
# CARDS

**Cancer: Rapid Diagnostics and Immune assessment for SARS-CoV-2 (COVID-19)**

## Statistical Analysis Plan

Version 1.0, 09.12.2021

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|---|---|
| **Protocol Title and CCR number:** | CARDS (CCR 5287) |
| **Protocol Version and Date:** | Protocol version v3.0, 10.07.2020 |
| **REC/MHRA Approval Date:** (pertaining to protocol version above) | 20.07.2020 |
| **Chief Investigator (or Delegate):** | Dr Sheela Rao |
| **Sponsor:** | The Royal Marsden NHS Foundation Trust |
| **Document Version:** | 1.0 dated 09.12.2021 |
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## Contents

## List of Abbreviations

| Acronym | Definition |
|---|---|
| AE | Adverse event |
| CI | Confidence Interval |
| COVID-19 | Coronavirus Disease 2019 |
| CRF | Case report form |
| D | Day number |
| eCRF | Electronic case report form |
| GMC | Geometric concentration |
| GMFR | Geometric fold rise |
| GMT | Geometric mean titres |
| HLA-KIR | Human leukocyte antigen, killer-cell immunoglobulin-like receptor |
| IgA | Immunoglobulin A |
| IgG | Immunoglobulin G |
| IgM | Immunoglobulin M |
| IQR | Interquartile range |
| N/A | Not applicable |
| REC | Research Ethics Committee |
| RECIST | Response Evaluation Criteria in Solid Tumours |
| RM | Royal Marsden NHS Foundation Trust |
| RNA | Ribonucleic Acid |
| RT-PCR | Reverse Transcription-Polymerase Chain Reaction |
| SAP | Statistical Analysis Plan |
| SARS | Severe acute respiratory syndrome |
| SD | Standard deviation |
| SUSAR | Suspected unexpected serious adverse reaction |
| TMG | Trial management group |

## Amendment history

| Date | Version | Timing in relation to analysis | Brief description of change | Justification for change |
|---|---|---|---|---|
| 09.12.2021 | 1.0 | First version prior to the final analysis | N/A | N/A |

# 1. Study Summary

CARDS is an observational study which will investigate how host cancer status and immune phenotype links to SARS-CoV-2 infection clinical presentation, seroconversion, and viral clearance. The study will also evaluate rapid antigen/antibody lateral flow tests.

# 2. Study objectives

## 2.1 Arm A objectives

- This is a prospective study which aims to define the clinical characteristics of SARS-CoV-2 infection in cancers patients. The aim is to collect clinical data, throat/nose swabs, saliva and blood longitudinally to investigate viral clearance and seroconversion.

## 2.2 Arm B objectives

- The arm of the study will investigate the prevalence of SARS-CoV-2 antibodies amongst patients with cancer. Clinical data and blood samples will be collected longitudinally to assess the impact of systemic therapy on SARS-CoV-2 antibody titres.

In parallel, the study will also evaluate an antigen (Arm A) and IgG/IgM antibody (Arms A & B): point of care test (lateral flow assay) for the development of rapid detection of SARS-CoV-2.

# 3. Study Methods

## 3.1 Study design

Patients with suspected acute COVID-19 infection will be recruited to Arm A. Throat/nose swabs, saliva and blood will be collected from patients undergoing investigation for suspected SARS-CoV-2 infection at the Royal Marsden NHS Foundation Trust on Day 0 (D0). Patients who are positive for SARS-CoV-2 by throat/nose RT-PCR or have a high clinical suspicion for acute COVID-19 will have further samples collected on D7 (if an inpatient), D14, D28, D42 and D56.

Asymptomatic patients with no clinical suspicion of COVID-19 will be recruited to Arm B. Blood will be collected from patients who do not have symptoms of COVID-19. Repeat blood will be collected on D28, D56 and D84.

Research tests including serology and antigen/antibody lateral flow tests will be performed at St George's University NHS Trust. Exploratory analyses including, but not limited to HLA-KIR profiling, blood immune cell activation and transcriptome and lateral flow testing will be carried out. Viral and immunological data will be collated in a link-anonymised manner and correlated to clinical outcome data. Samples will also be used to develop more rapid SARS-CoV-2 testing and assist in the future planning of ongoing or delayed treatment in cancer patients.

## 3.2 Sample Size

In Arm A, up to 60 patients with confirmed diagnosis of SARS-CoV-2 by RT-PCR will be recruited over the study period. In Arm B, up to 150 patients with no clinical suspicion of COVID-19 will be recruited.

# 4. Statistical Principles

## 4.1 Confidence Intervals and P values

No formal statistical hypothesis testing will be undertaken. All confidence intervals presented will be 95% and two-sided.

## 4.2 Adherence and protocol deviations

Protocol deviations will be reported as recorded on the relevant CRF.

## 4.3 Analysis populations

The analysis of the endpoints will involve the following populations:

- ***Patients with a completed blood test (Arm B)***

This population includes all patients who completed at least one blood test during the study.

- ***Unvaccinated patients (Arm B)***

This population includes all patients who did not receive a vaccine dose prior or during their last completed blood test in the study.

- ***Vaccinated patients (Arm B)***

This population includes all patients who received at least one vaccine dose prior to their D0 blood test.

- ***Vaccinated patients with a second dose (Arm B)***

This population includes all patients who received their second vaccine dose after their D0 blood test and prior to their last blood test in the study.

# 5. Study Population

## 5.1 Recruitment

A CONSORT flow diagram will be used to summarise the number of patients who were:

- registered
- lost to follow-up*
- withdrew*
- included in the final primary analysis
- excluded from the final primary analysis*

*reasons will be provided

## 5.2 Baseline patient characteristics

Patients will be described with respect to age, gender, cancer stage at diagnosis, anatomical site of malignancy, etc. Categorical data will be summarised by numbers and percentages. Continuous data will be summarised by mean, SD and range if data are normal and median, IQR and range if data are skewed. Minimum and maximum values will also be presented for continuous data.

# 6. Definitions of endpoints for analysis

Given that only one patient was recruited into Arm A, no analysis will be conducted on Arm A patients and all subsequent sections will refer to Arm B patients.

Pseudovirus neutralisation results are provided as continuous values, and currently there is limited research suggesting where the cut-off should lie in order to dichotomise the measurements into positive\negative neutralisation. Based on preliminary exploratory analysis run by the lab team at St. George's Hospital, we will dichotomise the pseudovirus neutralisation results using two different cuts-offs: 0.5 and 0.8. Any endpoint involving pseudovirus neutralisation will be reported using each different cut-off.

Descriptive analysis on pseudovirus neutralisation results (summary statistics and boxplot by timepoint) will be included in the report.

## 6.1 Primary endpoint

Proportion of patients at each sample timepoint (D0, D28, D56, D84) with a positive detection of IgG specific antibodies to SARS-CoV-2 (nucleocapsid and spike-protein). As part of a complete-case sensitivity analysis, proportions will be reported to include only patients who had a blood test at all timepoints.

## 6.2 Secondary endpoints

### 6.2.1 Unvaccinated patients

- Proportion of participants at each timepoint with a positive detection of IgG specific antibodies to SARS-CoV-2 (nucleocapsid and spike-protein).
- Geometric fold rise (GMFR) in geometric concentration (GMC) of spike-protein antibodies from the first timepoint with a positive antibody detection to each following timepoint, in antibody positive patients. **If data required to analyse this endpoint is not available at the time of primary analysis, the analysis for this endpoint will be postponed until the data is provided.**

### 6.2.2 Vaccinated patients

- Proportion of participants at each timepoint with a positive detection of (i) IgG specific antibodies to SARS-CoV-2 (spike-protein) and (ii) pseudovirus neutralisation. As part of a sensitivity analysis, proportions will be reported at each timepoint to include only patients who had not received a positive nucleocapsid detection up to and including that timepoint.

- Proportion of participants with a positive detection of (i) IgG specific antibodies to SARS-CoV-2 (spike-protein) and (ii) pseudovirus neutralisation at time periods adjusted to the no. of days from 1st vaccine date. As part of a sensitivity analysis, proportions will be reported at each time period to include only patients who had not received a positive nucleocapsid detection up to and including that time period.

- Geometric fold rise (GMFR) in geometric concentration (GMC) of spike-protein antibodies from the D0 sample timepoint to each post-D0 timepoint. **If data required to analyse this endpoint is not available at the time of primary analysis, the analysis for this endpoint will be postponed until the data is provided.**

## 6.3 Exploratory endpoints

### 6.3.1 Vaccinated/Unvaccinated patients

The following endpoints will be reported separately for (i) vaccinated and (ii) unvaccinated patients, and (iii) across all patients.

- Sensitivity: Proportion of participants with a positive detection of IgG specific antibodies to SARS-CoV-2 (spike protein) out of those who had a positive neutralisation detection from the pseudovirus assay, at each timepoint.

- Specificity: Proportion of participants at each timepoint with a negative detection of IgG specific antibodies to SARS-CoV-2 (spike protein) out of those who had a negative neutralisation detection from the pseudovirus assay, at each timepoint.

### 6.3.2 Vaccinated patients with a second dose

- Geometric fold rise (GMFR) in geometric concentration (GMC) of spike-protein antibodies from baseline (pre-second dose) to each timepoint (post-second dose). **If data required to analyse this endpoint is not available at the time of primary analysis, the analysis for this endpoint will be postponed until the data is provided.**

- GMFR of neutralising geometric mean titres (GMT) of spike-protein antibodies from baseline (pre-second dose) to each timepoint (post-second dose). **If data required to analyse this endpoint is not available at the time of primary analysis, the analysis for this endpoint will be postponed until the data is provided.**

### 6.3.3 Other exploratory endpoints

The following endpoints are also planned to be performed:

- Investigate antigen dynamics over time to compare with quantitative RT-PCR (Arm A)

- To examine HLA-KIR interactions that shape CD8 anti-viral responses (Arms A & B)
- To explore the relationship between host immune genotype (e.g., HLA-KIR) and peripheral blood (Arms A & B) immune cell phenotype with viral clearance (Arms A & B)
- To investigate blood immune cell activation and transcriptome in participants (Arms A & B)

**Due to the rapidly evolving nature of the COVID-19 pandemic, these exploratory endpoints are no longer relevant to this study. As the focus of the study has shifted towards exploring the serological responses following SARS-CoV-2 infection or vaccination (1,2), these exploratory endpoints will no longer be performed.**

# 7. Analysis methods for endpoints

## 7.1 Primary endpoint

At each sample timepoint (D0, D28, D56, D84), the proportion of patients with a positive detection of (i) nucleocapsid antibodies and (ii) spike-protein antibodies will be reported alongside an exact two-sided 95% confidence interval. For the sensitivity analysis, the proportions will be reported in a similar manner except the denominators and numerators used to calculate each proportion will only include patients who had blood test results available at all timepoints (D0, D28, D56, D84).

## 7.2 Secondary endpoints

### 7.2.1 Unvaccinated patients

- At each sample timepoint (D0, D28, D56, D84), the proportion of patients with a positive detection of (i) nucleocapsid antibodies and (ii) spike-protein antibodies will be reported alongside an exact two-sided 95% confidence interval.

- *Geometric fold rise (GMFR) in geometric concentration (GMC) of spike-protein antibodies from the first timepoint with a positive antibody detection to each following timepoint, in antibody positive patients.*), A scatterplot will be produced to plot the GMFR in GMC of antibodies against sample timepoints (D0, D28, D56, D84) in antibody positive patients. For each patient, the first data point to be plotted will be at the first sample timepoint at which they showed a positive antibody detection.

### 7.2.2 Vaccinated patients

- For all patients who received at least one dose prior to D0, at each sample timepoint (D0, D28, D56, D84), the proportion of patients with a positive detection of (i) spike-protein antibodies and (ii) pseudovirus neutralisation will be reported alongside exact two-sided 95% confidence intervals. For the sensitivity analysis, the proportions will be reported in a similar manner except the denominators and numerators used to calculate each proportion will only include patients who had not received a positive nucleocapsid detection up to and including that timepoint.

- At each time period (adjusted to the no. of days from 1st vaccine date), the proportion of patients with a positive detection of (i) spike-protein antibodies and (ii) pseudovirus neutralisation will be reported alongside an exact two-sided 95% confidence interval. For the sensitivity analysis, the proportions will be reported in a similar manner except the denominators and numerators used to calculate each proportion will only include patients who had not received a positive nucleocapsid detection up to and including that timepoint.

- *Geometric fold rise (GMFR) in geometric concentration (GMC) of spike-protein antibodies from the D0 sample timepoint to each post-D0 timepoint.* A scatterplot will be produced to plot the GMFR in GMC of spike-protein antibodies against sample timepoints (D0, D28, D56, D84). For each patient, the first data point to be plotted will be at their D0 sample timepoint.

## 7.3 Exploratory endpoints

### 7.3.1 Vaccinated/Unvaccinated patients

The following endpoints will be reported separately for (i) vaccinated and (ii) unvaccinated patients, and (iii) across all patients:

- Sensitivity: the proportion of participants at each sample timepoint (D0, D28, D56, D84) with a positive detection of spike-protein antibodies out of those who had a positive neutralisation detection from the pseudovirus assay. Proportions will be reported alongside an exact two-sided 95% confidence interval.

- Specificity: the proportion of participants at each sample timepoint (D0, D28, D56, D84) with a negative detection of spike-protein antibodies out of those who had a negative neutralisation detection from the pseudovirus assay. Proportions will be reported alongside an exact two-sided 95% confidence interval.

### 7.3.2 Vaccinated patients with a second dose

- *Geometric fold rise (GMFR) in geometric concentration (GMC) of spike-protein antibodies from baseline (pre-second dose) to each timepoint (post-second dose).* A scatterplot will be produced to plot the GMFR in GMC of spike-protein antibodies against sample timepoints (D0, D28, D56, D84). For each patient, the first data point to be plotted will be at the last sample timepoint prior to their second dose.

- *GMFR of neutralising geometric mean titres (GMT) of spike-protein antibodies from baseline (pre-second dose) to each timepoint (post-second dose).* A scatterplot will be produced to plot the GMFR in GMT of spike-protein antibodies against sample timepoints (D0, D28, D56, D84). For each patient, the first data point to be plotted will be at the last sample timepoint prior to their second dose.

The number and proportion of patients with major and minor protocol deviations, alongside deviation details will be reported.

## 7.4 Timing of analyses

Analyses for all endpoints will be performed and reported once the study has closed, final set of lab data have been received, and a database lock has been applied.

## 8. References

1) Walsh, Edward E et al. "Safety and Immunogenicity of Two RNA-Based Covid-19 Vaccine Candidates." The New England journal of medicine vol. 383,25 (2020): 2439-2450. doi:10.1056/NEJMoa2027906

2) Voysey M, Clemens SAC, Madhi SA, et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK [published correction appears in Lancet. 2021 Jan 9;397(10269):98]. Lancet. 2021;397(10269):99-111. doi:10.1016/S0140-6736(20)32661-1

## 9. Statistical Packages

All analyses will be performed using STATA 17.0 or subsequent versions.

## 10. Data Management & Central Statistical Monitoring Plan

All data prior to analysis should have been cleaned and checked as per SOP ST08 (Procedures for Statistical Analyses and Reporting of Clinical Trials, Final and Interim in RM/ICR Sponsored Trials) and SOP ST09 (Procedures for Central Statistical Monitoring in RM/ICR Sponsored Trials).

## 11. Changes to the protocol

As the COVID-19 pandemic evolved, several factors had an impact on the study, and consequently on some of the endpoints. These had to be taken into account when drafting the SAP, which led to the following changes:

1. Arm A: this arm will be excluded from any analysis since recruitment was halted, with only one Arm A patient registered to the study at the time of study closure.
2. Changes in technology\assay: the statistical report will only look at positive detection of IgG specific antibodies and not IgM antibodies as stated in the protocol.
3. PV neutralisation results will be used in both the sensitivity and specificity analyses, instead of lateral flow test results.
4. Some of the analyses will now take patients' vaccination status into account.
5. As the goal of the study has shifted towards analysing serological responses to previous SARS-CoV-2 infection and vaccination, the exploratory endpoints will no longer be performed.

**CARDS**

**Cancer: Rapid Diagnostics and Immune assessment for SARS-CoV-2 (COVID-19)**

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