BMJ Open

Efficacy of sofosbuvir as treatment for yellow fever: protocol for a randomised controlled trial (SOFFA study)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-027207
Article Type:	Protocol
Date Submitted by the Author:	18-Oct-2018
Complete List of Authors:	Figueiredo-Mello, Claudia; Instituto de Infectologia Emilio Ribas, Department of Education and Research; Faculdade de Medicina da Universidade de Sao Paulo, Department of Infectious and Parasitic Diseases Casadio, Luciana ; Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Department of Infectious and Parasitic Diseases Avelino-Silva, Vivian ; Faculdade de Medicina da Universidade de Sao Paulo, Department of Infectious and Parasitic Diseases Yeh-Li, Ho; Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Department of Infectious and Parasitic Diseases Sztajnbok, Jaques ; Instituto de Infectologia Emilio Ribas, Medical Division-Intensive Care Unit Joelsons, Daniel ; Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Intensive Care Unit of the Department of Infectious and Parasitic Diseases Antonio, Marilia ; Faculdade de Medicina da Universidade de Sao Paulo, Department of Infectious and Parasitic Diseases Pinho, João ; Faculdade de Medicina da Universidade de Sao Paulo, LIM 07 - Department of Gastroenterology Malta, Fernanda ; Faculdade de Medicina da Universidade de Sao Paulo, LIM 07 - Department of Gastroenterology Gomes-Gouvêa, Michele ; Faculdade de Medicina da Universidade de Sao Paulo, LIM 07 - Department of Gastroenterology Salles, Ana; Faculdade de Medicina da Universidade de Sao Paulo, LIM 07 - Department of Gastroenterology Corá, Aline ; Instituto de Infectologia Emilio Ribas, Clinical Lab Moreira, Carlos ; Instituto de Infectologia Emilio Ribas, Inpatient unit Ribeiro, Ana ; Instituto de Infectologia Emilio Ribas, Epidemiology Service Nastri, Ana ; Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Department of Infectious and Parasitic Diseases Malaque, Ceila ; Instituto de Infectologia Emilio Ribas, Medical Division-Intensive Care Unit Teixeira, Ralcyon ; Instituto de Infectologia Emilio Ribas, Medical Division-Director Borges, Luciana ; Instituto de Infectologia Emilio Ribas, Medical Emergency Department Gonzalez, Mario ; Instituto de Infectologia Emilio Ribas, Outpatients

	clinic Junior, Luiz ; Instituto de Infectologia Emilio Ribas, Department Director Souza, Tâmara ; Instituto de Infectologia Emilio Ribas, ER and Outpatient Service Director Song, Alice ; Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Disciplina de Transplante de Figado e Orgaos do Aparelho Digestivo, Departamento de Gastroenterologia D'Albuquerque, Luiz ; Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Disciplina de Transplante de Figado e Orgaos do Aparelho Digestivo, Departamento de Gastroenterologia Abdala, Edson; Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Department of Infectious and Parasitic Diseases; Instituto do Cancer do Estado de Sao Paulo, Infectious Diseases Andraus, Wellington ; Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Disciplina de Transplante de Figado e Orgaos do Aparelho Digestivo, Departamento de Gastroenterologia Martino, Rodrigo ; Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Disciplina de Transplante de Figado e Orgaos do Aparelho Digestivo, Departamento de Gastroenterologia Ducatti, Liliana ; Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Disciplina de Transplante de Figado e Orgaos do Aparelho Digestivo, Departamento de Gastroenterologia Andrade, Guilherme ; Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Intensive Care Unit, Department of Gastroenterology Malbouisson, Luiz ; Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Intensive Care Unit, Department of Gastroenterology Souza, Izabel ; Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Epidemiologic Surveillance Department of the Hospital das Clínicas Carrilho, Flair; Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Departamento de Gastroenterologia Sabino, Ester; Universidade de Sao Paulo, Instituto de Medicina Tropical; Fundação Pró-Sangue, Hemocentro de São Paulo Levin, Anna; Faculdade de Medicina da Universidade de Sao Paulo, Department of Infectious and Parasitic Diseases
Keywords:	Yellow Fever, Sofosbuvir, Randomized Controlled Trial, Viral Kinetics

Title:

Efficacy of sofosbuvir as treatment for yellow fever: protocol for a randomised controlled trial (SOFFA study)

Corresponding author:

Claudia Figueiredo-Mello

Postal address: Av. Doutor Arnaldo, 165 - 01246-900 - São Paulo - SP - Brazil

Email: claudiamello@ymail.com

Telephone number: +55 11 983019837

Co-authors:

Claudia Figueiredo-Mello, Department of Education and Research, Instituto de Infectologia Emilio Ribas, Department of Infectious and Parasitic Diseases, Faculdade de Medicina da Universidade de Sao Paulo, São Paulo, Brazil.

Luciana Vilas Boas Casadio, Department of Infectious and Parasitic Diseases, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.

Vivian Iida Avelino-Silva, Department of Infectious and Parasitic Diseases, Faculdade de Medicina da Universidade de Sao Paulo, São Paulo, Brazil.

Yeh-Li Ho, Department of Infectious and Parasitic Diseases, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.

Jaques Sztajnbok, Medical Division-Intensive Care Unit, Instituto de Infectologia Emilio Ribas, São Paulo, Brazil.

Daniel Joelsons, Intensive Care of the Department of Infectious and Parasitic Diseases, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.

Marilia Bordignon Antonio, Department of Infectious and Parasitic Diseases, Faculdade de Medicina da Universidade de Sao Paulo, São Paulo, Brazil.

João Renato Rebello Pinho, LIM 07 - Department of Gastroenterology, Faculdade de Medicina da Universidade de Sao Paulo, São Paulo, Brazil.

Fernanda de Mello Malta, LIM 07 - Department of Gastroenterology, Faculdade de Medicina da Universidade de Sao Paulo, São Paulo, Brazil.

Michele Soares Gomes-Gouvêa, LIM 07 - Department of Gastroenterology, Faculdade de Medicina da Universidade de Sao Paulo, São Paulo, Brazil.

Ana Paula Moreira Salles, LIM 07 - Department of Gastroenterology, Faculdade de Medicina da Universidade de Sao Paulo, São Paulo, Brazil.
Aline Pivetta Corá, Clinical Lab, Instituto de Infectologia Emilio Ribas, São Paulo, Brazil.
Carlos Henrique Valente Moreira, Inpatient Unit, Instituto de Infectologia Emilio Ribas, São Paulo, Brazil.
Ana Freitas Ribeiro, Epidemiology Service, Instituto de Infectologia Emilio Ribas, São Paulo, Brazil.
Ana Catharina de Seixas Santos Nastri, Department of Infectious and Parasitic Diseases, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.
Ceila Maria Sant'Ana Malaque, Medical Division-Intensive Care Unit, Instituto de Infectologia Emilio Ribas, São Paulo, Brazil.
Ralcyon Francis Azevedo Teixeira, Medical Division-Director, Instituto de Infectologia Emilio Ribas, São Paulo, Brazil.
Luciana Marques Sansão Borges, Medical Emergency Department, Instituto de Infectologia Emilio Ribas, São Paulo, Brazil.
Mario Peribañez Gonzalez, Outpatients clinic, Instituto de Infectologia Emilio Ribas, São Paulo, Brazil.
Luiz Carlos Pereira Junior, Department Director, Instituto de Infectologia Emilio Ribas, São Paulo, Brazil.
Tâmara Newman Lobato Souza, ER and Outpatient Service Director, Instituto de Infectologia Emilio Ribas, São Paulo, Brazil.
Alice Tung Wan Song, Disciplina de Transplante de Figado e Orgaos do Aparelho Digestivo, Departamento de Gastroenterologia, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.
Luiz Augusto Carneiro D'Albuquerque, Disciplina de Transplante de Figado e Orgaos do Aparelho Digestivo, Departamento de Gastroenterologia, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.
Edson Abdala, Department of Infectious and Parasitic Diseases, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.
Wellington Andraus, Disciplina de Transplante de Figado e Orgaos do Aparelho Digestivo, Departamento de Gastroenterologia, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.

Rodrigo Bronze de Martino, Disciplina de Transplante de Figado e Orgaos do Aparelho Digestivo, Departamento de Gastroenterologia, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.
Liliana Ducatti, Disciplina de Transplante de Figado e Orgaos do Aparelho Digestivo, Departamento de Gastroenterologia, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.
Guilherme Marques Andrade, Intensive Care Unit, Department of Gastroenterology, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.
Luiz Marcelo Sá Malbouisson, Intensive Care Unit, Department of Gastroenterology, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.
Izabel Marcilio de Souza, Epidemiologic Surveillance Department, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.
Flair José Carrilho, Department of Gastroenterology, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.
Ester Cerdeira Sabino, Instituto de Medicina Tropical, Universidade de Sao Paulo, São Paulo, Brazil.
Anna S Levin, Department of Infectious and Parasitic Diseases, Faculdade de Medicina da Universidade de Sao Paulo, Instituto de Medicina Tropical, Universidade de Sao Paulo, São Paulo, Brazil.

Word count:

2021

ABSTRACT

Introduction: An ongoing outbreak of yellow fever (YF) has been reported in Brazil with 1261 confirmed cases and 409 deaths since July 2017. To date, there is no specific treatment available for YF. Recently published papers describing *in vitro* and animal models suggest a potential effect of antiviral drugs approved for the treatment of hepatitis virus against flaviviruses, including YF. The primary aim of this study is to analyse the effect of sofosbuvir on viral kinetics and clinical outcomes among patients presenting with YF. This is a multicentre open-label randomised controlled trial with 1:1 individual allocation, stratified by severity and by recruiting centre.

Methods and analysis: Adults with suspected or confirmed YF infection and symptoms lasting up to 15 days are screened. Eligible and consenting patients are randomised to receive oral sofosbuvir 400mg daily for 10 days or standard clinical care. Viral kinetics are measured daily and the reduction in YF plasma viral load from the sample at inclusion to 72h after randomization will be compared between active and control groups. Clinical outcomes include severity meeting criteria for intensive care support, liver transplantation, in-hospital mortality and mortality within 60 days.

Ethics and dissemination: Ethics approval was obtained at the participating sites and at the national research ethics committee (CAAE 82673018.6.1001.0068). The trial has been submitted for ethical approval in additional potential recruiting centres. Results of the study will be published in journals and presented at scientific meetings.

Trial Registration: Registered at Brazilian Clinical Trials Registry (RBR-93dp9n). This manuscript corresponds to trial version 1 (02/20/2018).

Keywords: Yellow Fever, Sofosbuvir, Randomized Controlled Trial, Viral Kinetics

ARTICLE SUMMARY

Strengths and limitations of this study

- In this open-label 1:1 parallel group randomized controlled trial, we will address the effect of oral Sofosbuvir at 400mg daily dose compared to standard clinical care for patients with yellow fever (YF) infection referred for hospitalization during a YF outbreak in Brazil
- Randomization will be done using an electronic platform, stratified by clinical severity and recruiting centre
- Median change in plasma YF viral levels at 72h after inclusion and clinical outcomes (meeting severity criteria for intensive care support, liver transplantation, in-hospital mortality, mortality within 60 days) will be compared between active and control groups
- An independent data safety monitoring group will be designated to supervise severe adverse events related to the study medication and perform an interim analysis after the inclusion of 2/3 of predicted total sample size

INTRODUCTION

There is an ongoing outbreak of yellow fever (YF) in Brazil, with an increasing number of cases since 2016, notably in the Southern region. Between July 2017 and May 2018, 1261 confirmed YF cases were reported since, with 409 deaths (mortality rate of 32.4%). Nowadays, a major part of population at risk for YF resides in newly affected areas (58.3%), where vaccination was not previously recommended.[1]

YF has a wide clinical spectrum, from asymptomatic to severe haemorrhagic disease associated with liver and renal failure and high case fatality rate. Vaccine safety remains a concern, especially because the yellow fever vaccine-associated viscerotropic disease (YFV-AVD) is a rare condition but has a high case-fatality rate. To date, there is no specific treatment available for YF or YFV-AVD.[2]

Recently published papers describing *in vitro* and animal models suggest a potential effect of sofosbuvir (an antiviral approved for the treatment of hepatitis C virus) for other flaviviruses, such as dengue, zika and YF.[3–6]

A group of researchers showed that sofosbuvir inhibited YF virus replication in different lineages of human hepatoma cells and reduced the YF-induced mortality and lack of weight gain in neonatal mice and suggested that sofosbuvir should be considered for clinical use in YF infected individuals.[5]

Another research team tested the *in vitro* activity against YF and zika virus of several antivirals used for hepatitis C virus (ribavirin, daclastavir, sofosbuvir and ledispavir/sofosbuvir combination). All the tested drugs presented activity and selectivity against YF and zika virus in human hepatoma cells, but ledispavir/sofosbuvir combination and sofosbuvir showed the best antiviral activity towards both viruses (Pinho JRR, Identification of pre-approved HCV antiviral drugs Sofosbuvir and Ledispavir as compounds capable of eliminating Yellow fever infection in vitro).

Although there is some evidence of sofosbuvir activity against YF, there is no data from human studies on the effect of sofosbuvir on acute YF.

We present the protocol for a multicentre open-label randomised controlled trial to analyse the effect of sofosbuvir on YF viral kinetics and on clinical outcomes (severity meeting criteria for intensive support, liver transplantation, in-hospital mortality and mortality within 60 days among patients presenting with acute YF). This is a multicentre open-label

randomised controlled trial with 1:1 individual allocation, stratified by severity and by recruiting centre.

METHODS AND ANALYSIS

Study setting

The study will be conducted in secondary and tertiary healthcare units in Brazil. At this moment two recruiting centres are actively enrolling, both tertiary teaching hospitals in São Paulo city (São Paulo State, Brazil). Six additional recruiting centres in the States of São Paulo and Minas Gerais are waiting for protocol ethical approval.

The inclusion of participants has begun in March 2018 and the protocol is still in recruiting phase.

Eligibility criteria

Adults ≥ 18 years of age hospitalized at the recruiting centres with suspected or confirmed YF infection and symptoms lasting up to 15 days are being screened.

We are including patients with a history of fever (axillary temperature above 37.8°C), exposed to geographic areas with risk for YF transmission (according to updated epidemiological reports) and with transaminases (either alanine aminotransferase or aspartate aminotransferase) above 500 U/L.

We are excluding patients with amiodarone use in the last 72 hours, current pregnancy, women refusing to interrupt breastfeeding, current use of sofosbuvir for hepatitis C treatment, those with a negative plasma YF real-time polymerase chain reaction (RT-PCR) prior to inclusion and those with signs/symptoms suggesting other diagnoses on admission.

Interventions

Eligible participants are randomised to receive either sofosbuvir 400mg daily for 10 days or standard care. Patients should remain hospitalised for at least 10 days after the randomisation.

For those allocated to receive sofosbuvir, the oral route is preferred, although enteral tube administration is also acceptable.

The following criteria determine immediate interruption of sofosbuvir prescription: negative YF RT-PCR in a test performed at enrolment; amiodarone prescription; anaphylactic shock or grade III rash attributed to sofosbuvir; inability to receive oral or enteral tube medications.

In the case of sofosbuvir interruption, patients will continue to be followed-up for adverse events and outcomes according to the protocol.

In case of withdrawal of consent, the study medication and study procedures will be interrupted, but the participant will be asked to undergo safety evaluations according to the protocol if possible.

Outcomes

YF plasma viral load will be measured daily and median change in plasma YF viral levels at 72h after inclusion will be compared between active and control groups.

Clinical outcomes (severity meeting criteria for intensive support, liver transplantation, in-hospital mortality, mortality in 60 days) will also be compared between active and control groups. Time to YF RT-PCR clearance in biological samples and overall survival will also be evaluated.

Criteria for intensive support is defined by any of the following conditions: transaminases > 3000 U/L; international normalized ratio (INR) > 1.5; platelet counts > 90,000/mm³; renal impairment (creatinine > 1.2 mg/dL or >50% increase in baseline creatinine); bleeding; encephalopathy ≥ grade I [7] or seizures; mechanical ventilation; or hypotension requiring use of vasoconstrictive drugs.

The timeline of procedures is illustrated in Figure 1.

Figure 1. Timeline of procedures of SOFFA study.

Sample size

The sample size calculation considered the estimated effect of sofosbuvir in YF infected patients submitted to the compassionate use of this drug (Pinho JRR, Sofosbuvir's compassionate use for treatment of yellow fever during an epidemic).

Our primary outcome, median change in plasma YF viral levels at 72h after inclusion, will be indirectly measured by the cycle threshold value (Ct) of the RT-PCR.

Assuming a two-sided 5% significance level and a power of 80%, we estimated the sample size per group with different estimates of drug effect and dispersion (standard deviation) of the variable, as shown in Table 1.

Considering the presented estimates, the calculated sample size was defined as 90 participants (45 per group).

Table 1. Sample size based on different estimates of difference between groups (measured by increase in the cycle threshold value (Ct) at 72h after inclusion) and variant estimates of standard deviation.

Difference between groups	Standard deviation	Sample size (per group)
4	7.5	56
4	8.2	68
4.5	7.5	45
4.5	8.2	53
5	7.5	37
5	8.2	44
5.5	7.5	31
5.5	8.2	37

Recruitment

All consecutive eligible patients hospitalised in the participating centres with confirmed or suspected acute YF will be screened. The investigators will contact the hospital epidemiology surveillance team on a daily basis to capture all confirmed or suspected acute YF cases admitted in any unit of the hospital over the course of the study.

Assignment of interventions

The random allocation sequences were generated by one investigator not involved in participant screening or enrolment using the electronic platform Sealed Envelope.[8]
Allocation was created in a 1:1 proportion using permuted blocks of size 4 or 6 and stratified by recruiting centre and severity (moderate or severe strata).
The sequence was transferred to sequentially numbered, opaque, sealed envelopes that are maintained in locked cabinets accessible only to investigators performing screening and randomisation procedures.
Participants are randomised for severe stratum if they meet any criteria for intensive support.
Patients and healthcare providers will be unblinded regarding study allocation, but the intervention will not be disclosed to laboratory personnel running YF RT-PCR tests during the study.

Data collection methods

Clinical information and medical history will be obtained at enrolment directly from the participant, family members or medical charts. Laboratory test results will be retrieved from medical charts and laboratory reports.
Plasma concentrations of sofosbuvir will be determined in blood samples obtained daily during administration of sofosbuvir using liquid chromatography-tandem mass spectrometry (with a lower limit of quantification of 1 ng/mL).[9,10]
All study variables will be collected and managed using REDCap electronic data capture tools hosted at Faculdade de Medicina da Universidade de Sao Paulo.[11]

Data management

The electronic data collection tools will be developed using appropriate range checks and validation rules. Investigators will obtain access to the electronic forms using password-secure unique usernames. Internal data monitoring will be conducted during the trial for cross-validation of collected information.

Statistical Methods

The main analysis in the study will be based on the intention to treat principle. However, we will also include *per protocol* analysis and comparisons stratified by severity at

inclusion. Demographic and clinical characteristics will be presented using frequencies, means/medians, standard deviations and interquartile ranges with their corresponding 95% confidence intervals. Comparisons between groups will be made using the chi-square test or Fisher's exact test for categorical variables and T-test or Wilcoxon's rank-sum test for continuous variables as appropriate. The independent effect of sofosbuvir on the endpoints of viral kinetics and clinical outcomes will be analysed through multivariate models adjusted for the potential confounding factors not controlled by random allocation. The effect of Sofosbuvir on time to achieve undetectable YF RT-PCR in biologic specimens will be analysed using survival curves and Cox's proportional regression models adjusted for potential confounders.

Data monitoring

An independent Data Safety Monitoring Board (DMSB) was designated to oversee safety and welfare of study participants and provide recommendations based on reported adverse events (AE) and following interim analysis regarding whether the study should continue without change, be modified, or be terminated. The DSMB is composed of 3 consultants including one infectious diseases specialist, one intensive care physician and one epidemiologist. An interim analysis will be conducted after the inclusion of the first 60 participants. The DSMB will meet at the beginning of the study to determine specific methods for interim analysis and stopping guidelines.

Harms

Solicited and spontaneous adverse events will be monitored over the course of the study. Serious AE (SAE) will be reported to the DSMB and to local Institutional Review Board (IRB) as soon as identified by the study investigators. In addition, a report describing non-serious AE Grade ≥3 will be bimonthly provided to the DSMB and to the IRB. Additional information about the reported AEs, including the outcomes related to each AE, will be forwarded as soon as available.

We will consider as SAE those: resulting in death, representing life threat, requesting hospitalisation or extending current hospitalisation, resulting in persistent or significant disability, resulting in congenital abnormality or birth defect, or including important medical events, even if they do not represent life threat or hospitalisation, but that may be risky to the patient or may require intervention to prevent one or more those events listed above.

Patient and public involvement

There was no involvement of patients or public in the conception or conduction of this study. At any time, participants can be informed about study outcomes through the principal investigator.

ETHICS AND DISSEMINATION

This study will be conducted according to the Good Clinical Practices of the International Conference on Harmonization Good Clinical Practices (ICH-GCP), and to local and national regulation.

Ethics approval was obtained at the participating sites and at the national research ethics committee (CAAE 82673018.6.1001.0068). The trial is currently under evaluation by the research ethics committee at additional potential recruiting centres. Results of the study will be published in journals and presented at scientific meetings.

Written informed consent will be obtained for each participant. We will invite the patient directly to enrol in the study if possible, and a legal representative or family will be contacted to take part in the informed consent process if necessary.

Personal information about potential and enrolled participants will be collected and shared only through RedCap platform,[11] accessible only by authorized trial investigators. Personal identifier information will be linked to stored data or samples only by a protected master list kept under lock locally and not shared outside the study staff at the local partner site during and after the trial.

We used the SPIRIT checklist when writing this manuscript.[12]

The final trial dataset will be available only to investigators responsible for analysing the data.

Dissemination policy

Trial results will be communicated to healthcare professionals, the public, and other relevant groups via publication, reporting in results databases, or other data sharing arrangements as appropriate. Communication of trial results to participants will be done if a direct benefit or harm prevention is anticipated from disclosure of the study results.

Study sponsors will have no role in study design, data collection, analysis, interpretation of results, manuscript writing or decision to publish resulting reports.

REFERENCES

1 Ministério da Saúde. Monitoramento do Período Sazonal da Febre Amarela Brasil 2017-2018. http://portalarquivos2.saude.gov.br/images/pdf/2018/maio/09/Informe-FA.pdf) (accessed 26 Sep 2018).

2 Monath TP, Vasconcelos PFC. Yellow fever. *J Clin Virol* 2015;**64**:160–73. doi:10.1016/j.jcv.2014.08.030

3 Sacramento CQ, De Melo GR, De Freitas CS, *et al.* The clinically approved antiviral drug sofosbuvir inhibits Zika virus replication. *Sci Rep* 2017;**7**:1–11. doi:10.1038/srep40920

4 Bullard-Feibelman KM, Govero J, Zhu Z, *et al.* The FDA-approved drug sofosbuvir inhibits Zika virus infection. *Antiviral Res* 2017;**137**:134–40. doi:10.1016/j.antiviral.2016.11.023

5 de Freitas C, Higa L, Sacramento C, *et al.* Yellow fever virus is susceptible to sofosbuvir both in vitro and in vivo. *bioRxiv* 2018.

6 Xu HT, Colby-Germinario SP, Hassounah SA, *et al.* Evaluation of Sofosbuvir (β-D-2′-deoxy-2′-α-fluoro-2′-β-C-methyluridine) as an inhibitor of Dengue virus replication. *Sci Rep* 2017;**7**:1–11. doi:10.1038/s41598-017-06612-2

7 Blei AT, Ferenci P, Lockwood A, *et al.* Hepatic encephalopathy - Definition, nomenclature, diagnosis, and quantification: Final report of the Working Party at the 11th World Congresses of Gastroenterology, Vienna, 1998. *Hepatology* 2002;**35**:716–21. doi:10.1053/jhep.2002.31250

8 Sealed Envelope Ltd. Create a blocked randomisation list. 2017.https://www.sealedenvelope.com/simple-randomiser/v1/lists (accessed 8 May 2018).

9 Farnik H, El-Duweik J, Welsch C, *et al.* Highly sensitive determination of HCV protease inhibitors boceprevir (SCH 503034) and telaprevir (VX 950) in human plasma by LC-MS/MS. *J Chromatogr B Anal Technol Biomed Life Sci* 2009;**877**:4001–6. doi:10.1016/j.jchromb.2009.10.013

10 Jung BH, Rezk NL, Bridges AS, *et al.* Simultaneous determination of 17 antiretroviral drugs in human plasma for quantitative analysis with liquid chromatography-tandem mass spectrometry. *Biomed Chromatogr* 2007;**21**:1095–104. doi:10.1002/bmc.865

11 Harris PA, Taylor R, Thielke R, *et al.* Research electronic data capture (REDCap)-A

metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform* 2009;**42**:377–81. doi:10.1016/j.jbi.2008.08.010

12 Chan AW, Tetzlaff JM, Altman DG, *et al.* SPIRIT 2013 statement: Defining standard protocol items for clinical trials. Ann Intern Med. 2013;**158**:200–7. doi:10.7326/0003-4819-158-3-201302050-00583

AUTHORS´ CONTRIBUTIONS

Claudia Figueiredo-Mello, main writer of this manuscript, contributed to the conception and conduction of the work.

Luciana Vilas Boas Casadio, helped write the manuscript, contributed to the conception and conduction of the work.

Vivian Iida Avelino-Silva, helped write the manuscript, contributed to the conception and conduction of the work.

Yeh-Li Ho, helped write the manuscript, contributed to the conception and conduction of the work.

Jaques Sztajnbok, contributed to the conception and conduction of the work.

Daniel Joelsons, helped with the conduction of the work and assistance with the included patients.

Marilia Bordignon Antonio, helped including patients during the protocol.

João Renato Rebello Pinho, contributed to the conception and conduction of the work; planning and execution of molecular diagnosis.

Fernanda de Mello Malta, contributed to the conception and conduction of the work; planning and execution of molecular diagnosis

Michele Soares Gomes-Gouvêa, contributed to the conception and conduction of the work; planning and execution of molecular diagnosis.

Ana Paula Moreira Salles, contributed to the conception and conduction of the work; planning and execution of molecular diagnosis.

Aline Pivetta Corá, contributed to the conception and conduction of the work; planning and execution of molecular diagnosis.

Carlos Henrique Valente Moreira, contributed to the conception and logistic.

Ana Freitas Ribeiro, contributed to the conception and conduction of the work.

Ana Catharina de Seixas Santos Nastri, contributed to the conception and conduction of the work, helped with the assistance with the included patients.

Ceila Maria Sant'Ana Malaque, helped with the conduction of the work and assistance with the included patients.

Ralcyon Francis Azevedo Teixeira, contributed to the conception and conduction of the work.

Luciana Marques Sansão Borges, helped with the conduction of the work and assistance with the included patients.

Mario Peribañez Gonzalez, contributed to the conception and logistic.

Luiz Carlos Pereira Junior, contributed to the conception and logistic.

Tâmara Newman Lobato Souza, contributed to the conception of the study, the ICF and logistics.

Alice Tung Wan Song, contributed to the conception, conduction of the work, and assistance with the included patients

Luiz Augusto Carneiro D'Albuquerque, helped with the conduction of the work and assistance with the included patients.

Edson Abdala, helped with the conduction of the work and assistance with the included patients.

Wellington Andraus, helped with the conduction of the work and assistance with the included patients.

Rodrigo Bronze de Martino, helped with the conduction of the work and assistance with the included patients.

Liliana Ducatti, helped with the conduction of the work and assistance with the included patients.

Guilherme Marques Andrade, helped with the conduction of the work and assistance with the included patients.

Luiz Marcelo Sá Malbouisson, helped with the conduction of the work and assistance with the included patients.

Izabel Marcilio de Souza, helped including patients during the protocol and helped with writing the manuscript.

Flair José Carrilho, contributed to the conception and conduction of the work.

Ester Cerdeira Sabino, second main advisor of the work, guided all stages of the process.

Anna S Levin, main advisor of the work, guided all stages of the process, from conception of the study to revision of this manuscript.

FUNDING STATEMENT

This work was supported by FAPESP, São Paulo Foundation for Research [grant number 2016/01735-2] and Fundação Faculdade de Medicina USP.
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

COMPETING INTERESTS STATEMENT

Authors declare no financial and other competing interests for the overall trial design and execution.

	STUDY PERIOD											
	Enrolment	Allocation	Post-allocation									Close-out
TIMEPOINT	$-t_1$	0	D1	D2	D3	D4	D5	D6	D7	D10±1	D14±2	D28±3	D60±7
ENROLMENT:													
Eligibility screen	X												
Informed consent	X												
Allocation		X											
INTERVENTIONS:													
Active Group: Sofosbuvir + Standard care			←	—	—	—	—	—	—	→			
Control group: Standard care			←	—	—	—	—	—	—	—	—	—	→
ASSESSMENTS:													
Clinical evaluation			X	X	X	X	X	X	X	X	X	X	X
Adverse events			X	X	X	X	X	X	X	X	X	X	X
Plasma YF viral levels			X	X	X	X	X	X	X	X	X	X	
Urine YF viral levels			X	X	X	X	X	X	X	X	X	X	
Semen YF viral levels												X	
Sofosbuvir plasma levels			X	X	X	X	X	X	X	X	X	X	

YF: yellow fever. D1 corresponds to the allocation moment until 23h59; D2: from 0h until 23h59, D3: from 0h until 23h59, and so on. Clinical evaluation and adverse events assessments on D60 could be done by phone contact.

Figure 1. Timeline of procedures of SOFFA study.

254x142mm (300 x 300 DPI)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	4
	2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	3	Date and version identifier	4
Funding	4	Sources and types of financial, material, and other support	16
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1,2,3,14,15
	5b	Name and contact information for the trial sponsor	
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	16
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	6
	6b	Explanation for choice of comparators	6
Objectives	7	Specific objectives or hypotheses	6

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	6
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7,8
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	7,8
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	7,8
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7,8
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	8
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	8
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	8,9
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	9
Methods: Assignment of interventions (for controlled trials)			
Allocation:			

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	10
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	10
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	10
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	10

	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	10
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	10,11
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	10,11
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	11
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	12
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	12
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	12
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	12
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	17
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	12

Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	12
	31b	Authorship eligibility guidelines and any intended use of professional writers	14,15
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Efficacy of sofosbuvir as treatment for yellow fever: protocol for a randomised controlled trial (SOFFA study)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-027207.R1
Article Type:	Protocol
Date Submitted by the Author:	23-Jul-2019
Complete List of Authors:	Figueiredo-Mello, Claudia; Instituto de Infectologia Emilio Ribas, Department of Education and Research; Faculdade de Medicina da Universidade de Sao Paulo, Department of Infectious and Parasitic Diseases Casadio, Luciana ; Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Department of Infectious and Parasitic Diseases Avelino-Silva, Vivian ; Faculdade de Medicina da Universidade de Sao Paulo, Department of Infectious and Parasitic Diseases Yeh-Li, Ho; Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Department of Infectious and Parasitic Diseases Sztajnbok, Jaques ; Instituto de Infectologia Emilio Ribas, Medical Division-Intensive Care Unit Joelsons, Daniel ; Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Intensive Care Unit of the Department of Infectious and Parasitic Diseases Antonio, Marilia ; Faculdade de Medicina da Universidade de Sao Paulo, Department of Infectious and Parasitic Diseases Pinho, João ; Faculdade de Medicina da Universidade de Sao Paulo, LIM 07 - Department of Gastroenterology Malta, Fernanda ; Faculdade de Medicina da Universidade de Sao Paulo, LIM 07 - Department of Gastroenterology Gomes-Gouvêa, Michele ; Faculdade de Medicina da Universidade de Sao Paulo, LIM 07 - Department of Gastroenterology Salles, Ana; Faculdade de Medicina da Universidade de Sao Paulo, LIM 07 - Department of Gastroenterology Corá, Aline ; Instituto de Infectologia Emilio Ribas, Clinical Lab Moreira, Carlos ; Instituto de Infectologia Emilio Ribas, Inpatient unit Ribeiro, Ana ; Instituto de Infectologia Emilio Ribas, Epidemiology Service Nastri, Ana ; Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Department of Infectious and Parasitic Diseases Malaque, Ceila ; Instituto de Infectologia Emilio Ribas, Medical Division-Intensive Care Unit Teixeira, Ralcyon ; Instituto de Infectologia Emilio Ribas, Medical Division-Director Borges, Luciana ; Instituto de Infectologia Emilio Ribas, Medical Emergency Department Gonzalez, Mario ; Instituto de Infectologia Emilio Ribas, Outpatients

	clinic Junior, Luiz ; Instituto de Infectologia Emilio Ribas, Department Director Souza, Tâmara ; Instituto de Infectologia Emilio Ribas, ER and Outpatient Service Director Song, Alice ; Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Disciplina de Transplante de Figado e Orgaos do Aparelho Digestivo, Departamento de Gastroenterologia D'Albuquerque, Luiz ; Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Disciplina de Transplante de Figado e Orgaos do Aparelho Digestivo, Departamento de Gastroenterologia Abdala, Edson; Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Department of Infectious and Parasitic Diseases; Instituto do Cancer do Estado de Sao Paulo, Infectious Diseases Andraus, Wellington ; Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Disciplina de Transplante de Figado e Orgaos do Aparelho Digestivo, Departamento de Gastroenterologia Martino, Rodrigo ; Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Disciplina de Transplante de Figado e Orgaos do Aparelho Digestivo, Departamento de Gastroenterologia Ducatti, Liliana ; Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Disciplina de Transplante de Figado e Orgaos do Aparelho Digestivo, Departamento de Gastroenterologia Andrade, Guilherme ; Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Intensive Care Unit, Department of Gastroenterology Malbouisson, Luiz ; Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Intensive Care Unit, Department of Gastroenterology Souza, Izabel ; Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Epidemiologic Surveillance Department of the Hospital das Clínicas Carrilho, Flair; Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Departamento de Gastroenterologia Sabino, Ester; Universidade de Sao Paulo, Instituto de Medicina Tropical; Fundação Pró-Sangue, Hemocentro de São Paulo Levin, Anna; Faculdade de Medicina da Universidade de Sao Paulo, Department of Infectious and Parasitic Diseases
Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Research methods
Keywords:	Yellow Fever, Sofosbuvir, Randomized Controlled Trial, Viral Kinetics

Title:

Efficacy of sofosbuvir as treatment for yellow fever: protocol for a randomised controlled trial (SOFFA study)

Corresponding author:

Claudia Figueiredo-Mello

Postal address: Av. Doutor Arnaldo, 165 - 01246-900 - São Paulo - SP - Brazil

Email: claudiamello@ymail.com

Telephone number: +55 11 983019837

Co-authors:

Claudia Figueiredo-Mello, Department of Education and Research, Instituto de Infectologia Emilio Ribas, Department of Infectious and Parasitic Diseases, Faculdade de Medicina da Universidade de Sao Paulo, São Paulo, Brazil.

Luciana Vilas Boas Casadio, Department of Infectious and Parasitic Diseases, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.

Vivian Iida Avelino-Silva, Department of Infectious and Parasitic Diseases, Faculdade de Medicina da Universidade de Sao Paulo, São Paulo, Brazil.

Yeh-Li Ho, Department of Infectious and Parasitic Diseases, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.

Jaques Sztajnbok, Medical Division-Intensive Care Unit, Instituto de Infectologia Emilio Ribas, São Paulo, Brazil.

Daniel Joelsons, Intensive Care of the Department of Infectious and Parasitic Diseases, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.

Marilia Bordignon Antonio, Department of Infectious and Parasitic Diseases, Faculdade de Medicina da Universidade de Sao Paulo, São Paulo, Brazil.

João Renato Rebello Pinho, LIM 07 - Department of Gastroenterology, Faculdade de Medicina da Universidade de Sao Paulo, São Paulo, Brazil.

Fernanda de Mello Malta, LIM 07 - Department of Gastroenterology, Faculdade de Medicina da Universidade de Sao Paulo, São Paulo, Brazil.

Michele Soares Gomes-Gouvêa, LIM 07 - Department of Gastroenterology, Faculdade de Medicina da Universidade de Sao Paulo, São Paulo, Brazil.

Ana Paula Moreira Salles, LIM 07 - Department of Gastroenterology, Faculdade de Medicina da Universidade de Sao Paulo, São Paulo, Brazil.

Aline Pivetta Corá, Clinical Lab, Instituto de Infectologia Emilio Ribas, São Paulo, Brazil.
Carlos Henrique Valente Moreira, Inpatient Unit, Instituto de Infectologia Emilio Ribas, São Paulo, Brazil.
Ana Freitas Ribeiro, Epidemiology Service, Instituto de Infectologia Emilio Ribas, São Paulo, Brazil.
Ana Catharina de Seixas Santos Nastri, Department of Infectious and Parasitic Diseases, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.
Ceila Maria Sant'Ana Malaque, Medical Division-Intensive Care Unit, Instituto de Infectologia Emilio Ribas, São Paulo, Brazil.
Ralcyon Francis Azevedo Teixeira, Medical Division-Director, Instituto de Infectologia Emilio Ribas, São Paulo, Brazil.
Luciana Marques Sansão Borges, Medical Emergency Department, Instituto de Infectologia Emilio Ribas, São Paulo, Brazil.
Mario Peribañez Gonzalez, Outpatients clinic, Instituto de Infectologia Emilio Ribas, São Paulo, Brazil.
Luiz Carlos Pereira Junior, Department Director, Instituto de Infectologia Emilio Ribas, São Paulo, Brazil.
Tâmara Newman Lobato Souza, ER and Outpatient Service Director, Instituto de Infectologia Emilio Ribas, São Paulo, Brazil.
Alice Tung Wan Song, Disciplina de Transplante de Figado e Orgaos do Aparelho Digestivo, Departamento de Gastroenterologia, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.
Luiz Augusto Carneiro D'Albuquerque, Disciplina de Transplante de Figado e Orgaos do Aparelho Digestivo, Departamento de Gastroenterologia, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.
Edson Abdala, Department of Infectious and Parasitic Diseases, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.
Wellington Andraus, Disciplina de Transplante de Figado e Orgaos do Aparelho Digestivo, Departamento de Gastroenterologia, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.
Rodrigo Bronze de Martino, Disciplina de Transplante de Figado e Orgaos do Aparelho Digestivo, Departamento de Gastroenterologia, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.

Liliana Ducatti, Disciplina de Transplante de Figado e Orgaos do Aparelho Digestivo, Departamento de Gastroenterologia, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.
Guilherme Marques Andrade, Intensive Care Unit, Department of Gastroenterology, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.
Luiz Marcelo Sá Malbouisson, Intensive Care Unit, Department of Gastroenterology, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.
Izabel Marcilio de Souza, Epidemiologic Surveillance Department, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.
Flair José Carrilho, Department of Gastroenterology, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.
Ester Cerdeira Sabino, Instituto de Medicina Tropical, Universidade de Sao Paulo, São Paulo, Brazil.
Anna S Levin, Department of Infectious and Parasitic Diseases, Faculdade de Medicina da Universidade de Sao Paulo, Instituto de Medicina Tropical, Universidade de Sao Paulo, São Paulo, Brazil.

Word count:
2205

ABSTRACT

Introduction: An ongoing outbreak of yellow fever (YF) has been reported in Brazil with 1261 confirmed cases and 409 deaths since July 2017. To date, there is no specific treatment available for YF. Recently published papers describing *in vitro* and animal models suggest a potential effect of antiviral drugs (approved for the treatment of hepatitis virus) against flaviviruses, including YF. The primary aim of this study is to analyse the effect of sofosbuvir on viral kinetics and clinical outcomes among patients presenting with YF. This is a multicentre open-label randomised controlled trial with 1:1 individual allocation, stratified by severity and by recruiting centre.

Methods and analysis: Adults with suspected or confirmed YF infection and symptoms lasting up to 15 days are screened. Eligible and consenting patients are randomised to receive oral sofosbuvir 400mg daily for 10 days or to receive standard clinical care. Viral kinetics are measured daily and the reduction in YF plasma viral load from the sample at inclusion to 72h after randomization will be compared between active and control groups. Clinical outcomes include severity meeting criteria for intensive care support, liver transplantation, in-hospital mortality and mortality within 60 days.

Ethics and dissemination: Ethics approval was obtained at the participating sites and at the national research ethics committee (CAAE 82673018.6.1001.0068). The trial has been submitted for ethical approval at additional potential recruiting centres. Results of the study will be published in journals and presented at scientific meetings.

Trial Registration: Registered at Brazilian Clinical Trials Registry (RBR-93dp9n). This manuscript corresponds to trial version 1 (02/20/2018).

Keywords: Yellow Fever, Sofosbuvir, Randomized Controlled Trial, Viral Kinetics

ARTICLE SUMMARY

Strengths and limitations of this study

- In this open-label 1:1 parallel group randomized controlled trial, we will address the effect of oral Sofosbuvir at 400mg daily dose compared to standard clinical care for patients with yellow fever (YF) infection referred for hospitalization during a YF outbreak in Brazil
- Randomization will be done using an electronic platform, stratified by clinical severity and recruiting centre
- Median change in plasma YF viral levels at 72h after inclusion and clinical outcomes (meeting severity criteria for intensive care support, liver transplantation, in-hospital mortality, mortality within 60 days) will be compared between active and control groups
- An independent data safety monitoring group will be designated to supervise severe adverse events related to the study medication and to perform an interim analysis after the inclusion of 2/3 of predicted total sample size

INTRODUCTION

There is an ongoing outbreak of yellow fever (YF) in Brazil, with an increasing number of cases since 2016, notably in the Southern region. Between July 2017 and May 2018, 1261 confirmed YF cases were reported, with 409 deaths (mortality rate of 32.4%). Nowadays, a major part of population at risk for YF resides in newly affected areas (58.3%), where vaccination was not previously recommended.[1]

YF has a wide clinical spectrum, from asymptomatic to severe haemorrhagic disease associated with liver and renal failure and high case fatality rate. Vaccine safety remains a concern, especially because the yellow fever vaccine-associated viscerotropic disease (YFV-AVD) is a rare condition but has a high case-fatality rate. To date, there is no specific treatment available for YF or YFV-AVD.[2]

Recently published papers describing *in vitro* and animal models suggest a potential effect of sofosbuvir (an antiviral approved for the treatment of hepatitis C virus) for other flaviviruses, such as dengue, zika and YF.[3–6]

A group of researchers showed that sofosbuvir inhibited YF virus replication in different lineages of human hepatoma cells and reduced the YF-induced mortality and lack of weight gain in neonatal mice and suggested that sofosbuvir should be considered for clinical use in YF infected individuals.[5]

Another research team tested the *in vitro* activity against YF and zika virus of several antivirals used for hepatitis C virus (ribavirin, daclastavir, sofosbuvir and ledispavir/sofosbuvir combination). All the tested drugs presented activity and selectivity against YF and zika virus in human hepatoma cells, but ledispavir/sofosbuvir combination and sofosbuvir showed the best antiviral activity towards both viruses (Mendes EA, Pilger DRB, Santos Nastri ACS, Malta FM, Pascoalino BS, Carneiro D'Albuquerque LA, Balan A, Freitas Junior LH, Durigon EL, Carrilho FJ, Pinho JRR Sofosbuvir inhibits yellow fever virus in vitro and in patients with acute infection, 2018). Although there is some evidence of sofosbuvir activity against YF, there is no data from human studies as to the effect of sofosbuvir on acute YF.

We present the protocol for a multicentre open-label randomised controlled trial to analyse the effect of sofosbuvir on YF viral kinetics and on clinical outcomes (severity meeting criteria for intensive support, liver transplantation, in-hospital mortality and mortality within 60 days among patients presenting with acute YF). This is a multicentre open-label randomised controlled trial with 1:1 individual allocation, stratified by severity and by recruiting centre.

METHODS AND ANALYSIS

Study setting

The study will be conducted in secondary and tertiary healthcare units in Brazil. At this moment two recruiting centres are actively enrolling, both of whcih are tertiary teaching hospitals in São Paulo city (São Paulo State, Brazil). Six additional recruiting centres in the States of São Paulo and Minas Gerais are waiting for protocol ethical approval. The inclusion of participants has begun in March 2018 and the protocol is still in recruiting phase.

Eligibility criteria

Adults ≥ 18 years of age hospitalized at the recruiting centres with suspected or confirmed YF infection and with symptoms lasting up to 15 days are being screened. We are including patients with a history of fever (axillary temperature above 37.8°C), exposed to geographic areas with risk for YF transmission (according to updated epidemiological reports) and with transaminases (either alanine aminotransferase or aspartate aminotransferase) above 500 U/L.

We are excluding patients with amiodarone use in the last 72 hours, current pregnancy, women refusing to interrupt breastfeeding, current use of sofosbuvir for hepatitis C treatment, those already tested with a negative plasma YF real-time polymerase chain reaction (RT-PCR) prior to inclusion and those with another known aetiology that justified all signs and symptoms.

Interventions

Eligible participants are randomised to receive either sofosbuvir 400mg daily for 10 days or standard care. Patients should remain hospitalised for at least 10 days after the randomisation.

For those allocated to receive sofosbuvir, the oral route is preferred, although enteral tube administration is also acceptable.

The following criteria determine immediate interruption of sofosbuvir prescription: negative YF RT-PCR in a test performed at enrolment; amiodarone prescription; anaphylactic shock or grade III rash attributed to sofosbuvir; inability to receive oral or enteral tube medications.

In the case of sofosbuvir interruption, patients will continue to be followed-up for adverse events and outcomes according to the protocol.
In case of withdrawal of consent, the study medication and study procedures will be interrupted, but the participant will be asked to undergo safety evaluations according to the protocol if possible.

Outcomes

YF plasma viral load will be measured daily and median change in plasma YF viral levels at 72h after inclusion will be compared between active and control groups.
The YF RT-PCR assay employed has a sensitivity of 17000 copies/mL.
Clinical outcomes (severity meeting criteria for intensive support, liver transplantation, in-hospital mortality, mortality in 60 days) will also be compared between active and control groups. Time to YF RT-PCR clearance in biological samples and overall survival will also be evaluated.
Criteria for intensive support is defined by any of the following conditions: transaminases > 3000 U/L; international normalized ratio (INR) > 1.5; platelet counts > 90,000/mm³; renal impairment (creatinine > 1.2 mg/dL or >50% increase in baseline creatinine); bleeding; encephalopathy ≥ grade I [7] or seizures; mechanical ventilation; or hypotension requiring use of vasoconstrictive drugs.
The timeline of procedures is illustrated in Figure 1.

Figure 1**.** Timeline of procedures of SOFFA study.

Sample size

The sample size calculation considered the estimated effect of sofosbuvir in YF infected patients submitted to the compassionate use of this drug (Mendes EA, Pilger DRB, Santos Nastri ACS, Malta FM, Pascoalino BS, Carneiro D'Albuquerque LA, Balan A, Freitas Junior LH, Durigon EL, Carrilho FJ, Pinho JRR
Sofosbuvir inhibits yellow fever virus in vitro and in patients with acute infection, 2018).
Our primary outcome, median change in plasma YF viral levels at 72h after inclusion in the study, will be indirectly measured by the cycle threshold value (Ct) of the RT-PCR.
Assuming a two-sided 5% significance level and a power of 80%, we estimated the sample size per group with different estimates of drug effect and dispersion (standard deviation) of the variable, as shown in Table 1.
Considering the presented estimates, the calculated sample size was defined as 90 participants (45 per group).

Table 1. Sample size based on different estimates of difference between groups (measured by increase in the cycle threshold value (Ct) at 72h after inclusion) and variant estimates of standard deviation.

Difference between groups	Standard deviation	Sample size (per group)
4	7.5	56
4	8.2	68
4.5	7.5	45
4.5	8.2	53
5	7.5	37
5	8.2	44
5.5	7.5	31
5.5	8.2	37

Recruitment

All consecutive eligible patients hospitalised in the participating centres with confirmed or suspected acute YF will be screened. The investigators will contact the hospital epidemiology surveillance team on a daily basis to capture all confirmed or suspected acute YF cases admitted in any unit of the hospital over the course of the study.

Assignment of interventions

The random allocation sequences were generated by one investigator (not involved in participant screening or enrolment) using the electronic platform Sealed Envelope.[8] Allocation was created in a 1:1 proportion using permuted blocks of size 4 or 6 and stratified by recruiting centre and severity (moderate or severe strata).

The sequence was transferred to sequentially numbered, opaque, sealed envelopes which are maintained in locked cabinets accessible only to investigators performing screening and randomisation procedures.

Participants are randomised for severe stratum if they meet any criteria for intensive support.

Patients and healthcare providers will be not be blinded regarding study allocation, but the intervention will not be disclosed to laboratory personnel running YF RT-PCR tests during the study.

Data collection methods

Clinical information and medical history will be obtained at enrolment directly from the participant, family members or medical charts. Laboratory test results will be retrieved from medical charts and laboratory reports.

Plasma concentrations of sofosbuvir will be determined in blood samples obtained daily during administration of sofosbuvir using liquid chromatography-tandem mass spectrometry (with a lower limit of quantification of 1 ng/mL).[9,10]

All study variables will be collected and managed using REDCap electronic data capture tools hosted at the Faculdade de Medicina da Universidade de Sao Paulo (FMUSP).[11]

Data management

The electronic data collection tools will be developed using appropriate range checks and validation rules. Investigators will obtain access to the electronic forms using password-secure unique usernames. Internal data monitoring will be conducted during the trial for cross-validation of collected information.

Statistical Methods

The main analysis in the study will be based on the intention-to-treat principle. However, we will also include *per protocol* analysis and comparisons stratified by severity at inclusion. Demographic and clinical characteristics will be presented using frequencies, means/medians, standard deviations and interquartile ranges with their corresponding 95% confidence intervals. Comparisons between groups will be made using the chi-square test or Fisher's exact test for categorical variables and T-test or Wilcoxon's rank-sum test for continuous variables as appropriate. The independent effect of sofosbuvir on the endpoints of viral kinetics and of clinical outcomes will be analysed through multivariate models adjusted for the potential confounding factors not controlled by random allocation. The effect of Sofosbuvir on the time to achieve undetectable YF RT-PCR in biologic specimens will be analysed using survival curves and Cox's proportional regression models adjusted for potential confounders.

Data monitoring

An independent Data Safety Monitoring Board (DSMB) was designated to oversee the safety and welfare of study participants and to provide recommendations based on reported adverse events (AE). It will evaluate the interim analysis regarding whether the study should continue without change, be modified, or be terminated. The DSMB is composed of 3 consultants including one infectious diseases specialist, one intensive

care physician and one epidemiologist. An interim analysis will be conducted after the inclusion of the first 60 participants. The DSMB will meet at the beginning of the study to determine specific methods for interim analysis and stopping guidelines.

Harms

Solicited and spontaneous adverse events will be monitored over the course of the study. Serious AE (SAE) will be reported to the DSMB and to the local Institutional Review Board (IRB) as soon as identified by the study investigators. In addition, a report describing non-serious AE Grade ≥3 will be provided bimonthly to the DSMB and to the IRB. Additional information about the reported AEs, including the outcomes related to each AE, will be forwarded as soon as available.

We will consider as SAE those: resulting in death, representing life threat, requesting hospitalisation or extending current hospitalisation, resulting in persistent or significant disability, resulting in congenital abnormality or birth defect, or including important medical events, even if they do not represent life threat or hospitalisation, but that may be risky to the patient or may require intervention to prevent one or more of the events listed above.

Patient and public involvement

There was no involvement of patients or public in the conception or conduct of this study. At any time, participants can be informed about study outcomes through the principal investigator.

ETHICS AND DISSEMINATION

This study will be conducted according to the Good Clinical Practices of the International Conference on Harmonization Good Clinical Practices (ICH-GCP), and to local and national regulation.

Ethics approval was obtained at the participating sites (Comissão de Ética para Análise de Projetos de Pesquisa do Hospital das Clínicas da FMUSP and Comitê de Ética em Pesquisa do Instituto de Infectologia Emílio Ribas) and at the national research ethics committee (CAAE 82673018.6.1001.0068). The trial is currently under evaluation by the research ethics committee at additional potential recruiting centres. Results of the study will be published in journals and presented at scientific meetings.

Written informed consent will be obtained for each participant. We will invite the patient directly to enrol in the study if possible, and a legal representative or family will be contacted to take part in the informed consent process if necessary.

Personal information about potential and enrolled participants will be collected and shared only through RedCap platform,[11] accessible only by authorized trial investigators. Personal identifier information will be linked to stored data or samples solely by a protected master list kept under lock locally and will not be shared outside the study staff at the local partner site during and after the trial.

We used the SPIRIT checklist when writing this manuscript.[12]

The final trial dataset will be available only to investigators responsible for analysing the data.

Dissemination policy

Trial results will be communicated to healthcare professionals, the public, and other relevant groups via publication, reporting in results databases, or other data-sharing arrangements as appropriate. Communication of trial results to participants will be done if direct benefit or prevention of harm is anticipated from disclosure of the study results. Study sponsors will have no role in study design, data collection, analysis, interpretation of results, manuscript writing or decision to publish resulting reports.

REFERENCES

1 Ministério da Saúde. Monitoramento do Período Sazonal da Febre Amarela Brasil 2017-2018. http://portalarquivos2.saude.gov.br/images/pdf/2018/maio/09/Informe-FA.pdf) (accessed 26 Sep 2018).

2 Monath TP, Vasconcelos PFC. Yellow fever. *J Clin Virol* 2015;**64**:160–73. doi:10.1016/j.jcv.2014.08.030

3 Sacramento CQ, De Melo GR, De Freitas CS, *et al.* The clinically approved antiviral drug sofosbuvir inhibits Zika virus replication. *Sci Rep* 2017;**7**:1–11. doi:10.1038/srep40920

4 Bullard-Feibelman KM, Govero J, Zhu Z, *et al.* The FDA-approved drug sofosbuvir inhibits Zika virus infection. *Antiviral Res* 2017;**137**:134–40. doi:10.1016/j.antiviral.2016.11.023

5 de Freitas C, Higa L, Sacramento C, *et al.* Yellow fever virus is susceptible to sofosbuvir both in vitro and in vivo. *PLoS Negl Trop Dis* 2019;**13**:e0007072. doi: 10.1371/journal.pntd.0007072

6 Xu HT, Colby-Germinario SP, Hassounah SA, *et al.* Evaluation of Sofosbuvir (β-D-2′-deoxy-2′-α-fluoro-2′-β-C-methyluridine) as an inhibitor of Dengue virus replication. *Sci Rep* 2017;**7**:1–11. doi:10.1038/s41598-017-06612-2

7 Blei AT, Ferenci P, Lockwood A, *et al.* Hepatic encephalopathy - Definition, nomenclature, diagnosis, and quantification: Final report of the Working Party at the 11th World Congresses of Gastroenterology, Vienna, 1998. *Hepatology* 2002;**35**:716–21. doi:10.1053/jhep.2002.31250

8 Sealed Envelope Ltd. Create a blocked randomisation list. 2017.https://www.sealedenvelope.com/simple-randomiser/v1/lists (accessed 8 May 2018).

9 Farnik H, El-Duweik J, Welsch C, *et al.* Highly sensitive determination of HCV protease inhibitors boceprevir (SCH 503034) and telaprevir (VX 950) in human plasma by LC-MS/MS. *J Chromatogr B Anal Technol Biomed Life Sci* 2009;**877**:4001–6. doi:10.1016/j.jchromb.2009.10.013

10 Jung BH, Rezk NL, Bridges AS, *et al.* Simultaneous determination of 17 antiretroviral drugs in human plasma for quantitative analysis with liquid chromatography-tandem mass spectrometry. *Biomed Chromatogr* 2007;**21**:1095–104. doi:10.1002/bmc.865

11 Harris PA, Taylor R, Thielke R, *et al.* Research electronic data capture (REDCap)-A metadata-driven methodology and workflow process for providing

translational research informatics support. *J Biomed Inform* 2009;**42**:377–81. doi:10.1016/j.jbi.2008.08.010

12 Chan AW, Tetzlaff JM, Altman DG, *et al.* SPIRIT 2013 statement: Defining standard protocol items for clinical trials. Ann Intern Med. 2013;**158**:200–7. doi:10.7326/0003-4819-158-3-201302050-00583

CONTRIBUTORSHIP STATEMENT

CFM, LVBC, VIAS, YLH, JS, DJ, MBA, JRRP, FMM, MSGG, APMS, APC, CHVM, AFR, ACSSN, CMSM, RFAT, LMSB, MPG, LCPJ, TNLS, ATWS, LACA, EA, WA, RBM, LD, GMA, LMSM, IMS, FJC, ECS and ASL are the authors. CFM, CFM, LVBC, VIAS, YLH, JS, JRRP, FMM, MSGG, APMS, APC, CHVM, AFR, ACSSN, CMSM, RFAT, MPG, LCPJ, TNLS, ATWS, FJC, ECS and ASL conceived the idea of study and its design. CFM, LVBC, YLH, JS, DJ, MBA, CHVM, AFR, ACSSN, CMSM, RFAT, LMSB, ATWS, LACA, EA, WA, RBM, LD, GMA, LMSM, IMS are essential to data collection. CFM, LVBC and VIAS wrote the first draft of the manuscript. ECS and ASL provided advice and supervision. All authors met the criteria: (1) Substantial contributions to the conception or design of the work; or the acquisition, analysis or interpretation of data for the work. (2) Drafting the work or revising it critically for important intellectual content. (3) Final approval of the version to be published (4) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

ACKNOWLEDGEMENTS

We are thankful to Marjorie Shafferman for her review of the manuscript.

FUNDING STATEMENT

This work was supported by FAPESP, São Paulo Foundation for Research [grant number 2016/01735-2] and Fundação Faculdade de Medicina USP.
The funders had no role in study design, data collection and analysis, decision to publish, or in the preparation of the manuscript.

COMPETING INTERESTS STATEMENT

Authors declare no financial and other competing interests for the overall trial design and execution.

	STUDY PERIOD											
	Enrolment	Allocation	Post-allocation									Close-out
TIMEPOINT	$-t_1$	0	D1	D2	D3	D4	D5	D6	D7	D10±1	D14±2	D28±3	D60±7
ENROLMENT:													
Eligibility screen	X												
Informed consent	X												
Allocation		X											
INTERVENTIONS:													
Active Group: Sofosbuvir + Standard care			←	—	—	—	—	—	—	→			
Control group: Standard care			←	—	—	—	—	—	—	—	—	—	→
ASSESSMENTS:													
Clinical evaluation			X	X	X	X	X	X	X	X	X	X	X
Adverse events			X	X	X	X	X	X	X	X	X	X	X
Plasma YF viral levels			X	X	X	X	X	X	X	X	X	X	
Urine YF viral levels			X	X	X	X	X	X	X	X	X	X	
Semen YF viral levels												X	
Sofosbuvir plasma levels			X	X	X	X	X	X	X	X	X	X	

YF: yellow fever. D1 corresponds to the allocation moment until 23h59; D2: from 0h until 23h59, D3: from 0h until 23h59, and so on. Clinical evaluation and adverse events assessments on D60 could be done by phone contact.

Figure 1. Timeline of procedures of SOFFA study.

254x142mm (300 x 300 DPI)

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	4
	2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	3	Date and version identifier	4
Funding	4	Sources and types of financial, material, and other support	16
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1,2,3,14,15
	5b	Name and contact information for the trial sponsor	
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	16
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	6
	6b	Explanation for choice of comparators	6
Objectives	7	Specific objectives or hypotheses	6

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	6
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7,8
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	7,8
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	7,8
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7,8
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	8
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	8
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	8,9
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	9
Methods: Assignment of interventions (for controlled trials)			
Allocation:			

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	10
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	10
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	10
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	10

	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	10
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	10,11
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	10,11
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	11
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	12
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	12
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	12
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	12
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	17
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	12

Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	12
	31b	Authorship eligibility guidelines and any intended use of professional writers	14,15
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Efficacy of sofosbuvir as treatment for yellow fever: protocol for a randomised controlled trial in Brazil (SOFFA study)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-027207.R2
Article Type:	Protocol
Date Submitted by the Author:	30-Aug-2019
Complete List of Authors:	Figueiredo-Mello, Claudia; Instituto de Infectologia Emilio Ribas, Department of Education and Research; Universidade de São Paulo Faculdade de Medicina, Department of Infectious and Parasitic Diseases Casadio, Luciana ; Universidade de São Paulo Hospital das Clínicas, Department of Infectious and Parasitic Diseases Avelino-Silva, Vivian ; Universidade de São Paulo Faculdade de Medicina, Department of Infectious and Parasitic Diseases Yeh-Li, Ho; Universidade de São Paulo Hospital das Clínicas, Department of Infectious and Parasitic Diseases Sztajnbok, Jaques ; Instituto de Infectologia Emilio Ribas, Medical Division-Intensive Care Unit Joelsons, Daniel ; Universidade de São Paulo Hospital das Clínicas, Department of Infectious and Parasitic Diseases Antonio, Marilia ; Universidade de São Paulo Faculdade de Medicina, Department of Infectious and Parasitic Diseases Pinho, João ; Universidade de São Paulo Faculdade de Medicina, LIM 07 - Department of Gastroenterology Malta, Fernanda ; Universidade de São Paulo Faculdade de Medicina, LIM 07 - Department of Gastroenterology Gomes-Gouvêa, Michele ; Universidade de São Paulo Faculdade de Medicina, LIM 07 - Department of Gastroenterology Salles, Ana; Universidade de São Paulo Faculdade de Medicina, LIM 07 - Department of Gastroenterology Corá, Aline ; Instituto de Infectologia Emilio Ribas, Clinical Lab Moreira, Carlos ; Instituto de Infectologia Emilio Ribas, Inpatient unit Ribeiro, Ana ; Instituto de Infectologia Emilio Ribas, Epidemiology Service Nastri, Ana ; Universidade de São Paulo Hospital das Clínicas, Department of Infectious and Parasitic Diseases Malaque, Ceila ; Instituto de Infectologia Emilio Ribas, Medical Division-Intensive Care Unit Teixeira, Ralcyon ; Instituto de Infectologia Emilio Ribas, Medical Division-Director Borges, Luciana ; Instituto de Infectologia Emilio Ribas, Medical Emergency Department Gonzalez, Mario ; Instituto de Infectologia Emilio Ribas, Outpatients clinic Junior, Luiz ; Instituto de Infectologia Emilio Ribas, Department Director Souza, Tâmara ; Instituto de Infectologia Emilio Ribas, ER and

	Outpatient Service Director Song, Alice ; Universidade de São Paulo Hospital das Clínicas, Departamento de Gastroenterologia D'Albuquerque, Luiz ; Universidade de São Paulo Hospital das Clínicas, Departamento de Gastroenterologia Abdala, Edson; Universidade de São Paulo Hospital das Clínicas, Department of Infectious and Parasitic Diseases; Universidade de Sao Paulo Instituto do Cancer do Estado de Sao Paulo, Infectious Diseases Andraus, Wellington ; Universidade de São Paulo Hospital das Clínicas, Departamento de Gastroenterologia Martino, Rodrigo ; Universidade de São Paulo Hospital das Clínicas, Departamento de Gastroenterologia Ducatti, Liliana ; Universidade de São Paulo Hospital das Clínicas, Departamento de Gastroenterologia Andrade, Guilherme ; Universidade de São Paulo Hospital das Clínicas, Departamento de Gastroenterologia Malbouisson, Luiz ; Universidade de São Paulo Hospital das Clínicas, Department of Gastroenterology Souza, Izabel ; Universidade de São Paulo Hospital das Clínicas, Epidemiologic Surveillance Department Carrilho, Flair; Universidade de São Paulo Hospital das Clínicas, Departamento de Gastroenterologia Sabino, Ester; Universidade de Sao Paulo, Instituto de Medicina Tropical; Fundação Pró-Sangue, Hemocentro de São Paulo Levin, Anna; Universidade de São Paulo Faculdade de Medicina, Department of Infectious and Parasitic Diseases
Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Research methods
Keywords:	Yellow Fever, Sofosbuvir, Randomized Controlled Trial, Viral Kinetics

Title:

Efficacy of sofosbuvir as treatment for yellow fever: protocol for a randomised controlled trial in Brazil (SOFFA study)

Corresponding author:

Claudia Figueiredo-Mello

Postal address: Av. Doutor Arnaldo, 165 - 01246-900 - São Paulo - SP - Brazil

Email: claudiamello@ymail.com

Telephone number: +55 11 983019837

Co-authors:

Claudia Figueiredo-Mello, Department of Education and Research, Instituto de Infectologia Emilio Ribas, Department of Infectious and Parasitic Diseases, Faculdade de Medicina da Universidade de Sao Paulo, São Paulo, Brazil.

Luciana Vilas Boas Casadio, Department of Infectious and Parasitic Diseases, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.

Vivian Iida Avelino-Silva, Department of Infectious and Parasitic Diseases, Faculdade de Medicina da Universidade de Sao Paulo, São Paulo, Brazil.

Yeh-Li Ho, Department of Infectious and Parasitic Diseases, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.

Jaques Sztajnbok, Medical Division-Intensive Care Unit, Instituto de Infectologia Emilio Ribas, São Paulo, Brazil.

Daniel Joelsons, Intensive Care of the Department of Infectious and Parasitic Diseases, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.

Marilia Bordignon Antonio, Department of Infectious and Parasitic Diseases, Faculdade de Medicina da Universidade de Sao Paulo, São Paulo, Brazil.

João Renato Rebello Pinho, LIM 07 - Department of Gastroenterology, Faculdade de Medicina da Universidade de Sao Paulo, São Paulo, Brazil.

Fernanda de Mello Malta, LIM 07 - Department of Gastroenterology, Faculdade de Medicina da Universidade de Sao Paulo, São Paulo, Brazil.

Michele Soares Gomes-Gouvêa, LIM 07 - Department of Gastroenterology, Faculdade de Medicina da Universidade de Sao Paulo, São Paulo, Brazil.

Ana Paula Moreira Salles, LIM 07 - Department of Gastroenterology, Faculdade de Medicina da Universidade de Sao Paulo, São Paulo, Brazil.

Aline Pivetta Corá, Clinical Lab, Instituto de Infectologia Emilio Ribas, São Paulo, Brazil.
Carlos Henrique Valente Moreira, Inpatient Unit, Instituto de Infectologia Emilio Ribas, São Paulo, Brazil.
Ana Freitas Ribeiro, Epidemiology Service, Instituto de Infectologia Emilio Ribas, São Paulo, Brazil.
Ana Catharina de Seixas Santos Nastri, Department of Infectious and Parasitic Diseases, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.
Ceila Maria Sant'Ana Malaque, Medical Division-Intensive Care Unit, Instituto de Infectologia Emilio Ribas, São Paulo, Brazil.
Ralcyon Francis Azevedo Teixeira, Medical Division-Director, Instituto de Infectologia Emilio Ribas, São Paulo, Brazil.
Luciana Marques Sansão Borges, Medical Emergency Department, Instituto de Infectologia Emilio Ribas, São Paulo, Brazil.
Mario Peribañez Gonzalez, Outpatients clinic, Instituto de Infectologia Emilio Ribas, São Paulo, Brazil.
Luiz Carlos Pereira Junior, Department Director, Instituto de Infectologia Emilio Ribas, São Paulo, Brazil.
Tâmara Newman Lobato Souza, ER and Outpatient Service Director, Instituto de Infectologia Emilio Ribas, São Paulo, Brazil.
Alice Tung Wan Song, Disciplina de Transplante de Figado e Orgaos do Aparelho Digestivo, Departamento de Gastroenterologia, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.
Luiz Augusto Carneiro D'Albuquerque, Disciplina de Transplante de Figado e Orgaos do Aparelho Digestivo, Departamento de Gastroenterologia, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.
Edson Abdala, Department of Infectious and Parasitic Diseases, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.
Wellington Andraus, Disciplina de Transplante de Figado e Orgaos do Aparelho Digestivo, Departamento de Gastroenterologia, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.
Rodrigo Bronze de Martino, Disciplina de Transplante de Figado e Orgaos do Aparelho Digestivo, Departamento de Gastroenterologia, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.

Liliana Ducatti, Disciplina de Transplante de Figado e Orgaos do Aparelho Digestivo, Departamento de Gastroenterologia, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.
Guilherme Marques Andrade, Intensive Care Unit, Department of Gastroenterology, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.
Luiz Marcelo Sá Malbouisson, Intensive Care Unit, Department of Gastroenterology, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.
Izabel Marcilio de Souza, Epidemiologic Surveillance Department, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.
Flair José Carrilho, Department of Gastroenterology, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo HCFMUSP, São Paulo, Brazil.
Ester Cerdeira Sabino, Instituto de Medicina Tropical, Universidade de Sao Paulo, São Paulo, Brazil.
Anna S Levin, Department of Infectious and Parasitic Diseases, Faculdade de Medicina da Universidade de Sao Paulo, Instituto de Medicina Tropical, Universidade de Sao Paulo, São Paulo, Brazil.

Word count:
2248

ABSTRACT

Introduction: An ongoing outbreak of yellow fever (YF) has been reported in Brazil with 1261 confirmed cases and 409 deaths since July 2017. To date, there is no specific treatment available for YF. Recently published papers describing *in vitro* and animal models suggest a potential effect of antiviral drugs (approved for the treatment of hepatitis virus) against flaviviruses, including YF. The primary aim of this study is to analyse the effect of sofosbuvir on viral kinetics and clinical outcomes among patients presenting with YF. This is a multicentre open-label randomised controlled trial with 1:1 individual allocation, stratified by severity and by recruiting centre.

Methods and analysis: Adults with suspected or confirmed YF infection and symptoms lasting up to 15 days are screened. Eligible and consenting patients are randomised to receive oral sofosbuvir 400mg daily for 10 days or to receive standard clinical care. Viral kinetics are measured daily and the reduction in YF plasma viral load from the sample at inclusion to 72h after randomization will be compared between active and control groups. Clinical outcomes include severity meeting criteria for intensive care support, liver transplantation, in-hospital mortality and mortality within 60 days.

Ethics and dissemination: Ethics approval was obtained at the participating sites and at the national research ethics committee (CAAE 82673018.6.1001.0068). The trial has been submitted for ethical approval at additional potential recruiting centres. Results of the study will be published in journals and presented at scientific meetings.

Trial Registration: Registered at Brazilian Clinical Trials Registry (RBR-93dp9n). This manuscript corresponds to trial version 1 (02/20/2018).

Keywords: Yellow Fever, Sofosbuvir, Randomized Controlled Trial, Viral Kinetics

ARTICLE SUMMARY

Strengths and limitations of this study

- In this open-label 1:1 parallel group randomized controlled trial, we will address the effect of oral Sofosbuvir at 400mg daily dose compared to standard clinical care for patients with yellow fever (YF) infection referred for hospitalization during a YF outbreak in Brazil
- Randomization will be done using an electronic platform, stratified by clinical severity and recruiting centre
- Median change in plasma YF viral levels at 72h after inclusion and clinical outcomes (meeting severity criteria for intensive care support, liver transplantation, in-hospital mortality, mortality within 60 days) will be compared between active and control groups
- An independent data safety monitoring group will be designated to supervise severe adverse events related to the study medication and to perform an interim analysis after the inclusion of 2/3 of predicted total sample size

INTRODUCTION

There is an ongoing outbreak of yellow fever (YF) in Brazil, with an increasing number of cases since 2016, notably in the Southern region. Between July 2017 and May 2018, 1261 confirmed YF cases were reported, with 409 deaths (mortality rate of 32.4%). Nowadays, a major part of population at risk for YF resides in newly affected areas (58.3%), where vaccination was not previously recommended.[1]

YF has a wide clinical spectrum, from asymptomatic to severe haemorrhagic disease associated with liver and renal failure and high case fatality rate. Vaccine safety remains a concern, especially because the yellow fever vaccine-associated viscerotropic disease (YFV-AVD) is a rare condition but has a high case-fatality rate. To date, there is no specific treatment available for YF or YFV-AVD.[2]

Recently published papers describing *in vitro* and animal models suggest a potential effect of sofosbuvir (an antiviral approved for the treatment of hepatitis C virus) for other flaviviruses, such as dengue, zika and YF.[3–6]

A group of researchers showed that sofosbuvir inhibited YF virus replication in different lineages of human hepatoma cells and reduced the YF-induced mortality and lack of weight gain in neonatal mice and suggested that sofosbuvir should be considered for clinical use in YF infected individuals.[5]

Another research team tested the *in vitro* activity against YF and zika virus of several antivirals used for hepatitis C virus (ribavirin, daclastavir, sofosbuvir and ledispavir/sofosbuvir combination). All the tested drugs presented activity and selectivity against YF and zika virus in human hepatoma cells, but ledispavir/sofosbuvir combination and sofosbuvir showed the best antiviral activity towards both viruses (Mendes EA, Pilger DRB, Santos Nastri ACS, Malta FM, Pascoalino BS, Carneiro D'Albuquerque LA, Balan A, Freitas Junior LH, Durigon EL, Carrilho FJ, Pinho JRR Sofosbuvir inhibits yellow fever virus in vitro and in patients with acute infection, 2018). Although there is some evidence of sofosbuvir activity against YF, there is no data from human studies as to the effect of sofosbuvir on acute YF.

We present the protocol for a multicentre open-label randomised controlled trial to analyse the effect of sofosbuvir on YF viral kinetics and on clinical outcomes (severity meeting criteria for intensive support, liver transplantation, in-hospital mortality and mortality within 60 days among patients presenting with acute YF). This is a multicentre open-label randomised controlled trial with 1:1 individual allocation, stratified by severity and by recruiting centre.

METHODS AND ANALYSIS

Study setting

The study will be conducted in secondary and tertiary healthcare units in Brazil. At this moment two recruiting centres are actively enrolling, both of whcih are tertiary teaching hospitals in São Paulo city (São Paulo State, Brazil). Six additional recruiting centres in the States of São Paulo and Minas Gerais are waiting for protocol ethical approval. The inclusion of participants has begun in March 2018 and the protocol is still in recruiting phase.

Eligibility criteria

Adults ≥ 18 years of age hospitalized at the recruiting centres with suspected or confirmed YF infection and with symptoms lasting up to 15 days are being screened. We are including patients with a history of fever (axillary temperature above 37.8°C), exposed to geographic areas with risk for YF transmission (according to updated epidemiological reports) and with transaminases (either alanine aminotransferase or aspartate aminotransferase) above 500 U/L.

We are excluding patients with amiodarone use in the last 72 hours, current pregnancy, women refusing to interrupt breastfeeding, current use of sofosbuvir for hepatitis C treatment, those already tested with a negative plasma YF real-time polymerase chain reaction (RT-PCR) prior to inclusion and those with another known aetiology that justified all signs and symptoms.

Interventions

Eligible participants are randomised to receive either sofosbuvir 400mg daily for 10 days or standard care. Patients should remain hospitalised for at least 10 days after the randomisation.

For those allocated to receive sofosbuvir, the oral route is preferred, although enteral tube administration is also acceptable.

The following criteria determine immediate interruption of sofosbuvir prescription: negative YF RT-PCR in a test performed at enrolment; amiodarone prescription; anaphylactic shock or grade III rash attributed to sofosbuvir; inability to receive oral or enteral tube medications.

In the case of sofosbuvir interruption, patients will continue to be followed-up for adverse events and outcomes according to the protocol.
In case of withdrawal of consent, the study medication and study procedures will be interrupted, but the participant will be asked to undergo safety evaluations according to the protocol if possible.

Outcomes

YF plasma viral load will be measured daily and median change in plasma YF viral levels at 72h after inclusion will be compared between active and control groups.
The YF RT-PCR assay employed has a sensitivity of 17000 copies/mL.
Clinical outcomes (severity meeting criteria for intensive support, liver transplantation, in-hospital mortality, mortality in 60 days) will also be compared between active and control groups. Time to YF RT-PCR clearance in biological samples and overall survival will also be evaluated.
Criteria for intensive support is defined by any of the following conditions: transaminases > 3000 U/L; international normalized ratio (INR) > 1.5; platelet counts > 90,000/mm³; renal impairment (creatinine > 1.2 mg/dL or >50% increase in baseline creatinine); bleeding; encephalopathy ≥ grade I [7] or seizures; mechanical ventilation; or hypotension requiring use of vasoconstrictive drugs.
The timeline of procedures is illustrated in Figure 1.

Figure 1**.** Timeline of procedures of SOFFA study.

Sample size

The sample size calculation considered the estimated effect of sofosbuvir in YF infected patients submitted to the compassionate use of this drug (Mendes EA, Pilger DRB, Santos Nastri ACS, Malta FM, Pascoalino BS, Carneiro D'Albuquerque LA, Balan A, Freitas Junior LH, Durigon EL, Carrilho FJ, Pinho JRR
Sofosbuvir inhibits yellow fever virus in vitro and in patients with acute infection, 2018).
Our primary outcome, median change in plasma YF viral levels at 72h after inclusion in the study, will be indirectly measured by the cycle threshold value (Ct) of the RT-PCR.
Assuming a two-sided 5% significance level and a power of 80%, we estimated the sample size per group with different estimates of drug effect and dispersion (standard deviation) of the variable, as shown in Table 1.
Considering the presented estimates, the calculated sample size was defined as 90 participants (45 per group).

Table 1. Sample size based on different estimates of difference between groups (measured by increase in the cycle threshold value (Ct) at 72h after inclusion) and variant estimates of standard deviation.

Difference between groups	Standard deviation	Sample size (per group)
4	7.5	56
4	8.2	68
4.5	7.5	45
4.5	8.2	53
5	7.5	37
5	8.2	44
5.5	7.5	31
5.5	8.2	37

Recruitment

All consecutive eligible patients hospitalised in the participating centres with confirmed or suspected acute YF will be screened. The investigators will contact the hospital epidemiology surveillance team on a daily basis to capture all confirmed or suspected acute YF cases admitted in any unit of the hospital over the course of the study.

Assignment of interventions

The random allocation sequences were generated by one investigator (not involved in participant screening or enrolment) using the electronic platform Sealed Envelope.[8] Allocation was created in a 1:1 proportion using permuted blocks of size 4 or 6 and stratified by recruiting centre and severity (moderate or severe strata).

The sequence was transferred to sequentially numbered, opaque, sealed envelopes which are maintained in locked cabinets accessible only to investigators performing screening and randomisation procedures.

Participants are randomised for severe stratum if they meet any criteria for intensive support.

Patients and healthcare providers will be not be blinded regarding study allocation, but the intervention will not be disclosed to laboratory personnel running YF RT-PCR tests during the study.

Data collection methods

Clinical information and medical history will be obtained at enrolment directly from the participant, family members or medical charts. Laboratory test results will be retrieved from medical charts and laboratory reports.

Dialysis requirement will be assessed daily by the attending physicians and the evaluated criteria such as creatinine levels, urine output, metabolic acidosis, catecholamine infusion, mechanical ventilation encephalopathy grade and active bleeding will be recorded, as well as the receipt of renal replacement therapy.

Plasma concentrations of sofosbuvir will be determined in blood samples obtained daily during administration of sofosbuvir using liquid chromatography-tandem mass spectrometry (with a lower limit of quantification of 1 ng/mL).[9,10]

All study variables will be collected and managed using REDCap electronic data capture tools hosted at the Faculdade de Medicina da Universidade de Sao Paulo (FMUSP).[11]

Data management

The electronic data collection tools will be developed using appropriate range checks and validation rules. Investigators will obtain access to the electronic forms using password-secure unique usernames. Internal data monitoring will be conducted during the trial for cross-validation of collected information.

Statistical Methods

The main analysis in the study will be based on the intention-to-treat principle. However, we will also include *per protocol* analysis and comparisons stratified by severity at inclusion. Demographic and clinical characteristics will be presented using frequencies, means/medians, standard deviations and interquartile ranges with their corresponding 95% confidence intervals. Comparisons between groups will be made using the chi-square test or Fisher's exact test for categorical variables and T-test or Wilcoxon's rank-sum test for continuous variables as appropriate. The independent effect of sofosbuvir on the endpoints of viral kinetics and of clinical outcomes will be analysed through multivariate models adjusted for the potential confounding factors not controlled by random allocation. The effect of Sofosbuvir on the time to achieve undetectable YF RT-PCR in biologic specimens will be analysed using survival curves and Cox's proportional regression models adjusted for potential confounders.

Data monitoring

An independent Data Safety Monitoring Board (DSMB) was designated to oversee the safety and welfare of study participants and to provide recommendations based on reported adverse events (AE). It will evaluate the interim analysis regarding whether the study should continue without change, be modified, or be terminated. The DSMB is composed of 3 consultants including one infectious diseases specialist, one intensive care physician and one epidemiologist. An interim analysis will be conducted after the inclusion of the first 60 participants. The DSMB will meet at the beginning of the study to determine specific methods for interim analysis and stopping guidelines.

Harms

Solicited and spontaneous adverse events will be monitored over the course of the study. Serious AE (SAE) will be reported to the DSMB and to the local Institutional Review Board (IRB) as soon as identified by the study investigators. In addition, a report describing non-serious AE Grade ≥3 will be provided bimonthly to the DSMB and to the IRB. Additional information about the reported AEs, including the outcomes related to each AE, will be forwarded as soon as available.

We will consider as SAE those: resulting in death, representing life threat, requesting hospitalisation or extending current hospitalisation, resulting in persistent or significant disability, resulting in congenital abnormality or birth defect, or including important medical events, even if they do not represent life threat or hospitalisation, but that may be risky to the patient or may require intervention to prevent one or more of the events listed above.

Patient and public involvement

There was no involvement of patients or public in the conception or conduct of this study. At any time, participants can be informed about study outcomes through the principal investigator.

ETHICS AND DISSEMINATION

This study will be conducted according to the Good Clinical Practices of the International Conference on Harmonization Good Clinical Practices (ICH-GCP), and to local and national regulation.

Ethics approval was obtained at the participating sites (Comissão de Ética para Análise de Projetos de Pesquisa do Hospital das Clínicas da FMUSP and Comitê de Ética em Pesquisa do Instituto de Infectologia Emílio Ribas) and at the national research ethics committee (CAAE 82673018.6.1001.0068). The trial is currently under evaluation by the research ethics committee at additional potential recruiting centres. Results of the study will be published in journals and presented at scientific meetings.

Written informed consent will be obtained for each participant. We will invite the patient directly to enrol in the study if possible, and a legal representative or family will be contacted to take part in the informed consent process if necessary.

Personal information about potential and enrolled participants will be collected and shared only through RedCap platform,[11] accessible only by authorized trial investigators. Personal identifier information will be linked to stored data or samples solely by a protected master list kept under lock locally and will not be shared outside the study staff at the local partner site during and after the trial.

We used the SPIRIT checklist when writing this manuscript.[12]

The final trial dataset will be available only to investigators responsible for analysing the data.

Dissemination policy

Trial results will be communicated to healthcare professionals, the public, and other relevant groups via publication, reporting in results databases, or other data-sharing arrangements as appropriate. Communication of trial results to participants will be done if direct benefit or prevention of harm is anticipated from disclosure of the study results. Study sponsors will have no role in study design, data collection, analysis, interpretation of results, manuscript writing or decision to publish resulting reports.

REFERENCES

1 Ministério da Saúde. Monitoramento do Período Sazonal da Febre Amarela Brasil 2017-2018. http://portalarquivos2.saude.gov.br/images/pdf/2018/maio/09/Informe-FA.pdf) (accessed 26 Sep 2018).

2 Monath TP, Vasconcelos PFC. Yellow fever. *J Clin Virol* 2015;**64**:160–73. doi:10.1016/j.jcv.2014.08.030

3 Sacramento CQ, De Melo GR, De Freitas CS, *et al.* The clinically approved antiviral drug sofosbuvir inhibits Zika virus replication. *Sci Rep* 2017;**7**:1–11. doi:10.1038/srep40920

4 Bullard-Feibelman KM, Govero J, Zhu Z, *et al.* The FDA-approved drug sofosbuvir inhibits Zika virus infection. *Antiviral Res* 2017;**137**:134–40. doi:10.1016/j.antiviral.2016.11.023

5 de Freitas C, Higa L, Sacramento C, *et al.* Yellow fever virus is susceptible to sofosbuvir both in vitro and in vivo. *PLoS Negl Trop Dis* 2019;**13**:e0007072. doi: 10.1371/journal.pntd.0007072

6 Xu HT, Colby-Germinario SP, Hassounah SA, *et al.* Evaluation of Sofosbuvir (β-D-2′-deoxy-2′-α-fluoro-2′-β-C-methyluridine) as an inhibitor of Dengue virus replication. *Sci Rep* 2017;**7**:1–11. doi:10.1038/s41598-017-06612-2

7 Blei AT, Ferenci P, Lockwood A, *et al.* Hepatic encephalopathy - Definition, nomenclature, diagnosis, and quantification: Final report of the Working Party at the 11th World Congresses of Gastroenterology, Vienna, 1998. *Hepatology* 2002;**35**:716–21. doi:10.1053/jhep.2002.31250

8 Sealed Envelope Ltd. Create a blocked randomisation list. 2017.https://www.sealedenvelope.com/simple-randomiser/v1/lists (accessed 8 May 2018).

9 Farnik H, El-Duweik J, Welsch C, *et al.* Highly sensitive determination of HCV protease inhibitors boceprevir (SCH 503034) and telaprevir (VX 950) in human plasma by LC-MS/MS. *J Chromatogr B Anal Technol Biomed Life Sci* 2009;**877**:4001–6. doi:10.1016/j.jchromb.2009.10.013

10 Jung BH, Rezk NL, Bridges AS, *et al.* Simultaneous determination of 17 antiretroviral drugs in human plasma for quantitative analysis with liquid chromatography-tandem mass spectrometry. *Biomed Chromatogr* 2007;**21**:1095–104. doi:10.1002/bmc.865

11 Harris PA, Taylor R, Thielke R, *et al.* Research electronic data capture (REDCap)-A metadata-driven methodology and workflow process for providing

translational research informatics support. *J Biomed Inform* 2009;**42**:377–81. doi:10.1016/j.jbi.2008.08.010

12 Chan AW, Tetzlaff JM, Altman DG, *et al.* SPIRIT 2013 statement: Defining standard protocol items for clinical trials. Ann Intern Med. 2013;**158**:200–7. doi:10.7326/0003-4819-158-3-201302050-00583

CONTRIBUTORSHIP STATEMENT

CFM, LVBC, VIAS, YLH, JS, DJ, MBA, JRRP, FMM, MSGG, APMS, APC, CHVM, AFR, ACSSN, CMSM, RFAT, LMSB, MPG, LCPJ, TNLS, ATWS, LACA, EA, WA, RBM, LD, GMA, LMSM, IMS, FJC, ECS and ASL are the authors. CFM, CFM, LVBC, VIAS, YLH, JS, JRRP, FMM, MSGG, APMS, APC, CHVM, AFR, ACSSN, CMSM, RFAT, MPG, LCPJ, TNLS, ATWS, FJC, ECS and ASL conceived the idea of study and its design. CFM, LVBC, YLH, JS, DJ, MBA, CHVM, AFR, ACSSN, CMSM, RFAT, LMSB, ATWS, LACA, EA, WA, RBM, LD, GMA, LMSM, IMS are essential to data collection. CFM, LVBC and VIAS wrote the first draft of the manuscript. ECS and ASL provided advice and supervision. All authors met the criteria: (1) Substantial contributions to the conception or design of the work; or the acquisition, analysis or interpretation of data for the work. (2) Drafting the work or revising it critically for important intellectual content. (3) Final approval of the version to be published (4) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

ACKNOWLEDGEMENTS

We are thankful to Marjorie Shafferman for her review of the manuscript.

FUNDING STATEMENT

This work was supported by FAPESP, São Paulo Foundation for Research [grant number 2016/01735-2] and Fundação Faculdade de Medicina USP.
The funders had no role in study design, data collection and analysis, decision to publish, or in the preparation of the manuscript.

COMPETING INTERESTS STATEMENT

Authors declare no financial and other competing interests for the overall trial design and execution.

	STUDY PERIOD											
	Enrolment	Allocation	Post-allocation									Close-out
TIMEPOINT	$-t_1$	0	D1	D2	D3	D4	D5	D6	D7	D10±1	D14±2	D28±3	D60±7
ENROLMENT:													
Eligibility screen	X												
Informed consent	X												
Allocation		X											
INTERVENTIONS:													
Active Group: Sofosbuvir + Standard care			←	—	—	—	—	—	—	→			
Control group: Standard care			←	—	—	—	—	—	—	—	—	—	→
ASSESSMENTS:													
Clinical evaluation			X	X	X	X	X	X	X	X	X	X	X
Adverse events			X	X	X	X	X	X	X	X	X	X	X
Plasma YF viral levels			X	X	X	X	X	X	X	X	X	X	
Urine YF viral levels			X	X	X	X	X	X	X	X	X	X	
Semen YF viral levels												X	
Sofosbuvir plasma levels			X	X	X	X	X	X	X	X	X	X	

YF: yellow fever. D1 corresponds to the allocation moment until 23h59; D2: from 0h until 23h59, D3: from 0h until 23h59, and so on. Clinical evaluation and adverse events assessments on D60 could be done by phone contact.

Figure 1. Timeline of procedures of SOFFA study.

254x142mm (300 x 300 DPI)

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	4
	2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	3	Date and version identifier	4
Funding	4	Sources and types of financial, material, and other support	16
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1,2,3,14,15
	5b	Name and contact information for the trial sponsor	
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	16
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	6
	6b	Explanation for choice of comparators	6
Objectives	7	Specific objectives or hypotheses	6

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	6
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7,8
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	7,8
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	7,8
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7,8
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	8
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	8
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	8,9
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	9
Methods: Assignment of interventions (for controlled trials)			
Allocation:			

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	10
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	10
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	10
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	10

	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	10
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	10,11
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	10,11
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	11
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	12
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	12
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	12
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	12
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	17
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	12

Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	12
	31b	Authorship eligibility guidelines and any intended use of professional writers	14,15
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.