbering. The same code will be used to identify the biological material and health-related personal of each patient.

Only the Investigator and authorized personnel have access to the storage location of the coding key. The code may only be broken if it is necessary to avert an immediate risk to the health of the person concerned or to guarantee the rights of the person (e.g. in revoking the consent) or a legal basis exists for breaking the code.

10.4 Archiving and Destruction

All study data and relevant source documents will be maintained at the Investigator’s site for a minimum of 10 years after study termination or premature termination of the clinical trial.

11 PUBLICATION AND DISSEMINATION POLICY

Information on the study will be entered in the clinical trials database of the European Union Drug Regulating Authorities Clinical Trials (https://eudract.ema.europa.eu/index.html).

11.1 Publication of results

The results obtained are planned to be included in the publication of an original article. All data generated in this study will be the property of Dompé farmaceutici s.p.a. Publication of the results by the PI will be subject to mutual agreement between the PI and Dompé farmaceutici s.p.a.

12 REFERENCES

1. https://www.clinicaltrials.gov/ct2/show/NCT04324606
2. https://clinicaltrials.gov/ct2/show/NCT04516746
3. https://clinicaltrials.gov/ct2/show/study/NCT04470427
4. https://clinicaltrials.gov/ct2/show/NCT04283461
5. Huang, C.*, et al.* Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* **395**, 497-506 (2020).
6. Zhou, P.*, et al.* A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* **579**, 270-273 (2020).
7. Zhu, N.*, et al.* A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med* **382**, 727-733 (2020).
8. Ou, X., *et al.* Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. *Nat Commun* **11,** 1620 (2020).
9. Klein, S.L. & Flanagan, K.L. Sex differences in immune responses. *Nat Rev Immunol* **16**, 626-638 (2016).
10. Montopoli, M.*, et al.* Androgen-deprivation therapies for prostate cancer and risk of infection by SARS-CoV-2: a population-based study (n=4532). *Annals of Oncology* **31**(8):1040-1045 (2020).
11. Montopoli, M.*, et al.* Clinical outcome of SARS-CoV-2 infection in breast and ovarian cancer patients who underwent antiestrogenic therapy. *Annals of Oncology* **32**(5):676-677 (2021).
12. Stilhano RS, *et al*. SARS-CoV-2 and the possible connection to ERs, ACE2, and RAGE: Focus on susceptibility factors. FASEB J. **34**(11):14103-14119 (2020).
13. Bilezikian JP, *et al.* MECHANISMS IN ENDOCRINOLOGY: Vitamin D and COVID-19. Eur J Endocrinol. **183**(5):R133-R147 (2020).
14. Khan D, Ansar Ahmed S. The Immune System Is a Natural Target for Estrogen Action: Opposing Effects of Estrogen in Two Prototypical Autoimmune Diseases. Front Immunol. **6**:635 (2016)

13 APPENDICES

13.1 APPENDIX 1-SPONSOR APPROVAL PAGE

Prospective study to evaluate the effects of Raloxifene therapy on SARS-CoV-2 immunity after COVID-19 vaccination (Raloxivaxi)

Sponsor Medical Expert: _____ Date: _____/____/____

Printed Name, Title

13.2 APPENDIX 2-INVESTIGATOR'S SIGNATURE PAGE

Investigator's Statement

I have read study protocol RLX0121 (*Prospective study to evaluate the effects of Raloxifene therapy on SARS-CoV-2 immunity after COVID-19 vaccination*) and agree to conduct the study as outlined in the protocol, and in accordance with the Declaration of Helsinki, ICH-GCP and any local regulations, being responsible for personally supervise the study conduct and ensure study staff complies with protocol requirement.

Name of Principal Investigator (block letters):_____

Signature: _____ Date: _____