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Sponsor: Sanofi Pasteur	**Study Identifiers:** U1111-1183-5687, NCT03158233
Drug substance(s): Not applicable	**Study code:** VAG00001

Title of the study: Prospective Surveillance and Case Definition Study of Zika Virus Disease and Infection in Adolescents and Adults in Latin America in Preparation for an Efficacy Trial of a Zika Virus Whole Virion, Purified Inactivated Vaccine.

Study center(s): Multi-center in 4 countries in Latin America (Colombia, Honduras, Mexico, and Puerto Rico)

Study period:

Date first subject enrolled: 28/Apr/2017

Date last subject completed: 19/Dec/2018

Phase of development: Not applicable (observational study)

Objectives:

1) To identify suspected Zika virus disease (ZVD) cases among the study cohort in order to detect the occurrence of virologically-confirmed ZVD
2) To describe the clinical and epidemiological characterization of ZVD and "virologically-confirmed Zika" (VCZ) cases
3) To describe the Zika seroprevalence among the cohort at baseline and the Zika incidence of asymptomatic infection in the study cohort based on seroconversion
4) To validate the surveillance system implemented in the study and develop the operational capabilities for future Zika efficacy trial sites

Methodology:

This was a prospective, multi-center, cohort study in 2400 subjects aged 15 to 40 years at enrollment in 4 countries in Latin America: Colombia, Honduras, Mexico, and Puerto Rico.

The study involved active and passive surveillance for ZVD and determination of prevalence and seroconversion incidence in areas of Latin America experiencing Zika epidemic activity, as reported by their local/national health authorities. Description of occurrence of dengue virus (DENV) and chikungunya virus (CHIKV) infections in the cohort were provided as differential diagnosis of ZVD.

Surveillance

Active and passive surveillance was performed to detect the appearance of any of the following clinical signs/symptoms/syndromes which have been associated with symptomatic Zika: rash (pruritic or not), axillary temperature ≥ 37.5°C, conjunctivitis (non-purulent/hyperemic), arthralgia/arthritis/myalgia/peri-articular edema, or sign(s)/symptom(s) of a neurologic/neuroinflammatory disorder (such as acute disseminated encephalomyelitis [including site specific variants], cranial nerve disorders [including paralyses/paresis], Guillain-Barré syndrome [GBS]—including Miller Fisher syndrome [MFS] and other variants, immune-mediated peripheral neuropathies or plexopathies, optic neuritis, multiple sclerosis, narcolepsy, transverse myelitis, meningitis, or meningoencephalitis) not explained by other medical conditions.

Blood/Urine Sampling

All enrolled subjects provided blood samples at enrollment (Day 0) and at the end of the study (Day 365) to be tested for Zika virus (ZIKV) anti-nonstructural protein 1 (NS1) antibodies (and possibly anti-ZIKV and anti-DENV neutralizing antibodies).

Study participants with any of the signs/symptoms provided a blood sample within 7 days of the onset of signs/symptoms and blood and urine samples 7 to 14 days later*. These samples were taken for detection of ZIKV ribonucleic acid (RNA) by reverse transcription polymerase chain reaction (RT PCR). Any samples testing positive for ZIKV by RT PCR was defined as VCZ cases. Acute blood samples were also tested for DENV and CHIKV RNA as a differential diagnosis of interest. The acute and convalescent serum samples of subjects with signs/symptoms of a neurologic/neuroinflammatory disorder were additionally tested for anti- ZIKV NS1 antibodies.* Acute blood samples and convalescent blood and urine samples for all suspected ZVD cases should have been collected within the pre-specified timeframe described above. If this could not be accomplished, these samples were still to be obtained and sent for testing as soon as possible thereafter.

In addition, a blood sample was taken at the enrollment visit and a year after enrollment to determine the seroprevalence of Zika and the incidence of seroconversion* at the end of the 1-year follow-up in the study cohort, defined as the presence of anti-ZIKV NS1 antibodies in subjects who were seronegative at enrollment. Day 0 and Day 365 blood samples were also tested for anti-ZIKV neutralizing antibodies measured by microneutralization (MN) assay. These blood samples were additionally tested for anti-DENV in an effort to descriptively assess cross-reacting antibody responses and the possible effect of immunological experience with other flaviviruses on the immune response to ZIKV.

*Seroconversion: Subjects with Zika NS1 titers <10f at baseline (seronegative), who had Zika NS1 titers >10f post-baseline by Zika anti-NS1 assay or subjects with Zika NS1 titers <10f at baseline (seronegative), who had MN titers >100 post-baseline.

f: A titer >10 (minimum sample dilution) was considered to be the assay threshold for positive

Safety

Any serious adverse event (SAE) assessed as related to a study procedure by the investigator was reported to the Sponsor.

Number of subjects:	Planned: 2400
	Enrolled: 2400
	Completed: 2188
Evaluated:	Surveillance and case definition: 2400

Diagnosis and criteria for inclusion:

An individual must have fulfilled *all* of the following criteria in order to be eligible for study enrollment:

1) Aged 15 to 40 years on the day of inclusion, currently residing in the site zone, and planning to continue to reside in the site zone for the duration of the study
2) For subjects under the age of majority on the day of inclusion: the assent form (AF) has been signed and dated by the subject (if required by local regulations), and the informed consent form (ICF) has been signed and dated by the parent(s) or legal guardian(s).
   For subjects at or over the age of majority on the day of inclusion: the ICF has been signed and dated.
3) Subject (and parent/guardian if subject was under the age of majority) able to attend all scheduled visits and to comply with all study procedures
4) In good health, based on medical history and physical examination

Study treatments: Not applicable

Duration of observation: 1 year

Criteria for evaluation:

1) Identification of subjects with suspected ZVD and VCZ infection in the cohort:

- Suspected ZVD was defined as any of the following clinical signs/symptoms: rash (pruritic or not), axillary temperature ≥ 37.5°C, conjunctivitis (non-purulent/hyperemic), arthralgia/arthritis/myalgia/peri-articular edema, or sign(s)/symptom(s) of a neurologic/neuroinflammatory disorder (such as acute disseminated encephalomyelitis [including site specific variants], cranial nerve disorders [including paralyses/paresis], GBS—including MFS and other variants, immune-mediated peripheral neuropathies or plexopathies, optic neuritis, multiple sclerosis, narcolepsy, transverse myelitis, meningitis, or meningoencephalitis) not explained by other medical conditions
- VCZ case: a subject with suspected ZVD with detection of ZIKV RNA in blood or urine

Note: If a subject had a suspected ZVD episode that occurred > 15 days from the last day of the last sign/symptom of a previously reported suspected ZVD episode, the second episode was considered as an independent suspected ZVD episode, and the subject was requested to visit the site for subsequent acute and convalescent visits.

2) Description (occurrence, intensity, duration) of signs/symptoms accompanying suspected ZVD and VCZ cases in the study subjects
3) Description of the percentage of subjects seropositive to Zika (and possibly dengue, yellow fever and other flaviviruses) at baseline and of those who seroconverted to ZIKV by the end of the study
4) Assessment of the surveillance operational performance for Zika studies. The identification of study operational issues will be achieved through regular monitoring

Statistical methods:

Analyses:

Two analyses of the data were planned. A preliminary analysis was performed 6 months after the first visit of the first subject (FVFS) to help with the activities in preparation of the Zika purified, formaldehyde-inactivated virus (ZPIV) vaccine efficacy trial. A final analysis was done at the end of the study once all data were available and presented:

1) A description of the incidence of suspected ZVD cases and VCZ cases by age group, gender, site/country, and for all sites/countries combined.
2) A description of signs/symptoms accompanying suspected ZVD and VCZ cases.
3) A description of the number and percentage of Zika antibody positive subjects at baseline and of subjects who seroconverted to ZIKV by the end of study by country and in the overall population.
4) A qualitative and quantitative assessment of the sites' ability to conduct surveillance for suspected ZVD and all other operational procedures.

All the analyses were descriptive. For quantitative data, 95% confidence intervals (CIs) of point estimates were calculated using a normal approximation and an exact binomial distribution was used for the proportions (Clopper-Pearson method).

Summary:

Subject Disposition:

A total of 2400 subjects were enrolled across 4 countries: 500 subjects in Honduras, 700 subjects in Colombia, 400 subjects each at 2 sites (site 300 and site 320) in Mexico, and 400 subjects in Puerto Rico. A total of 2188 (91.2%) subjects completed the study: 467 (93.4%) subjects in Honduras, 638 (91.1%) subjects in Colombia, 356 (89.0%) subjects (site 300) and 385 (96.3%) subjects (site 320) in Mexico, and 342 (85.5%) subjects in Puerto Rico. A total of 212 (8.8%) subjects did not complete the study: 33 (6.6%) subjects in Honduras, 62 (8.9%) subjects in Colombia, 44 (11.0%) subjects (site 300) and 15 (3.8%) subjects (site 320) in Mexico, and 58 (14.5%) subjects in Puerto Rico. There were no early terminations due to an SAE or other adverse event (AE).

Data sets analyzed

The full analysis set (FAS) was used for the analyses of Outcome 4. The FAS and per-protocol analysis set (PPAS) were used for the analysis of Outcomes 1, 2, and 3.

Demographics

There were a total of 775 male subjects (32.3%) and 1625 (67.7%) female subjects among all the enrolled subjects. The overall ratio of male/female subjects was 0.48. There were more female subjects than male subjects across all centers. Subjects' ages were comparable between all the centers. The mean age at enrollment was 27.3 ± 6.97 years.

Outcomes

Outcome 1: Identification of Subjects with Suspected ZVD and VCZ Infection in the Cohort

A total of 681 subjects with at least 1 episode of suspected ZVD were reported in the study. Of these, 2 subjects were reported having virologically confirmed ZVD by RT PCR in the PPAS. Additionally, one subject was reported having virologically confirmed ZVD in the FAS. The highest number of suspected cases and the only 2 virologically confirmed cases in the PPAS was observed in one of the sites in Mexico (120 suspected cases; 2 confirmed cases) in the acute and convalescent blood samples. Of these, 1 confirmed case was virologically confirmed in the convalescent urine sample.

Of the 1760 suspected Zika episodes reported in the study, the sera of 41 cases were tested positive for DENV infection by RT PCR. The highest number of confirmed cases were observed in Honduras (23/742) followed by one of the sites (site 320) in Mexico (12/359). There were no cases of virologically confirmed CHIKV infections reported in the study.

Outcome 2: Description of Signs/Symptoms Accompanying Suspected ZVD and VCZ Cases in the Study Subjects

The most frequently reported clinical signs/symptoms captured during this surveillance study were axillary temperature ≥37°C (62.5%), myalgia (62.4%), and arthralgia (61.7%). There were 2 subjects with neurologic/neuroinflammatory disorders reported during the study. None of the subjects required hospitalization and recovered with persistent symptoms/sequelae.

There were 2 virologically confirmed ZVD (determined by RT PCR) cases reported during the study. The clinical symptoms of the 2 virologically confirmed ZVD cases were axillary temperature ≥37°C and arthralgia. None of the subjects with virologically confirmed ZVD reported any neurologic/neuroinflammatory disorders.

Outcome 3: Description of the Percentage of Subjects Seropositive to Zika (and Possibly Dengue, Yellow Fever and Other Flaviviruses) at Baseline and of Those who Seroconverted to ZIKV by the End of the Study

At baseline (Visit 1), a total of 2400 subjects provided blood samples for the Zika anti-NS1 BOB enzyme-linked immunosorbent assay (ELISA) testing and 2395 subjects provided blood samples for the Zika MN assay testing in the FAS. The percentage of subjects seropositive for Zika NS1 BOB ELISA was 52.6% (n=1262) and for Zika MN titers ≥100 was 56.0% (n=1342). Among the subjects in the PPAS, the percentage of subjects seropositive for Zika NS1 BOB ELISA was 52.0% (n=945) and for Zika MN titers ≥100 was 56.1% (n=1020). The highest seropositive rate at baseline was in one of the sites (site 300) in Mexico: 80.5% (n=227) detected by Zika anti-NS1 BOB ELISA and 85.1% (n=240) by Zika MN titers ≥100.

The final seroconversion definition was used to perform analysis on the FAS and PPAS. Zika anti-NS1 BOB ELISA (n=2400) and Zika MN (n=2395) assays were performed for subjects at baseline (Visit 01) in the FAS. Of these, the percentage of subjects seronegative for Zika NS1 BOB ELISA and Zika MN assays were 47.4% (n=1138) and 44.0% (n=1053), respectively and the percentage of subjects who were double seronegative for Zika NS1 BOB ELISA and Zika MN titers was 38.3% (n=917). The percentage of subjects who were double seronegative at baseline and seroconverted at Visit 02 (at D365 ± 14 day window) as determined by Zika anti-NS1 BOB ELISA testing and Zika MN titers were 0.5% (n=5).

Outcome 4: Assessment of the Surveillance Operational Performance for Zika Studies

There were a total of 1172 suspected Zika episodes reported throughout the study. The percentage of subjects with suspected ZVD and which were tested during the acute phase was 90.9% while the percentage of subjects with suspected ZVD and which were tested during the convalescent phase was 94.1%.

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