IIV Study: Monitoring & Evaluation of Immune Responses induced by SARS-CoV-2 vaccination in the general population in NL

Primary and secondary objectives *in short*:

Primary objective: Measuring the systemic antibody response induced by SARS-CoV-2 vaccination in the general population.

Secondary objectives:

- Monitoring of kinetics and longevity of antibody subclasses induced by SARS-CoV-2 vaccination
- Measuring vaccine-induced antibody capacity for (pseudo)neutralization of circulating SARS-CoV-2 subvariants, either dominant genotype or future mutations
- Assessment of the magnitude, characteristics and longevity of the cellular response induced by various SARS-CoV-2 vaccines
- Measuring the (IgA) antibody response in the oropharyngeal cavity, induced by, and if possible compared between, various vaccines
- Measuring the vaccine response in previously infected (SARS-CoV-2 or seasonal corona) individuals
- Comparison of systemic immune responses induced by various SARS-CoV-2 vaccines, incl. from novel platforms

Study design:

This is a longitudinal study where antibody responses will be measured at different time points throughout a 12-month period after vaccination:

Participants will be asked to donate blood samples by venapuncture and saliva samples for cellular and (functional) antibody analyses, during home visits by a research nurse (or if mentioned only blood is collected by fingerprick), at the following timepoints:

- T(0/ α) = pre-vaccination / baseline (vaccination is day '0'). First vaccination.
- T(1/ β) = 'innate' sample = day 2 ± 1 day after 1st Dose
- T(2) = 28 days after 1st dose. *Only fingerprick*. Second vaccination.
- Post completion of both vaccinations:
    - T(3/ γ) = 28 days.
    - T(4) = 6 months. *Only fingerprick*.
    - T(5/ δ) = 12 months

Also faeces samples will be collected at baseline.

Participants will be asked to donate a finger prick blood sample by self-sampling and fill in a questionnaire at baseline and at different time points after vaccination with a SARS-CoV-2 vaccine.

Table: Study objectives and parameters

	Objectives	Endpoints
Overall	Monitoring & Evaluation of immune responses induced by SARS-CoV-2 vaccination in the general population in NL	x
Primary	Measuring the systemic antibody response induced by SARS-CoV-2 vaccination in the general population.	Primary parameter of the study is vaccine (eg. Spike protein)-specific IgG GMC at day 28 after completion of SARS-CoV-2 vaccination, using the bead-based multiplex immune assay (MIA).
Secondary	Monitoring of kinetics and longevity of antibody subclasses induced by SARS-CoV-2 vaccination	IgG/IgA/IgM by MIA at baseline (T0) and at day 28 (T2), at 6 months (T3) and at 12 months (T4) after vaccination that can be represented as: - Seroconversion (generally defined as an increase >=4x compared to baseline) - Geometric mean concentrations (GMCs) (or antibody geometric mean titers (GMTs)) - Geometric mean fold-rise (GMFRs) vs. baseline titers); - Ratio seropositive/seronegative (positive being >LOD (limit of detection) or LOQ (limit of quatitation)) - Reverse Cumulative Distribution Curves
	Measuring immunogenicity after the 1st dose of vaccination	IgG GMC at day 28 following 1st Dose (T1) by MIA *Non inferiority analysis, compared to what, 1D?*
	Measuring the vaccine response in previously infected (SARS-CoV-2 or seasonal corona) individuals	IgG at baseline (T0) specific for SARS-CoV-2 and/or seasonal corona viruses; and stratified by age: related to vaccine-specific IgG GMC at day 21-28 following 1st Dose (T1) and day 28 following 2nd Dose (T2)
	Measuring vaccine-induced antibody capacity for (pseudo)neutralization of circulating SARS-CoV-2 subvariants, either dominant genotype or future mutations	-Pseudoneutralization in MIA at day 28 for all participants; - Neutralization of various genotypes in golden standard assay (IDS) at day 10 following vaccination (Tγ) in a subset of participants; *Neutralization assay will also validate pseudoneutralization by MIA in subset* (incl biobanking by IDS of 1 mL serum for future analyses / mutants)
	Assessment of the magnitude, characteristics and longevity of the adaptive cellular response induced by various SARS-CoV-2 vaccines	-Vaccine-specific number, phenotype and function of (effector, memory, regulatory) T-cells by in vitro stimulation of PBMC with viral antigen followed by ELISpot, supernatant analysis for cytokines and Fluorescence-Activated Cell Sorting (FACS; Tα, Tγ,

		Tδ); - Vaccine-specific memory B-cell cell frequency, phenotype and functional capacity, by in vitro stimulation of PBMC with viral antigen followed by ELISpot, supernatant analysis for secreted antibodies and FACS (Tα, Tγ, Tδ)
	Comparison of systemic immune responses induced by various SARS-CoV-2 vaccines, incl. from novel platforms	Vaccine (eg. Spike protein)-specific IgG GMC and (pseudo)neutralizing capacity by MIA at baseline (T0) and at day 28 (T2), at 6 months (T3) and at 12 months (T4) after vaccination, compared between vaccines; Vaccine-specific T- and B-cell responses 10 days after (completion of) vaccination (Tγ), compared between vaccines
	Measuring the (IgA) antibody response in the oropharyngeal cavity, induced by various SARS-CoV-2 vaccines; and compared between various vaccines	IgA1 and IgA2 in saliva at baseline (Tα) and 10 days after (completion of) vaccination (Tγ); and if possible compared between vaccines
Exploratory	Assessment of the (functional) characteristics of the innate response following vaccination and exploration of a mechanistic skewing of the ensuing adaptive response to vaccination in a subset of participants	Epigenetic / metabolic reprogramming of innate cells by gene expression profiling of PBMCs 2 days after vaccination (Tβ) and innate cytokine/chemokine markers in serum and in supernatant from stimulated PBMCs (Tβ) compared to baseline (Tα)
	Assessment of intestinal microbiome shortly prevaccination to explore the possible impact on vaccine responses / vaccine take	baseline (Tα)
	Assessment of functional capacity of antibody responses induced by SARS-CoV-2 vaccination; and comparison between vaccines	Vaccine specific antibody phagocytic score, antibody-mediated cellular cytotoxicity score and antibody-mediated complement deposition score; antibody glycosylation status; at day 28 (T2), and compared between vaccines
	Comparison between vaccines of local reactogenicity post vaccination to explore the possible interrelation between reactogenicity (pain, redness, swelling) and ensuing vaccine response	Local reactogenicity as reported in questionnaire following vaccination, related to eg. early serum cytokines, CRP and innate transcription profiles (Tβ) and T-cell phenotype and IgG GMC at (Tγ) and Tδ)

	Anti vector antibodies? *Immune repertoire sequencing?* *Neutralization vs. ADE?* *T cell programming; B cell programming / IGH-IGL gene sequencing?* *Biobanking?* *Added as objectives (actually about other studies)??:* *-Responses will be compared to responses in risk groups (ZonMW studies – protocols are harmonized via Nynke – this protocol can function as a default response / 'negative control')* *-Responses will be compared to responses induced by natural infection (eg. FFX study)*	