Statistical Analysis Plan

Study ID: 218459

Official Title of the Study: A Phase 1, observer-blind, randomized, placebo-controlled study to evaluate reactogenecity, safety and immune response of an HSV-targeted immunotherapy in HSV-2 seronegative Japanese participants aged 18-40 years

NCT Number: NCT05989672

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TITLE PAGE

Protocol Title: A Phase 1, observer-blind, randomized, placebo-controlled study to evaluate reactogenicity, safety and immune response of an HSV-targeted immunotherapy in HSV-2 seronegative Japanese participants aged 18-40 years.

Study Number: 218459

Compound Number: GSK3943104A

Abbreviated Title: TH HSV REC-004

Sponsor Name: GlaxoSmithKline Biologicals SA (GSK)

XBU Statistical Analysis Plan (SAP) Template v2.0 17 January 2022

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Version history

SAP Version	Approval Date	Protocol Version (Date) on which SAP is Based	Change	Rationale
SAP	29 Jan 2024	16 March 2023		Original version

1. INTRODUCTION

The purpose of this Statistical Analysis Plan (SAP) is to describe the planned analyses for TH HSV REC-004 (218459). A separate charter for the activities of the internal Safety Review Committee (iSRC) will be used.

1.1. Objectives, Estimands and Endpoints

Objectives	Endpoints and Estimands
Primary	
To evaluate the reactogenicity and safety of the HSVTI.	• Percentage of participants reporting each solicited administration site event (redness, pain, and swelling) within 7 days (Day 1-Day 7) post-each dose. • Percentage of participants reporting each solicited systemic event (fever, fatigue, headache, myalgia, arthralgia) within 7 days (Day 1-Day 7) post-each dose. • Percentage of participants reporting unsolicited AEs within 28 days (Day 1-Day 28) post-each dose. • Percentage of participants reporting MAEs from Dose 1 (Day 1) up to 28 days post-Dose 2 (Day 57). • Percentage of participants reporting SAEs from Dose 1 (Day 1) up to 28 days post-Dose 2 (Day 57). • Percentage of participants reporting newly diagnosed pIMDs from Dose 1 (Day 1) up to 28 days post-Dose 2 (Day 57). • Percentage of participants reporting exacerbation of pre-existing pIMDs from Dose 1 (Day 1) up to 28 days post-Dose 2 (Day 57). • Percentage of participants reporting any hematological and biochemical laboratory abnormalities at pre-study intervention administration (Day 1), post-Dose 1 (Day 8 and Day 29), and post-Dose 2 (Day 36 and Day 57).
Secondary	
To evaluate the humoral immune response induced by the HSVTI.	• CCI [redacted] antibody GMC and seropositivity rate (assessed by ELISA) at pre-study intervention administration (Day 1), 28 days post-Dose 1 (Day 29), and 28 days post-Dose 2 (Day 57).
To evaluate the cellular immune response induced by the HSVTI.	• Geometric mean of CCI [redacted] CD4+/CD8+ T-cells frequency expressing at least 2 activation markers (IFN-γ, TNF-α, IL-2, IL-13, IL-17, 4-1BB and/or CD40L) and including at least one cytokine (assessed by CFC) at pre-study intervention administration (Day 1), 28 days post-Dose 1 (Day 29) and 28 days post-Dose 2 (Day 57).
To evaluate the safety of the HSVTI up to the end of follow-up period.	• Percentage of participants reporting MAEs from Dose 1 (Day 1) up to study end (Day 209). • Percentage of participants reporting SAEs from Dose 1 (Day 1) up to study end (Day 209).

Objectives	Endpoints and Estimands
	• Percentage of participants reporting newly diagnosed pIMDs from Dose 1 (Day 1) up to study end (Day 209). • Percentage of participants reporting exacerbation of a pre-existing pIMDs from Dose 1 (Day 1) up to study end (Day 209).

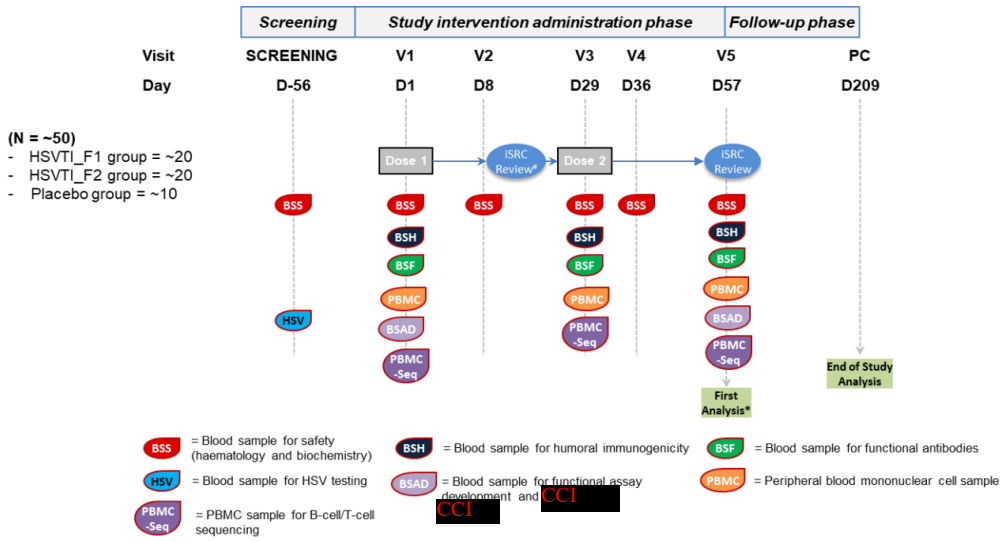
AE: adverse event; **CD4+/CD8+** : cluster of differentiation 4/8; **CD40L**: cluster of differentiation 40 ligand; **CFC:** cell flow cytometry; **ELISA**: enzyme-linked immunosorbent assay; CCI [redacted] **GMC**: geometric mean concentration; **HSVTI**: HSV-targeted immunotherapy; **IFN-γ** : interferon-gamma; **IL (IL-2, IL 13, IL 17)** : interleukin; **MAE**: medically attended event; **pIMD**: potential immune-mediated disease; **SAE**: serious adverse event; **TNF-α**: tumour necrosis factor-alpha.

1.2. Study Design

This is a Phase 1, randomized, placebo-controlled, observer-blind, single-country study with 3 parallel groups (refer to Figure 1):

- HSVTI_F1 group: approximately 20 HSV-2 seronegative participants aged 18 to 40 years will receive 2 doses of HSVTI CCI [redacted].
- HSVTI_F2 group: approximately 20 HSV-2 seronegative participants aged 18 to 40 years will receive 2 doses of HSVTI CCI [redacted].
- Placebo group: approximately 10 HSV-2 seronegative participants aged 18 to 40 years will receive 2 doses of placebo as control.

Figure 1 Study Design Overview



D: day; **HSVTI**: HSV-targeted immunotherapy **iSRC**: internal safety review committee; **N**: number of participants; **PC**: phone call; **V**: visit; **PBMC:** peripheral blood mononuclear cell

The iSRC evaluation will take place approximately once a month until all participants complete the Day 57 visit to review all accumulated safety data. *Ad hoc* iSRC evaluations can be triggered in case any safety concern is observed.

* A first analysis will be performed on all data available and as clean as possible, when data for at least primary and secondary endpoints up to Day 57 are available

2. STATISTICAL HYPOTHESES

No formal statistical hypotheses are to be tested. All analyses will be descriptive.

2.1. Multiplicity Adjustment

No formal statistical hypotheses are to be tested. In general, test statistics will not be adjusted for multiple comparisons.

3. ANALYSIS SETS

Analysis set	Description
Screened set	All participants who were screened for eligibility
Enrolled set	All participants who entered the study (who were randomized or received study intervention or underwent a post-screening procedure)
Exposed set (ES)	All participants who received at least 1 dose of the study intervention.
Per Protocol set for analysis of humoral immunogenicity (PPS_Immuno)	All eligible participants who received all doses as per protocol, have immunogenicity results post-dose, complied with dosing/blood draw intervals, without intercurrent conditions that may interfere with immunogenicity and without prohibited concomitant medication/vaccination. The analysis will be done according to the study intervention that participants received at dose 1.
Per Protocol set for analysis of cellular immunogenicity (PPS_CMI)	All eligible participants who received all doses as per protocol, have CMI results post-dose, complied with dosing/blood draw intervals, without intercurrent conditions that may interfere with immunogenicity and without prohibited concomitant medication/vaccination. The analysis will be done according to the study intervention that participants received at dose 1.

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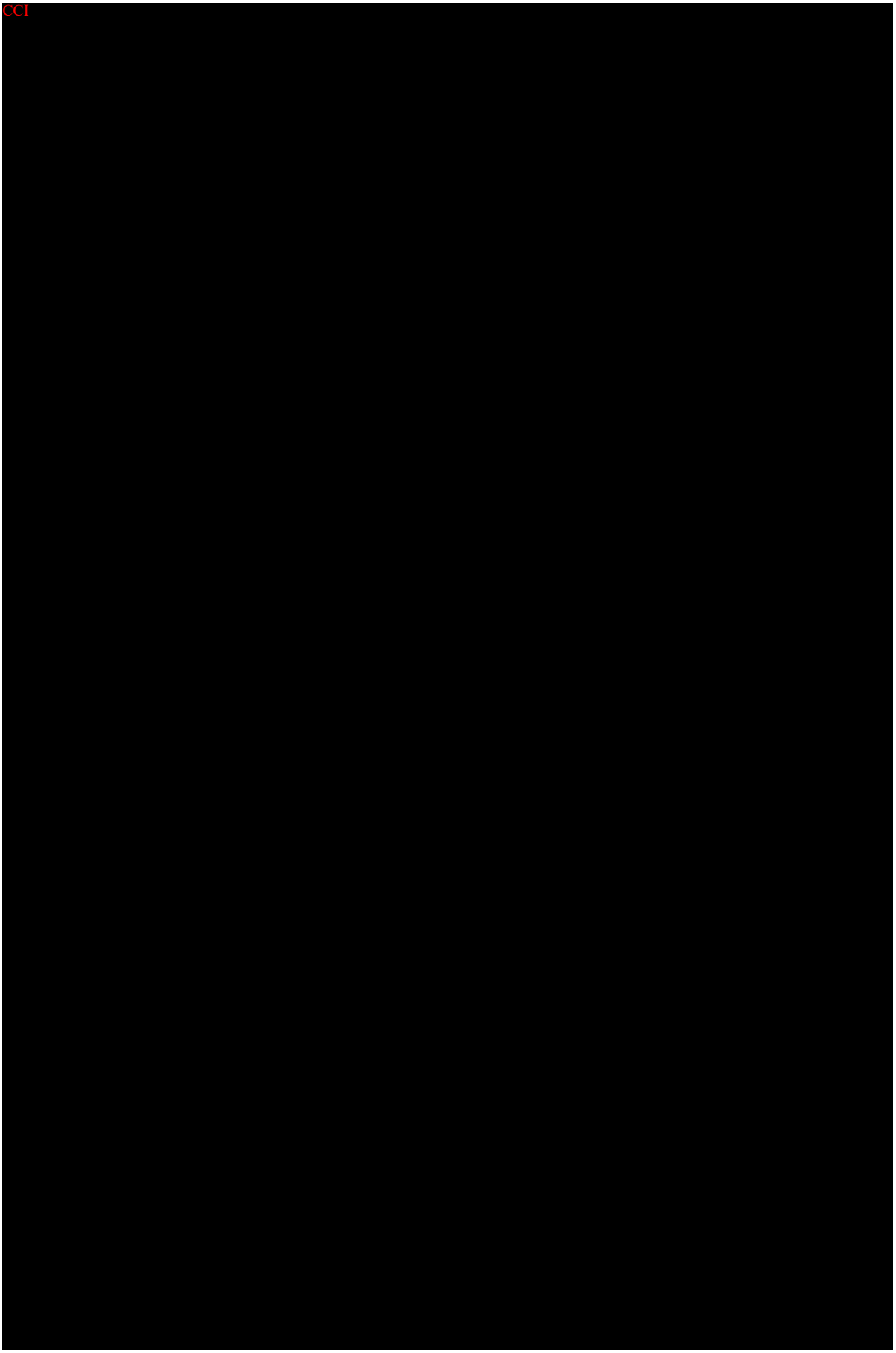

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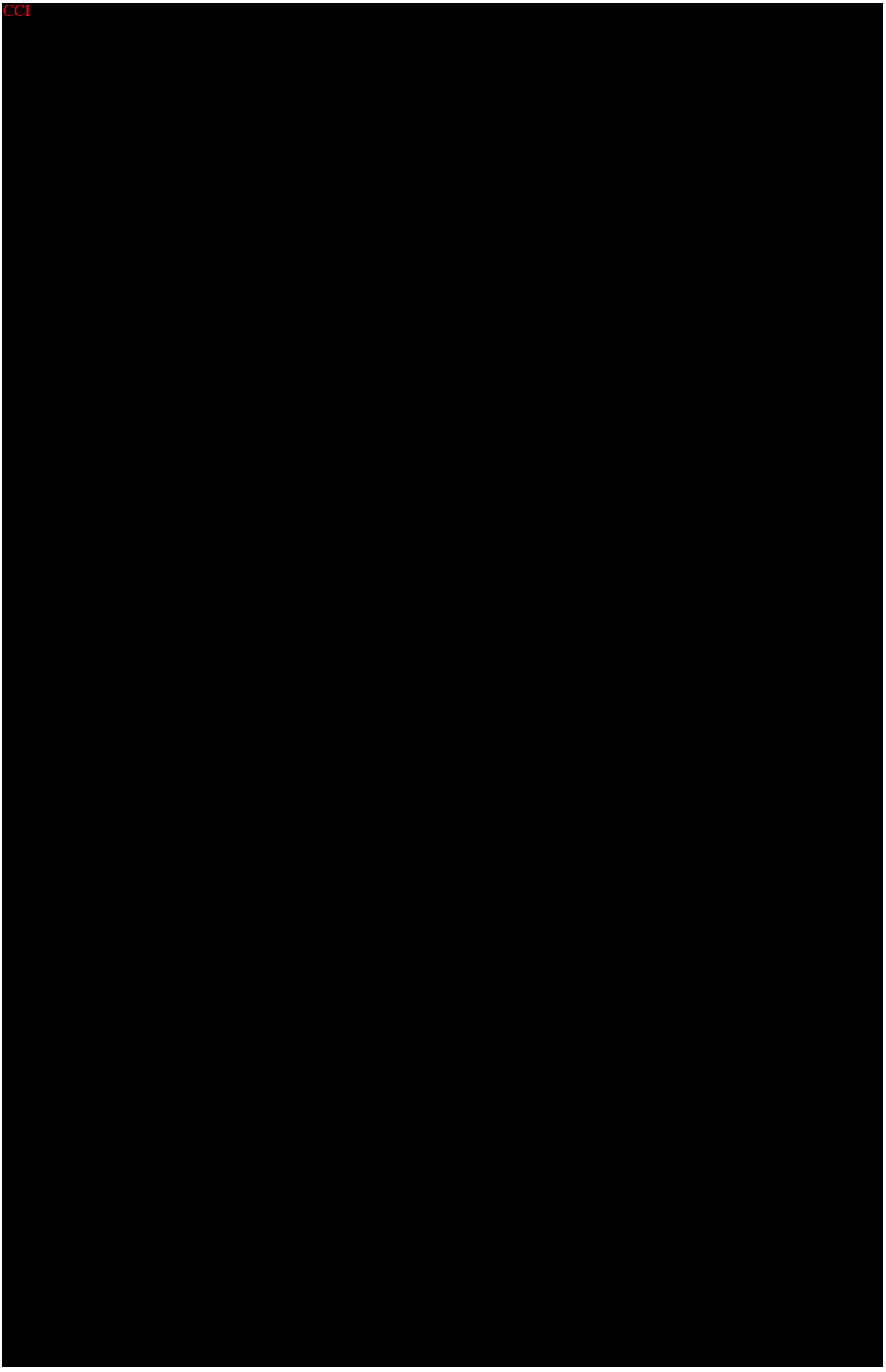

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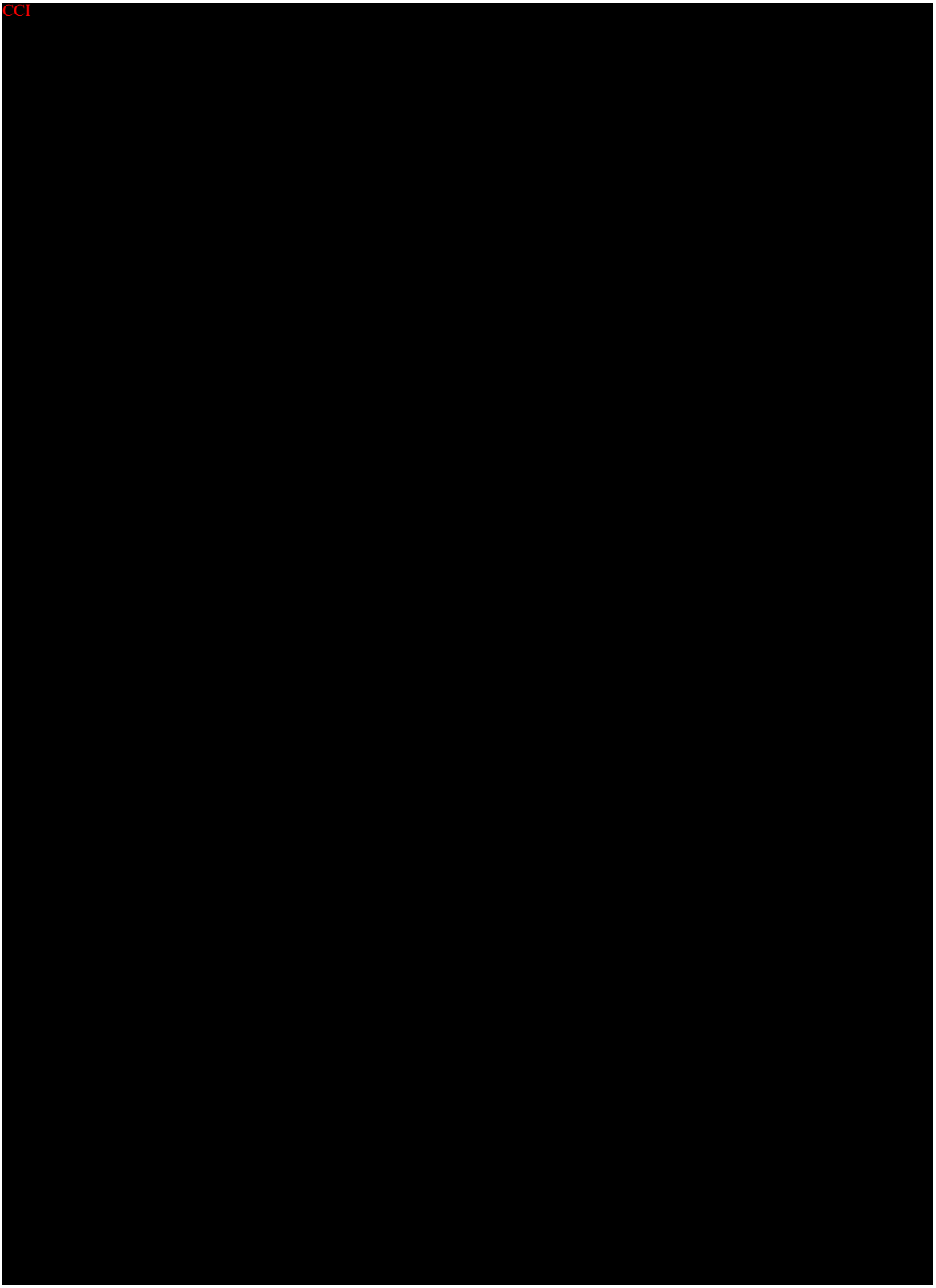

4. STATISTICAL ANALYSES

4.1. General Considerations

4.1.1. General Methodology

Enrolled participants who prematurely withdraw from study will not be replaced.

Re-screened subjects fulfilling the enrollment criteria will enter the study and be analyzed based on their latest screening results.

Confidence intervals (CIs) will use 95% confidence levels, unless otherwise specified.

In general, CIs for proportions will be calculated using the Clopper-Pearson interval method [Clopper, 1934]. All continuous data will be summarized using descriptive statistics (n, mean, standard deviation (SD), median, minimum and maximum) and categorical data will be summarized using the number and percentage of participants in each category.

4.1.2. Sequence of analyses

- A **primary analysis** will be performed on all data available and when data for at least primary and secondary endpoints up to Day 57 are available.

  At this point, the statistician will be unblinded (i.e., will have access to the individual participant treatment assignment). Investigators, participants and other remaining study personnel will stay blinded up to study end (i.e., day 209 and will not have access to the individual participant treatment assignment). It is possible however, due to the limited sample size, that unblinding occurs for a few participants having a specific AE or SAE (e.g., an AE/SAE occurring only in a single participant). Therefore, anyone having access to this analysis could become unblinded regarding those specific cases. This would be acceptable given the early/descriptive nature of the study and the limited number of expected unblinding. Investigators and participants will remain blinded up to study end (Day 209). The investigators will not be provided with the individual data listings or with the randomization listings until the end of study analysis.
- The **end of study analysis** will be performed when all data for primary and secondary endpoints up to study conclusion are available (Day 209). Individual listings will only be provided at this stage.
- If data for tertiary endpoints become available at a later stage, (an) additional analysis/analyses will be performed.

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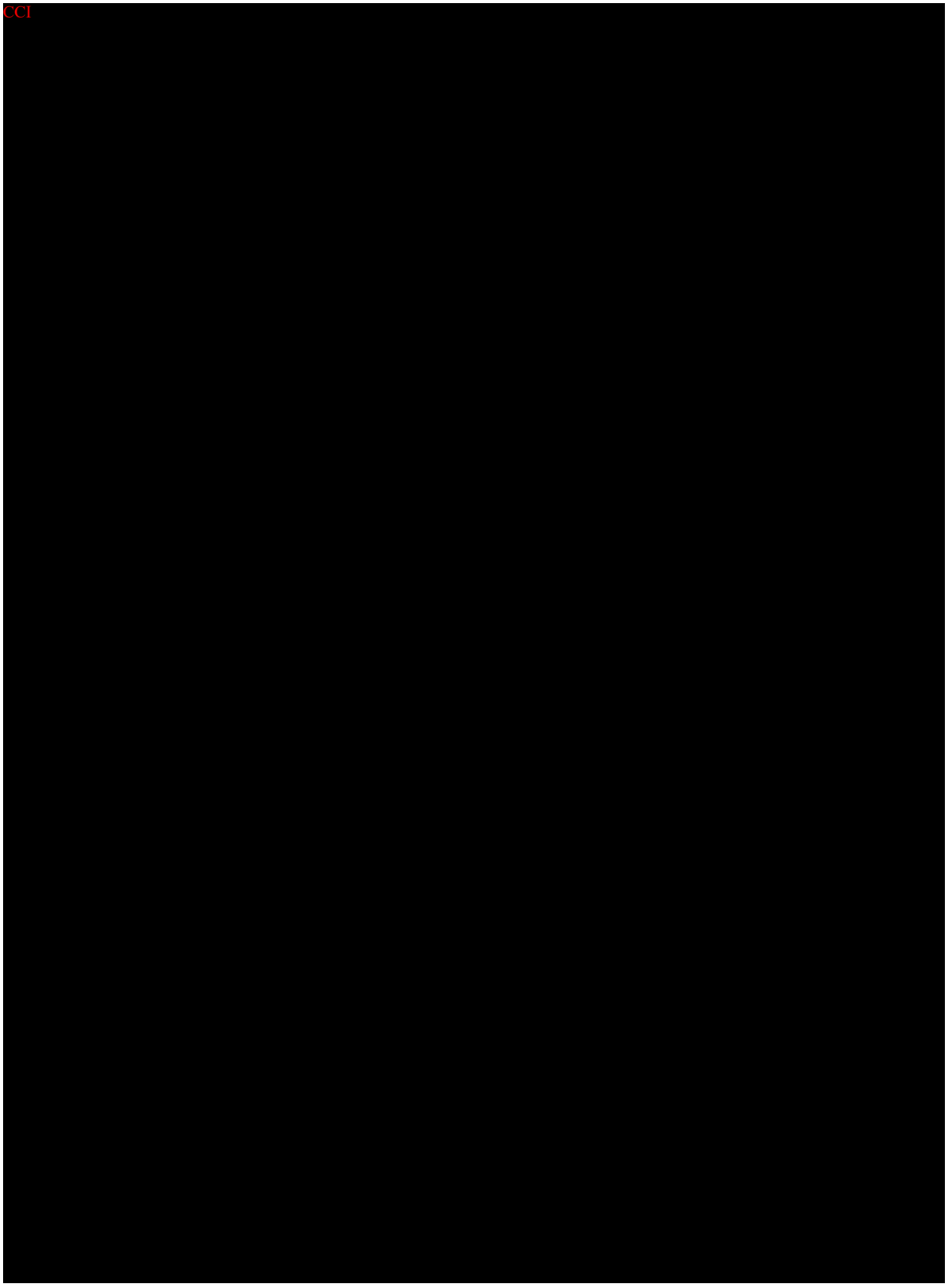

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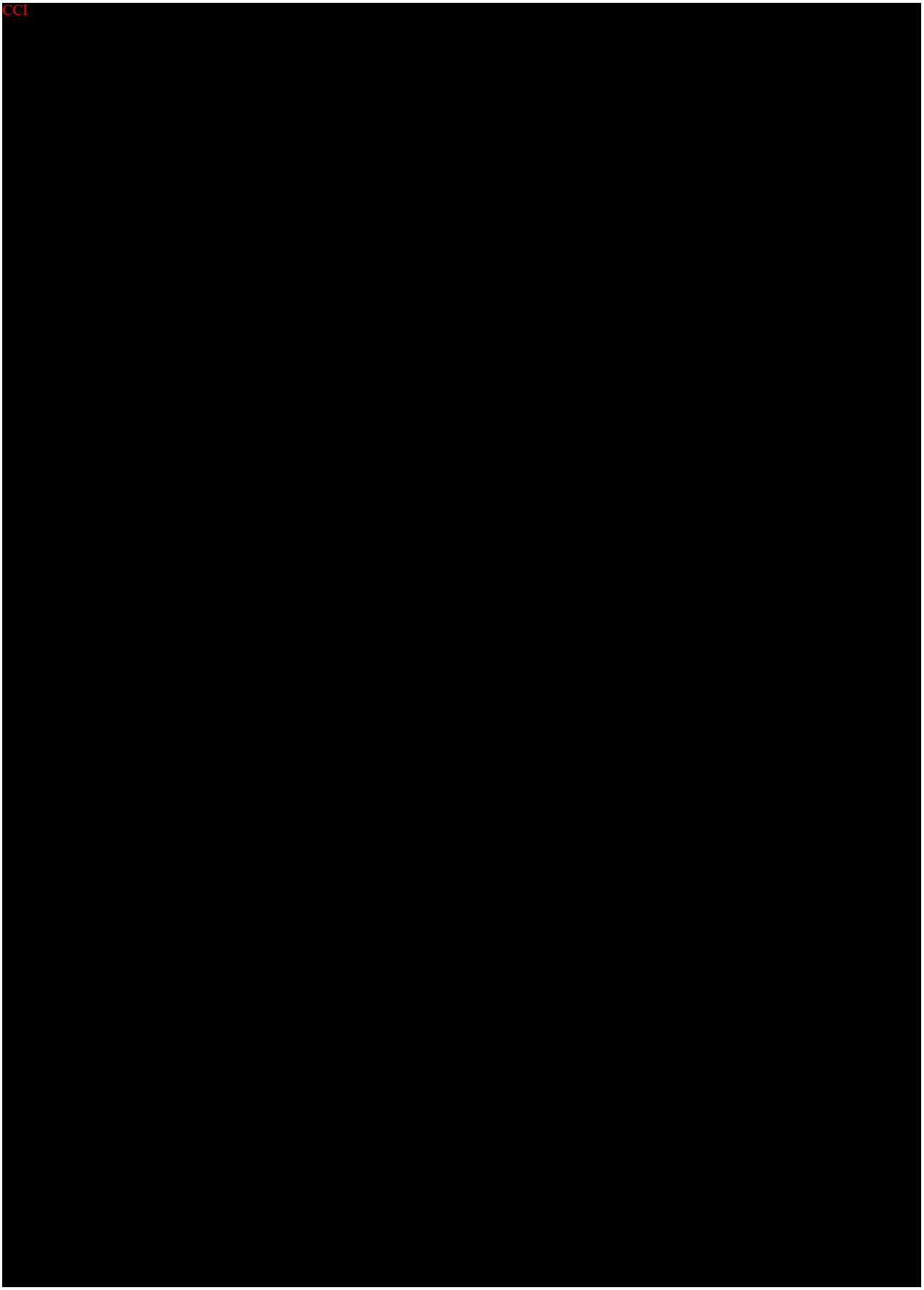

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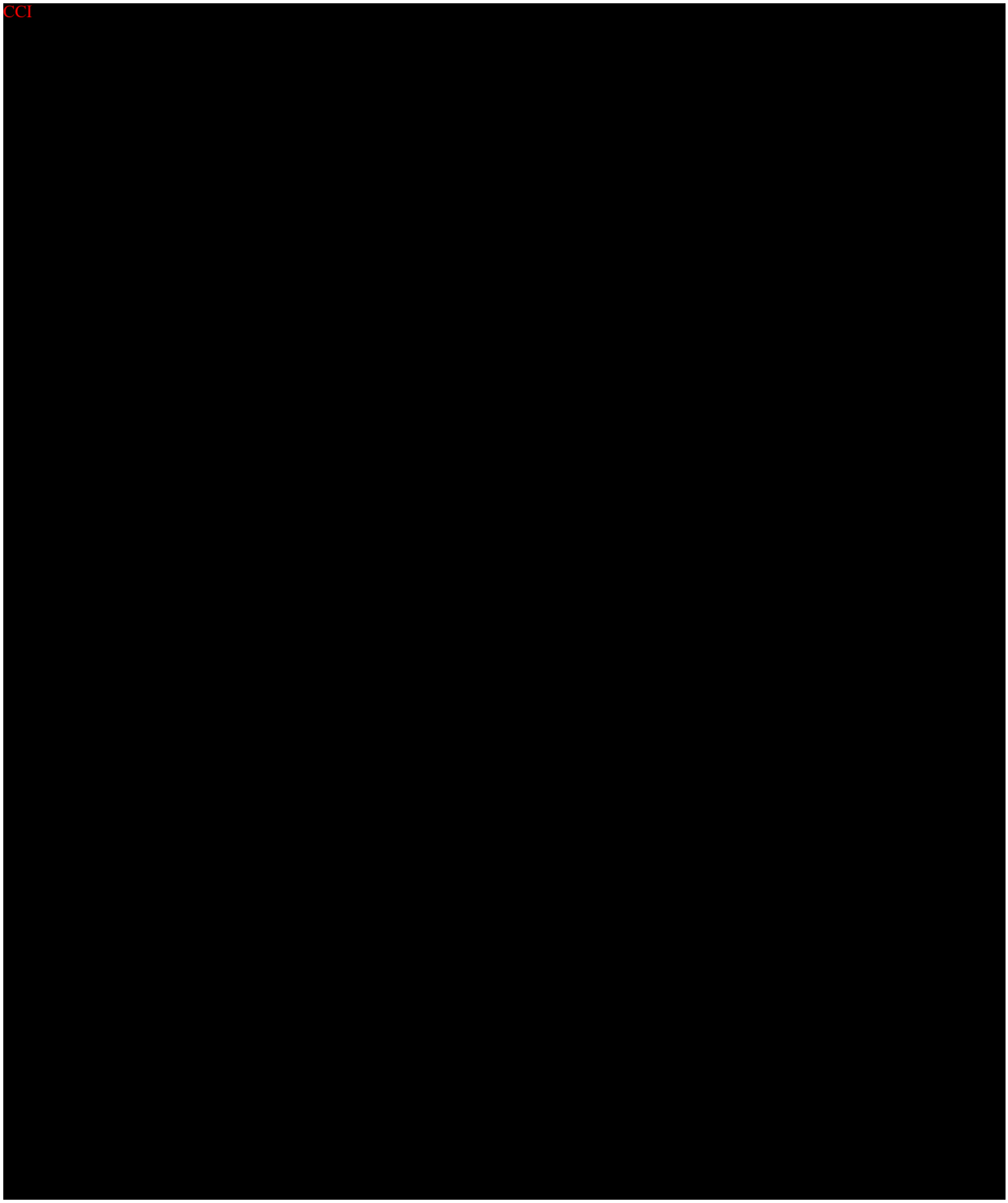

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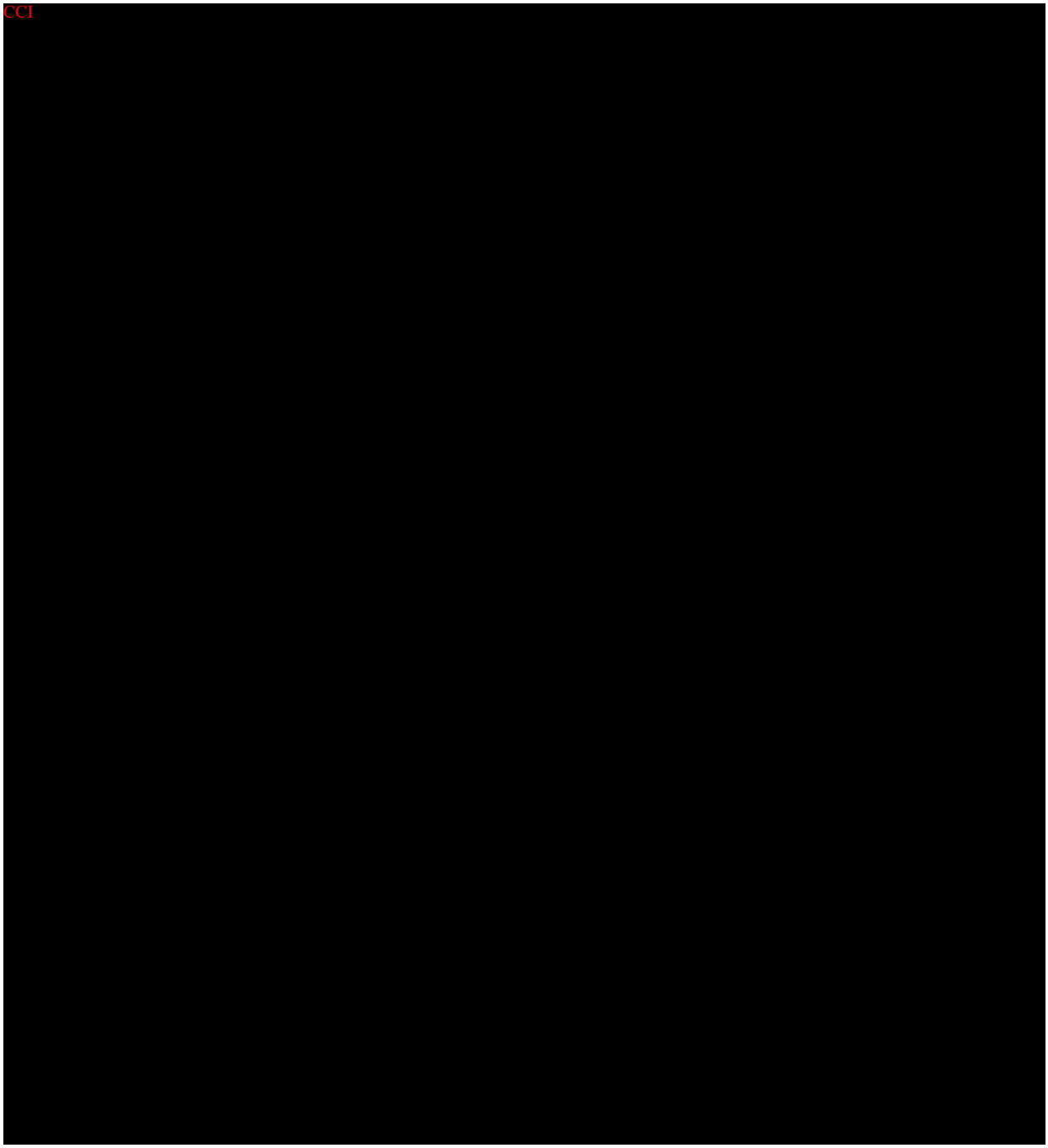

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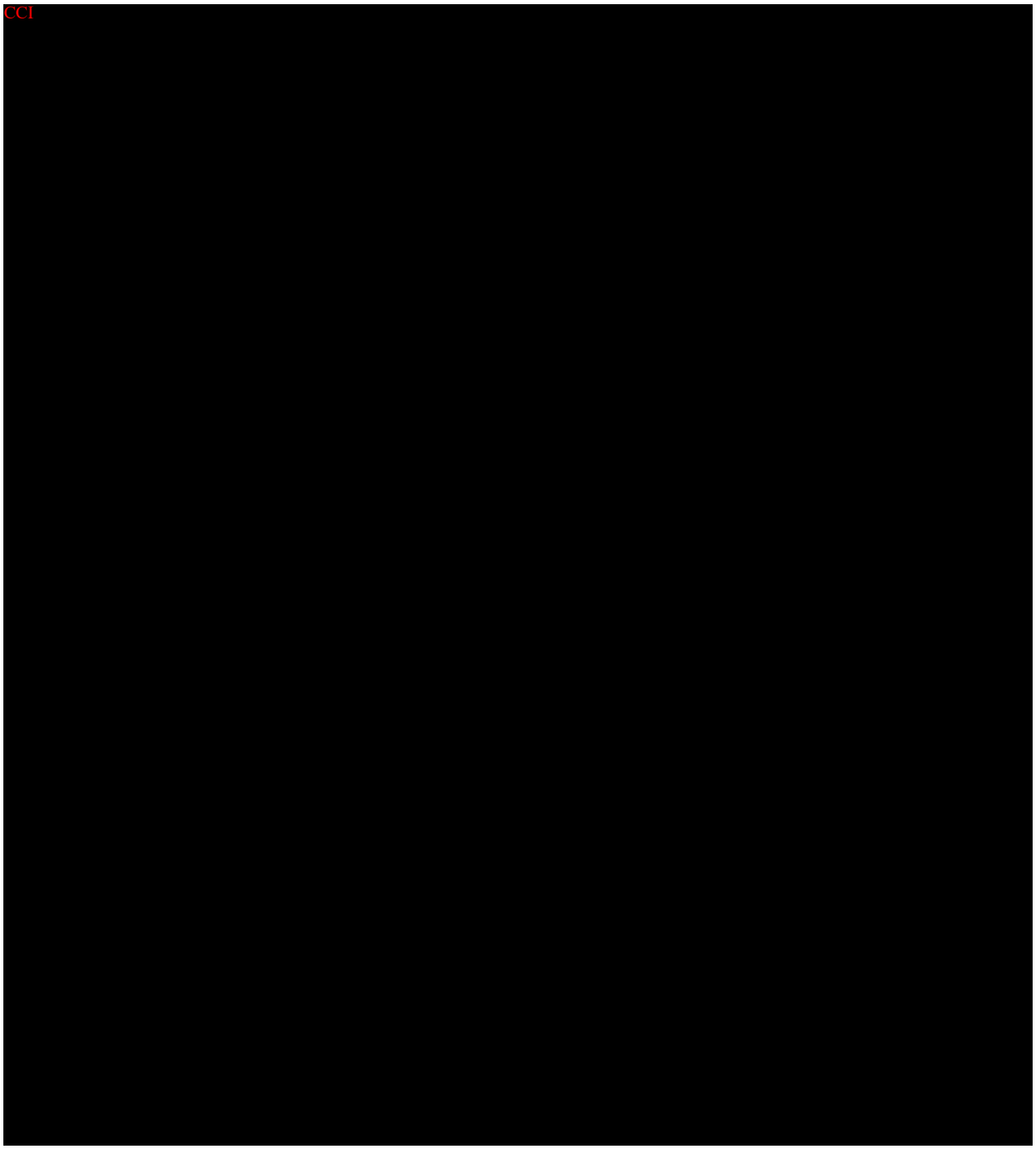

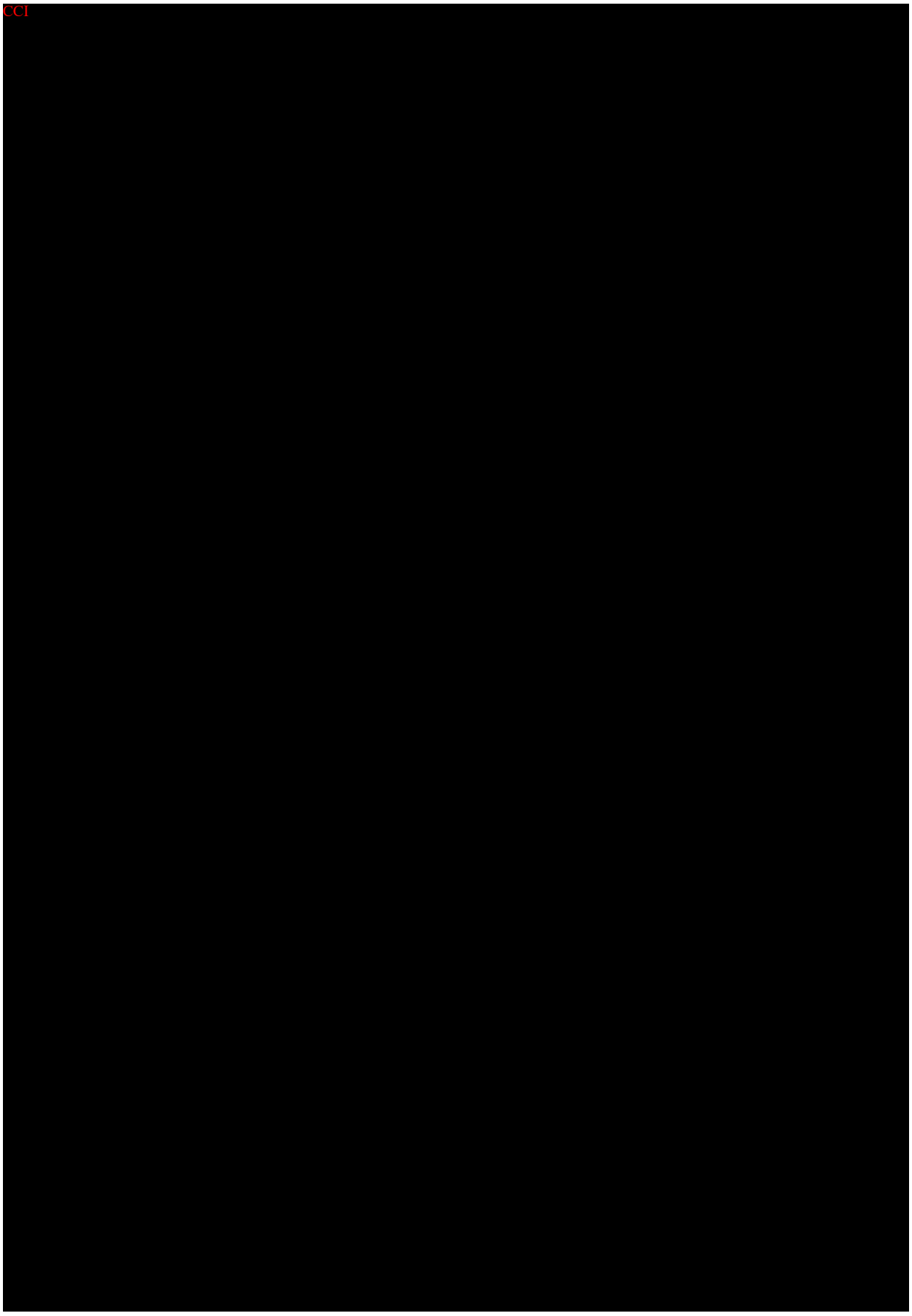
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4.6. Safety Analyses

The main safety analyses are described in Section 4.2

4.6.1. Additional Safety Assessments

4.6.1.1. Vital signs

Vital signs (systolic/diastolic blood pressure, and heart rate) and pre-intervention temperature reported before each study intervention administration (Visit 1 [Day 1] and Visit 3 [Day 29]) will be summarized by group using descriptive statistics.

4.6.1.2. COVID-19 Assessment

- COVID-19 cases will be tabulated as unsolicited events

4.7. Other Analyses

Not applicable.

4.8. Changes to Protocol- Defined Analyses

N/A

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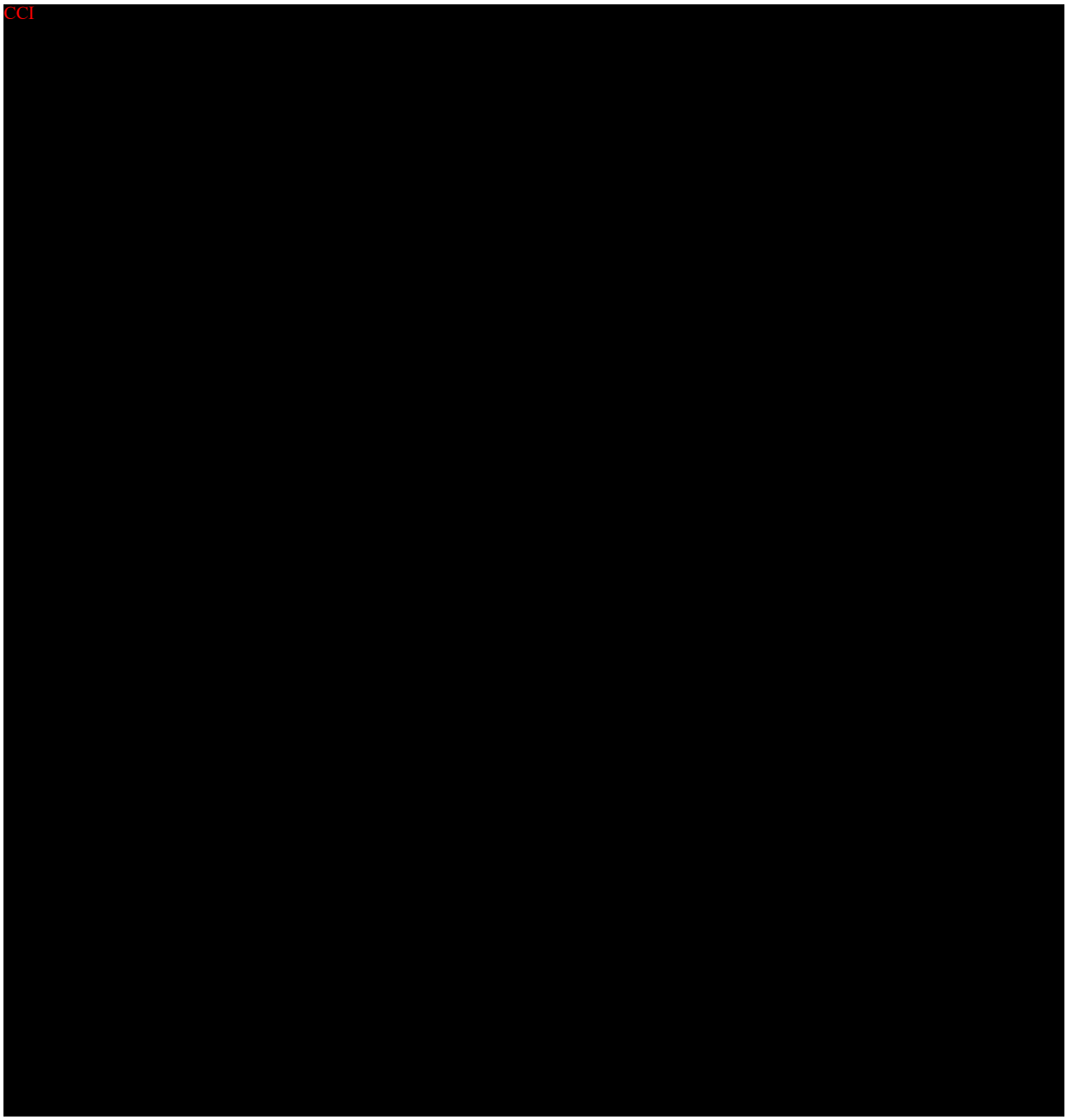

6. SUPPORTING DOCUMENTATION

6.1. Appendix 1 Study Population Analyses

6.1.1. Participant Disposition

The number of participants who are enrolled, received study intervention and in each study group will be tabulated.

In addition, the number of participants in each analysis set will be described, e.g., including the number of participants excluded from the PPS and the number who withdraw and reason for dropout.

6.1.2. Demographic and Baseline Characteristics

The median, mean, range and SD of age (in years) attime of screening, height (cm), weight (kg), and BMI (kg/m²) will be computed by group, distribution of participants in each CCI category (positive/negative), race, ethnicity, and sex composition will be summarised overall and by study intervention group. This analysis will be based on the ES, the PPS_Immuno and the PPS_CMI at Day 1.

6.1.3. Participant exposure

The number and percentage of participants who received each study intervention formulation or the placebo will be tabulated by group and by dose for the ES.

6.1.4. Concomitant Medications

Medications will be coded using the GSKDRUG dictionaries.

The number and percentage of participants using concomitant medication (any medication and any antipyretic) during the 7-day and the 28-day follow-up period after each dose and overall will be tabulated with exact 95% CI.

6.2. Appendix 2 Data Derivations Rule

This section contains standard rules for data display and derivation for clinical and epidemiological studies.

6.2.1. Attributing events to study intervention doses

The dose relative to an event is the most recent study dose given to a participant prior to the start of a given event. For example, if the start date of an AE is between Dose 1 and Dose 2, the relative dose will be Dose 1.

If an event starts on the same day as a study dose, the relative dose will be derived from the additional information provided in the eCRF using the contents of the flag indicating if the event occurred before or after study dose. If ‘after study dose’ is selected, the relative dose for the event will be the one administered on the start day of the event. If ‘before study dose’ is selected, the relative dose for the event will be the dose prior to this one.

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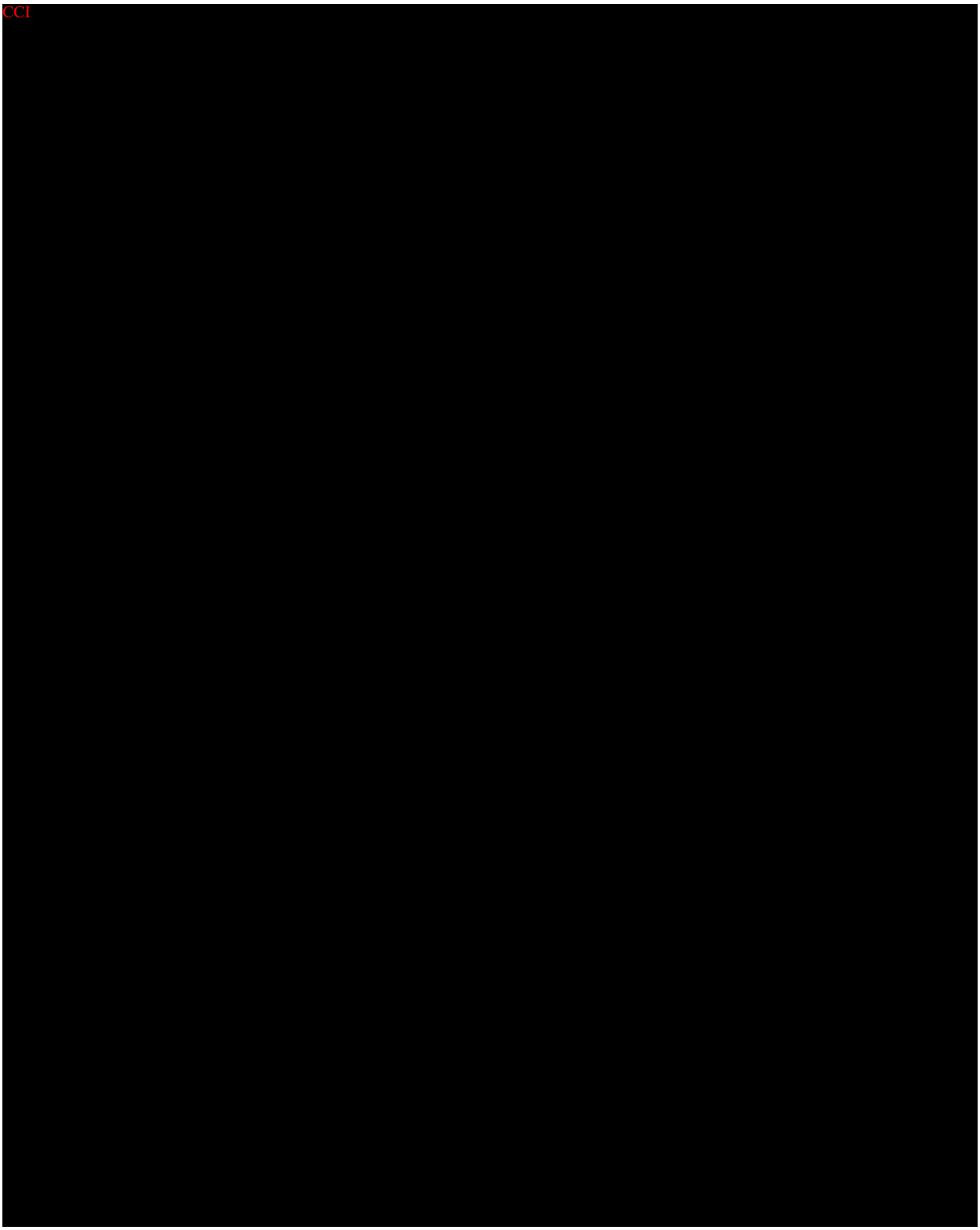

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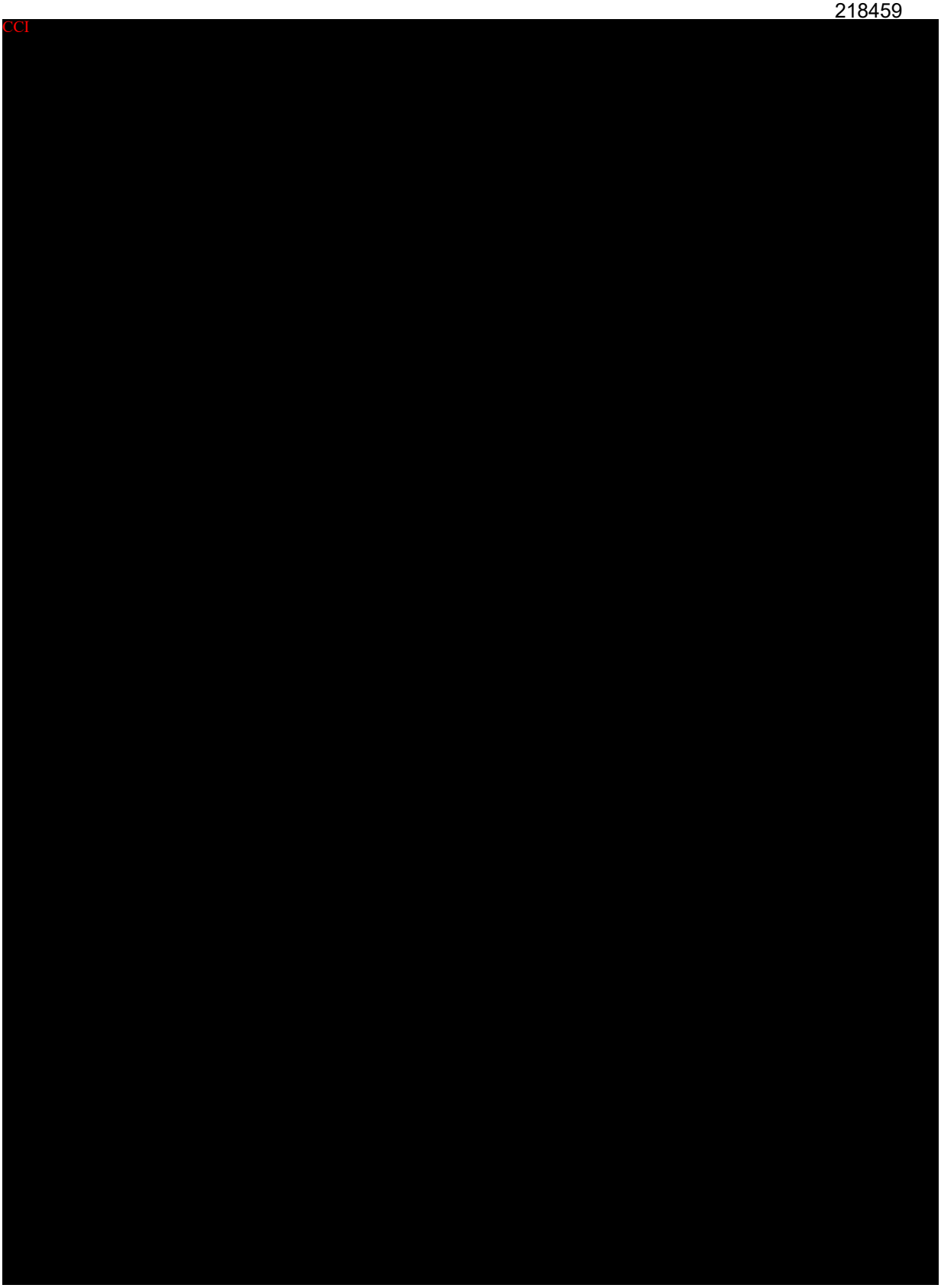

6.2.3. Data derivation

6.2.3.1. Age at time of screening in years

Age will be calculated as the number of years between the date of birth and the date at time of screening

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6.2.3.2. Temperature

Temperatures will be presented in degrees Celsius (°C). Temperatures reported in degrees Fahrenheit (°F) will be converted as follows:

Temperature (Celsius) = ((Temperature (Fahrenheit) - 32) x 5)/9

6.2.3.3. Numerical serology results

Numerical serology results will be derived from the content of IS.ISORRES in the SDTM dataset. For assays with a specific cut-off, the following derivation rules apply:

IS.ISORRES	Derived value
“NEG”, “-“, or “(-)”	cut-off/2
“POS”, “+”, or “(+)”	cut-off
“< value” and value is <= assay cut-off	cut-off/2
“< value” and value is > assay cut-off	value
“> value” and value is < assay cut-off	cut-off/2
“> value” and value is >= assay cut-off	value
“value” and value is < cut-off	cut-off/2
“value” and value is >= cut-off	Value
“value” and value is > ULOQ	ULOQ
All other cases	missing

6.2.3.4. Geometric mean titers (GMTs) and concentrations (GMCs)

Geometric Mean Titer (GMT) or Concentration (GMC) calculations are performed by taking the inverse logarithm of the mean of the log titer or concentration transformations. Non quantifiable antibody titers or concentrations will be converted as described in section 6.2.3.3 for the purpose of GMT/GMC calculation. Cut-off values are defined by the laboratory before the analysis.

6.2.3.5. Onset day

The onset day for an event (e.g. AE, concomitant medication/vaccination) is the number of days between the last (i.e., most recently received) study dose (i.e., it may be dose 1 or dose 2 of the study intervention) and the start date of the event plus one day. This is 1 for an event occurring on the same day as a study dose (and reported as starting after study dose) and e.g., 2 for an event occurring on the day after the study dose was given.

6.2.3.6. Duration of events

The duration of an event with a start and end date will be the difference between the start and end date plus one day, i.e. an event that starts on 3 March 2018 and ends on 12 March 2018 has a duration of 10 days.

The duration of solicited events will be calculated as the sum of the individual days with the AE reported at grade 1 or higher during the solicited event period.

6.2.3.7. Counting rules for combining solicited and unsolicited adverse events

For output combining solicited and unsolicited AEs, all SAEs will be considered systemic events since the administration site flag is not included in the expedited AE eCRF pages. Unsolicited AEs with missing administration site flag will also be considered systemic.

Multiple events with the same PT which start on the same day are counted as only one occurrence.

6.2.3.8. Counting rules for occurrences of solicited events

When the occurrences of solicited events are summarized, each event recorded as having occurred during a specific period will be counted as only one occurrence regardless of the number of days on which it occurs. Also, in the case of co-administered study interventions, an administration site event recorded for a participant following multiple study interventions will be counted as only one occurrence.

In addition, if a subject has multiple occurrences of a specific AE, data for each subject will be summarized according to the maximal severity observed during the follow-up period for each adverse event and each dose.

Table 4 Intensity grading scale for solicited events

Event	Intensity grade	Parameter
Pain at administration site	0	None
	1	Mild: Any pain neither interfering with nor preventing normal everyday activities.
	2	Moderate: Painful when limb is moved and interferes with everyday activities.
	3	Severe: Significant pain at rest. Prevents normal everyday activities.
Redness at administration site	0	< 25 mm
	1	25 - 50 mm
	2	51 - 100 mm
	3	> 100 mm
Swelling at administration site	0	< 25 mm
	1	25 - 50 mm
	2	51 - 100 mm
	3	> 100 mm
Temperature*	0	< 38.0°C (< 100.4°F)
	1	38.0°C (100.4°F) - 38.4°C (101.1°F)
	2	38.5°C (101.2°F) - 38.9°C (102.0°F)
	3	> 38.9°C (> 102.0°F)
Fatigue Headache Myalgia Arthralgia	0	None
	1	Mild: Symptom that is easily tolerated
	2	Moderate: Symptom that interferes with normal activity
	3	Severe: Symptom that prevents normal activity

*The route for measuring temperature can be oral, axillary or tympanic. Fever is defined as temperature ≥38.0°C regardless of the location of measurement.

6.2.3.9. Counting rules for occurrence of unsolicited adverse events

Unsolicited AE summaries are including SAEs unless specified otherwise.

As per CDISC Vaccines Therapeutic Area guide, the solicited events which continue beyond the observation period are stored in the AEs domain but they do not contribute to the summaries of unsolicited AEs.

Missing severity, relationship with study intervention, and outcome of unsolicited AEs will not be replaced and will appear as ‘UNKNOWN’ when displayed in a statistical output.

6.2.4. Display of decimals

6.2.4.1. Percentages

Percentages and their corresponding confidence limits will be displayed with one decimal except for 100% in which case no decimal will be displayed.

6.2.4.2. Differences in percentages

Differences in percentages and their corresponding confidence limits will be displayed with two decimals.

6.2.4.3. Demographic/baseline characteristics statistics

The mean, median, and SD for continuous baseline characteristics (height, weight, body mass index (BMI), pre-dose body temperature) will be presented with one decimal.

The minimum and maximum values and quartile values (if required) will be presented with the same number of decimals as the observed values.

The maximum and minimum of transformed body temperatures will be displayed with one decimal.

6.2.4.4. Serological summary statistics

For each assay, GMTs or GMCs and their confidence limits will be presented with one decimal, as well as GMT/GMC fold increase from pre-dose.

GMT or GMC group ratios and their confidence limits will be displayed with 2 decimals regardless of the actual values.

7. REFERENCES

Clopper CJ, Pearson E. The Use of Confidence or Fiducial Limits Illustrated in the case of the Binomial. *Biometrika.* 1934;26:404-13.

Dunnett, Charles W. "A multiple comparison procedure for comparing several treatments with a control." *Journal of the American Statistical Association* 50.272 (1955): 1096-1121.